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Stimulating microorganisms in the cultivation and improvement of vegetable plants

Domenico Prisa [1,*] and Giordano Menci [2]

[1] *CREA Research Centre for Vegetable and Ornamental Crops, Council for Agricultural Research and Economics, Via dei Fiori 8, 51012 Pescia, PT, Italy.*

[2] *Agrogen, Viale delle Fosse 16, Anagni (FR) 03012, Italy.*

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Abstract

Research objective: In this study, the possibility of using different microbial types inoculated into the growing medium was evaluated in order to assess the growth-promoting potential of certain vegetable species.

Materials and Methods: The plants were grown in pots under controlled conditions; 30 seedlings per thesis, divided into 3 replications of 10 plants each, were planted in early November 2023. The plants used in the trial were *Cichorium intybus* and *Allium ampeloprasum*. All plants were fertilised with a slow-release fertiliser (2 kg m-3 of Osmocote Pro® for 6 months) introduced into the growing medium at the time of transplanting. The six experimental groups in cultivation were: i) group without microorganisms, irrigated with water and previously fertilised substrate; ii) group with *Pacilomyces lilacinus*; iii) group with *Azospirillum* sp.; iv) group with *Glomus* sp.; v) group with *Trichoderma viride*; vi) group with mix *Bacillus subtilis*, *Pseudomonas* sp. and *Trichoderma viride.* On 11 January 2024, plant height, number of leaves, total leaf area per plant (mm^2), primary root length (mm), biomass of the aerial and root system, and number of dead plants were recorded.

Results and Discussion: The experiment showed that the use of microbial strains of various types can indeed significantly improve the vegetative and root growth of *Cichorium intybus* and *Allium ampeloprasum*. All treatments showed a significant improvement over the untreated control for the agronomic parameters analysed, but the *Azospirillum* sp. and *Glomus* sp. treatments were statistically the best. Improvements were also found in plant height, number of leaves, leaf area, vegetative and root biomass and root length. A very interesting aspect was also the ability of the microbial products to reduce plant mortality, particularly the application of *Trichoderma viride* in *Cichorium intibus* and all microbial treatments on *Allium ampeloprasum*.

Conclusions: This experiment highlights other interesting and innovative aspects of the use of microorganisms, which have already been highlighted in previous trials on various vegetable and ornamental species. Given the importance of the application of microbial inoculums in plants, the new agricultural experiments are very important because they could enable the development of new products to be used in organic and sustainable farming systems and enable better results.

Keywords: Plants growth; Sustainable applications; Microorganisms; Rhizosphere; Soil improvement

1. Introduction

The soil is a thin layer of the earth's surface that acts as an interface between the atmosphere, lithosphere and hydrosphere. It represents the natural reservoir of food, fodder, textile fibres, substances useful to the pharmaceutical industry, and renewable energy sources on which life on earth depends [1]. After the oceans, soil is the planet's largest

* Corresponding author: Domenico Prisa

store of carbon, and one third of all living species are found beneath its surface. In fact, soil is characterised by an extreme variability and complexity of environmental conditions, allowing it to host a very large number of living organisms that perform essential functions for humans, ecosystems and life itself. In a handful of agricultural soil, an average of 0.5 grams of living organisms can be found, many of which are not visible to the naked eye [2]. Ecosystem services are determined by soil functions, which in turn depend on the chemical and physical characteristics of soils and the physical, chemical and biological processes occurring in them. These processes, called addition, subtraction or removal, translocation and transformation of the different components within the soil, mean that the soil can be considered a living system in continuous evolution [3].

1.1. Soil and living organisms

Living organisms are part of the five soil-forming factors and perform multiple functions that influence the chemical and physical characteristics of horizons. For the purposes of the process of soilogenesis, we can distinguish two major classes of organisms in relation to their role in organic matter:

- Autotrophic organisms, which produce and release organic matter into the soil;
- Heterotrophic organisms that utilise soil organic matter.

The latter either break down and decompose the organic substance down to the starting inorganic molecules or rework it into complex, degradation-resistant molecules. Through the process of mineralisation, the microorganisms produce organic acids, lower the pH value and promote the mobility of iron and aluminium [4,5]. In fact, they actively contribute to pedogenesis through solubilisation and precipitation processes and oxidation-reduction reactions related to the breakdown of primary minerals. Furthermore, by promoting the production of humic colloids, they contribute to the formation of clay-humic complexes, which are fundamental in the regulation of nutrient exchanges between the soil matrix and the circulating solution, as well as in the formation of aggregates and thus the characteristics that influence the water balance [6]. The organic matter that reaches the soil can be of different nature, composition and degree of degradability. The C/N ratio of organic matter provides important indications of its degree of degradability or the overall degradation achieved by it in the soil [7]. Climate change and human action can strongly influence the fate of soil carbon. For example, soil management can influence and stimulate organic matter decomposition processes, e.g. by promoting soil oxygenation, contact of organic matter with microbial communities, initiation processes and surface erosion. Soils and their properties vary in space both vertically and horizontally depending on the factors of pedogenesis [8].

1.2. PGPR and BCA in soil

The term PGPR Plant Growth Promoting Rhizobacteria, introduced in 1978 by Kloepper and Schroth, refers to those microorganisms that, by colonising the rhizosphere, promote root and aerial growth, thus leading to increased plant development [9,10]. PGPRs can be divided into two broad categories:

- Plant growth-promoting bacteria that establish close symbioses with plants, which include rhizobia, bacteria that live in symbiosis with leguminous plants;
- Bacteria capable of promoting plant growth without establishing close symbiosis with them, which grow in the rhizosphere or on the surface of the roots they colonise, promoting plant growth through various mechanisms.

The term BCA 'Biological Control Agent' refers to an organism capable of counteracting the attack of another pathogenic organism. BCAs can be fungi or antagonistic bacteria; many viruses, too, can play the role of a BCA [11]. PGPRs and BCAs are of considerable interest in agriculture as PGPRs act as biofertilisers and biostimulants, while BCAs protect plants against pathogens. However, it is important to bear in mind that the same strain can behave as both PGPR and BCA at the same time [12]. A good PGPR or bacterial BCA must be able to colonise the root surface and rhizosphere quickly and stably, reaching a high density, thus establishing close physical contact with or proximity to the root surface. In some cases, the PGPR/BCA can even penetrate inside the roots and thus become an endophyte, which can reach the aerial parts of the plant through the vascular system [13]. Roots release root exudates and rhizo-deposition products resulting in the accumulation, around the roots themselves, of a complex series of molecules that act as nutrients or chemotaxis signals or even contribute to the constitution of polymeric matrices that protect and stabilise microbial biofilms [14]. The micro-organisms, in turn, activate a series of metabolic processes that enable them to colonise the roots of plants to a high and stable density. Once root colonisation has taken place, PGPRs and BCAs exert their action through various mechanisms, both direct and indirect [15,16].

In this study, the possibility of using different microbial types inoculated into the growing medium was evaluated in order to assess the growth-promoting potential of certain vegetable species.

Figure 1 Details of the plants used in the trial

2. Materials and methods

The plants were grown in pots under controlled conditions; 30 seedlings per thesis, divided into 3 replications of 10 plants each, were planted in early November 2023. The plants used in the trial were *Cichorium intybus* and *Allium ampeloprasum*. All plants were fertilised with a slow-release fertiliser (2 kg m^{-3} of Osmocote Pro® for 6 months) introduced into the growing medium at the time of transplanting.

The six experimental groups in cultivation were:

- Group without microorganisms (CTRL): (peat 70% + pumice 20%), irrigated with water and previously fertilised substrate;
- Group with *Pacilomyces lilacinus* (GL): (peat 70% + pumice 20%), irrigated with water and previously fertilised substrate, (4 x 10^6 spores/g) 1 g of product mixed per kg of substrate;
- Group with *Azospirillum* sp. (AZ): (peat 70% + pumice 20%), irrigated with water and previously fertilised substrate, (4 x 10^8 spores/g) 1g of mixed product per kg of substrate;
- Group with *Glomus* sp. (GS): (peat 70% + pumice 20%), irrigated with water and previously fertilised substrate, (3 x 10^9 spores/g) 1g of mixed product per kg of substrate;
- Group with *Trichoderma viride* (TV): (peat 70% + pumice 20%), irrigated with water and previously fertilised substrate, (3 x 10^8 spores/g) 1g of product mixed per kg of substrate;

Group with mix *Bacillus subtilis*, *Pseudomonas* sp. and *Trichoderma viride* (BPT): (peat 70% + pumice 20%), irrigated with water and previously fertilised substrate, (4 x 10^9 spores/g) 1g of product mixed per kg of substrate.

The plants were watered once a day and cultivated for 3 months. The plants were drip-irrigated. Irrigation was activated by a timer whose schedule was adjusted weekly according to weather conditions and leaching fraction. On 11 January 2024, plant height, number of leaves, total leaf area per plant (mm^2), primary root length (mm), biomass of the aerial and root system, and number of dead plants were recorded.

2.1. Statistics

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).

3. Results and Discussion

The experiment showed that the use of microbial strains of various types can indeed significantly improve the vegetative and root growth of *Cichorium intybus* and *Allium ampeloprasum* (Table 1 and Table 2). All treatments showed a significant improvement over the untreated control for the agronomic parameters analysed, but the *Azospirillum* sp. and *Glomus* sp. treatments were statistically the best (Figure 2 and Figure 3). Improvements were also found in plant height, number of leaves, leaf area, vegetative and root biomass and root length. A very interesting aspect was also the ability of the microbial products to reduce plant mortality, particularly the application of *Trichoderma viride* in *Cichorium intibus* and all microbial treatments on *Allium ampeloprasum*.

The market for microbial preparations has suffered from a lack of regulation at national and EU level. In current legislation, there is no unambiguous definition that identifies the category of microbial-based biopreparations, which, if not registered as biopesticides, fall under the category of 'mycorrhizal fungi inoculums' in Legislative Decree 75/2010, which regulates them as 'products with specific action on soil and plants', which are placed under the subcategory of biostimulants [17].

The element that characterises this type of product is the active ingredient, represented by live spores of beneficial microorganisms. Within this group, we can highlight, for example, bacteria of the genus *Bacillus*, *Azospirillum*, *Pseudomonas* and *Rhizobium*, as well as fungi of the genera *Beauveria*, *Gliocladium*, *Pochonia*, *Metarhizium* and *Trichoderma*, as well as actinomycetes of the genus *Streptomyces* [18-20]. These soil microorganisms are in close contact with plants, occupying a large part of the space known as the rhizosphere. Furthermore, in most cases, they are able to form a biofilm that protects the roots against pathogens [21,22]. The beneficial microorganisms are supported by the host plant, taking advantage of the carbon-rich compounds contained in the root exudates, such as sugars, amino acids, flavonoids, proteins and fatty acids that are essential for their nutrition [23-25]. Furthermore, exudates may have an antimicrobial action, inhibiting the growth of some species and favouring others, indicating a strong selective power towards microorganisms for colonising the rhizosphere [26]. Plants, therefore, play a key role in influencing the microbial composition of the native and inoculated rhizosphere community. It has been observed that depending on the growth phase of the plant, the signal molecules emitted by plants can influence the structure of the microbial community in the rhizosphere [27,28]. It has also been observed that depending on the growth phase of the plant, the signal molecules emitted by plants can influence the structure of the microbial community in the rhizosphere [29-31]. Furthermore, inoculums based on microorganisms are able to increase photosynthetic activity, salinity tolerance and the bioavailability of iron and other nutrients, as well as reduce disease [32-36]. As confirmed in this trial, the use of microorganisms resulted in improved plant growth and quality and reduced plant mortality. Also in other tomato trials on saline soils in the Naples area, the use of microorganisms with a prevalence of *Glomus* sp. resulted in a 49% increase in root colonisation and an increase in fruit yield and quality with regard to dry residue, soluble solids, mineral element concentration, lycopene, polyphenols and ascorbic acid [37]. On shallots (*Allium cepa* L. Aggregatum), the application of microgreens resulted in an increase in bulb production and quality, as well as in the concentration of mineral elements, ascorbic acid and antioxidant activity [38].

Table 1 Evaluation of microbial fertilisers on agronomic characters on plants of *Cichorium intybus*

Cychorium intybus	PH (cm)	LN (n°)	TLA (mm^2)	VW (g)	RW (g)	RL (cm)	DP (n°)
CTRL	12.48 e	4.43 d	54.47 e	35.60 e	22.54 e	4.67 f	4.60 a
GL	13.78 d	6.00 c	57.69 c	40.59 d	28.69 d	5.32 e	1.40 b
AZ	16.93 a	10.80 a	61.37 a	45.71 a	34.91 a	8.36 a	0.60 c
GS	15.56 b	9.00 b	59.49 b	43.60 b	32.58 b	7.35 b	0.60 c
TV	14.41 c	6.41 c	56.49 d	41.84 c	30.36 c	6.15 c	0.00 c
BPT	14.37 c	6.23 c	55.85 d	41.41 c	30.28 c	5.72 d	0.60 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). Parameters: PH = plant height (cm); LN = leaves number (cm); TLA = total leaves area (mm^2); VW = vegetative weight (g); RW = roots weight (g); RL = roots length (cm); DP= dead plants (n°). Treatments: CTRL=control; GL= *Pacilomyces lilacinus*; AZ=*Azospirillum* sp.; GS=*Glomus* sp.; TV=*Trichoderma viride*; BPT=*Bacillus subtilis*, *Pseudomonas* sp. and *Trichoderma viride*

Table 2 Evaluation of microbial fertilisers on agronomic characters on plants of *Allium ampeloprasum*

Allium ampeloprasum	PH (cm)	VW (g)	RW (g)	RL (cm)	DP (n°)
CTRL	8.68 d	20.19 d	16.50 e	5.88 e	1.80 a
GL	9.22 c	21.71 c	17.63 c	6.26 d	0.00 b
AZ	12.51 a	23.75 a	19.42 a	7.65 a	0.00 b
GS	11.70 b	22.88 b	18.36 b	7.22 b	0.00 b
TV	9.37 c	21.86 c	17.28 d	6.63 c	0.00 b
BPT	9.24 c	21.85 c	17.47 cd	6.14 d	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); Parameters: PH = plant height (cm); VW = vegetative weight (g); RW = roots weight (g); RL = roots length (cm); DP= dead plants (n°). Treatments: CTRL=control; GL= *Pacilomyces lilacinus*; AZ=*Azospirillum* sp.; GS=*Glomus* sp.; TV=*Trichoderma viride*; BPT=*Bacillus subtilis*, *Pseudomonas* sp. and *Trichoderma viride*

Figure 2 Effect of *Glomus* sp. on vegetative biomass of *Cichorium intybus* compared with fertilised control

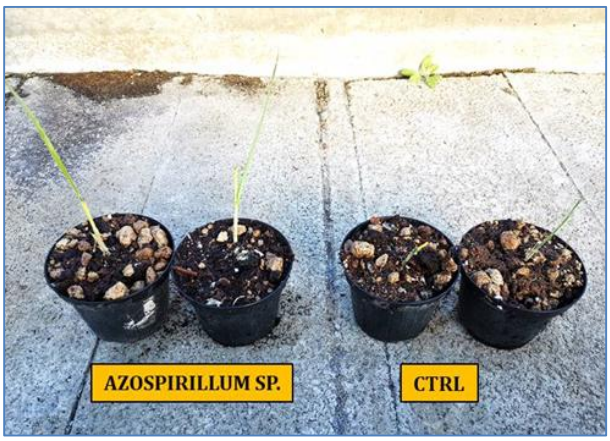

Figure 3 Effect of *Azospirillum* sp. on vegetative biomass of *Allium ampeloprasum* compared with fertilised control

4. Conclusion

Experiments have shown that the use of microorganisms can significantly improve the growth, vegetative and root biomass of *Cichorium intybus* and Allium *ampeloprasum*. The treatment also provides increased resistance to mortality that can occur in nursery cultivation.

This highlights other interesting and innovative aspects of the use of microorganisms, which have already been highlighted in previous trials on various vegetable and ornamental species. Given the importance of the application of microbial inoculums in plants, the new agricultural experiments are very important because they could enable the development of new products to be used in organic and sustainable farming systems and enable better results.

Compliance with ethical standards

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Disclosure of conflict of interest

The author declares no conflict of interest.

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