«VALorization of Mediterranean small-scale FARMs by cropping wild UnExploited species»

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Deliverable 3.6.1

Evaluation of novel biofertilizers and soil amelioration properties of WEPs

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Report on the progress of Work Package 3

This work package has started on M13. Specific tasks related to this WP include:

Task 3.1 Evaluation of PGPRs, PGPFs and AMFs as novel cultural practices for WEPs;

Task 3.2 Soil improving properties of WEPs;

Task 3.3 The effect of root types on soil weathering;

Task 3.4 The effect of root types of WEPs on functional and structural soil microbial diversity; and

Task 3.5 Evaluation of non-microbial biostimulants for WEPs cultivation.

The activities of the involved partners are described below:

University of Thessaly (UTH) is performing experiments related to Tasks 3.1-3.5.

- Field experiments regarding the use of manure in cultivation of *Cichorium spinosum, Crithmum maritimum, Portulaca oleracea*, *Sonchus oleraceus* within the context of incorporating the selected species in organic farming systems (Task 3.2-3.4).
- Pot experiments regarding the use of manure in cultivation of *Portulaca oleracea* and *Sonchus oleraceus* within the context of incorporating the selected species in organic farming systems (Task 3.2-3.4).
- Pot experiments with *Sonchus oleraceus* in soils contaminated with Cd and treated with biostimulants containing humic and fulvic acids aiming to evaluate the phytoremediation properties of the WEP and the stress alleviation effects of biostimulants (Tasks 3.3 and 3.5).
- Field and pot experiments to evaluate the effect of non-microbial biostimulants and biofertilizers on WEPs cultivation (Task 3.5).

The tested biostimulants include formulations provided by CSIC, as described in the following Table 1.

Table 1. Biostimulants used in field and pot experiments.

Code	**Biostimulant**
IMb1 (2*10^9 ufc/g)	*Entrosphora infrequens*
	Funneliformis geosporum
	Glomus fasciculatum
IMb2 (5*10^9 ufc/g)	*Bacillus megaterium*

	Bacillus altitudinis
	Bacillus subtilis
	Bacillus licheniformis
	Bacillus methylotrophicus
IMb3 (5000 spores/g)	*Rhizophagus irregularis*
	Glomus mosseae
	Glomus etunicatum
IMb4	*Trichoderma halzianum T78*

Moreover, the following commercially available biostimuant products were used in the experiments:

- CMB1: **Pentacil** (*Bacillus amyloliquefaciens, B.licheniformis, B. pumilus, B. simplex* και *B. Subtilis* 3×109 CFU/g)
- CMB2: **Bactiva** (*Trichoderma harzianum, T. reesei, T. viride, Gliocladium virens*, 10^8 CFU/g *Bacillus subtilis, B. polymyxa, B. megaterium, Pseudomonas fluorescens* 10^8 CFU/g)
- CMB3: **Phosbactin** (*Bacillus megaterium* vP, *Pseudomonas putida, P. fluorescence* 1 x10^{12} cfu*/lt; humic acids, aminoacids, sugars; natural phytoregulators)
- CMB4: **Azospir** (*Azospirillum, Azotobacter* 2 x10^{12} cfu*/lt; humic acids, aminoacids, sugars; natural phytoregulators)
- CMB5: **Micoseeds** (*Glomus* spp., *Trichoderma* sp., *Bacillus* spp. *Streptomyces* sp., *Pseudomonas* sp.; Microstym 100 spores/gr, Microtech TX 10^7 CFU/gr, PGPR 10^7 CFU/gr).

University of Wuppertal (BUW) is performing experiments related to Tasks 3.2, 3.3, and 3.4.

- Greenhouse pot experiments to examine the effect of soil type (artificial soil, arable soils forest soil, garden soil, and grassland soil), their properties and nutrients content on the growth and biomass of *Crithmum maritimum*, *Portulaca oleracea*, *Sonchus oleraceus* and the content of nutrients in the roots, stem, shoots, and leaves (Task 3.2).

- Field experiment to compare the growth of German and Greek wild *Portulaca* in the German arable soils (Task 3.2).
- Greenhouse pot experiments to examine the effect of *Crithmum maritimum*, *Portulaca oleracea*, *Sonchus oleraceus* cultivation on the soil microbial community and activity in different soils, as affected by soil type, properties and nutrients content in the rhizosphere soil of each plant (Task 3.4).
- Greenhouse pot experiments to examine the ability of *Crithmum maritimum*, *Portulaca oleracea*, *Sonchus oleraceus*, *Cichorium spinosum* to grow in two chemically degraded (toxic metals contaminated) soils and their potential to accumulate and translocate toxic metals (e.g., Ag, Al, As, Cd, Co, Cr, Cu, Fe, Mn, Hg, Ni, Pb, Sb, Se, Sn, V, and Zn) from soils (Task 3.3).

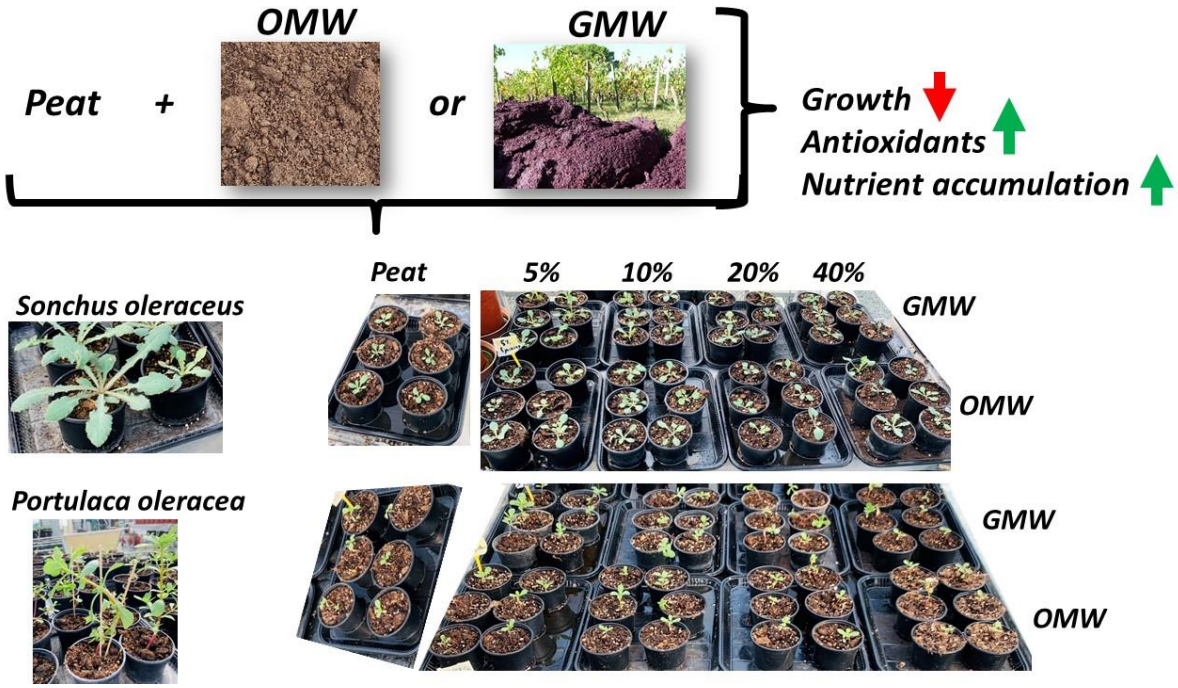
Cyprus University of Technology (CUT) is performing experiments related to Tasks 3.1 and 3.5.

- **Task 3.1:** CUT is going to contribute to mineral analysis for several experimental studies related to biostimulants, performed by UTH. CUT has recently received the samples from Greece, and is going to start the extractions of minerals, before analysis. The work is in progress.

- **Task 3.5:** CUT had evaluated the use of plant residues/wastes for the partial substitute of peat in growing media for both *Sonchus oleraceus* and *Portulaca oleracea.* It was examined the biostimulant and/or fertilizer role of Olive-mill waste (OMW), Grape-mill waste (GMW), *Origanum dubium* hydrodistilled waste (ODW), *Sideritis cypria* hydrodistilled waste (SCW) as a peat substitute at 0-5-10-20-40% rates.

Experiment 1

Olive-mill waste (OMW) and grape-mill waste (GMW) as a substitute growing media component for unexplored vegetables production (*Sonchus oleraceus* and *Portulaca oleracea.*)



The partial peat substitution with OMW or GMW for the production of purslane and sowthistle seedlings was examined. The OMW and GMW affected the physicochemical properties of the growing media, as the bulk density of the mixtures and available minerals to the plants were increased, while the available pore space was decreased. Purslane's growth parameters were affected by GMW and OMW, with the effects being more pronounced when plants grew at the high ratio of 40% and of ≥ 20%, respectively. In sowthistle, the addition of GMW up to 10% stimulated or had no effect on growth features as the plant height, leaf number, fresh weight and chlorophyll content. On the other hand, plants grew at ≥10% OMW revealed decrease, slower plant growth. Leaf stomatal conductance for both purslane and sowthistle, was decreased as the GMW and OMW ratio were increased into the growing media. Even though OMW and GMW decreased plant physiological attributes and plant growth, when they were applied in high ratios in the growing media, they both affected plant mineral accumulation. Moreover, the mixture with OMW ≥20% (and GMW at 40%) boosted the antioxidant content and the total phenolics in both species. The increased stress condition caused by the addition of the tested materials in the mixture was reflected by the cellular damage of the plant tissue, as it was measured by the increased lipid peroxidation and the production of hydrogen peroxide.

Concluding, the present study denotes that both GMW and OMW can be used in the growing media at low ratios of 10% and 5%, respectively, in order to produce the examined plants successfully, providing an appropriate balance among plant growth, production, nutritional value (minerals and antioxidants) and plant health, while reducing participation of peat in potting mixtures.

Experiment 2

Use of Medicinal and Aromatic Plant Residues for Partial Peat Substitution in Growing Media for *Sonchus oleraceus* Production

The present study evaluated the potential use of *Origanum dubium* Boiss. residues (ODR) and *Sideritis cypria* Post. residues (SCR) derived via distillation at different levels (0–5–10–20–40% *v*/*v*) for use in partial peat substitution in the production of *Sonchus oleraceus* L. (sowthistle) plants. Both ODR and SCR accelerated the pH, electrical conductivity, organic matter content, and mineral content of the growing media, but also negatively affected several of the physical characteristics of the media, such as the total porosity and aeration. This resulted in decreased plant growth, which was more noticeable at the high residue ratios. Plants responded to this by decreasing the leaf stomatal conductance, decreasing the chlorophyll content at 40% ODR and 20% SCR mixtures, and activating several non-enzymatic (phenols, flavonoids, and antioxidant capacity) and enzymatic (superoxide dismutase) mechanisms to challenge the observed stress conditions, as indicated by lipid peroxidation and the hydrogen peroxide increase. Plants grown in residue media exhibited changes in mineral accumulation, even though both ODR and SCR were rich in minerals. It may be concluded that ODR and SCR, when employed at low levels of 10% and 20%, respectively, have the potential for use in the preparation of growing media as they may increase plant material antioxidants, but further improvement of the growing media's properties is needed to ensure adequate yield.

Experiment 3

Soilless Cultivation of *Portulaca oleracea* Using Medicinal and Aromatic Plant Residues for Partial Peat Replacement

The present study evaluated the potential of using *Origanum dubium* wastes (ODW) and *Sideritis cypria* waste (SCW) obtained after EO distillation for partial peat substitution (0-5-10-20-40% v/v) in *Portulaca oleracea* production. Both ODW and SCW increased pH, electrical conductivity, organic matter, and mineral content, but negatively affected total porosity and aeration of the growing media. Plant growth was inhibited, especially when high

ratios of residues were used, and this was reflected by leaf stomatal conductance and chlorophylls decrease, as well as by the activation of several non-enzymatic (phenols, flavonoids, and antioxidant capacity) and enzymatic (catalase, superoxide dismutase, peroxidase) mechanisms and the increase of lipid peroxidation and hydrogen peroxide, indicating stress conditions. Despite both ODW and SCW were rich in minerals, but plants could not accumulate them. It can be concluded, that both ODW and SCW have the potential to be used in the growing media at low ratios up to 10%, with increased antioxidants content in the final product. Nonetheless, the growing media properties i.e. total pore space and aeration, still need to be improved to result in sufficient yields.

Consejo Superior de Investigaciones Científicas (CSIC) is performing experiments related to Tasks 3.1 and 3.5.

- Greenhouse experiments to study the effects of inoculation with different species of plant growth promoting bacteria's (**PGPRs**), arbuscular mycorrhizal fungi (**AMFs**) and plant growth promoting fungi's (**PGPFs**) on growth, yield, phenolic and flavonoids compounds, soil biological and chemical properties and rhizosphere dynamics of purslane (***Portulaca oleracea*** L.).

  The used treatments are:

  -PGPR species *Bacillus amyloliquefaciens* at concentration 10^5, 10^6, 10^7 ufc/g

  -PGPR species *Bacillus subtilis* at concentration 10^5, 10^6, 10^7 ufc/g

  -Mixture of *Bacillus* species (*B. megaterium, B. altitudinis*)

  -B. subtilis, B. licheniformis, B. methylotrophicus) at concentration 10^5, 10^6, 10^7 ufc/g

  -PGPF species *Trichoderma asperellum* at concentration 10^5, 10^6, 10^7 ufc/g

  -PGPF species *Trichoderma T78* at concentration 10^5, 10^6, 10^7 ufc/g

  -PGPF species *Trichoderma atroviride* at concentration 10^5, 10^6, 10^7 ufc/g

  -AMF species Funneliformis mosseae (5%)

  -AMF species Rhizophagus intraradices (5%)

The determination of parameters is in progress.

- Pot experiment under greenhouse conditions to study the effects of organic fertilization with different treatments on growth, yield, soil biological and chemical properties and rhizosphere microorganisms dynamics of purslane (*Portulaca oleracea* L.).

  The used treatments are:

-Biochar coming from citrus (0.5%, 1%, 2.5%)

-Biochar coming from vineyard wastes (0.5%, 1%, 2.5%)

-Compost from vineyard wastes (0.5%, 1%, 2.5%)

-Compost from beekeeping wastes (solid and liquid; treated with a decomposer fungi and without treated)

There is also a manuscript for the presentation of related scientific results under preparation.

- Field experiment to study the effects of organic compost, mineral fertilisation (300-0-0), inoculation with plant growth promoting bacteria's (PGPRs), arbuscular mycorrhizal fungi (AMFs), plant growth promoting fungi's (PGPFs) and a mixture of AMF+PGPR+PGPF on growth, yield, soil biological and chemical properties and rhizosphere microorganisms dynamics of purslane (*Portulaca oleracea* L.)