T04-P2

CHITOSAN-TRIPOLYPHOSPHATE NANOPARTICLES AS OLEA EUROPAEA EXTRACTS CARRIER: SYNTHESIS, CHARACTERIZATION, AND THEIR ANTIFUNGAL

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In this work, we investigated the *in vitro* antifungal activity of two leaf extracts obtained from *Olea europaea* plants and commercial oleuropein, both encapsulated in chitosan-tripolyphosphate nanoparticles and in solution, against the germination and development of *Fusarium proliferatum*. High performance liquid chromatography (HPLC) testing is performed to evaluate the relative concentration of bioactive molecules in the *Olea europaea* leaf extract. The antifungal effect was compared against *Fusarium proliferatum* strains by *in vitro* assays, looking at a number of different concentrations and preparations of phenolic compounds, both as such and encapsulated in chitosan nanoparticles. The aim was to individuate the most appropriate formulation between two leaf extracts and phenols-encapsulated chitosan nanoparticles to develop a safe, stable and efficient drug delivery system.

The nanoformulation of chitosan requires less fungicide and hence environmental friendly method for the control of phytopathogenic fungi. The phenols/nanoparticles showed greater efficacy at higher concentration when compared to extracts pure or commercial oleuropein against target species. Using such these biofungicides in a replacement for synthetics or in combination with other established disease management practices could help control *Fusarium proliferatum* in a more sustainable and eco-friendly way.

T04-P3

EVALUATION OF EFFECTS OF SPILOCAEA OLEAGINA DISEASE AND FUNGICIDE TREATMENTS ON OLIVE VEGETATIVE-REPRODUCTIVE ACTIVITIES

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Peacock eye is an olive disease which causes defoliations, but little information is available on the quantification of the effects of the disease on yield. Copper compounds represent the most used fungicides to control peacock eye. From some years, also dodine is used. The aims of this study were to evaluate the effects of peacock eye disease on the vegetative and reproductive activities of the trees and the efficiency of copper compounds or dodine in controlling the disease. The trial was carried out in central Italy in an olive orchard constituted of adult trees of the cultivar Moraiolo, spaced 5 x 5 m. Not treated trees were used as control. At the beginning of the trial about 15% of the leaves were symptomatic and about 50% of the asymptomatic ones were infected (NaOH test), for a total of 65% of infected leaves. The percentages of symptomatic leaves and of fallen ones remained constant up to the beginning of March. Then the percentage of symptomatic leaves remained almost constant on trees treated with dodine and increased up to 40-50% on control and trees treated with copper. The percentage of fallen leaves increased in all trees, but control and trees treated with copper showed a much higher defoliation. Trees treated with dodine had the highest number of inflorescences per node which were also the heaviest. Fuit set was similar in all treatments. Trees treated with dodine showed the highest yield. The olive oil content was similar in all treatments.

T04-P4

SCREENING OF BACTERIAL ISOLATES RELATED TO OLIVE ORCHARD INSECT PESTS IN TUNISIA AND STUDY OF THEIR BIOTECHNOLOGICAL USE

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This study investigated the bacterial diversity of dead olive insect pests collected from Tunisian olive orchards. In this context, we aimed to explore metabolic diversity, screen enzymatic activities for biotechnological applications and carry out preliminary bioassays for bio-insecticide development.

A total of 50 bacterial strains were randomly isolated from 4 different biotopes situated in Sfax region (Tunisia). 16S ribosomal RNA gene sequences of these isolates showed close similarities between twenty-four bacterial species within the genera *Alcaligenes*, *Bacillus*, *Brevundimonas*, *Lysinibacillus, Myroides, Paenalcaligenes, Proteus, Providencia, Serratia,* and *Staphylococcus*. The genus *Bacillus* was significantly the most prevalent in the 4 biotopes with $p<0.05$. Specific enzyme screening was assayed on agar plates for amylolytic, proteolytic and lipolytic activity. These latter were specifically secreted by *Bacillus* comparing to the other genera with significant differences. Our bacterial collection was also evaluated for antimicrobial potential against bacterial and fungal isolates. *Bacillus subtilis* B8-2 showed strong antibacterial activities and *Bacillus licheniformis* B2-3 as well as *Serratia marcescens* B10-1 showed the highest antifungal activity. The study of isolates' insecticidal effects on second instar larvae of the factitious host *Ephestia kuehniella* (Lepidoptera: *Pyralaidae*) showed that 12.5% among them caused a percentage of mortality superior to 70% such as *Providencia vermicola* B20-1 which causes 100% of mortality.

In conclusion, the selected species from olive orchards represent an antibacterial and antifungal broad-spectrum and could be considered promising sources with potential enzymatic activities and insecticidal effect, which could be used for future applications of industrial interest for biological control.

T04-P5

OLIVE MILD MOSAIC VIRUS CP AND P6 ARE SUPPRESSORS OF RNA SILENCING AND THEIR SILENCING CONFERS RESISTANCE AGAINST OMMV

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RNA silencing is an important defense mechanism in plants, yet several plant viruses encode proteins that suppress this mechanism. Here the genome of Olive mild mosaic virus (OMMV) was screened for silencing suppressors in the herbaceous host *Nicotiana benthamiana 16C* using a green fluorescent based transient suppression assay. The full OMMV cDNA and 5 different OMMV open reading frames (ORFs) were cloned into the Gateway binary destination vector pK7WG2, transformed into *Agrobacterium tumefaciens* C58C1 and agroinfiltrated into *N. benthamiana* 16C. Among all ORFs tested, CP and p6 showed suppressor activity, with CP showing a significant higher activity when compared to p6, yet lower than that of the full OMMV. This suggests that OMMV silencing suppression results from a complementary action of both CP and p6.

This is the first time that a silencing suppressor was found in a necrovirus and that two independent proteins act as silencing suppressors in a member of Tombusviridae family.

Such discovery led to the use of those viral suppressors in the development of OMMV resistant plants through pathogen-derived resistance (PDR) based on RNA silencing. Two hairpin constructs targeting each suppressor were agroinfiltrated in *N. benthamiana* plants which were then inoculated with OMMV RNA. When plants expressed both constructs, a highly significant reduction in viral accumulation and symptom attenuation was observed as compared to those of controls. These results indicate that the use of both OMMV viral suppressors as transgenes is a very efficient and promising approach to obtain olive and other hosts resistant to OMMV.

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