**SUPPORTING INFORMATIONS**

**The Enhancement of bioactive potential in *Vitis vinifera* leaves by application of microspheres loaded with biological and chemical agents**

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**Materials and methods**

***Preparation of Trichoderma viride isolates and spore suspension***

The culture of the isolate was grown in Petri dishes of 10 cm in diameter containing 20 ml of PDA and incubated in a humid chamber at 25 °C for 7 days until conidiation occurred. To obtain spore suspensions, the STP was grown in Erlenmeyer flasks containing potato dextrose broth (PDB). Flasks were inoculated with 5 PDA mycelial plugs. Incubation took place at 22 ºC under the constant aeration for 10 days under illumination. After this incubation period the liquid cultures with fungal biomass, consisting of hyphal segments, chlamydospores, and conidia, were filtrated by suction through filter paper (595 Schleicher & Schuell; Whatman International, Ltd., Kent, England) so that the major parts of mycelium was removed. The filtrates were stored in the freezer until microencapsulation took place. Nebulisation of microencapsulated spores was checked *in vitro* using an ultrasonic nebulizer (Omron Healthcare Europe, Netherlands).

***Microsphere preparation***

Microspheres loaded with calcium and magnesium cations, and *T. viride* were prepared by the ionic gelation technique. The microspheres were made by dripping 100 mL of sodium alginate (2.5% w/v) solution using BUCHI M B-390 encapsulator (with the diameter of the nozzle 1 mm) into (i) 100 mL of calcium chloride solution (1 mol dm-3 CaCl2) or (ii) mixture of calcium/magnesium chloride solution (1 mol dm-3 CaCl2|1.5 mol dm-3 MgCl2), or by dripping 100 mL mixture of sodium alginate (2.5% w/v) and *T. viride* into (iii) calcium chloride solution (1 mol dm-3 CaCl2), or (iv) mixture of calcium/magnesium chloride solutions (1 mol dm-3 CaCl2|1.5 mol dm-3 MgCl2). A mixture of sodium alginate solution and *T. viride* spores was made as further explained. *T. viride* spores were taken from the liquid growth media, poured into the 2.5% sodium alginate solution and homogenized. The number of spores was adjusted to 1.4·106/ ml. Then, the solution was filtered through the sterilized muslin cloth to filter out spores.Microspheres were formed in a cross-linking solution under mechanical stirring. The contact time between components was performed for about 30 minutes to give microspheres time to form and harden. Afterward, capsules were washed several times with sterilized water.

***Determination of calcium and magnesium in vine leaves***

Grapevine leaf samples were taken at the end of the growing period. Average samples were formed from healthy leaves, taken opposite to clusters from vine plants. Leaves were digested with concentrated HNO3 (Milestone 1200 Mega Microwave Digester). Calcium and magnesium were determined by the flame atomic absorption spectroscopy (Perkin Elmer) according to the atomic adsorption technique (AOAC 1995).

**Results and discussion**

***Vineyard site and plant material***

**Table S1.** Growing season average temperature, insolation and precipitation of observed vintage 2016

|  |  |
| --- | --- |
| Average temperature (°C) | 18.0 |
| Insolation (h) | 1523.1 |
| Precipitation (mm) | 518.9 |

**Table S2.** The average values of Ca (%) and Mg (%) in leaves (dry matter)

|  |  |  |
| --- | --- | --- |
| Microsphere | Ca  | Mg |
| ALG/Ca | 4.86 | 0.52 |
| ALG/(Ca+*Tv*) | 4.81 | 0.49 |
| ALG/(Ca+Mg+*Tv*) | 4.96 | 0.59 |
| ALG/(Ca+Mg) | 4.87 | 0.54 |
| Control | 4.75 | 0.44 |

**References**

AOAC (1995). Official method of Analysis of AOAC International, 16th Edition, Vol. I, Arlington, USA.