Valorization of *Nannochloropsis* spp. biomass residues grown on brewery wastewater as a fertilizer and its phytotoxicity assessment on plants

Natasha Waris[1], Martina Coletta[1], Marco Monticelli[1], Giovanni Caprioli[2], Marco Cespi[2], Elena Vittadini[1], Antonietta La Terza[1]

[1]School of Biosciences and Veterinary Medicine, University of Camerino, Via Gentile III da Varano 62032, Camerino (MC), Italy

[2]School of Pharmaceutical Sciences and Health Products, University of Camerino, Via Madonna delleCarceri, Varano 62032, Camerino (MC), Italy

Keywords: Microalgae, spent coffee grounds (SCG), fertilizer, waste management, circular economy

Presenting author email: natasha.waris@unicam.it

Introduction

The overreliance on mineral fertilizers due to the increasing population and decreasing agricultural land has deteriorated soil health and caused other issues, such as soil acidification and groundwater contamination. Microalgae are being studied due to their rich nutrient content and their potential to be repurposed for various applications, including their use as a fertilizer by growing them on wastewater to address waste management challenges, maximize nutrient recovery, and promote sustainable fertilization practices (Osorio-Reyes *et al.*, 2023).Within the framework of the EU ERA-NETs - SUSFOOD2 project, "AlgaeBrew," our study aims to formulate a microalgae-based fertilizer (MBF) from *Nannochloropsis spp.* cultivated on brewery wastewater and evaluate its phyto-toxicological effects on plants.

Materials and Methods

Nannochloropsis spp. biomass residues obtained from the University College Dublin (UCD), Ireland and Swansea University (UK) were used to create nine formulations using spent coffee grounds (SCG) and a 0.5% xanthan gum binder solution. The elemental analysis was done using an Elementar CHNS Vario Macro Cube analyzer. The EC (at 25 °C) and pH were measured in a 1:10 (w/v) sample-to-water ratio. Flowability was measured through an angle of repose (θ) calculated by a fixed funnel method (Anand, 2013). The Phytotoxkit solid sample (Ecotox LDS Srl, MI, Italy) was used for phytotoxicity tests. The water-holding capacity (WHC) was calculated before starting the test. Five different fertilizer doses (1.25%, 2.5%, 5%, 10%, and 15%) were administered and compared with the control soil (OECD soil). Three plant species were selected: *Sinapis alba*, *Lepidium sativum*, and *Sorghum saccharatum*. The obtained data was analyzed using R 4.2.0 software.

Results and Discussion

The C/N ratio of microalgae was 6.74, below the optimum range of 9 to 12 (Vu, 2023); therefore, a formulation containing 50% SCG and 50% microalgae with a C/N ratio of 11 was selected out of nine formulations.This selected MBF was assessed for flowability, and it was observed that the microalgae's angle of repose (θ) could not be calculated due to the occurrence of the ratholing phenomenon. In contrast, the flowability of SCG was found to be "passable-may hang up," whereas significant improvement in the flowability of MBF was observed as "fair-aid not needed," suggesting smooth handling of the material (Anand, 2013). Moreover, plants require optimum hydration for proper growth, and it was observed that the newly developed MBF,when added to the soil, increased the water-holding capacity (WHC), ensuring continuous hydration. Finally, to assess the potential phytotoxic effects of the newly developed MBFs on plants, tests were performed using the previously mentioned plant species.In the case of *S. saccharatum*, the highest germination index (GI) was observed at 10% MBF

application and the lowest at 5%. However, 1.25%, 2.5%, and 15% gave comparable results as the control. For *L. sativum*, a high GI was observed in the control and up to 5% fertilizer application, but a drastic reduction was observed at 10% and 15%. Similarly, for *S. alba*, the highest GI was observed at 1.25% and 2.5%, and the lowest at 10% and 15%. Moreover, an increase in fertilizer concentration led to a gradual decrease in mean root length, longest root length, and Elongation index in all tested plants, except for *S. alba* at 1.25% MBF application.Upon root length analysis (Figure 1), it is seen that in the case of all tested plant species, the 1.25% fertilizer application had a non-significant effect on root length compared with the control. In contrast, this non-significant effect is noted even at 2.5% concentration (in *S. alba* and *S. saccharatum*) and 5% (*S. saccharatum*). However, higher concentrations of fertilizer showed a reduction in the root length of all tested plants.

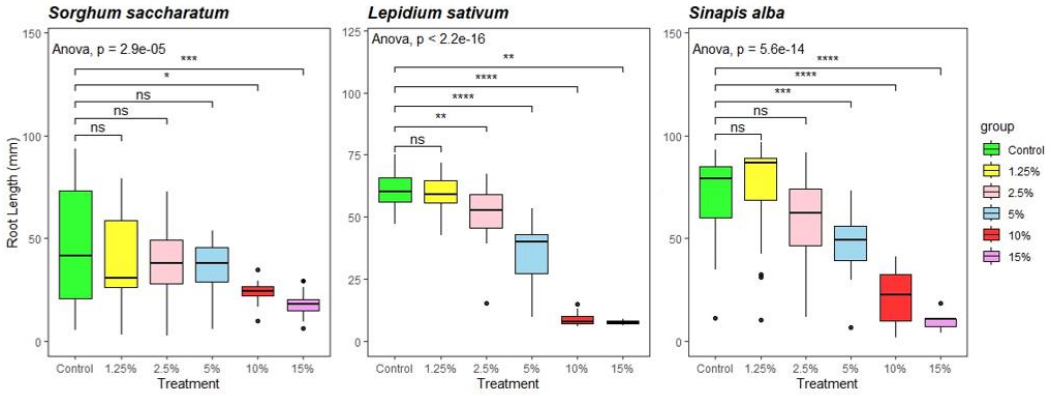

Figure 1.Root length (mm) of three plants grown in control soil and different concentrations of fertilizer. Significance codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

Conclusion

The novel MBF showed promising potential as a fertilizer because of its optimized C/N ratio, improved flowability, and ability to increase soil's water-holding capacity. Furthermore, it showed no toxic effect on seed germination, and the root length, especially at lower concentrations. Currently, other brewery wastes are also being considered for developing other fertilizers to promote circular economy and sustainable agricultural practices. In the future, plant pot experiments will also be conducted to evaluate its effects on plants' physical and nutritional attributes, alongside microbiome analysis by using 16S and 18S metabarcoding approaches.

References

Anand, C. (2013). Concept of fixed dose comination in development of dispersible tablets of tolfenamic acid and paracetamol. *Journal of Drug Delivery and Therapeutics*, *3*(2).

Osorio-Reyes, J. G., Valenzuela-Amaro, H. M., Pizaña-Aranda, J. J. P., Ramírez-Gamboa, D., Meléndez-Sánchez, E. R., López-Arellanes, M. E., ... & Martínez-Ruiz, M. (2023). Microalgae-based biotechnology as alternative biofertilizers for soil enhancement and carbon footprint reduction: Advantages and implications. *Marine Drugs*, *21*(2), 93.

Vu, T. Q. (2023). The potential of Plant Residues and Industrial Sewage Sludge as Organic Fertilizers, and their Effect on Soil pH and Moisture of Acid Sulfate Soil. *Malaysian Journal of Soil Science*, *27*, 1-7.

Acknowledgment: This study is part of the AlgaeBrew project funded under the ERA-NET SUSFOOD2 and FOSC Joint Call 2021 to A. La Terza