Int.J.Curr.Microbiol.App.Sci (2021) *10*(10): 67-76

International Journal of Current Microbiology and Applied Sciences
ISSN: 2319-7706 Volume 10 Number 10 (2021)
Journal homepage: http://www.ijcmas.com

Original Research Article https://doi.org/10.20546/ijcmas.2021.1010.010

Evaluation of Growth Parameters on *Carpobrotus edulis, Kalanchoe daigremontiana and Kalanchoe tubiflora* in Relation to Different Seaweed Liquid Fertilizer (SLF) as a Biostimulant

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ABSTRACT

The aim of this research was to evaluate the biofertilization capacity of five different species of algae, representative of the three main groups of macroalgae selected from Lake Ganzirri in Messina, Venice Lagoon and Norwegian coasts. The experiments, started in January 2021, were conducted in the greenhouses of CREA-OF in Pescia (Pt), Tuscany, Italy (43°54′N 10°41′E) on *Carpobrotus edulis*, *Kalanchoe daigremontiana* and *Kalanchoe tubiflora* from seed. The test showed a significant increase in the agronomic parameters analysed in plants treated with algae extracts on succulents cultivated. The test also showed increased control of the pathogen *Pythium debaryanum* in seedlings where the algae extracts were introduced and also an increase in the percentage of seed germination and a reduction in the average germination time. This research work has shown that algal species have great potential for use in the development of biofertilisers in sustainable agriculture in terms of cost-effectiveness, ecological role, possible reduction of synthetic fertilisers and plant protection products, increased soil fertility and microbiological diversity.

Keywords

Sustainable agriculture; Biofertilizers; Organic farming; Seaweed extracts; Biocontrol

Article Info

Accepted:
05 September 2021
Available Online:
10 October 2021

Introduction

Marine macroalgae are a source of various biomass use for very different purposes, such as the extraction of bioactive molecules used in pharmaceutical or cosmetics, used as food, feed and agriculture (Leandro *et al.,* 2019; Melo *et al.*, 2020). In the next few years the commercial interest in a product derived from totally sustainable biomass shifts the focus on some products to those of marine origin, in particular, macroalgae (Biris-Dorhoi *et al.*, 2020); where new products are tested and marketed (Costa *et al.*, 2021), especially in a biorefinery vision and zero waste focus (Zollman *et al.*, 2019).

In particular agriculture products, the majority of which are aimed at increasing the resistance of plants to adverse, in general, abiotic and biotic stress.

Algal extracts are prominent for plant growth and improved the defence from external biological agents for many reasons; the first is the presence in algae of appreciable amounts of trace elements indispensable to plant growth, such as Ca, P, Mg, Fe, Na and K (El-Said and El-Sikaily, 2013; Tuhy *et al.*, 2015); the second reason is the presence of many chemical compounds, naturally present of algae, belong to family of phytohormones (Crouch and Van Staden, 1993;Shoubaky and Salem, 2016); after all, algae have many phycocolloids that substances appreciated for various purposes, of which bio-stimulating activities for plants are also recognized (Kapoore *et al.*, 2021).

Is it important to point out that not all macroalgae have the same set of nutrients, phytohormones and phycocolloids, differentiated not only if look at large groups (brown, green and red algae) but also as regards the differences within the genus (eg *Ulva*) of which not yet the differences are well known (Ali *et al.*, 2021).

Moreover, even within the same species, the levels of nutrients and phytohormones could vary greatly depending on populations, seasonal periods and many other factors, including environmental and biotic factors (Nabil and Cosson, 1996; Khairy and El-Shafay, 2013). Also are important time and type of application, seed germination, foliar direct spray or inside the irrigation water (Vijayakumar *et al.*, 2019). In general, extracts of marine macroalgae are biostimulants for terrestrial plants and raw extracts from seaweed could be better than refined because they restrain many different types of molecules which in refined extracts will be lost (E L Boukhari *et al.*, 2020).

In this study, we aimed to test different seaweed extracts from five different species and test 5 extracts to know which of these was important for the growth of C*arpobrotus edulis* (Figure 1), *Kalanchoe daigremontiana*, *Kalanchoe tubiflora.*

The seaweed extracts used in the present study were prepared from five different species, representative of the three major macroalgal groups. For the Phaeophyceae (Brown algae) *Saccharina latissima* (A) and *Sargassum muticum* (C) were chosen, about Chlorophyta (Green algae) was chosen *Ulva ohnoi* (B) and for Rhodophyta (Red algae) *Hypnea cornuta* (D) and *Agardhiella subulata* (E). All these macroalgae were used because of utilization of unwanted biomass, alien or invasive species and, in the case of *S. latissima*, production waste for human consumption.

Materials and Methods

The experiments, started in January 2021, were conducted in the greenhouses of CREA-OF in Pescia (Pt), Tuscany, Italy (43°54′N 10°41′E) on *Carpobrotus edulis*, *kalanchoe daigremontiana* and *kalanchoe tubiflora* from seed. The seeds were placed in a 54 hole plateau, in potting soil for sowing (blond peat 40%, black peat 60%, silica sand 80 l/m^3, fertilization 500/m^3 NPK, pH 5.7); 60 seeds per thesis, divided into 6 replicas of 10 seeds each. The plants were watered 2 times per day, 7 days a week and grown for 6 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 June 15, 2021, seeds germination, average germination time, plants height, leaves number, vegetative and radical weight, were recorded. In addition, plant infection by *Pythium debaryanum* selected from *Solanum lycopersicum* seedlings and inoculated on succulents plants was evaluated in the experiment.

The algae were collected from different places all around Europe and for different purposes that have been mentioned above. *Saccharina latissima* from crop waste in Norway (58°17'33.3"N 6°38'58.3"E), after sampling, was quickly dring; *Sargassum muticum* from Venice lagoon (45°25’42.6" N 12°19'50.7"E) (Italy); *Ulva ohnoi*, *Hypnea cornuta* and *Agardhiella subulata* from Ganzirri lake (38°15'28" N 15°36'37” E) (Italy).

After sampling algae were brought to the laboratory and rapidly washed with fresh water to remove epiphytes and sand. The fresh alga was dried in the laboratory stove at 40°C for 48h and kept dried until extraction, preserving dryness through silica gel.

Dried macroalgae were prepared for extraction with the modified protocol from Rama, about the preparation of Liquid Seaweed Fertilizer (Rama 1990).

Five different solutions were prepared, each solution was prepared with 500 ml of distilled water and 25 g of dried seaweed (ratio 1:20 DW/V) at 80°C for 3h. The residue biomass was removed by the use of cotton cloth and the liquid solution gives, as a result, a 100% SLF (Seaweed Liquid Fertilizer), used with a different concentration on *Carpobrotus edulis*, *Kalanchoe daigremontiana, Kalanchoe tubiflora*. To preserve the fertilizer was add 1g/l of citric acid and keep at 4°C until dilution and use.

Results and Discussion

The test showed a significant increase in the agronomic parameters analysed in plants treated with algae extracts on *Carpobrotus edulis*, *Kalanchoe daigremontiana* and *Kalanchoe tubiflora*. The test also showed increased control of the pathogen *Pythium debaryanum* in seedlings where the algae extracts were introduced. There was also an increase in the percentage of seed germination and a reduction in the average germination time in the theses treated with algae. In particular, the trials showed a more significant effect on vegetative and root development in the theses treated with *Hypnea cornuta* and *Agardhiella subulata* on all three succulent species in cultivation. All plants treated with the algae extracts showed a significant increase in the height and number of leaves per plant, the vegetative and root weight of the plants, a reduction in attack by the pathogen *Pythium debaryanum*, an increase in seed germination and a reduction in the average germination time.

Specifically, in (Table 1), it is shown that on *Carpobrotus edulis*, treatment (E) significantly improved the plant height with 14.35 cm, compared to 13.81 cm (D), 11.17 cm (C), 10.49 cm (B), 9.61 cm (A) and 7.92 cm of the untreated control. The use of algae extracts also significantly increased the number of leaves per plant with 6.80 (E), 6.20 (D), 5.40 (A), 5.00 (B), 4.80 (C) and 4.60 of (CTRL). It also increased the vegetative weight, 27.71 g (E), 26.94 g (D), 24.89 g (B), 24.48 g (A), 24.27 (c) and 22.94 of the untreated control and root weight 20.27g (E), 19.93 g (D), 18.43 g (C), 18.33 g (B), 17.59 g (A) and 16.83 g (CTRL) (Figure 2). The trial also showed that treatments based on algae extracts can have a biocontrol effect on the *Pythium dabaryanum* pathogen. Indeed, in theses sprayed with algae extracts, 1.00 plant was affected by Pythium in (E), 1.20 in (D), 1.40 in (A) and (C), 2.20 in (B), 3.40 (CTRL). In relation to seed germination, theses (E) and (D) were the best with 8.20 and 7.80 seeds germinated, followed by (B) and (C) with 6.40, (A) with 5.60 and (CTRL) with 4.40. Regarding the mean germination time, (E) and (D) were the best theses with 18.60 and 18.80 days, (C) 21.80 days, (A) 21.40 days, (B) 21.00 days and the control with 24.20 (CTRL).

In (Table 2) on *Kalanchoe daigremontiana*, it is noted that treatments (E) and (D) significantly improved the plant height with 8.86 cm and 8.67 cm respectively, followed by (B) with 7.93 cm, (A) with 7.91 cm and (C) with 7.71 cm. The control was the treatment with the lowest plant height with 7.04 cm. Regarding the number of leaves, treatments (E) and (D) were the best with 8.60 and 8.00 leaves, followed by (C) with 6.40, (A) and (B) with 6.00 and the untreated control with 5.20. The application of algae extracts also resulted in an increase in vegetative weight (Figure 3), 18.83 g (E), 17.57 g (D), 16.09 g (C),15.48 g (A), 15.04 (CTRL) and 14.92 (b) and root weight 14.55 g (E), 13.85 g (D), 12.53 g (C), 12.19 g (D), 11.45 g (A) and 10.05 g (CTRL) (Figure 4). Also on *K. daigremontiana*, it was evident that the algae extracts showed a biocontrol effect on Pythium. In particular, in all the theses treated with the extracts there was a significant reduction in the pathogen's attack compared to the control. In terms of seed germination, thesis (D) was the best with 9.00 germinated seeds, followed by (E) with 8.80, (B) and (C) with 8.40, (A) with 8.00 and (CTRL) with 7.40. Regarding the mean germination time, (E) and (D) were the best theses with 15.30 and 16.40 days, (A) and (C) 17.20 days, (B) 17.60 days and the control with 18.00 (CTRL).

On *Kalanchoe tubiflora* in (Table 3), it can be seen that treatment (E) was the best for plant height with 8.87 cm, succeeded by (D) with 8.32 cm and (A), (C) and (B) with 7.73 cm, 7.64 cm and 7.58 cm respectively.

Finally, the untreated control with 7.05 cm. For the number of leaves, treatment (D) was the best with 8.60, then (E) with 8.40, (B) with 7.80, (A) and (C) with 7.40. The last one was the untreated control with 7.20. An increase in vegetative weight was also shown on *K. tubiflora* particularly in treatment (E) with 16.43 g, which was followed by (D) with 16.17 g, (C), (B) and (A) with 15.60 g, 15.42 g and 15.39 g respectively, lastly the control with 14.70 g. Treatment (E) was also the best for root weight with 14.68 g, closely followed by (D) with 14. 01 g, (C) and (B) with 13.55 g and 13.52 g respectively, (A) with 12.64 g and (CTRL) with 11.26 g. No treatment showed any significant effect on the control of *Pythium debaryanum*, while with regard to seed germination treatments (E) and (D) with 9.20 and 8.80 were the best, compared to the other plant extracts and the control. Treatment (E) was also the best in terms of average germination time with 13.20 days.

In modern years, there has been a tendency to increase efficiency in the use of synthetic fertilisers and a move back to the use of plant algae products to enhance plant quality and the ability to utilise plant nutrients (Thajuddin and Subramanian, 2005). Since the 1950s, the use of algae has been superseded by the use of commercial extracts that provide useful molecules for plants. The efficiency of algae as bio-stimulants is dependent on the content and composition of molecules that can improve plant metabolism under stressful conditions (Saadatnia and Riahi, 2009).

Some of the hormones most commonly found in algae extracts include cytokinins, auxins, gibberellins and abscisic acid, as well as other hormone-like substances (Song *et al.*, 2005).

The effects found in the use of algae in plant cultivation include: (i) increased porosity and production of adhesive substances; (ii) excretion of substances that stimulate the production of phytohormones (auxin, gibberellin); (iii) increased water retention due to the gelatinous structure of algae; (iv) increased soil biomass as a result of the death and desiccation of algal structures; (v) reduced soil salinity; (vi) reduced weed development; and (vii) increased phosphate and organic acid content in the soil (Sahu *et al.*, 2012; Rodriguez *et al.*, 2006; Wilson, 2006). Beneficial effects of algae inoculation in plants have been found on barley, oats, tomato, radish, cotton, sugarcane, maize, chilli and lettuce and also on ornamentals such as cacti and succulents (Saadatnia and Riahi, 2009; Liesack *et al.*, 2000; Nayak *et al.*, 2001; Prisa, 2019; Prisa, 2020).

Table.1 Evaluation of liquid seaweed fertilizer on agronomic and pathological characters of *Carpobrotus edulis*

Groups	PH (cm)	LN (n°)	VW (g)	RW (g)	PI (n°)	SG (n°)	GT (days)
CTRL	7,92 f	4,60 c	22,94 d	16,83 e	3,40 a	4,40 d	24,20 a
A	9,61 e	5,40 b	24,48 c	17,59 d	1,40 c	5,60 c	21,40 b
B	10,49 d	5,00 bc	24,89 c	18,33 c	2,20 b	6,40 b	21,00 b
C	11,17 c	4,80 bc	24,27 c	18,43 c	1,40 c	6,40 b	21,80 b
D	13,81 b	6,20 a	26,94 b	19,93 b	1,20 c	7,80 a	18,80 c
E	14,35 a	6,80 a	27,71 a	20,27 a	1,00 c	8,20 a	18,60 c
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; (A): *Saccharina latissima;* (B): *Ulva ohnoi*; (C): *Sargassum muticum*; (D): *Hypnea cornuta*; (E): *Agardhiella subulata*; PH: plant height; LN: leaves number; VW: vegetative weight; RW: roots weight; PI: plants infected by *Pythium debaryanum;* SG: number of seeds germinated; GT: germination time.

Table.2 Evaluation of liquid seaweed fertilizer on agronomic and pathological characters of *Kalanchoe daigremontiana*

Groups	PH (cm)	LN (n°)	VW (g)	RW (g)	PI (n°)	SG (n°)	GT (days)
CTRL	7,04 c	5,20 c	15,04 de	10,05 f	1,60 a	7,40 d	18,00 a
A	7,91 b	6,00 bc	15,48 d	11,45 e	0,40 b	8,00 c	17,20 a
B	7,93 b	6,00 bc	14,92 e	12,19 d	0,00 b	8,40 bc	17,60 a
C	7,71 b	6,40 b	16,09 c	12,53 c	0,40 b	8,40 bc	17,20 a
D	8,67 a	8,00 a	17,57 b	13,85 b	0,00 b	9,00 a	16,40 b
E	8,86 a	8,60 a	18,83 a	14,55 a	0,20 b	8,80 ab	15,30 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; (A): *Saccharina latissima;* (B): *Ulva ohnoi*; (C): *Sargassum muticum*; (D): *Hypnea cornuta*; (E): *Agardhiella subulata*; PH: plant height; LN: leaves number; VW: vegetative weight; RW: roots weight; PI: plants infected by *Pythium debaryanum;* SG: number of seeds germinated; GT: germination time.

Table.3 Evaluation of liquid seaweed fertilizer on agronomic and pathological characters of *Kalanchoe tubiflora*

Groups	PH (cm)	LN (n°)	VW (g)	RW (g)	PI (n°)	SG (n°)	GT (days)
CTRL	7,05 d	7,20 c	14,70 d	11,26 e	0,20 a	7,20 b	15,60 a
A	7,73 c	7,40 c	15,39 c	12,64 d	0,00 a	7,60 b	14,80 b
B	7,58 c	7,80 bc	15,42 c	13,52 c	0,00 a	7,20 b	14,20 bc
C	7,64 c	7,40 c	15,60 c	13,55 c	0,20 a	7,60 b	14,00 cd
D	8,32 b	8,60 a	16,17 b	14,01 b	0,20 a	8,80 a	13,40 de
E	8,87 a	8,40 ab	16,43 a	14,68 a	0,20 a	9,20 a	13,20 e
ANOVA	***	***	***	***	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; (A): *Saccharina latissima;* (B): *Ulva ohnoi*; (C): *Sargassum muticum*; (D): *Hypnea cornuta*; (E): *Agardhiella subulata*; PH: plant height; LN: leaves number; VW: vegetative weight; RW: roots weight; PI: plants infected by *Pythium debaryanum;* SG: number of seeds germinated; GT: germination time.

Fig.1 Detail of germination plateaus and germinated *Carpobrotus edulis* seedlings in nurseries at CREA-OF in Pescia

Fig.2 Effect on vegetative and roots biomass of *Carpobrotus edulis* by *Hypnea cornuta* (D) and *Agardhiella subulata* (E) compared to the untreated control (CTRL)

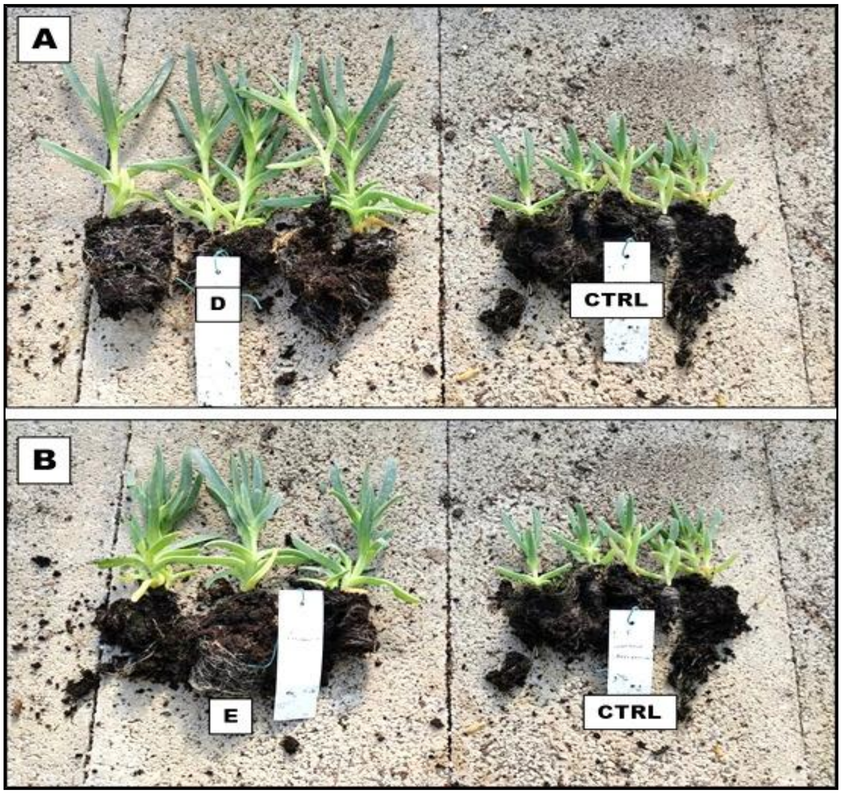


Fig.3 Effect on vegetative and roots biomass of *kalanchoe tubiflora* by *Hypnea cornuta* (D) and *Agardhiella subulata* (E) compared to the untreated control (CTRL)

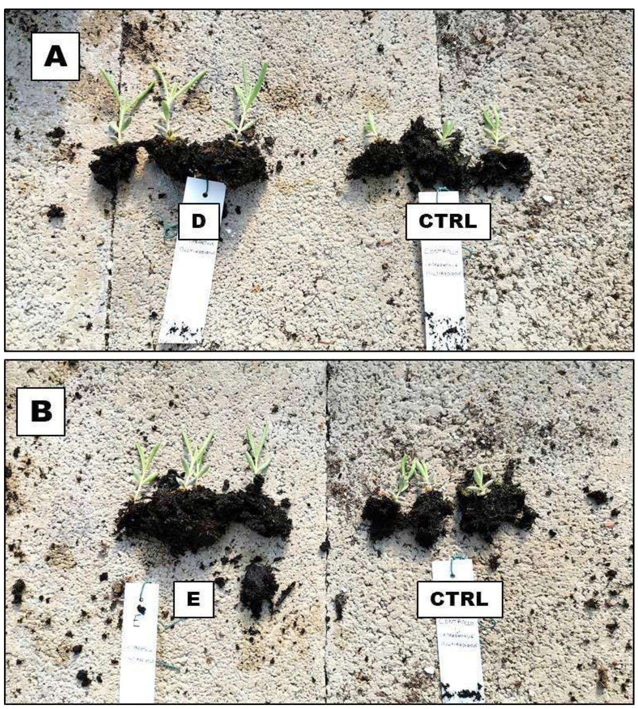
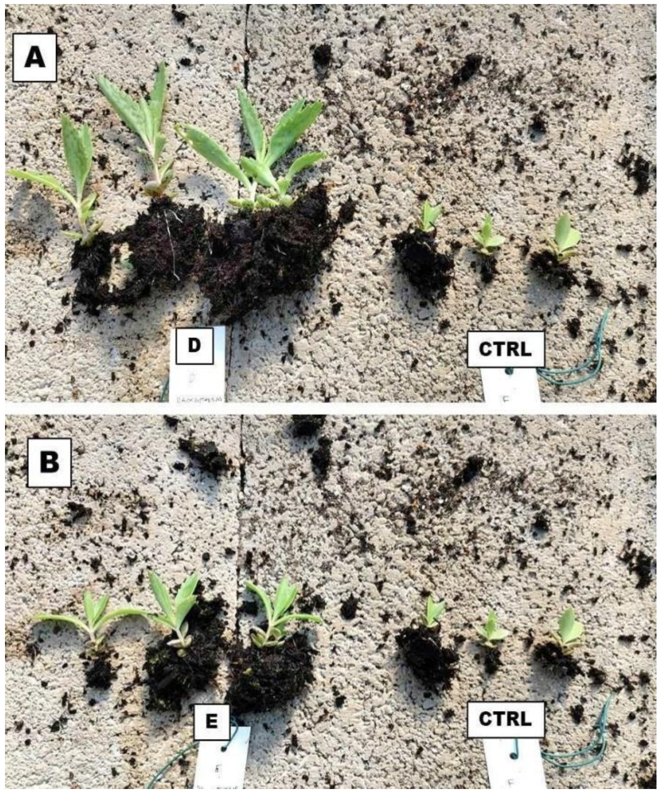


Fig.4 Effect on vegetative and roots biomass of *Kalanchoe daigremontiana* by *Hypnea cornuta* (D) and *Agardhiella subulata* (E) compared to the untreated control (CTRL)



In this experiment on *Carpobrotus edulis, Kalanchoe daigremontiana* and *Kalanchoe tubiflora*, the ability of selected algae extracts from Lake Ganzirri (ME), Venice Lagoon and Norwagian coasts, to significantly improve and increase plant quality, increase vegetative and root biomass, control pathogens such as *Pythium debaryanum* and improve seed germination is confirmed. Among all five algal extracts, two red algae present in Lake Ganzirri, proved effective in all measurements: *Hypnea cornuta* but, most of all, *Agardhiella subulata*.

Effects that can certainly be related to an increase in nutritional status due to increased nutrient uptake and the structural capabilities of the algae to provide greater water retention. The increased control of seedling pathogens can certainly be related to the structural and metabolic component of the algae that inhibits the development of fungi and bacteria (Rao and Burns, 1991).

Another aspect that should not be underestimated could be the possible modification of the microbial balance in the soil following the inoculation of blue-green algae or other types of algae (Ibrahim *et al.*, 1971; Acea *et al.,* 2001). Some studies have shown that algae inoculation can lead to an increase of up to eight times in microbial communities. The results suggest a carbon and energy surplus due to algae polysaccharides as one of the reasons behind the increase in heterotrophic microbial populations (Rogers and Burns, 1994; Anderson and Gray, 1991).

This research work has shown that algal species have great potential for use in the development of biofertilizers in sustainable agriculture in terms of cost-effectiveness, ecological role, possible reduction of synthetic fertilisers and plant protection products, increased soil fertility and microbiological diversity.

In particular, this trial is of particular interest for the selection of new algal products that can be used as plant biofertilizers to improve growth and reduce biotic and abiotic stresses in plants. in particular, were tested five different extracts and, as not expected result, *Agardhiella subulata* extract was the most effective biostimulant for all three plant tested at the expense of brown algae, with normally greater biostimulating capacities and green algae that produce flourishing biomass.

Further experiments will be carried out to evaluate the selection capabilities of new algal extracts detected in the Ganzirri Lake of Messina (Italy) in order to better understand the biostimulation potential and the possible reduction of water and salt stresses of plants grown in pots.

Acknowledgments

The research is part of the project "Micronaturale": innovative techniques with low environmental impact for the cultivation and protection of plants.

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How to cite this article:

Damiano Spagnuolo and Domenico Prisa. 2021. Evaluation of Growth Parameters on *Carpobrotus edulis, Kalanchoe daigremontiana and Kalanchoe tubiflora* in Relation to Different Seaweed Liquid Fertilizer (SLF) as a Biostimulant. *Int.J.Curr.Microbiol.App.Sci.* 10(10): 67-76. doi: https://doi.org/10.20546/ijcmas.2021.1010.010