Thyme-based nano-biocides exploring Calcium Carbonate and Cellulose Nanocrystals: the case studies of Xylella fastidiosa and Pseudomonas savastanoi

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Quarantine pathogens require the investigation of new tools for effective plant protection. Plant extracts and essential oils are natural sources of efficient biocides, such as phenols. These bioactives have some critical issues, such as hight volatility and poor solubility in water. Nanoencapsulation allows to overcome these limits, enhancing stability and bioactivity.

In the present studies, we have developed thyme-based nano-formulations investigating the encapsulation of thymol molecule and thyme essential oil. We have studied two types of nanomaterials: calcium carbonate nanoparticles and cellulose nanocrystals.

In particular, we obtained thymol nanoparticles through adsorption on CaCO3 nanocrystals, exploiting their carrier action. The in vitro test revealed an interesting synergistic action of thymol and nanocarriers, suggesting the potential application of thymol-nanoparticles as effective biocides to control Xylella fastidiosa infection.

Furthermore, oil in water nanoemulsions of thyme essential oil were formulated by high-speed homogenization using tween-80 as surfactant, calcium chloride as crosslinker and cellulose nanocrystals as stabilizers. Thyme essential oil was kindly provided by the society Licofarma s.r.l. Microscopic analysis detected a nanometric dispersed phase into very small droplets (radium < 20 nm). The nanoemulsion resulted very stable, as confirmed by Dynamic Light Scattering and Turbiscan analysis.

Nanoemulsion and free essential oil were evaluated on Pseudomonas savastanoi pv. Savastanoi. Firstly, the nanoformulation was in vitro tested by microdilution and a disk diffusion assay: the first test revealed the capability of fully inhibiting the bacterial growth after 48 h (at 0.5%), while the second one showed an inhibition halo of 8-10 mm at the concentrations of 0.5 and 1% respectively. Inoculated olive seedlings were treated with nanoemulsion at 1%. After 7- and 14- days post treatment the level of recovered epiphytic colonies was reduced, showing similar values to the copper treatment.

The current results highlight the possibility of exploiting thyme-derived bioactives as effective plants protection tools enhancing their activity through nanoencapsulation procedures.