Release of *Trichoderma viride* from microcapsules simultaneously loaded with chemical and biological agents

8th CASEE Conference
"Sustainable development in Europe –cooperation between science and practice. What's the position of Central and South Eastern Europe?"

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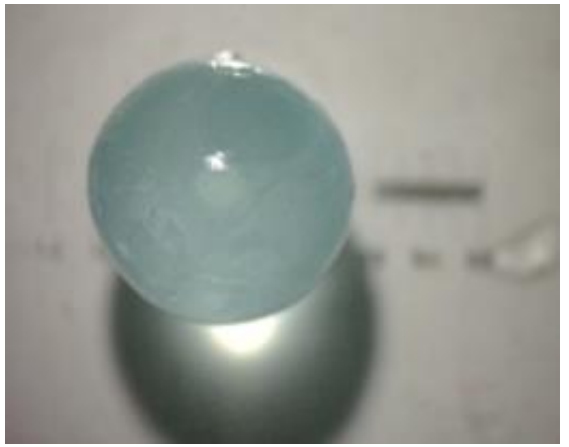
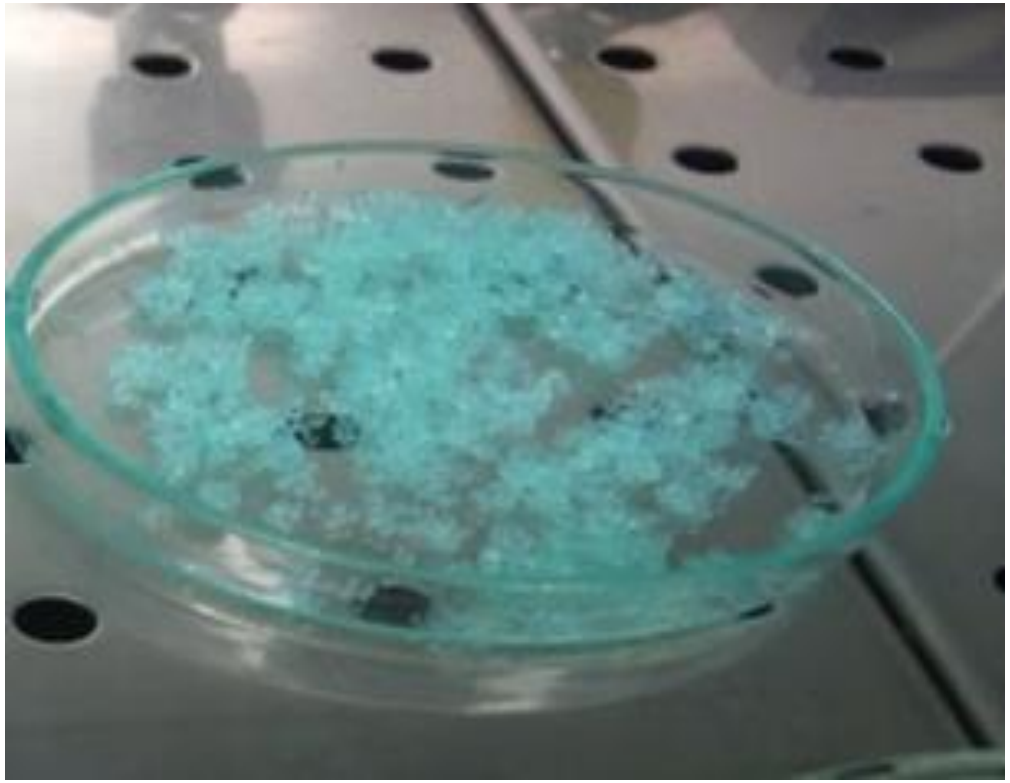
❑ Introduction

- Definition of encapsulation
- Objective of investigation



❑ Encapsulation processes

- Dripping / ionic gelation technologies

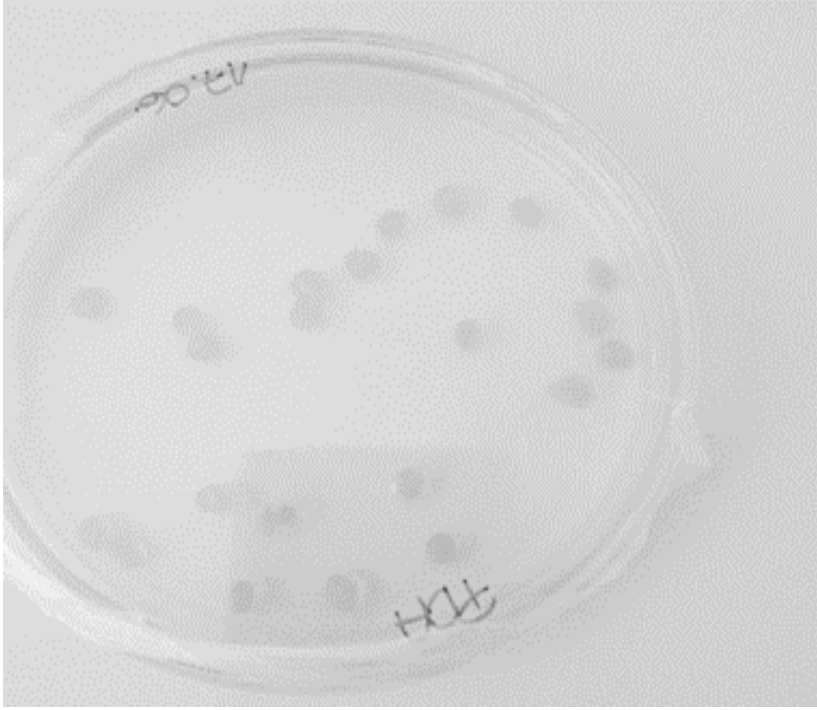


❑Characterizations

- Microcapsule size measurements,
- Microscopy (optical, confocal, SEM)
- Phytopatology (surviving of *Trichoderma viride*),
- Interactions between chemical and biological agents in mixture and microcapules (Fourier Transform Infrared Spectroscopy, FTIR),
- Quantification, release kinetic profiles.

❑Conclusion

What is encapsulation?



Encapsulation relates to technologies which enable to formulate **one active compound (or more)**, **inside individualized particles** with a specific geometry and properties.

Prepared microcapsules – 2 mm size

Microencapsulation usually refers to sizes ranging from 1 µm to a few mm.
The current presentation focuses on sizes ranging from 0.45 mm to 2 millimeters.

Objective of investigation

- preparation and characterisation of novel chitosan/alginate microcapsules simultaneously loaded with **copper ions** and ***Trichoderma viride***

The specific goals are:

(i) investigation of intermolecular interactions in systems with oppositely charged biopolymers,

(ii) investigation of interactions between bioactive agents and the delivery system,

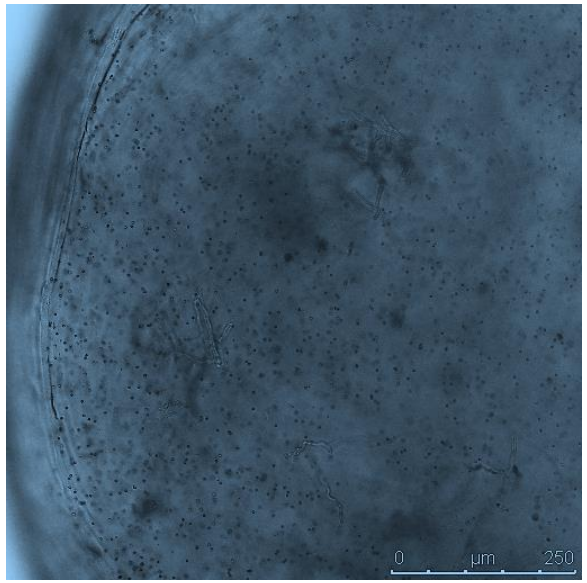
(iii) investigation of conditions for simultaneous encapsulation of biological and chemical agents,

(iv) laboratory investigations of optimal microcapsule formulations,

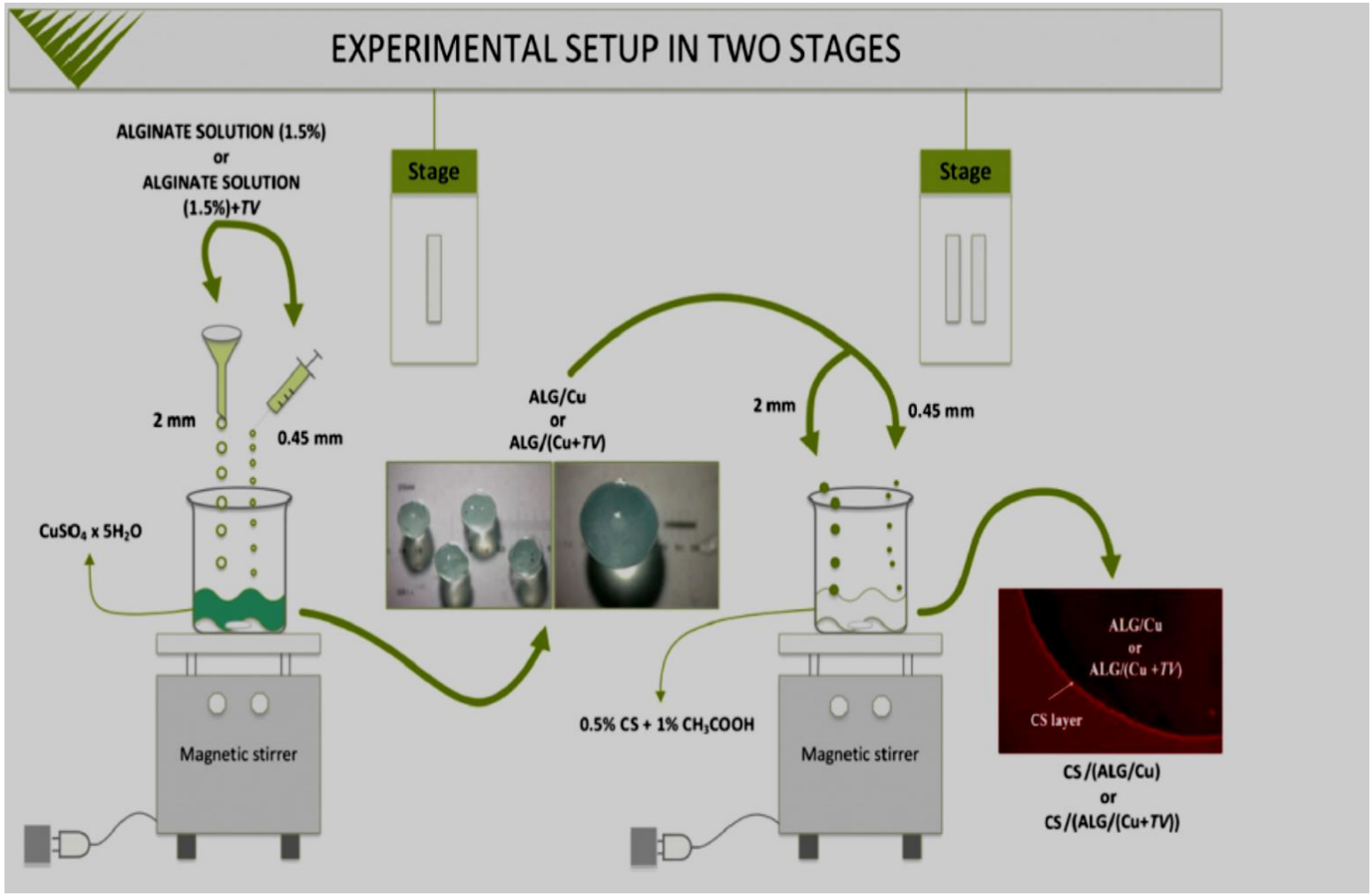
(v) in vivo testing of optimal microcapsule formulations on conventionally and hydroponically grown lettuce and tomato.

chemical agent

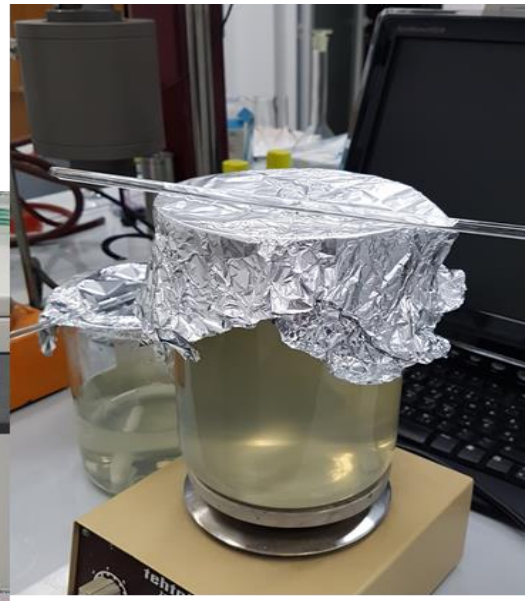
biological agent



Encapsulation processes



- Microcapsules were prepared by the **ionic gelation technique** at ambient temperature.
- Preparation is rapid and reliable, and microcapsules were obtained spontaneously under very mild conditions in two stages.
- **The first stage** comprises the formation of core microcapsules loaded with copper cations (ALG/Cu) or loaded with *Trichoderma viride* (ALG/(Cu+*TV*)).
- **The second stage** includes the coating of core microcapsules by chitosan.

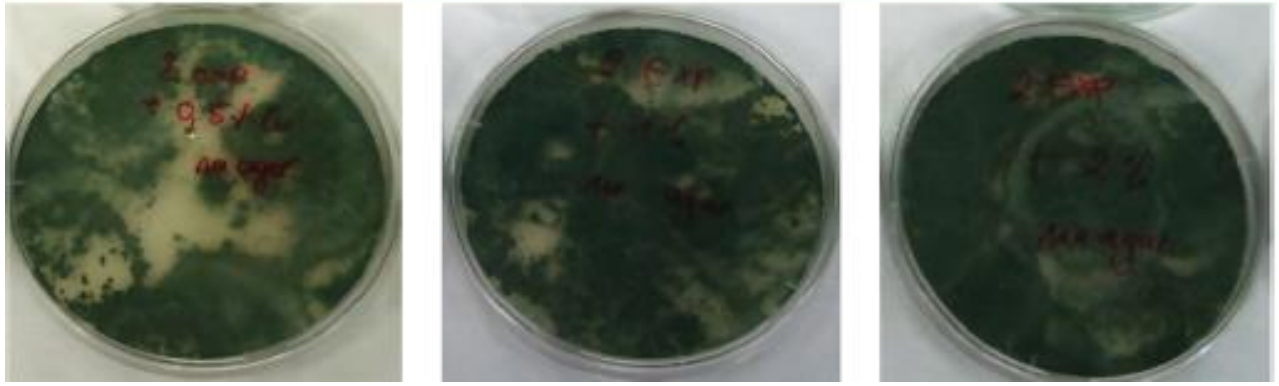


Characterizations

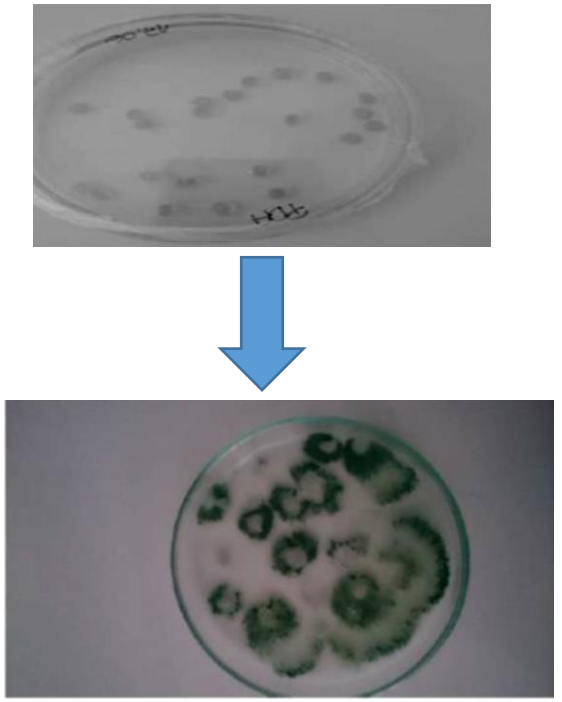
Phytopathology (surviving of *Trichoderma viride*)

Trichoderma viride (TV) is an opportunistic avirulent plant symbiont as well as a mycoparasite of plant pathogenic fungi.
Its agricultural importance is its good antagonistic abilities:

a) against soilborne plant pathogenic fungi thanks to different mechanisms of antagonism,
b) the production of antifungal metabolites (antibiosis), competition for space and nutrients,
c) induction of defense responses in plant, and mycoparasitism.

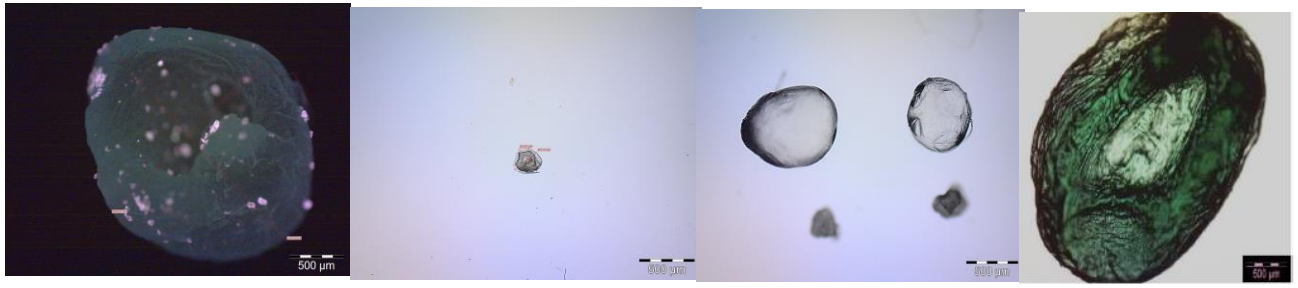


Microphotographs of the mycelial growth of *Trichoderma viride* spores sprayed with CCVD prepared at increasing initial copper cation concentrations (c_i) = 4.5, 9, and 18 mmol dm^{-3} (from left to right) taken after spraying

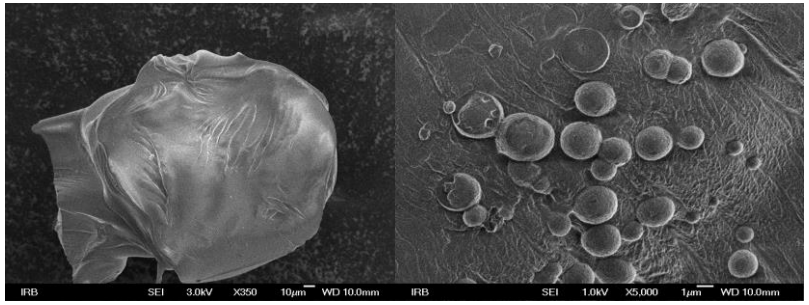


Microphotographs of prepared microcapsules filled with copper ions and *Trichoderma viride*.
Survival and growth of *Trichoderme viride* on nutrient substrate (0 day, 15 days)

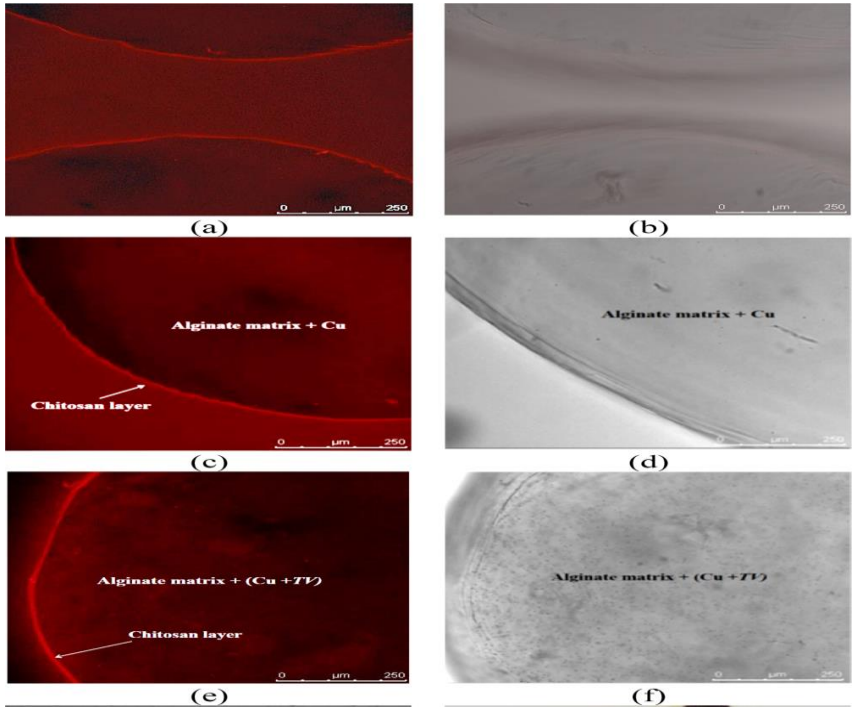
Microcapsule morphology

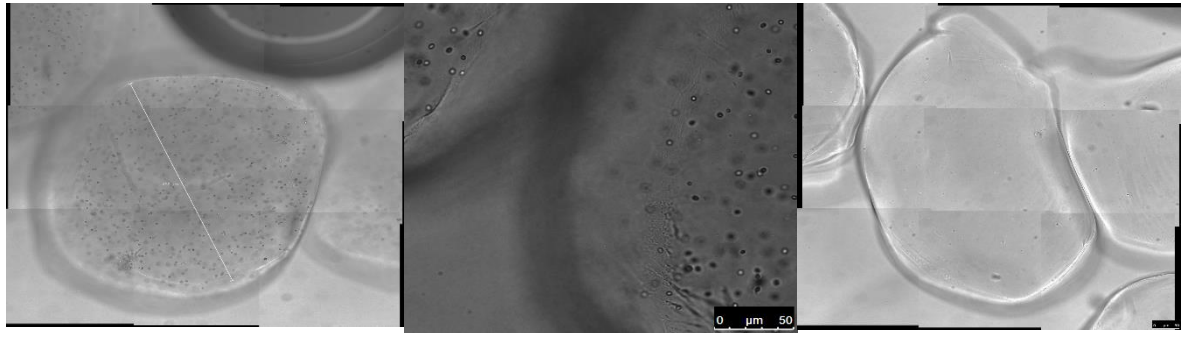


Microphotographs of microcapsules (wet and dry) ((ALG/(Cu+TV)/CS) obtained by optical microscope. The size of prepared wet microcapules were 2 mm and dry one 0,45 mm



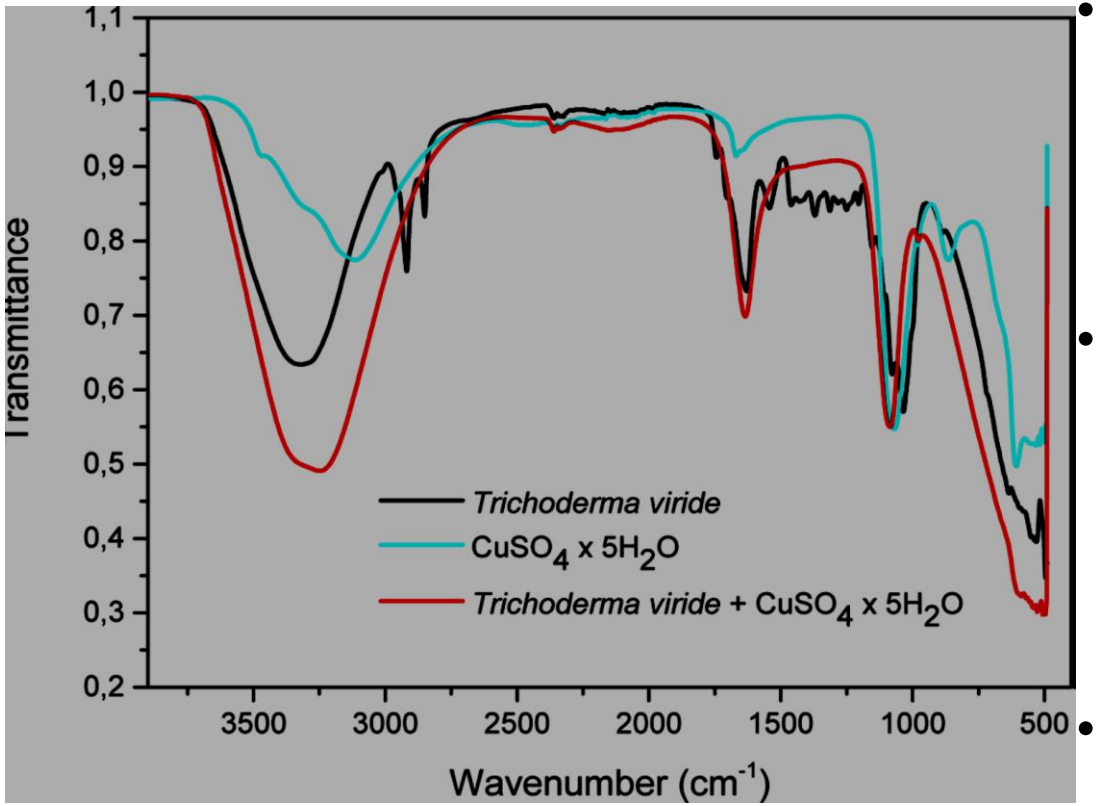
Microphotographs of prepared microcapsules (dry) ((ALG/(Cu+TV)/CS) obtained by SEM





Microphotographs of prepared microcapsules (wet) ((ALG/(Cu+TV)/CS) obtained by confocal microscope

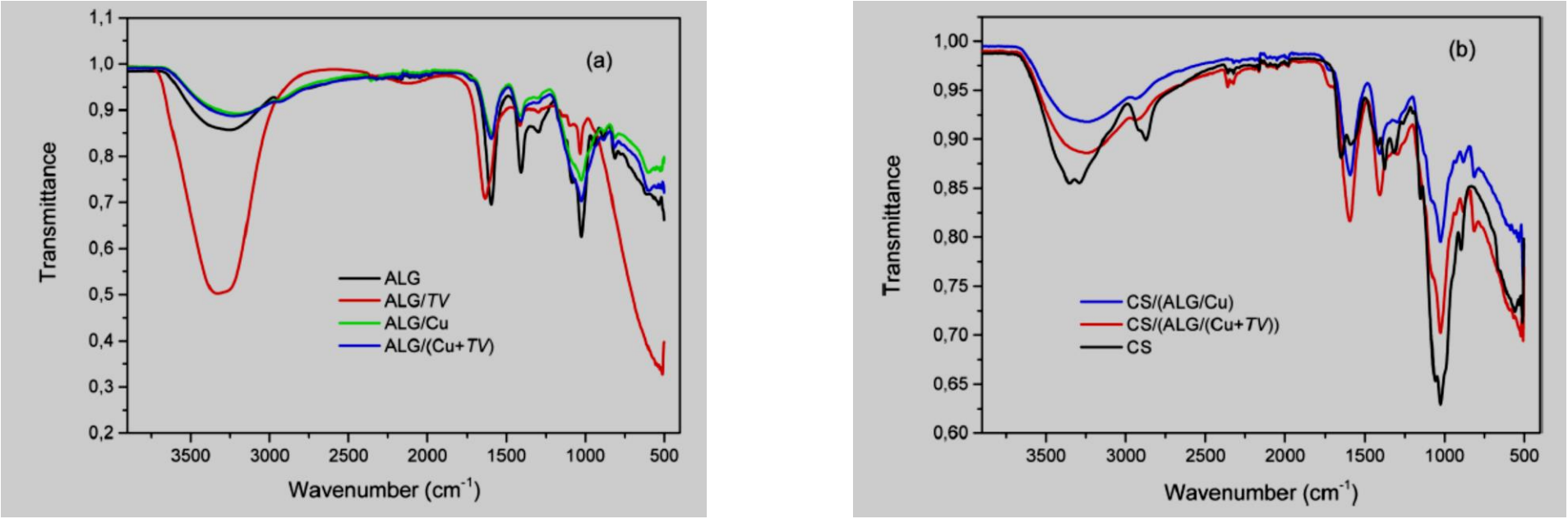
Interactions between chemical and biological agents in mixture



FTIR spectra of *T. viride* spores (**black line**), copper sulfate pentahydrate (**cyan line**), and their mixture (**red line**)

- The spectrum of *T. viride* with bound copper cations shows much more intense and broad −OH and −NH stretching vibration bands, the disappearance of *T. viride* bands at 2921, 2854, and 1545 cm^{-1}, and the absence of small peaks between 1452 and 1200 cm^{-1},
- The disappearance of bands and shifting of peaks toward the lower frequency (from 3321 to 3274 cm^{-1}) or toward higher frequency (from 1625 to 1635 cm^{-1}, from 1072 to 1087 cm^{-1}, and from 887 to 981 cm^{-1}) have suggested that at least amine, hydroxyl, carbonyl, and amide bonds are the major sites for binding of copper cation,
- Observation conducted by electron microscopy and cell fractionation studies (Anand et al., *Bioresource Technology* **2006**, *97*, 1018–1025) revealed copper cation location on the cell wall of *T. viride* spores, indicating this is the place where the interaction between *T. viride* and copper cations occurred.

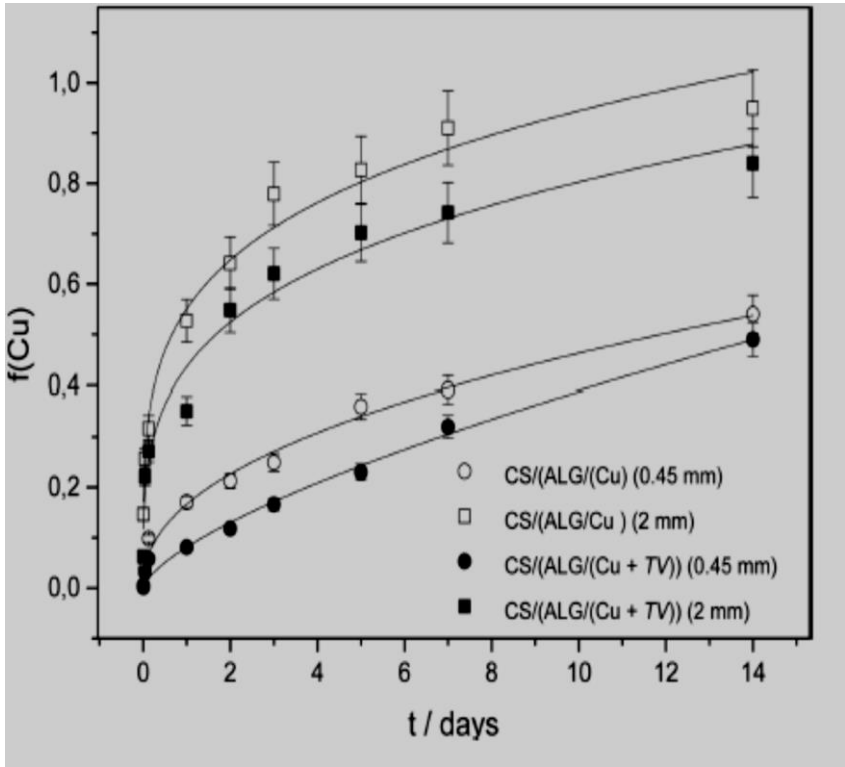
Interactions between chemical and biological agents in prepared microcapsules



FTIR spectra of (a) sodium alginate (ALG, black line), alginate and *T. viride* (ALG/TV, **red line**), core microcapsule (ALG/Cu, **green line**), and core microcapsule with copper cations and *T. viride* (ALG/(Cu+TV), **blue line**) and (b) chitosan (CS, **black line**), core microcapsule with copper cations (CS/ALG/Cu, **blue line**), and core microcapsule with copper cations and *T. viride* coated with chitosan (CS/ALG/(Cu+TV), red line).

- **elecrostatic interactions between chemical and biological agents, and oppositely charged biopolymers**
- **hydrogen bonding**

In Vitro Release of Active Agents



Fraction of released copper cations, f(Cu), from CS/(ALG/Cu) (open symbols) and CS/((ALG/(Cu+TV)) (solid symbols) microcapsules at initial copper cation concentration c(Cu)i = 18 mmoldm^{-3} with time (t)

The in vitro copper cation release profile was fitted to the **Korsmeyer–Peppas empirical model**. **Fickian diffusion** was found to be the **rate-controlling mechanism at smaller microcapsules**, whereas anomalous transport kinetics (a combination of the diffusion mechanisms and type II transport) **controlled release from larger microcapsules**. The copper cation release exhibited an **initial burst followed by a slower release**. It can be clearly seen that the amount of copper cations released depends on microcapsule size and loaded active agents.

All curves of ***in vitro*** **release** of copper ions can be described by the equation:

$$f(\mathrm{Cu}) = \frac{R_t}{R_{\mathrm{total}}} = kt^n$$

f(Cu) represents the fraction of released copper cations,

R_t is the amount of copper cations released at time t,

R_{total} is the total amount of Cu loaded in capsules,

k is a constant characteristic of the active agents/polymer system that considers structural and geometrical aspects of the system, and

the exponent (n) characterizes the transport mechanism of active agents through the microcapsule.

Values of the Release Constant (k) and Exponent (n) of Copper Cations Encapsulated in CS/(ALG/Cu) and CS/(ALG/(Cu+TV)) Microcapsules

microcapsule	size (mm)	k (day^{-1})	n
CS/(ALG/Cu)	0.45	0.167	0.45
CS/(ALG/Cu)	2.0	0.551	0.23
CS/(ALG/(Cu+*TV*))	0.45	0.081	0.68
CS/(ALG/(Cu+*TV*))	2.0	0.436	0.27

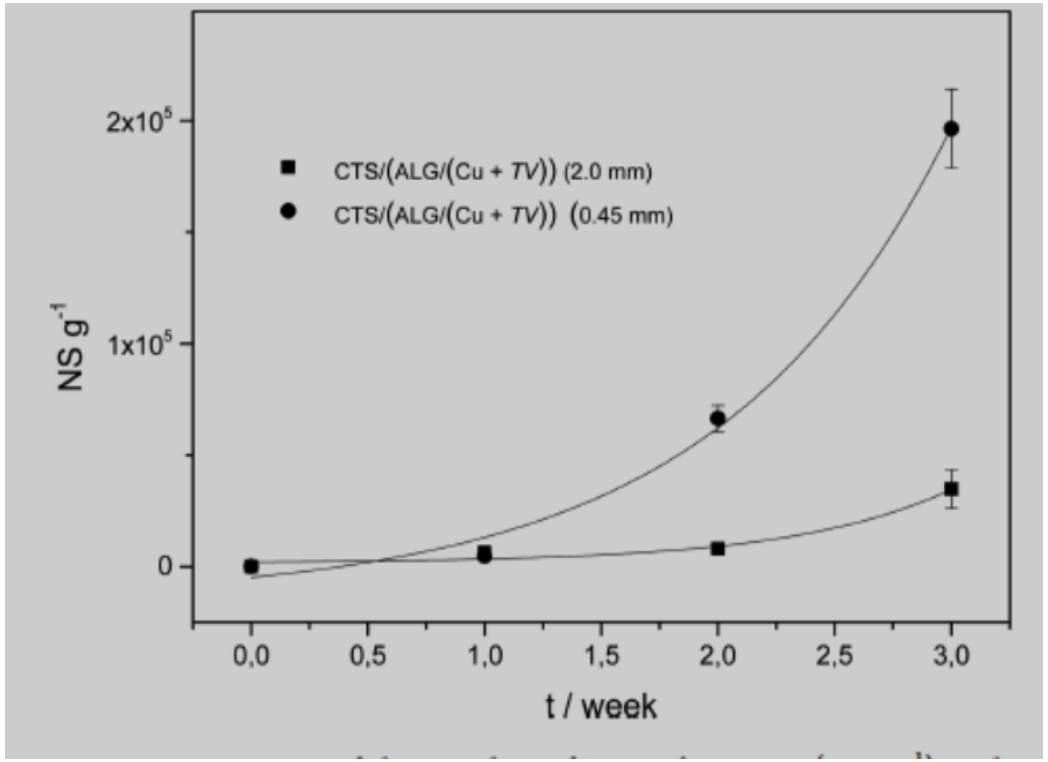
Large microcapsules - n<0.43

- the release mechanism of copper cation involved is controlled by a **classical Fickian diffusion**,

Smaller microcapsules – n>0.43

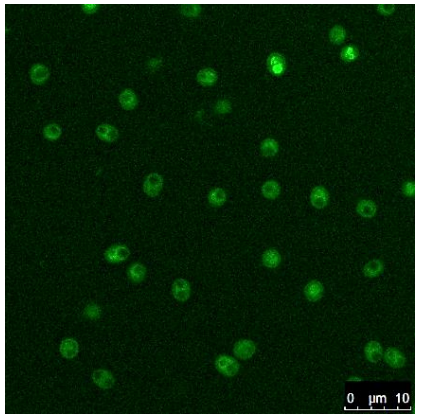
- copper cation release followed **non-Fickian kinetics**, due to rapid swelling and partial dissolution of microcapsules.

In Vitro Release of Active Agents



Variation of the number of *T. viride* spores (NS g^{-1}) with time (t). Microcapsule diameters are denoted in parentheses. The error bars indicate the standard deviation of the means

- ***T. viride*** spores release profile showed an exponential increase over initial lag time.
- A much slower release of ***T. viride*** spores at the early stage may be ascribed to their **larger size** in comparison with copper cations and intermolecular interactions with alginate and copper cation.



Confocal laser scanning image of *T. viride* spores in fluorescence mode (stained with Rhodamine 123)

Conclusions

- The results revealed chitosan/alginate microcapsules can simultaneously incorporate ***T. viride*** spores and **copper cations** without inhibiting their activities and even promoted ***T. viride*** germination

- Investigation of **intermolecular interactions** between oppositely charged biopolymers and bioactive agents using FTIR spectroscopy revealed interaction between **copper cations** and ***T. viride*** spore functional groups as well between alginate and bioactive agents

- The *in vitro* **copper cation** release profile was fitted to the Korsmeyer−Peppas empirical model. **Fickian diffusion** was found to be the rate-controlling mechanism at smaller microcapsules, whereas **anomalous transport kinetics** (a combination of the diffusion mechanisms and type II transport) controlled release from larger microcapsules

- The ***T. viride*** spores release profile showed an exponential increase over initial lag time. A much slower release of ***T. viride*** spores at the early stage may be ascribed to their larger size in comparison with copper cations and intermolecular interactions with alginate and copper cations

- After initial fast release or lag time, **encapsulated agents** could be delivered to the plant for a prolonged time by choosing the appropriate concentration of cross-linking cation and microcapsule size.

- Results obtained opened up perspectives for the future use of chitosan/alginate microcapsules simultaneously loaded with biological and chemical agents in the plant nutrition and protection.

Thank you☺