GSC Advanced Research and Reviews
eISSN: 2582-4597 CODEN (USA): GARRC2
Cross Ref DOI: 10.30574/gscarr
Journal homepage: https://gsconlinepress.com/journals/gscarr/

(Research Article)

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# Production and evaluation of new microbic strains on zucchini (*Cucurbita pepo* L.) plants and control of Powdery mildew and *Cladosporiosis*

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GSC Advanced Research and Reviews, 2024, 20(01), 338–348

Publication history: Received on 09 June 2024; revised on 22 July 2024; accepted on 24 July 2024

Article DOI: https://doi.org/10.30574/gscarr.2024.20.1.0275

## Abstract

**Research objective:** The main objective of this article is to report the results obtained from the use of new microbial strains on vegetable plants. In particular, the article will deal with two important topics: i) the study of the effect of microorganisms on the biomass of vegetable plants; ii) the possible control of mortality due to diseases such as powdery mildew and Cladosporiosis. The information reported in this research work can support the design of cropping systems in which agricultural sustainability is fundamental due to the presence of microorganisms with biostimulating activity and as a possible alternative to synthetic plant protection products.

**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 February 2024. The plants used in the trial were *Cucurbita pepo* L.. The six experimental groups in cultivation were: i) group without microorganisms, irrigated with water and previously fertilised substrate; ii) group with *Paecilomyces lilacinus*, irrigated with water and previously fertilised substrate, (4.5 x $10^6$ spores/ml), 5ml of product sprayed per kg of substrate; iii) group with *Azospirillum* sp., irrigated with water and previously fertilised substrate, (4 x $10^8$ spores/g), 1g of mixed product per kg of substrate; iv) group with *Glomus* sp., irrigated with water and previously fertilised substrate, (3 x $10^9$ spores/g), 1g of mixed product per kg of substrate; v) group with *Trichoderma viride*, irrigated with water and previously fertilised substrate, (3.6 x $10^8$ spores/ml), 5ml of product sprayed per kg of substrate; vi) group with mix *Bacillus subtilis*, *Pseudomonas* sp. and *Trichoderma viride*, irrigated with water and previously fertilised substrate, (2.3 x $10^8$ spores/ml) 5 ml of product sprayed per kg of substrate. On 5 June 2024, plant height, number of leaves, total leaf area per plant (mm2), primary root length (mm), biomass of the aerial and root system, number and weight of fruits, flowers number and the number of plants dead from powdery mildew and cladosporiosis attacks were recorded.

**Results and Discussion:** The experiment showed that the use of of different types of probiotic and plant defence microorganisms can indeed significantly improve the vegetative and root growth of *Cucurbita pepo* L.. All treatments showed a significant improvement over the untreated control for the agronomic parameters analysed. The *Bacillus subtilis*, *Pseudomonas* sp. and *Trichoderma viride* treatment with a mix of microorganisms was significantly the best in terms of increasing vegetative and root biomass. Improvements were also found in plant height, number of leaves, leaf area and root length. In addition, significant effects were found on the number of flowers and fruits as well as fruit weight. The trial also revealed the significant effect of the products on the control of powdery mildew and *Cladosporiosis*, in which case the product PL (*Paecilomyces lilacinus*) was the best on the two diseases.

**Conclusions:** Experiments have shown that the use of microorganisms can significantly improve the growth, vegetative and root biomass of *Cucurbita pepo* L.. The treatment also offers increased resistance to mortality that can occur in nursery cultivation. A variety of horticultural and ornamental species have already been tested with microorganisms in

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previous trials, which highlight other interesting and innovative aspects of the use of microorganisms. Due to the importance of using microbial inoculums in plants, the new agricultural experiments are extremely important as they may allow the development of new products for organic and sustainable farming systems and lead to better results.

**Keywords:** Microorganism; Sustainable applications; Plants; Rhizosphere; Plant-microbial interaction

## 1. Introduction

Increasing world population, rapid industrialization, and urbanization, as well as anthropogenic activities have impacted the terrestrial ecosystem and the hidden players that regulate it [1]. The United Nations reports that the global population is expected to reach approximately 9 billion by 2050. There will be 10 billion people by the year 2050, putting greater pressure on land to feed such huge populations [1,2]. Deforestation will also lead to several human interventions in ecosystem functioning as a result of increased world population, affecting soil water holding capacity and enhancing soil erosion. As a result, the world needs another green revolution, at the expense of extensive chemical fertilizer and pesticide use, which will negatively influence soil health and microbiome diversity [3]. In order to address these issues, multiple strategies are needed, such as using microbes that promote plant growth as biostimulants. As inoculum, microbial consortia or single microorganisms have previously been studied [4]. Although microbial inoculants can be successfully transferred from the lab to the field, this is still a challenging process. A variety of crops, varying environmental conditions between fields, and the exponential increase in microbial isolates are the primary reasons for this. The use of biostimulants requires a comprehensive strategy that includes "diagnosis" of the crop's genotype and its field environment (e.g., soil), good agricultural practices, screening for inoculations from available culture collections, promoting microbiome research, and finally integrating all of the above into large-scale industrial production [5]. Since the 1980s, many researchers have studied microbial inoculants, but with only patchy success. By identifying candidates or methods for inoculation that are more suitable for a given environment, it may be possible to identify stressors, genotype, and environmental variables that affect plant microbiota [6]. The plant microbiome is composed of a variety of organisms, including fungi, archaea, and bacteria [7,8]. Plant microbiomes are vast communities of microorganisms that live within and on plants. The presence of these microorganisms is crucial for the growth and health of plants [9]. Recent years have seen a rise in interest in investigating plant microbiomes to better understand the interactions between plants and microbes.

### 1.1. Plant microbiome

"Plant Microbiome" is a relatively new area of research that focuses on the many communities of microorganisms that live inside and outside of plant tissues and facilitate their growth and health. Bacteria, fungi, viruses, and others interact intricately with plants [10]. Plant microbiomes consist of phyllosphere, endosphere, spermosphere, rhizosphere, and soil-associated non-rhizospheric microbiomes [11]. Plant microbiomes play a crucial role in increasing plant growth and resistance to disease, nutrient deficiency, and drought. In mutualistic relationships between plants and soil microbes, nutrients are provided and deadly infections are defended [12,13]. Both agricultural and natural ecosystems benefit from microbial populations that are associated with plants and soil. In addition to helping plants uptake additional nutrients from the soil, beneficial soil bacteria provide resistance to biotic and abiotic stresses [14]. In exchange, microorganisms exploit plant waste byproducts for energy. Microbiomes also play an important role in defending plants against pathogens by engaging in niche competition, producing antibiotics, providing nutrients, releasing secondary metabolites (SMs), and activating the plant's defense system against initial pathogen attacks through systemic resistance [15]. Microbes also help plants by producing enzymes that reduce pathogens' harmful effects by producing antimicrobials such as hydrogen cyanide (HCN) and siderophore [16-18]. However, selective breeding, monoculture, and the use of agrochemicals in agroecosystems have harmed the relationship between beneficial microbiota and plants. Natural alternatives to synthetic fertilizers and chemical pesticides can be used as microbial inoculants, which have several benefits over conventional inputs. As an example, microbial inoculants can improve soil fertility and health while also being economical and environmentally friendly. Inoculants that are specifically adapted to different crop types and growing environments can also improve their effectiveness in promoting plant development and disease resistance [19,20]. In spite of the advantages of using the plant microbiome in agriculture, several issues remain. It is difficult to pinpoint the microorganisms that are most beneficial for crops and growth environments due to the complexity of the plant microbiome. The efficacy of microbial inoculants can also be significantly influenced by soil characteristics and crop management techniques, which can differ greatly across a wide range of agricultural systems.

### 1.2. A plant microbiome's role in regulating abiotic and biotic stress

Worldwide, abiotic stress, such as drought, salinity, and extremely high or low temperatures, is a significant problem for crop production. Plant microbiomes, which are comprised of numerous microbes found in plant tissues and rhizospheres, have become an important factor in regulating plant responses to abiotic stress. Among the most significant abiotic factors affecting crop yields is drought stress. To increase plant water efficiency, microbes in the plant microbiome control stomatal conductance, synthesize osmoprotectants, and promote root growth. Plants can be improved in their ability to withstand drought by certain Rhizobacteria and fungal endophytes that produce phytohormones and stimulate the expression of genes related to stress [21,22]. In addition to soil salt levels, another significant abiotic stress that limits crop productivity is salinity stress. Plant microbes can reduce salinity stress by decreasing the uptake of noxious ions and improving food availability. Rhizobacteria such as *Pseudomonas* spp., *Serendipita indica*, *Bacillus* spp., and arbuscular mycorrhizal fungi can enhance the availability of nutrients like nitrogen and phosphorus, which are crucial for plant growth, but decrease sodium ion accumulation in plants [23]. It is also possible for crops to be affected by extreme temperature stress, which includes both high and low temperatures. Plant microbes can help plants cope with the stresses caused by extreme temperatures by controlling plant physiology and metabolism. Some fungal endophytes and *Enterobacter* sp. SA187, for example, can help plants resist high temperatures by boosting antioxidant activity and shielding plant tissues from heat damage [24]. The ability of plants to cope with abiotic stress can be enhanced by the application of microbial inoculants. By enhancing plant-microbe interactions, regulating plant hormone levels, and improving nutrient uptake, microbial inoculants can improve plant growth and stress tolerance. Plant growth and drought tolerance can be enhanced by inoculating crops with certain rhizobacteria [25], for example. As a result, it can be concluded that plant microbiomes play a crucial role in determining how plants react to abiotic stress, including drought, salinity, and high temperatures. By advancing research and practices on plant microbiomes, agriculture can be more sustainable and the harmful environmental effects of existing agricultural practices can be reduced.

A significant global concern for crop productivity is biotic stress caused by pests and pathogens. Plant microbiomes are a collection of microbes living in plant tissues and rhizospheres, which regulate plant responses to biotic stresses. Insect pests can severely affect crop yield due to biotic stress. The microbes of plants can assist in controlling insect pests by generating insecticidal substances (such as *Bacillus thuringiensis*' Bt-toxin) or by improving plant defenses. As an example, some bacteria and fungi create poisonous substances for insects, while others assist plants in preparing insect defenses [26]. Fungal infections, such as Fusarium and Phytophthora species, can result in significant crop losses. Microbes in plant microbiomes can help manage fungal infections by producing antifungal chemicals, promoting plant resistance, or competing for nutrients and space. Some bacteria (*Bacillus*, *Pseudomonas*, *Klebsiella*, etc.) compete with certain fungi for resources, and certain bacteria and fungi may produce antifungal substances that prevent the growth of disease-causing fungi [27]. ,The agricultural industry can benefit from biocontrol agents, microorganisms that can control pests and infections. Biocontrol agents improve plant growth and control biotic stress by enhancing plant uptake of nutrients, controlling plant hormone levels, and improving plant-microbe interactions. Tomato, cucumber, and pepper crops can be controlled with biocontrol agents such as fungi and bacteria [28].

**Figure 1** Details of the plants used in the trial

The main objective of this article is to report the results obtained from the use of new microbial strains on vegetable plants.

In particular, the article will deal with two important topics: i) the study of the effect of microorganisms on the biomass of vegetable plants; ii) the possible control of mortality due to diseases such as powdery mildew and *Cladosporiosis*. The information reported in this research work can support the design of cropping systems in which agricultural sustainability is fundamental due to the presence of microorganisms with biostimulating activity and as a possible alternative to synthetic plant protection products.

## 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 February 2024. The plants used in the trial were *Cucurbita pepo* L.. 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 Paecilomyces lilacinus (PL): (peat 70% + pumice 20%), irrigated with water and previously fertilised substrate, (4.5 x 106 spores/ml), 5ml of product sprayed per kg of substrate;
- Group with Azospirillum sp. (AZ): (peat 70% + pumice 20%), irrigated with water and previously fertilised substrate, (4 x 108 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 109 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, (3.6 x 108 spores/ml), 5ml of product sprayed 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, (2.3 x 108 spores/ml) 5 ml of product sprayed per kg of substrate.

The plants were watered once a day and cultivated for 5 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 5 June 2024, plant height, number of leaves, total leaf area per plant ($mm^2$), primary root length (mm), biomass of the aerial and root system, number and weight of fruits, flowers number and the number of plants dead from powdery mildew and cladosporiosis attacks 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 of different types of probiotic and plant defence microorganisms can indeed significantly improve the vegetative and root growth of *Cucurbita pepo* L. (**Table 1**). All treatments showed a significant improvement over the untreated control for the agronomic parameters analysed. The BPT treatment with a mix of microorganisms was significantly the best in terms of increasing vegetative and root biomass. Improvements were also found in plant height, number of leaves, leaf area and root length (**Table 1** and **Figures 4-7**). In addition, significant effects were found on the number of flowers and fruits as well as fruit weight (**Figure 2**). The trial also revealed the significant effect of the products on the control of powdery mildew and *Cladosporiosis*, in which case the product PL (*Paecilomyces lilacinus*) was the best on the two diseases (**Figure 3**).

Active ingredients, such as live microorganism spores, are what characterize this type of product. We can highlight, for example, bacteria such as *Bacillus*, *Azospirillum*, *Pseudomonas*, and *Rhizobium*, as well as fungi such as *Beauveria*, *Gliocladium*, *Pochonia*, *Metarhizium*, and *Trichoderma* [29-35], as well as *Actinomycetes* such as *Streptomyces* [36,37]. As soil microorganisms occupy a large amount of the rhizosphere, they are close to plants, and they are also capable of forming biofilms that protect the roots against pathogens [38-42]. The beneficial microorganisms are supported by the host plant, taking advantage of the carbon-rich compounds contained in the root exudates, including sugars, amino acids, flavonoids, proteins and fatty acids that are crucial for their nutrition [43-45].

**Table 1** Evaluation of new microbic strains on agronomic characters on plants of *Cucurbita pepo* L

| ***Cucurbita pepo* L.** | **PH (cm)** | **LN (n°)** | **TLA (mm²)** | **VW (g)** | **RW (g)** | **RL (cm)** |
|---|---|---|---|---|---|---|
| CTRL | 30.71 f | 7.80 d | 112.80 e | 45.57 d | 33.24 d | 4.31 e |
| PL | 32.55 e | 8.80 c | 115.45 d | 46.39 c | 36.50 c | 5.34 d |
| AZ | 32.93 d | 9.20 c | 116.61 c | 46.58 bc | 36.62 c | 5.65 c |
| GS | 33.34 c | 13.00 b | 117.63 b | 46.54 c | 37.26 b | 5.74 c |
| TV | 33.88 b | 13.40 b | 118.14 b | 46.89 b | 37.67 b | 5.90 b |
| BPT | 36.33 a | 17.00 a | 121.57 a | 49.54 a | 39.67 a | 6.42 a |
| 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 ($P = 0.05$).; 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). Treatments: CTRL=control; PL= *Paecilomyces lilacinus*; AZ= *Azospirillum* sp.; GS= *Glomus* sp.; TV= *Trichoderma viride;* BPT= *Bacillus subtilis, Pseudomonas* sp. and *Trichoderma viride*

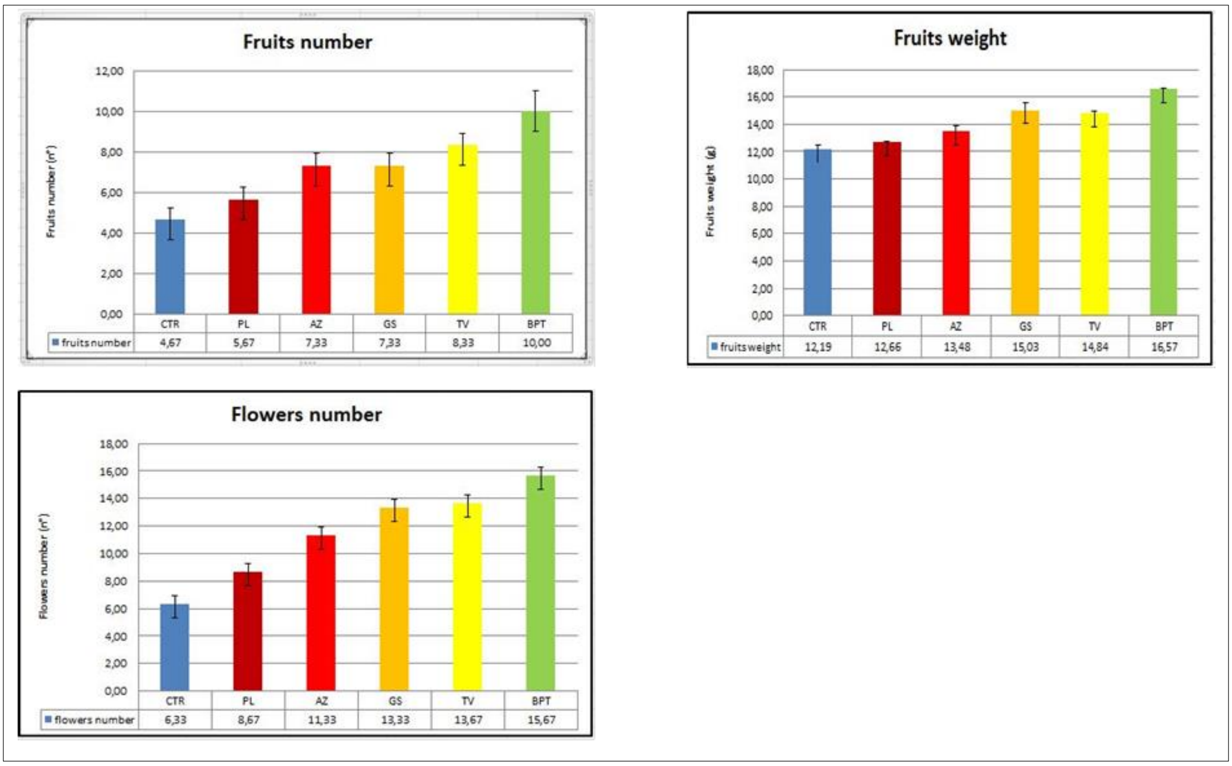


**Figure 2** Effect of new microbic strains on flowers and fruits number and fruits weight in *Cucurbita pepo* L. Legend: CTRL=control; PL= *Paecilomyces lilacinus*; AZ= *Azospirillum* sp.; GS= *Glomus* sp.; TV= *Trichoderma viride*; BPT= *Bacillus subtilis*, *Pseudomonas* sp. and *Trichoderma viride*

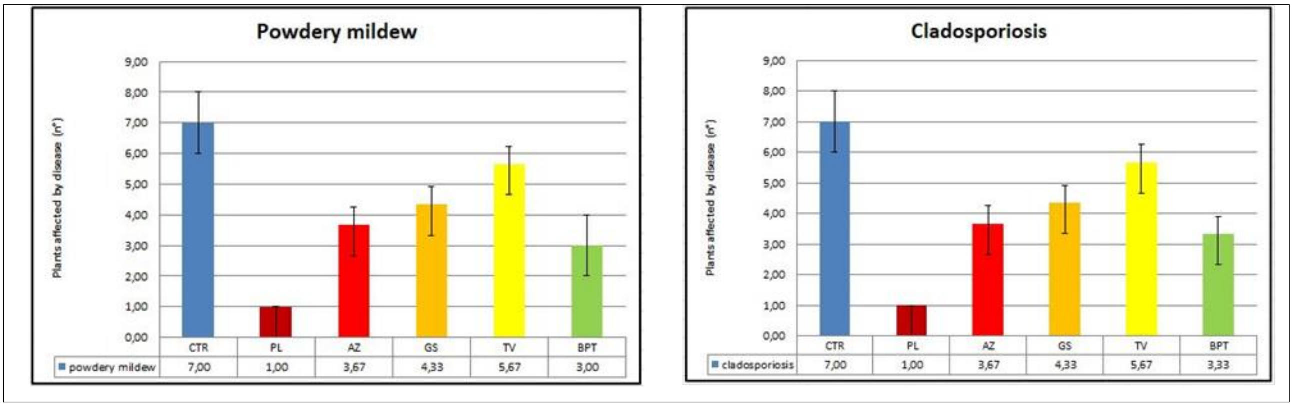


**Figure 3** Effect of new microbic strains on the control of *Powdery mildew* and *Cladosporiosis* in *Cucurbita pepo* L. Legend: CTRL=control; PL= *Paecilomyces lilacinus*; AZ= *Azospirillum* sp.; GS= *Glomus* sp.; TV= *Trichoderma viride*; BPT= *Bacillus subtilis*, *Pseudomonas* sp. and *Trichoderma viride*


**Figure 4** Effect of microbic strains on vegetative biomass of *Cucurbita pepo* L. compared with fertilised control. Legend: BPT: *Bacillus subtilis*, *Pseudomonas* sp. and *Trichoderma viride*; GS: *Glomus* sp.; TV: *Trichoderma viride*; CTRL: control


**Figure 5** Effect of microbic strains BPT (*Bacillus subtilis*, *Pseudomonas* sp. and *Trichoderma viride*) on radical biomass of *Cucurbita pepo* L. compared with fertilised control (CTRL)

Additionally, exudates may inhibit the growth of some species while favouring others, suggesting that the rhizosphere is highly selective for microbes. A plant's role in affecting the microbial composition of the native and inoculated rhizosphere community is therefore crucial [46]. A number of studies have shown that signal molecules emitted by plants can influence the microbial community in the rhizosphere based on the plant's growth phase [47-50]. As well, it has been observed that plant signal molecules can have an impact on the microbial community in the rhizosphere depending on the growth phase of the plant [51-53]. A microorganism-based inoculum can also increase photosynthetic activity, salinity tolerance, iron bioavailability, and reduce disease [54-57], as well as increase photosynthetic activity, salinity tolerance, and iron bioavailability. As confirmed in this trial, the use of microorganisms resulted in improved plant growth and quality and reduced plant mortality. In other tomato trials in the Naples area, Glomus sp. microorganisms were also used. Based on dry residue, soluble solids, mineral element concentration, lycopene, polyphenols, and ascorbic acid, there was a 49% increase in root colonization and fruit yield and quality [58]. The application of microgreens to shallots (*Allium cepa* L. *Aggregatum*) increased bulb production and quality, as well as mineral element concentrations, ascorbic acid concentrations, and antioxidant activity [59,60]. In this research work, the application of development-promoting and plant-protecting microorganisms led to a significant improvement in plant growth and increased protection against pathogens such as powdery mildew and cladosporiosis, confirming results obtained in other research by other authors.

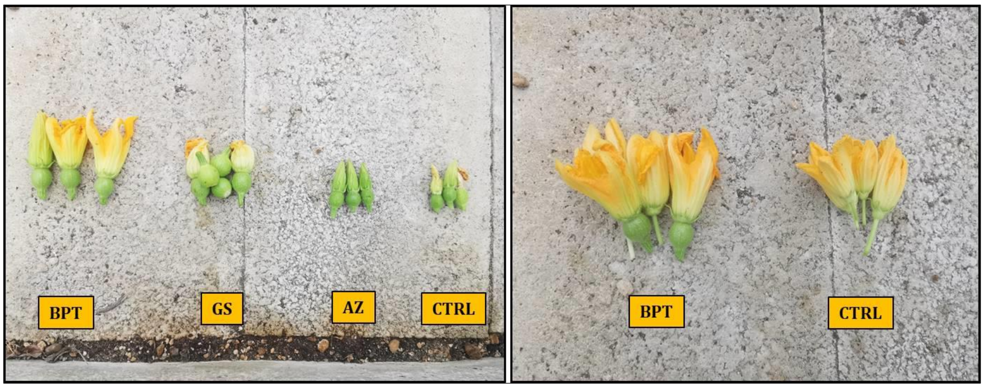


**Figure 6** Effect of microbic strains on flowers size of *Cucurbita pepo* L. compared with fertilised control (CTRL) Legend: BPT: *Bacillus subtilis*, *Pseudomonas* sp. and *Trichoderma viride*; GS: *Glomus* sp.; AZ: *Azospirillum* sp.; CTRL: control

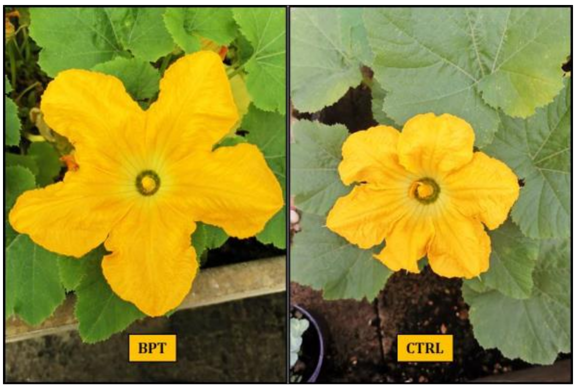


**Figure 7** Effect of microbic strains on flowers size of *Cucurbita pepo* L. compared with fertilised control (CTRL) Legend: BPT: *Bacillus subtilis*, *Pseudomonas* sp. and *Trichoderma viride*; CTRL: control

## 4. Conclusion

Experiments have shown that the use of microorganisms can significantly improve the growth, vegetative and root biomass of *Cucurbita pepo* L.. The treatment also offers increased resistance to mortality that can occur in nursery cultivation. A variety of horticultural and ornamental species have already been tested with microorganisms in previous trials, which highlight other interesting and innovative aspects of the use of microorganisms. Due to the importance of using microbial inoculums in plants, the new agricultural experiments are extremely important as they may allow the development of new products for organic and sustainable farming systems and lead to better results.

## Compliance with ethical standards

### *Acknowledgments*

The research is part of the 'PROVAMIC' project: Production and evaluation of microbic ceppes on plants vegetable and ornamental plants. I would like to thank the company Agrogen for their collaboration, in particular in the person of Dr. Giordano Menci, who actively collaborated in the Provamic project.

### *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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