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Integrated use of the next generation plant biostimulants for an enhanced sustainability of field vegetable high residue farming systems –STIM 4$^+$

RO-NO-2019-540

D. Constantinescu-Aruxandei, S. Brooks, A. Nicolescu, S. Shaposhnikov, F. Georgescu, L.A. Pairault, L. Marin, C. Deleanu, F. Oancea

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Partners

- National Institute for Research & Development in Chemistry and Petrochemistry—ICECHIM, Bucharest, Romania
- Norway Institute for Water Research—NIVA, Oslo, Norway
- Petru Poni Institute for Macromolecular Chemistry, Iasi, Romania
- Norgenotech AS, Skreia, Norway
- Enpro Soctech, Bucharest, Romania
- Amia Import-Export, Otopeni, Romania

Overall aims and objectives of the project.

The goal of the STIM4⁺ project is to develop (bio)technologies for the production and the integrated utilization of next generation plant biostimulants, for field vegetables grown into high residue farming systems.

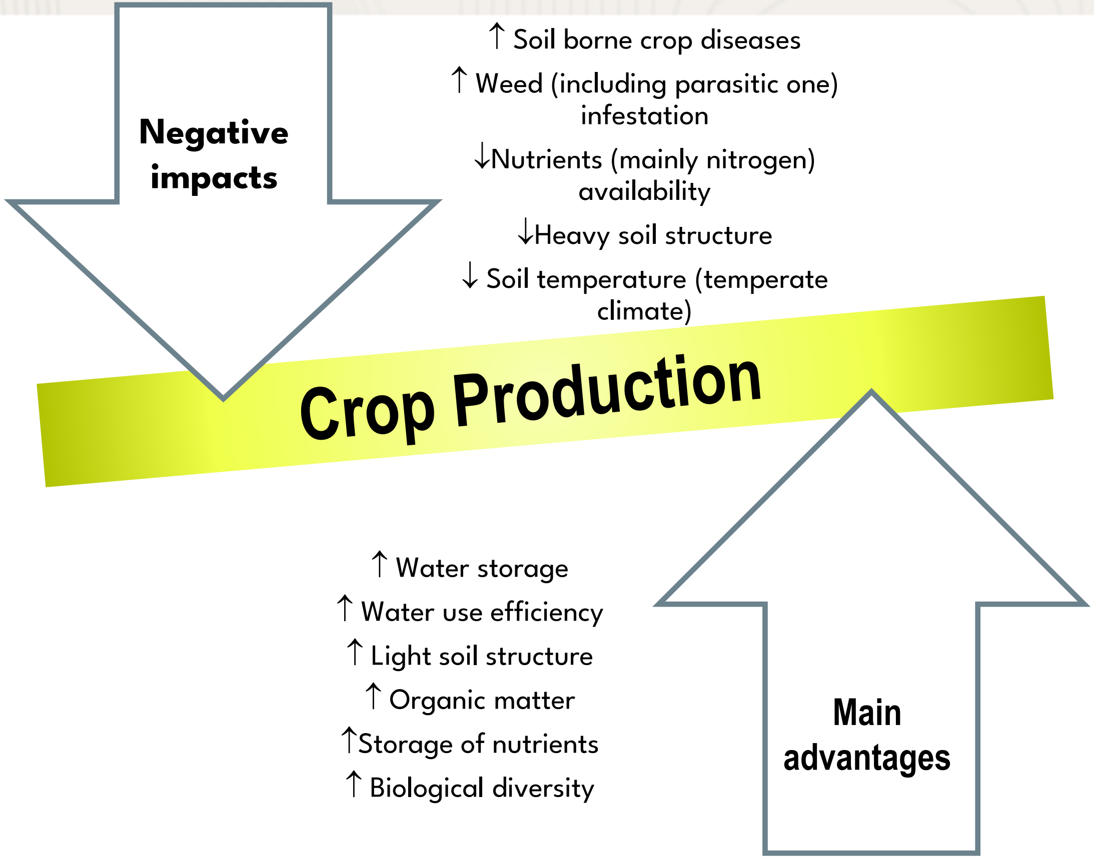
The STIM 4⁺ project objectives are:

(*i*) To develop next generation plant biostimulants intended to improve the resource use efficiency of high-residue grown vegetables;

(*ii*) To assess and to characterize the new plant biostimulants effects on vegetables and rhizosphere microorganism;

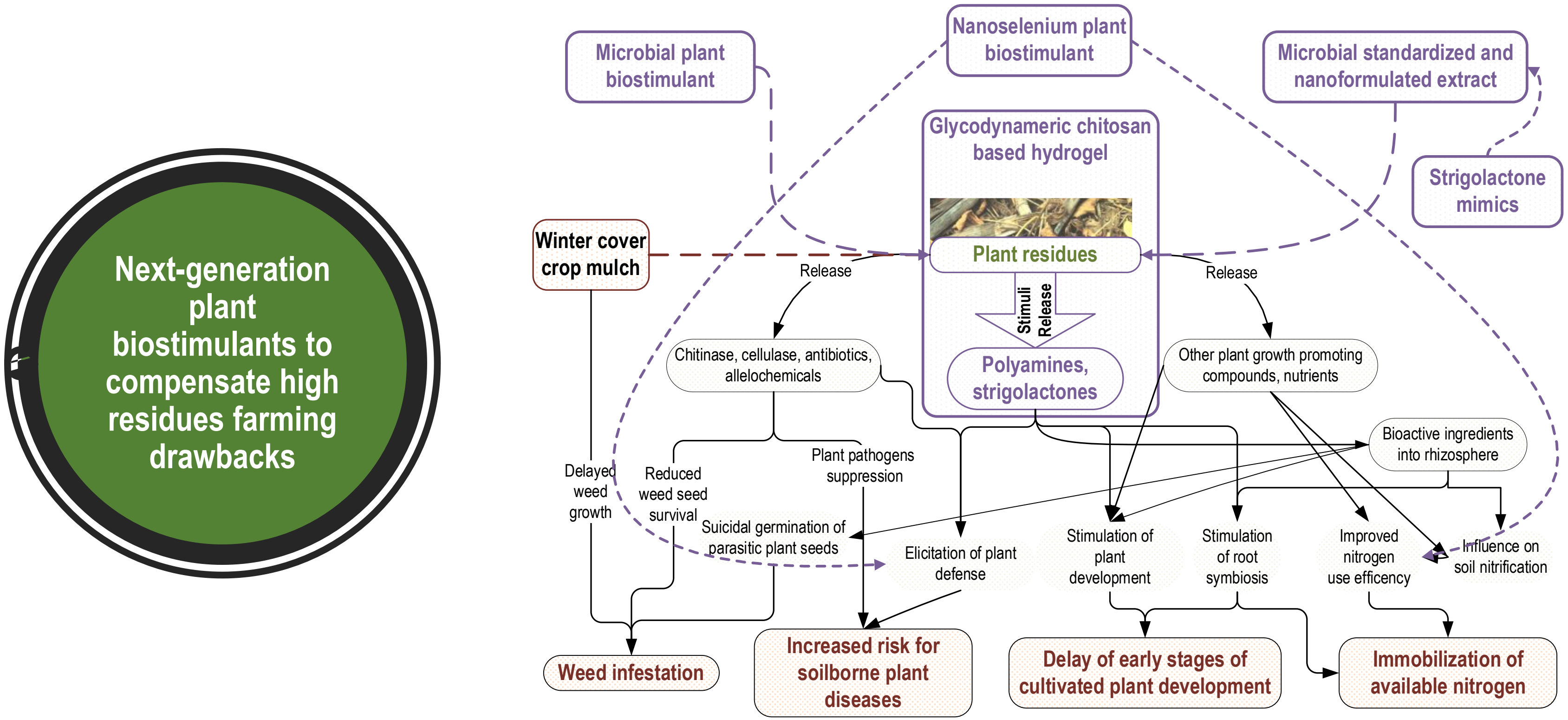
(*iii*) To investigate the safety and environmental impact of the next generation plant biostimulants.

Trade-off of the vegetable cultivation into high residues system - winter cover crop mulch

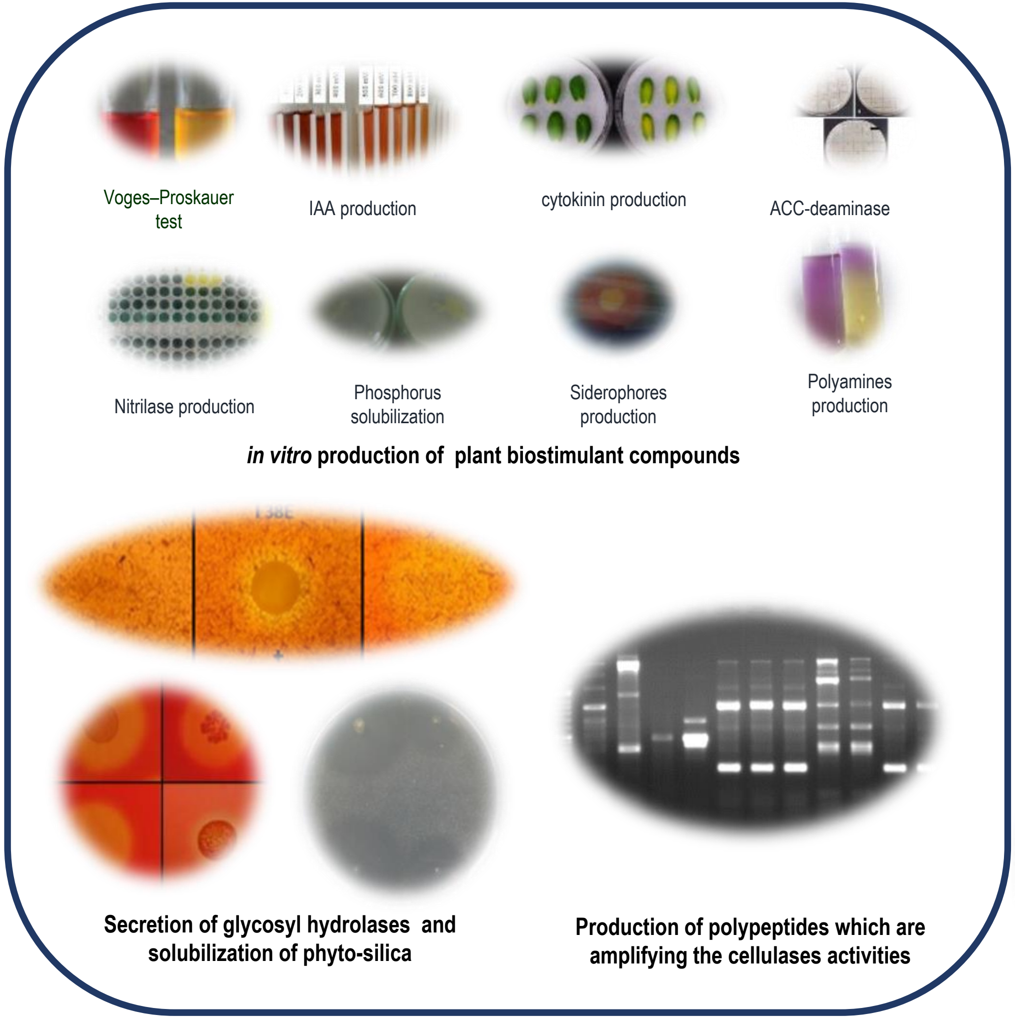


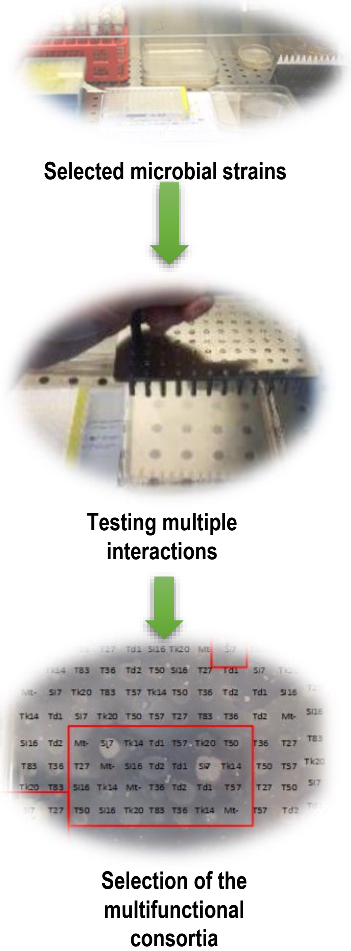
Next-generation plant biostimulants

(*i*) multi-functional *Trichoderma* strains-based plant biostimulants (a microbial plant biostimulants);
(*ii*) glycodynameric, chitosan based bioactive (micro)hydrogel formulation (organic plant biostimulants);
(*iii*) zerovalent selenium nanoparticles (inorganic plant biostimulant).
(*iv*) microalgae standardized extract, including phytohormones, polyamines and betaines (organic plant biostimulant),
fortified with a strigolactone mimic (bio-designed plant biostimulant);

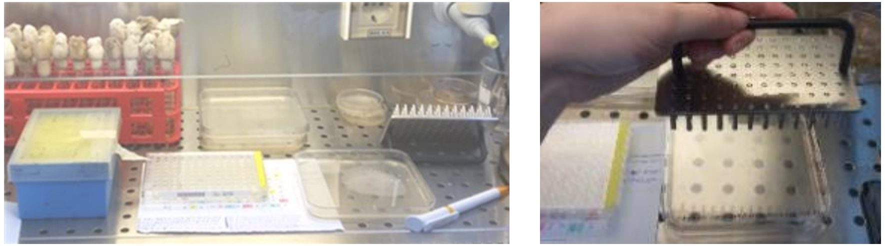

Next-generation plant biostimulants to compensate high residues farming drawbacks
Microbial plant biostimulant
Nanoselenium plant biostimulant
Microbial standardized and nanoformulated extract
Strigolactone mimics
Glycodynameric chitosan based hydrogel
Winter cover crop mulch
Plant residues
Stimuli
Release
Release
Release
Polyamines, strigolactones
Chitinase, cellulase, antibiotics, allelochemicals
Other plant growth promoting compounds, nutrients
Bioactive ingredients into rhizosphere
Delayed weed growth
Reduced weed seed survival
Plant pathogens suppression
Suicidal germination of parasitic plant seeds
Elicitation of plant defense
Stimulation of plant development
Stimulation of root symbiosis
Improved nitrogen use efficency
Influence on soil nitrification
Weed infestation
Increased risk for soilborne plant diseases
Delay of early stages of cultivated plant development
Immobilization of available nitrogen

Selection of multi-functional *Trichoderma* strains

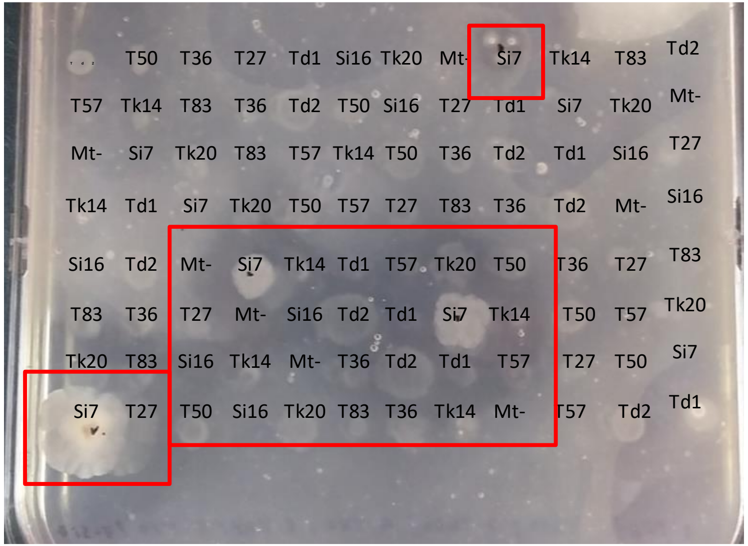




HTS to select *Trichoderma* consortium responding to strigolactone



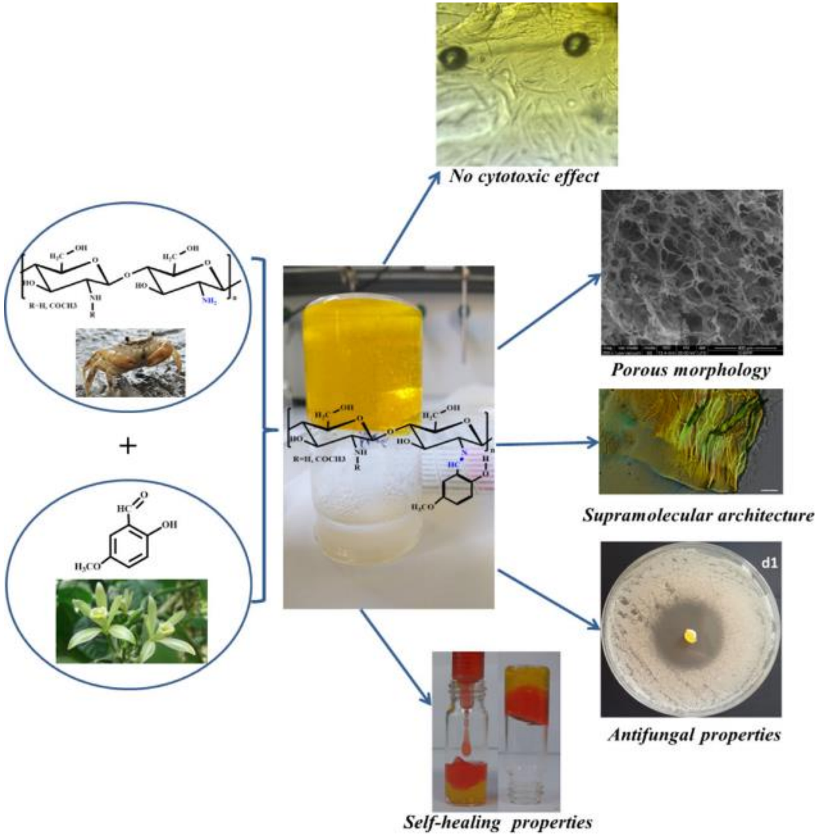
a



b

Consortium Si7- T27 - Tk20

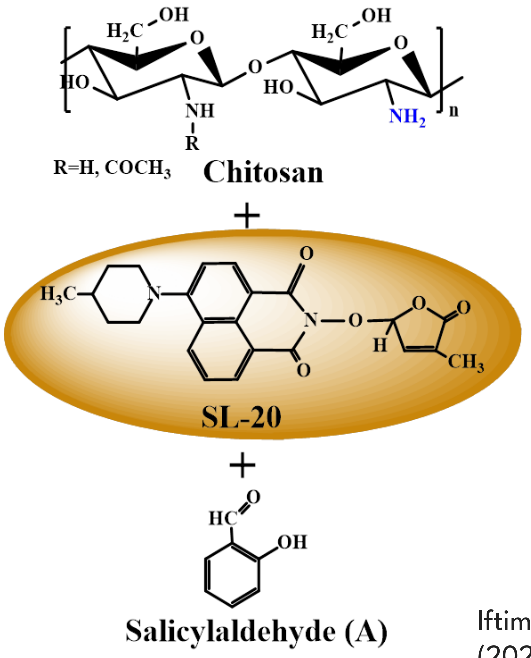
Patent RO131827 - PCT application

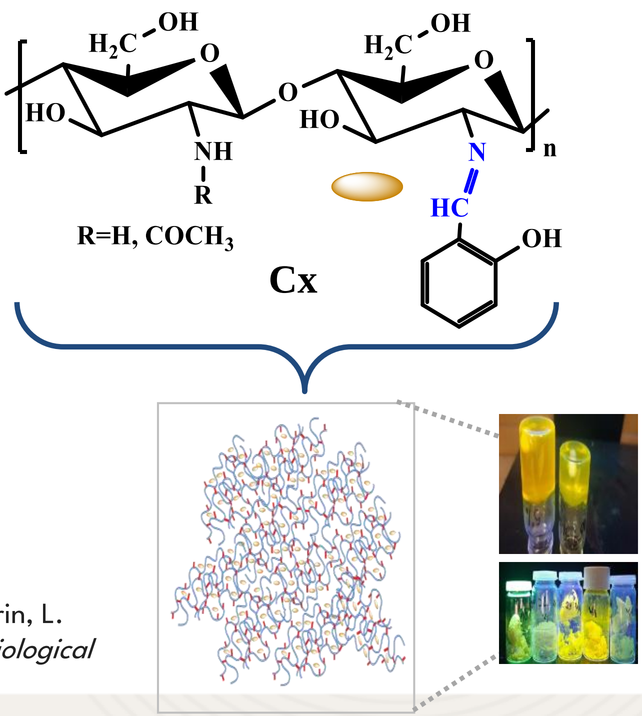


Glycodynameric, chitosan based bioactive (micro)hydrogel formulation

Iftime, M. M., Rosca, I., Sandu, A. I., & Marin, L. (2022). Chitosan crosslinking with a vanillin isomer toward self-healing hydrogels with antifungal activity. *International Journal of Biological Macromolecules*, *205*, 574-586.

Preparation of SL-20 -loaded chitosan-salicyl-imine hydrogels





Iftime, M. M., Tartau, L. M., & Marin, L. (2022). *International Journal of Biological Macromolecules*, *162*, 398-408.

Degradation in soil of the formulations and swelling capacity

Code	Time (days)	1 day	7 days	2 days	7/9 days
Co	8				
C1	8				
C2	9		 9 days		
C3	9				
C4	10				
C5	15				
CS-Sl20	4				

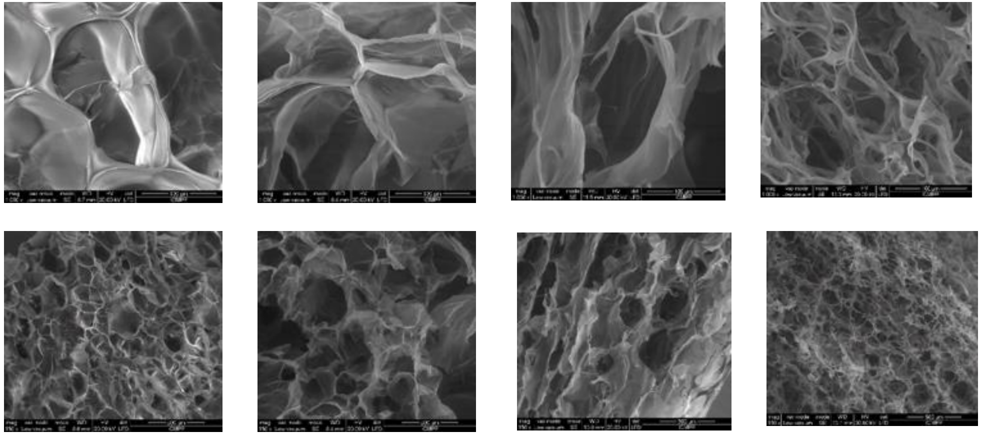
MES Probe	xerogels	pellets	Degradation time (days)	
	1 day		xerogels	pellets
Co	12	8	8	8
C1	16	10	8	8
C2	14	12	9	10
C3	13	11	9	10
C4	8	7	10	12
C5	5	4	15	22
CS-SL	29	-	4	1

Images of the formulations sample in soil, before and after degradation experiment

Glycodynameric, chitosan based bioactive (micro)hydrogel formulation

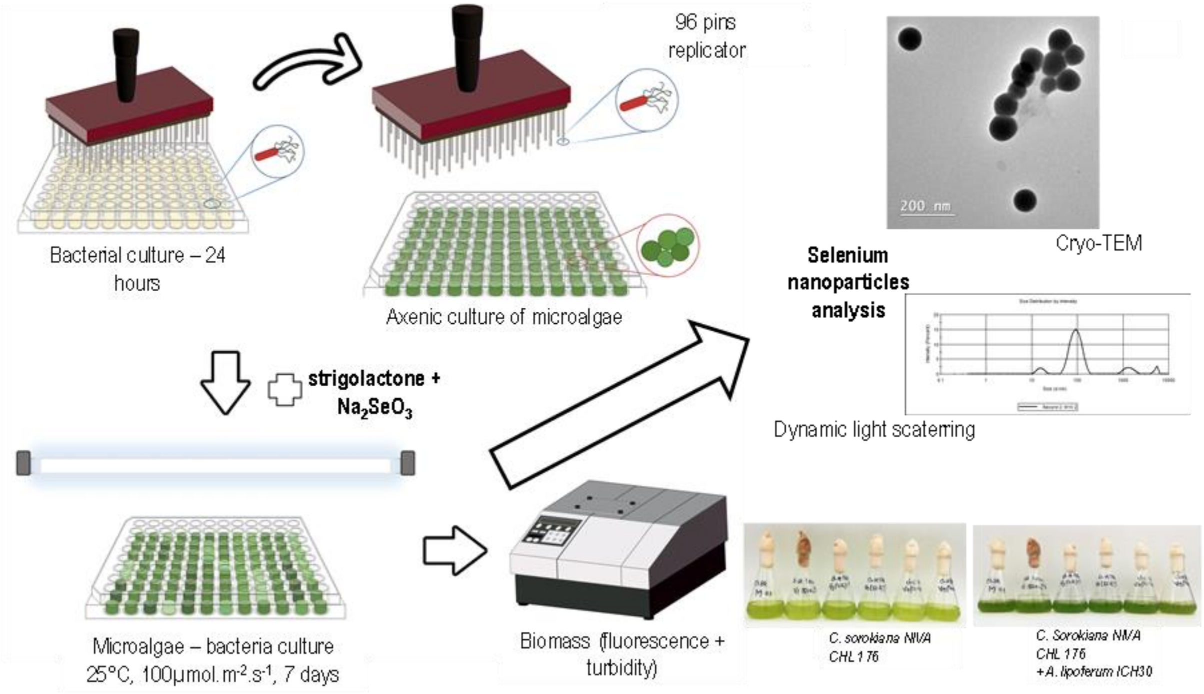
In order to choose the glycodinameric hydrogel for further developing of the biostimulant formulation, a series of properties, relevant for formulation obtaining and behaviour, were investigated. They are briefly presented below.

Hydrogel morphology influences their ability to retain water in soil, to encapsulate different bioactive compounds and to release them in a controlled manner. The morphology of the hydrogels was assessed by scanning electron microscopy. It was observed that they have a porous structure, with well delimited pores for the hydrogels with high crosslinking density, and a fibrous structure for those with lower crosslinking density.



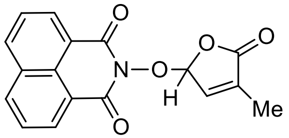
Representative SEM images of hydrogels at different magnifications a) 1000x and b) 150x

Novel, identified microbial strains which produce nanoselenium

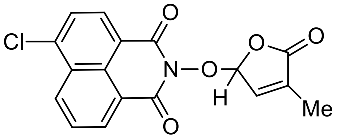


New fluorescent strigolactone mimics

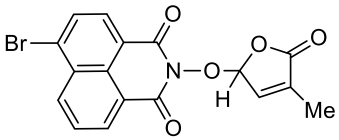
√ Starting from commercially available 1,8-naphthalic anhydrides:



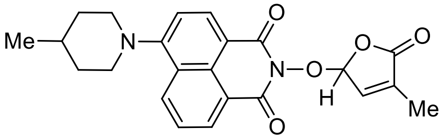
3 (SL-6)



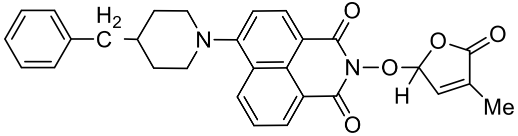
4 (SL-26)



5 (SL-FD1)

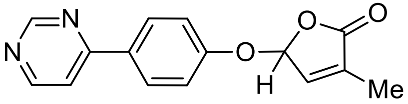


6 (SL-20)

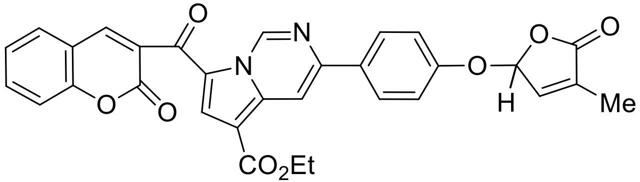


7 (SL-21)

√ Starting from 4-(4-hydroxyphenyl)pyrimidines:



8 (SL-13)



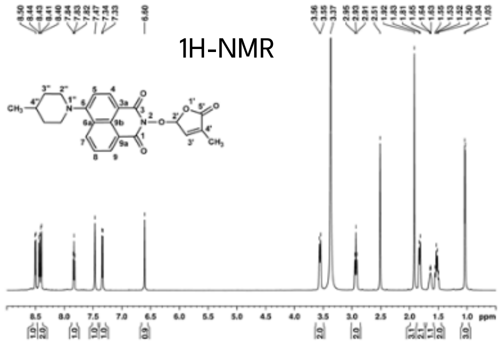
9 (SL-15)

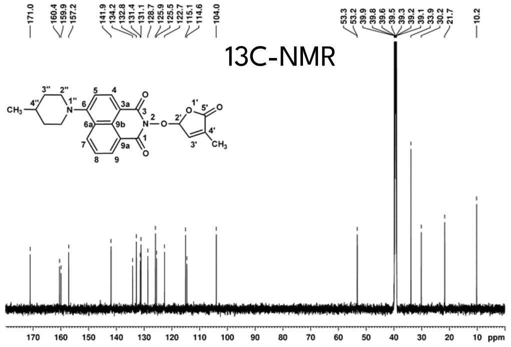
Solvatochromic effect

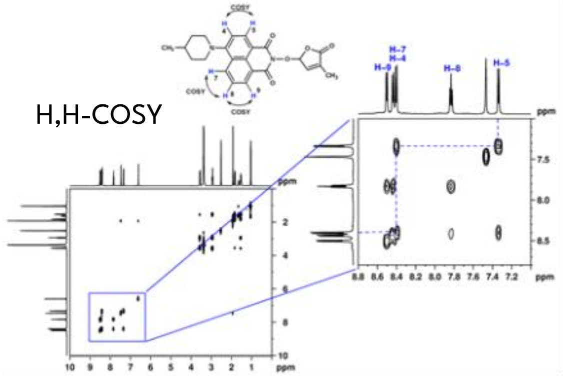
- Nicolescu, A., Airinei, A., Georgescu, E., Georgescu, F., Tigoianu, R., Oancea, F., & Deleanu, C. (2021). Synthesis, photophysical properties and solvatochromic analysis of some naphthalene-1, 8-dicarboxylic acid derivatives. *Journal of Molecular Liquids*, *303*, 112626.
- Tigoianu, R., Airinei, A., Georgescu, E., Nicolescu, A., Georgescu, F., Isac, D. L., Deleanu, C. & Oancea, F. (2022). Synthesis and solvent dependent fluorescence of some piperidine-substituted naphthalimide derivatives and consequences for water sensing. *International journal of molecular sciences*, *23*(5), 2760.

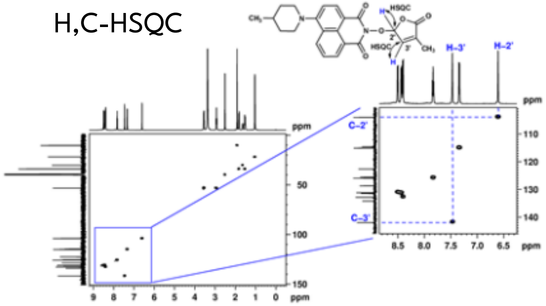
Advanced structural characterization of the newly synthesized strigolactone mimics

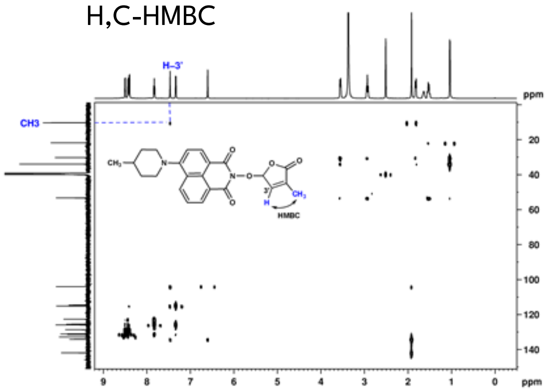
Types of NMR experiments performed during activity T2.1, exemplified for compound SL-20. All the experiments were recorded on a Bruker Avance NEO 600MHz spectrometer.

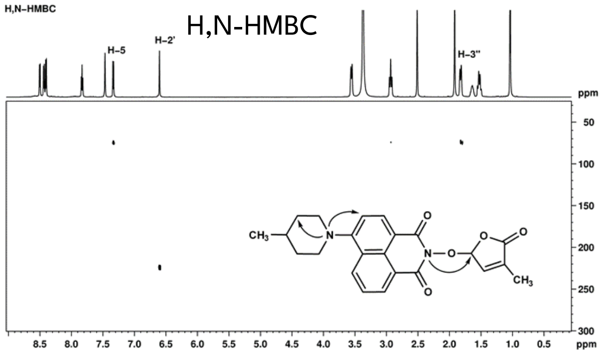








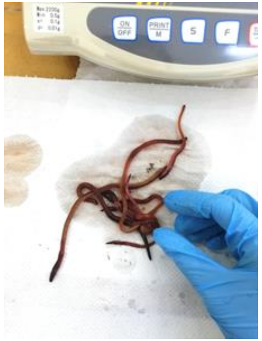




Acute toxicity on earthworm - ISO 11268-1:2012



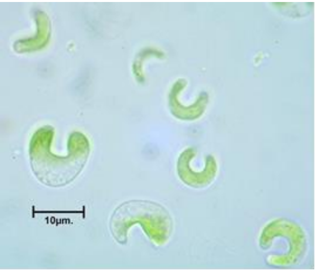

No effect at the applied concentration

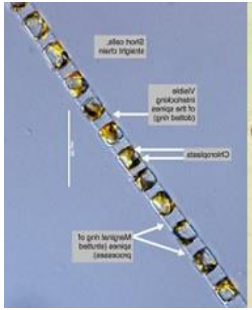
Ecotoxicity evaluation of Strigolactone (SL-6)

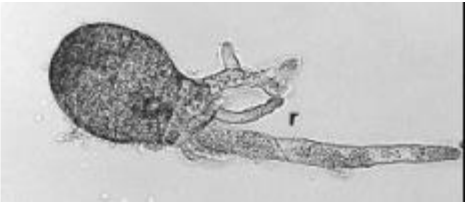
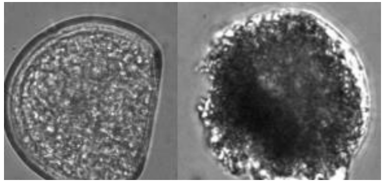
- Freshwater toxicity:
  - Unicellular algae growth inhibitio (OECD201)
  - *Daphnia magna* (OECD202)
  - Fish embryo test (OECD236)

- Marine toxicity:
  - Algal growth inhibition (ISO10253)
  - Fucus germling growth
  - Copepod (Tisbe) acute toxicity (ISO14699)
  - Oyster embryo bioassay (ASTM E724-89)

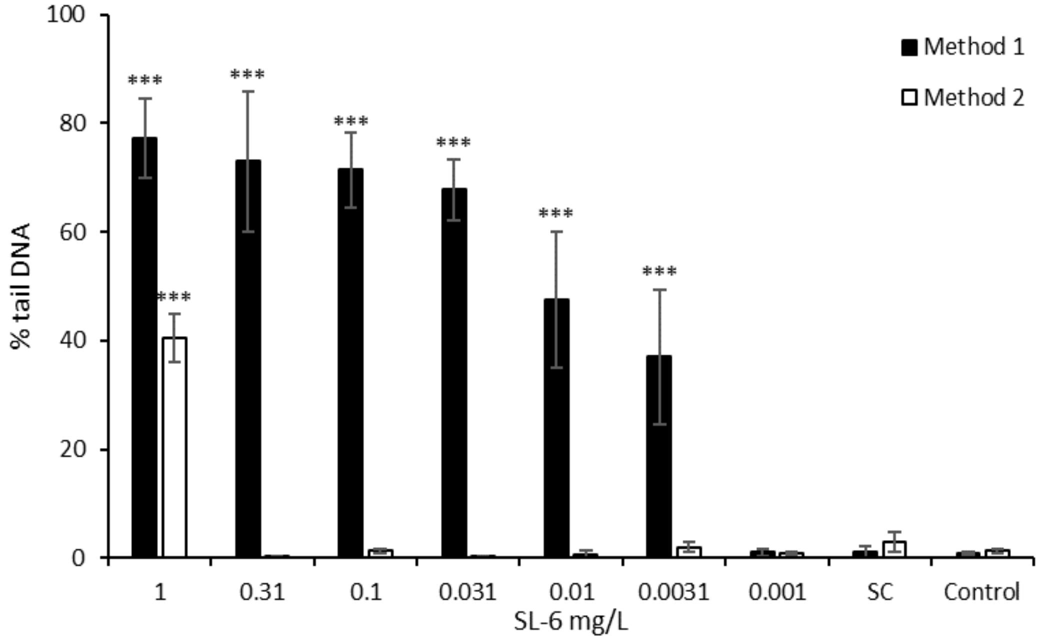






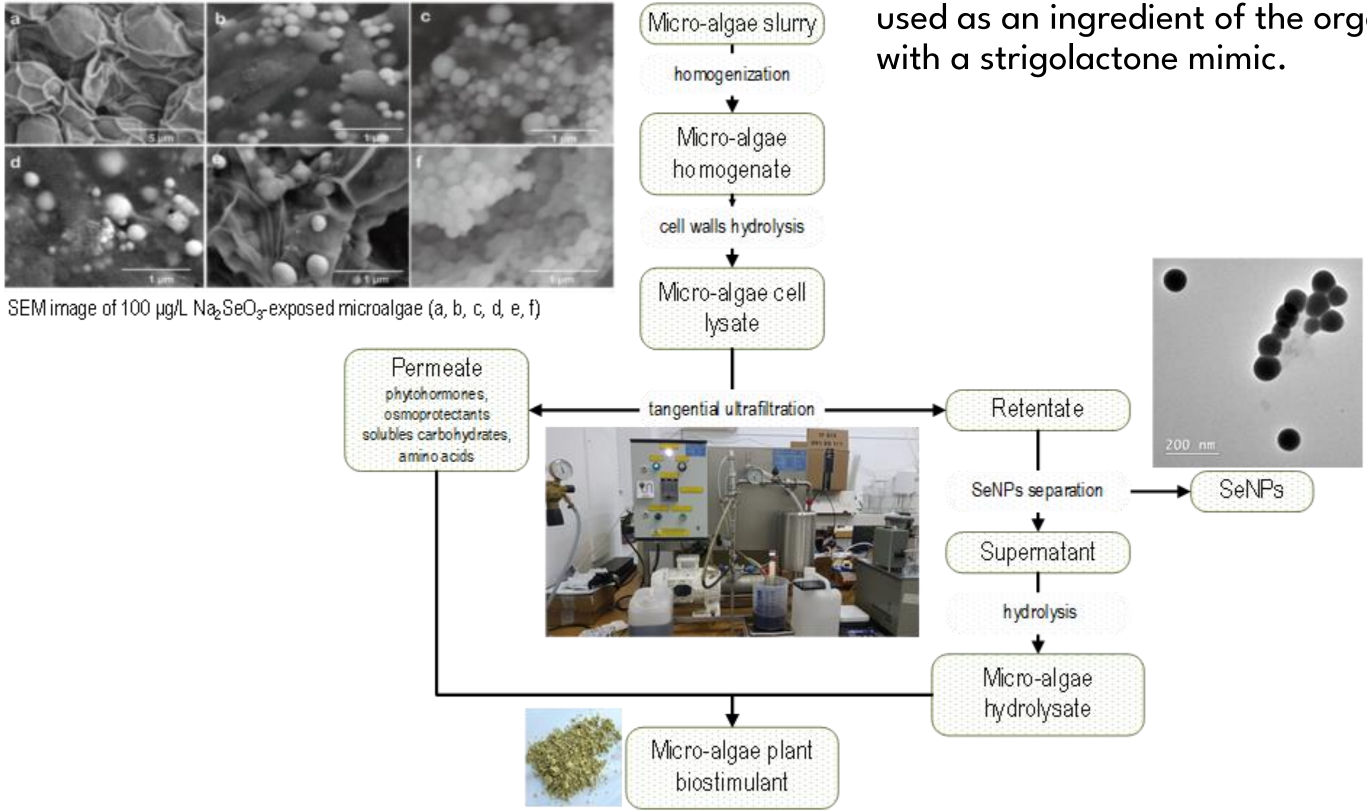
Genotoxicity of SL-6 in Microalgae model



Used concentration – 0.001 mg SL6

Cell genotoxicity (% tail DNA) of SL-6 to the microalgae model. C-control, SC-solvent control (SL-6 concentration in mg/L, mean ±SD). **Method 1**: Standard lysis, 2.5 M NaCl, 0.1 M Na_2EDTA, 10 mM Tris Base, 1% Triton-X-100, pH 10.**Method 2**: Alternative lysis, 300 mM NaOH, 2 mM Na2 EDTA, 0.01% SDS. Subsequent steps were as for the standard comet assay for strand breaks. Statistically significant increase (*$p < 0.05$, **$p < 0.01$, ***$p < 0.001$)

Presentation of the integrated process to produce nanoselenium and microalgae based plant biostimulants. The microalgae - bacteria are grown in the presence of strigolactone and 100 µg/L Na_2SeO_3 for 14 days. The biomass is harvested, SeNPs are separated from the cell components and cell components are used as an ingredient of the organic plant biostimulant, fortified with a strigolactone mimic.



SEM image of 100 µg/L Na_2SeO_3-exposed microalgae (a, b, c, d, e, f)

Patent RO135350
PCT application

Field experiment

Enhanced plant tolerance to stress

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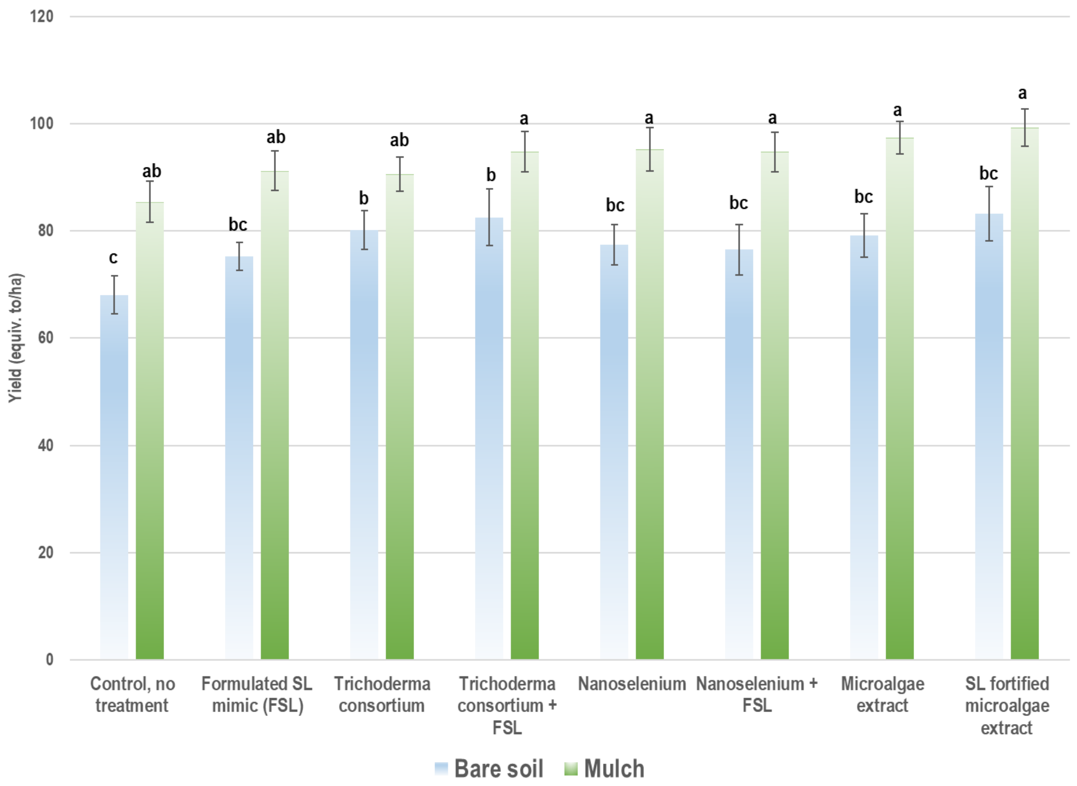

Increased yield of field grown tomatoes

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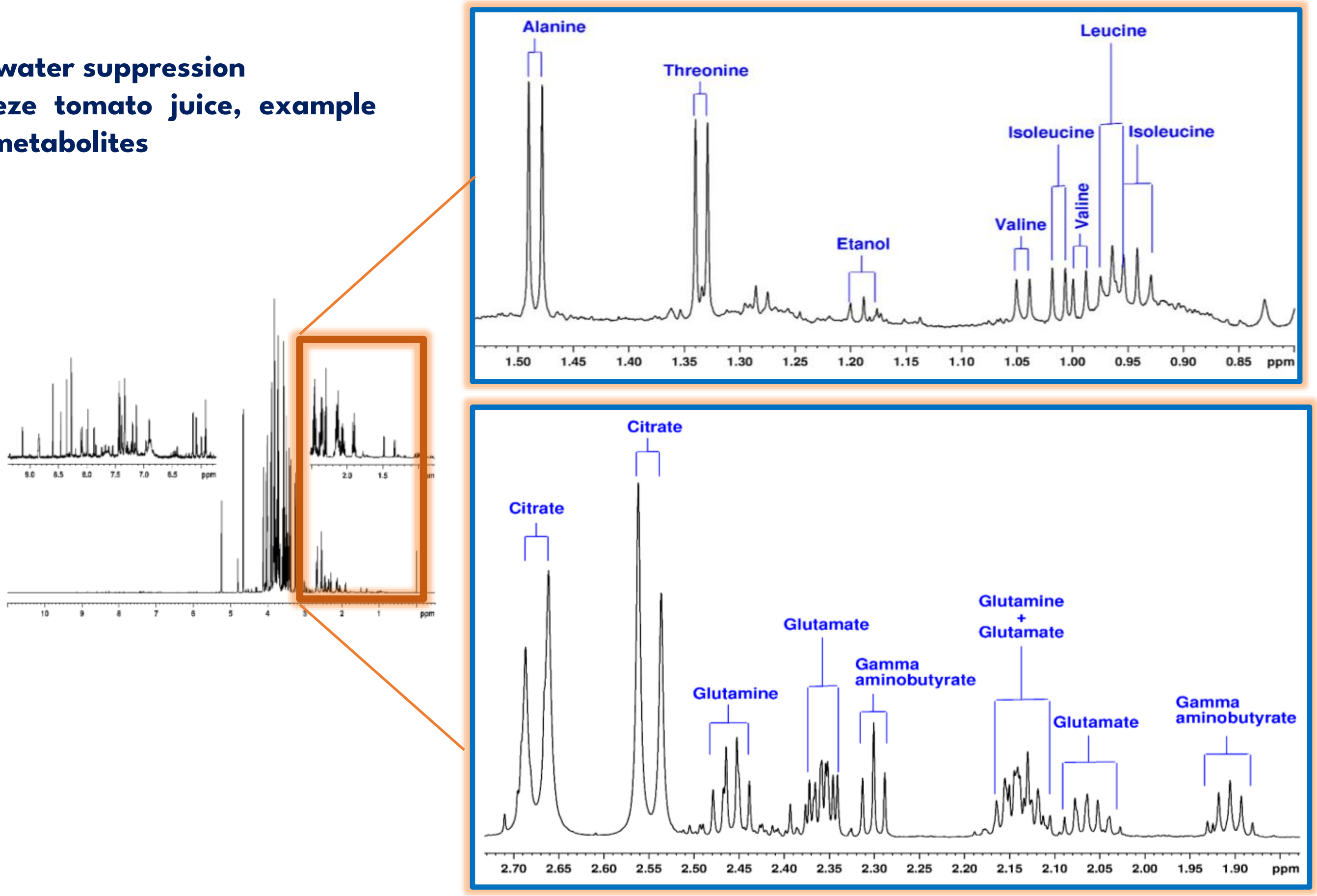
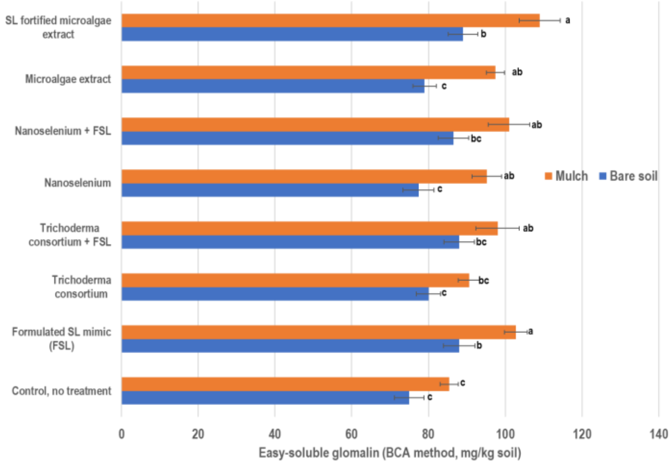
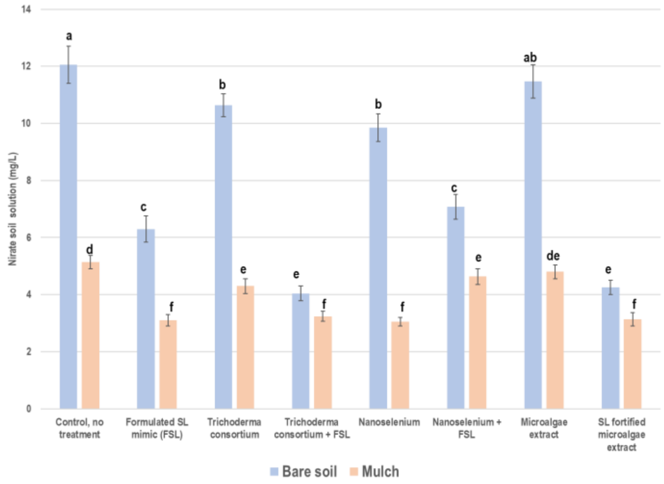
Improved fruit quality

1H NMR with water suppression
Freshly squeeze tomato juice, example of identified metabolites



Easy extractable glomalin and soil nitrate





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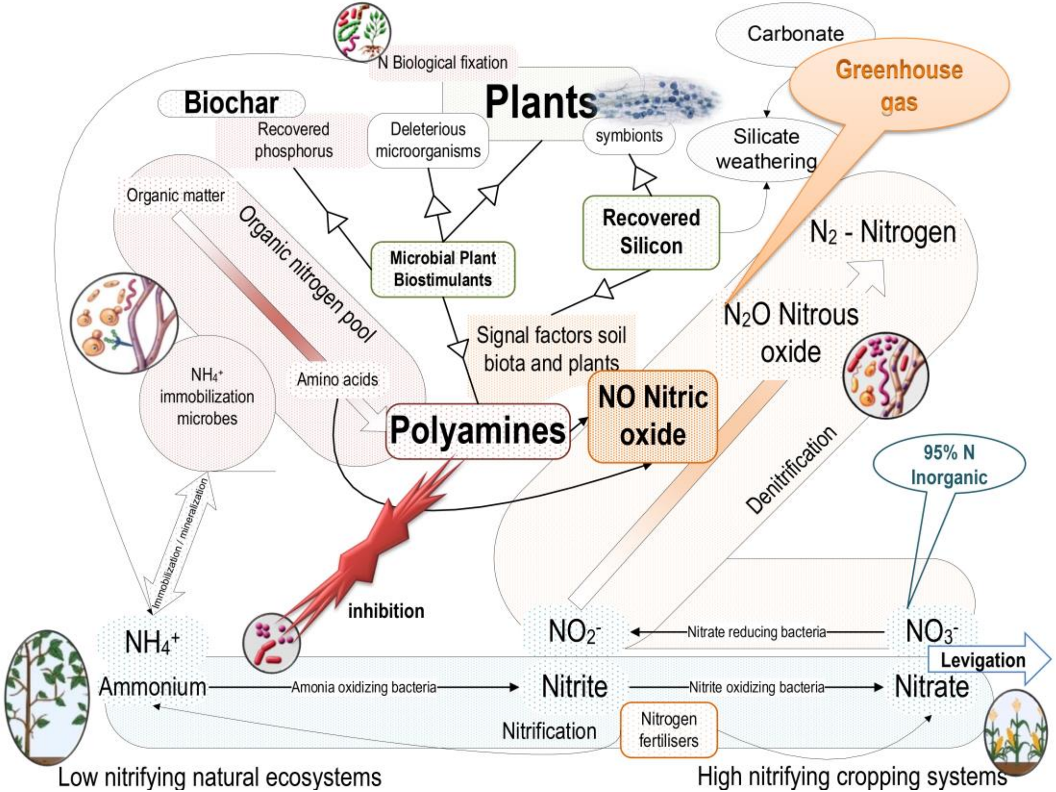

Polyamines and strigolactones - Stimulation of mycorrhizal symbiosis formation

Constantinescu-Aruxandei, D., & Oancea, F. (2023). Closing the Nutrient Loop—The New Approaches to Recovering Biomass Minerals during the Biorefinery Processes. *International Journal of Environmental Research and Public Health*, *20*(3), 2096.

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