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Impact of Different Tillage Systems on Soil Dehydrogenase Activity and Spring Wheat Infection

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ABSTRACT

Field trials were conducted at the Experimental Station (Brody) belonging to Poznan University of Life Sciences (NE Poland). The purpose was to evaluate the impact of different tillage systems and white mustard cover crop on soil enzymatic activity and severity of plant infection by pathogenic fungi in spring wheat. A randomized complete block design was set up with four replicates per treatment (conventional and no-tillage with and without cover crop). The results demonstrated higher enzyme activity in the soil treated post wheat harvest with herbicide Glyphosate at the rate of 4.0 L ha^{-1}, 360 g L^{-1} ai, [*N*-(phosphonomethyl) glycine] with adjuvant AS 500 SL 1.5 L ha^{-1} of the stubble, white mustard cover crop in direct sowing. Less activity was observed during spring time application of Glyphosate at the rate of 1.5 L ha^{-1}+adjuvant AS 500 SL 1.5 L ha^{-1}, followed by direct sowing of spring wheat. The main objective of this study was to evaluate the effects of tillage system and cover crop on soil dehydrogenase activity and plant health of spring wheat. Conducted investigation showed that there was no significant impact of the tillage system or left biomass on the eyespot (*Oculimacula acuformis*) and brown foot root (*Fusarium* sp.) diseases. Only with take-all (*Gaumannomyces graminis* var. *tritici*) there was significant impact of soil tillage system on the percentage of infected plants. During the tillering stage of the spring wheat significantly higher enzyme activities were observed on the treatment with cover crop and spring wheat cultivation in no tillage technology.

Keywords: Activity, Conventional tillage, Cover crop, Diseases, No-tillage, Soil.

INTRODUCTION

Soil tillage systems affect soil compaction, water dynamics, soil temperature and crop yield due to changes in soil microbiological activity and disease intensity. Soil quality depends on physical, chemical, and soil biological properties (Billalis *et al.,* 2012). Physical and chemical changes in soil are lower compared to changes in the soil microbiological properties including enzyme activities. The latter are usually measured for monitoring the soil quality and its degradation (Omidi *et al.,* 2008). Physical, chemical, and biological properties of the soil may be affected by changing soil tillage system from ploughing to ploughless with shallow cultivation or direct drilling (Kladivko, 2001; Małecka *et al.,* 2012). Biological, physical, and chemical processes continually interact with time, resulting in a diversely arranged mixture of soil minerals, organic matter, and pore spaces that together define soil structure (Blanco-Canqui *et al.,* 2005). This organic matter, with a variable level of decomposition, can enhance the microbial biomass and activity (Pankhurst, *et al.,* 2002). Concerning plant disease development, contrasting results have been

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observed (Bockus and Shroyer, 1998). The effect of limited tillage on plant pathogens greatly depends on specific regional crop–pathogen–environment interactions (Paulitz *et al.,* 2002). Even for the same pathosystem, different effects can be observed. For example, concerning take-all of wheat, Cook and Haglund, (1991) found an increase in disease severity with conservation tillage compared with conventional tillage, whereas de Boer *et al.,* (1993) reported that the incidence of take-all on plants was up to twice as high in a conventionally cultivated treatment as in a direct drill treatment.

From the perspective of sustainable crop production systems, it is essential to amend organic matter and to maintain soil cover by using different plants, as well as mulching process (Ferreira and Martin-Didonet, 2012). Intercrop, cover plants being left on the surface of a field as mulch positively affect properties of soil. Consequently its structures and water-air relations are improved (Błażewicz-Woźniak, 2005).

A good indicator of what is happening in the soil environment following the agrotechnical treatments is the activity of the enzymes responsible for the changes in the composition of the soil (Bielińska *et al.,* 2004). It reflects the changes in the specific abilities of the soil complex influenced by cultivation methods (Bandick and Dick, 1999; Hirzel and Matus, 2013). Enzymatic tests to analyze the functioning of the landscape structures allow estimating an efficiency of the agricultural landscape shaping recommendations (Bielińska and Węgorek, 2005).

Dehydrogenase enzymes are one of the most commonly used biological indicators used for evaluation of the ecological well-being of the soil environment. Total activity of the DHO's is the indicator of the redox system and measure of the respiratory activity of soil microorganisms (Kieliszewska-Rokicka, 2001; Bennicelli *et al.,* 2009). Marking the activity of the DHO's is the source of information about the population of living microorganisms in the soil and total microbiological activity of the soil (Makoi and Ndakidemi, 2008). Klikocka *et al*., (2012) found positive correlation between the biological activity of the soil and yield of crops, which confirms the validity of using enzymatic markers as assessment tests for soil fertility.

The main objective of this study was to evaluate the effects of tillage system and cover crop on soil dehydrogenase activity and plant health of spring wheat.

MATERIALS AND METHODS

Experimental Design

The experimental field was established in the Brody Research and Education Station of the Poznan University of Life Sciences, Poland (52° 25' N; 16° 18' E) on soil classified (WRB, 2007) as *Albic Luvisols* developed on loamy sands overlying loamy material (12% clay, 19% silt and 69% sand). The studies, carried out over 2009-2011, involved a static field experiment. The 0-20 cm soil layer had 1.61% organic matter, pH 6.2 (measured in 1M KCl), available contents of P, K and Mg, at 226, 170 and 42 mg kg^{-1}, respectively, at the beginning of the experiment.

Spring wheat cultivar, Vinjett, was sown at the rate of 500 seeds per 1 m^2 across all tillage systems. Two tillage systems were arranged in a randomized block design in four replications, resulting in a total of 8 plots. The size of each tillage plot was 10 m long and 5 m wide. The plots were separated by 0.3 m wide buffer strips and 6 m gaps between blocks for the tractor. The straw of spring wheat of previous crops was removed from all plots in all years. White mustard cover crop cultivar Salvo was sown at the rate of 20 kg ha^{-1} in the Conventional Tillage (CT) (after skimming) and No-Tillage (NT). Prior to planting, 4.0 L ha^{-1} of Glyphosate herbicide+1.5 L ha^{-1} adjuvant As 500 SL was applied to experimental area (Table 1).

Spring wheat was sown in the: (1) Conventional Tillage (CT), and (2) No-

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Table 1. Experimental treatment.

Treatment	Soil tillage system under spring wheat	Dose of Glyphosate ((*N*-(phosphonomethyl)glycine)) 360 g L^{-1}+As 500 SL	Soil tillage system under cover crop (White mustard)
1	CT (Spring plowing)	-	-
2	CT (Spring plowing)	-	CT (Skimming)
3	NT (No tillage)	1.5 l ha^{-1} +1.5 l ha^{-1} (before spring wheat sowing)	CT (Skimming)
4	CT (Spring plowing)	4.0 l ha^{-1}+1.5 l ha^{-1} (before white mustard sowing)	NT (No tillage)
5	NT (No tillage)	4.0 l ha^{-1}+1.5 l ha^{-1} (before white mustard sowing) 1.5 l ha^{-1} +1.5 l ha^{-1} (before spring wheat sowing)	NT (No tillage)
6	NT (No tillage)	1.5 l ha^{-1} +1.5 l ha^{-1} (before spring wheat sowing)	-

Tillage (NT) with and without white mustard cover crop (Table 1). The CT consisted of spring ploughing to a depth of 22 cm with three furrows reversible plough (the third week of March) and pre-sowing tillage for seedbed preparation with a field cultivator followed by harrowing an 8 cm depth (one week before sowing). The NT involved sowing directly into the stubble of the previous crop. The CT plots were drilled with a traditional grain drill (2.5 m wide, row distance of 15 cm) and NT plots with a double disk drill (Great Plains, Solid Stand 10' equipped in fluted coulter for residue cutting, double disk for seed placement, and single press wheel, 3.05 m wide, row distance 17.8 cm). Operating speeds used for ploughing and drilling were 1.5 and 1.8 m s^{-1} for other tillage treatments (cultivator, disk harrow). Sowing dates of spring wheat depended on soil water conditions and occurred between 23rd and 31st of March and sowing depth in all tillage systems were 3-4 cm.

Fertilization was uniform for all tillage systems and each experimental year (90 kg N ha^{-1}, 24 kg P ha^{-1}, 24 kg K ha^{-1}). The herbicide program for tillage systems consisted of pre-plant and post-emergence applications. Before sowing, 1.5 L ha^{-1} of Glyphosate herbicide+1.5 L ha^{-1} adjuvant As 500 SL was applied to all plots with no-tillage to control perennial weed and volunteer plants. For weed control, during the growing season post-emergence, Lintur 70 WG (dicamba 65.9%+triasulfuron 4.1%)+Chwastox Extra 300 SL (MCPA 300 g L^{-1}) herbicides were applied at the rate of 150 g ha^{-1}+1.0 L ha^{-1}. The seeds were treated with Raxil Extra 060 FS fungicide (0.06 L per 100 kg seeds) containing thiuram and tebuconazole. For disease control, Falcon 460 EC fungicide (spiroksamine 250 g L^{-1}+tebuconazole 167 g L^{-1}+triadimenol 43 g L^{-1}) at the rate of 0.6 L ha^{-1} was applied in all plots at BBCH 32 growth stage and Fury 100 EW insecticide (zeta-cypermetryne 100 g L^{-1}) at the rate of 0.1 L ha^{-1} at growth stage BBCH 61.

Sampling and Measurements

Dehydrogenase activity was determined colorimetrically in accordance with the methodology recommended by Thalmann (1968) on spectrophotometer Novaspec II (Pharmacia Biotech lack of wavelength 485 nm), using TTC (2.3.5-triphenylformazanchloride) as a substrate (μg TPF g^{-1} soil DM 24 h^{-1}). With the determination of dehydrogenases, soil pH was measured in 1M KCl with a pH-meter of Piccolo Hanna Instruments.

Measurements of enzyme activity were made four times during the vegetation

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season in three replicates (before sowing–control,) tillering (BBCH 22-23), earring (BBCH 55), and after harvest (BBCH 89).

Diseases

Samples from experimental plots were assessed for take-all root (caused by *Gaeumannomyces graminis* var. *tritici*), eyespot diseases (caused by *Oculimacula acuformis*,) and brown foot root (caused by *Fusarium* sp.). Assessments of diseases, based on the methods of Goulds and Polley (1990), were visually assessed at the early dough (BBCH 81) growth stage. Eyespot severity was classified as slight, moderate or severe, according to the number of shoots infected and the amount of girdling, leaf sheath penetration and stem softening (Scott and Hollins, 1974). From each plot a subsample of 25 plants with roots were sampled and disease incidence was recorded as the percentage of plants with root lesions. If eyespot lesions were not clearly visible, the internodes were split and checked for internal growth of a typical grayish, cottony mycelium. Plants were assessed into one of the following categories for stem lesion severity, where: 0- Uninfected, 1– Slight eyespot (one or more small lesions occupying at least half the circumference of the stem), 2– Moderate eyespot (one or more lesions occupying less than half the circumference of the stem), 3– Severe eyespot (stem completely girdled with lesions). The same assessment was used for brown foot rot.

Weather conditions varied in the years of the study. In the 2009 (March, May and June) and 2010 (March and May) season rainfall in the spring months was above the average for the multiplicity and further standard air temperature was higher than in the multi-year period. So this can be considered as a favorable growing season for the occurrence of fungal diseases. In the third year of study a lower rainfall at higher average daily air temperature was reported during the growing season of spring wheat, which is not conducive to the activity of pathogens.

Statistical Analysis

Analysis of variance was conducted in order to examine the impact of soil tillage methods on the activity of dehydrogenases in the soil, percentage of infections of the spring wheat plants by the take-all patch, and severity of incidence of the disease. When the impact of a factor proved to be statistically significant (P-value less than 0.01), the analysis was followed by post-hoc Tukey tests. The variables expressed as percentages were pre-transformed using the $z = \arcsin\sqrt{x}$ formula.

RESULTS AND DISCUSSION

Dehydrogenases Activity in Soil

A highly significant impact posed by the soil tillage system on the activity of dehydrogenases was observed (Table 2). Within each of the analyzed time periods, higher enzyme activity was noted in the soil treated post wheat harvest with Glyphosate 360 g L^{-1} e. i., [*N*-(phosphonomethyl) glycine] at the rate of 4.0 L ha^{-1} with adjuvant AS 500 SL 1.5 L per ha^{-1} of the stubble. White mustard cover crop in direct sowing was followed by springtime application of Glyphosate at the rate of 1.5 L ha^{-1}+adjuvant AS 500 SL 1.5 L ha^{-1}, followed by direct planting of spring variety of wheat. The time of collecting the soil samples was related to the growth stage of the spring wheat. The highest enzyme activity was observed during the earring period (Figure 1). The same result was confirmed in Brazilian soil tested by Andrea *et al.,* (2003), where dehydrogenase activity was slightly higher then at the beginning of the experiment after a month from glyphosate stimulated DHO activity, which means that the herbicide might stimulate the

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Table 2. Analysis of variance and Tukey's test.

Time of measurements /Analysis of variance (P-value)	Number of experimental treatment	Average activity of DHOs µg TPF g^{-1} dm soil 24 h^{-1}	Homogenous groups (Tukey test)[a]
Before sowing (BBCH 00) *P*-value< 0.001	2	2.074	A
	1	2.418	A
	3	2.646	AB
	4	4.136	ABC
	6	4.802	BC
	5	6.313	C
Tillering (BBCH 22-23) *P*-value< 0.001	2	2.136	A
	3	2.326	A
	1	2.517	A
	6	2.784	A
	4	4.000	A
	5	6.518	B
Earing (BBCH 55) *P*-value< 0.001	1	2.654	A
	2	3.304	AB
	3	4.84	BC
	4	5.842	CD
	6	6.826	CD
	5	7.344	D
After harvest (BBCH 89) *P*-value< 0.001	1	2.044	A
	6	2.978	AB
	2	3.013	AB
	3	3.441	AB
	4	4.594	BC
	5	6.074	C

[a] Different case letters among treatment means indicate significant differences according to Tukey's *LSD* test (P< 0.05).

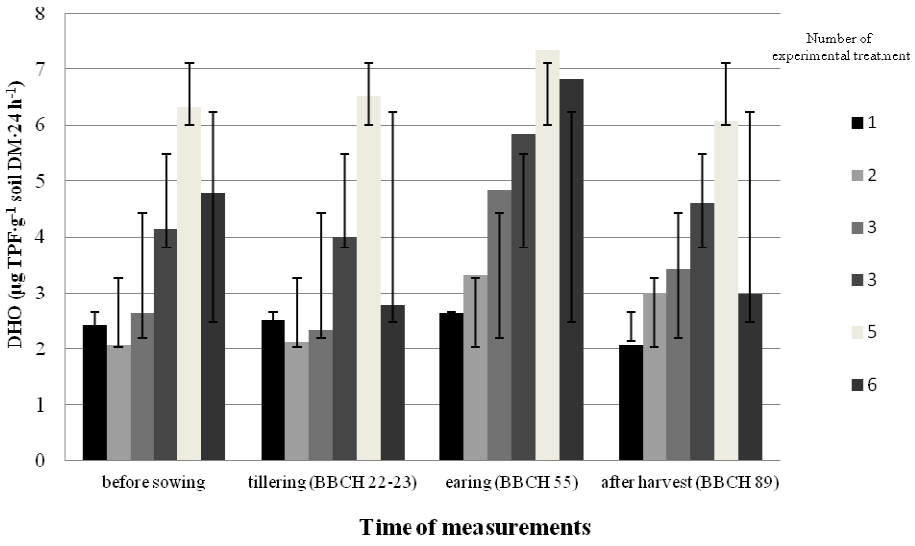

Figure 1. Average level of DHO activity during different measurement time mean values±standard errors.

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soil oxidative processes. Bennicelli *et al.,* (2009) confirmed that glyphosate can inhibit soil DHO activity by 80% relative to control soils (non-amended with Glyphosate). Stępniewska *et al.,* (2007) reported that the pesticide fonofos fall of dehydrogenase activity and influence of the on soil enzymatic activity started to be observed after one week of incubation, but from the 14^{th} day to the end of the experiment this effect was significant and noticeable. In turn Wolińska *et al.* (2015) shows significant lower values of DHO in cultivated soils while they reached much higher levels in control soils. Values of DHO noted in control soils were found to be 25-137% higher than cultivated soils.

Bielińska *et al.* (2008) and Mikanova *et al.* (2006) found significant impact of simplified farming on the increase of dehydrogenases activity (even by 50-70%). It indicates the usefulness of this group of enzymes to the evaluation of changes in the soil environment under a particular farming method. Observed stimulation coincided with notably higher content of organic C and organic N in the surface soil layers than under the traditional farming. This result confirms that the activity levels of soil enzymes are largely determined by the content of organic matter. Dynamic development of the microorganisms is related to the abundance of easily accessible and highly energetic material. The studies of Ciarkowska and Gambuś (2004) also reported that the activity of dehydrogenases corresponds to the organic carbon and nitrogen content in the soil. Roldan *et al.* (2005) noted higher activity of dehydrogenases in the zero tillage farming coincides with higher fraction of organic C. Decomposition of the harvest residues releasing nutrients, such as N, P and S important for plants and microorganisms. Celik *et al.* (2011) found higher activity of dehydrogenases under spring variety of wheat cultivation in direct sowing. Moreover, the enzymes' activity was directly correlated with TOC (Total Organic Carbon) and decreased with depth of the soil. Higher activity of the enzymes in the upper layers of soil was confirmed by other authors (Bielińska *et al.,* 2008; Wolińska and Stępniewska, 2012). Diosma *et al.* (2003) indicated the variability in microbiological activity in the soil cultivated and mineral fertilization dependency from growth stage during vegetation season. Koper *et al.* (1999) found positive correlation between the enzyme activity and organic C content in the soil under spring barley cultivation with crop rotation (r= 0.53). Samuel *et al.* (2009) also found higher activity of dehydrogenases in the soil where NT system was applied. However, Natywa *et al.* (2009) reported higher activity of those enzymes in the soil cultivated traditionally. Schulten *et al.* (1995) found that the increased size of particles and increased complexity of organic matter bindings coincided in the traditional tillage with the significant drop of enzymes activity (60-80%) that participated in the C, N, and P transformation cycle. Wolińska and Stępniewska (2012) claimed that the changes of enzyme activity during the vegetation season are probably caused by the content changes of the enzyme substrates in the soil, as well as by the fluctuations in the temperature or humidity. Nat Holland (1995) claimed that an increase of microbial biomass in the zero tillage system corresponds to the increase of root secretions.

The highest level of DHO activity observed in this study coincides with the earring stage (Figure 1). This would suggest an increased occurrence of physiologically active microorganisms, because these enzymes are present only inside the living microorganism cells. According to Kieliszewska-Rokicka (2001), their elevated levels confirm the presence of physiologically active microbes. The increased activity in this period may be related to the intensified root secretions, which is consistent with the results obtained by Włodarczyk (2000).

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Diseases of Spring Wheat

The results showed a mixed impact of no tillage cultivation on fungal diseases of spring wheat. With the eyespot (*Oculimacula acuformis*,) and brown foot root (*Fusarium* sp.) diseases, no significant impact of the tillage system or the left biomass on the incidence of these infections was observed (Figure 2). Only with take-all (*Gaeumannomyces graminis* var. *tritici*) there was significant impact of the soil tillage system on the percentage value of infected plants. Obtained results demonstrated lower levels of infection on the treatments with no tillage (3, 5, 6– below 0,2% in year 2010 and 0, 21% in year 2011) than the combination of ploughing tillage without cover crop with shallow tillage for preceding crop and normal for spring sowing (see Figure 2).

As with other soil borne diseases, take-all is difficult to control because resistant wheat varieties are not available. Crop rotation and tillage are effective controls, but because of the limited value of alternative crops in modern cereal-based production systems, 2 or 3 crops of wheat will often be grown before a break, increasing the incidence and severity of take-all. Suppressive soils are defined as "soils in which the pathogen does not establish or persist, establishes but causes little damage, or establishes and causes disease for a while but thereafter the disease is less important even though the pathogen may persist in the soil" (Weller *et al*., 2007). General suppression is a characteristic of essentially all soils to inhibit the growth and activity of soil borne pathogens to a limited extent, growing to the activity of the total microbial biomass in soil competing with the pathogen (Weller *et al.,* 2007). Specific suppression is highly effective and results from the activity of individual or selected groups of microorganisms. General suppression is not transferable between soils, but specific suppression is transferable by adding a small amount of suppressive soil to a conductive soil. Specific suppression can be eliminated by pasteurization (60°C, 30 minutes) or fumigation of the soil (Weller *et al.,* 2002;

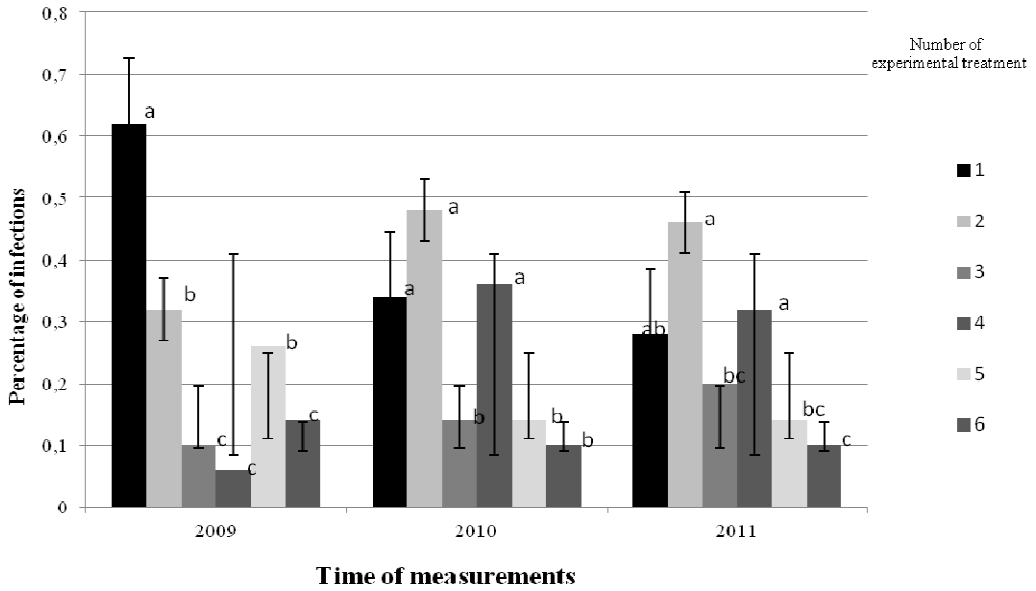

Figure 2. Percentage of infection with take all; mean values±standard errors; different letters denote statistical differences at level α= 0.05, n= 5. Different case letters among treatment means indicate significant differences according to Tukey's *LSD* test (P< 0.05).

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Weller *et al.,* 2007).

A number of studies claim that the impact of preceding crop on the fungal infections is much greater than that of the cultivation tillage (Krupinsky *et al.,* 2002). Residuals left over after the harvest of the preceding crop may transmit many infectious diseases and influence the species composition of saprophytes, including antagonistic organisms that help develop the pathogens. Introducing some regenerative plants into the crop rotation either as the main crop or a stubble cover crop may considerably lessen the degree of fungal infections (Krupinsky *et al*., 2002). Although all regenerative plants will improve the phytosanitary conditions of the soil (Turkington and Clayton, 2000), but most importantly among them are the *Fabaceae*, which not only provide the succeeding crop with the nitrogen accumulated in the post-harvest residuals but also, among other things, will reduce the infection incidence of fungal diseases of roots and stem bases of the cereals.

CONCLUSIONS

There was highly significant impact of tillage system on the soil dehydrogenase activity. In all analyzed tillering and earing of plant growth stages, higher soil enzyme activity was observed in soil under no-tillage variant. The highest enzyme activity during the vegetation season was noted during the earring stage of the spring wheat.

Soil tillage system and cover crop influenced the percentage of infection of spring wheat plant by take-all root disease, but did not affect the intensity of the eyespot occurrence or of the brown foot root.

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اثر سیتم های مختلف خاکورزی بر فعالیت دهیدروژناز خاک و بیماری گندم بهاره

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چکیده

آزمایشات مزرعه ای در ایستگاه تجربی (Brody) متعلق به دانشگاه پوزنان علوم حیاتی (NE لهستان) انجام شد. هدف این مطالعه، ارزیابی اثر سیستم های مختلف خاکورزی و پوشش گیاهی خردل سفید بر فعالیت آنزیمی خاک و شدت عفونت های گیاهی توسط پاتوژن های قارچی در گندم بهاره بود. طرح بلوک های کامل تصادفی با چهار تکرار در هر تیمار (معمولی و بدون خاکورزی با و بدون پوشش گیاهی) تعیین شد. نتایج، فعالیت آنزیمی بیشتری در خاک تیمار شده بعد از برداشت گندم با علف کش گلیفوزیت در مقدار۴ لیتر در هکتار، ۳۶۰ گرم بر لیتر -*N*)) (phosphonomethyl)glycine)) همراه با ادجوانت AS 500 SL 1.5 لیتر در هکتار کاه

پوشش گیاهی خردل سفید در کاشت مستقیم گندم نشان داد. فعالیت آنزیمی کمتر در زمان بهار و کار با علف کش در مقدار ۱.۵ لیتر در هکتار همراه با ادجوانت AS 500 SL، ۱.۵ لیتر در هکتار و پس از کاشت مستقیم بهاره گندم مشاهده شد. هدف اصلی این مطالعه محاسبه اثرات سیستم خاکورزی و پوشش گیاهی بر فعالیت دهیدروژناز خاک و سلامت گندم بهاره بود. تحقیقات انجام شده نشان داد که سیستم خاکورزی و بیومس به جا گذاشته تاثیر قابل توجهی بر روی بیماری لکه چشمی (Oculimacula acuformis) و بیماری فوزاریومی پوسیدگی ریشه قهوه ای (Fusarium sp) ندارد. فقط روی بیماری پاخوره گندم (Gaumannomyces graminis var. tritici) سیستم خاکورزی اثر معناداری بر درصد بوته های آلوده می‌گذارد. در مرحله پنجه زنی گندم بهاره، فعالیت آنزیمی بیشتری در تیمارهای با پوشش گیاهی وکشت گندم بهاره بدون سیستم خاکورزی مشاهده شد.

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