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MICROBIOLOGICAL AND BIOCHEMICAL PROPERTIES OF SOIL DEPENDING ON ADENINE AND AZOTOBACTERIN APPLIED

Jadwiga Wyszkowska, Mirosław Kucharski, Jan Kucharski

Chair of Microbiology
University of Warmia and Mazury in Olsztyn

Abstract

The aim of the study was to determine how adenine affected biological proprieties of soil. The performance of this precursor cytokinine was tested in a pot trial. The question posed was whether it was possible to improve efficacy of adenine by enlarging populations of bacteria from *Azotobacter* species in soil. The experiment was carried out on proper brown soil, formed from dust clay sand with pH_{KCl} 6,9. Pots were filled with 3.2 kg of soil. The investigations were performed in two series: with and without addition Azotobacterin to soil. Tow rates of nitrogen fertilisation: 0 and 50 mg $N \cdot kg^{-1}$ of soil were applied in test. Adenine was applied in the following quantities: 0; 5; 10 and 15 $mg \cdot kg^{-1}$ of soil. Radish, 6 plants per pot, was the test plant.

It was confirmed that the adenine had a significant effect on growth and development of radish. It positively affected microbiological and the biochemical proprieties of soil. The counts of total oligotrophic bacteria, oligotrophic sporulation bacteria, total copiotrophic bacteria, copiotrophic sporulation bacteria, ammonifying bacteria, immobilizing bacteria, celulolytic bacteria, *Azotobacter* sp., *Artrobacter* sp. and *Pseudomonas* sp. were increased, and the number of fungi diminished. Adenine also stimulated activities of dehydrogenase, urease and alkaline phosphatase, although it depressed the activity of acid phosphatase. The inoculation with bacteria from *Azotobacter* species applied to soil failed to improve efficacy of adenine. Nevertheless, it increased counts of these bacteria, which had a beneficial influence on the development of oligotrophic bacteria, immobilizing bacteria, celulolytic bacteria and actinomyces, while negatively affecting fungi, ammonifying bacteria and *Arthrobacter*.

K e y w o r d s: adenine, enzymes activity, microorganisms numbers.

prof. dr hab. Jan Kucharski, Chair of Microbiology, Univwersity of Warmia and Mazury, ul. pl. Łódzki, 10 -727 Olsztyn, Poland, phone: 089-49-38, e-mail: jan.kucharski@uwm.edu.pl.

MIKROBIOLOGICZNE I BIOCHEMICZNE WŁAŚCIWOŚCI GLEBY KSZTAŁTOWANE PRZEZ ADENINĘ I AZOTOBAKTERYNĘ

Abstrakt

Celem badań było określenie wpływu adeniny na biologiczne właściwości gleby. Działanie tego prekursora cytokinin testowano w doświadczeniu wazonowym. Sprawdzano możliwość wzmocnienia efektywności adeniny przez zwiększenie w glebie puli bakterii z rodzaju *Azotobacter*. Badania wykonano w próbkach gleby brunatnej właściwej, wytworzonej z piasku gliniastego pylastego o pH_{KCl} 6,9. W wazonach umieszczono po 3,2 kg gleby. Badania obejmowały dwie serie: bez i z dodatkiem do gleby szczepionki Azotobakteryny. W doświadczeniu zastosowano zróżnicowane nawożenie azotem: 0 i 50 mg $N \cdot kg^{-1}$ gleby. Adeninę stosowano w następującej ilości: 0; 5; 10 i 15 mg $\cdot kg^{-1}$ gleby. Rośliną doświadczalną była rzodkiewka – 6 roślin w wazonie.

Stwierdzono, że adenina istotnie wpływała na wzrost i rozwój rzodkiewki. Korzystnie oddziaływała na mikrobiologiczne i biochemiczne właściwości gleby. Zwiększała liczebność bakterii: oligotroficznych ogółem, oligotroficznych przetrwalnikujących, kopiotroficznych ogółem, kopiotroficznych przetrwalnikujących, amonifikacyjnych, immobilizujących azot, celulolitycznych, *Azotobacter* sp., *Arthrobacter* sp., *Pseudomonas* sp. oraz promieniowców, a zmniejszała liczbę grzybów. Stymulowała także aktywność dehydrogenaz, ureazy i fosfatazy alkalicznej, natomiast hamowała aktywność fosfatazy kwaśnej. Zastosowana szczepionka złożona z bakterii z rodzaju *Azotobacter* nie poprawiała efektywności adeniny. Zwiększała jednak liczbę tych bakterii w glebie, co wpłynęło korzystnie na rozwój bakterii oligotroficznych, immobilizujących azot, celulolitycznych oraz promieniowców, natomiast negatywnie – na grzyby oraz bakterie amonifikacyjne i bakterie z rodzaju *Arthrobacter*.

Słowa kluczowe: adenina, aktywność enzymów, liczebność drobnoustrojów, rzodkiewka.

INTRODUCTION

Phytohormones are synthesised by plants, lichens, mosses and by microorganisms (Ergün et al. 2002, Khalid et al. 2004). According to Jameson (2000), 80% of microorganisms isolated from the rhizosphere are capable of synthesising phytohormones. For this reason, plants can be supplied with growth regulators by introducing their precursors into the soil and by relying on the ability of soil microbes to synthesise phytohormones (Jameson 2000, Karadeniz et al. 2006, Kucharski et al. 1999, Wyszkowska, Kucharski 2001). Phytohormone biosynthesis is most intense in the rhizosphere due to higher availability of substrates in this environment as well as the quantitative and qualitative diversity of microorganisms. The rate of biosynthesis is determined by soil fertility and the availability of the appropriate precursors. Their synthesis can be enhanced by providing soil-dwelling microbes with optimal conditions for growth and development (Nieto, Frankenberger 1990, Taylor et al. 2006).

Cytokinins are an important group of growth regulators. They are produced by, among others, bacteria of the genera *Arthrobacter, Azospirillum, Azotobacter* and *Rhizobium*. They are also synthesised in large quantities by selected phytopathogens, such as *Corynobacterium fascians,*

Agrobacterium tumefaciens, Pseudomonas savastanoi), and fungi of the genera *Amanita, Boletus, Dictyostelium, Exobasidium, Glomus, Monilia, Nectria, Plasmodiophora, Rhizopagon, Suillus* and *Taphrina* (JAMESON 2000, KARADENIZ et al. 2006, NIETO, FRANKENBERGER 1990, TAYLOR et al. 2006). Cytokinin biosynthesis can also be enhanced through the application of adenine to soil (JAMESON 2000, KARADENIZ et al. 2006, KUCHARSKI et al. 1999, WIERZBOWSKA 2006, WYSZKOWSKA, KUCHARSKI 2001).

The above findings encouraged us to conduct a study investigating the effect of adenine (cytokinin precursor), interacting with nitrogen-fixing bacteria (*Azotobacter*), on the biological properties of soil.

MATERIALS AND METHODS

The study was carried out in 4 replications in a greenhouse at the University of Warmia and Mazury in Olsztyn. The experiment involved samples of typical brown soil developed from very fine, light loamy sand with pH_{KCl} of 6.9, hydrolytic acidity of 5.7 mmol(H^+)·kg^{-1}, total exchangeable bases of 68.5 mmol(+)·kg^{-1} and organic carbon content of 6.6 g·kg^{-1}. Prior to pot filling, soil was fertilised with the following macroelements in mg·kg^{-1} of soil (expressed as pure substance): P – 50 [K_2HPO_4]; K – 90 [KH_2PO_4+ KCl]; Mg – 20 [$MgSO_4 \cdot 7H_2O$]. Different rates of nitrogen fertiliser were applied at 0 and 50 mg·kg^{-1}. Mineral fertilisers were introduced into the soil in a single application before plant sowing, and they were thoroughly mixed with the entire soil material intended for 1 pot (3.2 kg of soil). The study covered two series of experiments: with and without Azotobacterin inoculation. Azotobacterin was applied in the form of a water suspension (one package was dissolved in 1 dm^3 of water and 20 cm^3 of the suspension was added per pot). Prior to the experiment, soil was mixed with mineral fertilisers and, in respective plots, it was combined with adenine which was administered in the following quantities: 0; 5; 10 and 15 mg·kg^{-1} soil.

Soil was placed in pots and kept at a moisture level of 60% of capillary water capacity throughout the entire experiment (42 days). The experimental plant was radish cv. Saxa (6 plants per pot). The plants were harvested on day 42 of the experiment. Soil samples were collected repeatedly at the same time, and they were subjected to microbiological and biochemical analyses.

Microbiological analyses involved the determination of the counts of: oligotrophic bacteria (Olig), spore-forming oligotrophic bacteria ($Olig_p$), copiotrophic bacteria (Cop) and spore-forming copiotrophic bacteria (Cop_p) – on ONT and HATTORI medium (1983); *Azotobacter* spp. (Az) – as described by FENGLEROWA (1965); *Arthrobacter* (Art), *Pseudomonas* (Ps), nitrogen-

immobilising bacteria (Im), ammonification bacteria (Am) and cellulose-decomposing bacteria (Cel) – on a medium described by WYSZKOWSKA et al. (2007); actionomyces (Act) – on Kuster and Williams medium with the addition of nystatin and actidione (PARKINSON et al. 1971); fungi (Fun) – on MARTIN medium (1950). Microorganisms were cultured on Petri dishes at a temperature of 28°C for 2 (*Azotobacter*) to 21 (oligotrophic bacteria) days. The counts of spore-forming oligotrophic and copiotrophic bacteria were determined in material pasteurised for 15 minutes at 85°C. The number of colony-forming units (cfu) was estimated with the use of a colony meter.

Biochemical analyses involved the determination of the activity of: dehydrogenases with TTC substrate (ÖHLINGER 1996), urease – by the method proposed by ALEF and NANNPIERI (1998), acid phosphatase and alkaline phosphatase – as described by ALEF et al. (1998). The activity of dehydrogenases was indicated in cm^3 H_2 required to reduce TTC to TFP, of urease – in mg N-NH_4 produced from hydrolysed urea, of phosphatases – in mmol p-nitrophenol (PNP) produced from sodium 4-nitrophenyl phosphate.

The results were verified statistically by Duncan's multiple range test with the use of a three-factorial analysis of variance (StatSoft, Inc....2005). This study presents only selected results obtained in respect of the investigated factors, but it does not illustrate the interactions observed between those factors because the reported interactions had no significant effect on the quality and reliability of the presented results.

RESULTS AND DISCUSSION

Adenine applied at a dose of 5 to 15 $mg \cdot kg^{-1}$ d.m. of soil significantly increased the abundance of oligotrophic, copiotrophic, ammonification, nitrogen-immobilising bacteria, *Azotobacter*, *Arthrobacter*, cellulose-decomposing bacteria, *Pseudomonas* and actionomyces (Table 1). Adenine had a strong inhibitory effect on fungi. The stimulating effect on the above microorganisms and the inhibitory effect on fungi increased at higher adenine doses. The highest increase was observed in respect of ammonification bacteria, and the lowest – in *Azotobacter* populations. The population size of ammonification bacteria increased 2.5-fold following the application of an adenine dose of 15 $mg \cdot kg^{-1}$ d.m. of soil.

Adenine also had a stimulating effect on the activity of dehydrogenases, urease and alkaline phosphatase in the soil, and it inhibited the activity of acid phosphatase (Table 2). Changes in enzymatic activity were intensified with an increase in adenine doses.

Table 1
Tabela 1

The effect of adenine on numbers of soil microorganisms (cfu kg^{-1} of d.m. soil)
Wpływ adeniny na liczebność drobnoustrojów glebowych (jtk · kg^{-1} s.m. gleby)

Microorganisms Drobnoustroje	Adenine dose (mg kg^{-1} of d.m. soil) Dawka adeniny mg · kg^{-1} s.m. gleby				*r*	$LSD_{0.01}$ $NIR_{0,01}$
	0	5	10	15		
Olig · 10^8	16.13	20.16	20.70	29.66	0.930	3.89
Oligp · 10^7	10.57	14.52	16.94	20.88	0.995	1.54
Cop · 10^8	47.94	54.12	58.16	66.67	0.990	4.11
Copp · 10^7	14.13	16.52	16.70	17.68	0.927	1.11
Am · 10^8	48.93	65.77	95.07	124.19	0.993	6.18
Im · 10^8	45.07	44.81	50.45	56.27	0.936	5.06
Cel · 10^7	47.40	51.26	56.72	60.93	0.998	4.63
Az · 10^4	16.16	17.62	18.53	20.11	0.995	1.96
Art · 10^7	20.43	23.03	27.06	29.12	0.993	3.47
Ps · 10^7	41.40	41.49	47.76	52.33	0.951	4.52
Act · 10^8	50.90	65.95	74.55	79.57	0.973	5.56
Fun · 10^6	54.75	46.33	41.31	37.37	-0.984	4.78

r – correlation coefficient – współczynnik korelacji; Olig – oligotrophic bacteria – bakterie oligotroficzne, $Olig_p$ – oligotrophic sporulation bacteria – bakterie oligotroficzne przetrwalnikujące, Cop – copiotrophic bacteria (bakterie kopiotroficzne), Cop_p – copiotrophic sporulation bacteria – bakterie kopiotroficzne przetrwalnikujące) Am – ammonifying bacteria – bakterie amonifikacyjne, Im – immobilizing nitrogen bacteria – bakterie immobilizujące azot, Cel – cellulolytic bacteria – bakterie celulolityczne, Az –*Azotobacter* sp., Art – *Artrobacter* sp., Ps – *Pseudomonas* sp., Act – actinomyces – promieniowce, Fun – fungi – grzyby

Adenine had a positive effect on the microbiological and biochemical properties of soil and it stimulated the growth and development of the analysed radish cultivar (Figure 1). Adenine increased the yield of both radish shoots and roots as higher doses of the substance were administered.

Azotobacterin had a much less pronounced effect on the biological properties of soil than adenine. The inoculum did not increase the abundance of all analysed microorganisms (Table 3). Azotobacterin stimulated the growth of oligotrophic bacteria, spore-forming copiotrophic bacteria, nitrogen-immobilising bacteria, cellulose-decomposing bacteria and actinomyces, while it reduced the total counts of copiotrophic bacteria, ammonification bacteria, *Arthrobacter* and fungi. The inoculum had no impact on bacteria of the genus *Pseudomonas*. For obvious reasons, Azotobacterin enriched the soil with *Azotobacter* bacteria whose population increased 89-fold.

Table 2
Tabela 2

The effect of adenine on soil enzymes activity (per kg^{-1} of d.m. soil)
Wpływ adeniny na aktywność enzymów w 1 kg s.m. gleby

Enzymes Enzymy	Adenine dose (mg kg^{-1} of d.m. soil) Dawka adeniny mg·kg^{-1} s.m. gleby				r	$LSD_{0.01}$ $NIR_{0,01}$
	0	5	10	15		
Dehydrogenases Dehydrogenazy (cm^3 H_2·d^{-1})	2.73	3.21	3.26	3.48	0.938	0.12
Urease Ureaza (mg N-NH_4·h^{-1})	6.90	7.20	7.56	8.16	0.986	0.18
Alkaline phosphatase Fosfataza alkaliczna (mmol PNP·h^{-1})	1.15	1.23	1.27	1.31	0.984	0.05
Acid phosphatase Fosfataza kwaśna (mmol PNP·h^{-1})	1.15	1.17	1.08	1.08	-0.793	0.05

r – correlation coefficient – współczynnik korelacji

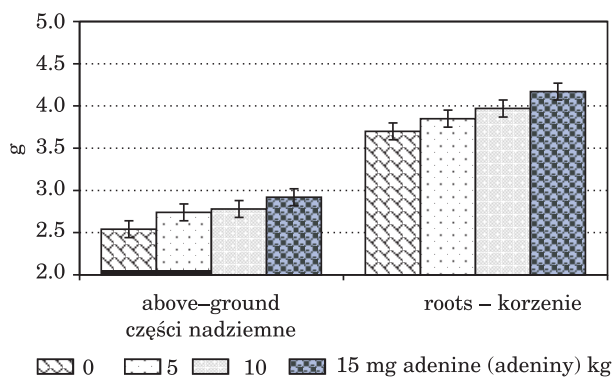

Fig. 1. Effect of adenine on radish yield (in g d.m. per pot)
Rys. 1. Wpływ adeniny na plon rzodkiewki (g s.m.·$wazon^{-1}$)

Azotobacterin had a completely different effect on the activity of soil enzymes than adenine (Table 4). It inhibited dehydrogenase and urease activity. Azotobacterin had a stimulating effect on acid phosphatase, but no interactions with alkaline phosphatase were observed. Azotobacterin did not affect the growth and development of radish (Figure 2).

The third investigated factor – nitrogen fertilisation – also influenced the biological properties of soil. Nitrogen had a varied impact, which was less explicit than that of adenine, but it was more pronounced than the influence of Azotobacterin. Nitrogen fertilisation enriched the soil with

Table 3
Tabela 3

The effect of Azotobacterin on numbers of soil microorganisms (cfu kg^{-1} of d.m. soil)
Wpływ Azotobakteryny na aktywność drobnoustrojów glebowych (jtk · kg^{-1} s.m. gleby)

Microorganisms* Drobnoustroje*	Without Azotobacterin Bez Azotobakteryny	With Azotobacterin Z Azotobakteryną	$LSD_{0.01}$ $NIR_{0,01}$
Olig · 10^8	20.07	23.26	2.76
Oligp · 10^7	14.38	17.07	1.09
Cop · 10^8	61.29	52.15	2.91
Copp · 10^7	15.81	16.70	0.78
Am · 10^8	87.86	79.12	4.37
Im · 10^8	43.64	54.66	3.58
Cel · 10^7	50.05	58.11	3.28
Az · 10^3	4.08	357.98	13.82
Art · 10^7	26.84	22.98	2.45
Ps · 10^7	46.42	45.07	n.s.
Act · 10^8	59.81	75.67	3.93
Fun · 10^6	51.66	38.22	3.38

* Explanations under Table 1 – Objaśnienia podano pod tabelą 1

Table 4
Tabela 4

The effect of Azotobacterin on soil enzymes activity (per kg^{-1} of d.m. soil)
Wpływ Azotobakteryny na aktywność enzymów w 1 kg s.m. gleby

Enzymes Enzymy	Without Azotobacter inoculum Bez Azotobakteryny	With Azotobacter inoculum Z Azotobakteryną	$LSD_{0.01}$ $NIR_{0,01}$
Dehydrogenases Dehydrogenazy ($cm^3 H_2 \cdot d^{-1}$)	3.32	3.02	0.08
Urease Ureaza (mg $N\text{-}NH_4 \cdot h^{-1}$)	7.92	6.99	0.13
Alkaline phosphatase Fosfataza alkaliczna (mmol PNP · h^{-1})	1.25	1.23	n.s.
Acid phosphatase Fosfataza kwaśna (mmol PNP · h^{-1})	1.06	1.18	0.04

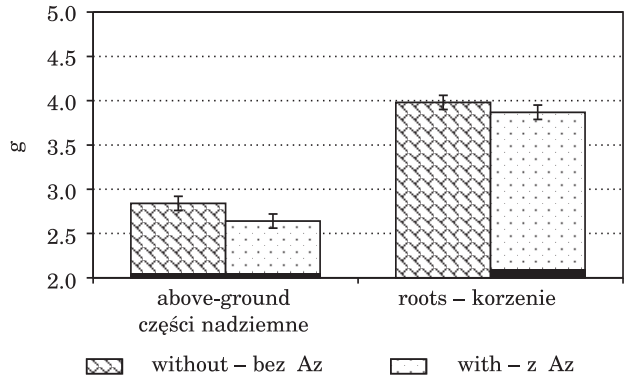

Fig. 2. Effect of Azotobacterin (Az) on radish yield (in g d.m. per pot)
Rys. 2. Wpływ Azotobakteryny (Az) na plon rzodkiewki (g s.m.·wazon^{-1})

spore-forming oligotrophic bacteria, copiotrophic bacteria, ammonification bacteria and *Arthrobacter*, and reduced the counts of nitrogen-immobilising bacteria, *Azotobacter*, *Pseudomonas*, actionomyces and fungi (Table 5). Nitrogen enhanced the activity of urease and phosphatases, but it inhibited dehydrogenase activity (Table 6). It created a favourable environment for radish growth and development (Figure 3). Nitrogen had a particularly beneficial impact on the growth of radish aboveground parts.

Various research studies (Arkipova et al. 2007, Kano, Fukuoka 1996, Muhammad et al. 2007, Nieto, Frankenberger 1990, Wierzbowska 2006) suggest that phytohormone precursors are capable of increasing plant biomass. Phytohormone precursors are converted by microorganisms into growth-regulators, and they have a beneficial effect on the microbiological properties of soil (Kucharski et al. 1999, Taylor et al. 2006, Wyszkowska, Kucharski 2001). These interactions most probably occurred in the discussed experiment. Adenine increased the total counts of soil microbes and stimulated the activity of most enzymes, thus creating favourable conditions for the growth and development of radish and, consequently, increasing radish yield.

Contrary to our expectations, the Azotobacterin inoculum and nitrogen fertilisation did not enhance adenine's positive effect on the microbiological and biochemical properties of soil. Although its stimulating impact on the effectiveness of adenine was not determined, Azotobacterin had a beneficial influence on the majority of the investigated bacteria and actionomyces.

Table 5
Tabela 5

The effect of urea fertility on numbers of soil microorganisms (cfu kg^{-1} of d.m. soil)
Wpływ nawożenia mocznikiem na liczebność drobnoustrojów glebowych (jtk · kg^{-1} s.m. gleby

Microorganisms* Drobnoustroje*	Without urea Bez mocznika	With urea Z mocznikiem	$LSD_{0.01}$ $NIR_{0,01}$
Olig · 10^8	21.15	22.18	n.s.
Oligp · 10^7	13.71	17.75	1.09
Cop · 10^8	53.05	60.40	2.91
Copp · 10^7	16.21	16.30	n.s.
Am · 10^8	67.83	99.15	4.37
Im · 10^8	55.29	43.01	3.58
Cel · 10^7	54.31	53.85	n.s.
Az · 10^3	209.28	152.78	13.82
Art · 10^7	23.07	26.75	2.45
Ps · 10^7	49.37	42.12	3.19
Act · 10^8	70.92	64.56	3.93
Fun · 10^6	51.66	38.22	3.38

* Explanations under Table 1 – Objaśnienia podano pod tabelą 1

Table 6
Tabela 6

The effect of urea fertility on soil enzymes activity (per kg^{-1} of d.m. soil)
Wpływ nawożenia mocznikiem na aktywność enzymów w 1 kg s.m. gleby

Enzymes Enzymy	Without urea Bez mocznika	With urea Z mocznikiem	$LSD_{0.01}$ $NIR_{0,01}$
Dehydrogenases Dehydrogenazy (cm^3 $H_2 \cdot d^{-1}$)	3.24	3.10	0.08
Urease Ureaza (mg $N\text{-}NH_4 \cdot h^{-1}$)	7.08	7.83	0.13
Alkaline phosphatase Fosfataza alkaliczna (mmol $PNP \cdot h^{-1}$)	1.15	1.33	0.04
Acid phosphatase Fosfataza kwaśna (mmol $PNP \cdot h^{-1}$)	1.06	1.18	0.04

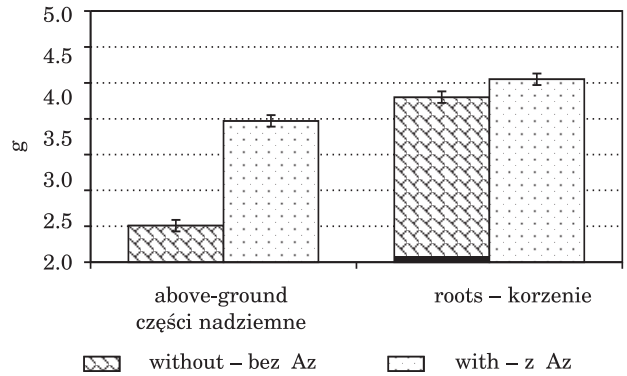

Fig. 3. Effect of urea fertility (N) on radish yield (in g d.m. per pot)

Rys. 3. Wpływ nawożenia mocznikiem (N) na plon rzodkiewki (g s.m.·wazon^{-1})

CONCLUSIONS

1. Adenine had a positive influence on soil bacteria, as it enhanced the activity of dehydrogenases, urease and alkaline phosphatase, and had an inhibitory effect on fungi and acid phosphatase.

2. The investigated inoculum improved the microbiological and biochemical properties of soil and positively affected radish growth and development.

3. Azotobacterin and urea fertilisation did not enhance the beneficial effect of adenine on the biological properties of soil.

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