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Sustainable Methods Based on Microbial Biofertilizers and Plant Repellent Extracts in the Cultivation of *Aloe Vera*

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ABSTRACT

This work aimed to develop a biological cultivation and defense protocol that can be used by companies growing medicinal succulent plants. The protocol was characterized by the use of microbial biostimulants and plant extracts with repellent action (plant growth-promoting rhizobacteria, in particular, Effective microorganisms and extracts of neem, propolis, and horsetail) able to improve the growth and quality of Aloe vera plants especially for the production of gels for cosmetic and medicinal use. The experimental trial at Welcare Industries S.P.A. (Orvieto) showed a significant improvement of agronomic parameters analyzed on Aloe vera plants treated with Effective microorganisms and a significant reduction of the presence of mealybugs following treatments with repellents of plant origin. The application of symbiotic microorganisms and plant extracts for defense in agricultural operations can therefore ensure higher production standards, with a possible improvement in the agronomic quality of plants, also reducing the use of water and fertilizers. This experiment may be of particular interest to farms that want to focus on the production of succulents for medicinal and cosmetic use where plant quality levels must be high.

Keywords: *Medicinal plants, Sustainable agriculture, Effective microorganisms, Succulent plants, Microbic biofertilizers*

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INTRODUCTION

The name *Aloe vera* was used for the first time by Linnaeus, as *Aloe perfoliata var. vera* (1753). However, Linnaeus' description of *Aloe vera* as a separate species is after Miller's description of *Aloe barbadensis* (1768), although they are surely specimens of the same species (Reynolds, 1966). Moreover, the name barbadensis was given by Miller to specimens collected on the island of Barbados, in the Lesser Antilles, surely descendants of some Aloe plant left on the island by Portuguese or Spanish navigators more than two centuries before. For this reason, according to the rules about the attribution of names, it must be used the name *Aloe vera Miller*, (not: Linnaeus) (Bassetti & Sala, 2003).

Before the binomial classification introduced by Linnaeus, the species was called *Aloe vera vulgo* or *Aloe vera Vulgaris* sive *sempervivum marinum*, and later *Aloe foliis spinosis confertis dentatis*, *vaginantibus planis maculatis*. This indicates that the name vera is to explain that the plant was considered by the "vulgo" the true Aloe (Miladi & Damak, 2008).

In the world, Aloe is mainly cultivated in Africa, Australia, North America, Central America, South America, Russia, Japan. There is a large production in the Dominican Republic and Mexico. In Europe and the Middle East, *Aloe vera* is cultivated in Spain, Italy, Greece, and Israel (Marshall, 1990). The use of Aloe is very ancient, as witnessed by the cuneiform text of some clay tablets found at the end of the nineteenth century by a group of archaeologists in the Mesopotamian city of Nippur, near Baghdad, Iraq, and dated back to about 2000 BC. Aloe was also known and used by the Egyptians for embalming preparations (hence "plant of immortality") or the care and hygiene of the body or as a cicatrizant and mentioned several times in the Bible as an aromatic plant or for the preparation of ointments before burial (Boudreau & Beland, 2006). Plants over the millennia have developed associations and interactions with different groups of microorganisms in the soil (Cakmakci *et al*., 2007). These associations can be different depending on the areas that are colonized near the roots, in the surface areas, or even within the plant tissues. Interactions between plants and bacteria can be positive or negative and based on these interactions, it is possible to highlight if they are beneficial effects on soil fertility, growth, productivity, and plant defense (Glick, 1995). Many microbial agents can lead to plant death and have determined the development of defense strategies in plants to control pathogens (Elsharkawy *et al*., 2015). Several are the known microorganisms that can influence the life cycle of plants (rhizosphere bacteria such as Effective microorganisms, endo- and ectomycorrhizae such as *Glomus sp.,* biocontrol agents such as *Trichoderma sp.*) (Condor *et al*., 2007).

The stability of such positive plant-microbe relationships results in the development of eco-sustainable agroecosystems and the improvement of the quality of plant growth and development, allowing a possible reduction of synthetic fertilizers and pesticides.

Aims/objectives of the study

This work aimed to develop a biological cultivation and defense protocol that can be used by companies growing medicinal succulent plants. The protocol was characterized by the use of microbial biostimulants and plant extracts with repellent action (plant growth-promoting rhizobacteria, in particular, Effective microorganisms and extracts of neem, propolis, and horsetail) able to improve the growth and quality of *Aloe vera* plants especially for the production of gels for cosmetic and medicinal use.

Figure 1. Cultivation of the *Aloe vera* plants in Orvieto

MATERIALS AND METHODS

The experiments, started in March 2021, were conducted in the greenhouses of Welcare Industries S.P.A., Orvieto (TR), Umbria, Italy (42°13′N 12°6′E) on Aloe vera (7-8-year-old plants) **(Figure 1)**. The plants were placed in soil, 21 plants per thesis, divided into 3 replicas of 7 plants each. All plants were fertilized with "Grena olive special", 150 g per plant in April, June, and October. In the treated thesis the propogem products based on propolis 1% + equisetum 1% (7 treatments every 30 days) and neem extract 1% (7 treatments every 20 days) of the company La Praglia have been used to control mealybug **(Figure 2b)**. A conventional mineral oil-based product was used in the control. While the microbial biofertilizer based on Effective microorganisms (6 treatments) was purchased from the company Emagea.

Preparation/activation effective microorganisms (Ema)

Ingredients:

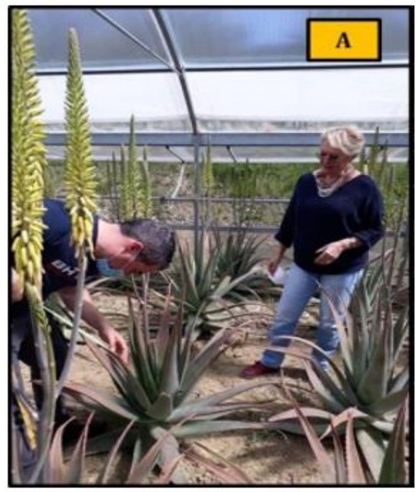
- 5% EM-1 (mother of microorganisms)
- 3% sugar cane molasses
- 50 L of water

Good quality water and work in aseptic conditions as possible. Use a fermenter (wine-beer) with aquarium heating element/serpentine. Fermenter with water at 30°C where molasses will be added, insert EM-1 and leave for 10 days at 30°C, degassing at least 2-3 times. This will yield 50L of Effective microorganisms. For the Aloe treatment 5L of Ema was taken from the 50L obtained, in 20L of water. From the 20L use 200ml x plant, once every 40 days. The remainder is kept in the fridge at 4°C and can be used every time for new multiplications with the procedure indicated above.

The experimental groups were:

- Group control (CTRL) irrigated with water, fertilized, and treated with mineral oil-based;
- Group with Effective microorganisms (EM) (peat 50% + pumice 50%) irrigated with water, fertilized, and treated with propolis, equisetum, and neem.

On November 8, 2021, leaves length **(Figure 2a)**, leaves width, pH gel, fresh leaves weight, fresh gel weight, optical density on gel nutrient and chemical composition were analyzed. In addition, the content of sugars, aloin, and proline, has been evaluated. 2 leaves per plant, 3 plants per treatment for the evaluation of sugars, proline, and aloin have been selected (Bates *et al.*, 1973). The presence of mealybugs was also evaluated on the leaves (total percentage of leaves per thesis in which the presence of the pathogen was detected).



a)

b)

Figure 2. Leaf length measurement (a) and treatments for mealybug control (b) with vegetal repellents on *Aloe vera* plants

Statistics

The experiment was done in a randomized complete block design. To analyze the collected data, one-way ANOVA was used through GLM univariate procedure, to assess significant ($P \leq 0.05$, 0.01, and 0.001) differences among treatments. Then, mean values were separated by LSD multiple-range test ($P = 0.05$). Graphics and statistics were supported by the programs Costat (version 6.451) and Excel (Office 2010).

RESULTS AND DISCUSSION

The experimental trial at Welcare Industries S.P.A. (Orvieto) showed a significant improvement of agronomic parameters analyzed on *Aloe vera* plants treated with Effective microorganisms and a significant reduction of the presence of mealybugs following treatments with repellents of plant origin. In particular, there was a significant improvement in leaf length and width, fresh weight of leaves, and gel. On the Em-treated gel, there was also a significant reduction in optical density (indicative of greater gel purity) and an increase in sugar content. In particular, there was a significant increase in the content of fructose, glucose, proline, and aloin. On the other hand, there were no significant differences between treatments in pH, soluble solids, and fiber content

In **(Table 1)**, there was a significant increase in leaf length in (EM) 54.86 cm compared to (CTRL) with 50.52 cm and leaf width 9.73 cm (EM) in comparison to 8.84 cm (CTRL) **(Figure 3)**. There was also a significant increase in leaf fresh weight, 382.93 g (EM) concerning 375.11 g (CTRL), and gel fresh weight 138.24 g (EM) in comparison to 132.03 of the untreated control **(Figure 4)**. In contrast, no differences were found in gel pH.

In **(Table 2)** there was a reduction in the optical density of the gel treated with Effective microorganisms 1.03 (abs) compared to the untreated control 1.09 abs, indicating higher purity. In terms of sugar content, the EM thesis was also better with 1336.59 mg/L compared to 1324.60 mg/L for the control. It was also shown that the treatment with plant repellents based on propolis, neem, and horsetail significantly reduced the presence of mealybug on the leaves, 9.56% (EM), compared to 12.61% (CTRL). There were no significant differences in soluble solids and fiber in the two treatments.

All treatments significantly increased fructose, glucose, proline, and aloin content compared with the untreated control **(Table 3)**. Specifically concerning fructose, the thesis (EM) with 80.75 (mg (g DW)$^{-1}$ was better than (CTRL) with 69.89 (mg (g DW)$^{-1}$. Also for glucose the thesis (EM) with 34.35 (mg (g DW)$^{-1}$ was better than (CTRL) with 32.24 (mg (g DW)$^{-1}$. The thesis (EM) also increased proline content with 0.63 (mg (g DW)$^{-1}$ compared to the untreated control 0.52 (mg (g DW)$^{-1}$ and aloin where the thesis (EM) with 147.01 (mg (g DW)$^{-1}$ was superior to (CTRL) with 143.12 (mg (g DW)$^{-1}$.

The rhizosphere is the fraction of soil that surrounds plant roots (Kennedy, 1999). This definition was introduced by the German agronomist Lorenz Hiltner in 1904. Today we know that in the rhizosphere lives a wide variety of microorganisms, fungi, bacteria, which can be useful or harmful for plant growth (Ortas *et al*., 2001). Despite the positive progress in the scientific field, the aspect and the role of 90% of the microbial species present in the soil is still unknown. The rhizosphere is undoubtedly actively influenced by the plant. Plant roots continually excrete a complex mixture of substances that can locally alter the soil (water and pH content, oxygen) and better capture nutrients. Shoots secrete up to 40% and adult plants up to 20% of their fixed carbon into the rhizosphere, increasing the viability of microbial colonies in the rhizosphere. The microbial flora around the plant root differs significantly from that of the surrounding soil in both its composition and activity (Apte & Thomas, 1997).

PGPR (Plant Growth-Promoting Rhizobacteria) represents a group of bacteria of the rhizosphere able to stimulate plant development, both by improving mineral nutrition and by producing biocontrol factors (Prisa, 2018). They include bacteria belonging to different genera: *Acinetobacter, Alcaligenes, Arthrobacter, Azospirillum, Azotobacter, Bacillus, Burkholderia, Enterobacter, Erwinia, Flavobacterium, Proteus, Pseudomonas, Rhizobium, Serratia, Xanthomonas.* Stimulation of plant growth occurs through direct and indirect mechanisms (Kawalekar, 2013; Olle, 2014).

PGPRs can act directly on the nutrients present in the soil making them available to plants, thus performing a biofertilizer action (Kour *et al*., 2019). Nitrogen, phosphorus, potassium, and iron are indispensable elements for plant survival and growth. Although they are particularly abundant in the soil, very often they are not directly usable by plants, as they are present in a non-assimilable form. The metabolic activity of numerous bacteria in the rhizosphere converts these molecules into soluble forms (Kumar *et al*., 2016). Direct mechanisms include nitrogen fixation, phosphorus solubilization, production of phytohormones (auxins, cytokines, and gibberellins), and production of siderophores (Nielsen *et al*., 2002). In this test, plants treated with (EM) showed significant improvement in agronomic parameters of *Aloe vera* plants and gel quality characteristics. In particular, there was a significant increase in sugars, fibers, and an improvement in gel purity. This improvement in the quality of Aloe plants caused by the activity of microorganisms has also been observed in previous trials on other vegetable and ornamental species (Prisa, 2019a, 2019b; Prisa & Gobbino, 2021a, 2021b). These aspects are probably related to the microbial influence on the stimulation of root growth, the efficiency of nutrient assimilation by the plant, and the increased solubility of mineral elements in the medium (Rodriguez *et al.,* 2006; Thomas & Singh, 2019). It is also known that microorganisms can improve plant resistance to abiotic stresses, in particular water and nutrient stress (Yadav *et al*., 2017). Plants have developed during their evolution the most varied defense mechanisms against fungi, bacteria, viruses, insects, and also herbivorous animals. The chemical substances recognized by the plant and that induce its defense reactions are called inducers and promote in the plant defense mechanisms capable of protecting it from the attacks of pathogenic microorganisms, without producing hypersensitivity reactions. Moreover, inducers are also able to act even in absence of the pathogenic agent. Numerous molecules of biological origin are being tested: from proteins to lipids, up to polysaccharides.

Among the plant, metabolites have effect SAR salicylic acid, linoleic acid, active on potato against *Phytophthora infestans*, galacturonic acid and m-hydroxybenzoic acid on cucumber for anthracnose, jasmonates (including jasmonic acid and methyl jasmonate) active on oat powdery mildew, potato blight, and tomato (El-Seedi *et al.,* 2012). Laminarins selected extracts of algae, seem to have an interesting activity against *Botritys cinerea* and *Plasmopara viticola* on the grapevine, *P. herpotrichoides*, *Septoria tritici*, *Septoria nodorum*, *Erysiphe graminis*, *Fusarium roseum* and *Puccinia recondita* on wheat and *Phytophthora infestans* on potato and *Venturia inaequalis* on an apple tree (Caballero-Gallardo *et al.,* 2011). Extracts of ivy (*Hedera helix*) applied to apple and Cotoneaster plants seem to have a certain SAR activity against *Erwinia amylovora* agent of the bacterial fire blight of pome fruits (Frances & Wirtz, 2005). Oligosaccharins are a small group of oligosaccharides that cause hormonal effects on plants (Elmhalli *et al*., 2009). They are effective at nanomolar to micromolar concentrations thus giving them the status of phytoregulators (Feuerstein *et al*., 1986). The effects that some of these carbohydrates produce include stem elongation, stimulation of ethylene production, auxin inhibitory action, and finally stimulation of various defensive actions that the plant can put in place (Chang *et al.,* 2001).

Also in this trial, the use of propolis, neem extracts, and horsetail allowed a significant reduction of the presence of mealybug on *Aloe vera* leaves. An interesting aspect is that the pathogen can often be lethal for Aloe and other succulents. The repellent effects are certainly to be found in the very strong-smelling volatiles that can repel insects, their antibiotic, antifungal, and antiviral action, and their resistance-inducing action in plants.

Table 1. Characteristics of fresh *Aloe vera* leaves and gel

Groups	LL (cm)	LW (cm)	FLW (g)	FGW (g)	pH
CTRL	50,52 b	8,84 b	375,11 b	132,03 b	4,33 a
EM	4,86 a	9,73 a	382,93 a	138,24 a	4,23 a
ANOVA	***	***	***	**	ns

One-way ANOVA; n.s. – non significant; *,**,*** – significant at P ≤ 0.05, 0.01 and 0.001, respectively; different letters for the same element indicate significant differences according to Tukey's (HSD) multiple-range test (P = 0.05). Legend: (CTRL): control; (EM): Effective microorganisms; LL: leaves length; LW: leaves width; FLW: fresh leaves weight; FGW: fresh gel weight

Table 2. Chemical properties and mealybug detection of *Aloe vera* leaves

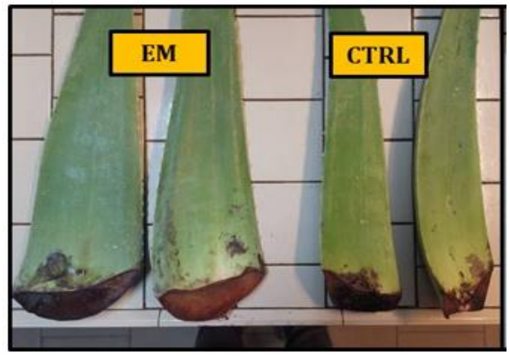
Groups	OD (abs)	SS (%)	S (mg/l)	FB (%)	MB (%)
CTRL	1,09 a	0,64 a	1324,60 b	0,08 a	12,61 a
EM	1,03 b	0,65 a	1336,59 a	0,07a	9,56 b
ANOVA	***	ns	***	ns	***

One-way ANOVA; n.s. – non significant; *,**,*** – significant at P ≤ 0.05, 0.01 and 0.001, respectively; different letters for the same element indicate significant differences according to Tukey's (HSD) multiple-range test (P = 0.05). Legend: (CTRL): control; (EM): Effective microorganisms; OD: Optical density; SS: Soluble solids; S: Sugars; F: Fibre; MB: mealybugs

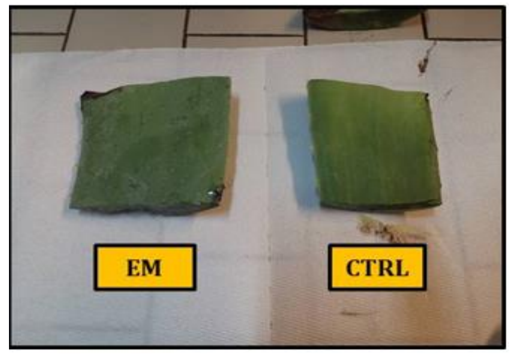
Table 3. Chemical properties of *Aloe vera* leaves

Groups	FR (mg (g DW)$^{-1}$	GL (mg (g DW)$^{-1}$	PR (mg (g DW)$^{-1}$	AL (mg (g DW)$^{-1}$
CTRL	69,89 b	32,24 b	0,52 b	143,12 b
EM	80,75 a	34,35 a	0,63 a	147,01 a
ANOVA	***	**	***	***

One-way ANOVA; n.s. – non significant; *,**,*** – significant at P ≤ 0.05, 0.01 and 0.001, respectively; different letters for the same element indicate significant differences according to Tukey's (HSD) multiple-range test (P = 0.05). Legend: (CTRL): control; (EM): Effective microorganisms; FR: Fructose; GL: Glucose; PR: Proline; AL: Aloin



a)



b)

Figure 3. Vegetative growth and width of *Aloe vera* leaves treated with Effective microorganisms

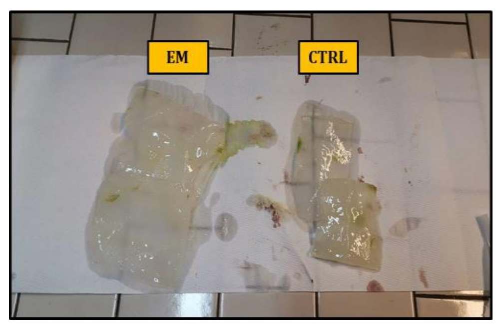


Figure 4. *Aloe vera* gel production with different treatments

CONCLUSION

The trial showed that the use of rhizosphere bio stimulating microorganisms can improve growth and leaf production in Aloe vera. In addition, the use of microorganisms can influence the quality of the gel produced by these plants and increase the presence of useful metabolites for cosmetic and pharmaceutical companies. The use of plant extracts in the defense of succulent plants can be a valid alternative to synthetic substances, especially in biological protocols. The repellent substances present in some plants can be used to control the presence of pathogenic insects, fungi, and viruses, strengthening plants in the presence of abiotic stresses such as the absence of water, saline soils, heat, and cold. The application of symbiotic microorganisms and plant extracts for defense in agricultural operations can therefore ensure higher production standards, with a possible improvement in the agronomic quality of plants, also reducing the use of water and fertilizers. This experiment may be of particular interest to farms that want to focus on the production of succulents for medicinal and cosmetic use where plant quality levels must be high.

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CONFLICT OF INTEREST: None

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ETHICS STATEMENT: None

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