

BIOLOGICAL EFFECTS OF L-TRYPTOPHAN AND THE VACCINE AZOTOBACTERYNA IN SOIL

Summary

The objective of this study has been to determine the effect of L-tryptophan on the biological properties of soil. The influence of L-tryptophan, a precursor of auxins, was tested in a pot experiment. Another aim has been to check whether it is possible to enhance the effect of L-tryptophan by increasing the pool of soil-borne *Azotobacter* spp. bacteria. The experiments were performed on samples of eutrophic brown soil developed from loamy sand of the pH_{KCl} equal to 6.9. Portions of soil weighing 3.2 kg were placed in pots. The research comprised two series: with and without the vaccine *Azotobacter* added to soil. L-tryptophan was applied in the following doses: 0; 0.5; 5; 50 and $mg\ kg^{-1}$ of soil. Onion was the tested plant. The results have demonstrated that L-tryptophan significantly affected the growth and development of onion. It had positive influence on the microbiological and biochemical properties of soil. L-tryptophan raised counts of the following bacteria: oligotrophic and their endospores, copiotrophic and their endospores, ammonifying, nitrogen-immobilizing, *Azotobacter*, and cellulolytic cells, as well as actinomycetes and fungi. It also stimulated the activity of dehydrogenases, urease, acid and alkaline phosphatase. In contrast, it decreased the counts of *Arthrobacter* and *Pseudomonas*. The vaccine *Azotobacter* increased the counts of such bacteria as *Azotobacter*, oligotrophs and their endospores, copiotrophs and their endospores, nitrogen-fixing bacteria, *Arthrobacter*, *Pseudomonas*, actinomycetes and fungi. The vaccine stimulated the activity of urease, but lowered the counts of ammonifying and cellulolytic bacteria as well as the activity of dehydrogenases.

Key words: L-tryptophane, enzymes activity, microorganisms numbers

BIOLOGICZNE SKUTKI DZIAŁANIA L-TRYPTOFANU I AZOTOBAKTERYNY W GLEBIE

Streszczenie

Celem badań było określenie wpływu L-tryptofanu na biologiczne właściwości gleby. Działanie tego prekursora auksyn testowano w doświadczeniu wazonowym. Sprawdzano możliwość wzmocnienia efektywności L-tryptofanu poprzez zwiększenie w glebie puli bakterii z rodzaju *Azotobacter*. Badania wykonano w próbkach gleby brunatnej eutroficznej typowej wytworzonej z piasku gliniastego (loamy sand) o pH_{KCl} 6,9. W wazonach umieszczono po 3,2 kg gleby. Badania obejmowały dwie serie: bez i z dodatkiem do gleby szczepionki *Azotobacter*. L-tryptofan stosowano w następującej ilości: 0; 0,5; 5 i 50 $mg\ kg^{-1}$ gleby. Rośliną doświadczalną była cebula. W wyniku przeprowadzonych badań stwierdzono, że L-tryptofan istotnie wpływał na wzrost i rozwój cebuli. Korzystnie oddziaływał na mikrobiologiczne i biochemiczne właściwości gleby. Zwiększał liczebność bakterii oligotroficznych i ich form przetrwalnych, koptroficznych i ich form przetrwalnych, amonifikacyjnych, immobilizujących azot, *Azotobacter*, celulolitycznych, promieniowców i grzybów oraz aktywność dehydrogenaz, ureazy, fosfatazy kwaśnej i fosfatazy alkalicznej, a zmniejszał - *Arthrobacter* i *Pseudomonas*. *Azotobacter* zwiększała liczebność bakterii: *Azotobacter*, oligotroficznych i ich form przetrwalnych, koptroficznych i ich form przetrwalnych, immobilizujących azot *Arthrobacter*, *Pseudomonas*, promieniowców i grzybów oraz stymulowała aktywność ureazy a zmniejszała liczebność bakterii amonifikacyjnych i celulolitycznych i aktywność dehydrogenaz.

Słowa kluczowe: L-tryptofan, aktywność enzymów, liczebność mikroorganizmów

1. Introduction

Auxins belong to basic phytohormones. They participate in all life processes of plants, including formation of radicles and cell divisions [7, 8, 10]. Apart from research on typical growth regulators, studies are now undertaken on possible use of saprophytic microorganisms to produce growth hormones from their precursors [1, 10, 12].

Free living microorganisms populating the rhizosphere support the growth of plants and are therefore referred to as PGPRs (plant growth-promoting rhizobacteria). Soil-borne microorganisms, for example bacteria of the genera *Pseudomonas*, *Arthrobacter*, *Azotobacter*, *Rhizobium*, *Acinetobacter* and *Azospirillum*, saprophytic and mycorrhizal fungi as well as cyanobacteria are able to synthesize auxins. They

produce indoleacetic acid from L-tryptophan [1, 3, 4, 15]. There are two hypothesis trying to explain the synthesis of IAA by soil microorganisms. The first one assumes that L-tryptophan is converted to indole-3-pyruvic acid with the help of transaminase and then undergoes decarboxylation to indole-3-acetic aldehyde, later oxidized to IAA. The second hypothesis suggests that L-tryptophan first undergoes decarboxylation to indole-3-acetamide and then is hydrolyzed to IAA [11].

The above considerations have encouraged the authors to carry out an experiment in order to determine the effect of L-tryptophan (precursor of auxins), co-acting with bacteria fixing free nitrogen from the air (*Azotobacter*) on biological properties of soil.

2. Material and methods

The trials, set up in four replications, were carried out in polyethylene pots kept in a greenhouse. Samples of soil collected from the arable humic horizon of eutrophic brown soil developed from loamy sand were used to in the experiment test. The soil had the following properties: pH_{KCl} 6.9, hydrolytic acidity $5.7 \text{ mmol}(\text{H}^+) \text{ kg}^{-1}$, total base exchangeable cations $68.5 \text{ mmol}(+) \cdot \text{kg}^{-1}$ and organic carbon content 6.6 g kg^{-1} . Prior to placing in pots (soil batches of 3.2 kg per pot), the soil was mixed in a polyethylene container with the following macronutrients, in mg kg^{-1} of soil, (expressed as pure element): N – 50 [$\text{CO}(\text{NH}_2)_2$], P – 50 [K_2HPO_4], K – 90 [$\text{KH}_2\text{PO}_4 + \text{KCl}$], Mg – 20 [$\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$]. The tests encompassed two series: with and without soil inoculation with Azotobakteryna produced by Biofood S.C. in Wałcz. Azotobakteryna (a soil inoculant) was applied to soil as aqueous solution (a packet of the vaccine was suspended in 1 dm^3 of water and 20 cm^3 of the suspension was added to each pot). Before setting up the experiment, the soil was mixed with mineral fertilizers and, in chosen pots, with L-tryptophan, which was added in the following doses: 0; 0,5; 5 and 50 mg kg^{-1} of soil.

Once placed in pots, the soil was watered until it reached the moisture level equal 60% of capillary water capacity. The same moisture level was maintained throughout the whole experiment (105 days). The experiment was conducted with cv. Wolska onion (4 plants per pot). The plants were harvested on 150 day of the experiment.

The microbiological assays included determination of counts of the following bacteria: oligotrophic and their endospores, copiotrophic and their endospores, Azotobacter, Arthrobacter, Pseudomonas, nitrogen-immobilizing, ammonifying and cellulolytic cells as well as actinomycetes and fungi. Colony forming unit (cfu) counts were determined using a colony counter.

The biochemical determinations included tests on the activity of dehydrogenases (EC 1.1), urease (EC 3.5.1.5) and acid (EC 3.1.3.2) and alkaline phosphatase (EC 3.1.3.1). The protocols for the performed microbiological and biochemical assays were explained in an earlier paper by Wyszowska et al. [14].

The results were processed statistically by Duncan's multiple range test, using two-factorial analysis of variance [13]. This paper contains only some of the results, namely the ones concerning particular factors, because interactions between the analyzed variables were weak and did not affect substantially the final results.

3. Results and discussion

Soil application of L-tryptophan and Azotobakteryna modified the biological activity of soil, as measured by counts of microorganisms and activity of enzymes (tabs 1-4). L-tryptophan added to soil in the doses of 0.5 to 50 mg kg^{-1} d.m. of soil significantly increased counts of oligotrophs ($r = 0.750$) and their endospores ($r = 0.908$), copiotrophs ($r = 0.757$) and their endospores ($r = 0.662$), ammonifying bacteria ($r = 0.909$), nitrogen-immobilizing bacteria ($r = 0.787$), Azotobacter ($r = 0.609$), cellulolytic bacteria ($r = 0.730$) and actinomycetes ($r = 0.511$) or fungi ($r = 0.745$). In contrast, it decreased numbers of a Arthrobacter ($r = -0.671$) and Pseudomonas ($r = -0.908$) (tab. 1). The higher the dose of L-tryptophan in soil, the stronger its

effect on microorganisms. A dose of 50 mg of L-tryptophan per kg^{-1} d.m. of soil most significantly raised counts of oligotrophic bacteria (a 78% increase), followed by spore-forming oligotrophs (71% more), nitrogen-immobilizing bacteria (a count higher by 68%), copiotrophs (55% more), copiotrophic spore forming bacteria (a 43% increase), fungi (42% more), Azotobacter (a count higher by 35%) and cellulolytic bacteria (a 34% increase). Actinomycetes and ammonifying bacteria were the least affected (an increase by 20% and 15%, respectively). In turn, the count of Arthrobacter spp. decreased by 42% in response to 50 mg L-tryptophan kg^{-1} d.m. of soil. The count of Pseudomonas fell down by 34%.

L-tryptophan stimulated the growth of most of the analyzed groups of microorganisms, as shown above. In addition, it stimulated the activity of dehydrogenases (by 61%), urease (by 31%) and acid (by 19%) and alkaline phosphatase (by 9%) (tab. 2). Changes in the enzymatic activity were larger in response to higher doses of L-tryptophan.

Recapitulating, the analyzed precursor of auxins had a beneficial effect on microbiological and biochemical properties of soil. Moreover, it promoted the growth and development of onion (fig. 1). In response to L-tryptophan, the yield of aerial parts and roots of onion increased. The increase was proportional to the increasing doses of L-tryptophan.

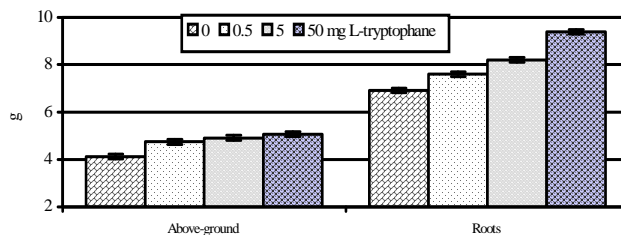


Fig. 1. Effect of L-tryptophane on onion yield (in g d.m. per pot)

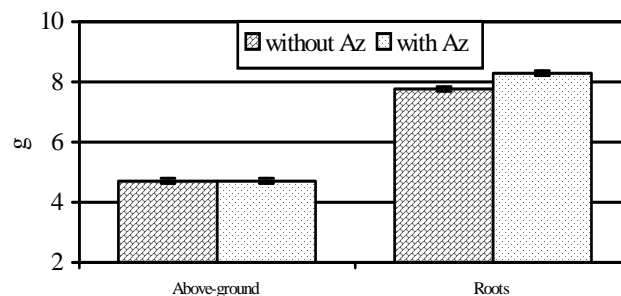


Fig. 2. Effect of Azotobacterin (Az) on onion yield (in g d.m. per pot)

The other analyzed factor, that is application of the vaccine Azotobakteryna, also affected the biological characteristics of soil (tabs 3-4). Under the influence of this vaccine, most of the analyzed microorganisms, except ammonifying and cellulolytic bacteria, increased in number. Thus, microbial counts rose by 125% (Arthrobacter), 86% (spore-forming oligotrophic bacteria), 51% (oligotrophic bacteria), 28% (spore-forming copiotrophic bacteria and fungi), 25% (copiotrophic bacteria), 23% (Pseudomonas), 12% (actinomycetes) and by 11% (nitrogen-fixing bacteria). For obvious reasons, Azotobakteryna also enriched the soil in Azotobacter cells. The count of these bacteria was 35-fold higher.

The effect of Azotobakteryna on soil enzymes was less unidirectional than that of L-tryptophan (tab. 4). The vaccine inhibited the activity of dehydrogenases (by 10%) but stimulated urease (by 10% as well). With respect to both phosphatases, its effect was negligible although statistically significant. Azotobakteryna did not produce any effect on the growth and development of onion's aerial organs, but resulted in a 7% increase in the growth of roots (fig. 2).

Table 1. The effect of L-tryptophane on numbers of soil microorganisms (cfu kg⁻¹ of d.m. soil)

| Microorganisms | L-tryptophane dose (mg kg ⁻¹ of d.m. soil) | | | | r | LSD |
|-------------------------------------|---|------------|------------|-------------|------------|-----------|
| | 0 | 0.5 | 5 | 50 | | |
| Olig x 10 ⁸ | 21.50 5 | 26.52 3 | 34.94 6 | 38.172 | 0.75 0 | 1.82 0 |
| Olig _p x 10 ⁸ | 0.860 | 0.949 | 1.183 | 1.469 | 0.90 8 | 0.15 9 |
| Cop x 10 ⁹ | 7.186 | 8.602 | 10.28 7 | 11.165 | 0.75 7 | 0.72 5 |
| Cop _p x 10 ⁹ | 1.089 | 1.391 | 1.461 | 1.563 | 0.66 2 | 0.11 0 |
| Am x 10 ⁸ | 89.60 6 | 94.62 4 | 95.69 9 | 103.22 6 | 0.90 9 | 4.68 9 |
| Im x 10 ⁸ | 42.29 4 | 43.19 0 | 63.79 9 | 70.789 | 0.78 7 | 4.21 0 |
| Cel x 10 ⁷ | 49.82 1 | 56.63 1 | 63.26 2 | 66.487 | 0.73 0 | 3.29 1 |
| Az x 10 ⁵ | 14.17 6 | 14.67 7 | 19.48 0 | 19.122 | 0.60 9 | 1.72 9 |
| Art x 10 ⁷ | 16.55 9 | 13.54 8 | 10.25 1 | 9.534 | - 0.671 | 1.67 5 |
| Ps x 10 ⁷ | 27.02 5 | 26.09 3 | 22.22 2 | 17.921 | - 0.908 | 2.06 7 |
| Act x 10 ⁸ | 80.28 7 | 93.36 9 | 94.44 4 | 96.057 | 0.51 1 | 6.74 6 |
| Fun x 10 ⁶ | 43.72 8 | 53.04 7 | 56.98 9 | 62.007 | 0.74 5 | 4.39 1 |

r – correlation coefficient;

Olig – oligotrophic bacteria, Olig_p – oligotrophic sporulation bacteria, Cop – copiotrophic bacteria, Cop_p – copiotrophic sporulation bacteria, Am – ammonifying bacteria, Im – immobilizing nitrogen bacteria, Cel – cellulolytic bacteria, Az – Azotobacter, Art – Artrobacter, Ps – Pseudomonas, Act – actinomyces, Fun – fungi.

Table 2. The effect L-tryptophane on soil enzymes activity (per kg⁻¹ of d.m. soil)

| Enzymes | L-tryptophane dose (mg kg ⁻¹ of d.m. soil) | | | | r | LSD |
|--|---|-------|-------|--------|-------|-------|
| | 0 | 5 | 10 | 15 | | |
| Dehydrogenases (mmol TFF h ⁻¹) | 6.545 | 7.508 | 9.914 | 10.540 | 0.734 | 0.385 |
| Urease (mmol N-NH ₄ h ⁻¹) | 0.617 | 0.668 | 0.677 | 0.806 | 0.961 | 0.036 |
| Alkaline phosphatase (mmol PNP h ⁻¹) | 1.120 | 1.167 | 1.218 | 1.218 | 0.598 | 0.040 |
| Acid phosphatase (mmol PNP h ⁻¹) | 1.026 | 1.043 | 1.201 | 1.222 | 0.711 | 0.031 |

r – correlation coefficient

Table 3. The effect of Azotobacter inoculum on numbers of soil microorganisms (cfu kg⁻¹ of d.m. soil)

| Microorganisms * | Without Azotobacter inoculum | With Azotobacter inoculum | LSD |
|-------------------------------------|------------------------------|---------------------------|-------|
| Olig x 10 ⁸ | 24.104 | 36.470 | 1.411 |
| Olig _p x 10 ⁸ | 0.779 | 1.452 | 0.131 |
| Cop x 10 ⁹ | 8.262 | 10.358 | 0.562 |
| Cop _p x 10 ⁹ | 1.205 | 1.546 | 0.085 |
| Am x 10 ⁸ | 99.462 | 92.115 | 3.634 |
| Im x 10 ⁸ | 52.240 | 57.796 | 3.263 |
| Cel x 10 ⁷ | 62.903 | 55.197 | 2.551 |
| Az x 10 ⁵ | 0.932 | 32.796 | 0.134 |
| Art x 10 ⁷ | 7.670 | 17.276 | 1.298 |
| Ps x 10 ⁷ | 20.896 | 25.735 | 1.602 |
| Act x 10 ⁸ | 86.022 | 96.057 | 5.228 |
| Fun x 10 ⁶ | 47.401 | 60.484 | 3.403 |

* - Explanations under table 1

Table 4. The effect of Azotobacter inoculum on soil enzymes activity (per kg⁻¹ of d.m. soil)

| Enzymes | Without Azotobacter inoculum | With Azotobacter inoculum | LSD |
|--|------------------------------|---------------------------|-------|
| Dehydrogenases (cm ³ H ₂ d ⁻¹) | 9.072 | 8.182 | 0.298 |
| Urease (mg N-NH ₄ h ⁻¹) | 0.660 | 0.724 | 0.028 |
| Alkaline phosphatase (mmol PNP h ⁻¹) | 1.163 | 1.199 | 0.031 |
| Acid phosphatase (mmol PNP h ⁻¹) | 1.092 | 1.154 | 0.024 |

Both own investigations and the relevant references [2, 5, 6, 9, 10, 14] suggest that phytohormone precursors can contribute to producing a larger biomass of plants. The effect is generated owing to the conversion of these compounds by microorganisms, which are typically more numerous in the rhizosphere than in soil outside the root system. A more intensive biosynthesis of phytohormones in the rhizosphere is dictated by a better availability of precursors and substrates in this environment [1, 3, 4, 15]. It is most probably this dependence that featured in the experiment reported herein. L-tryptophan added to soil raised counts of most soil-borne microorganisms and stimulated the activity of soil enzymes, which created more favourable conditions for the growth and development of onion and consequently led to harvesting higher onion yields.

Azotobakteryna did not enhance the positive effect of L-tryptophan on onion yields, as should have been expected, although it did have a beneficial effect on most of the microorganisms.

4. Conclusions

L-tryptophan raised significantly counts of the following bacteria: oligotrophs and their endospores, copiotrophs and their endospores, ammonifying, nitrogen-immobilizing, Azotobacter and cellulolytic ones, as well as actinomycetes and fungi. It also stimulated the activity of dehydrogenases, urease, acid and alkaline phosphatases. However, it reduced the counts of Arthrobacter and Pseudomonas.

Azotobakteryna raised the counts of such bacteria as Azotobacter, oligotrophs and spore-forming oligotrophs, copiotrophs and spore-forming copiotrophs, nitrogen immobilizing Arthrobacter and Pseudomonas as well as actin-

omycetes and fungi. It stimulated the activity of urease. However, it decreased counts of ammonifying and cellulolytic bacteria and depressed the activity of dehydrogenases.

L-tryptofan, by improving the microbiological and biochemical properties of soil, had a positive effect on the growth and development of onion.

The microbiological and biochemical properties of soil can be improved by soil application of phytohormone precursors and the inoculant *Azotobakteryna*.

5. References

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