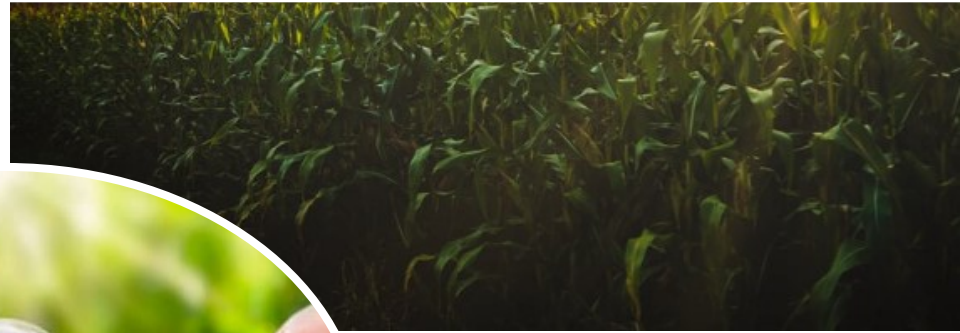


# Layman's report

## LIFE ALGARBBE

microALGae with ARomatic plants  
as Biostimulants with Biocide Effect



LIFE18 ENV/ES/000518

<http://www.algarbbelife.eu/>

LIFE  
ALGAR-BBE

## The problem...



Currently, pesticides and chemical fertilizers are abused in agricultural practices. 76% of the pesticides applied annually are used in the agricultural industry, which will be the sector addressed in this project.

According to Eurostat, almost 77 tons were sold in Spain in 2016, with fungicides and herbicides being the most used (50.5% and 19.75% respectively). Pesticides easily contaminate air, soil, and water.

Among other effects, it decreases and impoverishes both animal and plant biodiversity and enters the food chain, posing a health risk, due in part to the residues that remain in food. On the other hand, an excessive use of chemicals in fertilizers also has negative environmental consequences.



## ALGAR-BBE solution!

The Project aims to **develop new biostimulant products with biocide action**, initially tested on tomato and corn crops with the aim of reducing pesticides by almost 56%.

- ❑ The **biostimulant** action is achieved by adding microalgae
- ❑ The **biocidal** action is achieved thanks to extracts of **aromatic plants**



The Environment and Climate Action Program (LIFE) is the financial instrument of the European Union dedicated exclusively to the environment. Its general objective is based on catalyzing changes in the development and application of policies by providing solutions and best practices to achieve environmental and climate objectives, as well as by promoting innovative technologies in the field of environment and climate change.

The LIFE Program is divided into two subprograms with three priority areas each.

Environment Subprogram

- Environment and Efficiency in the Use of Resources
- Nature and Biodiversity
- Environmental Governance and Information

Climate Action Subprogram

- Climate Change Mitigation
- Climate Change Adaptation
- Climate Governance and Information

The LIFE ALGAR-BBE project is part of the Environment subprogramme, in the Environment and Efficiency in the Use of Resources.

# LIFE ALGARBBE PROJECT



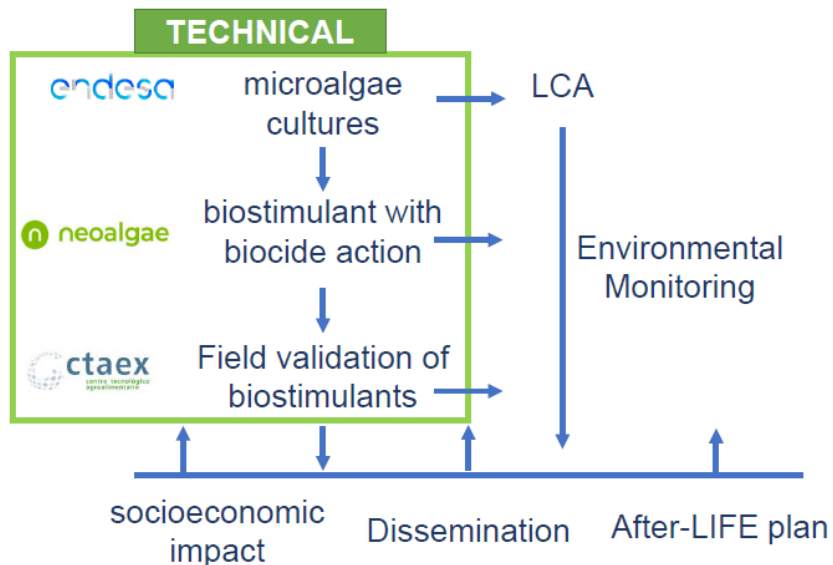
EU contribution:  
700.671 €



01 July 2019 to 30 June 2023



## ACTIVITIES CARRIED OUT



## MAIN OBJECTIVE

The main objective of the LIFE ALGAR-BBE project is **to mitigate the adverse effects** on the environment and human health of **pesticides of chemical origin that are currently used in tomato and corn cultivation.**

**The efficacy of 3 formulations** of natural, safe and sustainable origin will be demonstrated, **with biostimulant capacity and biocide action**, whose **active materials will be obtained from microalgae, extracts of aromatic plants, as well as residues from Spirulina extraction and residues from olive industry.**

Substitute pesticides of chemical origin to traditional fertilizers. The developed formulations may be marketed in the EU in the subsequent stages of the project.

With the expected results of this project, 56% of the pesticides currently used for the diseases and pests identified for the crops object of the project will be susceptible to substitution.

## What are microalgae?

Microalgae are **unicellular microorganisms** that have the ability to carry out **photosynthesis**. That is, they are capable of **generating organic biomass from CO<sub>2</sub> and light**, using water as an electron donor, oxidizing it to O<sub>2</sub>.

They are the basis of food webs and their large number of species and their versatility allow them to be used successfully in **different industrial fields**.

They are present in all environments with water (lakes, seas and rivers), but also in the soil and in most terrestrial environments, even the most extreme, which makes it possible to find them widely distributed in the biosphere, adapted to a large number of conditions

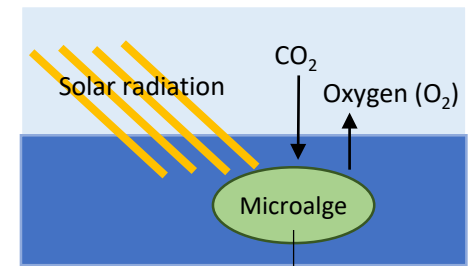
## Why do we use microalgae?

Microalgae **biomass performs beneficial biochemical and physiological functions** such as growth promotion, antioxidant, anti-inflammatory and immunomodulatory functions.

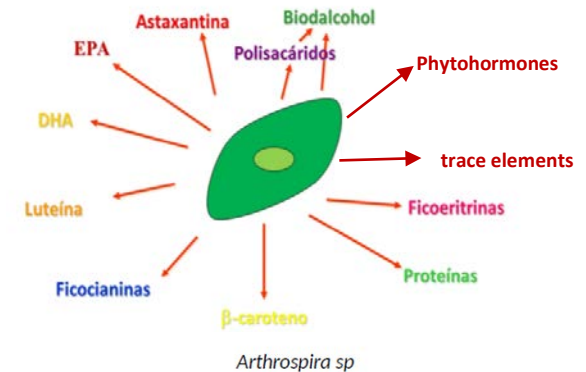
**In the field of agriculture they are especially beneficial** because they **promote plant growth and increase tolerance to different types of biotic** (ie insects, fungi, etc.) **and abiotic** (drought, salinity and any other adverse climatic factors) **stress**. They are capable of releasing several biologically active molecules such as phytohormones, polysaccharides, amino acids, so they have potential as a natural fertilizer.

## Benefits of microalgae

- ❑ **SUSTAINABLE AGRICULTURE:** their cultivation does not require agricultural land, so they do not compete with crops for food.
- ❑ **THEY GROW IN ALL TYPES OF WATER:** brackish, residual, fresh or salt water, they do not require drinking water.
- ❑ **HIGH PRODUCTIVITY:** Its rapid growth cycle and daily harvest guarantee the highest quality.
- ❑ **INEXHAUSTABLE SOURCE OF NUTRIENTS:** fatty acids, amino acids, proteins, antioxidants, pigments, etc... All of them are essential elements in all stages of life.
- ❑ **CAPTURE CO<sub>2</sub>** and convert it into oxygen: they form the most efficient ecosystem on the planet



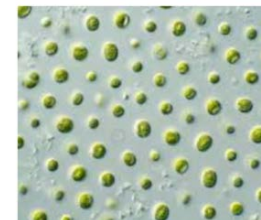
Photosynthesis, production and accumulation of compounds



*Arthrospira sp*



*Nannochloropsis gaditana*



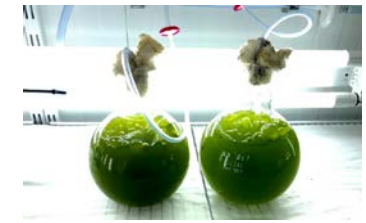
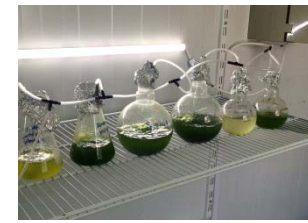
*Acutodesmus obliquus*



# CULTURE OF MICROALGAE IN THE PROJECT

## 1. CEPARIUM

The cultivation of microalgae, which is carried out in the pilot plant at ENDESA's Litoral Thermal Power Plant in Carboneras (Almería), begins in **small volumes** in the area known as the ceparium or inoculum area. At this stage, absolutely all the parameters are controlled (temperature, light, aeration, pH...).



## 2. SCALING UP AND CULTIVATION

These **small volumes** are gradually scaled up to reach **intermediate volumes** (1,000 liters approx.) in **flat-inclined photobioreactors**. The objective of these photobioreactors is to maintain and have high-quality cultures, with optimal cell density and in a sufficient volume to make the leap to the industrial production stage.



These photobioreactors are oriented towards the sun to make the most of its radiation, so that our microalgae can photosynthesize and grow in record time.

Subsequently, it is passed to the production stage in photobioreactors of greater volumetric capacity, ensuring the supply of the necessary quantities for the project. In the **"raceways"** the culture begins with a yellowish green hue due to the low cell concentration. After a few weeks the crop has reached its maximum concentration, the intense green color indicates that it is ready to produce.



## 3. DOWNSTREAM: HARVESTING AND DRYING

At this point part of the culture is removed from the reactor and replaced with water and nutrients. The removed crop is transferred to the harvesting and centrifugation area where **it will be concentrated, eliminating a large part of the water**, obtaining a microalgae concentrate.

The concentrate is now ready to go into **spray drying**. This process ensures that up to 97% of the water is removed, allowing the biomass to be stabilized and that it does not lose any of its qualities.

### SOME DATA....

- Cultured microalgae species: *Nannochloropsis gaditana* and *Acutodesmus obliquus*
- Optimization of culture protocols
- Generation of dry biomass for the production of biostimulants
- Decreased consumption of water and necessary nutrients
- Increase in CO<sub>2</sub> consumption

# MICROALGAE CULTIVATION IN THE PROJECT

## SUSTAINABILITY

### □ RECYCLING OF WATER

To promote the sustainability of microalgae cultivation, strategies for recirculating the excess water from the centrifuging process were studied to reduce both water and nutrient consumption.

- The amount of water supplied has been reduced by 60%
- The species *A. obliquus* tolerates a greater recirculation of water than the species *N. gaditana*
- Nutrient intake has been reduced by 55%

### □ CO<sub>2</sub> CAPTURE

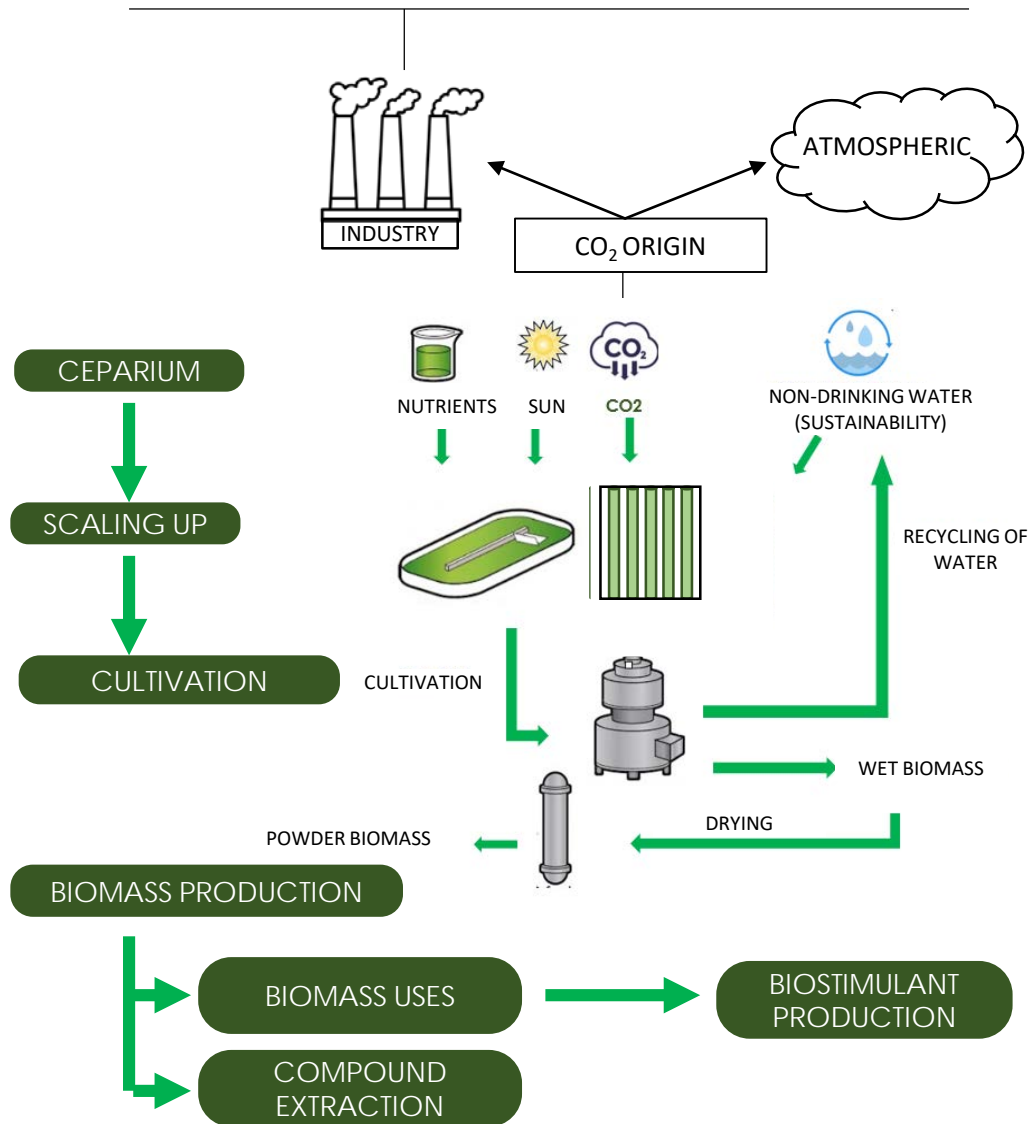
Given the photosynthetic nature of microalgae, they are capable of transforming light energy into biomass, with high efficiency rates. This conversion is carried out by consuming large amounts of carbon dioxide and releasing oxygen into the atmosphere, which allows mitigating greenhouse gas emissions.

During the project, a study was carried out on how the microalgae assimilated CO<sub>2</sub>, both of synthetic origin and the combustion gas of the thermal power plant.

As a result of the project, the optimization of the production process, as well as the industrialization of the scaling of the crops, made it possible to intensify both the injection of CO<sub>2</sub> and the generation of biomass.

Based on actual measurements of CO<sub>2</sub> uptake, consumption by crops is estimated to be 1.2Kg/m<sup>3</sup> and 1.9Kg/ m<sup>3</sup> in crops of *N. gaditana* and *A. obliquus*, that is, an average of 1.55 kg of CO<sub>2</sub> for each m<sup>3</sup> of microalgal culture.

Flue gas can be injected into microalgae cultures to increase CO<sub>2</sub> assimilation by biomass. But first, the gas must be treated to avoid damage to the microalgae due to high temperatures and the presence of other compounds that can be harmful to the cells.



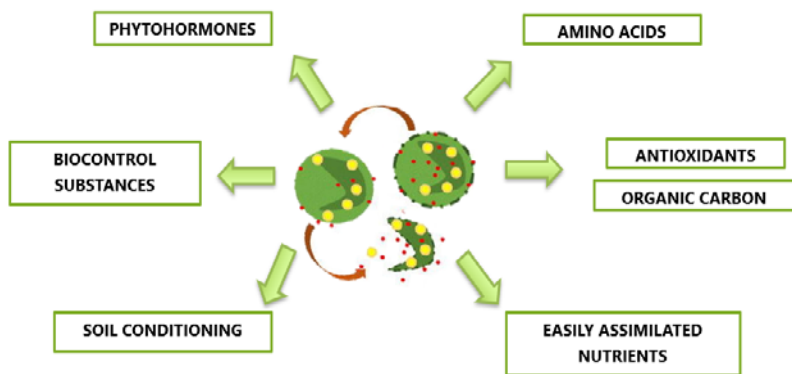
# WHAT ARE BIOSTIMULANTS?

Biostimulants are biological substances that act on the physiology of the plant in different ways and through different ways, improving the productive and growth capacity, the vigor of the crop, the yield and quality of the harvest. They contain substances and/or microorganisms whose function is to stimulate natural processes to improve the uptake, assimilation and efficiency of nutrients, tolerance to abiotic stress, and the quality of crops.

Therefore, they contribute to the reduction of inputs and mainly of nitrogen sources, which means reducing the use of fertilizers and getting closer to the goals suggested in the European Union with the "From Farm to Fork" strategy.

In addition, they try to respond to the new challenges facing agriculture, with the search for more sustainable solutions with the environment and improving the productivity of crops. It is an alternative already applied in agriculture 4.0

The formulations developed in the project are in liquid format, so they must be diluted with irrigation water in a certain dose (which varies with the type of crop) in order to be applied at the appropriate times.



- BENEFITS**
- TOLERANCE TO ABIOTIC STRESS**
  - Extreme temperatures
  - Drought / Floods
- TOLERANCE TO BIOTIC STRESS**
  - diseases and pests
- BETTER PERFORMANCE**
  - Root growth and branches
  - Improves flowering and fruits
- BEST POSTHARVEST**
  - Improves shelf life and storage
  - Improves nutritional composition



- APPLICATION MODE**
- FOLIAR**
  - Aspersión
  - Spray
  - Others
- ROOT**
  - Fertigation
  - Hydroponics

## DIFFERENCES BETWEEN FERTILIZER AND BIOSTIMULANT

FERTILIZER	BIOSTIMULANT
Essential nutrients for plants	Improve the incorporation of nutrients
They do not improve stress tolerance	Improve tolerance to pests or biotic stress
They do not improve the incorporation of nutrients	Its origin can be a by-product of organic origin
They are used in large quantities	They are applied in small quantities

# BIOSTIMULANTS WITH BIOCIDES ACTION IN THE PROJECT

## AROMATIC PLANTS

These plants are characterized by the production, from secondary metabolism, of a series of active principles that are stored in different leaf and flower structures, and that have applications in medicine, the food and aromatic industries, and agriculture.

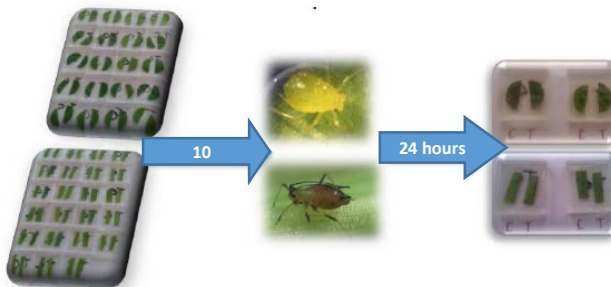
## AROMATIC PLANT EXTRACTS

Four aromatic plants (Oregano, Thyme, Chrysanthemum and Savory) were studied in the project. Environmentally sustainable extraction processes were carried out in order to obtain a combination of extracts, whose insecticidal and antifungal capacity was evaluated in vitro. The results are decisive to be able to decide the mixture of extracts that will be used in the formulations and at what doses they should be added.

Laboratory results:

- ❑ The selected mixture showed bactericidal activity, evidencing an inhibition capacity of 60% and 40% at doses of 10% and 5%.
- ❑ There was no evidence of activity against insects in vitro, although it was later seen in field trials.

An extraction process with environmentally sustainable solvents was developed and the main parameters were optimized.



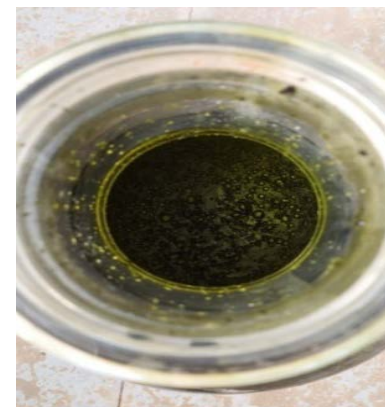
Activity tests against insects:

Insects are selected for the economic importance of their incidence, their transmission capacity and their availability and ease of breeding and maintenance in the laboratory.



Antifungal activity assays:

Petri dishes infected with the species of fungi to be studied are used and different doses of the extracts are applied, in addition to the control without extract.



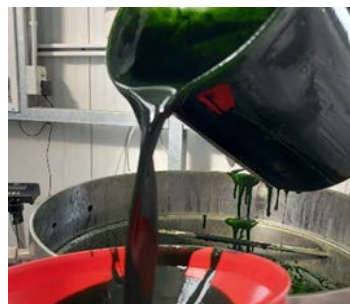
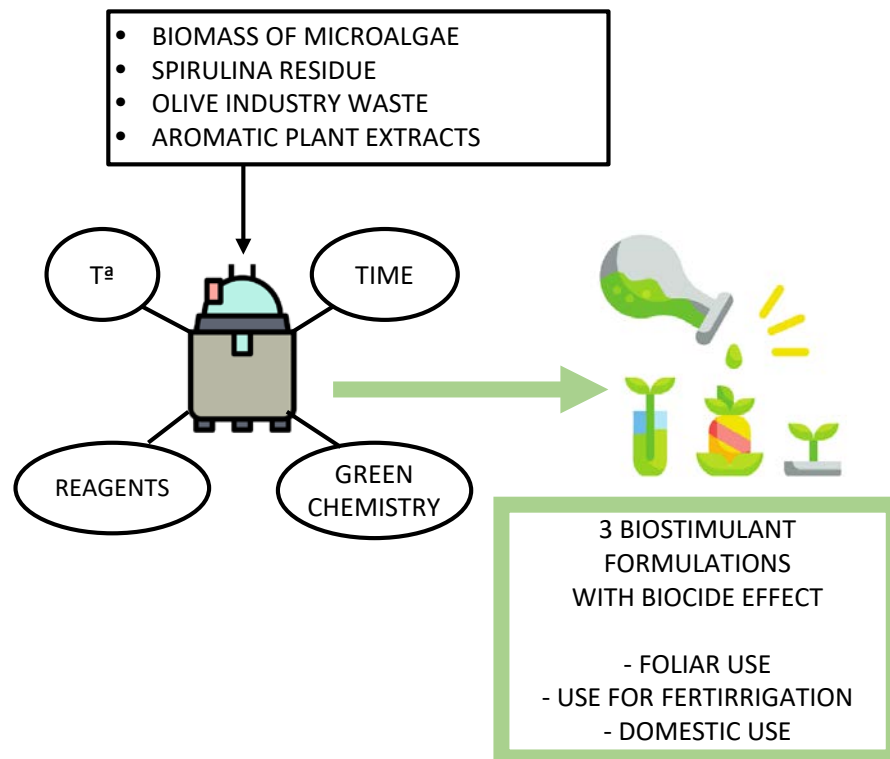


# BIOSTIMULANTS WITH BIOCIDAL ACTION IN THE PROJECT

The development of biostimulants during the project has been based on the following novelties:

- Using microalgae species that, although their efficacy as a biostimulant or biofertilizer has been demonstrated, are not the species typically used in commercial products
- Use residues from other processes (such as the residue from the extraction of the phycocyanin pigment from the Spirulina microalgae and residues from the olive industry), including the circular economy philosophy
- Include in the same product two concepts used separately: the biofertilizing capacity of microalgae and the biocidal capacity of extracts from aromatic plants

To achieve this, a new biotransformation process was developed that required the study of different parameters and at different scales, from the laboratory to a more industrial scale in order to produce the biostimulants necessary for field validation tests. What is achieved with this process is to treat the raw materials used to obtain a biostimulant with a high concentration of amino acids, oligosaccharides, trace elements and antioxidants, among other compounds, thanks to which the beneficial properties are increased.



## FIELD VALIDATION AND REPLICABILITY OF THE PROJECT

In order to validate the efficacy of the formulations developed from microalgae and aromatic plant extracts, field validation studies were carried out. The main crops studied were Tomato and Corn, with a total of 6 ha cultivated in the first trials carried out in 2020 and 2021.

The application mode of the formulations (liquid format) has been double:

- through localized irrigation in the roots by drip irrigation
- through foliar application

During the evolution of the crop, monitoring of the weather, the development and phenology of the plants, the health status and other possible effects of the applications per plot has been carried out.

CROP	YEAR	Ha Cultivated Formulated	Ha cultivated chemical fertilizers	Ha Formulated Replicability	Ha chemical replicability	Total ha Formulated	Total ha chemicals	TOTAL Ha
Tomato	2020	0,864	0,144	0	0	0,864	0,144	1,008
Corn	2020	0,864	0,144	0	0	0,864	0,144	1,008
Tomato	2021	0,8	0,2	0,8	0,2	1,6	0,4	2
Corn	2021	0,8	0,2	0,8	0,2	1,6	0,4	2
Tomato	2022	2,25	0,75	2,25	0,75	4,5	1,5	6
Corn	2022	2,25	0,75	2,25	0,75	4,5	1,5	6
Potat	2022	0,75	0,25	0	0	0,75	0,25	1
Pepper	2022	0,75	0,25	0	0	0,75	0,25	1
<b>TOTAL</b>		<b>9,328</b>	<b>2,688</b>	<b>6,1</b>	<b>1,9</b>	<b>15,428</b>	<b>4,5888</b>	<b>20,016</b>

It was possible to verify that the development of the plants has been significantly higher in the plots treated with the formulations, with a greater increase in the efficiency in the foliar applications with the highest percentage of extract of aromatic plants. Improvements have been seen in the following parameters:

- ❑ TOMATO: 20% more gross yield increase and almost 30% more raw material
- ❑ TOMATO: increase in the average weight of the fruit with the foliar formulation
- ❑ CORN: increase of up to 30% in productivity
- ❑ CORN: increase in the average weight of the ears, both in foliar and drip treatments
- ❑ Control of pests / diseases: in the plots in which no phytosanitary treatment has been applied, the damage has not increased, with which the effect of the formulations can be deduced



## FIELD VALIDATION AND REPLICABILITY OF THE PROJECT

It is important that the results obtained during the validation in the field are transferable to other crops and to other regions or countries with small changes in the planning of the applications or in the fertilization plan. Transferability is the ability of an experience to allow the repetition of its essential elements in a context other than that of its creation, with a high probability of success.

Thus, through this replicability and transferability Plan, carried out mainly during 2022, it has been possible to demonstrate the beneficial influence of those formulated regardless of the crop or growing area. Therefore, in 2022 the results of the project were transferred to maize and tomato at an industrial level in Portugal and in Extremadura to other crops such as potatoes and peppers. Trials have been carried out in an area of 6 ha for tomato and maize crops, respectively, and 1 ha in each case for pepper and potato crops.

These actions were based on the results of the first field validations and in order to increase the economic sustainability of the formulations, it was decided to lower the dose of the extracts of aromatic plants. Since the best formulation tested had been the foliar one, it was decided to test this type of formulation with four variations: (LT1) Control, (LT2) Formulated without extract, (LT2) Formulated with low extract, (LT3) Formulated with high extract. In all of them the dose tested was 3l/ha applied at key moments for the crop according to phenology, growth, flowering, fruit setting and fruit growth.

- ❑ As a final result of the validation tests, it can be concluded that the crops have improved their yields when applying those formulated with microalgae and extracts of aromatic plants. The following graphs show the comparisons in kg/ha produced (in blue, the gross yield and in orange, the net yield).
- ❑ **As for avoiding pests and diseases, it can be stated that the formulations have proven to be effective**, since the application of pesticides of chemical origin has not been necessary during the crops.



POTATO



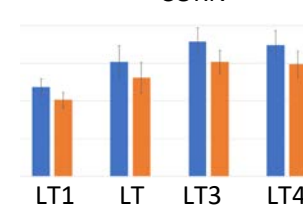
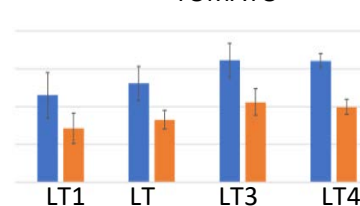
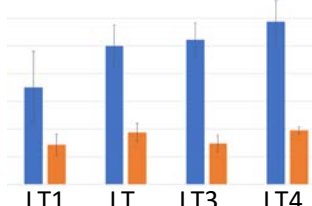
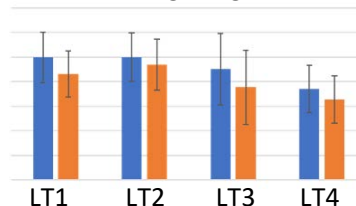
PEPPER



TOMATO



CORN



## ENVIRONMENTAL MONITORING

Chemical pesticides are one of the technological developments that made the so-called green revolution possible, and their use forms an important part of the agricultural practices that have supported it. On the one hand, it must be recognized that pesticides have made an important contribution to modern social development both from an economic point of view, since they have allowed a notable increase in world agricultural production, and from a health point of view, due to their application for the fight against disease vectors. But, on the other hand, chemical pesticides can be conceptualized as risk factors likely to negatively affect human health and the environment. Faced with this situation, it is necessary to reduce the amount of chemical products applied to crops and integrate alternative methods that allow good phytosanitary control and reduce environmental impacts.

During the project several natural formulations have been developed in which extracts of aromatic plants and microalgae are included in their formulation.

For all these reasons, it is important to carry out a direct evaluation of the contamination of the environment of the test fields used in the validation, through the identification and quantitative measurement of the pesticides to be replaced in samples of harvested raw material (corn and tomato), water and soil.

During the project, an evaluation of a series of environmental indicators, dependent on agronomic management, was carried out to establish the benefit of using the new formulations, in comparison with pesticides of chemical origin commonly used in tomato and corn crops. The aforementioned environmental benefit is based on the reduction of nitrate contamination of the soil solution, and on the reduction of pesticide residues of chemical origin in agricultural soil, infiltrated water and in the food produced.

As a result of the monitoring carried out, the following conclusions could be reached:

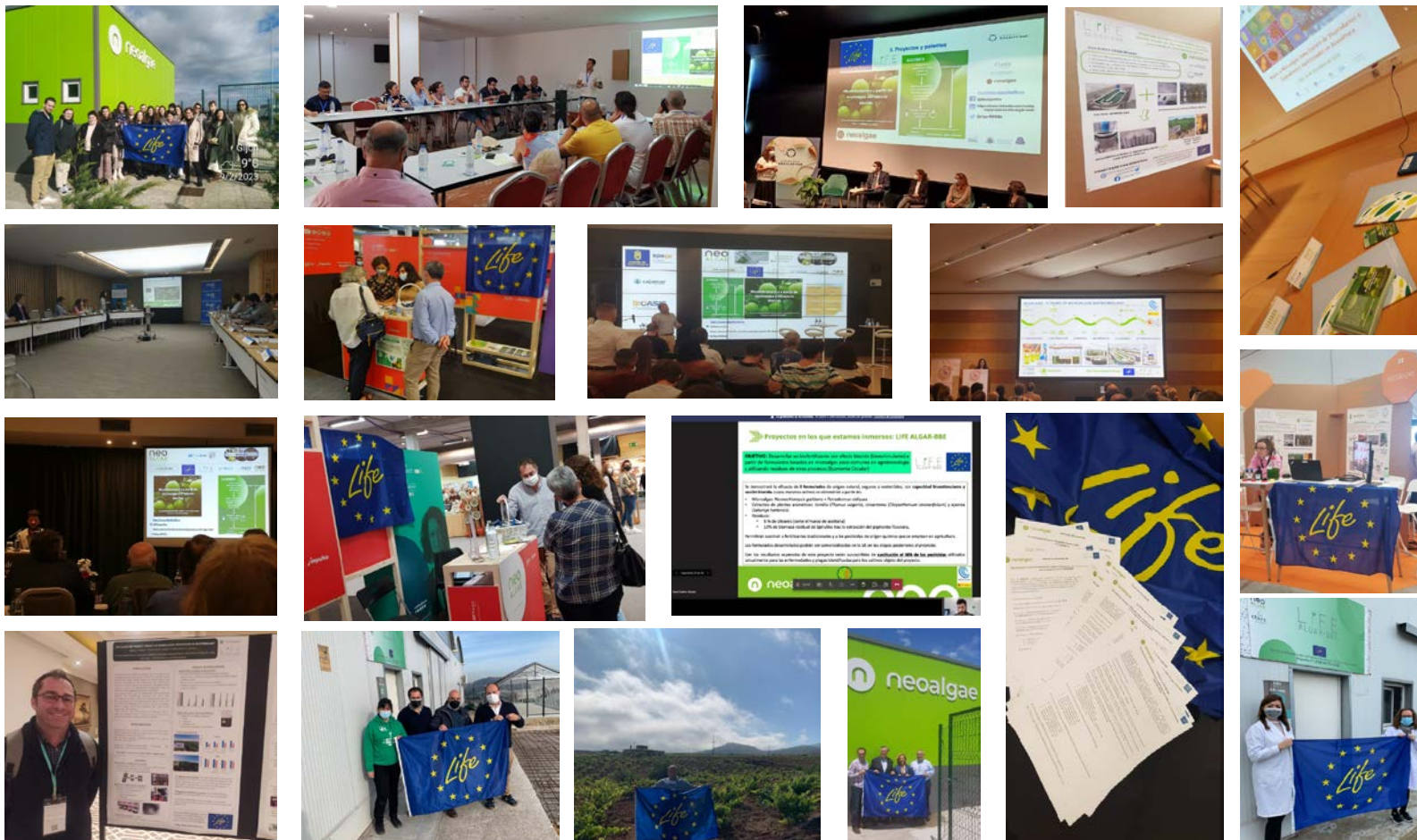
- ❑ The concentrations of nitrates in the soil solution decreased, and the reduction in nitrogen fertilization between the different theses during resulted in a decrease in nitrate concentrations.
- ❑ The use of the formulations developed in the project has led to a reduction in pesticide residues in the leachate water.
- ❑ In this project, a sustainable management has been achieved, attending exclusively to the surface of agricultural soil free of residues, which represents 93.2% with respect to the total surface tested
- ❑ No pesticide residues were detected in either the tomato or the corn produced in the campaigns studied, so the use of the active materials of chemical origin was correct, and the field application of the new formulations meets its objective from the food safety point of view



Different moments of environmental monitoring in agricultural trial plots

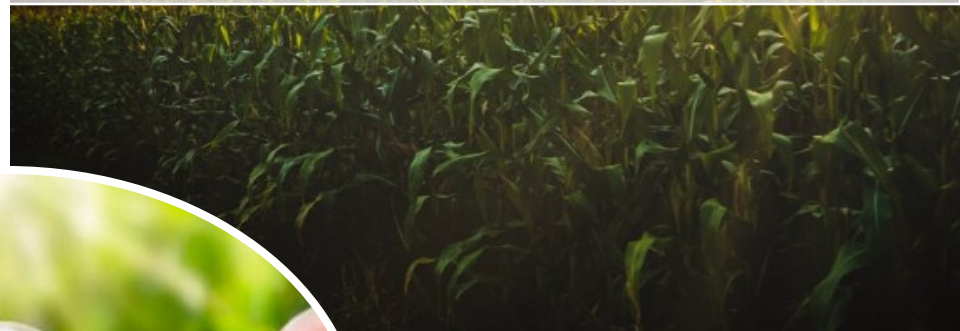
# DISSEMINATION AND DIFFUSION OF THE PROJECT

Throughout the project, dissemination activities have been constant and various actions have been carried out: visits to the plant, participation in different events (both online and in person), delivery of brochures and other project documentation, presentations at congresses, presentation of posters in congresses, conducting surveys, participation in fairs, appearance in the press and the media, newsletter dissemination, presence in social networks (Twitter, LinkedIn, website and Facebook), networking with other projects, etc. Below are examples of the activities carried out.





With the contribution of the LIFE financial instrument of the European Union  
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