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ASSESSMENT OF CATALASE SOIL ACTIVITY UNDER AMARANTH CULTIVATION NOT EXPOSED TO CHEMICAL PROTECTION METHODS

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ABSTRACT

The aim of the study was to determine the influence of habitat, cultivar and developmental growth stage on catalase activity in soil under two amaranth cultivars – Rawa (*Amaranthus cruentus* L.) and Aztek (*Amaranthus hypochondriacus* × *Amaranthus hybridus* L.). In a 3-year field experiment (2013–2015), amaranth's plants were grown in a wide-row spacing on the soil of the good wheat complex in south-eastern Poland (50°71'N, 23°04'E). The field experiment included 4 variable factors: weather conditions; selected amaranth growth stages (5-leaf, full flowering and seed maturity stages); NPK dose combinations (I: 40 kg N · ha^{-1}, 30 kg P · ha^{-1}, 30 kg K · ha^{-1}; II: 60 kg N · ha^{-1}, 40 kg P · ha^{-1}, 40 kg K · ha^{-1}; III: 80 kg N · ha^{-1}, 50 kg P · ha^{-1}, 50 kg K · ha^{-1}; IV: 120 kg N · ha^{-1}, 70 kg P · ha^{-1}, 70 kg K · ha^{-1}) and two cultivars ('Rawa' and 'Aztek'). No pesticides are applied in the cultivation due to the absence of pathogens and pests of this plant in Poland. Plant protection was limited to reducing weed infestation twice. The conducted research showed that weather conditions were the main factor affecting catalase activity in the soil under amaranth cultivation, followed by other factors, such as fertilization, cultivar and growth stage. All the analyzed factors proved to exert a significant impact on organic matter content in the soil, while only the applied NPK fertilization had effect on sorption capacity. Moreover, it was found that the cv. Aztek positively influenced the activity of catalase and humus accumulation in the soil in comparison to the cv. Rawa. The beneficial effect of amaranth on the soil environment and its enzymatic activity was ascribed to the lack of introduced pesticides.

Key words: amaranth, soil, catalase activity, non-chemical protection

INTRODUCTION

The growth, development and yielding of plants are significantly influenced not only by the physicochemical properties of soil, but also by its biological characteristics [Shahane and Shivay 2021]. Among them, enzymatic activities of bacteria, fungi and actinomycetes inhabiting the rhizosphere are of paramount importance [Gałązka et al. 2017, Huera-Lucero et al. 2020], because these microorganisms continuously participate in shaping soil fertility and plant health [Kaczmarek et al. 2008]. Biological soil properties, including biochemistry are considered irreplaceable elements conditioning its biological quality, i.e. the ability to support specific ecosystems, ensuring good crop productivity and maintaining appropriate environmental quality, as well as plant and animal health [Aon and Colaneri 2001, Jamiołkowska et al. 2021]. For these reasons, enzymatic and respiratory activity, microbial biomass, composition and species abundance are commonly used as indicators of soil state. Factors disturbing or even inhibiting vital processes of soil or-

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ganisms most often deregulate the proper functioning of the ecosystem. For these reasons, biological activity is widely used to determine soil quality and estimate changes caused by the application of potentially dangerous [Bünemann et al. 2018] or newly introduced factors. It seems important to determine soil enzymatic activity, because soil enzymes reflect disturbances in the ecosystem. They are now often utilized as indicators of biogeochemical cycles, organic matter degradation and soil remediation [Lee et al. 2020]. Hence, the type of aboveground vegetation and its cultivation practices undoubtedly play an important role in modeling soil biological features. It should be noted that not all plant species exert positive influence on the growth, abundance and activity of soil microflora [Skwaryło-Bednarz 2008]. On the contrary, numerous scientific studies have confirmed that certain plant species may have a negative effect on soil microorganisms through their root exudates. It is therefore important to assess the reaction of the soil environment to the newly introduced plant species. This is the case of amaranth (*Amaranthus* spp.), known in Poland as szarłat, belonging to the group of pseudo-cereal or universal plants. It had already been cultivated 4,000 BCE by the Aztecs and Mayans, but it was only in the second half of the 20th century when this plant have attracted the interest of scientists, when the exact chemical composition of its seeds was determined [Skwaryło-Bednarz et al. 2020]. The seeds turned out to be of high nutritional value [Skwaryło-Bednarz et al. 2020]. Several literature reports concerning the influence of amaranth on the activity of soil microflora have demonstrated that this plant has stimulating properties [Skwaryło-Bednarz 2008], probably due to the amaranth cultivation practices, not requiring chemical protection methods. It is possible due to the soil and climatic conditions of Poland, particularly unfavorable for the spread of amaranth – specific phytopatogens, which results in the absence of pest colonization [Skwaryło-Bednarz and Nalborczyk 2006]. Aphids occur on selected individuals only occasionally and can be controlled with biological preparations. Most problems are caused by heavy weed infestations, therefore manual and/or mechanical weeding should be carried out at least twice, until the plants completely cover the soil.

The aim of the study was to assess the influence of habitat and cultivar factors as well as the plant developmental stage on catalase activity and selected chemical parameters of the soil under amaranth cultivation unexposed to chemical protection methods.

The following research hypotheses were adopted during the preparations:

The null hypothesis (H_0) assumed that habitat, varietal factors and developmental stage of amaranth cultivated without pesticides did not affect catalase activity and selected chemical parameters of the soil.

The alternative hypothesis (H_1) was to prove that habitat, varietal factors and developmental stage of amaranth cultivated without pesticides affected catalase activity and selected chemical parameters of the soil.

MATERIALS AND METHODS

Experimental design

A 3-year field experiment was carried out in the years 2013–2015 in Bodaczów (50°71'N, 23°04') near Zamość, using a split-plot design, in which 4 variable factors were examined (Fig. 1):

1) weather conditions,

2) amaranth developmental stages – 5-leaf, full flowering and full seed maturity stages,

3) four NPK dose combinations:

I – 40 kg N · ha^{-1}, 30 kg P · ha^{-1}, 30 kg K · ha^{-1} (100 NPK)
II – 60 kg N · ha^{-1}, 40 kg P · ha^{-1}, 40 kg K · ha^{-1} (140 NPK)
III – 80 kg N · ha^{-1}, 50 kg P · ha^{-1}, 50 kg K · ha^{-1} (180 NPK)
IV – 120 kg N · ha^{-1}, 70 kg P · ha^{-1}, 70 kg K · ha^{-1} (260 NPK)
and control (0 NPK).

Nitrogen was used in the form of ammonium nitrate 32% N (Grupa Azoty) in two equal doses before sowing, and subsequently during intensive plant growth period. Phosphorus, as granulated simple superphosphate 19% P_2O_5, 28% SO_3, 10% CaO (Fosfan S.A.) and potassium in the form of potassium salt 60% K_2O (Luvena S.A.) were introduced before sowing;

4) two cultivars of the test plant – Rawa and Aztek. The plot area was 20 m^2.

Soil material. The experiment was set up on brown soil with high P, K and Mg contents and slightly acidic pH (mean log KCl pH – 5.74). Winter barley was the forecrop for amaranth.

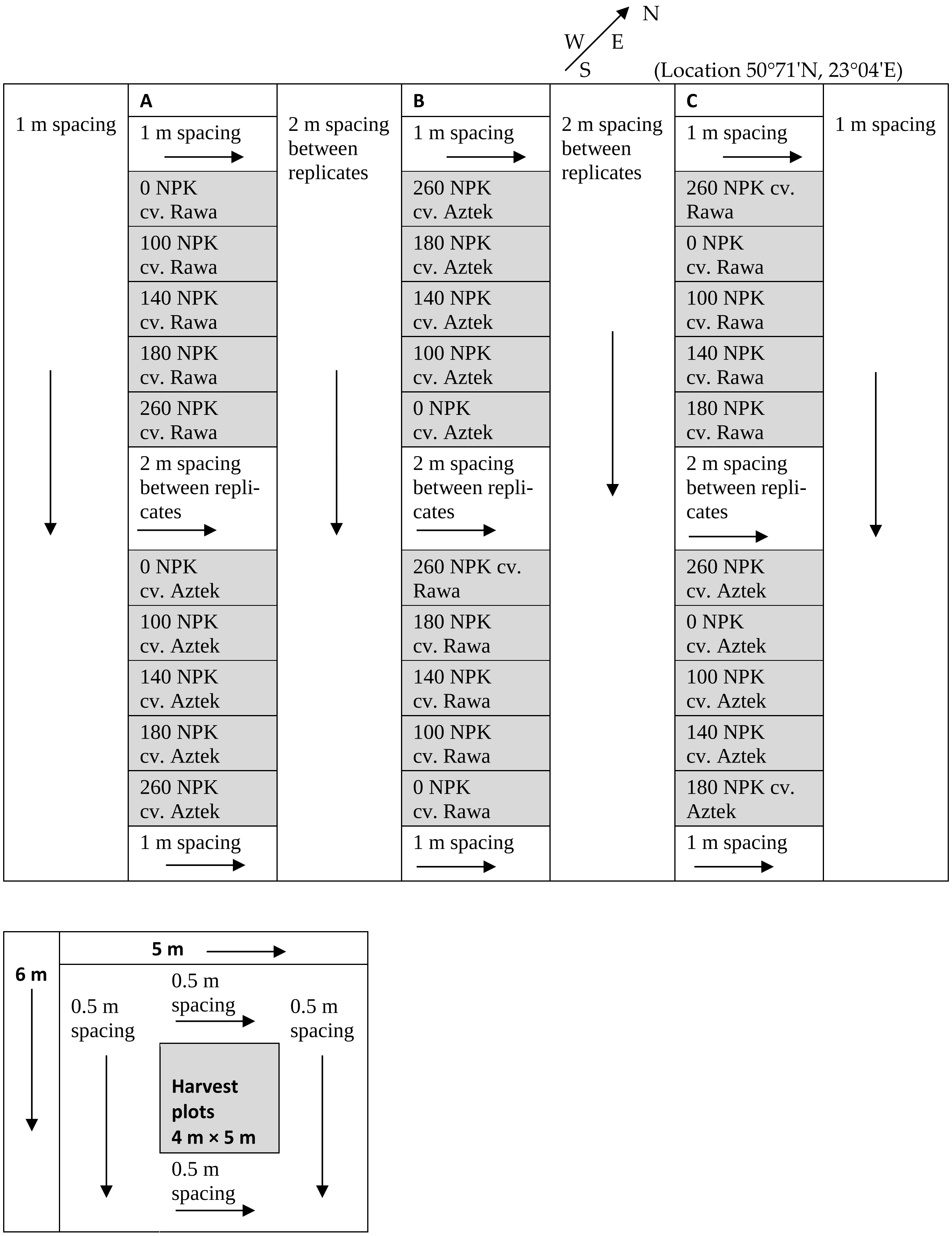

Fig. 1. Scheme of the field experiment

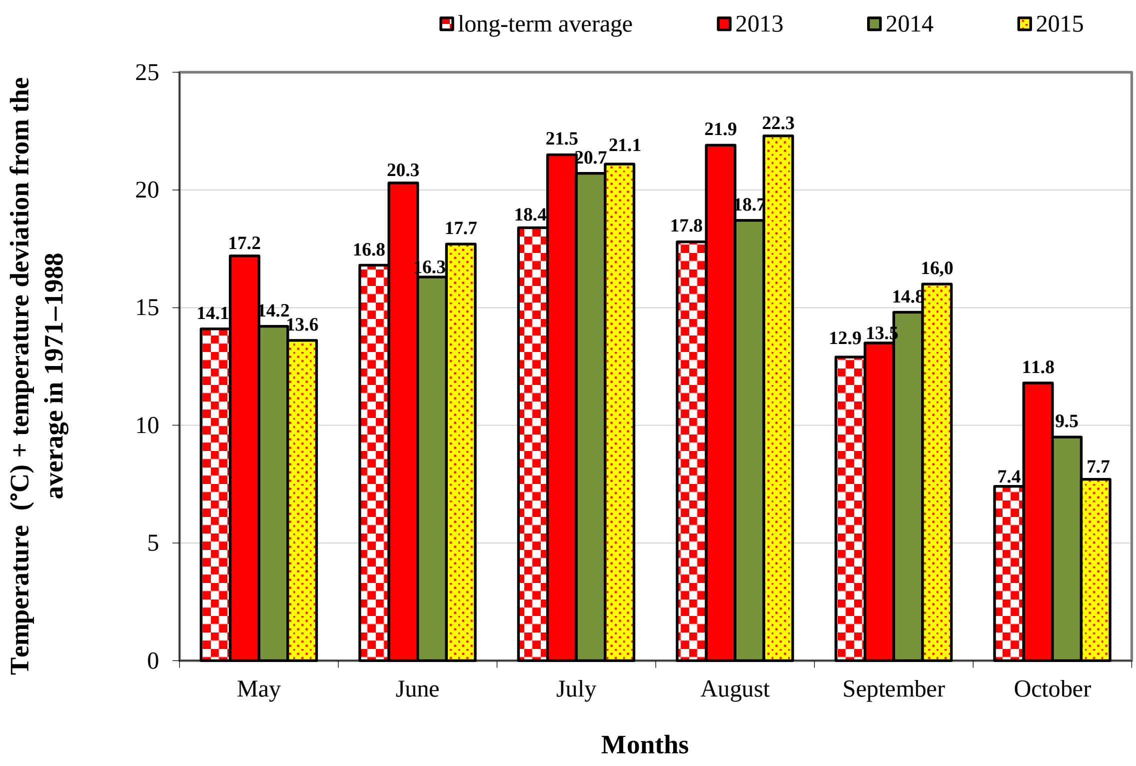

Fig. 2. Monthly mean temperature during the study period

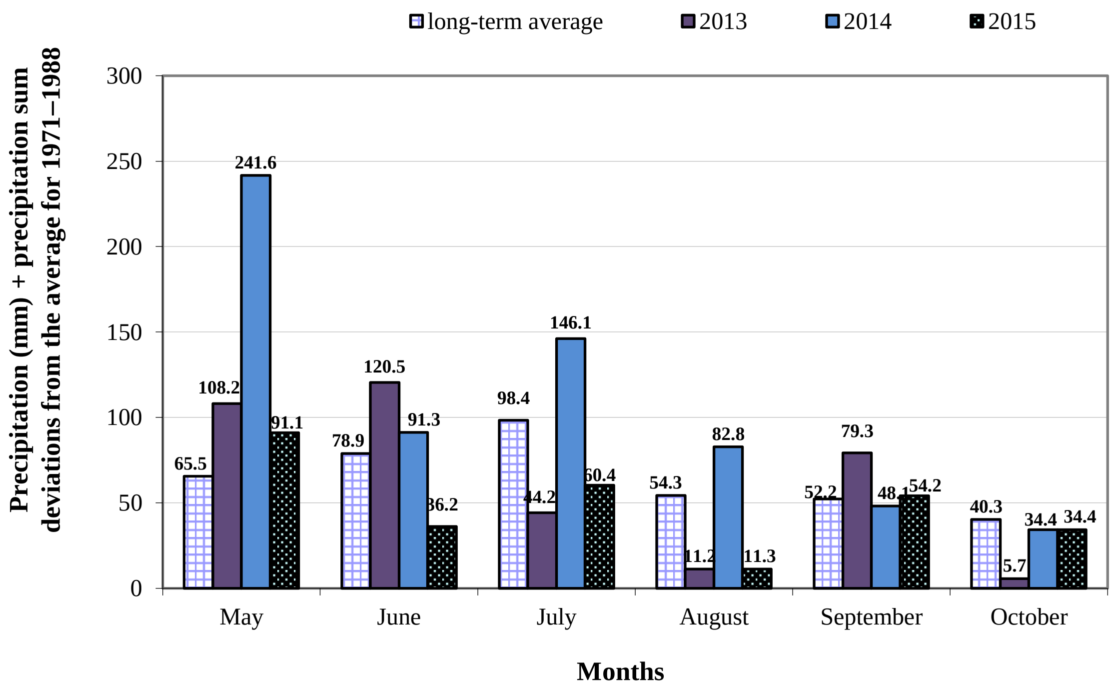

Fig. 3. Monthly mean precipitation during the study period

Field management. Soil preparation for sowing amaranth and its non-chemical care was carried out in accordance with the principles of good agricultural practice. Plantation care consisted of manual and mechanical weeding with the use of a weeder in the 2–4 leaf stage (BBCH12-14 stage) and in the shoot formation stage (BBCH31-33 stage).

Amaranth seeds were sown in the third decade of May in a wide-row spacing (every 60 cm).

Quantitative analyses. During the vegetation period, amaranth was assessed 3 times (5-leaf (BBCH 15), full flowering (BBCH 65) and full seed maturity stages (BBCH 89). During these stages, soil samples were also collected from the objects and the following parameters were determined:

– humus content [Ostrowska et al. 1991];

– soil sorption capacity [Ostrowska et al. 1991];

– catalase activity using the Johnson and Temple method [1964]. It involved soil incubation with the addition of hydrogen peroxide (natural substrate of the enzyme). H_2O_2 remaining in the soil, not decomposed by catalase, was titrated with potassium permanganate in an acidic environment. The results are given in units of catalase activity, equal to mg $H_2O_2 \cdot g^{-1}$ d.w. min^{-1}.

Statistical analyses. The results were statistically analyzed using the ANALWAR–5.2.FR Excel add-in tools. The analysis of variance was performed at the significance level of p = 0.05, and the significance of differences between the means was estimated using Tukey's test.

Individual years of research were characterized by varied weather conditions. Temperatures during the sowing period and initial growing season of the test plant were generally higher than the long-term average; they were lower than the values of long-term averages only in May 2015 and June 2014 (Fig. 2). Sowing amaranth in the third decade of May was difficult each year due to excessive rainfall. It should be emphasized that the afore-mentioned month was particularly wet in 2014, when the plants were in the emergence phase (Fig. 3). In 2013, excess rainfall was also recorded in June, when amaranth forms proper leaves, and in September, during plant maturation. On the other hand, a slight drought was recorder in September and October (full maturity period) of 2014. In 2015, a slight excess of rainfall occurred in September, while its deficiencies were recorded in the remaining months (Fig. 3).

RESULTS AND DISCUSSION

The result of the analysis of a 3-year study proved that the values of catalase activity and the tested chemical soil properties under amaranth cultivation (humus content and sorption capacity) were determined to a different extent by the studied factors, i.e. weather conditions, plant developmental stage, NPK fertilization dose and cultivar (Tab. 1).

Weather conditions during the research years significantly differentiated humus content in the soil under the cultivation of two amaranth cultivars. Significantly more humus in the soil under amaranth was found in the second (32.82 $g \cdot kg^{-1}$) compared to the first (less by approx. 0.74 $g \cdot kg^{-1}$) and third year (less by approx. 0.69 $g \cdot kg^{-1}$) of cultivation (Tab. 1). It can be concluded that amaranth cultivation is conducive to the accumulation of organic matter in the soil, which positively affects its physicochemical properties, such as sorption and buffer capacity, and determines biological transformation processes important for the functioning of the habitat. High content of organic matter in soils is a factor stabilizing their structure, reducing susceptibility to compaction and degradation as a result of water and wind erosion [Krasowicz et al. 2011, Stuczyński et al. 2007]. According to Pranagal [2004], the slightly lower humus content in the extreme years of the study (2013, 2015) could have been caused by the lower growth rate and biomass size in the soil during soil sampling. It could also have resulted from the intensification of soil organic matter mineralization [Sapek and Sapek 2006]. Carbon dioxide and methane emissions, as well as leaching of soluble organic carbon are also important causes of soil organic carbon (SOC) depletion [Sapek 2009, Corsi et al. 2012], as is mobile bond formation of soil minerals with soluble organic carbon, especially under aerobic conditions [Coward et al. 2018, Possinger et al. 2020]. Dissolved organic carbon (DOC), being part of dissolved organic matter (DOM), is one of the most sensitive indicators of changes in the soil environment, while DOM is among the most sensitive markers of alternations in the soil environment. It is considered the most mobile and active soil component and is an easily accessible source of nutrients and energy for microorganisms and other living organisms [Neff and Asner 2001, Smreczak and Ukalska-Jaruga 2021].

Table 1. Selected soil chemical parameters and catalase activity (means for years and factors)

Factor	Humus ($g \cdot kg^{-1}$)	T ($mmol(+) \cdot kg^{-1}$)	Catalase activity ($mg\ H_2O_2 \cdot g^{-1}$ d.w. min^{-1})
2013	32.08 a	98.86 a	0.0382 b
2014	32.82 b	99.04 a	0.0420 c
2015	32.13 a	99.37 a	0.0346 a
LSD (α = 0.05)	0.66	0.834	0.0033
F^0 value	4.4570	1.0930	14.7872
p value	0.014	0.339	$2.96 \cdot 10^{-6}$
5-leaf stage	31.85 a	99.01 a	0.0349 a
full flowering stage	32.32 ab	99.21 a	0.0384 b
full seed maturity stage	32.86 b	99.07 a	0.0415 bc
LSD (α = 0.05)	0.65	0.843	0.00337
F^0 value	7.053	0.169	11.0023
p value	0.001	0.845	$5.41 \cdot 10^{-5}$
Combination I (100 NPK)	31.71 ab	100.09 d	0.0356 ab
Combination II (140 NPK)	32.27 bc	99.19 c	0.0386 bc
Combination III (180 NPK)	33.00 cd	98.34 b	0.0404 cd
Combination IV (260 NPK)	33.58 d	97.13 a	0.0432 d
Control (0 NPK)	31.16 a	100.72 e	0.0336 a
LSD (α = 0.05)	0.67	0.43	0.0048
F^0 value	32.840	166.735	9.8136
P value	$1.53 \cdot 10^{-16}$	$2.24 \cdot 10^{-39}$	$3.42 \cdot 10^{-6}$
Rawa	31.99 a	98.74 a	0.0358 a
Aztek	32.70 b	99.23 a	0.0408 b
LSD (α = 0.05)	0.45	0.575	0.002327
F^0 value	10.059	2.799	17.9060
p value	0.002	0.098	$5.68 \cdot 10^{-5}$

Hydrothermal conditions did not have a significant effect on soil sorption capacity under amaranth cultivation, despite its slight increase in the study years (98.86–99.37 $mmol(+) \cdot kg^{-1}$) (Tab. 1).

Statistical analysis showed that weather conditions in 2013–2015 had a significant impact on the variation in catalase activity values under amaranth cultivation (Tab. 1), which could probably be related to temperature distribution and total rainfall in the study years. The highest value of catalase activity was recorded in 2014 (0.0420 $mg\ H_2O_2 \cdot g^{-1}$ d.w. min^{-1}), and the lowest in 2015 (0.0346 $mg\ H_2O_2 \cdot g^{-1}$ d.w. min^{-1}) (Tab. 1).

It can be concluded that the higher moisture in 2014 increased the value of catalase activity, and the low amount of rainfall in 2015 decreased it (Fig. 3, Tab. 1). The relationships between soil moisture and enzymatic activity were also reported by other authors [Nowak and Kaklewski 2003]. According to Brzezińska [2006], soil irrigation with clean water usually resulted in smaller, insignificant changes in catalase activity in the soil under energy willow, poplar and grass cultivation.

Our research also demonstrated significant relationships between the developmental stage of amaranth and the amount of humus (Tab. 1). A significantly higher value of this parameter was recorded at the full seed maturity stage (32.86 g · kg^{-1}), and a lower value at the 5-leaf stage (31.85 g · kg^{-1}) (Tab. 1). No significant differences were found between humus content at the full flowering stage (32.32 g · kg^{-1}) and at the 5-leaf and the full seed maturity stages (Tab.1).

This was most likely due to the higher accumulation of organic matter at the end of the vegetation period and the applied NPK fertilization. Meteorological conditions, crop species and the level of organic and mineral fertilization are among the most frequently mentioned factors that may cause changes in the content of soil organic matter [Kulig et al. 2004].

However, no significant relationship was found between the developmental stage of the test plant and sorption capacity of the soil (Tab. 1).

Catalase activity increased gradually from the 5-leaf to full seed maturity stage, when its highest value was recorded – 0.0415 mg H_2O_2 · g^{-1} d.w. min^{-1} (Tab. 1). The obtained value of this parameter differed significantly between the extreme phases and the 5-leaf and full flowering stages; however, no significant differences were found between the full flowering and full seed maturity stages (Tab. 1). This was consistent a scientific report, which emphasized that the highest levels of enzyme activity were most often observed in summer and autumn, and the lowest in winter [Yuan and Yue 2002].

The application of increasing NPK fertilization doses influenced the accumulation of humus in the soil (Tab. 1). The introduction of the highest NPK dose (combination IV, 260 NPK) resulted in the significantly highest content of organic matter in the soil (33.58 g · kg^{-1}) in comparison to lower amounts of macronutrient application and control. Studies found that NPK fertilization applied alone or as a supplement, contributed to a significant increase in organic carbon, and thus humus content in reclaimed light soils [Bęś and Warmiński 2015]. Humus content was higher in all objects with additional NPK fertilization compared to non-fertilized objects.

The increasing doses of applied fertilizers had a significant effect on soil sorption capacity. Its values gradually decreased with increasing NPK fertilization and humus content. The highest value of sorption capacity (100.72 mmol(+) · kg^{-1}) was found in the control plots (0 NPK). It was significantly higher than in the plots with fertilization levels I (100 NPK), II (140 NPK), III (180 NPK) and IV (260 NPK) (less by approx.: 0.63; 1.53; 2.38; 3.59 mmol(+) · kg^{-1} respectively) (Tab. 1). The obtained results were consistent with the literature data. According to Filipek-Mazur et al. [2018], the significantly highest value of sorption capacity in the first year of the experiment was found in non-fertilized soil, and the applied macronutrient fertilization decreased the value of sorption capacity even by 9–21%. The relationships between the soils of individual objects, with lower absolute values of this parameter, were similar after the second year of research to those after the first year.

The application of increasing NPK doses caused a significant the gradual raise in soil catalase activity under amaranth cultivation in relation to the control object (without fertilization, 0 NPK) (Tab. 1). The highest catalase activity was observed in the plots with the 4th level of fertilization – 260 NPK (0.0432 mg H_2O_2 · g^{-1} d.w. min^{-1}), which, in turn, did not differ from the 3rd level (180 NPK). The lowest activity of this enzyme was found in the soil of the control plots – 0 NPK (0.0336 mg H_2O_2 · g^{-1} d.w. min^{-1}) (Tab. 1). Enzymes present in soil can be used as bioindicators of the quality of the soil environment [Symanowicz et al. 2018, Adetunji et al. 2017]. Each type of soil is characterized by a specific level of enzymatic activity [Piotrowska-Długosz et al. 2021], that is influenced by the type of mineral, natural and organic fertilization [Chu et al. 2007, Symanowicz et al. 2014, Szymanowicz et al. 2018]. In addition to the type of fertilization, its dose is also important [Natywa et al. 2014]. Catalase is one of the enzymes found in the soil environment, it is present in animal, plant and bacterial cells. Most anaerobic bacteria (except *Propionibacteria shermanii*) and certain aerobic bacteria, e.g. *Baccillus popillie* and *Mycoplasma pneumonice*, do not have this enzyme [Switala and Loewen 2002, Ścibor and Czeczot 2006]. Catalase protects cells against toxic effects of hydrogen peroxide and other derivatives. It converts hydrogen peroxide to molecular oxygen and water [Ścibor and Czeczot 2006]. Thus, it reduces the effects of oxidative stress [Nandi et al. 2019]. The activity of catalase in soil depends on the content of organic matter, biomass, oxygen uptake, carbon dioxide release, as well as the activity of dehydrogenases, glucosidase amidase and phosphodiesterase [Riffaldi et al. 2002, Dinesh et al. 2004]. The activity of this enzyme is also affected by fertilization with increasing NPK doses. Studies conducted in the post-mining land and soils from these areas showed that mineral fertilization was the factor stimulating catalase activity [Skwaryło-Bednarz and Krzepiłko 2009].

The specific properties of amaranth cultivars exerted a significant impact on humus content and catalase activity in the soil. Significantly higher humus content was found in the cultivation of the amaranth cultivar Aztek compared to the cultivar Rawa (a difference of 0.71 g · · kg^{-1}) (Tab. 1). The cultivar Aztek was also characterized by significantly higher catalase activity compared to the cultivar Rawa (a difference of 0.005 mg $H_2O_2 \cdot g^{-1}$ d.w. min^{-1}) (Tab. 1). A more beneficial effect of the cultivar Aztek on soil biological quality compared to the cultivar Rawa has been described in previous scientific reports [Skwaryło-Bednarz 2008].

According to Bartosz [2003], the rate of hydrogen peroxide decomposition by a soil sample depended on organic compounds showing antioxidant and mineral activity (heavy metal oxides, transition group metal ions – Fe^{2+}, Cu^{1+}) and microorganisms containing catalase. This process also changes under the influence of fertilization, cultivation system and crops. Research showed a positive effect of amaranth on soil enzymatic activity and potential application in soil bioremediation [Skwaryło-Bednarz 2008]. The use of pesticides for amaranth protection may be one of the factors affecting the reduction of soil activity under the cultivation of this plant. Numerous studies confirmed the negative effect of pesticides, especially herbicides, such as Triflurotox 250 EC or acetamipiride biocide, used in plant protection, on the soil environment [Wyszkowska and Kucharski 2004, Yao et al. 2006]. In the author's own research, no sudden decrease in catalase activity has been found, which was usually observed in soil contaminated with heavy metals or chemical compounds [Skwaryło-Bednarz et al. 2018]. The observed differences in the activity most probably resulted from soil moisture conditions, fertilization, plant species and variety, as well as natural variability of microorganisms during the plant growing season.

CONCLUSIONS

In the present study an alternative hypothesis (H_1) was assumed that the habitat and variety factors, as well as the developmental stage of amaranth cultivated without pesticides, would affect catalase activity and selected chemical soil parameters.

A 3-year field experiment involving cultivation of amaranth cultivars Rawa and Aztek in the soil and climatic conditions of the Zamość region, demonstrated that most of the analyzed research factors had a positive effect on catalase activity and selected chemical parameters of the soil under amaranth cultivation without the use of pesticides.

1. The factors that differentiated catalase activity in the soil under amaranth cultivation to the greatest extent were: moisture and thermal conditions, followed by the combination of NPK fertilization, cultivar and developmental stage.

2. All analyzed factors had a significant impact on humus content, while only the increase of NPK fertilization affected the value of soil sorption capacity under amaranth cultivation.

3. The cultivar Aztek exerted a more favorable effect on catalase activity and favored higher humus accumulation in the soil compared to the cultivar Rawa.

4. It can be assumed that non-chemical protection of amaranth plantation, without pesticides applications, could have a positive effect on soil enzymatic quality.

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