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RESPONSE OF CATALASE TO MICROBIOLOGICAL AND CHEMICAL SOIL INDICATORS IN SOLE AND INTERCROPPED CABBAGE CULTIVATION

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Abstract

The influence of microbiological and chemical soil indicators on catalase activity in soils under sole and intercropped cabbage cultivation with leek, beans, tagetes, flower mix, and dill has been established. The highest catalase was observed in intercropping cabbage with tagetes (1.63 ml 0_2/30 min.), while the lowest was found in cabbage and leek (1.20 ml 0_2/30 min.). A high correlation was found between catalase and the total microflora in the soil (r=0.90), mainly determined by the activity of non-spore-forming bacteria (r=0.71) and mold fungi (r=0.82). A moderate correlation exists between mineralization activity and catalase (r=0.39), as well as between total microflora and mineralization coefficient (r=0.36). The relationship between catalase and ammonium (r=0.53) and potassium ions (r=0.33) is moderate, while no such relationship was found with phosphorus (r=0.02). The dependence between Fe and catalase is also moderate. A weak influence on catalase activity in the variants was observed from the quantity of bacilli (r=0.30) and actinomycetes (r=0.27), as well as from the content of humus (r=0.18) and carbon (r=0.17). There is no positive correlation between pH and catalase (r=-0.24).

Key words: *catalase, soil microflora, agrochemistry, co-planting, correlations.*

INTRODUCTION

Catalase is a respiratory enzyme, a sensitive biochemical marker indicative of the activity of soil microorganisms. Many soil microorganisms produce the enzyme catalase - bacteria, actinomycetes, micromycetes.

In agrogenic soils, many authors have established a stronger development of bacteria (especially non-spore-forming) and a weaker development of mycelial microorganisms (actinomycetes and molds), mainly aerobic and mesophilic microorganisms (Naskova et al., 2016; Plamenov et al., 2016; Yankova et al., 2016; Malcheva et al., 2018; 2019; Malcheva, 2021; Frene et al., 2022; Guo et al., 2022; Mladenova et al., 2023; Mladenova et al., 2024; Koleva et al., 2024).

These groups of microorganisms produce the enzyme catalase, which decomposes hydrogen peroxide to water and oxygen, releasing oxygen and an additional water molecule for Fe (heme) catalases (Chabot et al., 2020). Microbiological, mineralization and catalase activity depend on a complex of factors: amount and composition of soil microflora, soil temperature and moisture, sampling depth, content of organic matter and nutrients in the soil, soil type, mechanical composition, soil pH, type of vegetation, research methods, applied agrotechnical measures, the presence of inhibitors and other factors (Uzun and Uyanöz, 2011; Naskova et al., 2015; 2016; Malcheva et al., 2018; Malcheva et al., 2019; Malcheva, 2021).

In addition to microbial origin, there is also catalase of plant origin (Malcheva et al., 2018; Malcheva et al., 2019; Malcheva, 2021), higher in the co-cultivation of vegetables (Mladenova et al., 2023, 2024), as well as in broadleaf vegetation compared to coniferous vegetation (Malcheva et al., 2023).

Combined cultivation increases enzyme activities, including catalase activity, and the effect depends on the category of the enzyme, the type of plant, and other factors (Curtright and Tiemann, 2021; Mladenova et al., 2023).

Catalase contains iron in its non-protein component and some authors indicate a dependence of catalase on the iron content in the soil, as well as on the humidity and organic matter content in the soils (Grozeva and Nustorova, 1995; Mladenova et al., 2024).

Some authors have found a positive correlation between catalase activity and soil potassium and phosphorus levels, but the relationship with nitrogen is statistically insignificant (Türkay et al., 2024).

Catalase has a biological origin (organic matter, living and dead inhabitants) and abiotic origin (soil-forming rocks containing iron and manganese) (Grozeva and Nustorova, 1995; Hristov, 2009).

Soil catalase activity is determined as a function of the state of soil aeration, with its activity decreasing under hypoxic conditions (Brzezińska et al., 2005). Catalase is a key biological marker reflecting microbial activity and soil aeration for early diagnosis and assessment of soil health, and guiding sustainable soil management practices (Türkay et al., 2024).

In a comparison of garden soils and permafrost and high-altitude soils, Chabot et al. (2020) found that microbial communities experiencing higher levels of natural oxidative stress had higher basal intracellular catalase concentrations and catalase specific biomass activities. Other studies have shown that agronomic practices such as fertilization affect catalase activity, mainly indirectly, by affecting the development and activity of soil microorganisms (Naskova et al., 2016; Malcheva et al., 2018; Malcheva et al., 2019; Malcheva, 2021). While desert soils are dominated by Cyanobacteria and Proteobacteria (Mogul et al., 2017), garden, well-irrigated soils are typically dominated by Actinobacteria, Proteobacteria, and Firmicutes (Kim et al., 2013; Maksimova et al., 2016), with the former having two times lower catalase activity (Chabot et al., 2020).

Soil microbial parameters and enzyme activity are key indicators of soil health and fertility, and changes in land use and management practices affect them (Meena and Rao, 2021). Microbial diversity and catalase activity are key diagnostic indicators of the biological and biochemical state of soils.

The aim of the present study was to analyze the response of catalase to the influence of microbiological and chemical soil parameters in sole and intercropped cabbage cultivation.

MATERIALS AND METHODS

Agrogenic soils were studied, with sole and intercropped cultivation of cabbage, from the area of the Educational and Experimental Field of the Forestry University in the district Vrazhdebna, Sofia. The variants are as follows:

► V0 - control - soil without vegetation, before starting the experiment;

► V1 - sole cabbage cultivation - *Brassica oleraceae* L. var. *capitate* L.;

► V2 - intercropped cabbage cultivation - cabbage and leek - *Brassica oleraceae* L. var. *capitate* L. and *Allium porrum* L.;

► V3 - intercropped cabbage cultivation - cabbage and beans - *Brassica oleraceae* L. var. *capitate* L. and *Phaseolus vulgaris* L.;

► V4 - intercropped cabbage cultivation - cabbage and tagetes *Brassica oleraceae* L. var. *capitate* L. and *Tagetes patula* L.;

► V5 - intercropped cabbage cultivation - cabbage and flower mix (calendula *Calendula officinalis* L., borage *Borago officinalis* L., blue cornflower *Centaurea cyanus* L., phacelia *Phacelia* sp. Jus., buckwheat *Fagopyrum esculentum* Mill.);

► V6 - intercropped cabbage cultivation - cabbage and dill - *Brassica oleraceae* L. var. *capitate* L. and *Anethum graveolens* L.

The samples were taken from the root zone, at a depth of about 25 cm, and for microbiological analyses, the sampling was carried out sterilely. Microbiological analyses were carried out by the method of limiting dilutions and subsequent cultures on solid nutrient media, specific for each of the studied groups of microorganisms- Meat peptone agar for non-spore-forming bacteria and bacilli, Czapek-Dox agar for mold fungi and Actinomycete isolation agar for actinomycetes and bacteria assimilating mineral nitrogen (Gushterov et al., 1977; Mishustin & Emtsev, 1989). The cultures are cultivated in a thermostat at 25 °C. The total microflora (sum of bacteria, actinomycetes and micromycetes) and the mineralization coefficient (bacteria assimilating mineral nitrogen/(non-spore-forming bacteria + bacilli) were determined by calculation (Mishustin & Runov, 1957; Malcheva & Naskova, 2018).

Catalase was determined by the titration manganometric method (Khaziev, 1976).

Nitrogen was determined by the Kjeldahl method, phosphorus by the Petko Ivanov method, humus by the Tyurin method, total carbon - by calculation (Donov et al., 1974). Potassium and iron were determined by atomic absorption spectrophotometer. The active reaction was determined potentiometrically in an aqueous extract.

Correlation analysis was used as a statistical method, implemented using the Excel 2010 program.

RESULTS AND DISCUSSIONS

The biogenicity of the studied soils was higher when cabbage was grown together with: beans, tagetes, flower mix and dill (higher than the control) than when cabbage was grown alone and in combination with leeks (lower than the control) (Table 1).

Table 1. Quantity and composition of soil microflora (CFU/g)

Variants	Total micro-flora	Non-spore-for-ming bacteria	Bacilli	Acti-nomycetes	Micro-mycetes	Bacteria assimi-lating mineral nitrogen
V 0	1909940	1085800	76540	694200	53400	4722933
V 1	1260720	778940	406650	53350	21780	2292630
V 2	1093200	447300	561790	73460	10650	1021750
V 3	2328120	982160	1198440	94720	52800	3615520
V 4	2809775	1885230	770650	104775	49120	2160040
V 5	2390740	1686150	628130	50550	25910	1179310
V 6	2618440	1926930	606950	35600	48960	1958040

The total microflora is highest in the combined cultivation of cabbage and tagetes (V4), and lowest in cabbage and leeks (V2). In all variants, the amount of bacteria is higher than that of mycelial forms (actinomycetes and molds). In V1 (cabbage), V4 (cabbage and tagetes) and V5 (cabbage and flower mix) the distribution of microorganisms in the composition of the total microflora follows the descending order: non-spore-forming bacteria > bacilli > actinomycetes > molds. A similar distribution of the main groups of microorganisms in agrogenic soils is also indicated in other studies (Naskova et al., 2016; Plamenov et al., 2016; Yankova et al., 2016; Malcheva et al., 2018; 2019; Malcheva, 2021; Frene et al., 2022; Guo et al., 2022; Mladenova et al., 2023; 2024; Koleva et al., 2024). While in V2 (cabbage and leek) and V3 (cabbage and beans) the amount of bacilli is higher than that of non-spore-forming bacteria, in V6 (cabbage and dill) molds are more than actinomycetes. In the control, before the start of the experiment - V0, the amount of actinomycetes is higher than that of bacilli. The percentage of non-spore-forming bacteria in V4, V5 and V6 is about 70%, in V1 and V0 about 60%, in V2 and V3 about 40%. The participation of bacilli is as follows: about 50% in V2 and V3, about 30% in V1, V4 and V5, about 20% in V6 and only 4% in the control. The bacilli are spore-forming and adapt better to adverse conditions and changes in the environment. The lower percentage of bacilli in V0 is due to a higher percentage of actinomycetes - 36%. In the other variants, the amount of actinomycetes varies from 1 to 7%, and that of micromycetes is 1-2 in the variants with cultures and 3% in the control. The mineralization coefficient (MC) was determined from the ratio of bacteria assimilating mineral nitrogen and the sum of ammonifying bacteria (non-spore-forming bacteria and bacilli) (Figure 1). The results show that mineralization activity does not depend only on the amount of microorganisms.

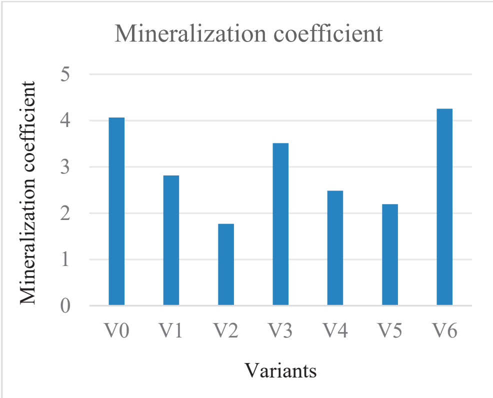

Figure 1. Values of mineralization coefficient

The highest MC values are at V6 (cabbage and dill), followed by V0 (control) and V3 (cabbage and beans), and the lowest rate of organic matter decomposition is at V2 (cabbage and leek). Mineralization activity also depends on a group of factors: composition and quantity of microorganisms, soil humidity and temperature, humus and nutrient content, vegetation type and other factors (Naskova et al., 2015; 2016; Plamenov et al., 2016; Yankova et al., 2016; Malcheva et al., 2018; 2019; Malcheva, 2021; Mladenova et al., 2023; 2024; Koleva et al., 2024). The enzymatic activity of soil microorganisms is represented by the values of the enzyme catalase - an exoenzyme that contains iron. The values of catalase and iron are presented in Figure 2.

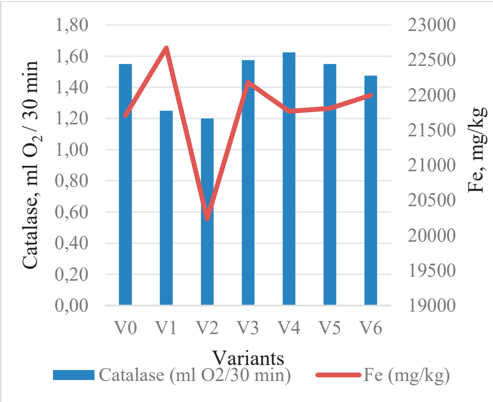

Figure 2. Dependence of catalase on iron

Catalase activity of soil microorganisms is highest in V4 (cabbage and tagetes) and lowest in V2 (cabbage and leek). This trend correlates with iron content to some extent in V4, while in V2 iron has the lowest value. Mineralization activity and enzyme activity are related to the humus and carbon content of the soils, the values of which are presented in Figure 3.

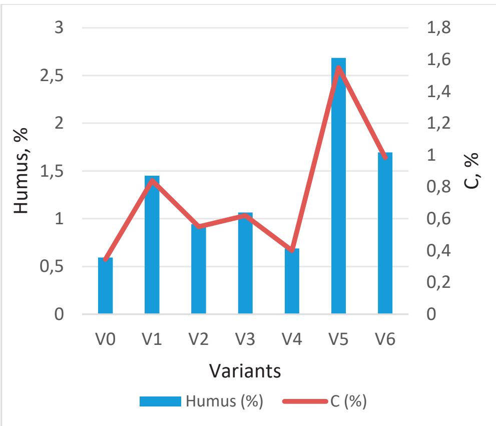

Figure 3. Humus and carbon content (%)

The organic matter content is higher in the variants with agricultural crops compared to the control, without vegetation. The highest values of humus and carbon are in V5 (cabbage and flower mix), followed by V6 (cabbage and dill) and V1 (cabbage), and the lowest in V4 (cabbage and tagetes) and V0. Catalase activity depends on a complex of factors - amount and composition of soil microflora, soil humidity and temperature, iron and nutrient content, type of vegetation, independent and joint cultivation of agricultural crops and other factors (Grozeva and Nustorova, 1995; Uzun and Uyanöz, 2011; Naskova et al., 2015; 2016; Malcheva et al., 2018; Malcheva et al., 2019; Malcheva, 2021; Mladenova et al., 2023; 2024). The nutrient content is presented in Table 2.

Table 2. Nutrient content in soils

Variants	N, %	NH_4^+-N (mg/kg)	NO_3^--N (mg/kg)	P (mg/kg)	P_2O_5 (mg/100 g)	K (mg/kg)	K_2O (mg/100 g)
V0	0.134	36.84	13.18	899.33	43.85	2255	19.17
V1	0.128	12.18	8.50	899.33	51.23	2317	14.90
V2	0.112	18.42	13.88	865.77	45.08	2071	16.00
V3	0.108	14.73	11.90	899.33	43.44	2296	16.60
V4	0.108	24.65	0.00	856.06	43.85	2112	14.90
V5	0.116	34.29	7.93	885.91	49.59	2194	17.90
V6	0.101	21.82	5.67	892.62	50.41	2235	14.80

The studied soils are well-stocked with nutrients. The content of total nitrogen is highest in the control and lowest in the co-cultivation of cabbage and dill (V6). The values of the available forms of nitrogen are highest in V0 and V5 for ammonium ions, V2 and V0 for nitrate ions. Total phosphorus has the highest values in V0, V1 and V3, and the lowest in V4. The share of available phosphorus is highest in V1 and V6, and lowest in V3, V4 and V0. The content of total potassium is highest in the independent cultivation of cabbage (V1) and lowest in the co-cultivation of cabbage and leek (V2). The available potassium has the highest values in the control, followed by the combined cultivation of cabbage and flower mix (V5), and the lowest values in the combined cultivation of cabbage and dill (V6), cabbage and tagetes (V4) and the independent cultivation of cabbage (V1). These discrepancies in the trends for total and available values are also due to the activity of soil microorganisms. The correlation dependences of catalase on the studied indicators are presented in Table 3.

Table 3. Values of correlation coefficient

Indicator	TM	Bacteria	Bacilli	Act.	Mold	B-N	MC	Cat.	pH	Humus	C	Fe	N	NH_4	NO_3	P	P_2O_5	K
TM	1.00																	
Bacteria	0.91	1.00																
Bacilli	0.42	0.15	1.00															
Act.	-0.08	-0.14	-0.63	1.00														
Mold	0.73	0.53	0.18	0.43	1.00													
B-N	0.11	-0.12	-0.18	0.79	0.72	1.00												
MC	0.36	0.28	-0.14	0.46	0.78	0.71	1.00											
Cat.	0.90	0.71	0.30	0.27	0.82	0.43	0.39	1.00										
pH	0.10	0.13	0.68	-0.99	-0.40	-0.77	-0.49	-0.23	1.00									
Humus	0.11	0.27	-0.40	0.21	-0.07	-0.02	0.16	0.17	-0.28	1.00								
C	0.11	0.27	-0.40	0.21	-0.07	-0.01	0.17	0.17	-0.28	1.00	1.00							
Fe	0.34	0.34	0.11	-0.05	0.43	0.37	0.49	0.34	0.03	0.23	0.23	1.00						
N	-0.53	-0.45	-0.74	0.68	-0.15	0.48	0.05	-0.20	-0.70	0.34	0.34	0.17	1.00					
NH_4	0.35	0.42	-0.49	0.62	0.26	0.21	0.12	0.53	-0.64	0.64	0.64	-0.16	0.32	1.00				
NO_3	-0.68	-0.80	-0.21	0.39	-0.28	0.30	0.05	-0.42	-0.40	0.16	0.16	-0.36	0.43	-0.02	1.00			
P	-0.12	-0.17	-0.16	0.29	0.26	0.55	0.67	0.02	-0.34	0.50	0.50	0.62	0.41	-0.03	0.45	1.00		
P_2O_5	-0.12	0.20	-0.23	-0.46	-0.40	-0.48	0.01	-0.38	0.37	0.52	0.52	0.41	0.01	-0.16	-0.21	0.33	1.00	
K	0.00	-0.07	-0.02	0.18	0.35	0.58	0.64	0.11	-0.21	0.32	0.32	0.84	0.37	-0.19	0.20	0.93	0.33	1
K_2O	-0.04	-0.15	-0.36	0.74	0.16	0.49	0.14	0.33	-0.74	0.59	0.59	-0.15	0.56	0.75	0.57	0.34	-0.34	0.1

Legend: TM - Total microflora; Act. - Actinomycetes; B-N - Bacteria assimilating mineral nitrogen; MC - Mineralization coefficient; Cat. - Catalase.

A high correlation was found between catalase and the total microflora in the soil (r=0.90), mainly determined by the activity of non-spore-forming bacteria (r=0.71) and mold fungi (r=0.82). A moderate correlation exists between total microflora and mineralization coefficient (0.36), and total mineralization activity and catalase (r=0.39). The relationship between catalase and ammonium (r=0.53) and potassium ions (r=0.33) is moderate, while no such relationship was found with phosphorus (r=0.02). Iron is involved in the structure of catalase and the dependence between Fe and the enzyme is also moderate (r=0.342). A weak influence on catalase activity in the variants was observed from the quantity of bacilli (r=0.30) and actinomycetes (r=0.27), as well as from the content of humus (r=0.18) and carbon (r=0.17). There is no positive correlation between pH and catalase (r=-0.24). The pH value in the control was 7.2, and in all other variants - 7.6, i.e. there was no variation in the value in the variants with agricultural crops. Some authors found a positive correlation between catalase activity and the levels of potassium and phosphorus in the soil, but the relationship with nitrogen was statistically insignificant (Türkay et al., 2024). Other studies indicate that catalase activity depends significantly on the humidity of arable soils with vegetable cultivation (Mladenova et al., 2024).

CONCLUSIONS

Catalase activity is very much dependent on the amount and composition of the soil microflora. It is mainly determined by the development of non-spore-forming bacteria and mold fungi, despite the fact that the former have a high percentage participation, and the latter the lowest percentage participation in the composition of the total microflora. The total microflora and catalase activity are highest in the combined cultivation of cabbage and tagetes, and lowest in cabbage and leek.

There is a moderate correlation between iron and catalase. The relationship between catalase and ammonium and potassium ions is moderate, while such a relationship has not been established for phosphorus. A weak influence on the activity of catalase in the variants is observed by the quantity of bacilli and actinomycetes, as well as the content of humus and carbon. The dependence between the rate of mineralization and catalase is moderate. The mineralization activity does not depend entirely and solely on the quantity of microorganisms (moderate correlation) - the highest values of the mineralization coefficient are in cabbage and dill, and the lowest in cabbage and leek. There is no correlation between catalase and soil pH.

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