

(RESEARCH ARTICLE)



## Microbic and Algae biofertilizers in *Aloe barbadensis* Miller

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### Abstract

The aim of this work was to develop an organic and sustainable cultivation protocol, based on the use of microbial biofertilizers (Plant Growth Promoting Rhizobacteria, *Trichoderma spp.*, arbuscular mycorrhizae and biostimulant algae) able to improve the growth and quality of *Aloe barbadensis* Miller plants. The experimental trial at CREA-OF in Pescia showed a significant improvement in the agronomic parameters analysed on *Aloe barbadensis* Miller plants treated with microbial and algae-based biofertilizers. In particular, there was a significant improvement in the number of leaves per plant, new shoots, vegetative fresh weight, root weight and gel weight. On the leaves in the treated theses, there was a significant increase in leaf length and width and an improvement in gel quality (optical density). The trial also showed a significant improvement in soluble solids, sugars and fibre content in the theses inoculated with microbial products and a significant increase in fructose, glucose, proline and aloin. These improvements in plant growth following the use of microbial biofertilizers and algae have also been found in previous trials in other vegetables and ornamental crops, but few trials have been carried out with Aloe. The application of symbiotic microorganisms in agricultural operations can therefore ensure higher production standards, with a possible improvement in the agronomic quality of the plants, while 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 ornamental and fruit cacti and succulents under organic farming methods.

**Keywords:** Succulent plants; Plant growth promoting rhizobacteria; Sustainable agriculture; Medicinal plants; Aloaceae

## 1. Introduction

### 1.1. Aloe characteristics

The botanical genus Aloe has always been classified in the Liliaceae family, although Tom Reynolds, a London-based researcher, has drawn up a new classification, Aloe has been placed in a new botanical family, the Aloaceae. Genus of evergreen, shrub-like, perennial and climbing plants with fat foliage and elongated flowers varying in colour from orange to scarlet [1,2]. The Aloaceae family comprises some three hundred and fifty plant varieties throughout the planet. In 1955, 132 species were listed in South Africa alone. We can distinguish three groups of Aloes: acauleas (without trunk), subcauleas (presence of visible but reduced trunk), cauleas (presence of extended and branched trunk) [3]. In the first group are contained the plants that do not have a trunk, or if present, it is very short, soft and thick, covered by the leaves arranged in a circular rose-like pattern, which rise from outside the basal stem. *Aloe barbadensis* Miller, *Aloe saponaria* and *Aloe aristata* belong to this first group. In the second group, *Aloe succotrina* and *Aloe chinensis* belong. The short, woody stem, which is easily visible, can reach a length of several tens of centimetres [4]. Finally, in

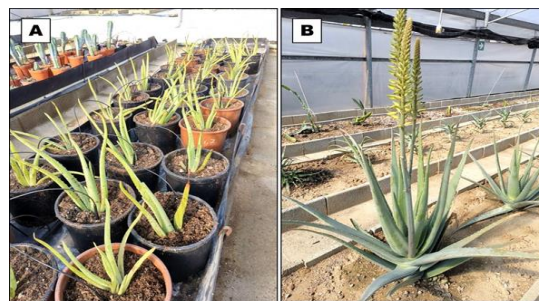
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the third group, in which the woody trunk branches out to form bushy shrubs that reach heights of up to several metres, species such as *Aloe ferox* and *Aloe arborescens* belong. The botanical classification of Aloe is complex due to the extreme ease with which these plants hybridize; it is believed that there are over 300 species, with at least 300 natural hybrids [5]. The leaf of the Aloe plant is structured with an outer covering, called the peel, which is about 2 millimetres thick and consists of elastic, impermeable, light green membranes that surround the pulp, a transparent, gelatinous, colourless mass that is very rich in nutrients. Aloe plants reproduce by means of pollination, which takes place thanks to birds and insects; this is because the plant is unable to fertilize itself independently and must receive pollen from another plant, so the fertilized flower matures, loses its petals and turns into a fruit that will later release its seeds, which are then blown around by the wind [6]. The Aloe plant also reproduces by means of stolons or shoots that grow at the base of the main plant, forming colonies. Areas intended for commercial cultivation of *Aloe vera* must have a cold climate, where the temperature during the winter does not fall below 8°C. 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. 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].

## 1.2. Microorganisms in sustainable agriculture

The microbiological part of the rhizosphere performs various functions to ensure plant life. Various rhizobacteria improve growth and resistance to diseases and are called PGPR (plant growth promoting rhizobacteria), microorganisms that through the synthesis of certain substances allow an increase in the vegetative and root development of plants; they are classified as biofertilizers or bioprotectors [8,9]. These microorganisms are able to promote plant growth by improving nutrient uptake by the roots following hormonal stimulation. The bacteria that colonise the plant rhizosphere are important because they have the ability to promote growth by improving nutrient cycling and reducing the use of chemicals [10,11]. Many rhizosphere bacteria are used extensively as biofertilizers in organic agriculture [12], and are well known and commercially exploited by governmental and private organizations worldwide [13]. Fungi residing in various plant surfaces (roots, leaves, stem, rhizosphere and phyllosphere) are also used for their ability to support plant growth promotion by activating mechanisms critical for plant development, resistance to diseases or abiotic stresses [14]. Interactions between plants and fungi in the rhizosphere and phyllosphere are able to promote plant development and the induction of resistance (ISR) to pathogens. A large number of fungi from different habitats have the ability to enhance plant growth promotion [15]. Important fungal genera recorded to have PGPF traits are *Aspergillus*, *Fusarium*, *Penicillium*, *Pyriformospora*, *Phoma*, *Trichoderma* and many others [16]. Mycorrhizae are fungi that can perform symbiotic activities with plant roots, improving water and nutrient uptake [17]. The mycorrhizosphere represents a significant environmental niche for various microbial communities. It is currently known that the density of bacteria in the mycorrhizosphere is higher (4–5 times) than in the plant rhizosphere. Arbuscular mycorrhizal association (AM) is the vital mutualistic interaction that has a significant beneficial impact worldwide, and more than 65% of known terrestrial plants have this association [18]. Plant-associated AM fungi have played a crucial role in plant evolution [19]. The aim of this work was to develop an organic and sustainable cultivation protocol, based on the use of microbial biostimulants (Plant Growth Promoting Rhizobacteria, *Trichoderma spp.*, arbuscular mycorrhizae and biostimulant algae) able to improve the growth and quality of *Aloe barbadensis Miller* plants (Figure 1).



**Figure 1** Cultivation in pots (A) and details of the *Aloe barbadensis Miller* plants (B)

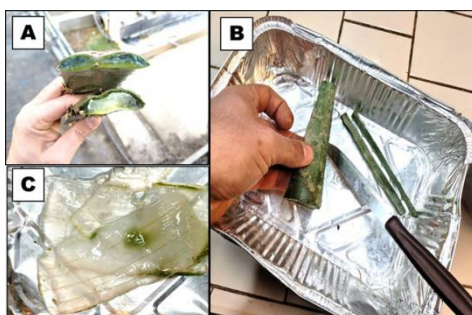
## 2. Material and methods

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 barbadensis Miller* (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 m<sup>-2</sup>

<sup>3</sup> 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 Tricorr<sup>PS</sup> 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 Mycorr<sup>Max</sup> 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 4 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 March 16, 2021, number of leaves per plant, number of plantlets per plant, fresh leaf weight, fresh gel weight, fresh root 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 [20], aloin [21] and proline [22], has been evaluated. 3 leaves per plant, 3 plants per treatment for the evaluation of sugars, proline and aloin have been selected.



**Figure 2** Field selection of *Aloe barbadensis* Miller leaves (A), cutting (B) and gel production (C)

### 2.1. Statistics

The experiment was carried out in a randomized complete block design. Collected data were analyzed 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 and discussion

The experimental trial at CREA-OF in Pescia showed a significant improvement in the agronomic parameters analysed on *Aloe barbadensis* Miller plants treated with microbial and algae-based biostimulants. In particular, there was a significant improvement in the number of leaves per plant, new shoots, vegetative fresh weight, root weight and gel weight. On the leaves in the treated theses, there was a significant increase in leaf length and width and an improvement in gel purity (optical density). The trial also showed a significant improvement in soluble solids, sugars and fibre content in the theses inoculated with microbial products and a significant increase in fructose, glucose, proline and aloin.

In (Table 1), in *Aloe barbadensis* there was a significant increase in the number of leaves per plant in (EM) 20.20, (MICO) with 19.80 and (ASCO) with 19.20 compared to (TRICHO) with 17.80 and to (CTRL) with 16.60. There was also a significant increase in the number of shoots per plant, 2.20 (EM), 1.40 (MICO), 1.00 (ASCO), compared to 0.80 in (TRICHO) and (CTRL).

In terms of vegetative weight, thesis (EM) was the best with 398.02 g, followed by (MICO) with 384.32 g and (ASCO) 382.22 g succeeded by (TRICHO) with 378.10 g and (CTRL) with 369.39 (Figure 3). The same trend for root weight where (EM) showed a weight of 373.87 g, (ASCO) with 369.36 g and (MICO) with 367.55 g followed by (TRICHO) with 356.60 g and (CTRL) with 355.19 g (Figure 4). In terms of fresh gel weight, (EM) was the best thesis with 143.29 g which was succeeded by (ASCO) with 138.61 g, (MICO) with 134.98 g, (TRICHO) with 131.75 g and (CTRL) with 126.53 g.

In terms of leaf characteristics of Aloe Vera (Table 2), thesis (EM) showed the leaves with the longest length 54.81 cm, followed by thesis (MICO) with 50.66 cm, (ASCO) with 44.61 cm, (TRICHO) with 41.65 cm and (CTRL) with 44.42 cm (Figure 5). In terms of leaf width, thesis (EM) was also the best with 10.26 cm, succeeded by (ASCO) with 9.31 cm, (MICO) with 9.00 cm and (TRICHO) with 8.84 cm, and finally the (CTRL) with 8.18 cm. The optical density value, which can be identified on the denaturation process of Aloe gel, showed a lower value in the theses (EM), (MICO) and (ASCO) with 1.027, 1.028 and 1.031 respectively. Higher values and thus more degradation in thesis (TRICHO) with 1.036 and (CTRL) with 1.043. There were no significant differences in gel pH in the treated theses compared to the control (Table 3). However, there were significant differences in soluble solids with a higher value in (EM) with 0.73%, compared to (MICO) and (ASCO) with 0.72% and 0.71% followed by (TRICHO) and (CTRL) with 0.70%. There was a significant increase in sugar content in Aloe leaves of thesis (EM), 1394 mg/L, compared to (MICO) with 1356.40 g, (ASCO) with 1351.41 g, (TRICHO) with 1338.22 g and 1322.61 of the untreated control. No significant differences existed in the fibre content of the leaves. All treatments significantly increased the fructose, glucose, proline and aloin content compared to the untreated control. Among the treatments Effective microorganisms was the thesis with the highest significant finding, while the treatment (TRICHO) was the worst (Table 4).

**Table 1** Evaluation of biostimulant treatments on the agronomic characters of *Aloe barbadensis* Miller.

Groups	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)
CTRL	16,60 c	0,80 bc	369,39 d	355,19 c	126,53 e
EM	20,20 a	2,20 a	398,02 a	373,87 a	143,29 a
TRICHO	17,80 b	0,80 c	378,10 c	356,60 c	131,75 d
MICO	19,80 a	1,40 b	384,32 b	367,55 b	134,98 c
ASCO	19,20 a	1,00 bc	382,22 b	369,36 b	138,61 b
ANOVA	***	***	***	***	***

One-way ANOVA; n.s. – non significant; \*, \*\*, \*\*\* – significant at  $P \leq 0.05$ , 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 mychorryzae; (ASCO): *Ascophyllum nodosum*

**Table 2** Characteristics of fresh *Aloe barbadensis* Miller leaves.

Groups	Leaf length (cm)	Leaf width (cm)	Optical density (abs)
CTRL	44,42 c	8,18 d	1,043 a
EM	54,81 a	10,26 a	1,027 cd
TRICHO	41,65 d	8,84 c	1,036 b
MICO	50,66 b	9,00 bc	1,028 cd
ASCO	44,61 c	9,31 b	1,031 c
ANOVA	***	***	***

One-way ANOVA; n.s. – non significant; \*, \*\*, \*\*\* – significant at  $P \leq 0.05$ , 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 mychorryzae; (ASCO): *Ascophyllum nodosum*

**Table 3** Chemical properties of *Aloe barbadensis* Miller gel

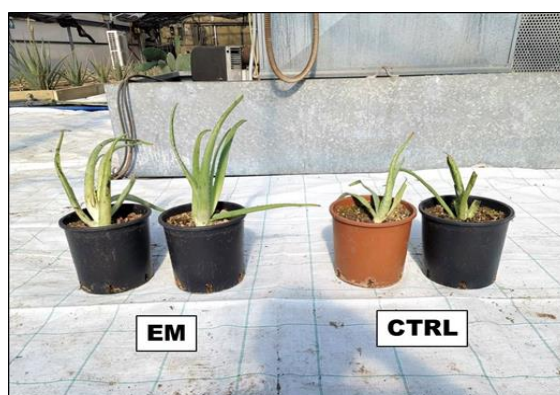
Groups	Ph	Soluble Solids (%)	Sugars (mg/L)	Fibre (%)
CTRL	4,44 a	0,70 c	1322,61 e	0,070 a
EM	4,42 ab	0,73 a	1394,00 a	0,072 a
TRICHO	4,40 b	0,70 c	1338,22 d	0,070 a
MICO	4,44 a	0,72 b	1356,40 b	0,073 a
ASCO	4,42 ab	0,71 b	1351,41 c	0,073 a
<i>ANOVA</i>	*	***	***	ns

One-way ANOVA; n.s. – non significant; \*, \*\*, \*\*\* – significant at  $P \leq 0.05$ , 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 mychorryzae; (ASCO): *Ascophyllum nodosum*

**Table 4** Influence of biostimulants on sugars, proline and aloin on plants of *Aloe barbadensis* Miller.

Groups	Fructose (mg (g DW) <sup>-1</sup> )	Glucose (mg (g DW) <sup>-1</sup> )	Proline (mg (g DW) <sup>-1</sup> )	Aloin (mg (g DW) <sup>-1</sup> )
CTRL	77,62 d	30,61 d	0,57 d	147,66 e
EM	86,86 a	35,80 a	0,78 a	157,23 a
TRICHO	77,13 d	30,73 cd	0,65 c	149,52 d
MICO	82,07 b	31,53 bc	0,72 b	152,67 c
ASCO	80,07 c	32,14 b	0,69 bc	154,60 b
<i>ANOVA</i>	***	***	***	***

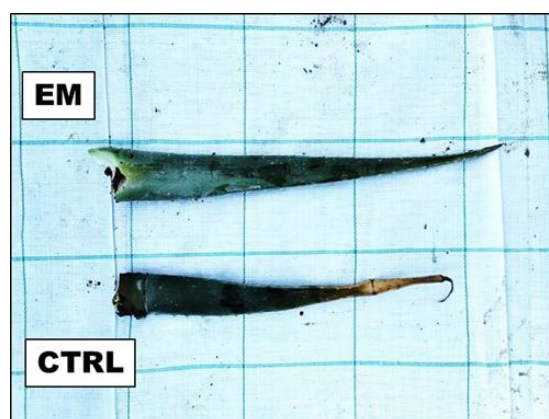
One-way ANOVA; n.s. – non significant; \*, \*\*, \*\*\* – significant at  $P \leq 0.05$ , 0.01 and 0.001, respectively; different letters for the same element indicate significant differences according to Tukey's (HSD) multiple-range test ( $P = 0.05$ ). Legend: (CTRL): control; (EM): Effective microorganisms; (TRICHO): *Trichoderma spp.*; (MICO): Arbuscular mychorryzae; (ASCO): *Ascophyllum nodosum*

**Figure 3** Comparison between Effective microorganisms (EM) and control (CTRL) on vegetative growth of *Aloe barbadensis* Miller





**Figure 4** Effect of *Arbuscular mycorrhizae* (MICO) on roots growth of *Aloe barbadensis* Miller



**Figure 5** Effect of Effective microorganisms on length leaves of *Aloe barbadensis* Miller

#### 4. Discussion

The rhizosphere is a complex habitat, where the plant through the exudates of its roots can promote microbial growth and metabolism. Soil microbiology has a significant influence on plant physiology and growth, but also on protection against deleterious pathogenic microorganisms [23]. In the soil, in fact, you can find a considerable number of microorganisms that perform different functions. Some are beneficial microorganisms, others can cause damage to plants and agricultural production. Among the beneficial microorganisms are the Plant Growth Promoting Rhizobacteria (PGPR), which can live close to the roots or inside specialized cells and are able to stimulate plant growth through various mechanisms. The best known mechanisms include: i) bio-fertilization, i.e. 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, i.e. the ability of certain bacteria to stimulate plant defences; iv) the production of phytohormones and signal molecules. Rhizobacteria through their activity are able to alleviate the negative effects of stress on plant growth [24, 25, 26, 27]. They can produce plant hormones and siderophores, regulating plant metabolism and the availability of nutrients in the soil, in particular iron, copper, zinc and manganese. There are several phytohormones influenced by PGPR, such as abscissic acid, auxins and cytokinins [28]. PGPR can produce several metabolites that can control reactive oxygen species under stress [29, 30]. PGPR can also produce antibiotics necessary for symbiosis with their host and for competition with other microorganisms in the rhizosphere [31]. In recent years there has been a trend towards greater efficiency in the use of synthetic fertilizers and a return to the use of algae based products to improve plant quality and the ability to use nutrients [32]. Since the fifties, the use of algae has been supplanted by the use of commercial extracts capable of providing useful molecules to plants. The effectiveness of algae as biostimulants depends on the composition and concentration of compounds that can improve plant metabolism under stressful conditions. Among the hormones most commonly found in algae extracts are cytokinins, auxins, gibberellins and abscissic acid as well as other hormone-like substances [33,34].

In this test, plants treated with (EM) and biofertilizers based on microorganisms and algae showed a significant improvement in the agronomic parameters of *Aloe barbadensis* plants and in the quality characteristics of the gel. In particular, there is a significant increase in sugars, fibres and a slowing down of the gel denaturation process.

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 [25,26,35,36,37,38]. 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, in particular water and nutrient stress [26,39,40].

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## 5. Conclusion

The test showed how the use of biostimulating microorganisms and algae can improve growth and gel quality in *Aloe barbadensis* plants. In particular, the use of these biostimulants resulted in a significant increase in the agronomic characteristics of the Aloe leaves and the quality of the gel, in particular the fibre content, soluble solids, sugars and a reduction in the degradation process. These improvements in plant growth following the use of microbial biofertilizers and algae have also been found in previous trials in other vegetables and ornamental crops, but few trials have been carried out with Aloe. The application of symbiotic microorganisms in agricultural operations can therefore ensure higher production standards, with a possible improvement in the agronomic quality of the plants, while 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 ornamental and fruit cacti and succulents under organic farming methods.

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## Compliance with ethical standards

### *Acknowledgments*

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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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