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ORIGINAL ARTICLE

Impact of tillage and no-tillage cultivation on the occurrence of entomopathogenic fungi in soil with integrated plant protection

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Abstract

This study aimed to evaluate the impact of tillage and no-tillage cultivation on entomopathogenic fungi in the soil. The research was carried out in 2021-2022 on plots at the Field Experimental Station of the Institute of Plant Protection – National Research Institute in Winna Góra, Poland. Winter oilseed rape, pea and winter wheat were grown on the plots. Entomopathogenic fungi were isolated using the trapping insect method and their occurrence intensity was assessed. The results demonstrated a positive effect of no-tillage cultivation on the presence of entomopathogenic fungi in the soil. In May 2021, the greatest numbers of trapped insect larvae infected by fungi were in the pea plots (90%) with no-tillage systems, while with tillage it was 43% lower. In June 2022, the greatest number of larvae infected by fungi were in the pea plots (96.7%) with no-tillage systems, while with tillage it was 23% lower. Among the isolated entomopathogenic fungi, the dominant genus was *Cordyceps* in all cultivation technologies.

Keywords: entomopathogenic fungi, integrated plant protection, no-tillage cultivation, tillage

Introduction

Agrotechnical treatments used by farmers have a significant impact on both plant cultivation and microbiological processes in the soil. Soil cultivation techniques contribute to the development of soil fertility and health (Brzezińska 2009; Klimek *et al*. 2010). Soil is a part of the dynamic, living, natural terrestrial ecosystem. Organic matter is one of the most important components of soil (Magdoff and van Es 2021). Among the beneficial microorganisms in the soil, entomopathogenic fungi (EPF) play an essential role in reducing the population of many plant pests. EPF are a special group of soil-dwelling microorganisms that infect and kill insects by penetrating their cuticle. They are currently used as biocontrol agents against insect pests and play a vital role in their management. However, human activities have led to soil degradation, which negatively affects its biodiversity (Holland *et al*. 2017). Awareness of soil biodiversity and its importance for sustainable food production has markedly increased in recent years. In particular, the loss of soil biodiversity due to of intensive agriculture, land degradation and climate change has raised concerns about its expected negative impacts on ecosystem services, food security and human health (Pulleman *et al*. 2022).

The European Union (EU) has responded to consumers' expectations by developing various strategies to prevent further environmental devastation of the soil (EU 2023). One such initiative is the EU's "Biodiversity Strategy for 2030", an ambitious long-term plan aimed to protect nature and reverse the degradation of

ecosystems. This strategy aims to put Europe's biodiversity on a path to recovery by 2030. Another related Strategy, "The Farm to Fork" seeks to reduce the overall use of chemical pesticides and the associated risks by 50% by 2030 with a focus on minimizing the use of more hazardous pesticides. These strategies are still under discussion and each Member State aims to agree on conditions for the use of pesticides based on their specific needs.

The reduction of chemical plant protection products is expected to increase interest in alternative, particularly non-chemical, methods of plant protection, with biological control being priority. However, the range of biopesticides remains limited, especially in field crops in Poland (Sosnowska 2018). Therefore, in this context, conservation biological control, which involves creating suitable conditions for the development of beneficial microorganisms such as EPF. Such strategies require a solid understanding of the ecology of the species involved at the individual, community and landscape scale (Holland *et al*. 2017). Conservation biological control with EPF includes the manipulation of both the crop environment and habitats outside the crop (Pell *et al*. 2010). The fungal community structure is strongly influenced by conservation tillage, though how conservation tillage systems affect the soil fungal community structure remains poorly understood (Gao *et al*. 2022).

This research, based on a long-term experiment, aimed to determine the effect of tillage and no-tillage cultivation systems and plants on the occurrence of entomopathogenic fungi in soil in the western part of Poland.

Materials and Methods

Field experimental design

The research was conducted at the Field Experimental Station of the Plant Protection Institute – National Research Institute in Winna Góra (Poland) (52°12'17"N 17°26'48"E). The trials with conventional tillage (CT) and no-tillage (NT) techniques were established as a part of a long-term experiment, during two growing seasons, 2020–2021 and 2021–2022. Three crop designs were used: winter oilseed rape (R1), winter wheat (R1-in monoculture as forecrop before winter oilseed rape), and pea (R4). R1 meant the recommended standard for chemical protection was applied with a 3-year simplified crop rotation with a 2-year monoculture of winter wheat (winter oilseed rape – winter wheat – winter wheat) (crop rotation commonly used on large-scale farms). R4 meant that there was reduced chemical protection and a 3-year crop rotation (winter oilseed rape – winter wheat – pea). The field experiment was carried out using a split-block design with three replications. The experiment analyzed the effect of two factors:

- factor I – two cultivation systems: tillage and no-tillage,
- factor II: three plants: winter oilseed rape, winter wheat and pea.

The distance between crop variants was 4 meters and the distance between plots within a variant was 2 meters. Three replicates were used. In both 2021 and 2022, soil was collected from plots where winter oilseed rape (Artoga variety), pea (Hubal variety), and winter wheat (mix of Arkadia and Wilejka varieties) were grown. Recommended chemical protection (a protection based on full doses of herbicides, fungicides, and insecticides) was applied to the oilseed rape and wheat, while only herbicides (50% of the recommended dose) were used on the pea plots. The crops were grown on plots under both standard tillage (deep ploughing) and no-tillage using a cultivation unit for intensive but shallow soil processing systems. The CT plots were tilled on August 20, 2020 and August 17, 2021, to a depth of 25 cm. Winter oilseed rape was sown on August 28, 2020 and August 27, 2021 (3.6 kg/ha). Winter wheat was sown on October 5, 2020 and October 8, 2021 (350 kg/ha); while peas were sown on March 26, 2021 and March 28, 2022 (300 kg/ha). The chemical plant protection products used are listed in Tables 1 and 2. The same recommended NPK fertilization was applied to both CT and NT designs (Table 3). The following machines were used: tractor + plow, disc harrow, subsoiler, seeder and suspended tractor sprayer.

Soil sampling and entomopathogenic fungi isolation

Soil samples were collected monthly from May to August in both seasons (2021, 2022). From each plot, samples were taken from 10 randomly selected locations (edge effect was eliminated) with an area of 120 m^2 using a soil corer to a depth of 15 cm. The soil corers were sterilized with ethanol before using in each replication. Each variant had a separate soil corer. Soil samples were not sieved but put into plastic bags. These samples were mixed to create a single composite for each plot. About 3 kg of soil was taken from each variant. A total of three soil samples was taken from the three replicates of each variant, resulting in nine samples from plots with tillage and nine from plots with no-tillage systems. Proximity to plants was considered during sampling. Entomopathogenic fungi were isolated using the trap method (Zimmermann 1986). In the laboratory, 10 *Galleria mellonella* L. (Lepidoptera, Pyralidae) larvae (L3–L4 old) were placed in each soil container which was filled with soil up to lid (about 300 g), and their mortality was observed. In the

Table 1. Plant protection products used in growing season 2020/2021 (Winna Góra)

Cultivated species	Insecticides (a.i.) – application timing and dose [g · ha^{-1}]	Fungicides (a.i.) – application timing and dose [g · ha^{-1}]	Herbicides (a.i.) – application timing and dose [g · ha^{-1}]
Winter oilseed rape (R1)	11.09.2020 lambda-cyhalothrin (6)	31.03.2021 (mepiquat chloride + metconazole) + tebuconazole (105 + 15) + 125	21.09.2020 (halauxifen-methyl + picloram) + (metazachlor + aminopyralid picloram) (2.5 + 12) + (300 + 3.2 + 8)
	31.03.2021 lambda-cyhalothrin (6.25)		12.04.2021 quizalofop-P-ethyl (50)
	8.05.2021 cypermethrin (25)		13.07.2021 glyphosate (1080)
	13.05.2021 (acetamiprid + lambda-cyhalothrin) (20 + 6)		
Winter wheat (R1)	20.05.2021 cypermethrin (25)	13.03.2021 (fenpropimorph + epoxconazole) + epoxiconazole (200 + 67.2) + 375	28.10.2020 (diflufenican + flufenacet) (140 +140)
Pea (R4)	–	–	29.03.2021 (dimethenamid–P + pendimethalin) (510 + 600)

a.i. – active ingredient
R1 – chemical protection program (recommended number of treatments and pesticide doses)
R4 – limited chemical protection program (in terms of the number of treatments and pesticide doses)

Table 2. Plant protection products used in growing season 2021–2022 (Winna Góra)

Cultivated species	Insecticides (a.i.) – application timing and dose [g · ha^{-1}]	Fungicides (a.i.) – application timing and dose [g · ha^{-1}]	Herbicides (a.i.) – application timing and dose [g · ha^{-1}]
Winter oilseed rape (R1)	11.09.2021 (acetamiprid + lambda-cyhalothrin) (30+9)	28.03.2022 (mepiquat chloride +metconazole) + tebuconazole (105 +1 5) + 100	02.10.2021 (halauxifen-methyl +picloram) + (metazachlor + aminopyralid + picloram) (2.5 + 12) + (375 + 4 +1 0)
	24.03.2022 (acetamiprid + lambda-cyhalothrin) (20 + 6)		25.10.2021 quizalofop-P-ethyl (40)
	06.05.2022 lambda-cyhalothrin (7.5)		14.07.2022 glyphosate (1080)
Winter wheat (R1)	–	24.03.2022 (fenpropimorph + epoxiconazole) (150 + 50.4)	29.04.2022 (2,4D+aminopyralid+florasulam) + fenoxaprop-P-ethyl (180 + 10 + 5) + 69
Pea (R4)	–	–	14.05.2022 (bentazone + imazamox) (480 + 22.4)

a.i. – active ingredient
R1 – chemical protection program (recommended number of treatments and pesticide doses)
R4 – limited chemical protection program (in terms of the number of treatments and pesticide doses)

Table 3. Fertilization (N-P-K) in cultivated species in both growing seasons (2020–2021 and 2021–2022) –Winna Góra

Growing season	Application timing	Fertilizer doses [kg · ha^{-1}]								
		Winter oilseed rape (R1)			Winter wheat (R1)			Pea (R4)		
		N	P	K	N	P	K	N	P	K
2020–2021	21.08.2020	24	80	120	–	–	–	–	–	–
	02.10.2020	–	–	–	21	70	105	–	–	–
	02.03.2021	80	–	–	60	–	–	–	–	–
	18.03.2021	–	–	–	–	–	–	15	50	75
	04.04.2021	51	–	–	41	–	–	–	–	–
	Total	155	80	120	122	70	105	15	50	75
2021–2022	20.08.2021	32	80	120	–	–	–	–	–	–
	20.09.2021	–	–	–	24	60	90	–	–	–
	04.03.2022	75	–	–	60	–	–	–	–	
	28.03.2022		–	–				15	50	75
	01.04.2022	46	–	–	37					
	Total	153	80	120	121	60	90	15	50	75

middle of the surface of each container cover there was a 4 cm diameter mesh, so that the larvae had access to air. The soil was sprayed with distilled water when it dried out. Food for *G. mellonella* was not added. Containers with trapped insects were placed in a temperature-controlled chamber at 25°C in the dark. The first check for larval mortality was performed 7 days after setting up the experiment, and the next one after 10 days. A total of 30 larvae per treatment (field crop) was observed. Dead individuals were placed in Petri dishes on a glass slide with damp paper in a temperature-controlled chamber at 25°C. When mycelium appeared, slides were prepared, and spore-forming structures and spores were examined under a Olympus BX 40 microscope (40x magnification). Entomopathogenic fungi were identified microscopically based on the morphology of their microstructures by determining the size and shape of conidia, conidiogenic cells, and colony morphology using standard identification keys (Rehner and Buckley 2005; Rehner *et al.* 2011; Humber 2012; Inglis *et al.* 2012). Since only morphological methods were used to identify the fungi, they were classified at the genus level. As demonstrated by recent phylogenetic studies based on DNA sequencing (Brkič *et al.* 2004; Bischoff *et al.* 2006, 2009; Kepler *et al.* 2017), there are numerous species of fungi within the genera *Beauveria*, *Cordyceps* and *Metarhizium*, which are often indistinguishable without molecular methods.

Meteorological data

To determine the variable environmental factors influencing the growth of entomopathogenic fungi in soil, meteorological data from the Field Experimental Station of the Plant Protection Institute – National Research Institute in Winna Góra (Poland) were utilized. The average values of air temperature and precipitation are presented in Figures 1 and 2.

Statistical analysis

The experiment was carried out using a split-block design with three replications. Statistical analysis was performed using a two-way analysis of variance (ANOVA). Normality of the model's residuals was assessed with the Shapiro-Wilk test, and the homogeneity of variance was tested with Levene's test. Tukey's HSD test was used for post-hoc comparisons. The analyses were performed at a significance level of 0.05. Calculations were made using the Statistica 12 program.

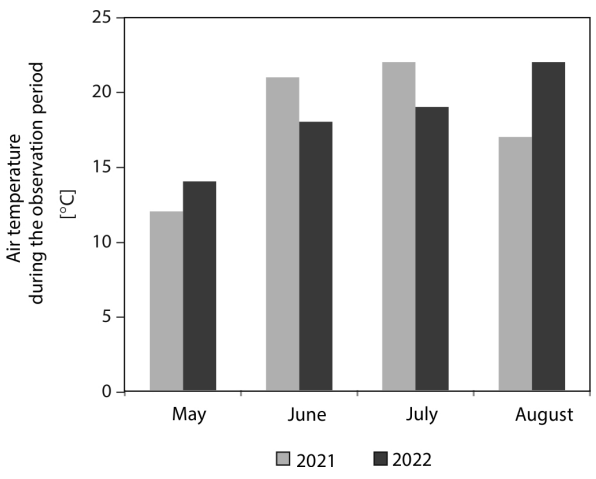

Fig. 1. Average of air temperature [°C] during the observation period in Winna Góra (2021–2022)

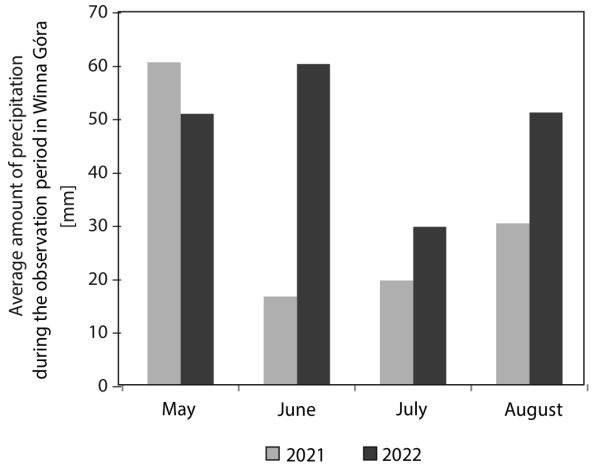

Fig. 2. Average amount of precipitation (mm) during the observation period in Winna Góra (2021–2022)

Results

In 2021, the highest intensity of entomopathogenic fungi occurrence was observed in no-tillage cultivation plots. The greatest numbers of trapped insect larvae infected by fungi were found in May in the pea plots (90%). Larval mortality decreased subsequently, reaching 73% in June, 33% in July, and 37% in August (Table 4). With tillage, larval mortality in May was significantly lower than with no-tillage cultivation ($F_{(1,2)} = 96.43$, $p = 0.0102$). In plots with peas, mortality was 43% lower in May, 40% in June, 20% in July and 24% in August which was comparable to no-tillage cultivation (Table 4). Similar trends were observed in 2022. The highest mortality of *G. mellonella* larvae occurred in plots with peas grown under the no-tillage system. In May, the mortality rate was 56.7%, reaching nearly 97% in June, 70% in July and the lowest rate of 26.7% was in August (Table 5). Compared to 2021 the high larval mortality in June and July may have been related to the increased precipitation during these months – 45 mm higher in June 2022 than in 2021, and 10 mm higher in July (Fig. 2). Conversely the average temperature in June and July 2021 was higher than in 2022 (Fig. 1). Heavy rainfall in May 2021 (above 60 mm), was associated with the highest *G. mellonella* larval mortality caused by EPF, reaching up to 90% in plots with peas grown in the no-tillage technology (Table 5). In contrast, tillage did not have this effect.

Table 4. Percent of dead *Galleria mellonella* larvae (%) in soils with tillage and no-tillage technology in different months and different plants (Winna Góra 2021)

Field crops	% of dead larvae (Mean ± SD)			
	May	June	July	August
Technology				
Tillage (CT)	20 ± 21.21 a	15.6 ± 20.68 a	6.7 ± 11.18	8.9 ± 10.54
No-tillage (NT)	70 ± 24.49 b	58.9 ± 28.92 b	27.8 ± 29.91	28.9 ± 24.72
F	96.43	18.78	6.94	3.86
p	0.0102	0.0493	0.1189	0.1885
Variants				
Winter rape R1	33.3 ± 35.02 a	21.7± 22.29	18.3± 36.01	21.7 ± 29.27
Pea R4	68.3 ± 27.14 b	53.3 ± 37.77	23.3 ± 21.6	25 ±1 8.71
Winter wheat R1	33.3 ± 31.41 a	36.7 ± 34.45	10 ± 12.65	10 ± 12.65
F	10.14	3.37	0.35	1.07
p	0.0272	0.1389	0.7251	0.4239
Technology * Variants				
Tillage * Winter rape R1	6.7 ± 5.77	6.7 ± 5.77	0 ± 0	6.7 ± 5.77
Tillage * Pea R4	46.7 ± 11.55	33.3 ± 30.55	13.3 ± 15.28	13.3 ± 15.28
Tillage * Winter wheat R1	6.7 ± 5.77	6.7 ± 5.77	6.7 ± 11.55	6.7 ± 11.55
No-tillage * Winter rape R1	60 ± 30	36.7 ± 23.09	36.7 ± 47.26	36.7 ± 37.86
No-tillage * Pea R4	90 ± 17.32	73.3 ± 37.86	33.3 ± 25.17	36.7 ± 15.28
No-tillage * Winter wheat R1	60 ± 17.32	66.7 ± 15.28	13.3 ± 15.28	13.3 ± 15.28
F	0.11	2.8	0.57	0.84
p	0.8957	0.1736	0.6068	0.4964

SD – Standard Deviation;
Different letters indicate statistically significant differences

When rainfall dropped to 15 mm in June 2021, the efficacy of EPF declined, resulting in 18% lower *G. mellonella* larval mortality in plots with peas without tillage. In the following month it dropped to 33%, which was also related to lower rainfall. In 2022, the average precipitation in June, July and August was higher than in 2021 (Fig. 2). During these months, the greatest infection rates of *G. mellonella* larvae reached 96.7% in June, 70% in July and decreased to 26.7% in August in the pea plots with no-tillage technology (Table 5).

The results of observations conducted in 2021–2022 clearly indicate the positive impact of no-tillage cultivation on the occurrence of entomopathogenic fungi in the soil. In May 2021, the average the mortality of larvae with no-tillage cultivation was 50% higher, in June by 43%, in July by 21% and in August by 20% than with tillage (Table 4). These differences were statistically significant in May and June ($F_{(1,2)} = 96.43$, $p = 0.0102$, $F_{(1,2)} = 18.78$, $p = 0.0493$). Similarly in 2022 larval mortality was 10% higher in May without tillage than with tillage ($F_{(1,2)} = 27$, $p = 0.0351$), 18% higher in June ($F_{(1,2)} = 19.69$, $p = 0.0472$), 29% in July, and in August by 21% (Table 5).

Among the isolated fungi, the dominant species belonged to the genus *Cordyceps*. *Cordyceps* species was most frequently isolated from pea plots under both no-tillage and tillage systems in each year of observation. In May 2021, it caused 90% of larval mortality with no-tillage pea cultivation while in June 2022 it was responsible for over 96% of larval infections in the same crop (Tables 6, 7). Fungi of the genus *Beauveria* constituted a small percentage of the isolated species. In June 2021 it caused only 6.7% of larval mortality in no-tillage pea plots (Table 7), while in 2022 it was not isolated at all. *Metarhizium* fungi were not isolated in 2021, but in June 2022 they caused 36.7% of larval mortality in tillage cultivated pea plots (Table 7).

In 2021 an insecticide was applied to winter oilseed rape plots in March (lambda-cyhalothrin) and two insecticides in May (cypermethrin; acetamiprid, lambda-cyhalothrin) (Table 1). A fungicide was applied to these plots in March pentamethyleneimine, metconazole) and an herbicide in April (quizalofop-p-ethyl) (Table 1). In the winter wheat plots, a fungicide (fenpropimorph, epoxconazole) was applied in March 2021, and insecticide (cypermethrin) in May. All chemical products were applied at recommended doses. No herbicide was applied. Analyzing the intensity of EPF occurrence in the soil on these plots, no differences were observed in the mortality of *G. mellonella* larvae. In both variants it was 33% of dead larvae in May on plots with winter oilseed rape and

Table 5. Percent of dead *Galleria mellonella* larvae (%) in soils with tillage and no-tillage technology in different months and different plants (Winna Góra 2022)

Field crops	% of dead larvae			
	May	June	July	August
Technology				
Tillage (CT)	38.9 ± 23.69 a	53.3 ± 23.45 a	12.2 ± 19.86	2.2 ± 4.41
No-tillage (NT)	48.9 ± 20.88 b	71.1 ± 23.15 b	41.1 ± 32.57	23.3 ± 20.62
F	27	19.69	5.08	6.94
p	0.0351	0.0472	0.1529	0.1189
Variants				
Winter rape R1	30 ± 14.14	51.7 ± 19.41a	10 ± 10.95 a	6.7 ± 5.16
Pea R4	51.7 ± 26.39	85 ± 23.45 b	50 ± 35.78 b	13.3 ± 15.06
Winter wheat R1	50 ± 20.98	50 ± 12.65 a	20 ± 25.3 ab	18.3 ± 27.87
F	2.34	14.27	10.4	1.14
p	0.2120	0.0151	0.0260	0.4061
Technology * Variants				
Tillage * Winter rape R1	30 ± 10	40 ± 10	6.7 ± 11.55	3.3 ± 5.77
Tillage * Pea R4	46.7 ± 35.12	73.3 ± 30.55	30 ± 26.46	0 ± 0
Tillage * Winter wheat R1	40 ± 26.46	46.7 ± 15.28	0 ± 0	3.3 ± 5.77
No-tillage * Winter rape R1	30 ± 20	63.3 ± 20.82	13.3 ± 11.55	10 ± 0
No-tillage * Pea R4	56.7 ± 20.82	96.7 ± 5.77	70 ± 36.06	26.7 ± 5.77
No-tillage * Winter wheat R1	60 ± 10	53.3 ± 11.55	40 ± 20	33.3 ± 35.12
F	0.47	0.21	1.03	1.21
p	0.6537	0.8196	0.4354	0.3879

SD – Standard Deviation;
Different letters indicate statistically significant differences

Table 6. Percentage of dead *Galleria mellonella* larvae [%] in soils with and without tillage (Winna Góra, 2021) caused by various entomopathogenic fungi

Mortality factor	% of dead *G. mellonella* larvae in soils with tillage system											
	May			June			July			August		
	winter rape	pea	winter wheat	winter rape	pea	winter wheat	winter rape	pea	winter wheat	winter rape	pea	winter wheat
Cordyceps spp.	6.6	40	6.7	6.7	33.3	13.3	0	13.3	6.7	0	3.3	0
Beauveria spp.	0	6.7	0	0	0	0	0	0	0	0	0	0
Metarhizium spp.	0	0	0	0	0	0	0	0	0	0	0	0
	% of dead *G. mellonella* larvae in soils with no-tillage system											
Cordyceps spp.	53.3	90	50	36.7	66.7	53.3	36.7	33.3	13.3	26.7	16.7	3.3
Beauveria spp.	6.7	0	3.3	0	6.7	0	0	0	0	0	0	0
Metarhizium spp.	0	0	0	0	0	0	0	0	0	0	0	0

Table 7. Percentage of dead *Galleria mellonella* larvae [%] in soils with and without tillage (Winna Góra, 2022) caused by various entomopathogenic fungi

Mortality factor	% of dead *G. mellonella* larvae [%] in soils with tillage system											
	May			June			July			August		
	winter rape R1/R4	pea	winter wheat	winter rape R1/R4	pea	winter wheat	winter rape R1/R4	pea	winter wheat	winter rape R1/R4	pea	winter wheat
Cordyceps spp.	23.3/16.7	43.3	40	40/23.3	36.7	46.7	6.7/10	30	0	3.3/0	0	3.3
Beauveria spp.	0/0	0	0	0/0	0	0	0/0	0	0	0/0	0	0
Metarhizium spp.	0/0	0	0	0/0	36.7	0	0/0	0	0	0/0	0	0
	% of dead *G. mellonella* larvae in soils with no-tillage system											
Cordyceps spp.	26.7/50	56.7	60	63.3/50	96.7	53.3	13.3/23	70	40	10/37	26.7	30
Beauveria spp.	0/0	0	0	0	0	0	0	0	0	0/0	0	0
Metarhizium spp.	3.3/3.3	0	0	0	0	0	0	0	0	0/0	0	3.3

R1/R4 – chemical protection/half doses of chemical protection

winter wheat (Table 4). In the pea plots, the mortality of larvae reached 68% (Table 4) in May and was 35% higher than in the winter oilseed rape and winter wheat crops. In pea plots, only half of the recommended herbicide (dimethenamide, pendimethalin) dose was applied in March 2021. In May 2021 the differences between larval mortality were significant ($F_{(2,4)} = 10.14$, $p = 0.0272$).

In 2022, insecticides were applied to winter oilseed rape plots in March (acetamiprid, lambda-cyhalothrin) and May (lambda-cyhalothrin), and an herbicide (quizalofop-p-eth-1) in April (Table 2). In contrast, only herbicide spraying (aminopyralid+fonoxaprop-p--ethyl) was used in April on plots with winter wheat. Where there were more chemical treatments we observed 30% of dead *G. mellonella* larvae in May (winter oilseed rape) in tillage and no-tillage systems, and in plots where there were less chemicals 40% of dead larvae were observed with tillage, and 60% with no-tillage (winter wheat) (Table 5).

Discussion

Conservation biological control relies on modifying the environment or management practices to protect and encourage natural enemies within the system. This approach requires a solid understanding of the ecology of the species concerned at the individual, community and landscape scale (Pell *et al*. 2010). Conservation biological control also includes agricultural practices compatible with the maintenance of natural enemy populations. Such agricultural practices that affect microorganisms in the soil are tillage and no-tillage systems.

Various studies indicate the superiority of no-tillage farming over tillage farming. Most importantly, no-tillage significantly protects fields from excessive soil erosion, allows organic matter accumulation by decreasing decomposition, and improves overall soil health (Kladivko 2001; Małecka-Jankowiak *et al*. 2016;

Smith *et al*. 2016; Srour *et al*. 2020; Liu *et al*. 2021). The higher moisture content of soils cultivated in no-tillage systems favors the development of entomopathogenic fungi. This research showed a greater intensity of EPF in soils cultivated without tillage as evidenced by higher parasitism of *G. mellonella* larvae. This finding agrees with Tkaczuk (2008) who found that the use of manure and no-tillage cultivation systems affects the species and quantitative composition of EPF in the soil. Sosa-Gomez *et al*. (2001) observed a higher incidence of entomopathogenic fungi in no-tillage systems. Under these experimental conditions, *Metarhizium anisopliae* Sorokin (Hypocreales, Clavicipitaceae) and *Beauveria bassiana* (Balsamo, Vuillemin) (Hypocreales, Cordicipitaceae) were more prevalent than *Purpureocillium lilacinum* (formerly *Paecilomyces lilacinus*) (Ophiocordycipitaceae).

Wang *et al*. (2016) found that no-tillage improved soil microbiological properties. In Portugues olive groves Oliveira *et al*. (2013) showed higher occurrence, diversity, and abundance of entomopathogenic fungi under no-tillage systems than tilled ones. Recently Korniłłowicz-Kowalska *et al.* (2022) demonstrated a significantly higher total number of rhizosphere fungi in the no-tillage system than in the tillage system, with the dominant group of fungi being the order Hypocreales (Ascomycota). In South Brazil Dorr de Quadros *et al*. (2012) recorded higher microbial diversity without tillage, particularly in plots with cereal crops (oat and maize).

This research revealed that in May and June the greatest mortality rate of *G. mellonella* larvae caused by entomopathogenic fungi occurred in no-tillage systems with pea cultivation. In contrast, in tillage systems, larval mortality was much lower. Legumes, such as peas, are valuable agricultural crops worldwide. Their nutritional properties likely contribute to the greatest occurrence of entomopathogenic fungi in the soil (Ofuya and Akhidue 2005; Brkič *et al*. 2004; Knight 2012; Faligowska *et al*. 2022).

Entomopathogenic fungi occur naturally in the soil and play an essential role in regulating insect populations. However, soil parameters such as texture, humidity, temperature, and organic matter influence the abundance of fungal species (Quesada-Moraga *et al*. 2007; Sun and Liu 2008; Garrido-Jurado *et al*. 2011). In the observations recorded here three types of entomopathogenic fungi were identified: *Beauveria*, *Metarhizium* and *Cordyceps*. The dominant genus in the western part of Poland was *Cordyceps*. Tkaczuk *et al*. (2014) recorded that *M. anisopliae* was the most frequent fungus in soils from both organic and conventional fields in the eastern part of Poland. It also infected the largest number of *G. mellonella* larvae. *Cordyceps fumosorosea* (Wize) Kepler, B. Shrestha & Spatafora (Hypocreales, Cordicipitaceae) formed more colony forming units (CFU) in conventionally cultivated soils. According to Vänninen (1995), *M. anisopliae* is the most tolerant species to agricultural practices like tillage and the use of chemical protection, and it can tolerate the periodic absence of potential hosts. In the plots observed in Winna Góra, the dominant genus was *Cordyceps*, while *Metarhizium* spp. was rarely isolated.

In Portugal, studies by Sharma *et al*. (2021) showed that the use of pesticides in the protection of Portuguese vines had no significant effect on the presence of entomopathogenic fungi which were isolated to a similar extent from both herbicide-treated and untreated research sites. Sosnowska (2000) indicated that herbicides had no effect on nematopathogenic fungi in beetroot plots in the central part of Poland. A greenhouse experiment by Clifton *et al.* (2015) when fungicides and herbicides were applied to the soil surface, no significant effects on entomopathogenic fungi were observed. They suggested, that fungicides and herbicides may not be toxic to soil-borne entomopathogenic fungi as originally thought. The same trend was observed in the results given here, namely, that the greater intensity of EPF occurrence was in plots with fewer chemical treatments. A laboratory study by Fiedler and Sosnowska (2017) when fungicides and insecticides were used against different EPF showed, that all tested insecticides can be applied together with *B. bassiana*. They even stimulate sporulation of this fungus at the recommended dose. In the case of fungicides, they observed inhibition of growth and sporulation of *B. bassiana*. The studies reported here showed that in pea plots where only herbicides were applied at half of the recommended dose, EPF intensity was higher than in winter oilseed rape and wheat plots where insecticides, fungicides and herbicides were used.

Weather conditions may also influence the occurrence of fungi in the soil. The higher precipitation in May 2021 than in 2022 was associated with greater larval mortality in no-tillage pea plots. Inglis *at al*. (2001) noted, that precipitation has beneficial effects on fungal epizootics, and plays a vital role in conidia dispersion. Heavy rainfall can remove large quantities of spores and conidia from the pests' cuticles. The present observations showed a greater abundance of entomopathogenic fungi (higher larval mortality) in the soil after heavy rainfall. Changes were observed in the intensity of fungi occurrence in the month with heavy rainfall. Searle and Doberski (1984) reported that environmental conditions such as temperature and humidity have a strong impact on the virulence and success of entomopathogenic fungi, confirming what was observed in the presented results.

Ultimately this study demonstrated that no-tillage cultivation can be a valuable strategy for promoting the occurrence of entomopathogenic fungi in soil.

This has implications for sustainable agriculture and natural pest control. Future research could explore the specific mechanisms through which no-tillage benefits entomopathogenic fungi and investigate the long-term effects of different cultivation practices on their populations.

Conclusions

- The no-tillage system positively influenced the occurrence of entomopathogenic fungi in the soil.
- In soils with pea cultivation under the no-tillage system, the greatest intensity of entomopathogenic fungi and the highest mortality rates of *G. mellonella* trap larvae were observed.
- Chemical protection can influence the occurrence of entomopathogenic fungi in soil conditions.
- Changes in rainfall intensity can affect the entomopathogenic fungi. Greater precipitation is associated with higher effectiveness of entomopathogenic fungi against *G. mellonella* larvae.
- Among the isolated entomopathogenic fungi, the dominant genus was *Cordyceps* in all cultivated technologies.

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References

Bischoff J.F., Rehner S.A., Humber R.A. 2006. *Metarhizium frigidum* sp. nov.: a cryptic species of *M. anisopliae* and member of the *M. flavoviride* complex. Mycologia 98 (5): 737–745. DOI: https://doi.org/10.3852/mycologia.98.5.737

Bischoff J.F., Rehner S.A., Humber R.A. 2009. A multilocus phylogeny of the *Metarhizium anisopliae* lineage. Mycologia 101 (4): 512–530. DOI: https://doi.org/10.3852/07-202

Brkič S., Milakovič Z., Kristek A., Antunovič M. 2004. Pea yield and its quality depending on inoculation, nitrogen and molybdenum fertilization. Plant, Soil and Environment 50: 39–45. DOI: https://doi.org/10.17221/3640-PSE

Brzezińska M. 2009. Wykorzystanie ekofizjologicznych wskaźników mikrobiologicznych do oceny jakości gleby. Postępy Nauk Rolniczych 1: 39–51.

Clifton Eric H., Stefan T. Jaronski, Erin W. Hodgson, Aaron J. Gassmann. 2015. Abundance of soil-born entomopathogenic fungi in organic and conventional fields in the midwestern USA with an emphasis on the effect of herbicides and fungicides on fungal persistence. Plos One 10 (7): 1–17. DOI: https://doi.org/10.1371/journal.pone.0133613

Dorr de Quadros Patricia, Kateryna Zhalnina, Austin Davis-Richardson, Jennie R. Fagen, Jennifer Drew, Cimelio Bayer, Flavis A.O. Camargo, Eric W. Triplett. 2012. The Effect of tillage system and crop rotation on soil microbial diversity and composition in a subtropical Acrisol. Diversity 4 (4): 375–395. DOI: https://doi.org/10.3390/d4040375

EU. 2023. Farm to Fork strategy 2023. Available on: [https://food.ec.europa.eu/horizontal-topics/farm-fork-strategy_en]

Faligowska A., Kalembasz S., Kalembasa D., Panasiewicz K., Szymańska G., Ratajczak K., Skrzypczak G. 2022. The nitrogen fixation and yielding of pea in different soil tillage systems. Agronomy 12 (2): 352. DOI: https://doi.org/10.3390/agronomy 12020352

Fiedler Ż., Sosnowska D. 2017. Side effects of fungicides and insecticides on entomopathogenic fungi *in vitro*. Journal of Plant Protection Research 57 (4): 355–360. DOI: https://doi.org/10.1515/jppr-2017-0048

Gao Ming, Haiyu Li, Meng Li. 2022. Effect of no tillage system on soil fungal community. structure of cropland in mollisol: a case study. Frontiers in Microbiology 13: 1–9. DOI: https://doi.org./10.3389/fmicb.2022.847691

Garrido-Jurado J., Torrent J., Barron V., Corpas A., Quesada-Moraga E. 2011. Soil properties affected the availability, movement, and virulence of entomopathogenic fungi conidia against puparia of *Ceratis capitate* (Diptera: Tephritidae). Biological Control 58 (3): 277–285. DOI: https://doi.org/10.1016/j.biocontrol.2011.05.017

Holland J.M., Douma J.C., Crowley L., James L., Kor L., Stevanson D.R.W., Smith B.W. 2017. Semi-natural habitats support biological control, pollination and soil conservation in Europe. A review. Agronomy for Sustainable Development 37 (4): 31–54.

Humber A.R. 2012. Identification of entomopathogenic fungi. p. 151–187. In: "Manual of Techniques in Invertebrate Pathology" (L.A. Lacey. ed.). Academic Press–London, UK, 504 pp. DOI: https://doi.org/10.1016/B978-0-12-386899-2.00006-3

https://eurlex.europa.eu?legalcontent/EN/TXT/?qid=1590574123338&uri=CELEX%3A52020DC0380

Inglis G.D., Enkerli J., Goettel M.S. 2012. Laboratory techniques used for entomopathogenic fungi: Hypocreales. p. 189–253. In: "Manual of Techniques in Invertebrate Pathology" (L.A. Lacey ed.). Academic Press–London, UK, 504 pp. DOI: https://doi.org/10.1016/B978-0-12-386899-2.00007-5

Inglis G.D., Goettel M.S., ButtT.M., Strasser H., Helen E.R., Fernando E.V., Dave C., Mark S., Goettel J.P., Eric W. 2001. Fungi as biocontrol agents: progress, problems and potential. Springer: 23–69. DOI: https://doi.org/10.1079/97808511993560.0023

Kepler R.M., Luangsa-ard J.J., Hywel-Jones N.L., Quandt C.A., Sung G.H., Rehner S.A., Aime M.C., Henkel T.W., Sanjuan T., Zare R., Chen M., Li Z., Rossman A.Y., Spatafora J.W., Shrestha B. 2017. A phylogenetically-based nomenclature for *Cordycipitaceae* (*Hypocreales*). IMA Fungus 8 (2): 335–353. DOI: https://doi.org/10.5598/imafungus.2017.08.02.08

Kladivko E.J. 2001. Tillage systems and soil ecology. Soil and Tillage Research 61 (1–2): 61–76. DOI: https://doi.org/10.1016/SO167-1987(01)00179-9

Klimek B., Stefanowicz A.M., Woch M.W., Jaźwa M. 2010. Czy istnieje związek między bioróżnorodnością roślin i mikroorganizmów glebowych? Kosmos 59: 589–598. DOI: https://kosmos.ptpk.org/index.php/Kosmos/article/view/860/845 (in Polish)

Knight J.D. 2012. Frequency of field pea in rotation impacts biological nitrogen fixation. Canadian Journal of Plant Science 92: 1005–1011. DOI: https://doi.org/10.4141/cjps2011-274

Korniłłowicz-Kowalska T., Andruszczak S., Bohacz J., Kraska P., Możejko M., Kwiecińska-Poppe E. 2022. The effect of tillage and no-tillage system on culturable fungal communities in the rhizosphere and soil of two spelt cultivars. Applied Soil Ecology 174. DOI: https://doi.org/10.1016/j.apsoil.2022.104413

Magdoff F., van Es H. 2021. Organic matter – the key to healthy soils. p. 3–49. In: "Building Soils for Better Crops. Ecologi-

cal Management for Healthy Soils" (F. Magdoff, H. van Es, eds.). 4th ed. University of Maryland, USA, 381 pp.

Małecka-Jankowiak I., Blecharczyk A., Swędrzyńska D., Sawinska Z., Piechota T. 2016. The effect of long-term tillage systems on some soil properties and yield of pea (*Pisum sativum* L.). Acta Scientinarum Polonorum Agricultura 15 (1): 37–50.

Ofuya Z.M., Akhidue V. 2005. The role of pulses in human nutrition: A Review. Journal of Applied Sciences and Environmental Management 9: 99–104. DOI: http://www.bioline.org.br/ja

Oliveira I., Pereira J., Quesada-Moraga E., Lino-Neto T., Bento A., Baptista P. 2013. Effect of soil tillage on natural occurrence of fungal entomopathogens associated to *Prays oleae* Bern. Scienta Horticulturae 159: 190–196. DOI: https://doi.org/10.1016/j.scienta.2013.05.009

Pell J.K., Hannam J.J., Steinkraus D.C. 2010. Conservation biological control using fungal entomopathogens. Biological Control 55 (1): 187–198. DOI: https://doi.org/10.1007/s10526-009-9245-6

Pulleman Mirjam M., Wietse de Boer, Ken E. Giller, Thom W. Kuyper. 2022. Soil biodiversity and nature-mimicry in agriculture; the power of metaphor? Outlook on Agriculture 51 (1): 75–90. DOI: https://doi.org/10.1177/00307270221080180

Quesada-Moraga E., Navas-Cortes J.A., Maranhoo E.A., Ortiz-Urquiza A., Santiago-Alvarez C. 2007. Factors affecting the occurrence and distribution of entomopathogenic fungi in natural and cultivated soils. Mycological Research 111 (8): 947–966. DOI: https://doi.org/10.1016/j.mycres.2007.06.006

Rehner S.A., Buckley E.P.A. 2005. *Beauveria* phylogeny inferred from nuclear ITS and EF1-α sequences: evidence for cryptic diversification and links to *Cordyceps teleomorphs*. Mycologia 97 (1): 84–98. DOI: https://doi.org/10.3852/mycologia.97.1.84

Rehner S.A., Minnis A.M., Sung G.H., Luangsa-ard J.J., Devotto L., Humber R.A. 2011. Phylogeny and systematics of the anamorphic, entomopathogenic genus *Beauveria*. Mycologia 103 (5): 1055–1073. DOI: https://doi.org/10.3852/10-302

Searle T., Doberski J. 1984. An investigation of the entomopathogenic fungus *Beauveria bassiana* (Bals.) Vuill. As a potential biological control agent for *Oryzaephilus surinamensis* (L.). Journal of Stored Products Research 20: 17–23. DOI: https://doi.org/10.1016/0022-474X(84)90031-6

Sharma L., Oliveira I., Gonçalves F., Raimundo F., Singh R.K., Torres L., Marques G. 2021. Effect of soil chemical properties on the occurrence and distribution of entomopathogenic fungi in Portuguese grapevine fields. Pathogens 10 (2): 137. DOI: https://doi.org/10.3390/pathogens10020137

Smith Ch.R., Blair P.L., Boyd Ch., Cody B., Hazel A., Hedrick A., Kathuria H., Khurana P., Kramer B., Muterspaw K., Peck Ch., Sells E., Skinner J., Tegeler C., Wolfe Z. 2016. Microbial community responses to soil tillage and crop rotation in a corn/soybean agroecosystem. Ecology and Evolution 6: 8075–8084.

Sosa-Gomez Daniel R., Katiaires Delpin, Flavio Moscardi, Jose R.B. Farias. 2001. Natural occurrence of the entomopathogenic fungi *Metarhizium*, *Beauveria* and *Paecilomyces* in soybean under till and no-till cultivation systems. Neotropical Entomology 30 (3): 407–410. DOI: https://doi.org/10.1590/S1519-566X2001000300011

Sosnowska D. 2000. Wpływ herbicydów na rozwój grzybów nicieniobójczych. Progress in Plant Protection/Postępy w Ochronie Roślin 40 (2): 758–760.

Sosnowska D. 2018. Konserwacyjna metoda biologiczna wsparciem integrowanej ochrony roślin i rolnictwa ekologicznego. Progress in Plant Protection 58 (4): 288–293. DOI: https://doi.org/10.14199/ppp-2018-040

Srour A.Y., Ammar H. A., Subedi A., Pimentel M., Cook R.L., Bond J., Fakhoury A.M. 2020. Microbial communities associated with long-term tillage and fertility treatments in a corn-soybean cropping system. Frontiers in Microbiology 11. DOI: https://doi.org/10.3389/fmicb.2020.01363

Sun B.-D., Liu X.-Z. 2008. Occurrence and diversity of insect associated fungi in natural soils in China. Applied Soil Ecology 39 (1): 100–108. DOI: https://doi.org/10.1016/j.apsoil.2007.12.001

Tkaczuk C. 2008. Występowanie i potencjał infekcyjny grzybów owadobójczych agrocenoz i środowisk seminaturalnych w krajobrazie rolniczym. Rozprawy Naukowe 94. Wyd. APSiedlce, 160 pp. (in Polish)

Tkaczuk C., Król A., Majchrowska-Safaryan A., Nicewicz Ł. 2014. The occurrence of entomopathogenic fungi in soils from field cultivated in a conventional and organic system. Journal of Ecological Engineering 15 (4): 137–144. DOI: https://doi.org/10.12911/22998993.1125468

Vänninen L. 1995. Distribution and occurrence of four entomopathogenic fungi in Finland: effect of geographical location, habitat type and soil type. Mycological Research 100: 93–101.

Wang Z.T., Liu L., Chen Q., Wen X.X., Liao Y.C. 2016. Conservation tillage increases soil bacterial diversity in the dryland of northern China. Agronomy for Sustainable Development 37 (44). DOI: https://doi.org/10.1007/s13593-016-0366-x

Liu Z., Cao S., Sun Z., Wang H., Qu S., Lei N., He J., Dong Q. 2021. Tillage effect on soil properties and crop yield after land reclamation. Scientific Reports 11. Agriculture number 4611. DOI: https://doi.org/10.1038/s41598-021-84191-z.

Zimmermann G. 1986. The „Galleria bait method" for detection of entomopathogenic fungi in soil. Journal of Applied Entomology. DOI: https://doi.org/10.1111/j.1439-0418.1986.tb00912.x