Biological treatments for quality improvement and production of Aloe vera gel

Domenico Prisa ¹, * and Marco Gobbino ²

¹ CREA Research Centre for Vegetable and Ornamental Crops, Council for Agricultural Research and Economics, Via dei Fiori 8, 51012 Pescia, PT, Italy.
² Welcare Research srl, Via San Giovanni sul Muro 18, 20121 Milano, MI, Italy.

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Abstract

**Research goal:** The aim of this work was to develop a sustainable and innovative organic cultivation protocol, usable by local Italian companies, based on the use of microbial biostimulants (beneficial bacteria and fungi, arbuscular mycorrhizae and algae) able to improve the growth and quality production of the medicinal gel of Aloe vera.

**Materials and Methods:** The experiments, started in December 2020, were conducted in the greenhouses of CREA-OF in Pescia (Pt), Tuscany, Italy (43°54′N 10°41′E) on Aloe vera (4 year old plants). The experimental groups were: i) group control, irrigated with water and substrate previously fertilized; ii) group with Effective microorganisms irrigated with water and substrate previously fertilized; iii) group with Trichoderma spp. irrigated with water and substrate previously fertilized; iv) group with arbuscular mycorrhizae irrigated with water and substrate previously fertilized; v) group with Ascophyllum nodosum irrigated with water and substrate previously fertilized.

**Results and Discussion:** The experiment showed a significant improvement in agronomic parameters and physical, chemical and microbiological characteristics analysed on plants treated with microbial and algae-based biofertilizers. In particular, there was a significant improvement in the number of leaves per plant, new shoots, fresh vegetative weight, root and gel weight and the inflorescences number. On the leaves of the treated theses, there was a significant increase in leaf length and width and an improvement in gel pureness (optical density). There was also an increase in the number of microorganisms in the treated substrates and a lowering of the pH of the growing medium. The test also showed a lowering of the pH of the gel and a significant increase in soluble solids, sugars and fiber content in the theses inoculated with Effective microorganisms and a significant increase in fructose, glucose, proline and aloin.

**Conclusions:** The trial confirms the significant results already obtained in other trials on vegetable, ornamental and Aloe plants by applying biofertilizing microorganisms and algae. The aim of this work was to develop an organic cultivation protocol based on microbial and algae treatments that can be used to improve the quality of Aloe vera plants. This protocol can be applied in general by those companies that are dedicated to the production of ornamental and fruit cacti and succulents and want to reduce or even eliminate the use of plant protection products

**Keywords:** Sustainable agriculture; Beneficial microorganisms; Medicinal extracts; Succulent plants; Biofertilizers

1. Introduction

The name of the plant Aloe vera is derived from the Arabic word "Alloeh" and means "sparkling sour substance", while "Vera" is derived from Latin and means "genuine". Belonging to the Aloaceae family, it can reach 100 cm in height and...
The classification of Aloe is complex due to the extreme ease when blown around by the wind. Its growth mostly in waterless area or region of America, Europe, Asia, Africa and other warm area [5]. Aloe plants have more than 250 species but only two species now commonly, Aloe barbadensis Miller and Aloe arborescens are the most famous. The ideal growing temperatures are around 20-24°C. By their very nature, these plants are drought-tolerant, while they have no tolerance for waterlogging [6]. In their natural habitats they always grow on well-drained slopes and never at the bottom of valleys or in concave places. The pH of the soil should be slightly acidic and the irrigation water should have a very low sodium content [7].

1.1. Biofertilizers improve plants growth

The development of organic agriculture and the growing demand for healthy food and long-term sustainability has led to the increasing development of new alternatives to synthetic fertilizers [8]. The application of biofertilizers in plant breeding can result in numerous benefits for improving plant and soil quality and productivity, as they can enhance nutrient transfer from soil to plants, improve soil microbial biodiversity, stabilize soil aggregates, and reduce fossil fuel use [9]. algae possess several properties: increase soil porosity and production of adhesive substances that aggregate soil particles; presence of hormones (auxin, gibberellin), vitamins, and amino acids; ability to retain water in their gelatious structures; increase in biomass as a result of their desiccation and decomposition; decrease soil salinity; reduce weed development; and increase in soil phosphorus as a result of excretion of organic acids. Benefits from the use of biofertilizing algae have been reported in several cereal, vegetable, and ornamental crops [10].

1.2. Effective Microorganisms in sustainable agriculture

Effective micro-organisms are a commercial microbial selection containing a mixture of coexisting beneficial microorganisms collected from the natural environment. This selection was developed at the University of Ryukyus, Japan, in early 1980 by Prof. Teruo Higa. About 80 different microorganisms are able to positively influence the decomposing organic substance in such a way as to transform it into a process of “promoting life” [11].

The main species involved in EM include:

- Lactic acid bacteria – Lactobacillus plantarum, L. casei, Streptococcus lactis;
- Photosynthetic bacteria – Rhodopseudomonas palustris, Rhodobacter spaoeroides;
- Yeast – Saccharomyces cerevisiae, Candida utilis;
- Actinomycetes – Streptomyces albus, S. griseus;
- Fermenting fungi – Aspergillus oryzae, Mucor hiemalis.

EM is a fermented mixed culture of naturally occurring species of micro-organisms coexisting in an acid environment (pH less than 3, 5). Microorganisms in EM improve crop health and yield by increasing photosynthesis, producing bioactive substances such as enzymes and metabolites, accelerating the decomposition of organic materials and controlling soil diseases [12]. Effective micro-organisms can be used as herbal insecticides to control insects and pathogenic microorganisms and can also be used as plant growth inducers [13]. Soil micro-organisms have an important influence on soil fertility and plant health. EMs interact with the soil–plant ecosystem by controlling plant pathogens and disease agents, solubilizing minerals, increasing plant energy availability, stimulating the photosynthetic system, maintaining the microbiological balance of the soil, fixing biological nitrogen [14]. A characteristic of this mixture is the coexistence of aerobic and anaerobic micro-organisms. After Higa's research in Japan, the characteristics of EM have been studied in many countries [15,16]. Studies have shown positive effects of the application of EM on soils and plants on soil quality and nutrient supply, plant growth, crop yield and crop quality [17]. However, in some studies no positive effects were found [14].

The aim of this work was to develop a sustainable and innovative organic cultivation protocol, usable by local Italian companies, based on the use of microbial biofertilizers (beneficial bacteria and fungi, arbuscular mycorrhizae and algae) able to improve the growth and quality production of the medicinal gel of Aloe vera (Figure 1).
2. Material and methods

2.1. Greenhouse experiment and growing conditions

Material and methods The experiments, started in December 2020, were conducted in the greenhouses of CREA-OF in Pescia (Pt), Tuscany, Italy (43°54′N 10°41′E) on Aloe vera (4 year old plants). The plants were placed in ø 20 cm pots; 30 plants per thesis, divided into 3 replicas of 10 plants each. All plants were fertilized with a controlled release fertilizer (3 kg m3 Osmocote Pro®, 9-12 months with 190 g/kg N, 39 g/kg P, 83 g/kg K) mixed with the growing medium before transplanting. The experimental groups were:

- Group control (CTRL) (peat 50% + pumice 50%), irrigated with water and substrate previously fertilized;
- Group with Effective microorganisms (EM) (peat 50% + pumice 50%) irrigated with water and substrate previously fertilized, dilution 1:100 (1L of EM inoculum dilution 1:100 was used for each 10L of peat), treatment every 20 days;
- Group with Trichoderma spp. (TRICHO) (peat 50% + pumice 50%) irrigated with water and substrate previously fertilized, 1g of TNC TricorrP5 with every 5 litres of growing medium;
- Group with Arbuscular mycorrhizae (MICO) (peat 50% + pumice 50%) irrigated with water and substrate previously fertilized, 50g of TNC MycorrMax into every 15 litres of growing medium;
- Group with Ascophyllum nodosum (ASCO) (peat 50% + pumice 50%) irrigated with water and substrate previously fertilized (for the algae product, Ecoalga® was used added to the growing substrate), 100g of Ecoalga® into every 10 litres of growing medium;

The plants were watered 2 times a week and grown for 10 months. The plants were irrigated with drip irrigation. The irrigation was activated by a timer whose program was adjusted weekly according to climatic conditions and the fraction of leaching. On September 15, 2021, leaves number per plant, plantlets number per plant, inflorescences number, substrate microbial count, pH, fresh leaves weight, fresh gel weight, fresh roots weight, weight leaf length, leaf width and optical density on 5 leaves of the plant, gel nutrient and chemical composition were analyzed (Figure 2). In addition the content of sugars, aloin and proline, has been evaluated. 3 leaves per plant, 3 plants per treatment for the evaluation of sugars [18], proline [19] and aloin [20] have been selected.

2.2. Statistics

The experiment was carried out in a randomized complete block design. Collected data were analysed by one-way ANOVA, using GLM univariate procedure, to assess significant ($P \leq 0.05, 0.01$ and $0.001$) differences among treatments. Mean values were then separated by LSD multiple-range test ($P = 0.05$). Statistics and graphics were supported by the programs Costat (version 6.451) and Excel (Office 2010).
3. Results

The experiment at the CREA-OF greenhouse in Pescia showed a significant improvement in agronomic parameters and physical, chemical and microbiological characteristics analysed on *Aloe vera* plants treated with microbial and algae-based biofertilizers. In particular, there was a significant improvement in the number of leaves per plant, new shoots, fresh vegetative weight, root and gel weight and the inflorescences number.

On the leaves of the treated theses, there was a significant increase in leaf length and width and an improvement in gel pureness (optical density). There was also an increase in the number of microorganisms in the treated substrates and a lowering of the pH of the growing medium. The test also showed a lowering of the pH of the gel and a significant increase in soluble solids, sugars and fiber content in the theses inoculated with Effective microorganisms and a significant increase in fructose, glucose, proline and aloin.

Table 1 Evaluation of biofertilizer treatments on the agronomic characters of *Aloe vera*

| Group | Number of leaves per plant (n°) | Number of plantlets per plant (g) | Fresh leaf weight (g) | Fresh weight of roots (g) | Fresh gel weight (g) | Inflorescences number (n°) |
|-------|---------------------------------|----------------------------------|-----------------------|---------------------------|----------------------|---------------------------|
| CTRL  | 23.00 d                         | 3.20 c                           | 393.23 c              | 361.11 d                  | 129.37 e             | 0.40 b                    |
| EM    | 36.00 a                         | 7.20 a                           | 462.79 a              | 405.67 a                  | 154.93 a             | 1.40 a                    |
| TRICH | 26.00 c                         | 4.00 c                           | 397.95 c              | 376.71 c                  | 135.95 d             | 0.80 ab                   |
| MICO  | 26.00 c                         | 5.60 b                           | 415.26 b              | 393.18 b                  | 139.62 c             | 0.80 ab                   |
| ASCO  | 29.00 b                         | 5.60 b                           | 423.24 b              | 390.75 b                  | 143.60 b             | 0.80 ab                   |
| 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; (TRICH): *Trichoderma* spp.; (MICO): *Arbuscular mycorrhiza*; (ASCO): *Ascophyllum nodosum*

In *Aloe vera* there was a significant increase in the number of leaves per plant in (EM) 36.00, (ASCO) with 29.00 and (TRICH) and (MICO) with 26.00, compared to 23.00 in (CTRL). There was also a significant increase in the number of shoots per plant, 7.20 (EM), 5.60 (MICO) and (ASCO), compared to 4.00 in (TRICHO) and 3.20 (CTRL). In terms of vegetative weight, the (EM) thesis was the best with 462.79 g, followed by (ASCO) and (MICO) with 423.24 g and 415.26 g respectively and (TRICHO) with 397.95 g and (CTRL) with 393.23 g (Figure 2). The same trend for root weight where (EM) showed a weight of 405.67 g, (MICO) and (ASCO) 393.18 g and 390.75 g respectively, ((TRICHO) with 376.71 g followed by (CTRL) with 361.11 g (Figure 3-4). In terms of fresh gel weight, (EM) was the best thesis with 154.93 g, followed by (ASCO) with 143.60 g, (MICO) with 139.62 g, (TRICHO) with 135.95 g and (CTRL) with 129.37 g (Figure 5C-5D). There was also a positive increase in the number of inflorescences in the thesis treated with Effective microorganisms.

In terms of leaf characteristics of *Aloe Vera* (Table 2), thesis (EM) showed the leaves with the longest length 59.98 cm, followed by thesis (MICO) with 53.28 cm, (ASCO) with 50.21 cm, (TRICH) with 47.20 cm and (CTRL) with 46.16 cm (Figure 5A). In terms of leaf width, thesis (EM) was also the best with 13.69 cm, followed by (ASCO) with 12.59 cm, (MICO) with 11.79 cm and (TRICH) with 10.78 cm, and finally the (CTRL) with 10.52 cm (Figure 5B). The optical density value, which can be identified on the denaturation process of Aloe gel, showed a lower value in theses (EM), (ASCO) and (CTRL) with 1.033, 1.038 and 1.039 respectively. Higher values and thus more degradation in thesis (MICO) with 1.042 and (TRICH) with 1.056. The analysis of the culture medium showed a significant increase in the number of microorganisms in thesis (EM) 3.7x10³ (cfu/g), followed by (MICO) with 3.5x10³ (cfu/g), (TRICH) with 3.3x10³ (cfu/g), (ASCO) with 5.6 x 10² (cfu/g) and finally (CTRL) with 3.4 x 10² (cfu/g). There was also a lowering of the pH (5.9) of the substrate in the thesis treated with Effective microorganisms.
Table 2 Characteristics of fresh *Aloe vera* leaves and microbiological activity of the substrate

| Groups | Leaf length (cm) | Leaf width (cm) | Optical density (abs) | Microbial count (cfu/g) | Substrates pH |
|--------|-----------------|-----------------|-----------------------|------------------------|---------------|
| CTRL   | 46.16 d         | 10.52 d         | 1.039 bc              | 3.4 x 10^2 e           | 6.6           |
| EM     | 59.98 a         | 13.69 a         | 1.033 c               | 3.7 x 10^3 a           | 5.9           |
| TRICHO | 47.20 d         | 10.78 d         | 1.056 a               | 3.3 x 10^3 c           | 6.2           |
| MICO   | 53.28 b         | 11.79 c         | 1.042 b               | 3.5 x 10^3 b           | 6.3           |
| ASCO   | 50.21 c         | 12.59 b         | 1.038 bc              | 5.6 x 10^2 d           | 6.4           |

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; (TRICHO): *Trichoderma* spp.; (MICO): *Arbuscular mychorrhizae*; (ASCO): *Ascophyllum nodosum*.

Table 3 Chemical properties of *Aloe vera* gel

| Groups | Gel pH | Soluble Solids (%) | Sugars (mg/L) | Fibre (%) |
|--------|--------|---------------------|---------------|-----------|
| CTRL   | 4.2    | 0.73 b              | 1347.56 d     | 0.073 c   |
| EM     | 3.9    | 0.83 a              | 1404.89 a     | 0.080 a   |
| TRICHO | 4.1    | 0.72 b              | 1395.54 b     | 0.076 bc  |
| MICO   | 3.9    | 0.72 b              | 1394.43 bc    | 0.074 bc  |
| ASCO   | 4.2    | 0.74 b              | 1389.02 c     | 0.077 ab  |

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; (TRICHO): *Trichoderma* spp.; (MICO): *Arbuscular mychorrhizae*; (ASCO): *Ascophyllum nodosum*.

Table 4 Influence of biofertilizers on sugars, proline and aloin on plants of *Aloe vera*

| Groups | Fructose (mg (g DW)^{1} | Glucose (mg (g DW)^{1} | Proline (mg (g DW)^{1} | Aloin (mg (g DW)^{1} |
|--------|--------------------------|--------------------------|-------------------------|-----------------------|
| CTRL   | 77.69 d                  | 30.70 d                  | 0.57 d                  | 149.19 c              |
| EM     | 89.81 a                  | 36.88 a                  | 0.82 a                  | 161.00 a              |
| TRICHO | 71.11 e                  | 30.47 d                  | 0.62 c                  | 148.71 c              |
| MICO   | 85.51 b                  | 32.73 c                  | 0.74 b                  | 157.33 b              |
| ASCO   | 80.54 c                  | 33.23 b                  | 0.64 c                  | 157.37 b              |

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; (TRICHO): *Trichoderma* spp.; (MICO): *Arbuscular mychorrhizae*; (ASCO): *Ascophyllum nodosum*.

There was evidence of lower gel pH in the treated theses compared to the control, particularly in (EM) and (MICO) (Table 3). There were also significant differences in soluble solids with a higher value in (EM) with 0.73%, compared to (ASCO) with 0.74% and (CTRL) with 0.73% finally (TRICHO) and (MICO) with 0.72%. There was a significant increase in sugar content in Aloe leaves of the thesis (EM), 1404.89 mg/L, compared to (TRICHO) with 1395.54 g. (MICO) with 1394.43 g. (ASCO) with 1389.02 g and 1347.56 of the untreated control. There were no significant differences in leaf...
fiber content although the thesis (EM) shows a higher content. All treatments significantly increased the content of fructose, glucose, proline and aloin compared to the untreated control (Table 4). In particular, the Effective microorganisms thesis proved to have the greatest influence on the increase in these parameters. The control thesis was the worst.

**Figure 2** Treatments comparison in the vegetative growth of *Aloe vera*. Legend: (CTRL): control; (EM): Effective microorganisms; (TRICHO): *Trichoderma spp*.; (MICO): Arbuscular mychorryzae; (ASCO): *Ascophyllum nodosum*

**Figure 3** Effective microorganisms effect (EM) on roots growth of *Aloe vera*
4. Discussion

A number of plants and plant extracts are now being researched to prevent disease, with the aim of reaching a healthy old age. Various strategies are being adopted to prevent the onset of degenerative diseases and reduce the degree of suffering they entail, through diet, exercise and supplements [21].
Aloe vera has been used worldwide for 6000 years for its medicinal value, and has been used in various degenerative diseases, cancer treatment and AIDS. It is used in the treatment of wounds, inflammation, constipation and stress. It also has an antiseptic effect due to its bactericidal, virucidal and antifungal activity. Its nutraceutical properties have earned it much popularity worldwide, particularly for the many phytochemicals that have the power to improve human health. This succulent plant is also used to reduce indoor air pollution in homes, due to its ability to remove benzene, formaldehyde and the like [22].

Aloe vera is cultivated in the tropics and warmer regions of the world, including the southern United States, Mexico, Arabia and the west Indies, although today its cultivation is spreading to Europe and Italy because of its many properties [23]. It is propagated by means of suckers that develop at the base of the plant, tolerates fairly high pH values and grows fastest on fertile, water-rich soil. Flowering of the plants starts in mid-April and ends in mid-May with a peak observed in the last week of April. The fruiting season is observed between July and the end of August. It can be grown in full sun or light shade [24]. The seeds are delicate and should therefore be handled with care. There are many chemical constituents derived from Aloe vera such as: acidic galactan, arabinans, glucogalactomannan, glucomannan, polyuronide, cellulose, 7-hydroxylain, aloemodin, aloeaponarin I-II, aloin A and B (barbaloin), anthranol, beta barbaloin, chrysophanol, chrysophanol glucoside, isobarbaloin, capric acid, hexadecadienoic acid, palmitic acid, stearic acid, β-carotene, choline, folic acid, vitamin K, vitamin D, vitamin E, arginine, glutamic acid, magnesium, calcium, zinc, copper, amylase, catalase, echitamine, picrinine [3].

In this trial, the aim was to develop a biological protocol for the production of Aloe gel for medicinal use. In particular, it is shown that plants treated with Effective microorganisms and biofertilizers based on mycorrhizae, Trichoderma spp. and algae showed a significant improvement in the agronomic and qualitative parameters of Aloe vera plants and on the chemical and physical characteristics of the gel. In particular, a significant increase in sugars, fibers and a slowing down of the gel denaturation process was found. The improvement in the quality and growth of Aloe plants is particularly noticeable in those substrates where microorganisms were inoculated, which, with their mineral solubilization and pH lowering activity, allow a greater uptake of water and nutrients by the roots. Aspects also observed in previous trials on other plant and ornamental species and in Aloe vera.

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. It is also known that microorganisms can improve plant resistance to abiotic stresses, particularly water and nutrient stress [25]. In this experiment, soil microbial activity not only influenced the vegetative and root development of plants, but also the sugar and fiber content of leaves and the weight and purity of the gel. All these aspects can be related to the increase in the number of beneficial microorganisms in the treated theses, which by living close to the roots or within specialised cells are able to stimulate plant growth through various mechanisms. The best known mechanisms are: i) biofertilization, the ability of certain bacteria to make certain nutrients more available; ii) biocontrol, the activity of suppressing certain diseases through the use of certain bacteria; iii) induction of resistance, the ability of certain bacteria to stimulate plant defences; iv) production of phytohormones and signal molecules [26]. The rhizosphere is a complex habitat, where the plant through the exudates of its roots can promote microbial growth and metabolism. Soil microbial activity can also alleviate the negative effects of stress on plant growth and can stimulate the production of plant hormones and siderophores in plants, which can regulate plant metabolism and soil nutrient availability, particularly iron, copper, zinc and manganese [27]. In recent years, there has been a trend towards greater efficiency in the use of synthetic fertilizers and a return to the use of algal products to improve plant quality and nutrient utilization capacity. Since the 1950s, the use of algae has been superseded by the use of commercial extracts that provide useful molecules for plants. The effectiveness of algae as biofertilizers depends on the composition and concentration of compounds that can improve plant metabolism under stressful conditions [28]. Among the hormones most commonly found in algae extracts are cytokinins, auxins, gibberellins and abscisic acid, as well as other hormone-like substances [29]. In this trial, plants treated with Ascophyllum nodosum showed an increase in growth parameters and an improvement in the characteristics of the Aloe gel, confirming as in other trials that the use of biofertilizing algae can significantly influence and speed up the growth rate and quality of plants.

5. Conclusion

The application of biofertilizing microorganisms and algae to the Aloe vera growing medium has shown that the use of these products can significantly increase the vegetative and root growth of the plants and improve the quality of the leaf gel. It is also pointed out that the increase of microorganisms in the pot cultivation substrate, in particular the application of Effective microorganisms can influence the content of fibres, soluble solids, sugars and the reduction of the degradation process of the Aloe gel. The trial confirms the significant results already obtained in other trials on vegetable, ornamental and Aloe plants by applying biofertilizing microorganisms and algae. The aim of this work was
to develop an organic cultivation protocol based on microbial and algae treatments that can be used to improve the quality of *Aloe vera* plants. This protocol can be applied in general by those companies that are dedicated to the production of ornamental and fruit cacti and succulents and want to reduce or even eliminate the use of plant protection products.

**Compliance with ethical standards**

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**Disclosure of conflict of interest**

The author declares no conflict of interest.

**Statement of ethical approval**

The present research work does not contain any studies performed on animals/humans subjects.

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