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BENEFICIAL INTERACTION BETWEEN ALGAE AND RHIZOBACTERIA IN THE CULTIVATION AND DEFENSE OF POTTED SUCCULENT PLANTS

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ABSTRACT

Research goal: *The aim of this research was therefore to evaluate whether there are interactions in the growing medium in the simultaneous use of algae and beneficial microorganisms, to assess the possible improvement of flower quality and growth of succulent plants and the possible bioncontrol against pathogens present in the farm soils.*
Materials and Methods: *The experiments, started in March 2021, were conducted in the greenhouses of CREA-OF in Pescia (PT), Tuscany, Italy (43°54'N 10°41'E) on Aichryson punctatum, Lewisia cotyledon, Crassula sarcocaulis, Kalanchoe orgyalis. The experimental groups were: i) group control, irrigated with water and substrate previously fertilized; ii) group with algae irrigated with water and substrate previously fertilized; iii) group with beneficial bacteria irrigated with water and substrate previously fertilized; iv) group with algae and beneficial bacteria irrigated with water and substrate previously fertilized.*
Results and Discussion: *The trial showed a significant improvement of agronomic parameters analyzed on plants of Aichryson punctatum, Lewisia cotyledon, Crassula sarcocaulis, Kalanchoe orgyalis treated with algae, bacteria and using a mixture of algae with bacteria. In particular, the trial showed that the use of algae and bacteria can increase plant height, number of leaves, vegetative, root and flower weight, and flower duration. In addition, the treatment with algae and microorganisms showed how the use of algae and bacteria can increase the protection from the attack on plants, of pathogens such as Rhizoctonia solani and Fusarium oxysporum. It is shown that the combined treatment algae plus microorganisms was significantly better than using the two treatments individually.*
Conclusions: *Scientific evidence confirms that the use of algae and microorganisms can play a particularly important role in improving the physical, chemical and microbiological quality of the soil and in biofertilization, biostimulation and plant protection.*

KEY-WORDS: *Cyanobacteria; Sustainable agriculture; Beneficial bacteria; Succulent plants; Algae-microorganisms interaction*

INTRODUCTION

Algae Biostimulants for Agricultural Crops

A product with biostimulant action means a fertilizer able to contribute positively to the improvement of plant nutrition and development, regardless of the presence of nutrients, with the exclusion of phytoregulators, which are not allowed, and products with declared and specific phytosanitary function [1]. The most commonly used biostimulants in modern agriculture solve, or contribute to solve, mainly the problems related to abiotic factors, such as high and low temperatures, salinity, excesses and water shortages; but they also contribute to optimize the productive capacity of the plant in normal environmental conditions, thanks to several effects on quality and quantity of crops [2].

Biostimulants represent a complement to the ordinary nutrition with nutritive elements, allowing for example the plant to elaborate necessary metabolites with less energy expenditure, or to acquire in greater quantities useful compounds that are precursors of substances normally elaborated by it [3]. They usually belong to the categories of amino acids, humic extracts, algae extracts, plus some other types of products with different characteristics and functions such as phosphites (or phosphonates) and the so-called elicitors, which, however, play a more defensive action. The use of algae in agriculture has ancient origins, even dating back to the times of Ancient Rome. In the sixteenth century seaweeds, washed in order to remove salt, were used near the coasts of Scotland and in France as an organic fertilizer. Although the initial use was as a simple organic fertilizer, their use immediately showed a stimulation of growth and production of crops that was not attributable to nutrients (especially microelements), present in their composition [4]. Algae have a biostimulating effect that leads to a

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greater absorption and translocation of nutrients in the plant, a greater resistance to diseases and stresses, a greater resistance to frost and a better shelf-life of productions [5,6]. In their composition are in fact found oligosaccharides, such as laminarin, which has an "elicitor" effect on the plant, that is it stimulates the production of phytoalexins, which are a sort of natural antibiotics. Algae contain polyamines, including putrescine, spermidine and spermine, which control the phenomena of cell division and organogenesis, important in flowering, fertilization and fruit set, and betaine, which behave in certain conditions as cytokinins [7,8]. These are natural hormones (cytokinins, indolacetic acid, gibberellins) that act as biological coordinators: that is, they regulate and stimulate the development and growth of plant organs [9]. Algae contain amino acids and mineral elements, among which numerous microelements, which, as natural additives, increase the content in the plant tissues of these substances indispensable to life; and colloids, such as alginic acid and mannitol, which as natural adjuvants favor absorption and translocation, thanks to their chelating action. Today the use of algae as they are has been replaced by that of powder or liquid extracts obtained from fresh or dehydrated algae [10,11].

Plant-Microorganisms Interaction

Plants determine the growth and balance of soil-poor bacterial colonies through the production of root exudates specific to the crop plant species [12]. Telluric microorganisms use these substances for their growth and multiplication and are of fundamental importance to plant biology as they produce substances with an action similar to plant hormones, inducing cell differentiation, root development, and changes in root hair growth [13]. Colonization of roots by microorganisms can result in the initiation of a symbiosis or cause disease [14]. As verified in numerous researches, microorganisms in the rhizosphere are decisive in plant growth and defense (10). Rhizobacteria can be classified as extracellular organisms (ePGPR) that live mainly in the rhizosphere, i.e. the portion of soil close to the roots, and intracellular organisms (iPGPR) that can colonize the internal structures of the roots with consequences on the metabolic profile of the plant. Several plant growth-promoting rhizobacteria may be present in soil, the most important of which include Nitrotobacter, Bacillus, Pseudomonas, Azospirillum, Agrobacterium, Burkholderia, Bradyrhizobium, Rhizobium, Frankia, Erwinia, Flavobacterium, Chromobacterium, Caulobacter, Arthrobacter, Allorhizobium, Mesorhizobium. The presence of a rich telluric microfauna determines the improvement of soil characteristics and fertility, thus favoring the cultivation of plants [15]. Normally, root colonization can occur through the formation of siderophores, solubilization of mineral phosphates, fixation of atmospheric nitrogen, and production of phytophores. thanks to the use of microbial inoculants, it is now possible to improve crop quality by improving plant growth, increasing defense against biotic and abiotic stresses, and stimulating soil fertility [16]. A very interesting aspect is to investigate the possible interaction between the use of biostimulant algae and the activity of soil microorganisms or those inoculated in the growing media. There are some researches in literature on this topic, but some aspects still remain unknown [17,18,19].

OBJECTIVES

The aim of this research was therefore to evaluate whether there are interactions in the growing medium in the simultaneous use of algae and beneficial microorganisms, to assess the possible improvement of flower quality and growth of succulent plants and the possible bioncontrol against pathogens present in the farm soils.

Figure 1 - Particulars of *Aichryson punctatum* flowers

MATERIAL AND METHODS

The experiments, started in March 2021, were conducted in the greenhouses of CREA-OF in Pescia (PT), Tuscany, Italy (43°54′N 10°41′E) on *Aichryson punctatum* (Figure 1), *Lewisia cotyledon*, *Crassula sarcocaulis*, *Kalanchoe orgyalis*. The plants were placed in pots ø 12 cm; 30 plants per thesis, divided into 3 replicas of 10 plants each. Plants were fertilized with a controlled release fertilizer (2 kg m^{-3} 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 60% + pumice 40%), irrigated with water and substrate previously fertilized;
- group with algae (**AG**) (peat 60% + pumice 40%) irrigated with water and substrate previously fertilized, dilution 1:1000 once a week (Kelpak biostimulant, *Ecklonia maxima,* Kelp products International);

- group with beneficial bacteria (**BAC**) (peat 60% + pumice 40%) irrigated with water and substrate previously fertilized, (TNC Bactorr^{s13}: *Bacillus amyloliquefaciens, B. brevis, B. cirulans, B. coagulans, B. firmus, B. halodenitrificans, B. laterosporus, B. licheniformis, B. megaterium, B. mycoides, B. pasteuri, B. polymyxa, B. subtilis* (1.3×10^{11} cfu/kg); Mix 1.5g (approx 1/2 tsp) with every litre of soil;
- group with algae + beneficial bacteria (**AGBA**) (peat 60% + pumice 40%) irrigated with water and substrate previously fertilized.

The plants were watered 2 times a week and grown for 7 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 October 10, 2021, plant height, leaves number, vegetative weight, roots weight, flowers weight, flowers life, were analysed. In addition, the mortality of plants caused by *Rhizoctonia solani* and *Fusarium oxysporum* was evaluated.

Statistical analysis

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).

RESULTS AND DISCUSSION

Results

The trial showed a significant improvement of agronomic parameters analyzed on plants of *Aichryson punctatum*, *Lewisia cotyledon*, *Crassula sarcocaulis*, *Kalanchoe orgyalis* treated with algae, bacteria and using a mixture of algae with bacteria. In particular, the trial showed that the use of algae and bacteria can increase plant height, leaves number, vegetative, root and flower weight, and flower life. In addition, the treatment with algae and microorganisms showed how the use of algae and bacteria can increase the protection from the attack on plants of pathogens such as *Rhizoctonia solani* and *Fusarium oxysporum*. It is shown that the combined treatment algae plus microorganisms was significantly better than using the two treatments individually.

In (Table 1), in *Aichryson punctatum* there was a significant increase in plant height in (AGBA) with 38.72 cm compared to 36.14 cm in (AG), 36.01 cm in (BAC) and 32.56 cm in (CTRL). For leaf number, the (AGBA) thesis was the best with 41.80, followed by (BAC) with 36.42, (AG) 32.00 and (CTRL) with 26.11 (Figure 2). Regarding vegetative weight, the thesis (AGBA) showed an increase compared to the others. In fact in (AGBA) the weight was 96.67 g compared to 95.30 g (BAC), 93.62 g (AG) and finally 90.20 g of the control (CTRL) (Figure 2A). The same trend for root weight where (AGBA) showed a weight of 80.36 g, (BAC) 76.72 g, (AG) 75.70 g and (CTRL) 72.62 g (Figure 2B). In terms of flower weight, the (AGBA) thesis was also the best with 27.97 g, compared to 25.37 g (BAC), 23.46 g (AG) and 20.97 g (CTRL). The trial also showed that the application of algae and microorganisms can increase flower life. In fact, in the thesis (AGBA) flower duration was 8.61 days, compared to 7.43 and 7.42 days of (BAC) and (AG) respectively and 6.44 days of the untreated control. On *Aichryson punctatum*, there was a significant reduction in the attack of the pathogens *F. oxysporum* and *R. solani* following treatment with algae and microorganisms.

In (Table 2), *Lewisia cotyledon* shows a significant increase in plant height in thesis (AGBA) with 18.69 cm followed by (BAC) with 17.59 cm and (AG) with 16.91 cm, finally the untreated control with 15.53 cm. For the number of leaves, the thesis (AGBA) was the best with 25.82, followed by (BAC) with 23.84, (AG) 21.86 and (CTRL) with 17.24. For vegetative weight, the (AGBA) thesis was the best with 49.41 g, followed by (BAC) with 47.06 g, (AG) 45.12 g and (CTRL) with 40.75 g. Also for root weight, the thesis (AGBA) showed a weight of 38.93 g, (BAC) 37.94 g, (AG) 36.94 g and (CTRL) 34.79 g. In terms of flower weight also in this situation, thesis (AGBA) was also the best with 28.73 g, compared to 26.76 g (BAC), 25.84 g (AG) and 22.55 g (CTRL) (Figure 3). Also in *Lewisia cotyledon* there was a significant increase in flowering duration determined by the use of algae and microorganisms. It was also evident how this type of treatment can increase the control of fungal pathogens. In (Table 3), in *Crassula sarcocaulis*, a significant plant height of the thesis (AGBA) with 44.54 cm is noted followed by (BAC) with 43.40 cm and (AG) with 42.08 cm, finally the untreated control with 40.44 cm. For the number of leaves, the thesis (AGBA) was the best with 62.21, followed by (BAC) with 57.24, (AG) 52.81 and (CTRL) with 45.00. Also for vegetative weight, the (AGBA) thesis was the best with 141.62 g, followed by (BAC) with 139.24 g, (AG) 137.27 g and (CTRL) with 135.29 g (Figure 4A). There was also a parallel significant increase in root weight in the thesis (AGBA) with 120.53 g, followed by (BAC) 116.73 g, (AG) with 115.33 g and the control with 106.82 g (Figure 4B). In terms of flower weight also on *Crassula sarcocaulis*, the (AGBA) thesis was the best with 22.97 g, compared to 21.54 g (BAC), 20.59 g (AG) and 18.69 g (CTRL). As with the other plant species in the trials, the use of algae and microorganisms also resulted in a significant increase in flower life and a significant reduction in the attack of pathogenic fungi such as *F. oxysporum* and *R. solani* on plants. In (Table 4), in *Kalanchoe orgyalis* the effect of algae-associated microorganisms (AGBA) on plant height was evident with 44.46 cm followed by (BAC) with 42.14 cm and (AG) with 40.13 cm, finally the untreated control with 36.33 cm. For the number of leaves, the thesis (AGBA) was the best with 33.46, followed by (BAC) with 31.34, (AG) 28.81 and (CTRL) with 23.00. Also for vegetative weight, the thesis (AGBA) was the best with 142.31 g, followed by (BAC) with 138.51 g, (AG) 136.25 g and (CTRL) with 132.80 g (Figure 5). There was also a significant increase in root weight in the thesis (AGBA) with 111.56 g, followed by (BAC) 109.39 g, (AG) with 108.33 g and the control with 103.04 g. In terms of flower weight also on *Kalanchoe orgyalis*, the (AGBA) thesis was the best with 20.62 g, compared to 19.62 g (BAC), 19.46 g (AG) and 17.78 g (CTRL). Also on this plant species, the combined use of algae and microorganisms resulted in a significant increase in flower life and increased plant resistance to biotic stresses.

Discussion

Microalgae are characterized by a great physiological and metabolic diversity, being able to synthesize numerous molecules of high biological value [20]. Just in the agricultural sector, the use of microalgae and cyanobacteria is experiencing a renewed interest, with the aim of stimulating the microbial activity in the soil, improve nutrient availability, increase soil fertility and enhance plant growth and productivity [21]. The high content of micro- and macronutrients makes algae biomass an important source of biofertilizers [22,23]. Several microalgae species have been studied for their potential application as biofertilizers, with effects on soil stabilization and increased nutrient content and increased water retention capacity [20]. Algal biomass can probably be degraded by the microbiome of the rhizosphere in order to release constituent nutrients, as found in this trial where the association and interaction between algae and microorganisms increased the effects on plant growth or be subject to natural degradation to allow for sustained nutrient release over time. In addition to their ability to provide nutrients, algae can act as phytostimulant hormones by positively influencing photosynthesis, respiration, nucleic acid synthesis, and nutrient assimilation [24]. Also as found in this experiment the use of algae-based biomass can increase resistance to biotic and abiotic environmental stresses [25]. Agricultural applications of algae and cyanobacteria have often focused on their use as biofertilizers and soil conditioners, and their effects on crops are generally attributed to the improvement of physical, chemical and biological soil fertility. In recent years, however, it has been discovered that positive physiological responses by plants cannot be attributed solely to the increase in available nutrients, but probably to the action of a wide range of bioactive molecules (phytohormones, vitamins, polyphenols) that affect plant life at concentrations lower than those of the main macro-nutrients such as nitrogen, phosphorus and potassium [26]. The possibility of algae to be able to produce these bioactive molecules using reduced quantities of product, has determined the interest of the scientific community [12]. Among the most noticeable effects is an increase in vegetative growth and quality of several vegetable and ornamental species, as also evidenced in this experiment. Increased plant growth may be associated with stimulation of nitrogen and carbon metabolism with increased protein, carbohydrate and photosynthetic pigment content. Significant increases in roots development following the use of algal and cyanobacterial extracts have been observed in many studies [27,28]. Stimulation effects have been seen following treatments of seeds by increasing germination, roots or leaves. The application of biostimulants can also trigger biochemical processes that lead to the formation of important metabolites useful for the quality and shelf life of the final product. he application of algae and cyanobacteria has not only proved useful to increase the quality and growth of plants but also to induce an enhancement of antioxidant defenses of plant tissues [29]. In particular by increasing the activity of enzymes such as catalase, peroxidase and superoxide dismutase in the presence of salt stress or the ability to sequester metal ions and sodium ions reducing the absorption by plants. In addition, the association with microorganisms can increase the defense of plants against pathogenic fungi, through mechanisms related to the occupation of sites of infection, sequestration of nutrients used for example by pathogenic fungi, stimulation of plant defense systems [24,12]. In this trial it was evident how the use of algae and beneficial microorganisms colonizing the rhizosphere was able to improve the productive quality of growing succulent plants, increasing vegetative and root parameters, flower life and defense against pathogens such as *Fusarium oxysporum* and *Rhizoctonia solani*. Aspects and results also recur in other plant species, but not too frequently dealing with the possible interaction that there can be between algae and symbiotic microorganisms. Biofertilizers especially those based on algae can be a viable alternative to chemical fertilizers, as they contain natural components that do not harm plants and soil. They can also protect crops from disease development, fungal attack and free pollutants [14].

Table 1 - Evaluation of algae and microorganisms interactions and on agronomic characters and pathogen protection on *Aichryson punctatum*

Groups	PH (cm)	LN (n°)	VW (g)	RW (g)	FW (g)	FL (days)	RS (n°)	FO (n°)
CTRL	32,56 c	26,11 d	90,20 d	72,62 d	20,97 d	6,44 c	1,82 a	1,21 a
AG	36,14 b	32,00 c	93,62 c	75,70 c	23,46 c	7,42 b	1,23 b	0,23 b
BAC	36,01 b	36,42 b	95,30 b	76,72 b	25,37 b	7,43 b	0,44 c	0,00 b
AGBA	38,72 a	41,80 a	96,67 a	80,36 a	27,97 a	8,61 a	0,00 c	0,00 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; (AG): *Ecklonia maxima* ; (BAC): TNC Bactorr^{s13}; (AGBA): *Ecklonia maxima* + TNC Bactorr^{s13}; PH: plant height; LN: leaves number; VW: vegetative weight; RW: roots weight; FW: flowers weight; FL: flowers life; RS: plants affected by *Rhizoctonia solani*; FO: plants affected by *Fusarium oxysporum*

Table 2 - Evaluation of algae and microorganisms interactions and on agronomic characters and pathogen protection on *Lewisia cotyledon*

Groups	PH (cm)	LN (n°)	VW (g)	RW (g)	FW (g)	FL (days)	RS (n°)	FO (n°)
CTRL	15,53 c	17,24 d	40,75 d	34,79 d	22,55 d	11,00 d	1,21 a	1,12 a
AG	16,91 b	21,86 c	45,12 c	36,64 c	25,84 c	13,11 c	0,44 b	0,00 b
BAC	17,59 b	23,84 b	47,06 b	37,94 b	26,76 b	14,23 b	0,00 b	0,00 b
AGBA	18,69 a	25,82 a	49,41 a	38,93 a	28,73 a	15,61 a	0,00 b	0,00 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; (AG): *Ecklonia maxima* ; (BAC): TNC Bactorr^{s13}; (AGBA): *Ecklonia maxima* + TNC Bactorr^{s13}; PH: plant height; LN: leaves number; VW: vegetative weight; RW: roots weight; FW: flowers weight; FL: flowers life; RS: plants affected by *Rhizoctonia solani*; FO: plants affected by *Fusarium oxysporum*

Table 3 - Evaluation of algae and microorganisms interactions and on agronomic characters and pathogen protection on *Crassula sarcocaulis*

Groups	PH (cm)	LN (n°)	VW (g)	RW (g)	FW (g)	FL (days)	RS (n°)	FO (n°)
CTRL	40,44 a	45,00 d	135,29 d	106,82 a	18,69 d	4,82 c	1,82 a	1,64 a
AG	42,08 c	52,81 c	137,27 c	115,33 b	20,59 c	6,42 b	0,00 b	0,00 b
BAC	43,40 b	57,24 b	139,24 b	116,73 b	21,54 b	6,43 b	0,24 b	0,00 b
AGBA	44,54 a	62,21 a	141,62 a	120,53 a	22,97 a	7,51 a	0,00 b	0,00 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; (AG): *Ecklonia maxima* ; (BAC): TNC Bactorr^{s13}; (AGBA): *Ecklonia maxima* + TNC Bactorr^{s13}; PH: plant height; LN: leaves number; VW: vegetative weight; RW: roots weight; FW: flowers weight; FL: flowers life; RS: plants affected by *Rhizoctonia solani*; FO: plants affected by *Fusarium oxysporum*

Table 4 - Evaluation of algae and microorganisms interactions and on agronomic characters and pathogen protection on *Kalanchoe orgyalis*

Groups	PH (cm)	LN (n°)	VW (g)	RW (g)	FW (g)	FL (days)	RS (n°)	FO (n°)
CTRL	36,33 c	23,00 d	132,80 a	103,04 c	17,78 c	3,62 c	0,00 a	0,00 a
AG	40,13 b	28,81 c	136,25 c	108,33 b	19,46 b	4,21 bc	0,00 a	0,00 a
BAC	42,14 ab	31,34 b	138,51 b	109,39 b	19,62 b	4,84 ab	0,00 a	0,00 a
AGBA	44,46 a	33,46 a	142,31 a	111,56 a	20,62 a	5,23 a	0,00 a	0,00 a
ANOVA	***	***	***	***	***	***	ns	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; (AG): *Ecklonia maxima* ; (BAC): TNC Bactorr^{s13}; (AGBA): *Ecklonia maxima* + TNC Bactorr^{s13}; PH: plant height; LN: leaves number; VW: vegetative weight; RW: roots weight; FW: flowers weight; FL: flowers life; RS: plants affected by *Rhizoctonia solani*; FO: plants affected by *Fusarium oxysporum*

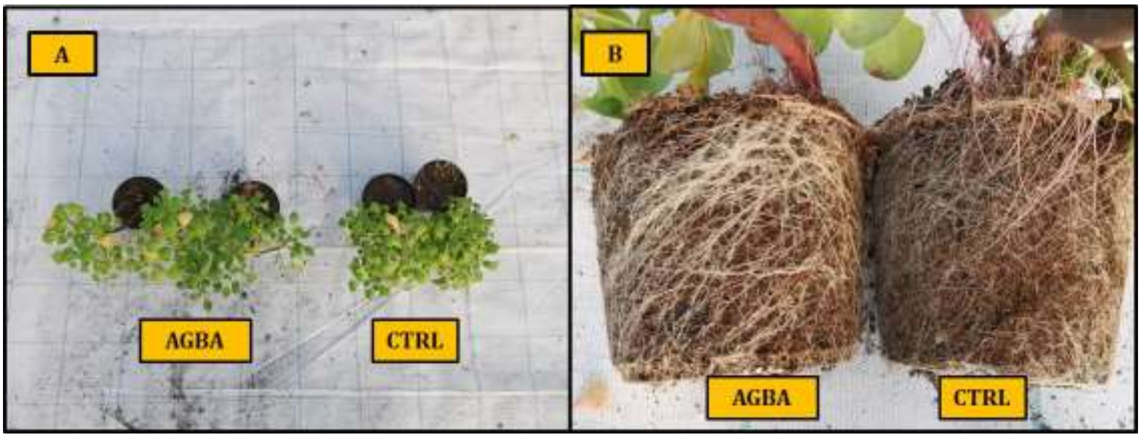

Figure 2 - Comparison between algae+beneficial microorganisms (AGBA) and control (CTRL) on vegetative (A) and roots growth (B) of *Aichryson punctatum*

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Figure 3 - Effect of algae (AG) on flowers production of *Lewisia cotyledon*

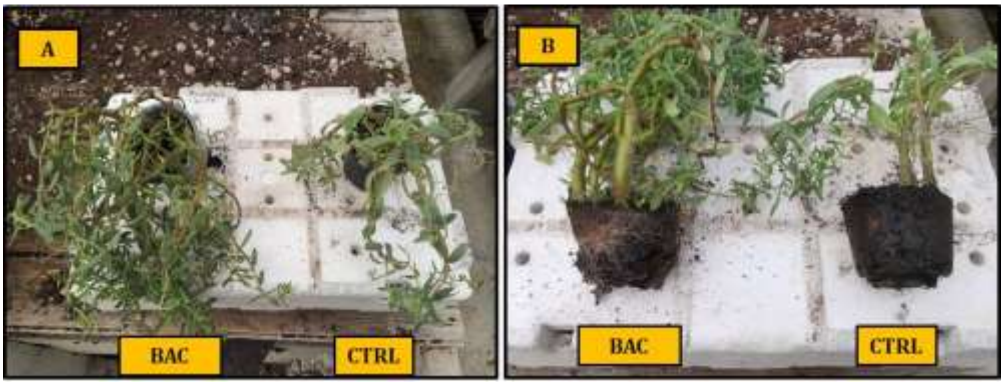

Figure 4 - Comparison between of beneficial microorganisms (BAC) and control (CTRL) on vegetative (A) and roots growth (B) of *Crassula sarcocaulis*

Figure 5 - Effect of algae+beneficial microorganisms (AGBA) on vegetative growth of *Kalanchoe orgyalis*

CONCLUSION

Scientific evidence confirms that the use of algae and microorganisms can play a particularly important role in improving the physical, chemical and microbiological quality of the soil and in biofertilization, biostimulation and plant protection. The use of these products is most interesting in crops grown in low fertility soils and with repeated applications. Currently, they are also of great interest in the world of ornamental species where the quality of the product marketed must be of first choice. Their use in organic farming also allows a further reduction in the use of chemical products with a consequent reduction in environmental impact.

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Conflict of interest
The author declares that there are no conflicts of interest

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