Nano 2020 Innovation Conference & Exhibition
Rome, 15-18 September

4° Workshop AgriNanoTechniques

Nanomaterials for products and application in agriculture

Polymeric nanoparticles as carriers for bioactive compounds: the case of crop protection from pathogens

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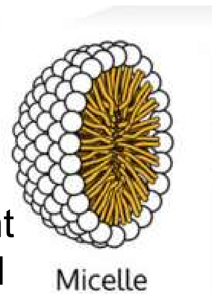
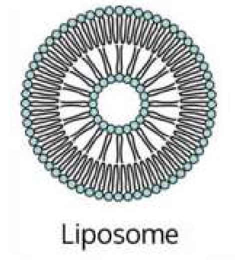
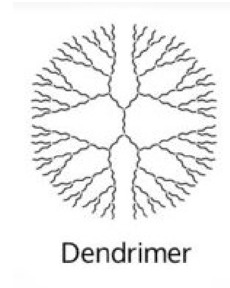
UNIVERSITÀ DEGLI STUDI FIRENZE

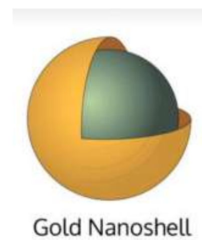
Nanotechnology

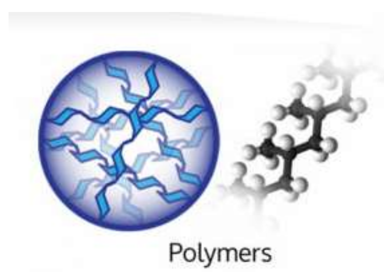
is extremely promising and could be a tool to improve the agriculture sector by increasing the efficiency of agricultural inputs and offering solutions to agricultural and environment problems for improving food productivity and security.



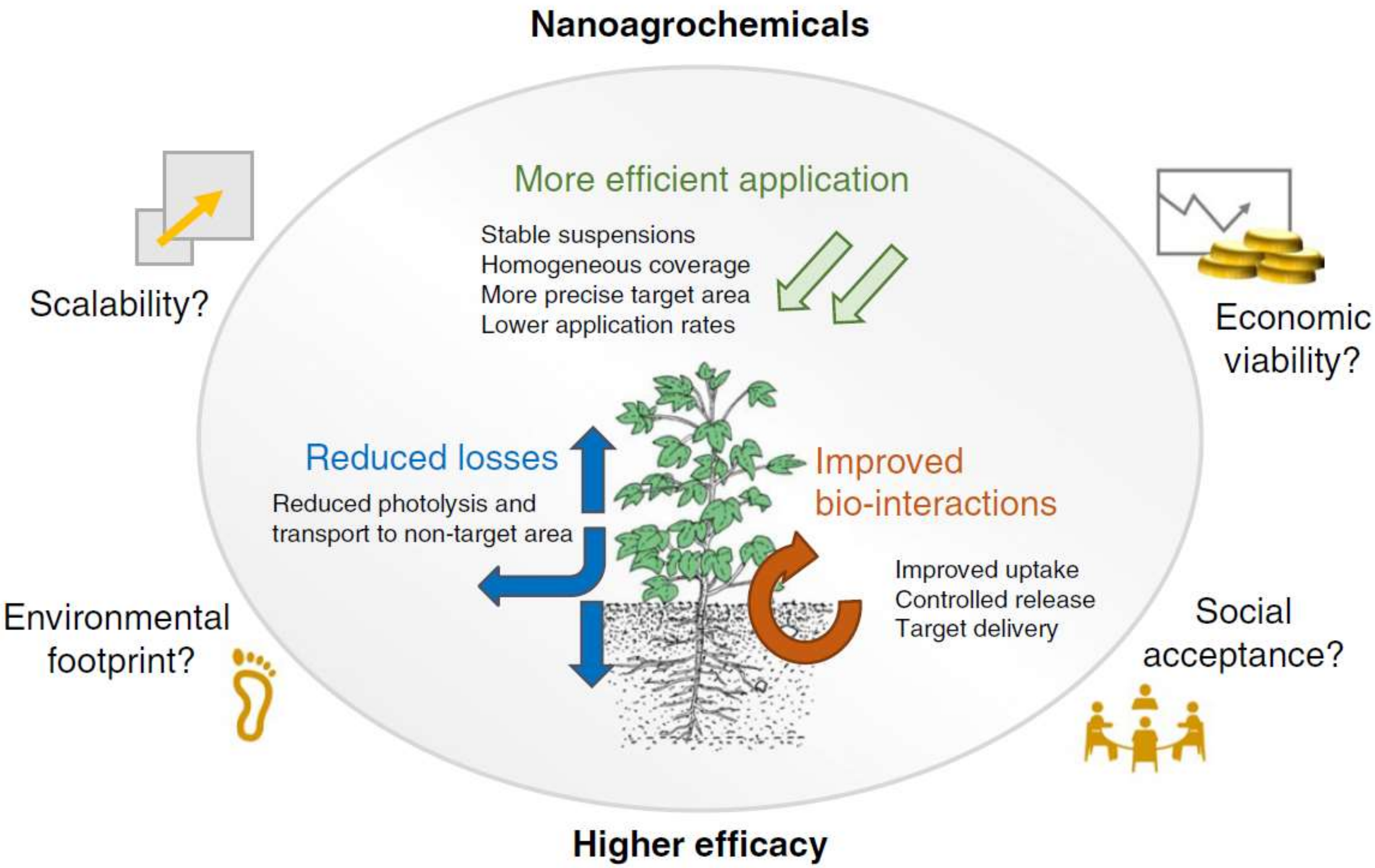








Key drivers and expectations

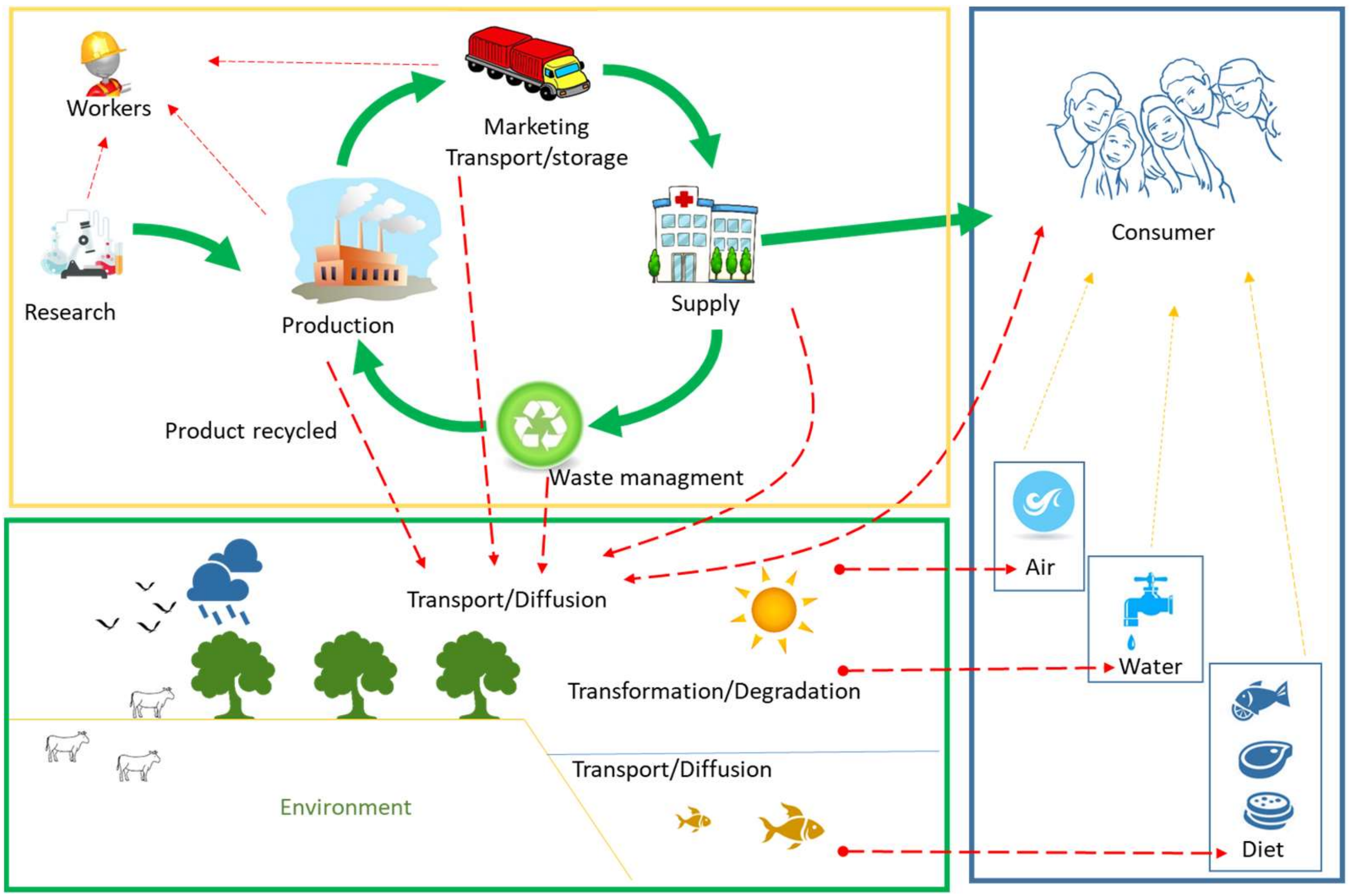


Sustainable nanotechnology

Recently, environmental concerns have led to a more **sustainable approach** for nanoparticles design such as renewable starting materials, less toxic solvents, and biodegradable compounds.

These procedures are important especially when nanoparticles move from the laboratory scale to **large-scale manufacturing**.

This is particularly true for agricultural use, the latest frontier of nanoscience applications.



Possible strategies to achieve
a more sustainable attitude in nanotechnology:

Life Cycle Assessment

Life Cycle Assessment (LCA) is standardized for the evaluation of the environmental aspects and potential impacts of any production process.

It runs throughout the whole product's life from raw materials extraction, functionalization, assembly, distribution, use and final disposal or recycling.

The LCA method is described by **ISO** (International Organization for Standardization) according to the **14040** and **14044** standards.

The ISO framework for LCA can be applied to nanomaterials, even though environmental data on the manufacturing, release, and impacts on this subject are still somehow scarce.

Different strategies to ensure that specific environmental issues are included within a single LCA

The **OECD** published a Guidance Manual towards the integration of **Risk Assessment** (RA) into **LCA** of nano-enabled applications. This is possible because RA focuses on the management of a toxicological threat under specific conditions of exposure, while LCA overviews the environmental impacts, including also non-toxicological and non-chemically detrimental effects.

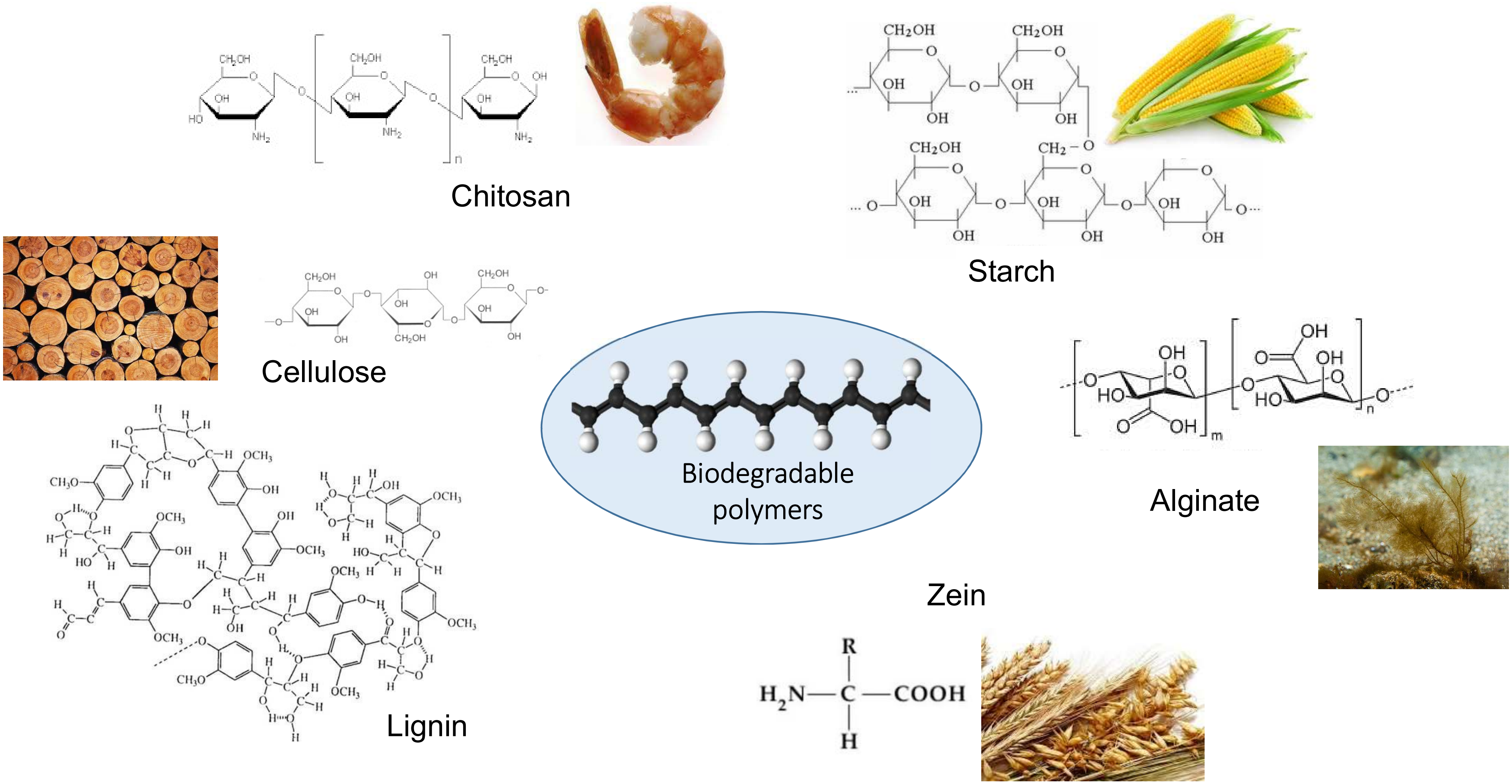
A recent interesting perspective proposes the integration of the two approaches at the methodological level explaining the main advantages and challenges this offers for decision-makers. (Nat. Nanotechnol., 2017, 12, 734–739.)

Another kind of approach has been proposed where the two methods are applied in parallel, integrating them only after obtaining independent results. (Nat. Nanotechnol., 2017, 12, 740–743.)

Possible alternatives to conventional solvents

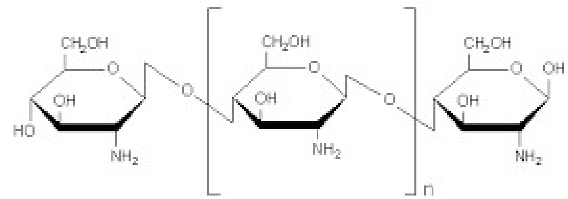
Conventional solvent	Greener option
Pentane, Haxane	Heptane
Diisopropyl ether or ether, dioxane, dimethoxyethane	2-MeTetraHydroFuran or tert-butyl methyl ether
Chloroform, dichloroethane, or carbon tetrachloride	DiChloroMethane
Dimethylformamide, Dimethylacetamide or N-Methyl-2-pyrrolidone	Acetonitrile
Pyridine	Triethylamine Et_3N (if pyridine used as base)
DiChloroMethane (extractions)	Ethyl acetate, MTBE, toluene, 2-MeTHF,
DiChloroMethane (chromatography)	Ethyl acetate or heptanes
Benzene	Toluene

Polymeric nanomaterials used in agriculture



Chitosan

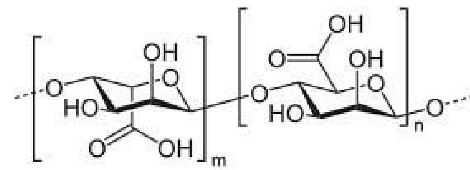
- Polymer that can be obtained by treating chitin from shrimp and other crustaceans with a base (alkaline substance), producing a polymeric β-glucan;
- Elicitor of defence responses in plants and it possesses antifungal properties that make it very attractive for applications in plant protection;
- It can be used as a carrier for pesticides when synthesized in the form of nanoparticles, either alone or in combination with other polymers.;
- This double function as nanocarrier and active substance itself, in addition to its origin from a waste by-product from the fishing industry, turns chitosan into a promising material for nanoformulating natural compounds.



Chitosano

Vurro et al.Pest Manag Sci 2019; **75**: 2403–2412 www.soci

Alginate



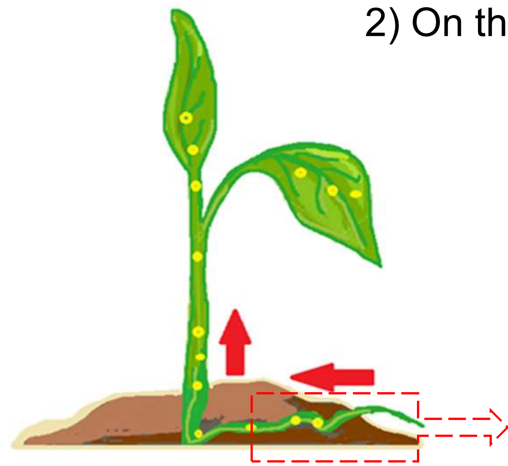
Alginato

- linear β-linked polysaccharide isolated form the brown algae group, Phaeophyceae, commonly known as seaweed.
- can be treated in several ways to produce hydrogels, microspheres, nanoparticles and nanocapsules, and can be combined with other polymers such as chitosan, producing a highly versatile system for nanoformulation of agrochemicals.
- Experiments with insecticides (imidacloprid) have shown that a reduction in the dose of the active ingredient can be achieved while the effectiveness of the treatment is not compromised and is even improved over time.

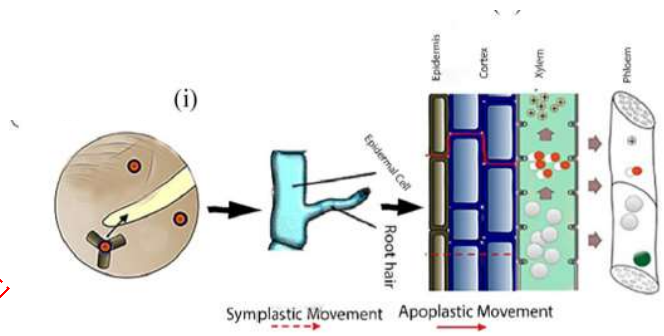
Vurro et al.Pest Manag Sci 2019; **75**: 2403–2412 www.soci

Administrations

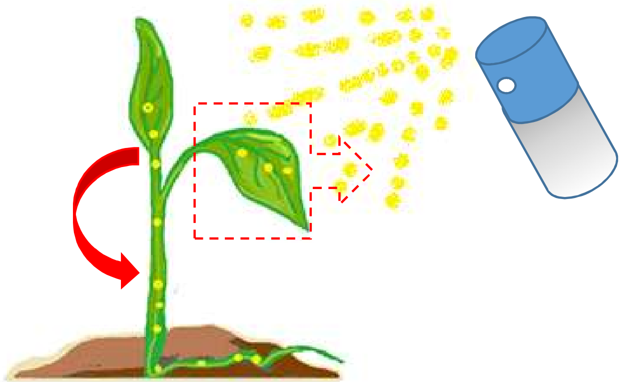
Through irrigation: 1) On seeds;

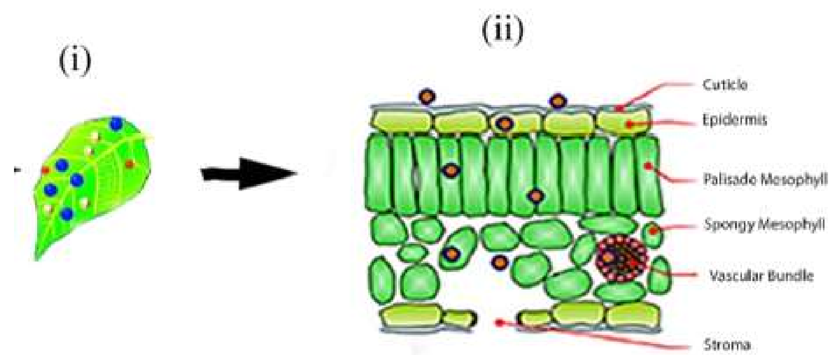


2) On the root system;

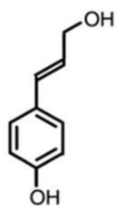


Foliage Application:

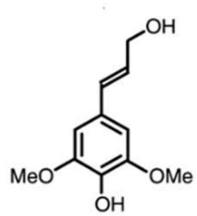




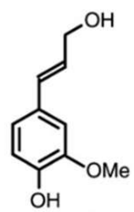
Lignin



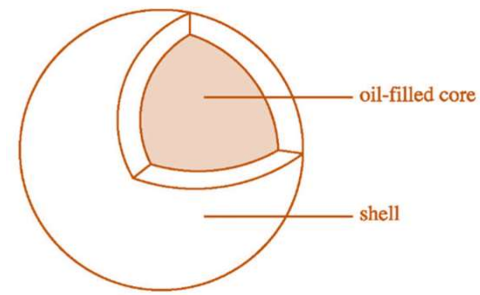
Unità H



Unità S



Unità G



Lignosolfonates

Lignin organosolv

Kraft Lignin

- **High number of phenolyc -OH**
- **High MW;**
- **Soluble in alkaline solution or organic solvents.**

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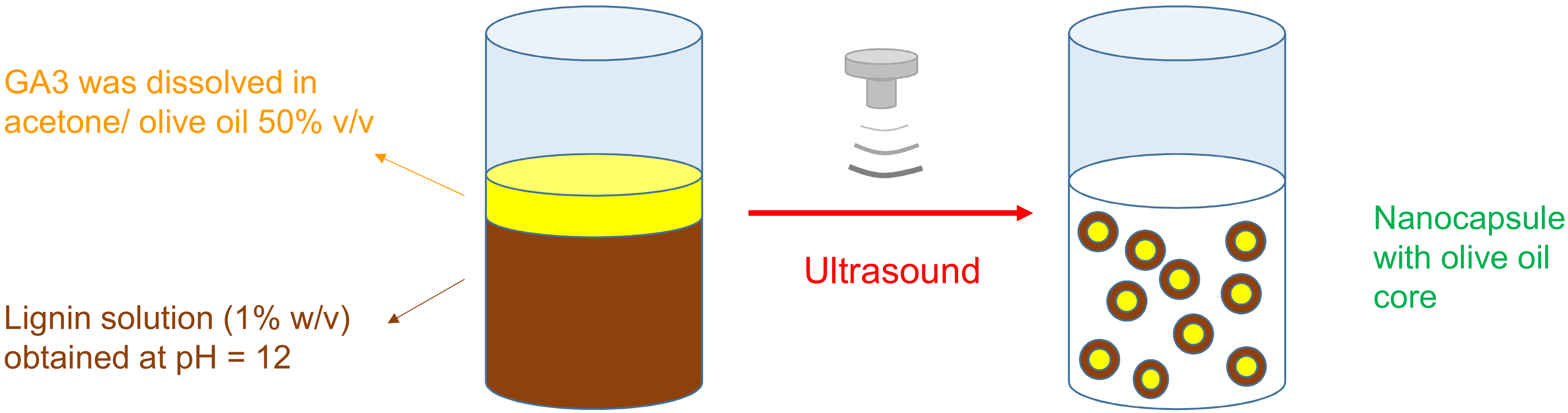
Questions?

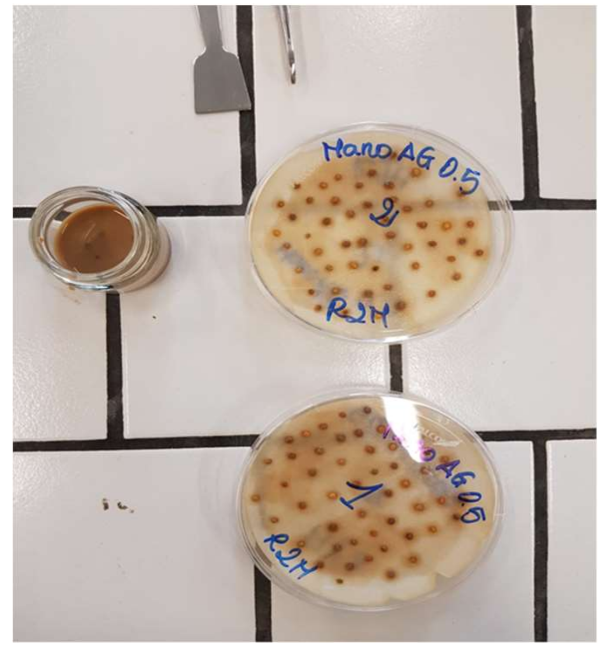
Nanocapsule preparation



In order to produce lignin nanocapsules (LNCs), we used a high-intensity ultrasound technology as a tool to emulsify an oil phase in a lignin aqueous solution and encapsulate GA3 into the lignin shell.

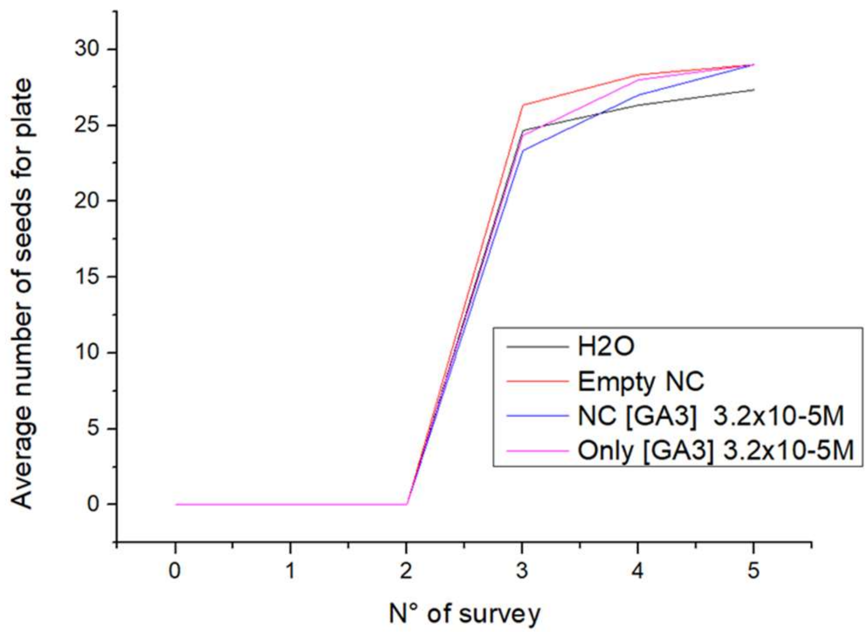
In vivo experiments are conducted on *Eruca sativa* and *Solanum lycopersicum L.*

- NC solutions are sterilized by UV rays and then diluted 1:2 in sterile water;
- The day before the experiment, the seeds have been sterilized by a solution of ethanol 70% and then in sodium hypochlorite 10%. After water washing, seeds are stored overnight in sterile water for rehydration.
- The day of the experiment, seeds have been immersed in NC solutions for 30 minutes and then planted on bibula paper where Nanocapsules preparations have been added.

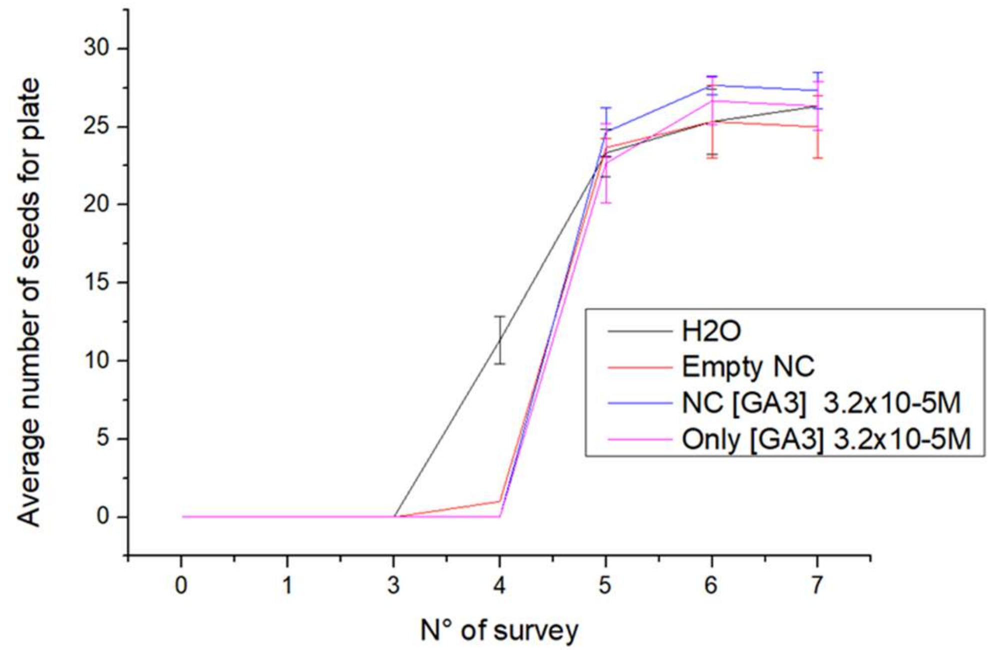


In vivo results

Germination curves of the ***Eruca sativa*** seeds treated with NC loaded with 0.5 mg/ml ($3.2x10^{-5}M$) compared with the controls



Germination curves of the ***Solanum lycopersicum L.*** seeds treated with NC loaded with 0.5 mg/ml ($3.2x10^{-5}M$) compared with the controls



Preliminary *in vitro* results

Germination percentage of Solanum Lycopersicum L. seeds after <u>one week</u>:

- Empty Nanocapsules: 46.6%
- NC [GA3] 0.5 mg/ml ($3.2x10^{-5}$M): 76.6 %
- Only [GA3] 0.5 mg/ml ($3.2x10^{-5}$M): 60%
- Water: 53.3%

Germination percentage of Solanum Lycopersicum L. seeds after <u>three weeks</u>:

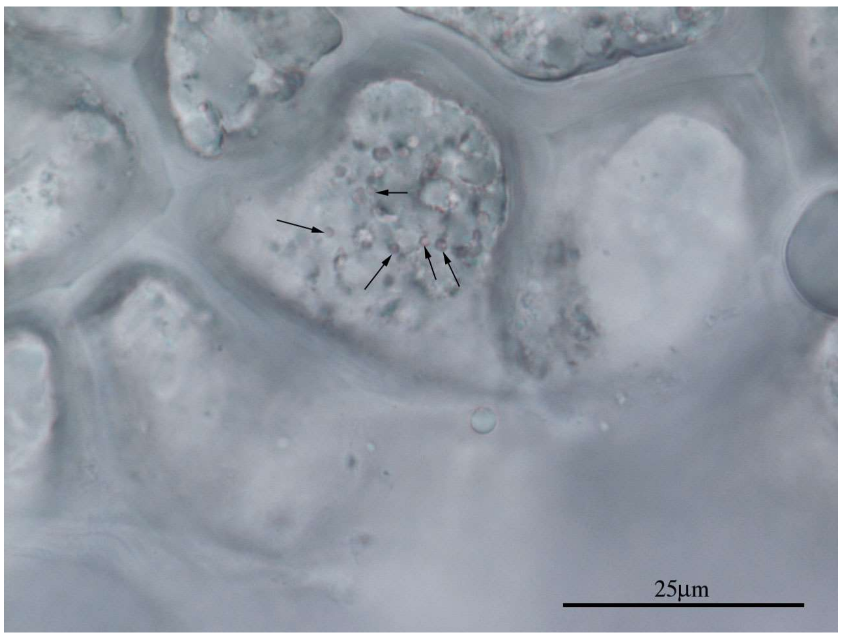
- Empty Nanocapsules: 93.3%
- NC [GA3] 0.5 mg/ml ($3.2x10^{-5}$M): 100%
- Only [GA3] 0.5 mg/ml ($3.2x10^{-5}$M): 90%
- Water: 83.3%

Only [GA3] 0.5 mg/ml after three weeks

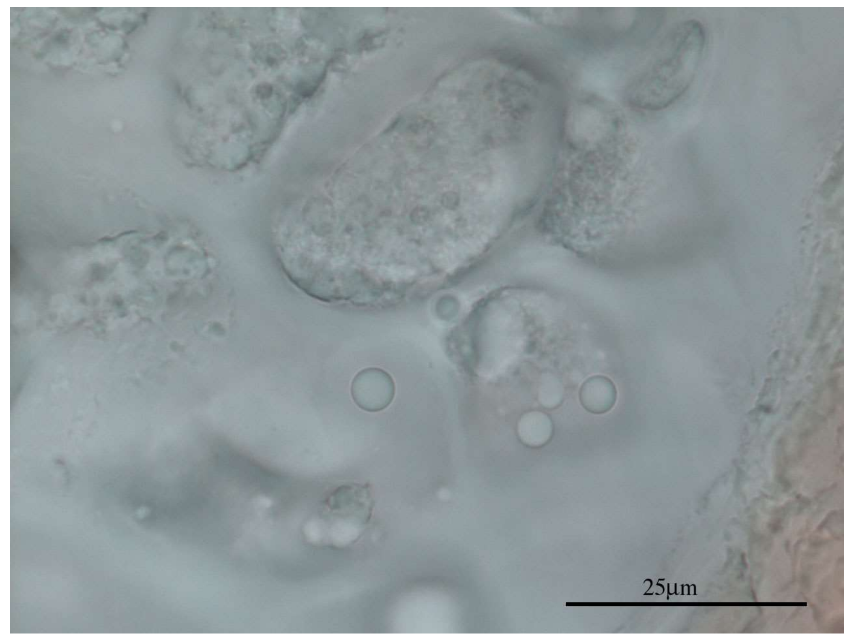
NC + [GA3] 0.5 mg/ml after three weeks

Cytologic and Histologic studies on *Solanum Lycopersicum L.* seeds

Nanocapsules loaded with GA3 0.5mg/mL

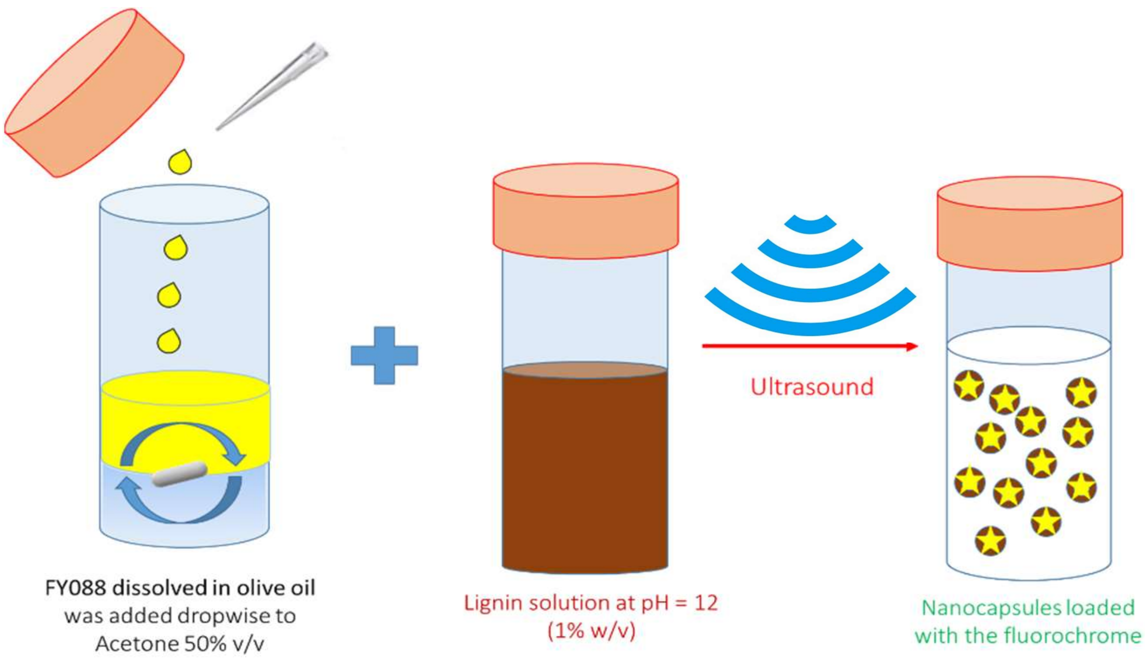


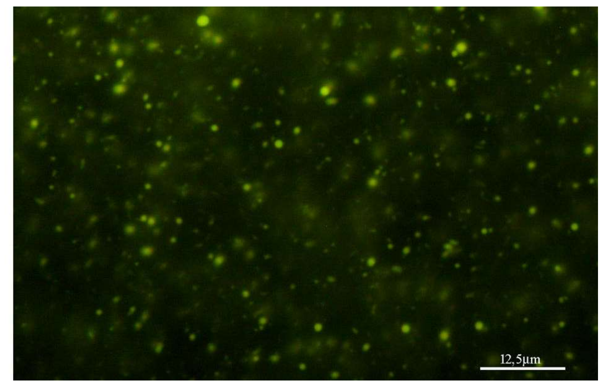
Only water



Lignin shell of the nanocapsules was marked with phloroglucinol

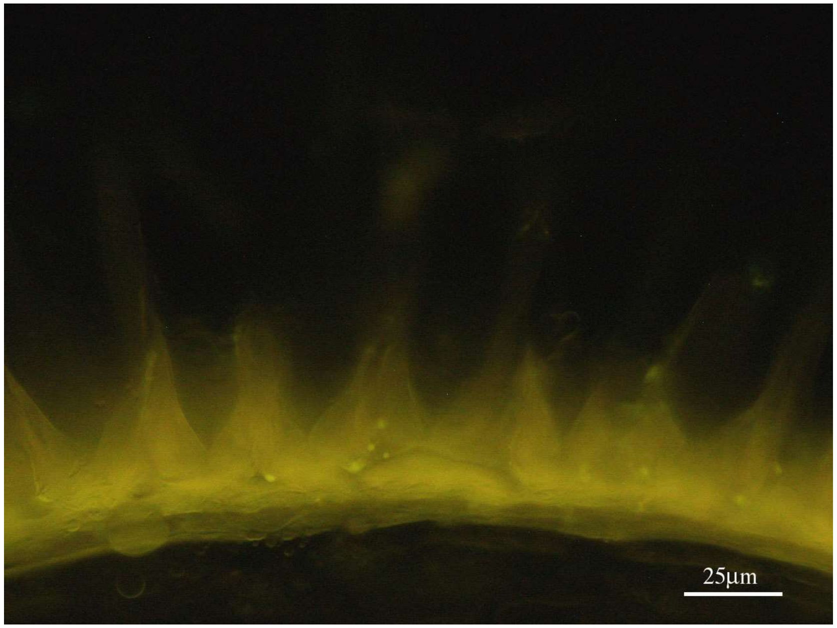
Lignin Nanocapsules loaded with FY088





Nanocapsules on the *Solanum lycopersicum L.* seeds

After 24 h from the initial treatment

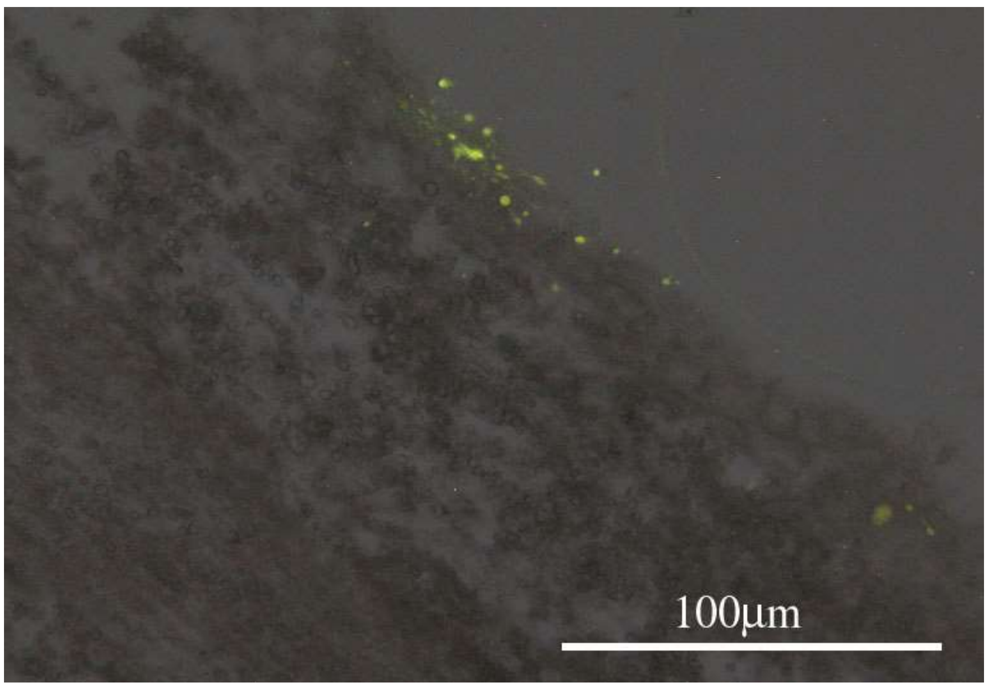


External tegument of the seed

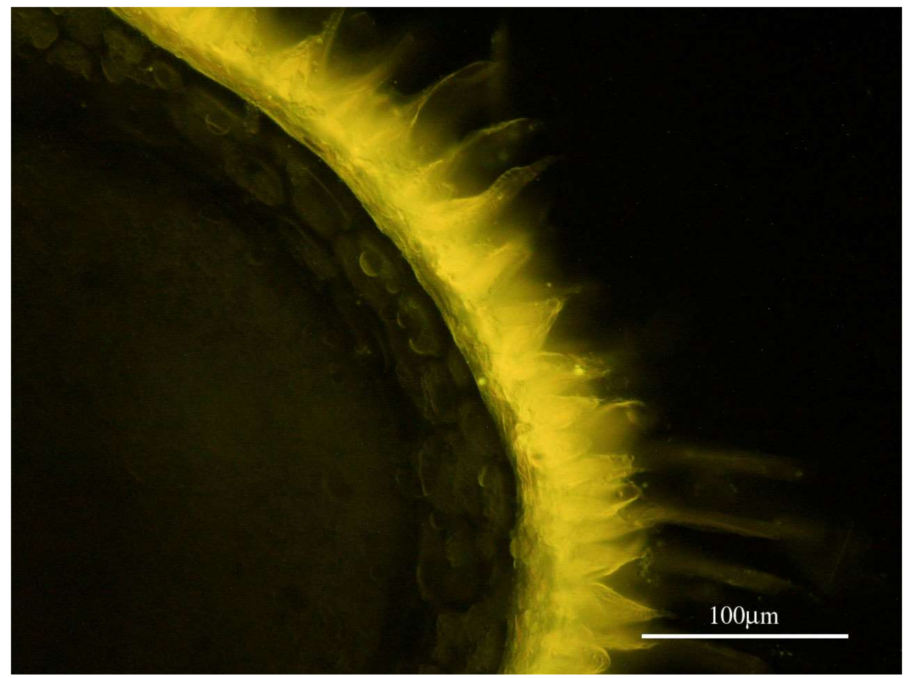
Endosperm of the seed

Nanocapsules on the *Solanum lycopersicum L.* seeds

After 48 h from the initial treatment



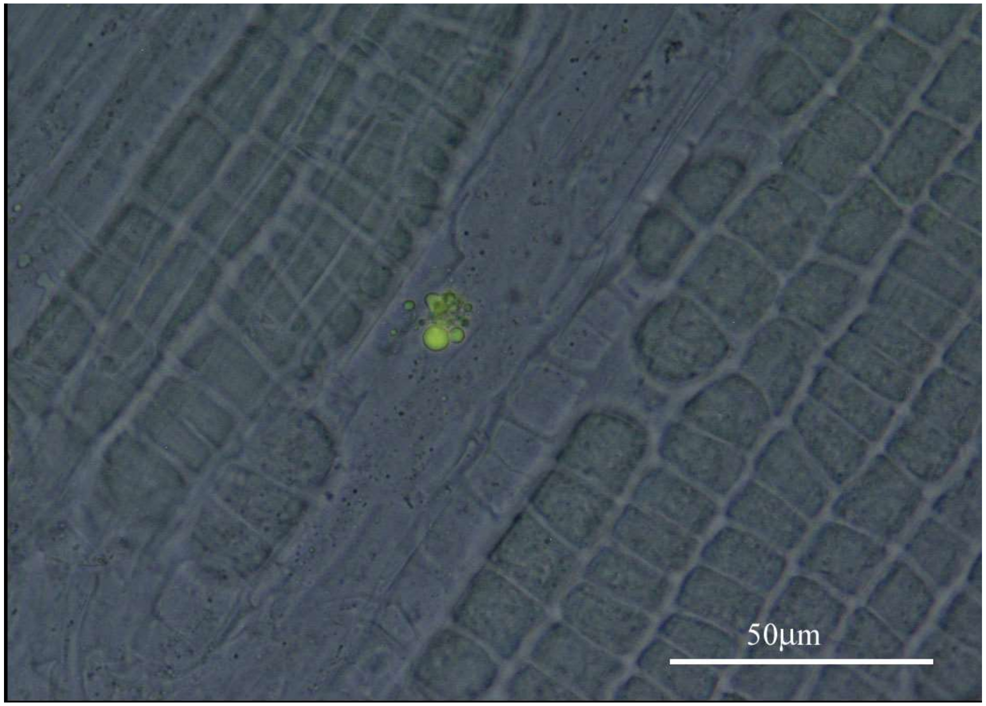
Root



External tegument of the seed

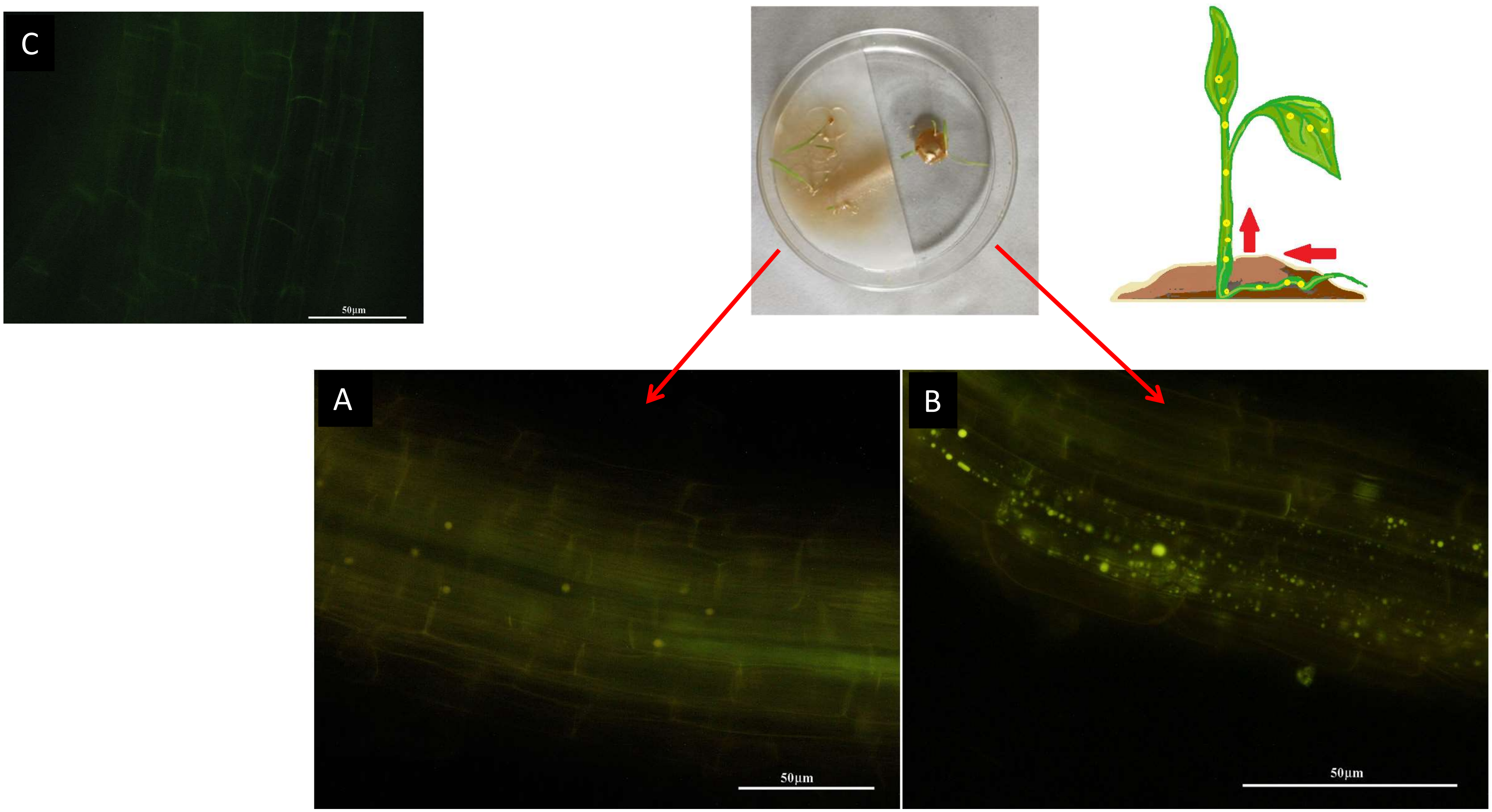
Nanocapsules on the *Solanum lycopersicum L.* seeds

After 72 h from the initial treatment



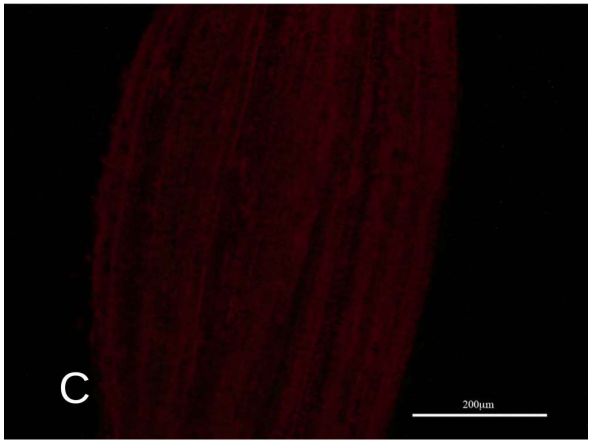
Root

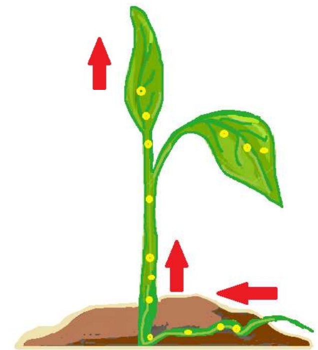
Nanocapsules on the *Eragrostis tef* plantlets

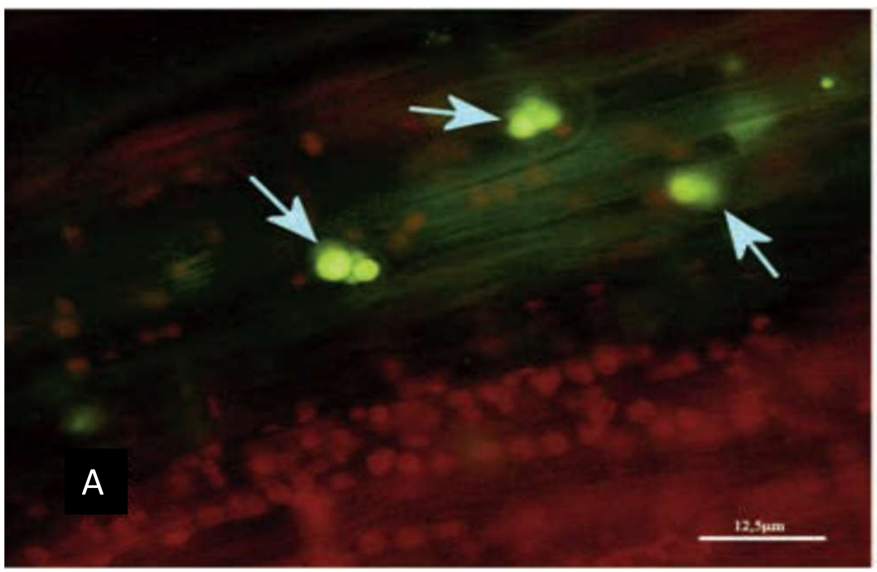


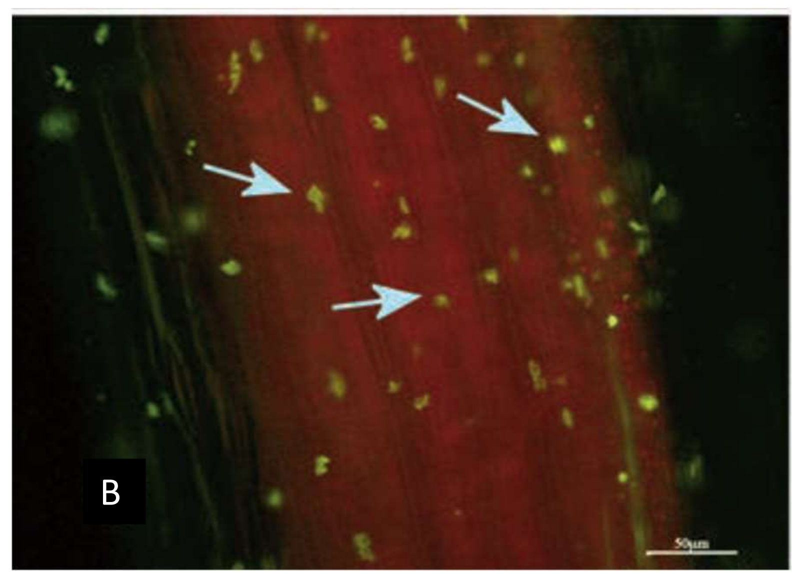
Closer to the xylem vessels

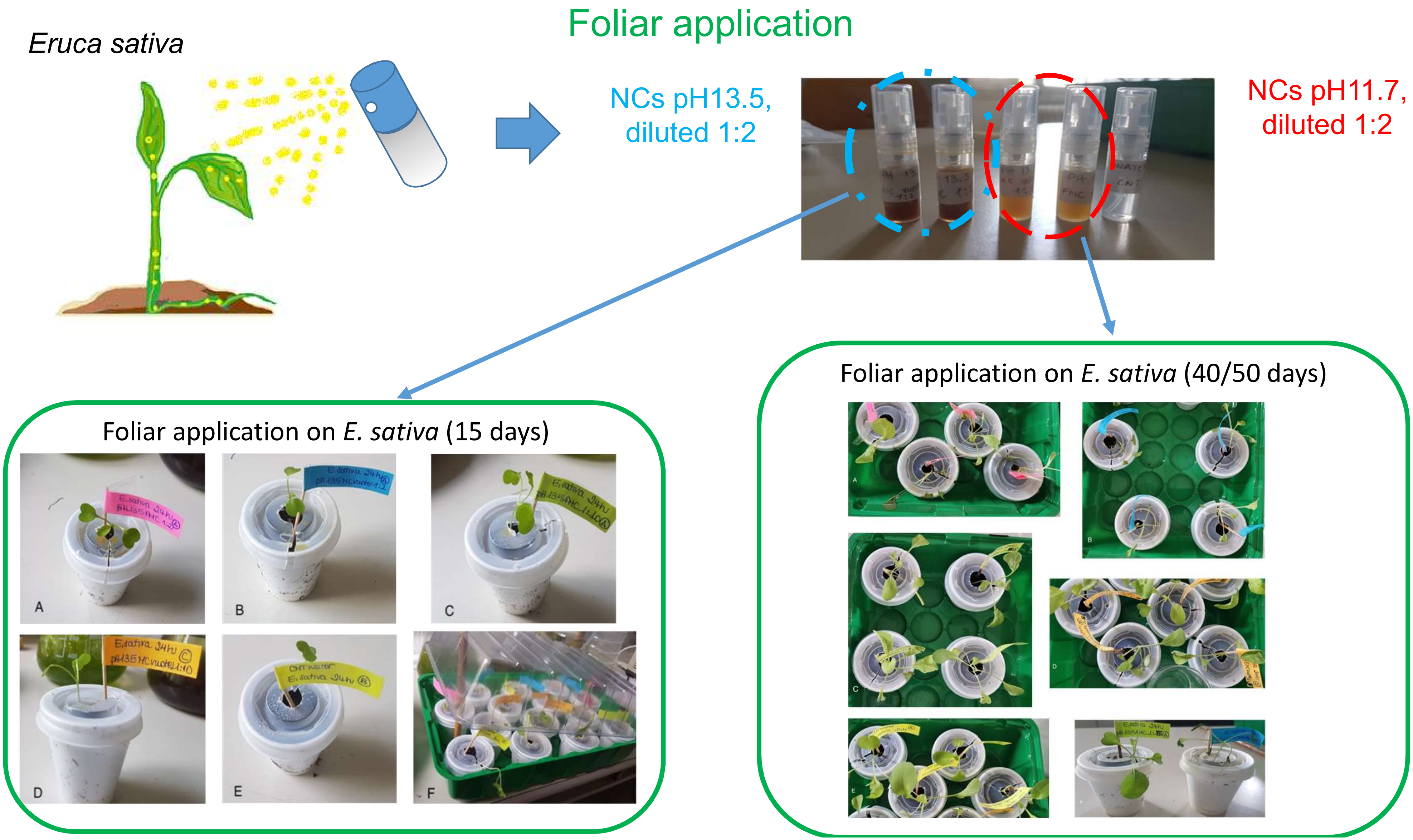
Nanocapsules on the *Eragrostis tef* plantlets



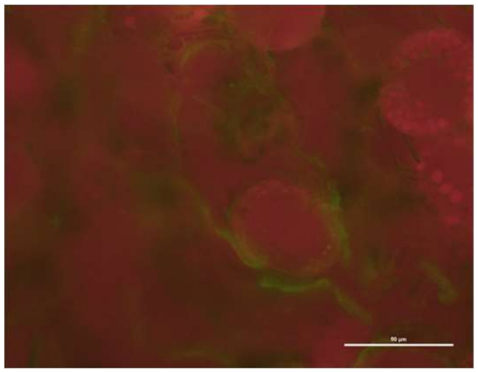




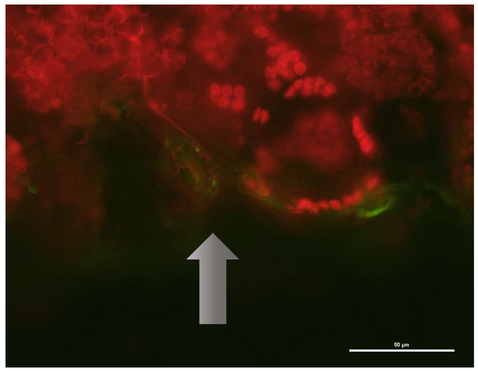



Eruca sativa
Foliar application
NCs pH13.5, diluted 1:2
NCs pH11.7, diluted 1:2
Foliar application on E. sativa (15 days)
Foliar application on E. sativa (40/50 days)
A
B
C
D
E
F

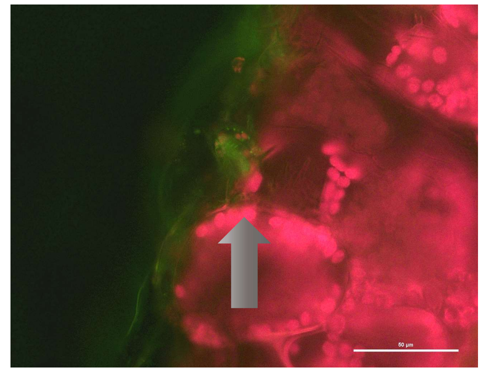
Uptake of nanocapsules pH 13.5 on *E. sativa* plantlets (15 days)



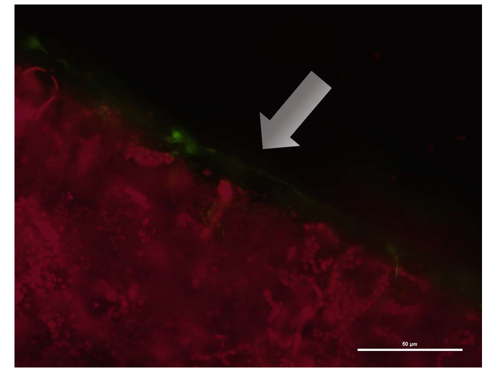
Cotyledon upper page 24h



Cotyledon cross section 24h



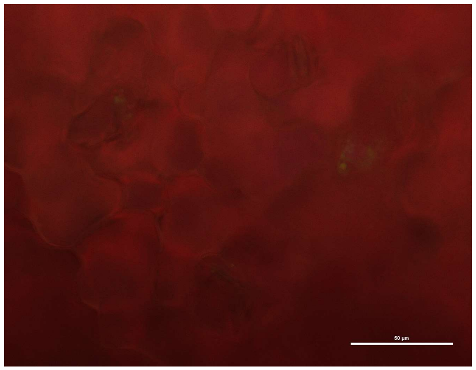
Cotyledon cross section 3h



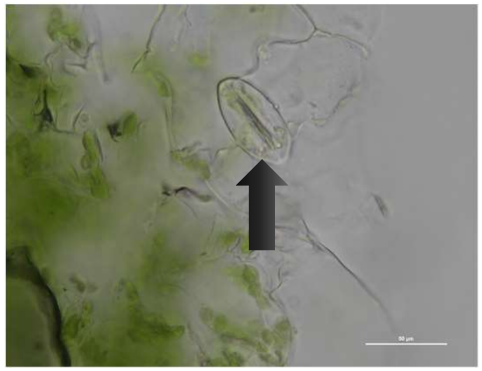
Cotyledon cross section 3h

E. sativa plantlets (15 days) after 24h administration of nanocapsules pH 13.5 (dil 1:2

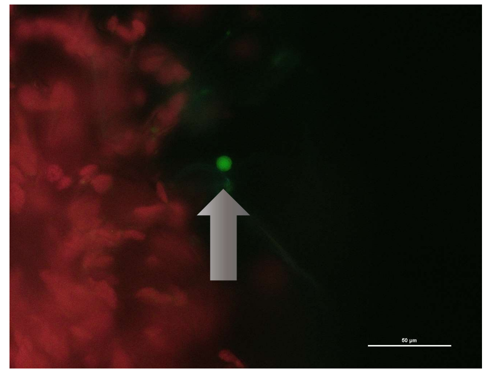
NCs Uptake of nanocapsule pH 13.5 on *E. sativa* plantlets (40 days)



Leaf upper page 24h

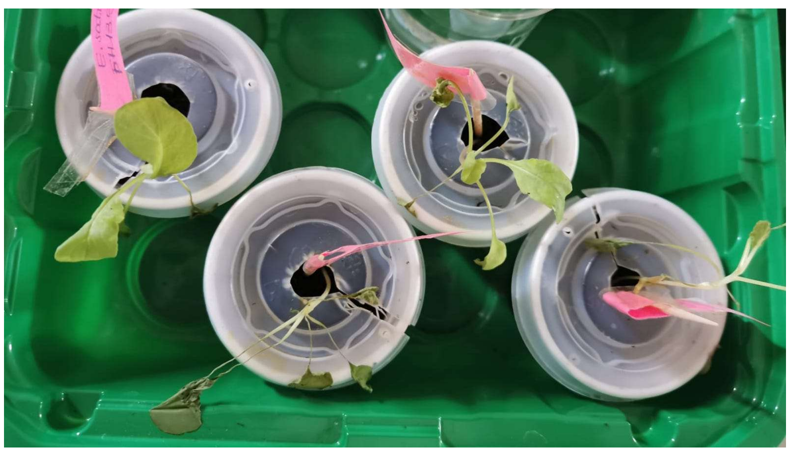


Leaf cross section 24h



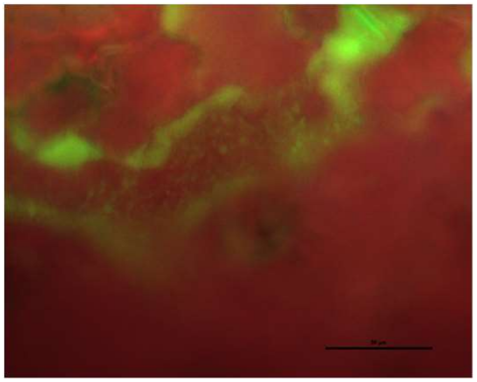
Leaf cross section 24h

Leaf longitudinal section 24h

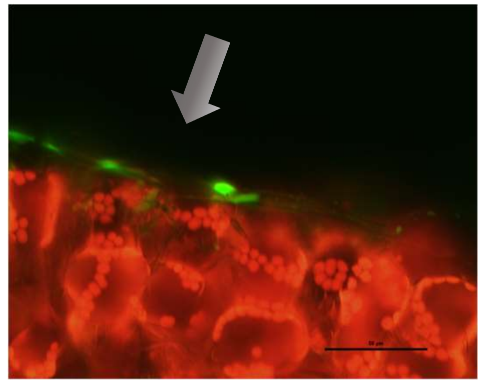


E. sativa plantlets (40 days) after 24h administration of nanocapsules pH 13.5 (dil 1:2)

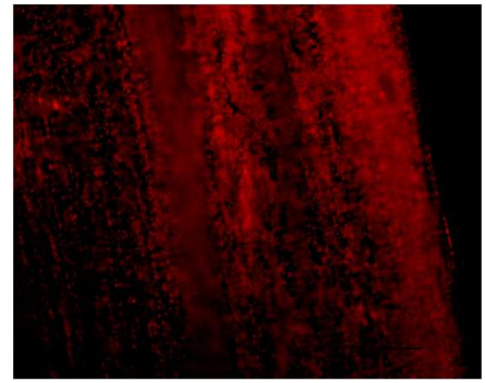
NCs Uptake of nanocapsule pH 11.7 on *E. sativa* plantlets (40 days)



Leaf upper page 24h



Leaf cross section 24h



Petiole longitudinal section 24h

E. sativa plantlets (40 days) after 24h administration of nanocapsules pH 11.7 (dil 1:2)

Conclusions

- Sustainable agriculture to meet one of the Agenda 2030 goals;
- Nanotechnology extremely promising to increase agricultural inputs improving food productivity and security;
- Possible strategies to implement nanotechnology sustainability: Life Cycle Assessment and Green Chemistry;
- Biodegradable polymers: Alginate, Chitosan, Lignin
- Lignin used for an innovative formulations for bioactive compound delivery through irrigation or foliage application.

References:

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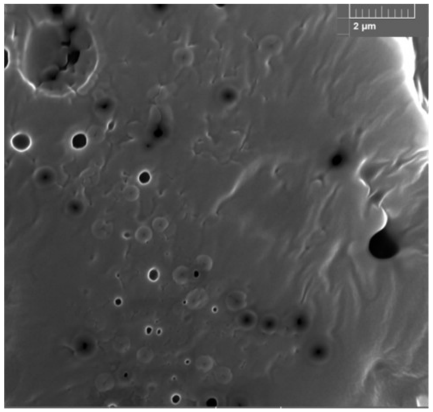
- A new method for the direct tracking of in vivo lignin nanocapsules in Eragrostis tef seedlings, European Journal of Hystochemistry, 2020, 64, 2, 114-119. doi:10.4081/ejh.2020.3112.

- Safe nanotechnologies for increasing the effectiveness of environmentally friendly natural agrochemicals, *Pest Manag Sci* 2019; **75**: 2403–2412.

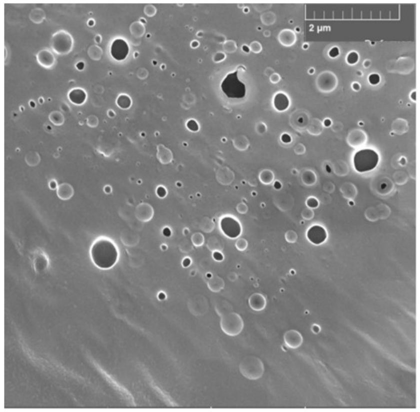
Dinamic Light Scattering (DLS)

Nanocapsule	Average Diameter (nm)	Polidispersity
Empty	295 nm± 20	0.38
[GA3] $6.54x10^{-5}$M	240nm±10	0.22
[GA3] $1.3x10^{-4}$M	175nm±10	0.17
[GA3] $1.97x10^{-4}$M	220nm±20	0.28

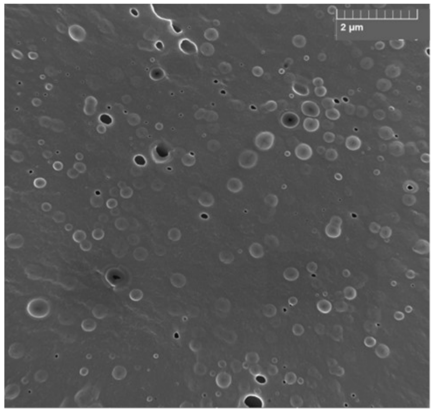
Scanning Electron Microscopy (SEM)



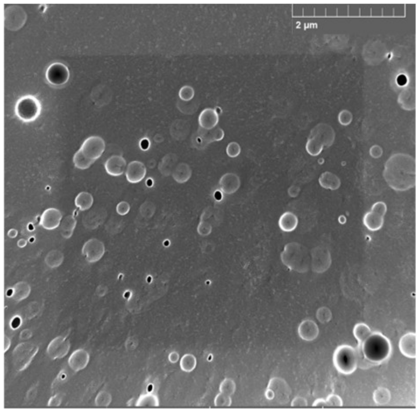
Empty nanocapsules



Nanocapsules [GA3] 0.5 mg/ml ($6.4x10^{-5}$M)



Nanocapsules [GA3] 1.0 mg/ml($1.3x10^{-4}$M)



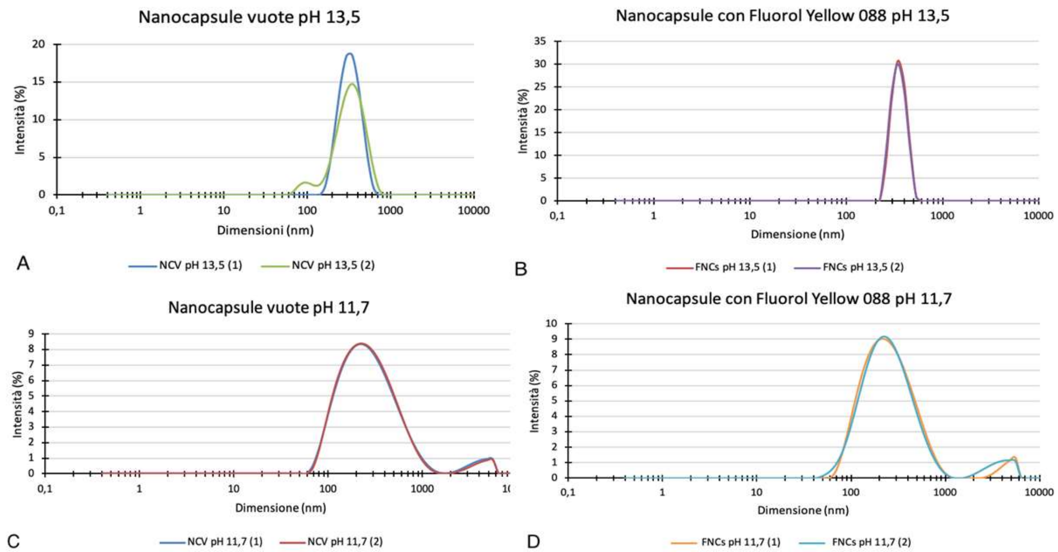
Nanocapsules [GA3] 1.5 mg/ml ($1.9x10^{-4}$M)

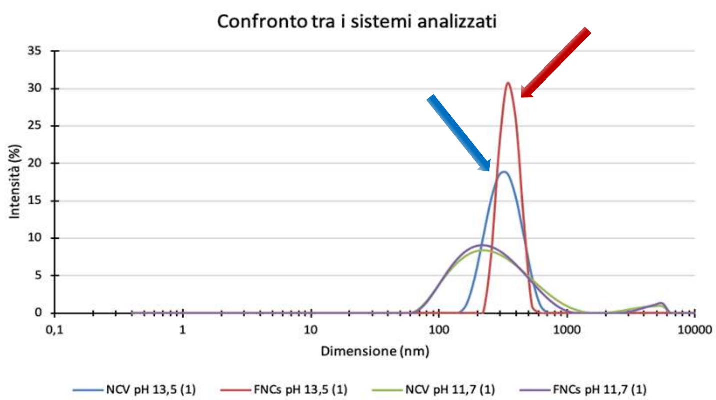
2. Caratterizzazione chimico-fisica:

Analisi al DLS (*Dynamic Light Scattering*)

Su campioni diluiti 1:100

Nanocapsule	Diametro medio (nm)	Polidispersità
Vuote a pH 13,5	330 nm	0,25
Con FY088 a pH 13,5	355 nm	0,01
Vuote a pH 11,7	304 nm	0,32
Con FY088 a pH 11,7	274 nm	0,32



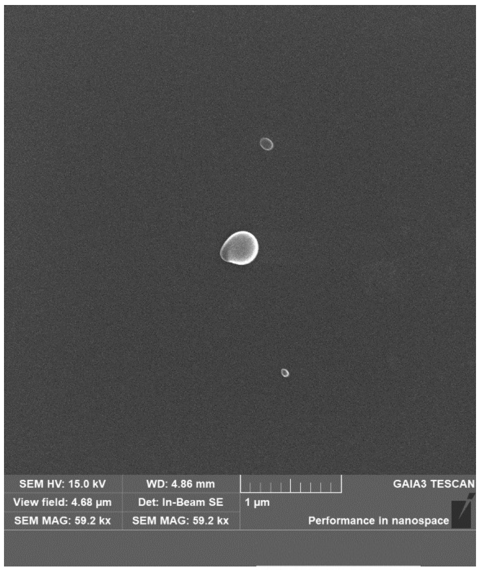


Solubilità *LIGNINA*
risulta maggiore nella preparazione a pH 13.5

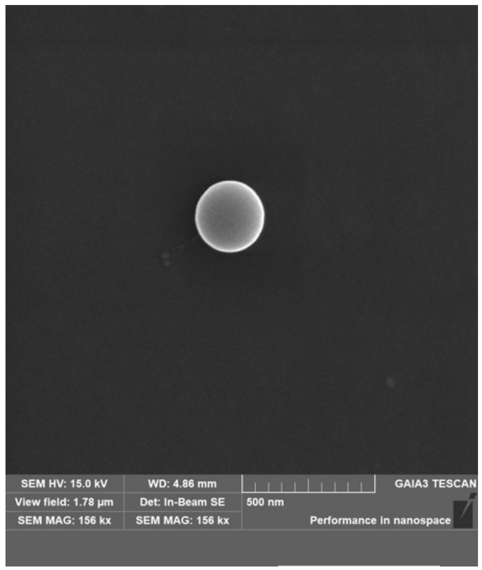
2. Caratterizzazione chimico-fisica:

Analisi al SEM (*Microscopio Elettronico a Scansione*)

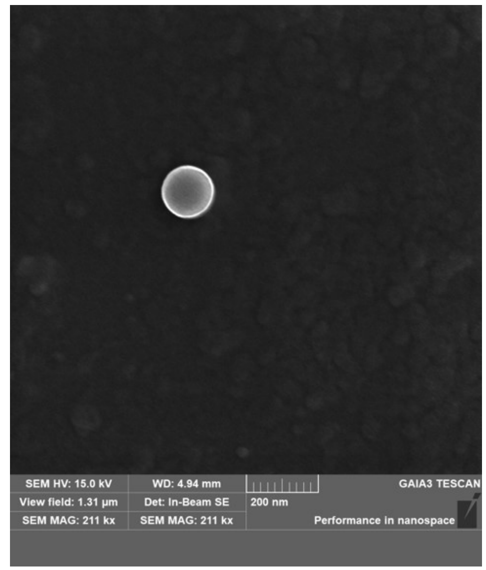
Su campioni diluiti 1:100



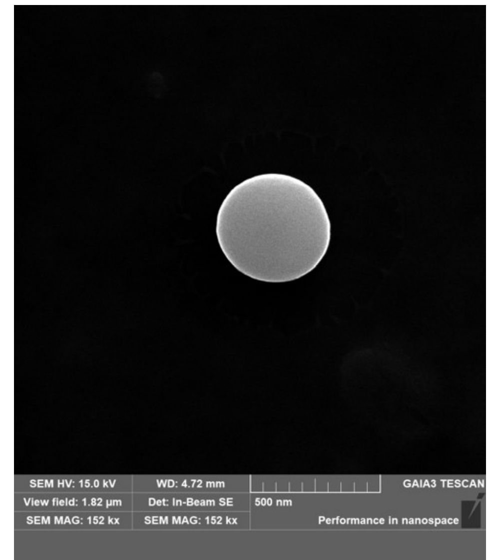
Nanocapsule vuote a pH 13.5



Nanocapsule con FY088 a pH 13.5



Nanocapsule vuote a pH 11.7



Nanocapsule con FY088 a pH 11.7