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THESIS OF

DOCTORAL (PhD) DISSERTATION

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MOSONMAGYARÓVÁR

2024

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DEPARTMENT OF PLANT SCIENCES

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SUBMITTED BY

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Introduction

Global challenges stemming from the escalating demands for food, water, and energy necessitate innovative and sustainable approaches to enhance agricultural productivity while preserving the environment. Conventional agricultural practices heavily reliant on agrochemicals have resulted in ecological damage, prompting the exploration of alternative strategies. A promising solution involves harnessing the collaborative potential of microalgae and beneficial bacteria to develop eco-friendly products like biofertilizers, biostimulants, and biopesticides. These products aim to reduce dependence on agrochemicals, mitigate negative environmental impacts, and promote sustainable agriculture on various scales.

Addressing the unreliability of single-strain inoculations in the rhizosphere can be achieved through the utilization of multispecies consortia, presenting a promising strategy to boost plant growth and soil fertility. Microalgae and cyanobacteria, acting as primary producers, combine with bacteria to constitute the uppermost layer of soil recognized as the biological soil crust. This complex ecosystem plays a crucial role in enhancing soil fertility and, in turn, significantly contributes to the overall improvement of crop productivity in a sustainable way.

Objective of the study

The present study was conducted to evaluate the potential impact of two-member consortia, comprising cyanobacterial biomass and plant growth-promoting bacteria (PGPB) strains, on maize (Z. mays L.) growth and soil fertility.

- To determine the effect on the growth and development of the maize crop
- To evaluate the composition of the soil fertility
- To evaluate the microbial biomass of the rhizosphere microorganisms

Methodology

The field trial spanned the years 2021, 2022, and 2023. The study followed a design of completely randomized block design (CRBD) with four replication and a total of 9 treatments. The experimental design included two main factors, which were: Cyanobacterium (MACC-612, *Nostoc linckia*) biomass and plant growth promoting bacteria (PGPB) (such as *Azospirillum lipoferum (*strain NF5) and *Pseudomonas fluorescens (*strain NCAIM B01666). The three levels of the cyanobacterial biomass (control, 0.3 g/L of MACC-612, and 1.0 g/L of MACC-612) and three levels of bacteria strains (control*, A. lipoferum (NF5)*, and *P. fluorescens (*NCAIM B01666)) were used for the experiment.

The solution of the microalgae (*N. linckia)* and PGPB treatments were introduced to the soil using a 15 L manual knapsack sprayer (pump sprayer garden pressure spray) during the sowing process, at an application rate of 300 L/ha.

Sowing was conducted using a row spacing of 75 cm, a plant spacing of 20 cm, and a sowing depth of 6 cm.

Data collection and measurements

Agronomic and physiological measurements, including chlorophyll content, NDVI, plant fresh weight, and plant dry weight, were recorded. The assessment of chlorophyll content and NDVI took place on three occasions at 50, 65 and 80 days after sowing (DAS) on 5 randomly selected maize plants from each plot, and the values were averaged. While the plant biomass measurement was conducted at 50 and 65 DAS. Furthermore, after the maturity phase of maize, leaves and seeds were collected from each plot, and the plant samples were oven-dried and ground before analyzing for total nitrogen content.

The research involved a thorough evaluation of multiple parameters post-maturation of maize, with four plants chosen from each central plot for detailed measurements, encompassing plant height, grain count per ear, thousand grains weight, and grain yield.

Soil samples were obtained from the experimental field, specifically focusing on the uppermost 0-20 cm layer of the productive soil stratum. Soil pH, humus, nitrate-nitrite nitrogen, total nitrogen, phosphorus (P), and potassium (K) underwent a comprehensive analysis following established procedures.

Microorganisms, including bacteria and actinomycetes, were assessed using the agar-plate method. This predominant cultural approach is employed for evaluating soil microbial populations, facilitating their identification and quantification.

All statistical computations and the creation of visual representations were conducted using R studio. The outcomes from all the experiments, which exhibited a normal distribution pattern, underwent two-way analysis variance (ANOVA). Following this, Tukey's HSD post-hoc analysis was applied at a significance level of $P \leq 0.05$.

Result and Discussion

Discovering that the application of both *N. linckia* and PGPB in soil treatment resulted in a significant (p<0.05) enhancement in chlorophyll levels, vegetation index, yield components, soil microbial population, and soil fertility. However, there was no significant (p<0.05) impact on the nitrogen content of plant biomass. The efficacy of biofertilizer treatment for plant growth and soil fertility dependent on the concentration rate of *N. linckia* and the specific PGPB strains employed.

We found that the combined application of *N. linckia* and PGPB strains positively influenced the chlorophyll content and overall green health vegetation of maize crops. Our investigation revealed that the peak chlorophyll content and NDVI throughout the experiment period, was achieved through the joint use of *N. linckia* at a concentration of 0.3 g/L in conjunction with *A. lipoferum*, while the minimum chlorophyll content was noted in the control group. In the year 2021, at 50 days after sowing (DAS), the chlorophyll content and NDVI exhibited a diminished level relative to the preceding consecutive years due to imposition of stress on the plants during the vegetative phase.

The combined inoculation of *N. linckia* and PGPB exhibited a significant enhancement in dry leaf weight, ranging between 35.56% to 107.32% and 29.58% to 49.77% at 50 and 65 DAS, respectively compared to control levels. Moreover, the combined application of *N. linckia* and PGPB led to a notable rise in dry root weight, exhibiting increases of 75.59-130.19% at 50 DAS and 56.08-69.93% at 65 DAS compared to the control group. Hence, the most effective approach was the joint use of *N. linckia* and PGPB, followed by the alone application of *N. linckia* or PGPB, which also showed notable improvements compared with the control group.

Our research demonstrated that the application of *N. linckia* and PGPB, either individually or in combination, significantly enhanced maize growth, leading to an increase in seeds per ear, higher thousand seed weight, and elevated yield. Specifically, our study revealed that the inoculation of *N. linckia* at the concentration of 0.3 g/L along with A. *lipoferum* positively influenced yield of maize, leading to a significant enhancement in grain yield by 7.09 $tonha^{-1}$ (33.20%) during 2021, 7.71 $tonha^{-1}$ (31.53 %) in season 2022, and 8.62 $tonha^{-1}$ (32.34%) in season 2023, as compared to the control. The improvement in various plant growth factors is likely a consequence of plants being better able to absorb essential nutrients from the soil.

The use of either *N. linckia* or PGPB, as well as their combined application, resulted in a notable improvement in soil pH, humus content, (NO_3^-+ NO_2^-)-N, and total nitrogen levels. Apart from 2022, it is evident from the data that the joint utilization of *N. linckia* and PGPB did not result in statistically significant (p<0.05) changes in pH values throughout the remaining seasons. Moreover, the combined use f *N. linckia* at a concentration of 0.3 g/L, in along with *A. lipoferum*, yielded noteworthy enhancements in humus content, with increments of 31.67%, 20.24%, and 15.71% observed for the respective years 2021, 2022, and 2023, against untreated control trials. Our study revealed that the combined application of *N. linckia* at the concentration of 1 g/L with *A. lipoferum* resulted increases the (NO_3^-+ NO_2^-)-N content by 27.05%, 59.20%, and 51.54% during the years 2021, 2022, and 2023, respectively, when compared to the control levels. Additionally, the studies demonstrate that the synergistic application of *N. linckia* at a concentration of 0.3 g/L, in conjunction with *A. lipoferum*, led to significant improvements in total nitrogen levels, registering increments of 40%, 20.69%, and 27.59% for the years 2021, 2022, and 2023, respectively, when compared to untreated control trials.

The application of either *N. linckia* or PGPB, whether individually or in conjunction, resulted in notable variations in bacterial and actinomycete populations. It is noteworthy that the control group consistently demonstrated the lowest levels of these populations throughout the entire duration of the research.

Conclusion

The study underscores that the combined use of *N. linckia* biomass and PGPB was the most effective strategy. The applied strains exhibited a high level of compatibility for co-growth. However, the most effective synergistic combination was identified by incorporating both *N. linckia* biomass at a concentration of 0.3 g/L along with *A. lipoferum*. This combination resulted in the highest enhancement of chlorophyll content, maize growth, yield, soil fertility, and microbial populations.

Novel Scientific Results of Doctoral Research

- The joint application of *Nostic linckia* biomass and plant growth-promoting bacteria increased chlorophyll and green vegetation content in maize, demonstrating potential for enhancing plant photosynthesis.
- It was found that the joint application of *Nostic linckia* biomass and plant growth-promoting bacteria significantly enhanced maize growth, resulting in increased fresh and dry shoot and root biomass.
- The combined use of *Nostic linckia* and plant growth-promoting bacteria significantly boosted maize productivity, resulting in more seeds per ear, increased thousand seed weight, and higher overall yield.
- The combined use of *Nostic linckia* biomass and plant growth-promoting bacteria synergistically improved soil pH, humus content, nitrate-nitrite nitrogen, total nitrogen, and microbial

populations, paving the way for sustainable and eco-friendly agricultural practices.

- Optimal synergistic groupings were identified by combining *N. linckia* biomass at a concentration of 0.3 g/L with *A. lipoferum*, leading to enhanced maize growth, increased yield, improved soil fertility, and increased microbial populations.

Publications

- **Solomon, W**., Janda, T., and Molnár, Z. (2024). Unveiling the significance of rhizosphere: Implications for plant growth, stress response, and sustainable agriculture. *Plant Physiology and Biochemistry* **206**, 108290. https://doi.org/10.1016/j.plaphy.2023.108290

- **Solomon, W**.; Mutum, L.; Rakszegi, M.; Janda, T.; Molnár, Z. (2023). Harnessing the Synergy of the Cyanobacteria-Plant Growth Promoting Bacteria for Improved Maize (*Zea mays*) Growth and Soil Health. *Sustainability*, *15*, 16660. https://doi.org/10.3390/su152416660

- **Solomon, W**., Mutum, L., Janda, T., Molnar Z. (2023). Potential benefit of microalgae and their interaction with bacteria to sustainable crop production. *Plant Growth Regulation*. https://doi.org/10.1007/s10725-023-01019-8

- Mutum, L., **Solomon, W**., Janda, T., Molnar Z. (2023). Time of application and cultivar influence on the effectiveness of microalgae biomass upon winter wheat (*Triticum aestivum* L.). *CEREAL RESEARCH COMMUNICATIONS*. https://doi.org/10.1007/s42976-023-00443-w

- Molnár, Z.; **Solomon, W**.; Mutum, L.; Janda, T. (2023). Understanding the Mechanisms of Fe Deficiency in the Rhizosphere to Promote Plant Resilience. *Plants*, 12, 1945. https://doi.org/10.3390/plants12101945
- Molnár, Z., Lamnganbi, M., **Solomon, W**.,& Jan da, T. (2023). Chitosan and cyanobacterial biomass accounting physiological and biochemical development of winter wheat (*Triticum aestivum* L.) under nutrient stress conditions. *Agrosystems, Geosciences & Environment*, 6, e20428. https://doi.org/10.1002/agg2.20428

- **Kabato, W.,** Ergudo, T., Mutum, Janda, ., Molnár, Z., (2022). Response of wheat to combined application of nitrogen and phosphorus along with compost. *J. Crop Sci. Biotechnology*. 25, 557–564. https://doi.org/10.1007/s12892-022-00151-7