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Application of innovative biostimulants for growth and quality improvement in vegetable and ornamental crops

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Abstract

Research objective: This research aims to evaluate the biostimulant potential of some innovative products that can improve germination, growth and protection from biotic stresses on some vegetable and ornamental species.

Materials and Methods: The experiments, which started in October 2022, were conducted in the CREA-OF greenhouses in Pescia (Pt), on *Cichorium intybus* and *Crassula rupestris* plants.

The experimental groups were: i) group control, irrigated with water and previously fertilised substrate; ii) biofertiliser Ecklonia maxima, dilution 1:1000, 5 ml of this dilution once a week per plant; iii) Elixir lite, irrigated with water and substrate previously fertilised; iv) Immuno pro group, irrigated with water and substrate previously fertilised; Harvest boost group, irrigated with water and substrate previously fertilised. On 23 January 2024, plant height, number of leaves, vegetative weight, root volume and length, number of microorganisms and pH of the substrate were determined. In addition, the mortality of the plants in the nursery was assessed.

Results and Discussion: The experiment showed that the use of biostimulants can significantly improve the vegetative and root growth of *Cichorium intybus* and *Crassula rupestris* plants.

In general, a significant increase in plant height, vegetative and root weight and root length was observed, particularly in the Elixir lite treatment. There was also a significant increase in the microbial colonisation of the substrate with all innovative biostimulant treatments, while the pH remained practically unchanged. The Immuno pro treatment was the best in terms of reducing plant mortality in all two plant species. Biostimulants are widely used in horticulture because we often work with short-cycle crops, varying from a few weeks to a few months. The rapid succession of different crops implies a constant supply of nutrients and intensive use of soil. More intensive horticulture in particular often benefits from the effect of biostimulant treatments due to the increased resource use efficiency of the crops. Foliar biostimulants are mainly used by farmers to increase the production of both leaf and fruit vegetable crops. The positive effects of their use are exerted both on increasing the content of secondary metabolites and on a general improvement of nutrient uptake efficiency. These biological effects can be attributed to the presence in biostimulants of polysaccharides, extracted from algae, such as alginates and carrageenan, which are responsible for two important biological actions: sequestering or slowly releasing nutrients. In fruit vegetables, biostimulants can also improve the homogeneity of flowering and fruit size, as reported for peppers. Commercial biostimulants have increased the unit yield of many leafy vegetable species by acting on nutrient uptake by the plant.

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Conclusions: In horticultural production, in open field and in protected crops, the application of biostimulants is aimed at achieving one or more of the following objectives: to favour a rapid emergence of seedlings in direct sowing crops or a rapid overcoming of the transplanting crisis; to precociate the entry into production; to increase growth, flowering, fruit set and fruit growth; to improve product quality; to increase the efficiency of nutrient use and tolerance to environmental stresses.

Keywords: Vegetables; Microorganisms; Plant growth; Biofertilizers; Rhizosphere

1. Introduction

In recent years, particular attention has been paid to the influence of biostimulant products on plant physiology [1]. Numerous studies have attempted to identify the mechanisms of action through which these products act, and the biologically active components present in their formulations. The work of scholars engaged in this area of research has consisted in characterising the chemical-physical properties of the products under investigation and in developing bioassays capable of evaluating their biostimulant activity [2]. With regard to chemical characterisation, it is of fundamental importance to determine the macro- and micro-nutrient content of the products in order to ascertain that their application to the plant does not induce a fertilising, rather than a biostimulating, effect [3]. Among the elements to be quantified, carbon occupies a special role because its content is generally used to determine the dose of the biostimulant to be distributed. Determining the correct dose is indispensable in order to achieve maximum effectiveness of action of the biostimulant. Excessive application of the biostimulant may, in fact, cause phytotoxic effects; conversely, too low a dose may not exert significant effects on crop productivity [4]. Another element that is necessarily quantified in biostimulant products is nitrogen (total, nitric and ammoniacal N) as it is absorbed by the plant in the form of nitrate and/or ammonium ions, translocated and then transformed into amino acids and proteins [5]. Biostimulants also stimulate plant defence responses to stress and the accumulation of phenolic compounds such as anthocyanins, flavonoids and phytoalexins. Phenolic compounds are a class of secondary metabolites characterised by the presence of an aromatic ring with one or more hydroxyl substituents [6]. The biological and ecological importance of these compounds is widely recognised. As constituents of lignins and suberins, phenolic polymers are an integral part of cell walls and, therefore, their function is that of mechanical support and barrier against microbial invasion [7]. These substances can influence competition between plants through a phenomenon called allelopathy. Certain phenols present in the formulation of biostimulant products themselves have a stimulating effect on growth processes when supplied to the plant in physiological concentrations. Their quantification in the products of interest is therefore useful. A further category of molecules that play a crucial role in regulating plant physiology are hormones [8]. These compounds are also referred to as chemical messengers because they transmit signals that can regulate important metabolic pathways by acting in extremely low micromole quantities. Among the various classes of hormones, those that have been studied in order to understand the action of biostimulants in plants include auxins, gibberellins and cytokinins [9]. However, recent studies have also highlighted the important role of ethylene and abscisic acid in the signalling pathways activated by biostimulant products. The mechanisms of action of most biostimulants remain largely unknown [10]. This is mainly due to the heterogeneous nature of the raw materials used for production and the complex mixtures of components contained in biostimulant products, which make it almost impossible to identify exactly which are actually responsible for the biological activity and thus determine their mode of action [11]. Therefore, there is an increasing tendency to define the 'mechanisms of action' of biostimulants in terms of their general impact on plant productivity, through the increase of processes such as photosynthesis, modulation of the hormonal response, nutrient and water uptake and activation of genes responsible for resistance to abiotic stresses [12].

1.1. Research Objectives

This research aims to evaluate the biostimulant potential of some innovative products that can improve germination, growth and protection from biotic stresses on some vegetable and ornamental species (Figure 1).

Figure 1 Details of the experiment at CREA-OF

2. Materials and methods

The experiments, which started in October 2022, were conducted in the CREA-OF greenhouses in Pescia (Pt), Tuscany, Italy (43°54′N 10°41′E) on *Cichorium intybus* and *Crassula rupestris* plants. The plants were placed in 12-diameter pots, 10 plants per 3 replicates, for a total of 30 plants per experiment.

All plants were fertilised with a controlled-release fertiliser (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 sowing. The experimental groups were:

- Group control (CTR) (100% peat) irrigated with water and previously fertilised substrate;
- Biofertiliser (BIOAL) group (peat 80%+ pumice 20%), irrigated with water and substrate previously fertilised; in addition, an algae-based biofertiliser (Kelpak biostimulant, Ecklonia maxima, Kelp products International) was used, dilution 1:1000, 5 ml of this dilution once a week per plant;
- Elixir lite (EL) group (peat 80%+ pumice 20%), irrigated with water and substrate previously fertilised; liquid organic fermented by Sanasoil microorganisms and earthworms; 5 ml of this product once a week per plant;
- Immuno pro (IP) group (peat 80%+ pumice 20%), irrigated with water and substrate previously fertilised; organic substitute for chemical fungicides and insecticides; 5 ml of this ptoduct once a week per plant;
- Harvest boost (HB) group (peat 80%+ pumice 20%), irrigated with water and substrate previously fertilised; a microbial inoculant dominated by photosynthesizing bacteria which improves the growing capabilities of plants therefore increasing harvest yields; 5 ml of this product once a week per plant;

The plants were watered once a day and grew for five months. The plants were drip-irrigated. Irrigation was activated by a timer whose programme was adjusted weekly according to the weather conditions and the leaching fraction.

On 23 January 2024, plant height, number of leaves, vegetative weight, root volume and length, number of microorganisms and pH of the substrate were determined. In addition, the mortality of the plants in the nursery was assessed.

2.1. Analysis methods

Microbial count: directly determining total microbial count by microscopy cells contained in a known sample volume using counting chambers (Thoma chamber). The surface of the slide is etched with a grid of squares, with the area of each square known. Determination of viable microbial load after serial decimal dilutions, spatula seeding (1 ml) and plate counting after incubation [13];

2.2. 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 ($P \le 0.05$, 0.01 and 0.001) differences among treatments. Mean values were then separated by the LSD multiple-range tests ($P = 0.05$). Statistics and graphics were supported by the programs Costat (version 6.451) and Excel (Office 2010).

3. Results

The experiment showed that the use of biostimulants can significantly improve the vegetative and root growth of *Cichorium intybus* and *Crassula rupestris* plants.

Table 1 Evaluation of the use of biostimulants on the agronomic characteristics of *Cichorium intybus*

Groups	Plant height (cm)	Leaves number (n°)	Substrate total Bacteria (Log CFU/g soil)	Vegetative weight (g)	Roots volume (cm^3)	Roots length (cm)	Substrate pH	Dead plants (n°)
CTR	11.38 d	4.40 d	1.67 d	35.55 e	22.54 e	4.74 e	6.84 b	5.20 a
BIOAL	13.82 c	6.40 c	2.24 c	40.64 d	28.69 d	5.34 d	7.14 a	1.60 b
EL	17.26 a	11.40 a	3.68 a	46.26 a	35.41 a	8.52 a	6.88 b	0.80 bc
IP	14.55 c	6.80 c	2.08 c	41.88 c	30.76 c	6.18 c	7.04 ab	0.00 c
HB	15.60 b	9.20 b	3.18 b	44.13 b	32.68 b	7.46 b	6.90 b	0.80 bc
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: (CTR) control; (BIOAL) *Ecklonia maxima*; (EL) Elixir lite; (IP) Immuno pro; (HB) Harvest boost

Table 2 Evaluation of the use of biostimulants on the agronomic characteristics of *Crassula rupestris*

Groups	Plant height (cm)	Leaves number (n°)	Substrate total bacteria (Log CFU/g soil)	Vegetative weight (g)	Roots volume (cm^3)	Roots length (cm)	Substrate pH	Dead plants (n°)
CTR	14.41 e	10.40 c	1.77 e	40.30 e	24.75 e	4.64 e	6.84 b	4.60 a
BIOAL	15.34 d	14.00 b	2.30 c	42.40 d	30.52 d	5.83 d	7.14 a	2.00 b
EL	17.78 a	18.40 a	3.76 a	47.27 a	35.70 a	9.28 a	6.88 b	0.40 c
IP	15.92 c	14.00 b	2.10 d	43.24 c	31.71 c	6.19 c	7.04 ab	0.00 c
HB	16.27 b	17.00 a	3.22 b	45.82 b	33.38 b	8.28 b	6.90 b	0.40 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: (CTR) control; (BIOAL) *Ecklonia maxima*; (EL) Elixir lite; (IP) Immuno pro; (HB) Harvest boost

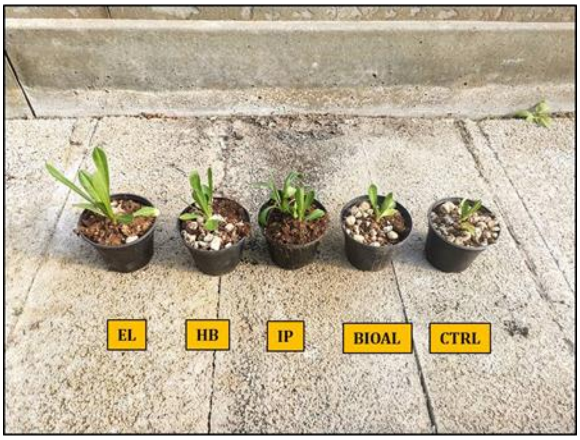
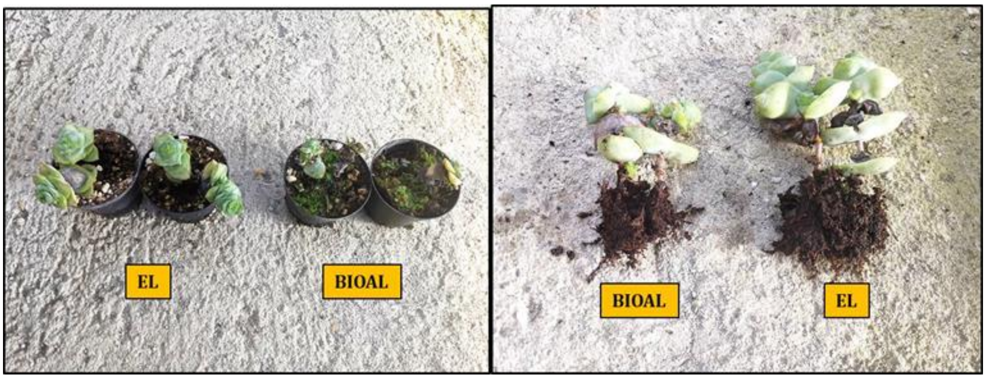


Figure 2 Comparison of different biostimulants on the vegetative development of *Cichorium intybus*

In general, a significant increase in plant height, vegetative and root weight and root length was observed, particularly in the Elixir lite (EL) treatment (Figure 2 and Figure 3). The Harvest boost thesis also shows a significant improvement in agronomic parameters compared to the control and biofertiliser with *Ecklonia maxima*. There was also a significant increase in the microbial colonisation of the substrate with all innovative biostimulant treatments, while the pH remained practically unchanged. The Immuno pro (IP) treatment was the best in terms of reducing plant mortality in all two plant species.



***F*igure 3** Effect of Elixir lite (EL) treatment compared with Ecklonia maxima (BIOAL) on the vegetative and root growth of *Crassula rupestris*

4. Discussion

Biostimulants are widely used in horticulture because we often work with short-cycle crops, varying from a few weeks to a few months [14]. The rapid succession of different crops implies a constant supply of nutrients and intensive use of soil. More intensive horticulture in particular often benefits from the effect of biostimulant treatments due to the increased resource use efficiency of the crops. Foliar biostimulants are mainly used by farmers to increase the production of both leaf and fruit vegetable crops [15]. The positive effects of their use are exerted both on increasing the content of secondary metabolites and on a general improvement of nutrient uptake efficiency [16]. These biological effects can be attributed to the presence in biostimulants of polysaccharides, extracted from algae, such as alginates and carrageenan, which are responsible for two important biological actions: sequestering or slowly releasing nutrients. In fruit vegetables, biostimulants can also improve the homogeneity of flowering and fruit size, as reported for peppers [17,18]. Commercial biostimulants have increased the unit yield of many leafy vegetable species by acting on nutrient uptake by the plant. These qualitative and quantitative benefits have been found in vegetables such as rocket and lettuce. In rocket, for example, it has been possible to decrease the growing nutrient solution by 75% compared to the standard solution, due to the increase in the plant's ability to absorb nutrients; it has also been shown that this increase in efficiency also corresponds to an increase in chlorophyll content. Increasing the concentration of chlorophyll in leafy vegetables contributes to improving the aesthetic quality of the product [19]. The leaf pigment content, in fact, is a very important quality parameter for leafy vegetables, also because it is the only one that the consumer can directly evaluate and is often associated with the freshness of salads [20]. The increase in nutrient use efficiency is linked both to the greater development of the root system of treated plants compared to control plants, and to the better use efficiency of solar radiation. The positive effect of biostimulants can also be observed in short-cycle vegetables, when the plant metabolism functions without environmental limitations. Conversely, under abiotic stress conditions, biostimulants normalise plant growth and development, thus enabling standardised growth and crop planning [21]. Vernieri et al. (2006) found an increase in root biomass following treatments with biostimulants; this response could be linked to the presence of compounds capable of inducing hormone-like effects, modifying the plant's hormonal balance. The increased growth of the root system in plants treated with biostimulants, administered both by foliar and root route, may lead to a reduction in transplant stress, speeding up rooting and growth [15]. Biostimulating effect of algae extracts was confirmed on lettuce in protected culture and endive. Tomato and pepper plants treated with commercial products showed an increase in secondary metabolites and improved root system growth and secondary root formation [22-24]. On pepper, the application of grape skin extract and an alfalfa hydrolysate resulted in an increase in leaf biomass and weight of harvested green ripening peppers, while increasing the growth and number of harvested red ripening peppers [25-29]. Experiments on hibiscus plants, treated with hydrolysed substances obtained from green compost and municipal solid waste fraction, showed an increase in photosynthetic activity resulting in a higher relative growth rate and biomass accumulation under both optimal growth conditions and nutrient deficiency in the root zone. Humic substances extracted from vermicompost and other organic compounds can be used to stimulate rooting in several agamically propagated species such as *Hibiscus rosa-sinensis* L., *Codianeum variegatum* L. and *Sanchezia nobilis* L., popular shrub species. In a study by Yazdani et al. (2014), the application of humic acids resulted in a significant improvement in root architecture, increased nutrient uptake efficiency and more flowers harvested. In gladiolus, chitosan treatments on the corms before transplanting extended the shelf-life of the flowers [30]. This trial confirms the positive effect of using biostimulants in the growth and defence of certain vegetable plants such as *Cichorium intybus* and ornamentals such as *Crassula rupestris*. Furthermore, as found in other trials, a significant increase in useful soil microbiology is noted [13,23].

5. Conclusion

In horticultural production, in open field and in protected crops, the application of biostimulants is aimed at achieving one or more of the following objectives: to favour a rapid emergence of seedlings in direct sowing crops or a rapid overcoming of the transplanting crisis; to precociate the entry into production; to increase growth, flowering, fruit set and fruit growth; to improve product quality; to increase the efficiency of nutrient use and tolerance to environmental stresses. The achievement of these objectives depends not only on the type of biostimulant used, the method of application and the dose applied, but also on the interaction of the biostimulant with genetic, agronomic and environmental factors.

Compliance with ethical standard

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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 animal/humans subjects

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