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# INTERNSHIP REPORT

## The Impacts of "Effective Microorganisms" on Potted *Vicia faba* and a Fodder Crop Field


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Summary:

Microbe-biome based solutions have high potential in addressing biodiversity loss and greenhouse gas emissions induced by agricultural activities. “Effective Microorganisms” (EM) are one such example of microbial products that could enhance crop yield and quality. This report presents the initial phase of a project aiming to assess the potential of EM in agriculture through a pot experiment and a field experiment. The first was set up with a culture of *Vicia faba* and the second with a fodder crop consisting of oats *(Avena sativa)*, barley *(Hordeum vulgare)* and the leguminosa *Hedysarum coronarium.* We suggest different measurements to assess the effect of EM on biomass, yield and root structure for the crops.

Introduction:

Agricultural production systems are an important cause of greenhouse gas emissions worldwide through their use of synthetic fertilisers (IPCC, 2019 ; Ray et al., 2020). Unsustainable agriculture and land mismanagement are also a direct driver of land degradation and pollution, leading to subsequent biodiversity loss (IPBES, 2018). The need for a more sustainable agriculture that would be less reliant on fossil fuels for the production of fertilisers and more aware of ecological needs is therefore key in averting climate change disasters and loss of habitat.

Microbiome-based approaches for sustainable agriculture are a source of hope in tackling these issues (Naik et al., 2019 ; Ray et al., 2020). Microorganisms have indeed proved to be useful in enhancing crop resistance to abiotic stress, as well as improving plant and livestock health through the use of probiotics (Olmo et al., 2022). “Biostimulants” are a type of soil enrichment product that do not serve directly as a source of nutrition for the crop but instead help it in its absorption of nutrients or in its tolerance to stress and may enhance the quality of the crop (Castiglione et al., 2021). Microbial biostimulants are currently the only soil additive recognised at the European level that are based on microorganisms in the EU fertiliser regulation 2019/1009: the legally authorised substances listed for these biostimulants are limited to nitrogen-fixating bacteria and mycorrhizal fungi (Barros-Rodriguez et al., 2020). In the case of Italy, the “*decreto legislativo*” 75/2010 also recognises their existence and establishes a list of the officially accepted biostimulants contained in the *Registro dei fertilizzanti*, in particular those applicable to organic farming. They rely on mycorrhizal fungi and rhizobacteria known to have effects on plant growth (Ray et al., 2020 ; Olmo et al., 2022). Although an emphasis on safety should be kept at all times (Barros-Rodriguez et al., 2020), this taxonomic approach to the possible organisms contained in biostimulants is quite restrictive and may lead to a halt in innovation (Castiglione et al., 2021).

In order to test one such microbiome-based approach that is currently not covered in the legal description of biostimulants, we conducted two experiments to assess the impact of the commercial product “Effective Microorganisms” (EM) on plant growth. EM were first introduced in the 1990s (Higa and Parr, 1994) and are composed of photosynthetic bacteria (*Rhodopseumonas palustris* and *Rhodobacter sphaeroides*), lactobacilli useful in the fermentation process (*Lactobacillus plantarum*, *L. casei* and *Streptococcus lactis*), yeasts (*Saccharomyces spp.*) and actinomycetes (*Streptomyces spp.*) (Olle and Williams, 2013). EM do not fit into any category of the EU regulation 2019/1009 establishing the different types of soil fertiliser or in the Italian *decreto* 75/2010. However, they are believed to enhance crop quality and yield (Olle and Williams, 2013) and are an inexpensive type of microbiome-based approach potentially relevant for sustainable agriculture. We therefore wanted to test their effect on crops and assess their potential for sustainable agricultural practices.

Our experiments were conducted in Bronte, Sicily, as part of an ongoing project to support local farmers, carried out by the Giacche Verdi Bronte association and the Manfred-Hermsen-Stiftung foundation. There were two parts to the experiment: a field test of EM and a potted-plant experiment allowing for more control over conditions and enabling us to test a different concentration of the product. In the field experiment, the objective was to test the effect that EM might have on a fodder crop composed of a mixture of oats *(Avena sativa)*, barley *(Hordeum vulgare)* and a leguminosa species (*Hedysarum coronarium*). In the potted-plant experiment, the EM were applied to faba beans (*Vicia faba*). We considered the effect of the microbial product on plant biomass and yield as well as the plants' root systems. Indeed, in previous studies into biostimulant research, these variables are recurrent in assessing the effect of bacteria on plants (Hungria et al., 2010 ; Rondina et al., 2020).

Materials and methods:

The field experiment took place at Praca (37°757942′ N, 14°777765′ E) in the town of Bronte, Sicily, Italy, in the valley of the Simeto river, the area of the proposed UNESCO biosphere reserve "Terre della Biosfera". The field is composed of 8 terraces containing mostly olive and almond trees as well as a few citrus trees and other species (Fig. 1). It is regularly used as a site for grazing for the local farmers' livestock (cows and sheep). The terraces used for our experiment were terraces 1 and 2.

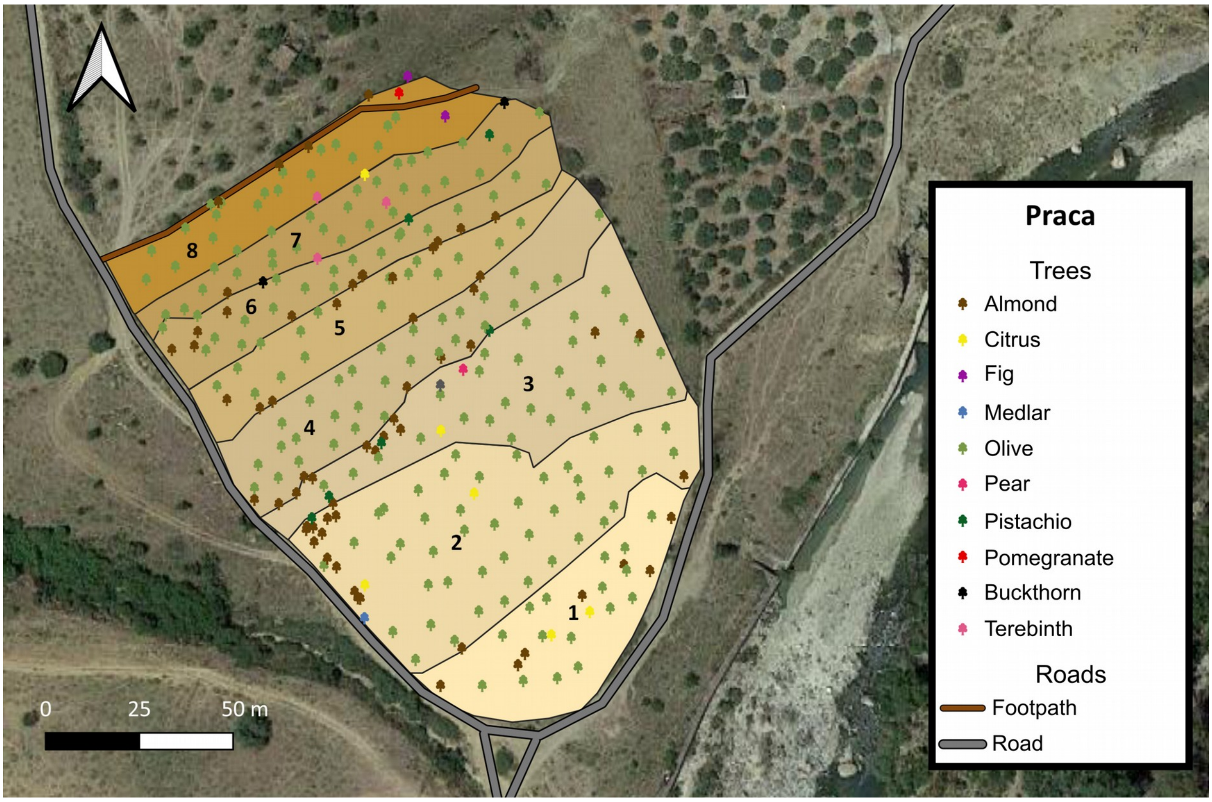


*Figure 1: Map of the Praca field with its eight terraces, its tree species and the paths leading to it.*

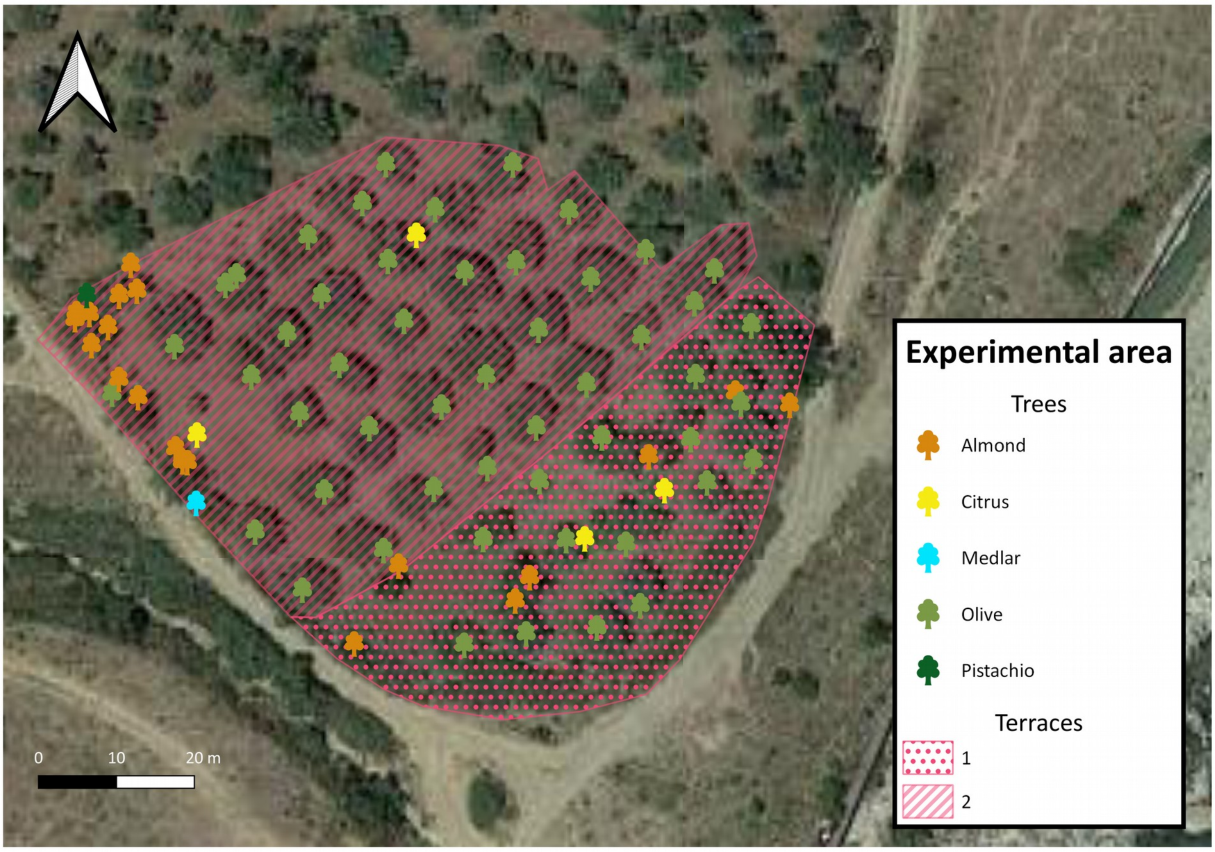


*Figure 2: Map of the area sowed with the fodder crop in terraces 1 and 2 used for the experiment, and the different tree species present.*

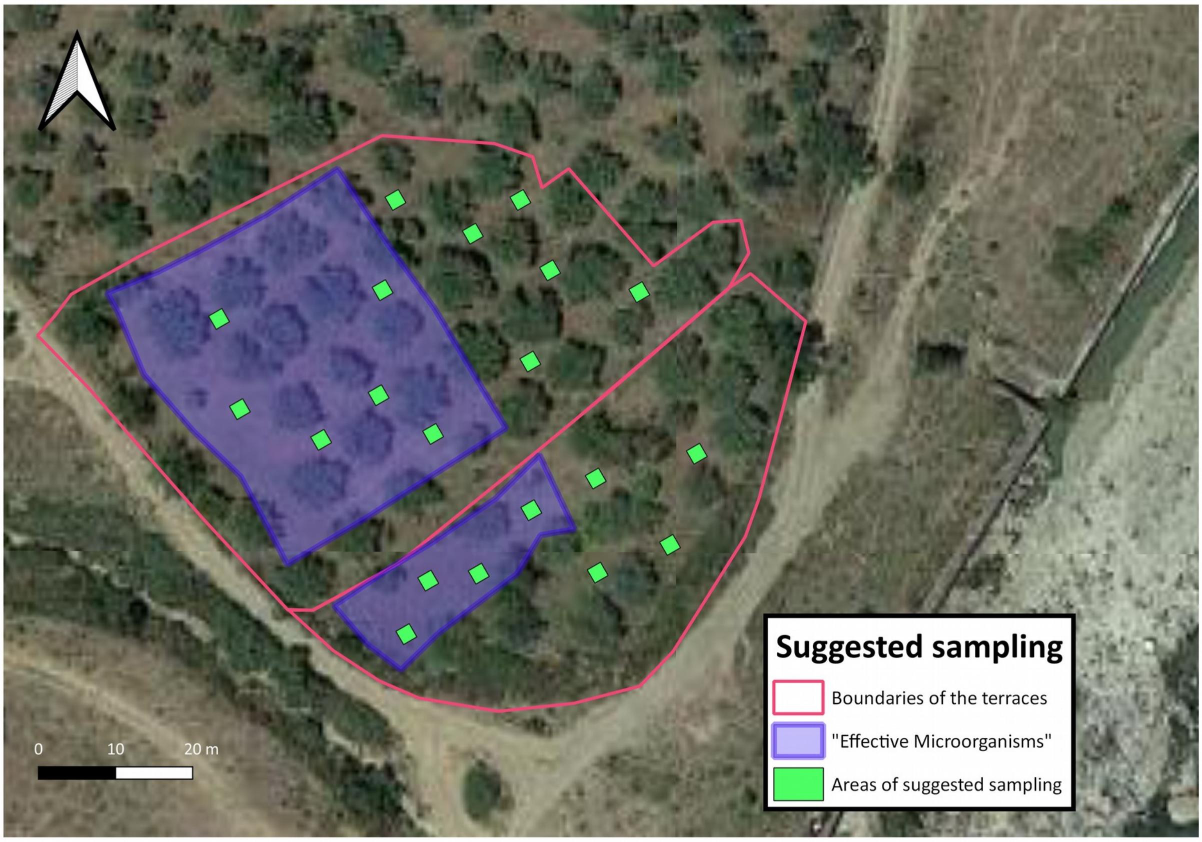


*Figure 3: Map of the areas sprayed with "Effective Microorganisms" (blue) within terraces 1 and 2 (red boundaries). The surfaces within the boundaries that are not coloured in blue are the control areas (fodder crop and no microorganisms). The green areas correspond to the suggested sampling spots for the future measurements to be conducted in 2023 (see p.7).*

The potted-plant experiment was conducted in a garden located in the town centre of Bronte (37°783241' N, 14°832654' E).

The fodder crop of the field experiment consisted of an equal proportion of *Avena sativa, Hordeum vulgare* and *Hedysarum coronarium* covering terraces 1 and 2 (Fig. 2). It was sowed on December 3rd 2022. The faba beans on the other hand were purchased from the Veredarredo & Co company in Bronte and were sowed on December 11th in 36 different bags with a surface area just under 200 cm² containing around 2 kg of soil collected from a several year-old pile of horse manure having undergone compostation, at La Difesa, Bronte (37°805396' N, 14°868860' E). One seed was inserted into each bag at around 3 cm of depth.

The "Effective Microorganisms" used in the experiment were purchased from the company EMIKO, under the product name EM1. On November 16th 2022, 600 ml of EM1 solution were added to water at a temperature of 34°C containing 600 ml of organic sugar cane treacle: the end product was 20 litres, kept at a constant temperature of 24°C. The pH of the final solution used was 4.1.

The EM were applied to the faba bean pots on December 11th with around 4 ml of the "activated" EM solution (water + treacle + EM1) added to each of the 18 bags subjected to microorganisms as in Cui et al.: these 4 ml were diluted in 80 ml of water and added to each bag and 80 ml of water were then added to the 18 control bags. Bags contained in crates 1 and 2 were submitted to microorganisms whereas crates 3, 4 and 5 act as control (Additional Photo 1).

The EM were applied to the field in Praca on December 14$^{th}$ in two separate areas of the field: one in terrace 1 covering around 310 m² and another in terrace 2 covering 1390 m² (Fig. 3). The concentration of the activated EM was of 170 litres per hectare in terrace 1 and 100 litres per hectare in terrace 2.

<u>Suggested measurements:</u> *to be conducted in 2023*

For the potted plants, all plants having germinated could be considered. The following measurements could be carried out between April and May 2023:

- growth variables should be assessed just before harvest (~April): stem diameter (at the base of the plant), number of branches and plant height (Cui et al., 2020) can be measured.
- biomass parameters should be assessed at harvest (~April/May): they should be separated between overall aboveground biomass and underground biomass (the whole underground root system should be thoroughly cleaned), and the aboveground weights should also be separated between leaf, stem (Cui et al., 2020) and fruit weights for each plant (Hungria et al., 2010). The dried weight should be established after the plant parts are submitted to a temperature between 60 and 70 °C until they reach constant weight, which can take up to 48-72h (Hungria et al., 2010 ; Cui et al., 2020 ; Rondina et al., 2020).
- measures of yield conducted at harvest: the number of pods per plant, the number of seeds per pod and finally the weights of the seeds should be assessed (Alharbi and Adhikari, 2020).
- root system parameters should be assessed at harvest, before drying: the number of root nodules per plant, the root system volume (roots are submerged in a large container with water) and the total length of the roots following the modified line-intersection technique (Tennant, 1975) could be assessed after filtering roots through a 0.5 mm sieve (Rondina et al., 2020).

For the field project, 10 replicate areas of around 2 m x 3 m (Hungria et al., 2010) should be randomly selected in each of the two terrace areas submitted to EM, and each of the two terrace areas used as control without EM (Fig. 3). The following measurements could be carried out between March and June:

- species distribution: what are the proportions of oats, barley and *Hedysarum coronarium* in each area? Are other species present? This could be carried out by using the Braun-Blanquet scale for species cover in each replicate (Braun-Blanquet, 1932).

- yield measurements: as the covering crop's main purpose is to serve as fodder, a measurement of the overall aboveground dry weight of the plants in the replicate areas seems appropriate. As extra information, the quantity of seeds of a few of the oats and barley and their weight could serve as information on oat and barley yield relevant for consumption (Drygas et al., 2021).
- root system parameters: these could be assessed as in the pot experiment on 4 random plants contained in each area for *Avena sativa, Hordeum vulgare* (no measurement of number of nodules) and *Hedysarum coronarium* (Rondina et al., 2020).

The GPS coordinates for the suggested sampling points are registered in the Giacche Verdi Bronte's Garmin 62s GPS. They were taken on 20th December 2022. The corresponding names for the points are as follows:

- SA1 to SA4: control points in terrace 1
- SA5 to SA8: EM treatment in terrace 1
- SB1 to SB6: EM treatment in terrace 2
- SB7 to SB12: control points in terrace 2

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## *Additional Photos*


*Photo 1: Bags containing the fava bean seeds. Crates 1 and 2 contain the bags submitted to EM while the others are control.*

*Photo 2: Preparation of the bags.*

*Photo 3 : The Praca field where the experiment was conducted with the covering crop subjected to EM.*

*Photo 4: Applying the EM in Praca.*