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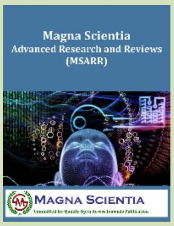

(RESEARCH ARTICLE)

Microorganisms and algae (GEA soil) use in the cultivation of onion (*Allium cepa* L.) and garlic (*Allium sativum* L.)

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Abstract

Research objective: This research aims to evaluate the biostimulant potential of an innovative microbial product on the development of onion (*Allium cepa* L.) and garlic (*Allium sativum* L.) bulbs and roots under controlled conditions

Materials and Methods: The experiments began in early July 2022 (mean temperature 22.5 °C), were carried out price experimental greenhouses of the CREA-OF of Pescia (Pt), Tuscany, on bulbs of onion (cv "Rossa di Tropea") and garlic (cv "Bianco Polesano"). The bulbs were placed in pots ø 14 cm; 40 bulbs for thesis divided into replicas of 20 bulbs each, for all onion and garlic.

The three experimental theses in cultivation were: i) group control irrigated with water and previously fertilised substrate; biofertiliser *Ecklonia maxima* irrigated with water and previously fertilised substrate; Gea soil (GEA) group irrigated with water and substrate previously fertilised.

On 26 January 2024, bulbs weight, bulb diameter, roots weight, number of microorganisms were determined. In addition, the mortality of the bulbs in the nursery was assessed.

Results and Discussion: The experiment showed that the use of biostimulants based on microorganisms and algae can significantly improve the size, weight and root growth of onion and garlic bulbs. In particular, the GEA soil thesis showed a significant increase in bulb length, weight and root growth compared to *Ecklonia maxima* and the untreated control thesis. There was also a significant increase in microbial colonisation of the substrate with GEA soil, a very interesting aspect that certainly had an effect on the growth of onion and garlic bulbs. The micro-organisms also by direct stimulation on the plants or by colonisation of the substrate significantly reduced bulb mortality in Gea soil, compared to BIOAL and the control. In recent years, horticulture has had to face the challenge of economic and environmental sustainability, reducing fertiliser use while adopting strategies to increase water use efficiency. Numerous forecasting models have shown that in the next 30 years many production areas could become semi-arid and among these is the Mediterranean basin, where many horticultural crops are grown. Increasing water use efficiency in this context appears essential for the development of sustainable horticulture. Many microorganisms or fungi can promote the plant's utilisation of nutrients and water in the soil. There are numerous strains of bacteria that promote root growth and are classified according to whether they act at the rhizosphere and rhizoplane level.

Conclusions: Microbial biostimulants can be a valuable strategy to mitigate environmental stresses that have been emphasised by climate change in recent years. Microbial biostimulants, by increasing the biodiversity of the agro-ecosystem and creating long-lasting symbiotic relationships with the plant, are, in fact, a sustainable and effective

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solution to prevent the reduction in productivity caused by abiotic stresses and to optimise the use efficiency of human inputs in the agrarian ecosystem.

Keywords: Vegetables; Microorganisms; Algae; Biofertilizers; Rhizosphere

1. Introduction

The use of beneficial microorganisms as an integral component of agricultural practices can contribute to increasing crop productivity in a sustainable manner by minimising the impact of biotic and abiotic stresses on cultivated plants and increasing nutrient availability [1]. The importance of the biotic component for soil fertility had been understood since ancient times. In recent years, the study of plant-microorganism interactions on a biochemical, physiological and molecular level has revealed that, in addition to the contribution of associated microorganisms, the plant itself is stimulated by its association with microbial symbionts to activate direct responses against stresses [2]. An example of these synergistic interactions is the development in the plant of a state of 'induced systemic tolerance', which consists of an increased tolerance to abiotic stresses, acquired through metabolic and structural adaptations developed in concert with symbiotic microorganisms [3]. There are currently more than a hundred products on the market based on live microorganisms, such as beneficial fungi and bacteria, that have been isolated and characterised from environments such as soil, plants, plant residues, water and composted manure [4]. Most of these products are based on biocontrol agents and growth promoters that enhance nutritional availability. Some of these have a direct effect, others express their action through interaction with the plant, e.g. by stimulating its defences or increasing its tolerance to stresses, and thus fall under the definition of biostimulants [5]. Compared to synthetic agrochemicals, microbial biostimulants are safe for the environment and humans, degrade easily and quickly, and have a reduced chance of inducing resistance in pathogenic microorganisms. However, the development of new biostimulant products presents some specific difficulties [6]. Firstly, the procedure for commercial registration is usually complex, and there is no harmonised international legislation. Secondly, a biostimulant may have different efficacy from crop to crop. When developing a plant-stimulant product, it is therefore advisable to consider the availability of microorganisms that have some form of communication and symbiosis with the specific plant to be treated. Indeed, the ability to establish a relationship with the host plant may favour, or even be an essential prerequisite for, the effectiveness of its action [7]. Finally, the study of the most correct formulation is necessary to ensure that the product is active at the time of treatment and maintains high efficacy regardless of the environmental or cultivation conditions in which it is used. As far as formulation is concerned, biostimulants containing Gram-positive bacteria have an undoubted advantage. Indeed, the production of spores and tolerance to desiccation make it possible to produce powder formulations with high stability over time [8]. The microorganisms contained in biostimulants can colonise both the root system and the aerial organs of the plant. The relationship with the plant host can be more or less close. In fact, the micro-organisms can reside in the area near the roots, on the root surface or in the aerial part, or they can show endophytic behaviour by colonising plant tissue internally. The bacteria used as biostimulants, generally called Plant Growth Promoting Rhizobacteria (PGPR), have the ability to influence many aspects of life, from growth to nutrition, morphogenesis and development, response to biotic and abiotic stresses, and interaction with other microorganisms within the agroecosystem [9,10]. Among the most studied and characterised genera are *Burkholderia*, *Bacillus*, *Pseudomonas*, *Serratia* and *Streptomyces*. As far as fungal biostimulants are concerned, endosymbionts are particularly interesting and much studied [11]. These types of fungi induce immune pre-activation responses in plants; they also improve their nutrient efficiency, growth and morphogenesis, alleviate nutritional stresses and control photosynthesis allocation. The genus *Trichoderma* is probably the most studied and used. Its phytostimulation mechanism involves different levels of communication with roots and shoots [12]. Interestingly, in some cases, growth-promoting effects by a fungal biocontrol agent have been imputed to its associated microbial consortium. Another group of fungi of agricultural interest is the arbuscular mycorrhizae. These endomycorrhizae, of the division Glomeromycota, are frequently associated with agricultural and horticultural plants, and form branching structures of interchange with cortex cells in the root of the host plant [13]. Despite beneficial plant properties, such as increased nutritional efficiency and protection from biotic and abiotic stresses, the use of mycorrhizae in commercial formulations is hampered by the difficulty of in vitro propagation and lack of understanding of the determinants of host specificity and population dynamics of mycorrhizae in the agroecosystem [14].

1.1. Research Objectives

This research aims to evaluate the biostimulant potential of an innovative microbial product on the development of onion (*Allium cepa* L.) and garlic (*Allium sativum* L.) bulbs and roots under controlled conditions

Figure 1 Details of the bulbs used in the trial

2. Material and methods

The experiments began in early July 2022 (mean temperature 22.5°C), were carried out price experimental greenhouses of the CREA-OF of Pescia (Pt), Tuscany, Italy (43°54′N 10°41′E) on bulbs of onion (cv "Rossa di Tropea") and garlic (cv "Bianco Polesano"). The bulbs were placed in pots ø 14 cm; 40 bulbs for thesis divided into replicas of 20 bulbs each, for all onion and garlic.

All bulbs were fed with the same amount of nutrients supplied through controlled release fertilizer (5 kg m^{-3} of Osmocote Pro® 6-9 months containing 190 g/kg N, 39 g/kg P, 83 g/kg K) blended with the growing medium before transplant.

The three experimental theses in cultivation were:

- Group control (CTR) (peat 80% + pumice 20%) 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;
- Gea soil (GEA) group (peat 80%+ pumice 20%), irrigated with water and substrate previously fertilised; The main species involved in GEA suolo include Lactic acid bacteria: *Lactobacillus plantarum, L. casei, Streptoccus lactis; Photosynthetic bacteria: Rhodopseudomonas palustrus, Rhodobacter spaeroides; Yeast: Saccharomyces cereviasiae, Candida utilis; Actinomycetes: Streptomyces albus, S. griseus*; Fermenting fungi: Aspergillus oryzae, Mucor hiemalis; Glomus sp.; Spirulina platensis. Dilution 1:100, 10 ml of this dilution once a week per bulb;

The lighting of the greenhouse at the plant level was about 12,000 lux with high pressure sodium lamps. The plants were lit for 16 hours a day. A minimum daytime temperature of 10 °C and a night-time temperature of 25 °C were maintained in the greenhouse. The plants were watered once a day every four days and grew for seven 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 26 January 2024, bulbs weight, bulb diameter, roots weight, number of microorganisms were determined. In addition, the mortality of the bulbs 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 [14];

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 ($P \leq 0.05$, 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 based on microorganisms and algae can significantly improve the size, weight and root growth of onion and garlic bulbs (Table 1 and Table 2). In particular, the GEA soil thesis showed a significant increase in bulb length, weight and root growth compared to *Ecklonia maxima* and the untreated control thesis (Figure 2 and Figure 3). There was also a significant increase in microbial colonisation of the substrate with GEA soil, a very interesting aspect that certainly had an effect on the development of onion and garlic bulbs. In fact, microorganisms have a decisive effect on the absorption of water and nutrients and the stimulation of the root system. The micro-organisms also by direct stimulation on the plants or by colonisation of the substrate significantly reduced bulb mortality in Gea soil, compared to BIOAL and the control.

Table 1 Evaluation of the use of biostimulants on the agronomic characteristics of onion cv "Rossa di Tropea"

Groups	Bulb lenght (mm)	Bulb weight (g)	Roots weight (g)	Substrate total Bacteria (Log CFU/g soil)	Dead plants (n°)
CTR	38.73 c	42.79 c	28.32 c	1.71 c	2.00 a
BIOAL	41.58 b	44.29 b	30.35 b	2.09 b	0.80 b
GEA	43.78 a	48.79 a	32.59 a	3.77 a	0.20 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: (CTR) control; (BIOAL) *Ecklonia maxima*; (GEA) *Lactobacillus plantarum, L. casei, Streptoccus lactis, Rhodopseudomonas palustrus, Rhodobacter spaeroides, Saccharomyces cereviasiae, Candida utilis, Streptomyces albus, S. griseus, Aspergillus oryzae, Mucor hiemalis, Glomus sp., Spirulina platensis*.

Table 2 Evaluation of the use of biostimulants on the agronomic characteristics of garlic cv "Bianco Polesano"

Groups	Bulb lenght (mm)	Bulb weight (g)	Roots weight (g)	Substrate total Bacteria (Log CFU/g soil)	Dead plants (n°)
CTR	17.60 c	24.02 c	34.21 c	1.25 c	2.11 a
BIOAL	20.26 b	26.45 b	36.05 b	2.08 b	0.84 b
GEA	24.62 a	28.51 a	40.17 a	2.96 a	0.22 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: (CTR) control; (BIOAL) *Ecklonia maxima*; (GEA) *Lactobacillus plantarum, L. casei, Streptoccus lactis, Rhodopseudomonas palustrus, Rhodobacter spaeroides, Saccharomyces cereviasiae, Candida utilis, Streptomyces albus, S. griseus, Aspergillus oryzae, Mucor hiemalis, Glomus sp., Spirulina platensis*.

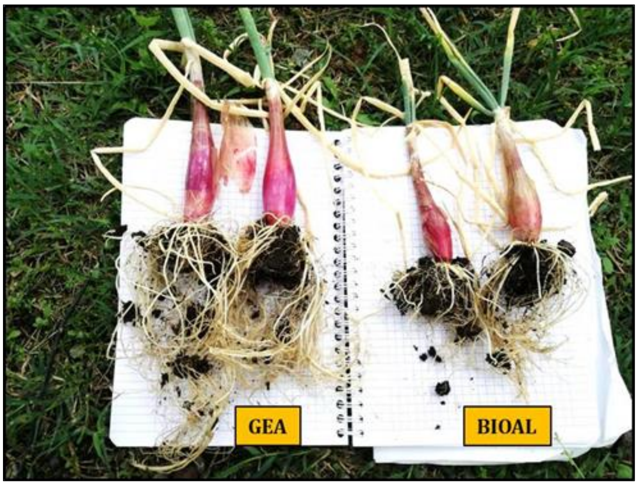

Figure 2 Comparison of onions treated with GEA soil and *Ecklonia maxima* cv "Rossa di Tropea"

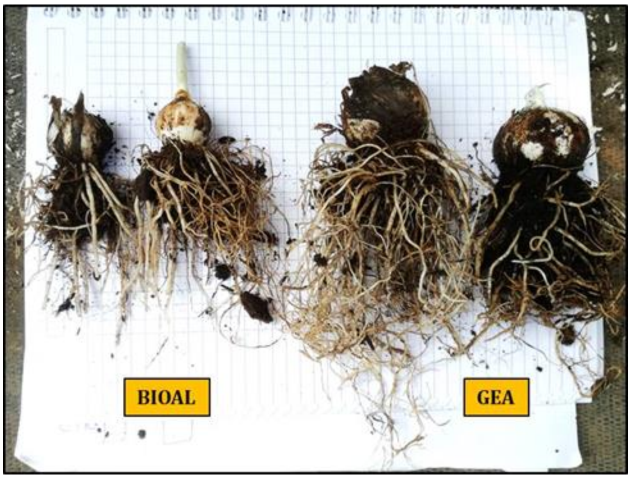

Figure 3 Comparison of garlic treated with GEA soil and *Ecklonia maxima* cv "Bianco Polesano"

4. Discussion

In recent years, horticulture has had to face the challenge of economic and environmental sustainability, reducing fertiliser use while adopting strategies to increase water use efficiency. Numerous forecasting models have shown that in the next 30 years many production areas could become semi-arid and among these is the Mediterranean basin, where many horticultural crops are grown [15]. Increasing water use efficiency in this context appears essential for the development of sustainable horticulture. Many microorganisms or fungi can promote the plant's utilisation of nutrients and water in the soil [16]. There are numerous strains of bacteria that promote root growth and are classified according to whether they act at the rhizosphere and rhizoplane level. Many studies with growth-promoting bacteria, which have yielded interesting results in a controlled environment or in vitro, have not appeared so effective in field crops in promoting the growth of vegetable species [17]. The inoculation technique for PGPR bacteria generally involves inoculating 'root dippind of seedlings' during the nursery stages or before or during transplanting.

In the case of broccoli (*Brassica oleracea* var. italica) grown in open field, inoculation with two bacterial species, *Brevibacillus rueszeri* and *Rhizobium rubi*, increased the unit yield, the overall plant weight and the diameter of the inflorescences [17]. The inoculated bacteria also resulted in an increase in the concentration of chlorophyll in the leaves and the uptake of micro- and macroelements by the plant. In lettuce, an increase in growth was observed in the presence of poorly fertile soils due to the inoculation of *Rhizobium leguminosarum biovar phaseoli*. Also in lettuce, the root-level inoculation of *Serratia proteamaculans* in protected culture reduced the negative effects of salt stress by increasing photosynthetic efficiency and chlorophyll content. The biostimulating effect of the bacterium also increased the leaf area, the fresh weight of the head and the content of macronutrients and certain enzymes responsible for the detoxification of reactive oxygen species [18]. Bacterial species capable of promoting the growth of vegetable plants include those of the genus *Bacillus*. Studies on peppers in open field have shown an increase in root biomass and stem weight. The increase in root biomass coincided with an elongation of the root system and an increase in root tips [19]. Also in fruiting vegetable species, the addition of *Bacillus subtilis* and *Bacillus amyloliquefaciens*, inoculated in a formulation with 2.5% chitin at the transplanting stage, protected the roots from root pathogens, anticipating the harvest phase and increasing total plant production. On strawberry, species of the genus *Pseudomonas* and *Bacillus* administered at both root and foliar level showed a positive effect on overall plant growth and total production. Also in this case, the PGPR bacteria increased the chlorophyll and water concentration in the leaves and improved the uptake of micronutrients [20]. A positive effect was also observed in the reduction of symptoms related to salt stress. The mode of action of PGPR bacteria clearly differs between genera; indeed, not all genotypes possess the same mechanisms of action and interaction with the plant [21]. Mechanisms of action of biostimulants include: changes in phytohormone levels, production of volatile compounds, increased availability of nutrients by the root and tolerance to abiotic stresses. Microorganisms that stimulate the growth and resistance of horticultural species also include arbuscular mycorrhizal fungi. They are effective in nutrient uptake but also in improving tolerance to abiotic stresses [22]. The following fungi are particularly used in horticulture: *Rhizophangus intraradicens* and *Funneliformis mossae* known as *Glomus*. Most of the mycorrhizal fungi available on the market today have demonstrated a positive action on uptake, phosphorus availability and mobilisation of bi- and trivalent cations. The main action of mycorrhizal fungi is to increase the absorption surface area of the root system and allow plants to explore a greater volume of soil in search of nutrients and water [23].

Mycorrhizae have provided very positive effects on artichoke plants, as well as in many other vegetable species. The improved quality of the plant in the nursery phase also has an effect on its behaviour in the subsequent cultivation phase. Indeed, mycorrhizae ensure a symbiotic relationship with the host plant, which makes it possible to overcome transplant stress and reduce the acclimatisation period. Colonised roots are protected from attacks by root pathogens by the barrier effect of the mycorrhiza itself, which prevents physical contact of the pathogen with the root. All these aspects result in greater plant homogeneity during cultivation even in abiotic stress situations [24,25].

5. Conclusion

Microbial biostimulants can be a valuable strategy to mitigate environmental stresses that have been emphasised by climate change in recent years. Microbial biostimulants, by increasing the biodiversity of the agro-ecosystem and creating long-lasting symbiotic relationships with the plant, are, in fact, a sustainable and effective solution to prevent the reduction in productivity caused by abiotic stresses and to optimise the use efficiency of human inputs in the agrarian ecosystem. The use of these formulations still has several limitations, mainly linked to the fact that their action is less rapid than synthetic agrochemicals. Moreover, being based on living organisms, their use is more complex than a xenobiotic molecule and is therefore more subject to crop variables. Indeed, it is important to emphasise that in the agroecosystem it is not only plants that are subject to the stresses exacerbated by climate change, but also the communities associated with them. For all these reasons, biostimulants are often not the first choice of growers. Several complementary strategies can help the spread of microbial biostimulants. Firstly, future programmes for the development of microbial products should consider the stress tolerance of the microbes themselves, as well as their beneficial effects on plant hosts. In addition, the use of microbial biostimulants must be accompanied by other techniques that aim to maintain the ecological balance of the agroecosystem, for example, by minimising the use of toxic pesticides and heavy metals such as copper, which also have a harmful effect on the active microorganisms of biostimulant products. Finally, intensive training of farmers on the benefits of these compounds and the specificity of their use is necessary.

Compliance with ethical standards

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Disclosure of conflict of interest

The author declares no conflict of interest.

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