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

Project Number: 1436

Project Acronym: Valuefarm

6th Semestrial report

Period covered by the report: from 01/03/2022 to 31/08/2023 (M31-M36)

Periodic report: 2nd

Partners:

- University of Thessaly (UTH), **Greece**
- Instituto Politécnico de Bragança (IPB), **Portugal**
- Cyprus University of Technology (CUT), **Cyprus**
- Dokuz Eylul University (DEU), **Turkey**
- Ege University (EGE), **Turkey**
- Consejo Superior de Investigaciones Científicas (CSIC), **Spain**
- Bergische Wuppertal University (BUW), **Germany**
- Benha University (BU), **Egypt**
- University of Mostaganem (UM), **Algeria**

1 Explanation of the work carried per WP

1.1 Work Package 1

Working package 1 is related to the administration of the project, starting in M1 and ending in M48.

The 3rd technical meeting took place in Braganca, Portugal on April 26th, while a joint event of the PRIMA projects (Valuefarm and Pulping) took place on 2th of April in the same city.

In the meeting all the WP leaders presented the activities related to the corresponding WP, while a discussion followed regarding the progress of the project, the ongoing and future activities and the organization of the next in-person technical meeting. All the partners decided that the next meeting will be held in Cyprus on January-February, 2023, while the final meeting will be organized together with the final conference by CSIC in Spain on April-May, 2023.

For WP1 all the pending deliverables have been submitted to the MEL platform, while the public deliverables have been also uploaded in the project website.

1.2 Work package 2

This working package is related to the evaluation of WEPs under innovative farming systems, starting in M1 and ending in M36, after the acceptance of our request to extend the whole project for 12 months. The specific task related to this semestrial report is **Task 2.3: Mixed cropping and intercropping systems, and short-term crop rotation systems**, as well as to Deliverables D2.3 Selection of the most environmentally sustainable WEP for each country conditions and D2.4 Selection of the most sustainable WEP and legume combinations for each country conditions both due on M36. However, some additional experiments were scheduled in order to address the evaluator's comment for better validating some of the obtained results of Task 2.2: **Agronomical characterization of WEPs under various cultivation conditions.** All the research activities were concluded in August 2023 which is the ending date of WP2

The specific activities of each project partner are described below:

Task 2.2: The partners involved in this Task are UTH, CUT, DEU, EGE, BU and CSIC. The 2nd round of experiments is completed and the results have been integrated in deliverables D2.3 and D2.4.

- **University of Thessaly (UTH)** performed the following experiments:

  (a) Field experiments regarding the use of mulching with plastic films in the cultivation of *Crithmum maritimum* and *Cichorium spinosum.*
  (b) field experiments regarding the evaluation of irrigation requirements of *Cichorium spinosum* and *Crithmum maritimum*

Plant and soil samples from both activities will be collected for chemical analyses that will be performed within the framework of WP4.

- **Consejo Superior de Investigaciones (CSIC)**
  (a) Pot experiments to study the effect of salinity with two levels (0 and 500 mM), and two types of fertilization (Mineral fertilizer and compost) with different doses (0.08, 0.16, 0.24 and 0.32 g of pure nitrogen) on yield of purslane (*Portulaca oleracea*), soil quality and rhizosphere microbial communities.

- **Cyprus University of Technology (CUT)** has performed the following experiments for the evaluation of the agronomic performance of the selected species:
  (a) Greenhouse (hydroponic-NFT) experiment regarding the potassium (K) and phosphorus (P) levels for *Sonchus oleraceus* and *Portulaca oleracea*.
  Experiments are completed. Analysis of samples is in progress.

- **Consejo Superior de Investigaciones (CSIC)** has performed the following experiment:
  (a) A pot experiment where the effect of organic (compost extracts) and inorganic fertilization (different ratios of N-P-K) on the growth of *Sonchus oleraceus* and *Porulaca oleracea* was evaluated.

- **Dokuz Eylul University (DEU)**
  - Pot experiments were repeated to investigate the agronomic and morphological characterization of *Portulaca oleracea* under soil heavy metal stress.
    Three different heavy metal levels were applied in soils with different organic matter content (1.8%-original, 3%, and 5%) (Table 1). Organic matter content of soils are arranged by addition of goat manure and peat. Heavy metal levels were arranged by addition of mine waste obtained from an abondoned Zn-Pb mine in Balya, Balikesir, Türkiye. However, high concentrations of Al, As, Cd, Cr, Cu, Mn, Ni were also available in mine waste and soil levels of these elements were also raised. Control pots (no mine waste addition) for each set having different OM contents were also applied.
    - Table1: Initial Heavy Metal Concentrations of Soils

Average Initial HM Concentrations					
OM 1.8%		OM 3%		OM 5%	
Pb(mg/kg)	Zn(mg/kg)	Pb(mg/kg	Zn(mg/kg)	Pb(mg/kg	Zn(mg/kg)
5163,5	5347,1	4228,9	4715,7	4228,7	5006,0
820,9	1302,2	886,4	1337,7	1882,8	1170,0
263,3	374,9	230,6	309,6	228,8	404,7
44,2	113,9	100,0	277,0	48,9	114,6
15,2	79,1	36,1	67,9	10,2	55,6

20 seeds of Portulaca were planted in each pot, having 8 L of volume, and 15 days of germination period was applied. The germinations in each pot were strictly followed, where greenhouse temperature and humidity were also recorded on hourly basis. 75% of field capacity was used for irrigation of pots.
The harvesting was applied when the plants flowered. The roots and aerial parts are separated, weighed and dried at room temperature. The data is now under statistical processing.
Greenhouse pot experiments have been planned to evaluate the effect of drought stress on the growth of *Crithmum maritimum* and *Scolymus hispanicus* plants in next seasons.

- **Ege University (EGE)**
  (a) Field and pot experiments have been performed to investigate the agronomic and morphological characterization of *Portulaca oleracea*, *Crithmum maritimum* and *Scolymus hispanicus* plants under drought conditions.

- **Benha University (BU)**
  (a) Pot experiments are being performed to study the effect of salinity on the growth and chemical composition of *Portulaca oleraceae* plants
  (b) Pot experiments are being performed to study the effect of drought on the growth and chemical composition of *Portulaca oleraceae* plants.

Task 2.3. The involved partners (UTH, CUT, DEU, EGE, BU and CSIC) are performing the second series of experiments related to this task. BUW and CSIC will receive soil samples that will be analyzed and the results will be used in WP3.
In particular, the following activities are/will be performed by the individual partners:

- **University of Thessaly (UTH)** performed the following experiments for the evaluation of the agronomic performance of the selected species:
  a) field experiments regarding the use of *Cichorium spinosum*, *Sonchus oleraceus*, *Scolymus hispanicus* and *Portulaca oleracea*, in crop rotation systems, following the cultivation of *Phaseolus vulgaris* and *Pisum sativum.*
  b) field experiments with *Portulaca oleracea* where the effect of intercropping with common bean and crop rotation is tested in comparison to sole cropping systems.

  Plant and soil samples from both activities will be collected for chemical analyses that will be performed within the framework of WP4.

- **Consejo Superior de Investigaciones (CSIC)**
  (a) Pot experiments to study the effect of salinity with two levels (0 and 500 mM), and two types of fertilization (Mineral fertilizer and compost) with different doses (0.08, 0.16, 0.24 and 0.32 g of pure nitrogen) on yield of purslane (Portulaca oleracea), soil quality and rhizosphere microbial communities.

(b) Field experiment that is an expansion of last year's crop systems. We are testing the effect of different cropping systems of purslane with legumes (Peas and cowpeas) on purslane yield, soil quality and rhizosphere microbial communities

The treatments used were:

- Control purslane (Monoculture of purslane)
- Control cawpea (Monoculture of cawpea)
- Rotation purslane (Purslane plants planted in summer after peas)
- Rotation cawpea (Cawpea plans planted in summer after peas)
- Intercropping (Purslane plants planted in summer along with cawpeas)
- Intercropping + Rotarion (Purslane plants planted in summer along with cawpeas, after peas)

- **Cyprus University of Technology (CUT)**

(a) has scheduled field experiments/demonstration and contacted local farmers in order to evaluate the species under field conditions and different cropping systems. The experimental set up is scheduled for late Autumn 2023.

- **Dokuz Eylul University (DEU)**
  - For green manuring, biodegradable vegetable and fruit sorting waste from university dining hall was collected and composted in a two-stage reactor. The total composting period of 42 days was applied and the compost left for maturation for 15 days afterwards. The product was then screed from 10 mm screen and bagged for the use in greenhouse trials. The characterization of produced compost is continuing. Pot trials where the effect of green manuring, crop rotation and intercropping will be started in August 2023.

- **Ege University (EGE)**

(a) will repeat the field trial for the purpose of mixed planting and co-planting system of purslane (*Portulaca oleracea*), sea fennel (*Crithmum maritimum*) and Scolymus (*Scolymus hispanicus* L.) plants under field conditions.

- **Benha University (BU)**

(a) is performing field trails for the second growing period with *Portulaca oleracea* in mixed cropping, intercropping and short-term crop rotation systems with legumes and other crops to define the most suitable cultivation systems.

1.3 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; Task 3.5 Evaluation of non-microbial biostimulants for WEPs cultivation**

- **University of Thessaly (UTH)** is performing experiments related to Tasks 3.1-3.5.
  (a) 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).
  (b) 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).
  (c) 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 our 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).

- **Cyprus University of Technology (CUT)**
  (a) Task 3.1. CUT will progress mineral analysis to various experimental studies performed by UTh, contributing to the different biostimulant related experiments.
  (b) Pot experiment, evaluating the plant residues/wastes from olive-mill and grape-mill wastes as a growing media for both *Sonchus oleraceus* and *Portulaca oleracea* (Task 3.5).
  Experiments and analysis is now completed.
  (c) Pot experiment, evaluating the plant residues/wastes from Medicinal and Aromatic Plants residues, as component in growing media for both *Sonchus oleraceus* and *Portulaca oleracea* (Task 3.5). The experiment is completed, and a large part of the analysis is ongoing.

(a) **Consejo Superior de Investigaciones (CSIC)** has performed and scheduled the following experiments related to Tasks 3.1-3.4:

(a) 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 treatments used have been:

-PGPR species *Bacillus amyloliquefaciens* concentration 10^5, 10^6, 10^7 ufc/g
-PGPR species *Bacillus subtilis* concentration 10^5, 10^6, 10^7 ufc/g
-Mixture of *Bacillus* species (*B. megaterium*, *B. altitudinis*,
B. subtilis, *B. licheniformis*, *B. methylotrophicus)* 10^5, 10^6, 10^7 ufc/g
-PGPF species *Trichoderma asperellum* concentration 10^5, 10^6, 10^7 ufc/g
-PGPF species *Trichoderma T78* concentration 10^5, 10^6, 10^7 ufc/g
-PGPF species *Trichoderma atroviride* concentration 10^5, 10^6, 10^7 ufc/g
-AMF species Funneliformis mosseae (5%)
-AMF species Rhizophagus intraradices (5%)

Parameters determination is in progress.

(b) Pot experiment in 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 treatments used have been:

-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)

(b) 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.)

- **Bergische Wuppertal University (BUW)**

a) Finished with the analyses of the macro, micro, and toxic elements in the soils and plants samples of the first experiment (Task 3.2)
b) Analyzing the PLFA in the rhizosphere soil samples in the first experiment (Task 3.2 and 3.4)
c) Finished the second pot experiment with Portulaca, Sonchus, Scolymus, and Plantago using two degraded contaminated soils (used instead of the eroded soils in Germany) in four replicates (Task 3.3)
d) Harvested the plants, separated to roots and shoots, air dried, and recorded the fresh and dry biomass (Task 3.3)
e) Collected soil samples from all pots, air dried, crushed, and sieved to be ready for extraction and analyses (Task 3.3)
f) Analyzed soil properties (pH, soil salinity, total organic carbon content, particle size distribution, oxides content) (Task 3.3)

Scheduled activities
Finishing the extraction of the pot experiment soil and plant samples as follows:

a) Extracting the root and shoot samples (March-April, 2023) (Task 3.3),
b) Extraction of the total content of macro-nutrients (C, P, K, Ca, Mg, S) in the soil samples (May-June, 2023) (Task 3.3),
c) Extraction of the available (DTPA) content of macro-nutrients (C, P, K, Ca, Mg, S) in the soil samples (June-July, 2023) (Task 3.3),
d) Microwave extraction of the total content of trace and toxic elements (Al, Ag, As, Cd, Co, Cr, Cu, Fe, Mn, Ni, Pb, Sb, Se, Sn, Tl, V, and Zn) in the soil samples (July- August, 2023) (Task 3.3),
e) Extraction of the available (DTPA) content of trace and toxic elements (Al, Ag, As, Cd, Co, Cr, Cu, Fe, Mn, Ni, Pb, Sb, Se, Sn, Tl, V, and Zn) in the soil samples (August-September 2023) (Task 3.3),
f) Analyses of the macro, micro, and toxic elements in the root and shoot samples (May-August, 2023) (Task 3.3),
g) Analyses of PLFA in the rhizosphere soil samples (May-August, 2023) (Task 3.2 and Task 3.4).

- **Dokuz Eylul University (DEU)**
  - Task 3.2: The pH and EC of soils and lime contents were measured prior the experiments in the pots (OM content of 1.8%, 3%, and 5%) (n:5 each) to follow the impacts of soil alkalinity on purslane growth. The irrigation was obtained as 75% of the field capacity.
    Acid digestion procedure was applied for soils and plant parts obtained from the trials conducted with three different initial heavy metal levels in soils. The results from ICP-OES analysis of the extracts are expected from the laboratory. The findings will be evaluated to determine the plant bioaccumulation factors (soil to roots, soil to aerial parts), and translocation factors (root to aerial parts). In previous set of experiments with high soil heavy metals conducted in fall and winter period, it was found that bioaccumulation factor increases with increasing soil organic matter. However, plant biomass was poor due to the low temperatures. In this new set, high biomass was obtained in the pots, especially in the ones with higher organic matter and lower heavy metal concentrations. Therefore, it will be possible to compare and discuss the results and having a clear conclusion in this part of the study.
    Crithmum maritimum and *Scolymus hispanicus* will be studied in future sets with same scientific aims.

  - Task 3.3: Soil samples from the pots having different organic matter content were collected before and after Portulaca growth and sieve analysis were conducted. The soil samples were collected around plant roots during harvesting. Root length and mass were also recorded and be used in data processing. Sieve analysis data was currently under statistical evaluation.

- **Ege University (EGE)**
  n/a
- **Benha University (BU)** has planned to perform the following experiments related to Task 3.1 and 3.5:
  a) The effect of bacterial strains *viz*, *Azotobacter chroococcum*, *Paenibacillus polymyxa* GQ375783.1 on plant growth and chemical compositions of *Portulaca olearacea* (Task 3.1).
  b) *Portulaca olearacea* plants were irrigated with different concentrations of saline water: 1000, 2000, 3000,4000, 5000, 6000 ppm and control, while they were also sprayed with plant growth stimulants such as melatonin, proline and salicylic acid at different concentrations to evaluate the amelioration effects of these treatments on salinity stress related damage (Task 3.5).

1.4 Work package 4

This WP is related to the evaluation of quality, environmental footprint and nutritional value of WEPs and includes the following activities: **Task 4.1 Characterization of physical properties and quality; Task 4.1 Characterization of physical properties and quality; Task 4.3 Determination of individual bioactive compounds; Task 4.4 Environmental footprint for WEPs; Task 4.5 Statistical analysis and interpretation of the obtained data.**

The following activities are planned or ongoing:

- **University of Thessaly (UTH)**
  (a) UTH has collected and sent samples to IPB for analyses related to quality and nutritional value of WEPs. Moreover, the data for LCA analysis have been sent to CUT for the evaluation of the environmental footprint of WEPs.

- **Cyprus University of Technology (CUT)**
  (a) CUT has finalized the mineral analysis (N, K, P, Mg, Ca, and Na) for the experiments related to WP2.
  (b) CUT is going to analyse samples for minerals from IPB.
  (c) CUT has received relevant info from UTH for several experiments and is performing data analysis for the Environmental footprint. A manuscript has been prepared and already submitted to a journal (under review).

- **Instituto Politécnico de Bragança (IPB)**

As the lead beneficiary of WP4, IPB has begun the assessment of the nutritional value and chemical composition, as foreseen in task 4.3., of the samples obtained from *Scolymus hispanicus*, *Cichorium spinosum, Sonchus oleraceus*, *Portulaca olearacea,* and *Crithmum maritimum,* treated with different fertilization treatments, different irrigation treatments, pot and field experiments provided by the project coordinator (UTH). In due time the evaluation of the bioactivity profile and phenolic compounds of samples provided by the partners involved in WP2 (namely the samples *Portulaca oleracea* and *Sonchus oleraceus* from the CUT partner) will be performed within the next months.

Nowadays, the total number of obtained samples from IPB amounts to is 159 (one hundred fifty-nine).

Regarding the samples from Greece, nutritional value including total fat, crude protein, ash, total dietary fiber, and carbohydrates (by difference) was evaluated following AOAC methods. Energy was calculated according to the equation: energy (kcal per 100 g) = 4 x (g protein + g carbohydrate) + 2 x (g total dietary fiber) + 9 x (g fat). The total fat, crude protein, ash of the samples has already been done: eleven roots samples of *Scolymus hispanicus* from pot experiments with different fertilization regimes and field experiment with different irrigation regimes; fourteen samples of *Crithmum maritimum* from pot experiments with different fertilizers. The chemical composition and bioactivities properties were also evaluated by IPB partner. In terms of chemical composition (free sugars, tocopherols, fatty acids, organic acids), the samples of four species mentioned before have been injected, and they will be identification and quantification as soon as possible. The mineral content in terms of potassium, sodium, calcium, magnesium, iron, manganese, copper, and zinc will be performed in the next semester to one hundred twenty-five samples.

Regarding the samples from Cyprus, the hydroethanolic extracts have been performed and in the next months the bioactivity profile and phenolic compounds of these samples will be evaluated.

As further advances, complete nutritional and chemical profile will be performed in *Scolumys hispanicus* and *Crithmum maritimum* hydroethanolic extracts, while the samples from Cyprus will be prepared and the following bioactive properties will be evaluated:

a) The phenolic profile will be obtained by HPLC-DAD/ESI-MS and identified through available standard compounds and literature information. Quantification will be performed using calibration curves obtained from available commercial standard compounds.
b) The antioxidant activity will be evaluated through the lipid peroxidation inhibition using porcine brain cell homogenates by using the thiobarbituric acid reactive substances (TBARS) assay and oxidative hemolysis inhibition assay (OxHLIA).
c) The antimicrobial activity will be evaluated using five Gram-negative bacteria: *Escherichia coli*, *Enterobacter Cloacae*, *Salmonella enterocolitica*, *Pseudomonas aeruginosa*, and *Yersinia enterocolitica* and three Gram-positive bacteria: *Bacillus cereus*, *Listeria monocytogenes*, and *Staphylococcus aureus*, which are strains of food interest. Strains of clinical interest were also tested namely *Escherichia coli*, *Klebsiella pneumoniae*, *Morganella morganii*, *Proteus mirabilis*, *Pseudomonas aeruginosa* (Gram-negative bacteria), and three Gram-positive bacteria (*Enterococcus faecalis*, *Listeria monocytogenes* and methicillin-resistant *Staphylococcus aureus* (MRSA)). The antifungal activity on the fungi *Aspergillus brasiliensis* and *Aspergillus fumigatus* was also carried out.
d) The anti-inflammatory potential will be evaluated through the production of nitric oxide formed by lipopolysaccharides from the rat macrophage cell line RAW 264.7.
e) The cytotoxic was performed by the sulforrhodamine B (SRB) assay and four human tumor cell lines will be tested: CaCo2 (human colorectal adenocarcinoma), MCF-7 (breast carcinoma), VERO (renal epithelial cells extracted from a monkey) and AGS (Human gastric adenocarcinoma). Finally, for the hepatotoxic effect on non-tumor cell lines, a cell culture prepared from pig liver obtained from a local slaughterhouse (PLP2) was used.

- **Benha University (BU)**
  (a) BU has collected and sent samples to IPB for analyses related to quality and nutritional value of WEPs.

- **University of Mostaganem (UM)**
  a) The samples will be dispatched by IPB after the determination of bioactive properties.
  b) UM has performed the first series of analyses related to the *in vivo* anti-inflammatory activity.
  c) Statistical analysis and interpretation of the obtained data
  d) The *in vivo* anti-inflammatory activity – histological parameters (The results are presented in Annex 1

1.5 Work package 5

This working package is related to the communication activities of the project which include the following tasks: **Task 5.1 Information and communication campaigns; Task 5.2 Organization of awareness events; Task 5.4 Establishment of physical and living lab platforms;**

Regarding the performed activities:

a) IPB organized a public event where the coordinators of two PRIMA projects (e.g., Valuefarm and Pulping) presented to the public the research activities of their projects, while all the consortia members presented their ongoing research projects.

b) A member of the Cypriot team (Dr. Antonios Chrysargyris) visited Instituto Politécnico de Bragança, Portugal. During the 2 weeks visit, Dr. A. Chrysargyris proceeded with the analysis of the Cypriot experiments of vegetables of high nutritive value related to ValueFarm project, through new methods and techniques. Additionally, Dr. Chrysargyris initiated the first event of Coffee@CIMO of 2023, with a lecture on ‘’Medicinal and Aromatic Plants (MAPs): The Connection between Cultivation Practices and Biological Properties’’.

1.6 Work package 6

The activities related to WP6 include **Task 6.1 Development of physical labs; Task 6.2 Planning of Dissemination Activities; Task 6.3 Implementation of Dissemination Activities.**

SOCIAL MEDIA

Facebook

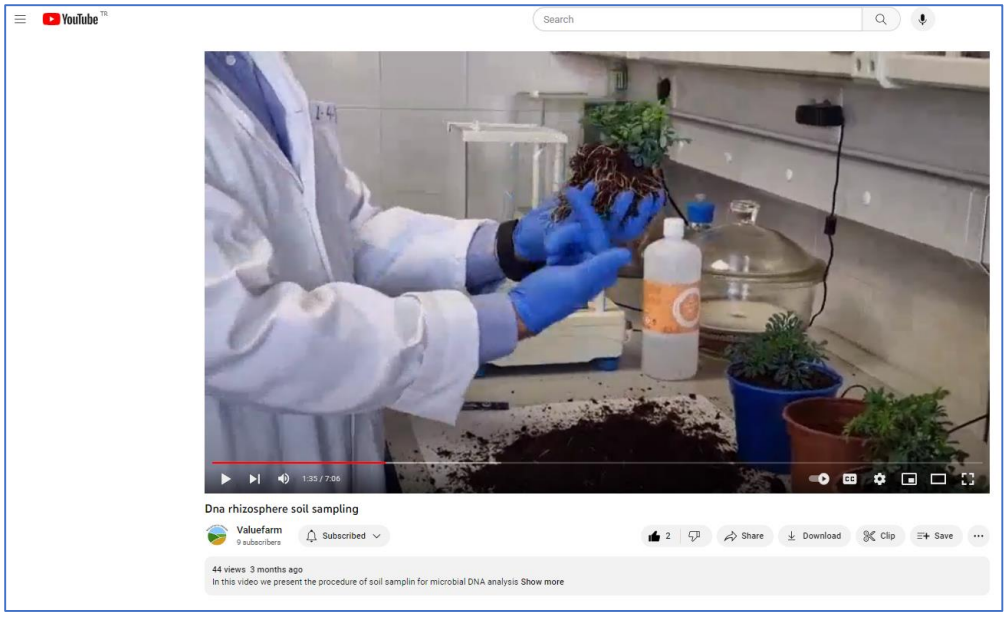
https://www.facebook.com/profile.php?id=100069454143411

DNA Rhizosphere Sampling Video announcement is published

YouTube

https://www.youtube.com/watch?v=TGULSDdIvDY

DNA Rhizosphere Sampling Video is published

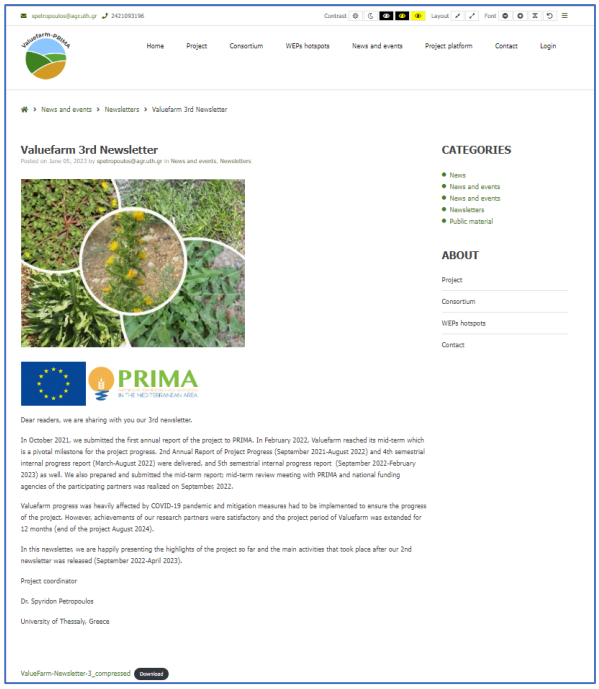


Project web-site

http://valuefarm-prima.agr.uth.gr/

Project 3rd newsletter was published

http://valuefarm-prima.agr.uth.gr/valuefarm-3rd-newsletter/



Regarding the dissemination of the project, the completed activities are presented below:

Scientific publications

1. Nikolaos Polyzos, Beatriz Paschoalinotto, Maria Compocholi, Maria Inês Dias, Lillian Barros, Spyridon A. Petropoulos. 2022. Fertilization of Pot Grown Cichorium spinosum L.: How it can affect plant growth, chemical profile and bioactivities of edible parts? Horticulturae 8(890): 1-22. https://doi.org/10.3390/horticulturae8100890
2. Beatriz H. Paschoalinotto, Nikolaos Polyzos, Maria Compocholi, Youssef Rouphael, Alexios Alexopoulos, Maria Inês Dias, Lillian Barros, Spyridon A. Petropoulos. 2022. Domestication of Wild Edible Species: The Response of Scolymus hispanicus Plants to Different Fertigation Regimes. Horticulturae 9, 103. https://doi.org/10.3390/horticulturae9010103.
3. Angel Carrascosa, Jose Antonio Pascual, Margarita Ros, Spyridon A. Petropoulos, Mª del Mar Alguacil García. 2023. Agronomical practices and management for commercial cultivation of Portulaca oleracea as a crop: A Review. Plants 12, 1246. https://doi.org/10.3390/plants12061246
4. Antonios Chrysargyris, Christos Goumenos and Nikolaos Tzortzakis. 2023. Use of Medicinal and Aromatic Plant Residues for Partial Peat Substitution in Growing Media for Sonchus oleraceus Production. Agronomy 2023, 13, 1074. https://doi.org/10.3390/agronomy13041074
5. Antonios Chrysargyris, Stavros Louka, Spyridon A Petropoulos, Nikolaos Tzortzakis. 2023. Soilless cultivation of Portulaca oleracea using medicinal and aromatic plants residues for partially peat replacement. Horticulturae 9, 474 https://doi.org/10.3390/horticulturae9040474
6. Carrascosa A., Pascual J.A., López-García A., Romo-Vaquero M., De Santiago A., Ros M., Petropoulos S.A., Alguacil M.M. 2023. Effects of inorganic and compost tea fertilizers application on the taxonomic and functional microbial diversity of the purslane rhizosphere. Frontiers in Plant Science 14: 1159823 https://doi.org/10.3389/fpls.2023.1159823
7. Carrascosa A., Pascual J.A., López-García A., Romo-Vaquero M., Ros M., Petropoulos S.A., Alguacil M.M. 2023. Different Functional and Taxonomic Composition of the Microbiome in the Rhizosphere of Two Purslane Genotypes rhizosphere. Agronomy 13, 1795. https://doi.org/10.3390/agronomy13071795

The publications are presented in D6.1.1.

Conference proceedings:

1. Conference proceedings: Beatriz H. Paschoalinotto, Miguel A. Prieto, Nikolaos Polyzos, Maria Compocholi, Spyridon Petropoulos, Isabel C.F.R. Ferreira, Maria Inês Dias, Lillian Barros. Análise do perfil nutricional de partes coméstiveis da Portulaca oleracea L. produzida pela técnica de rotação de culturas. III Colóquio Nacional de Plantas Aromáticas e Medicinais. Castelo Branco, Portugal, 24-26 March, 2022. Type of dissemination: oral
2. Conference proceedings: Beatriz H. Paschoalinotto, Miguel A. Prieto, Nikolaos Polyzos, Maria Compocholi, Spyridon Petropoulos, Isabel C.F.R. Ferreira, Maria Inês Dias, Lillian Barros. "Impacto de diferentes métodos de produção agrícola no perfil nutricional de beldroega (Portulaca oleracea L.)". Ciência 2022 – Encontro com a ciência e Tecnologia em Portugal. Lisboa, Portugal, 16-18 maio de 2022. Type of communication: poster

3. Conference proceedings: Nikolaos Polyzos, Beatriz Paschoalinotto, Maria Compocholi, Maria Inês Dias, Lillian Barros, Spyridon A. Petropoulos. The effects of fertilization regime on growth parameters and bioactive properties of pot grown Cichorium spinosum L. plants. 1st International Electronic Conference on Horticulturae (on-line). 16-30 April, 2022. Type of communication: poster
4. Conference proceedings Ángel Carrascosa, José Antonio Pascual, Margarita Ros, Spyridon Petropoulos, María del Mar Alguacil. The effect of fertilization regime on growth parameters of Sonchus oleraceus and two genotypes of Portulaca oleracea. 1st International Electronic Conference on Horticulturae (on-line). 16-30 April, 2022. Type of communication: poster
5. Conference proceedings Maria Inês Dias, Beatriz H. Paschoalinotto, Nikolaos Polyzos, Spyridon Petropoulos, Lillian Barros. Tailor-made fertilization regimes as strategies to increase phenolic composition: the case study of pot grown Cichorium spinosum L. Polyphenols Applications 2022. Valencia, Spain, 28-30 September, 2022. Type of communication: oral
6. Conference proceedings: Beatriz H. Paschoalinotto, Miguel A. Prieto, Nikolaos Polyzos, Maria Compocholi, Spyridon Petropoulos, Isabel C.F.R. Ferreira, Maria Inês Dias, Lillian Barros. Crop rotation and irrigation experiment effects the nutritional and chemical profile of C. spinosum. Ciência 2022 – XVI Encontro de Química dos Alimentos Castelo Branco, Portugal, 23-26 outubro de 2022. Type of dissemination: oral
7. Conference proceedings: Beatriz H. Paschoalinotto, Miguel A. Prieto, Nikolaos Polyzos, Maria Compocholi, Spyridon Petropoulos, Isabel C.F.R. Ferreira, Maria Inês Dias, Lillian Barros. "Functionality assessment of Scolymus hispanicus (golden thistle) for its daily-basis incorporation in the Mediterranean diet". Ciência 2022 – XVI Encontro de Química dos Alimentos Castelo Branco, Portugal, 23-26 outubro de 2022. Type of dissemination: poster
8. Conference proceedings: Ángel Carrascosa, José Antonio PascuaL, Álvaro López-García, Margarita Ros, Spyridon A Petropoulos, María del Mar Alguacil. Microbial community structure in purslane (Portulaca oleracea) rhizosphere after different organic and inorganic fertilizer rates. 13th International Scientific Agriculture Symposium "AGROSYM 2022". Jahorina, Bosnia and Herzegovina, October 6-09, 2022. Type of communication: poster
9. Paraskevi Katsimantou, Stefania-Fani Plitsi, Chrysanthi Foti, Ourania Pavli, Spyridon A. Petropoulos. Seed priming enhances seed germination and seedling growth of five wild edible species. 13th International Scientific Agriculture Symposium "AGROSYM 2022". Jahorina, Bosnia and Herzegovina, October 6-9, 2022. Type of communication: poster
10. Conference proceedings: Nikolaos Polyzos, Maria Kompocholi, Alexios Alexopoulos, Maria Ines Diaz, Beatriz Paschoalinotto, Lillian Barros, Spyridon A. Petropoulos. The effect of fertilization regimes on growth and chemical composition of Cichorium spinosum plants. 13th International Scientific Agriculture Symposium "AGROSYM 2022". Jahorina, Bosnia and Herzegovina, October 6-09, 2022. Type of communication: poster
11. Conference proceedings: Beatriz H. Paschoalinotto, Miguel A. Prieto, Maria Compocholi, Nikolaos Polyzos, Spyridon Petropoulos, Isabel C.F.R Ferreira, Maria Inês Dias, Lillian Barros. Avaliação da influência da adubação via solução nutritiva no perfil nutricional de Scolymus hispanicus L. IV Congresso das Escolas Superiores Agrárias, Santarem, Portugal, November 3-4, 2022. Type of dissemination: oral
12. Conference proceedings: Beatriz H. Paschoalinotto, Miguel A. Prieto, Nikolaos Polyzos, Maria Compocholi, Spyridon Petropoulos, Isabel C.F.R. Ferreira, Maria Inês Dias, Lillian Barros. "Impacto del riego en el perfil nutricional y químico de las partes comestibles del cardo dorado (Scolymus hispanicus L.). III Congreso Nacional de Jóvenes Investigadores en Ciencia,

Ingeniería y Tecnología de los Alimentos, Salamanca, Spain, 10-11 November, 2022. Type of dissemination: oral

13. Conference proceedings: Paschoalinotto B. H., Prieto M.A., Compocholi M.; Polyzos N.; Pires, T.C.S.P.; Petropoulos S.; Ferreira I.C.F.R.; Dias M.I.; Barros L. Estudo integrado da influência do tipo de cultivo e irrigação nas propriedades bioativas de Cichorium spinosum L. XXVI Encontro Galego-Português de Química, Santiago de Compostela, Espanha, 16-18 November, 2022. Type of dissemination: oral
14. Conference proceedings: Paschoalinotto B. H., Prieto M.A., Compocholi M.; Polyzos N.; Pires, T.C.S.P.; Petropoulos S.; Ferreira I.C.F.R.; Dias M.I.; Barros L. Combinação de diferentes regimes de fertilização e irrigação para a produção de cardo dourado (Scolymus hispanicus L.) de alto valor nutricional e mineral. XXVI Encontro Galego-Português de Química, Santiago de Compostela, Espanha, 16-18 November, 2022. Type of dissemination: oral
15. Conference proceedings: Polyzos N., Papaioannou E., Paschou M., Petropoulos S.A. Commercial exploitation of Sonchus oleraceus: the response of plants to fertilization regimes. 4th Mediterranean Forum, Chania, Greece, 4-7 December, 2022. Type of communication: poster
16. Chrysargyris A, Xylia P, Tzortzakis N, 2022. Olive-mill and grape-mill waste as a substitute growing medium component for unexplored vegetables production in nurseries. 9th International Conference on Sustainable Solid Waste Management, CORFU2022. 15-18 June 2022, Corfu, Greece. Type of communication: oral
17. Chrysargyris A, Hajisolomou E, Tzortzakis N, 2022. Origanum dubium and Sideritis cypria plant waste as a substitute growing medium component for Portulaca oleracea production in nurseries. 9th International Conference on Sustainable Solid Waste Management, CORFU2022. 15-18 June 2022, Corfu, Greece. Type of communication: poster
18. Tzortzakis N, Goumenos C, Xylia P, Chrysargyris A. 2023. Exploring Medicinal and Aromatic Plant residues after distillation as a peat substitute component in growing media for Sonchus oleraceus production. 10th International Conference on Sustainable Solid Waste Management, CHANIA2023. 21-24 June 2023, Chania, Greece. Type of communication: oral
19. Carrascosa A, Pascual JA, López-García A, Romo-Vaquero M, Ros M, Petropoulos SA, Alguacil MM. Effects of inorganic and compost tea fertilizers application on the taxonomic and functional microbial diversity of the purslane rhizosphere. 12th workshop en Investigación agraria para jóvenes agricultores, Cartagena (Spain) 9 May, 2023.
20. Thesis communication: “Uso combinado de prácticas agronómicas y microorganismos beneficiosos para el cultivo de especies vegetales silvestres comestibles (WEPs) de la cuenca Mediterránea” 11th workshop en Investigación agraria para jóvenes agricultores, Cartagena (Spain), 20 June, 2022.

The material of all the conferences is presented in D6.1.1.

Scheduled activities:

Manuscript preparations:

1. Aspasia Grammenou**,** Spyridon A. Petropoulos, Georgios Thalassinos, Jörg Rinklebe, Sabry M. Shaheen, Vasileios Antoniadis. 2023. Biostimulants in the soil-plant interface: Agro-environmental implications-A review. *Earth Systems and Environment* (submitted for publication).
2. Vassilis D. Litskas, Antonios Chrysargyris, Nikolaos Tzortzakis, Menelaos C. Stavrinides, Spyridon A. Petropoulos. 2023. Can the commercial cultivation of wild edible species contribute to sustainable food production? A case study of golden thistle (*Scolymus hispanicus* L.). *Journal of Cleaner Production* (submitted for publication)
3. Effect of ammonium to total nitrogen ratio on *Portulaca oleraceae* grown in hydroponics. Under preparation.
4. Can the commercial cultivation of wild edible species contribute to sustainable food production? A case study of golden thistle (*Scolymus hispanicus* L.). (submitted for publication).
5. A review paper regarding the chemical composition of wild edible species
6. A review paper regarding the integration of wild edible species in Mediterranean farming systems
7. Crop rotation with legume improves purslane production and change the soil microbial community
8. Effect of an organic fertilizer derived from apiculture waste “beekeeping wastes” on purslane plants and soil microbial structure

Scheduled participation in conferences

1. Nikolaos Polyzos, Beatriz H. Paschoalinotto, Maria Compocholi, Miguel A. Prieto, Maria Inês Dias, Lillian Barros, Spyridon A. Petropoulos. Drought stress effect on crop development and chemical composition of field grown *Scolymus hispanicus* L. plants. 1**8th International Conference on Environmental Science and Technology**. Athens, Greece, 30 August-3 September, 2023.
2. Nikolaos Polyzos, Vasileios Antoniadis, Spyridon A. Petropoulos. The effect of manure and zeolite on the morphological traits and yield of *Portulaca oleraceae.* **XIV International Agriculture Symposium "AGROSYM 2023"**. Jahorina, Bosnia and Herzegovina, 5-8 October, 2023.
3. Ángel Carrascosa, José Antonio PascuaL, Margarita Ros, Jessica Cuartero, Ana De Santiago, Spyridon A. Petropoulos, María del Mar Alguacil. Crop rotation with legumes improves purslane production and change the soil microbial community. **XIV International Agriculture Symposium "AGROSYM 2023"**. Jahorina, Bosnia and Herzegovina, 5-8 October, 2023.
4. Beatriz H. Paschoalinotto, Miguel A. Prieto, Tânia C.S. Pires, Ricardo Calhelha, Nikolaos Polyzos, Spyridon A. Petropoulos, Lillian Barros, Maria Inês Dias. 2023. Effects of Drought Stress on the Chemical Composition and Bioactive Properties of *Cichorium spinosum* L. **The 3rd International Electronic Conference on Agronomy**, 27 October–10 November, 2023.
5. Beatriz H. Paschoalinotto, Antonios Chrysagyris, Spyridon A. Petropoulos, Nikolaos Tzortzakis, Miguel A. Prieto, Maria Inês Dias, Lillian Barros. 2023. Impact of Ammonium

to Total Nitrogen Ratio Fertilization in the Centesimal and Chemical Profiles of Purslane Produced under Hydroponic Conditions. **The 4th International Electronic Conference on Applied Sciences (on-line**), 27 October–10 November, 2023.

6. Nikolaos Polyzos, Spyridon A. Petropoulos. The effect of manure on plant development and yield of the wild edible species *Cichorium spinosum*, *Scolymus hispanicus* and *Sonchus oleraceus.* **The 2nd International Electronic Conference on Agriculture (on-line**), 1-15 November, 2023.

Table 4. List of deliverables of Valuefarm project, displaying re-scheduled delivery months (in bold font). Completed indicates that the deliverable has been reached.

#	Deliverable name	WP	Lead	Type	Dissemination level	Due month	New due date
1.1	**Technical meeting organisation**	**1**	**UTH**	**OTHER**	**CO**	**2**	**Completed**
1.2	**Constitution of the Steering Group**	**1**	**UTH**	**OTHER**	**CO**	**2**	**Completed**
1.3	**Semestrial internal progress report**	**1**	**UTH**	**R**	**CO**	**6**	**Completed**
1.4	**Technical meeting organisation**	**1**	**UTH**	**OTHER**	**CO**	**12**	**Completed**
1.5	**Semestrial internal progress report**	**1**	**UTH**	**R**	**CO**	**12**	**Completed**
1.6	**Annual report**	**1**	**UTH**	**OTHER**	**CO/PU**	**12**	**Completed**
1.7	**Semestrial internal progress report**	**1**	**UTH**	**R**	**CO**	**18**	**Completed**
1.8	**Technical meeting organisation**	**1**	**UTH**	**OTHER**	**CO**	**24**	**Completed**
1.9	**Semestrial internal progress report**	**1**	**UTH**	**R**	**CO**	**24**	**Completed**
1.10	**Annual report**	**1**	**UTH**	**R**	**CO/PU**	**24**	**Completed**
1.11	Semestrial internal progress report	1	UTH	R	CO	30	**Completed**
1.12	Technical meeting organisation	1	UTH	OTHER	CO	33	**Completed**
1.13	Semestrial internal progress report	1	UTH	R	CO	36	36
1.14	Final report	1	UTH	R	CO/PU	36	48
2.1.1	**A DATABASE map of the selected WEPs in the participating countries**	**2**	**CSIC**	**DEC**	**PU**	**6**	**Completed**
2.1.2	**A DATABASE map of the selected WEPs in the participating countries**	**2**	**CSIC**	**DEC**	**PU**	**6**	**Completed**
2.2	**Multilingual electronic handbook of technical information and best practice guides of the selected WEPs**	**2**	**CSIC**	**R**	**PU**	**8**	**Completed**
2.3	Selection of the most environmentally sustainable WEP for each country conditions	2	CSIC	OTHER	PU	24	36
2.4	Selection of the most sustainable WEP and legume combinations for each country conditions	2	CSIC	OTHER	PU	24	36

3.1	Report for soil chemical properties in soil samples collected from the second field experiment	3	BUW	R	CO	30	42
3.2	Determination of plant nutrients and potentially toxic elements in both soil and plant samples collected from the first experiment	3	BUW	R	CO	30	42
3.3	Report for soil chemical properties in soil samples collected from the second field experiment	3	BUW	R	CO	30	42
3.4	Determination of plant nutrients in both soil and plant samples collected from the second experiment	3	BUW	R	CO	30	42
3.5	Determining the soil microbial community in soil samples collected from the second field experiment	3	BUW	R	CO	36	48
3.6.1	Reports	3	BUW	R	CO, PU	36	36
3.6.2	Reports	3	BUW	R	CO, PU	36	48
3.7	Publications in peer-reviewed scientific journals in an open access mode	3	BUW	OTHER	PU	36	48
4.1	Proximate composition and chemical profile of the different WEP's	4	IPB	R	PU	18	36
4.2	Report with the most suitable extraction conditions and mathematical models obtained by RSM) of the dependent variables used in the optimization of the extraction of the bioactive compounds from WEP's	4	IPB	R	CO	20	38
4.3	Overall composition description reports of the different WEP's	4	IPB	R	PU	24	42
5.1.1	**Public meetings**	**5**	**CUT**	**DEC**	**PU**	**12**	**Completed**
5.1.2	**Public meetings**	**5**	**CUT**	**DEC**	**PU**	**24**	**Completed**
5.1.3	Public meetings	5	CUT	DEC	PU	36	36
5.2	**Project website**	**5**	**CUT**	**DEC**	**PU**	**4**	**Completed**
5.3.1	**Communication and Publicity material**	**5**	**CUT**	**DEC**	**PU**	**6**	**Completed**
5.3.2	Communication and Publicity material	5	CUT	DEC	PU	33	45

5.4	Establish physical and living labs	5	CUT	DEM	PU	6	36
6.1.1	Scientific publications	6	DEU, EGE	R	PU	24	36
6.1.2	Scientific publications	6	DEU, EGE	R	PU	30	42
6.1.3	Scientific publications	6	DEU, EGE	R	PU	36	48
6.2.1	Video/DVD material	6	DEU, EGE	DEC	PU	24	36
6.2.2	Video/DVD material	6	DEU, EGE	DEC	PU	36	48
6.3.1	Handbooks	6	DEU, EGE	R	PU	18	30
6.3.2	Handbooks	6	DEU, EGE	R	PU	36	48
6.4.1	**Publicity material**	**6**	**DEU, EGE**	**DEC**	**PU**	**6**	**Completed**
6.4.2	**Publicity material**	**6**	**DEU, EGE**	**DEC**	**PU**	**12**	**Completed**
6.4.3	**Publicity material**	**6**	**DEU, EGE**	**DEC**	**PU**	**18**	**Completed**
6.4.4	Publicity material	6	DEU, EGE	DEC	PU	24	36
6.4.5	Publicity material	6	DEU, EGE	DEC	PU	30	42
6.4.6	Publicity material	6	DEU, EGE	DEC	PU	36	48

Table 5. List of milestones of the Valuefarm project, showing re-scheduled due month. Completed indicates that the deliverable has been reached. New milestones are highlighted with yellow color.

Milestone number	Milestone name	Related work package(s)	Means of verification	Due date (in month)	New due date
M1.1.1	**Reports on technical meetings**	**1**	**Reports available on the project website (PW)**	**2**	**Completed**
M1.1.2	**Reports on technical meetings**	**1**	**Reports available on the project website (PW)**	**12**	**Completed**
M1.1.3	**Reports on technical meetings**	**1**	**Reports available on the project website (PW)**	**24**	**Completed**
M1.1.4	Reports on technical meetings	1	Reports available on the project website (PW)	33	**Completed**
M1.1.5	Reports on technical meetings	1	Reports available on the project website (PW)	45	45
M1.2	**Establishment of steering committee**	**1**	**List of the Steering Committee members available on the PW**	**2**	**Completed**
M1.3.1	**Semestrial and annual progress reports completed**	**1**	**Reports available on the PW and sent to PRIMA**	**6**	**Completed**
M1.3.2	**Semestrial and annual progress**	**1**	**Reports available on the PW and sent to PRIMA**	**12**	**Completed**

	reports completed				
M1.3.3	**Semestrial and annual progress reports completed**	**1**	**Reports available on the PW and sent to PRIMA**	**18**	**Completed**
M1.3.4	**Semestrial and annual progress reports completed**	**1**	**Reports available on the PW and sent to PRIMA**	**24**	**Completed**
M1.3.5	**Semestrial and annual progress reports completed**	**1**	**Reports available on the PW and sent to PRIMA**	**30**	**Completed**
M1.3.6	Semestrial and annual progress reports completed	1	Reports available on the PW and sent to PRIMA	36	36
M1.3.7	Annual progress reports completed	1	Reports available on the PW and sent to PRIMA	48	48
M1.4	Final report completed	1	Final report on the website and sent to PRIMA	36	48
M3.1	Development of experimental design and implementation for the first field and pot experiment	3	Field and pot experiments have been done, agronomic measurements have been done, soil and plant samples collected, and ready for analyses	24	36
M3.2	Providing data of the first experiment	2	Analyses have ben finished and the results are collected, validated, and available	30	42
M3.3	Development of experimental design and implementation for the second field experiment	2	Field experiment results	30	42
M3.4	Providing data of the second experiment	2	Analyses have been finished and the results are collected, validated, and	33	45

			available for publication		
M3.5	Preparing scientific paper(s)	1,2,4	Submitted/Published paper	33	45
M4.1	Up-scalable and eco-friendly extraction processes for bioactivities compounds	4	High yields in the procedure of extraction	18	36
M4.2	Optimized conditions for bioactive compounds profiling and fingerprinting	4	Parametric estimation responses resulting from RSM	24	36
M5.1	Establishment of physical and living labs	5	On-line and printed questionnaires filled by users, comments through the project website	6	36
M5.2	Video material prepared	5	Video material available in project website and the Internet	33	45