Supporting Information for:

Encapsulation of Biological and Chemical Agents for Plant Nutrition and Protection: Chitosan/Alginate Microcapsules Loaded with Copper Cations and *Trichoderma viride*

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S.2.3.1. Preparation of CS/(ALG/Cu) microcapsules viscous dispersion (Procedure I). Copper cations loaded CS/(ALG/Cu) microcapsules viscous dispersions (abbreviation CCVD) were prepared by modifying known procedure[1] as described below.

Sodium alginate was solubilized in water at 0.2% concentration. Copper cations solution (1, 1.5 and 2 %) was dripped in sodium alginate solution and stirred for 2 hours at 25 °C. Chitosan was solubilized in 0.5% acetic acid solution at 0.032% concentration and injected dropwise by funnel (diameter of 2 mm) into ALG/Cu dispersion by continuous stirring (magnetic stirrer) for one hour at 25 °C. The weight ratio between the ALG/Cu dispersion and CS solution was 1:1. The viscous dispersion was stored at 4 °C until spraying on fungal isolate STP.

S2.3.2.1. Preparation of copper cations *loaded alginate/chitosan microcapsules (CS/(ALG/Cu)) (Procedure II).* The CS/(ALG/Cu) microcapsules preparation was carried out by the ionic gelation technique in two stages.

The first stage: The core microcapsules (ALG/Cu) were made by dropping 50 ml of sodium alginate (1.5 % (w/v) solution using funnel with the diameter of 2 mm (Procedure - PIIa) or needle with the diameter of 0.45 mm (Procedure - PIIb), respectively) into 50 ml of copper sulfate pentahydrate solution (1, 1.5 and 2%). The contact time between components was performed for about 30 minutes to give core microcapsules time to form. Core microcapsules were formed in the cross-linking solution under mechanical stirring, then washed several times with sterilized water and filtered through Büchner funnel. Afterward the solution was filtered and the core microcapsules were washed three times with water.

The second stage: Washed ALG/Cu core microcapsules were dispersed in 50 ml chitosan solution (0.5% CS in 1.0% CH_3COOH) under constant stirring (magnetic stirrer). The contact time between core microcapsules and chitosan solution was about 30 minutes to give chitosan time to form a layer around the core microcapsule. Microcapsules were filtered, washed with

deionized water and phosphate saline buffer, and stored in deionized water at 4 °C until further studies. A number of the microcapsules were allowed to air-dry at room temperature to reach their equilibrium moisture content.

S2.3.2.2. Preparation of copper cations and Trichoderma viride spores loaded chitosan/alginate microcapsules (CS/(ALG/(Cu+TV)) (Procedure III). Two differently sized microcapsules simultaneously loaded with copper cations and *T. viride* (CS/(ALG/(Cu+*TV*)) were prepared by basically the same procedure as that for CS/((ALG/Cu) microcapsules. Two procedures differ only in the first step. 10 ml of *T. viride* stock solution (number of spores adjusted to 1.4 x 10^6 /ml) was dispersed in 40 ml of sodium alginate (1.5% (w/v) and stirred (magnetic stirrer). The mixture was dropped into 50 ml of copper sulfate pentahydrate solution (1, 1.5 and 2%) using a funnel (with a diameter of 2 mm; Procedure - PIIIa) or a needle (diameter of 0.45 mm; Procedure - PIIIb)), respectively. Afterward the procedure follows steps as described in Procedure II.

S2.5. Characterization of delivery systems. *2.5.3. Encapsulation efficiency, loading capacity, swelling degree and in vitro active agents releasing.*

S2.5.3.1. Encapsulation efficiency. The encapsulation efficiency (EE) is defined as the ratio between the content of loaded copper cations in the microcapsules (c_{load}) versus the initial amount of copper cations (c_{tot}).[2] Encapsulation efficiency (expressed as the percentage of total available copper cations that was actually incorporated into microcapsules) was calculated by the equation:

$$EE = (\frac{c_{load}}{c_{tot}}) \times 100 \qquad \text{(S1)}.$$

The measurements were replicated three times.

Spores of *T. viride* were not detected in filtrate after core microcapsule separation indicating the encapsulation efficiency was almost 100%. The measurements were replicated three times.

S2.5.3.2. Loading capacity. Loading capacity (LC) is defined as the content of loaded copper cations per gram of dry microcapsules. Wet microcapsules loaded with bioactive agent/agents were air-dried at room temperature for several days until all the liquid evaporated. The copper cations content was determined by dissolving of 10 mg of dry microcapsules in 5 ml of a mixture of 0.2 M $NaHCO_3$ and 0.06 M $Na_3C_6H_5O_7$ x $2H_2O$ at pH 8.[3] The resulting solution was filtered and the concentration of copper ions in the filtrate determined by UV-VIS spectrophotometer at 795 nm (Shimadzu, UV-1700, Japan). Loading capacity (LC) expressed as a copper ions mmol per 1 g of dry microcapsules was calculated by the equation:

$$LC = (c_{Cu} \times V/w_c) \qquad \text{(S2)},$$

where c_{Cu} is a concentration of copper ions in sample, V is a volume of the sample and w_c is a weight of microcapsules. The measurements were replicated three times.

T. viride content was determined by dissolving of 4 g of dry microcapsules (0.45 and 2 mm) in 100 ml of a mixture of 0.2 M $NaHCO_3$ and 0.06 M $Na_3C_6H_5O_7$ x $2H_2O$. The resulting solution was filtered and the concentration of *T. viride* (the number of spores) in the filtrate determined by UV-VIS spectrophotometer at 550 nm (Shimadzu, UV-1700, Japan). Loading capacity (LC) expressed as the number of spores (NS) per 1 g of dry microcapsules was calculated by the equation:

$$(LC)_{TV} = (c_{NS} \times V/w_c) \qquad \text{(S3)},$$

where c_{NS} is concentration of spores in sample, V is a volume of the sample and w_c is a weight of microcapsules. The measurements were replicated three times.

S2.5.3.3. Swelling measurements. The swelling of CS/ALG microcapsules depends on several factors, such as properties of dissolution medium[4] the properties of chitosan, including the molecular weight and concentration as well as the membrane formation time and technology of preparation.[5] In order to avoid the influence of electrolytes from buffer solutions, the swelling degree (S_w) was determined on microcapsules dispersed in deionized water.

Microcapsules (10 mg) were dispersed in a glass vial containing 10 ml of deionized water and allowed to swell at room temperature during three hours. The wet weight of the swollen microcapsules was determined by blotting them with filter paper to remove moisture adhering to the surface, immediately followed by weighing.[6] The swelling degree (S_w) was then calculated using equation:

$$S_w\% = \frac{w_t - w_0}{w_0} \qquad \text{(S4)},$$

where w_t is the weight of the swollen microcapsules, and w_0 is their initial weight. The measurements were replicated three times.

S2.5.3.4. In vitro active agents releasing. The *in vitro* release studies of copper cations from the CS/(ALG/Cu) and (CS/(ALG/(Cu+*TV*)) were performed by dispersing microcapsules in deionized water and left to stand without stirring at room temperature. Samples were prepared by dispersing microcapsules (4 g) in 50 ml of deionized water. The release of copper cations produced characteristic blue coloring of dispersion. At appropriate time intervals, dispersion was stirred for 60 sec, aliquots were withdrawn and copper ions concentration was determined spectrophotometrically at 795 nm.

The release studies of *Trichoderma viride* spores from the (CS/(ALG/(Cu + *TV*)) microcapsules were carried out at room temperature. Microcapsules (4 g) loaded with copper cations and *Trichoderma viride* spores were dispersed in 100 ml of deionized water and allowed to stand without stirring. At appropriate intervals dispersion was stirred for 60 sec, aliquots were withdrawn and the spore concentration was determined spectrophotometrically at 550 nm by the method of Waghunde *et al.*[7] The measurements were replicated three times.

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