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Zeolites Encapsulated with Organic Matrices in Vegetable and Ornamental Plants Fertilization

Domenico Prisa*

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

**Corresponding author*

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ABSTRACT

The aim of this research was to evaluate the fertilizing capacity of an innovative zeolite product, characterised by an encapsulated structure with an organic matrix on vegetable and ornamental plants and the interaction with soil microorganisms present in the cultivation substrates. The experiments, started in January 2021, were conducted in the greenhouses of CREA-OF in Pescia (PT), Tuscany. The experimental groups were: i) group control, irrigated with water and substrate previously fertilized; ii) group with zeolite 21% ammoniacal nitrogen; iii) group with zeolite coated with Ecoat; iv) group with zeolite 21% ammoniacal nitrogen, SO_3 57.7% with nitritification inhibitor dcd (dicyandiamide) and DMPP 3,4 dimethylpyrazole-phosphate. The trial carried out on strawberry and *Polygala myrtifolia* actually showed how the use of zeolite can improve the fertilizing properties of the substrate. In particular, the use of encapsulated zeolite resulted in an increase in plant height, vegetative and root weight, number and flowers life, number and weight of fruits in strawberry and *Polygala myrtifolia*. In addition, there were changes in substrate pH, microbiological count and nitrogen, phosphorus and potassium content depending on the type of zeolytic product used. Research has shown that the use of loaded zeolite can significantly improve the agronomic and production quality of strawberry and *Polygala myrtifolia* plants. For example, Ecoat treatment with zeolite encapsulated with organic matrices in strawberries resulted in a pot production of 39.61 fruits and a weight of 36.39 g/fruit, compared to 24.21 and 26.48 g/fruit for the untreated control. While in *Polygala myrtifolia* the same treatment (Ecoat) resulted in 48.00 flowers per plant and a flowers life of 9.20 days compared to 34.86 flowers and 6.20 of the control. The trial also showed that treatment with Ecoat can promote the development of microbial colonies in the substrate, 3.5×10^4 cfu/g compared with 2.3×10^2 cfu/g in the control in strawberries and 3.2×10^4 cfu/g compared with 2.6×10^2 cfu/g in *Polygala myrtifolia*. In addition, the application of these aluminosilicates in substrates can influence the pH and the microbial component that is essential for the cultivation and defence of plants. The Ecoat product that performed best in the trial can play the role of both a nitrogen-based fertiliser and, thanks to its organic matrix, of stimulating microbial development in the substrate in which the plants are grown.

Introduction

Origin and structure of zeolites

Zeolites are special minerals with unique properties. These incredible minerals were discovered in 1756 by A. F. Constedt, who published a scientific article entitled “Observation and description of an unknown kind of rock to be named “zeolites”. Since then there have been numerous studies of these minerals. Their numerous applications have led to researchers from numerous companies all over the world, and numerous scientists have described their chemical and physical properties, and artificially create new ones (Mumpton, 1978; Prisa, 2020a; Prisa, 2020b). Zeolites are minerals belonging to the silicate group The zeolite family consists of 52 different mineralogical species. characterised by having:

Very open structure, with cavities ranging from 30% to 50% of the mineral volume;

Presence of channels linking the cavities of the mineral and the outside of the crystal.

The general chemical formula of zeolites is schematically as follows:

Ma Bb [Al(a+2b) Sin-(a+2b) O2n] x mH2O

Zeolites have hydrothermal or diagenetic genesis (Gottardi and Galli, 1985).

Chemical and physical properties of zeolites

Because of their particular structure and chemical composition, zeolites have unique properties

High and selective cation exchange capacity (CSC)

Cations within the interstices (cavities and channels) can easily be removed and replaced by other elements. maintaining the balance of positive cationic charges.

Reversible dehydration

Zeolites are hydrated minerals; the water content of zeolites varies from 10% to 20% of the mineral's weight. When the mineral is heated to 350 - 400°C, the water is completely lost. The process of rehydration is infinitely reversible, because it does not damage the molecular structure of the mineral. In addition rehydration always takes place in such a way as to bring the zeolite a water content in balance with the humidity of the environment.

Molecular adsorption

Interstices (structural channels and cavities) can be made available for the selective adsorption of liquid or gaseous molecules. The adsorption capacity increases with higher pressure and lower temperature.

Catalytic behaviour

Closely linked to the crystal structure are the extraordinary catalytic properties of these minerals. The enormous internal surface area useful for catalysis (Alexiev and Djourova, 1988).

Applications in agriculture and floriculture

In agriculture, zeolitites can be used either in their natural state, together with traditional fertilisers (manure, slurry, soluble nitrogen, potassium and phosphorous salts) or after natural or artificial enrichment (potassium, ammonium). The beneficial effects of zeolitites are related to the specific soil, and the quantity and quality depend on the kind of crop (Galli and Passaglia 2011).

The addition of natural zeolites to open field or greenhouse soils ensures: the neutralisation of excess soil acidity; the release of plant-nutrient potassium present in the zeolites; the capture of potassium and ammonia from fertilisers so that they are not immediately lost; the increase in the amount of nutrients to the crop and the reduction by leaching; the release of phosphorus and the slowing down of the retrogradation process of monocalcium phosphate from fertilisers; reducing the uptake of harmful and radioactive elements by crops; in sandy soils increasing cation exchange capacity, increasing water retention while maintaining the same degree of permeability; reducing the temperature range in the soil; in clay soils increasing the degree of soil aeration and permeability (Gualtieri *et al.*, 1999; Prisa, 2019a).

The commercial use of natural zeolites is still in its beginning stage, but more than 300,000 tonnes of zeolite-rich tuff is mined annually in the United States, Japan, Bulgaria, Hungary, Italy, Yugoslavia, Korea, Mexico, Germany and the Soviet Union. Natural zeolites have found applications as fillers in the paper industry, as lightweight aggregates in construction, cement and concrete, as ion exchangers in water and municipal effluent purification, as traps for radioactive species in nuclear plant wastewater, in oxygen production, as catalysts for oil fields, as acid-resistant absorbents and in natural gas drying and purification (Passaglia *et al.*, 1997).

In this experiment, new zeolite products were evaluated, as these minerals have several interesting characteristics for use in agriculture, particularly in horticulture, the use of zeolites in open field and greenhouse led to an increase in total product yield and improved plant development on table tomatoes (Bazzocchi *et al.*, 1996) celery (Prisa, 2019a), courgettes and melons (Passaglia and Poppi, 2005a), vegetables and fruits (Passaglia and Poppi, 2005b; Prisa, 2019b). In floriculture, the use of zeolites resulted in improved growth and increased resistance to biotic and abiotic stresses in several ornamental species (Prisa, 2019c,d,e).

The objective of this research was to evaluate the fertilising capacity of an innovative zeolite product (Figure 1), characterised by an encapsulated structure with an organic matrix on vegetable and ornamental plants and the interaction with soil microorganisms present in the cultivation substrates.

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 strawbarry (*Fragaria x ananassa* cv Candonga®Sabrosa) (Figure.2A) and *Polygala myrtifolia* plants (Figure.2B). The plants were placed in pots ø 20 cm; 30 plants per thesis, divided into 3 replicas of 10 plants each. Plants in the control thesis, were fertilized with a controlled release fertilizer (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 transplanting. The experimental groups were:

group control (CTRL) (peat 60% + pumice 40%), irrigated with water and substrate previously fertilized;

group with zeolite 21% ammoniacal nitrogen (2 g per litre of substrate) (WH);

group with zeolite coated with Ecoat (mix of minerals with high cation exchange and natural activators) (2 g per litre of substrate) (BR); group with zeolite 21 % ammoniacal nitrogen, SO_3 57.7 % with nitritification inhibitor dcd (dicyandiamide) and DMPP 3,4 dimethylpyrazole-phosphate (2 g per litre of substrate) (BL).

The plants were watered 2 times a week and grown for 5 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 May 13, 2021, plants height, vegetative and roots weight, flowers number and life, fruits number and weight, substrate microbial count, pH, nitrogen, phosphorus and potassium content of the substrate were analysed.

Analysis methods

pH: For the ph measurement 1 kg of substrate was taken from each thesis, 50 g of the mixture was placed inside a beaker with 100 ml of distilled water. After 2 hours the water was filtered and analysed;

microbial count: direct determination of total microbic charge by microscopy of cells contained in a known volume of sample through the use of counting chambers (Thoma chamber). The surface of the slide is etched with a grid of squares of which the area of each square is known. Determination of viable microbial load following serial decimal dilutions, spatula seeding (1 ml) and plate counts after incubation;

total phosphorus, potassium and nitrogen analysis: Analyses are performed using a light source with a 3-wavelength narrow-band interference filter, which is fast and accurate (4 minutes). At the end of the measurement the result can be transferred to the PC.

Analysis equipment

IP67 PHmeter HI99 series – Hanna instruments

Lysimeter for collecting solution samples from soil (HI83900) - Hanna instruments
Benchtop photometer for nutrient analysis in agriculture - HI83325 - Hanna instruments

Combined test kit for soil analysis - HI3896 - Hanna instruments

microbial diversity of culturable cells (Vagnerova and Macura, 1974)

Statistical analysis

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).

Results and Discussion

The trial carried out on strawberry and *Polygala myrtifolia* actually showed how the use of zeolite can improve the fertilising properties of the substrate. In particular, the use of encapsulated zeolite resulted in an increase in plant height, vegetative and root weight, number and flowers life, number and weight of fruits in strawberry and *Polygala myrtifolia*. In addition, there were changes in substrate pH, microbiological count and nitrogen, phosphorus and potassium content depending on the type of zeolytic product used.

In (Table 1), in strawberry cv Candonga®Sabrosa there was an increase in plant height in (BR) with 38.82 cm compared to 38.12 cm in (WH), 37.56 cm in (CTRL) and 37.40 cm in (BL). Regarding vegetative weight, thesis (BR) was the best with 285.05 g, followed by (BL) with 258.07 g, (WH) 262.19 g and (CTRL) with 243.57 g (Figure 3A, 3C). The same trend for root weight where (BR) showed a weight of 165.02 g, (BL) 156.05 g, (WH) 144.45 g and (CTRL) 132.54 g (Figure 4A). In terms of number of fruits, (BR) was the best thesis with 39.61, followed by (BL) with 34.42 and (WH) with 32.46, finally (CTRL) 24.21. In terms of fruit weight, (BR) was the best thesis with 36.39 g, followed by (WH) with 31.43 g, (BL) with 30.41 g and the untreated control with 26.48 g.

In (Table 2), *Polygala myrtifolia* showed significant increase in plant height in thesis (BR) with 46.62 cm followed by (BL) with 43.33 cm and (WH) with 43.31 cm, finally the untreated control with 40.90 cm. For vegetative weight, thesis (BR) was the best with 377.52 g, followed by (WH) with 371.56 g, (BL) 370.39 g and (CTRL) with 367.57 g (Figure.3B, 3D). Also for root weight, (BR) showed a weight of 262.07 g, (BL) 256.25 g, (WH) 252.00 g and (CTRL) 244.17 g (Figure 4B). In terms of number of flowers, (BR) was the best thesis with 48.00, followed by (WH) with 42.11 and (BL) with 39.23, finally (CTRL) with 34.86. Also in respect of flowering duration theses (BR) and (BL) were the best with 9.20 and 8.60 days respectively. They were followed by (WH) with 7.80 days and the untreated control with 6.20 days.

In (Table 3), the results concerning the mineral and microbiological content of the different strawberry test plots were shown. As far as the thesis (BR) was the one that showed a lower pH with 6.5, also the highest number of microorganisms 3.5 x 10^4 cfu/g was found. Regarding the nitrogen content, the thesis (BR) and (WH) were the best with 1.33 mg/kg and 1.27 mg/kg respectively. For phosphorus content, thesis (BR) was also the best with 28.41 mg/kg, followed by (WH) with 27.61 mg/kg, (BL) with 16.15 mg/kg and finally the control with 24.36 mg/kg. For potassium, the best thesis was (BR) with 100.24 mg/kg, followed by (WH) with 98.90 mg/kg, (BL) with 92.43 mg/kg and finally the untreated control with 90.33 mg/kg.

In (Table 4), the results concerning the mineral and microbiological content of the different experimental theses of *Polygala myrtifolia* were shown. As in strawberry, the lowest pH found in *Polygala myrtifolia* was 6.5 in (BR), and the highest number of microorganisms 3.2 x 10^4 cfu/g was found in this thesis. With regard to the nitrogen content, the thesis (BR) was the best with 1.56 mg/kg. For the phosphorus content, the thesis (BR) was also the best with 29.46 mg/kg, followed by (WH) with 27.54 mg/kg, (BL) with 26.57 mg/kg and finally the control with 25.33 mg/kg. For potassium, the best thesis was (BR) with 102.53 mg/kg, followed by (WH) with 95.04 mg/kg, (BL) with 92.25 mg/kg and finally the untreated control with 89.05 mg/kg.

Zeolite are called open-structure aluminosilicates due to their unique crystal structure in which molecular-sized channels pass through the crystal lattice giving it a high degree of porosity. Depending on their charge and size, cations can be weakly retained in the pore structure where they can be replaced by other competing ions depending on the interactions between the aluminosilicate structure, the ions and the ionic properties of the external solution. Zeolites are used in various sectors such as water purification, petrochemical industry, animal breeding and as biostimulants in horticulture, because they are real molecular sieves (Leggo *et al.*, 2001).

In agriculture, the use of granular zeolites (0-3 mm and 3-6 mm) introduced in soil or open field cultivation substrates can improve various aspects of cultivation such as seed germination, rooting of cuttings, plant growth for greater efficiency in the use of water, greater resistance to water and salt stress (Prisa, 2019f).

Table.1 Evaluation of zeolites on agronomic characters of strawberry cv Candonga®Sabrosa

Groups	Plant height (cm)	Vegetative weight (g)	Roots weight (g)	Fruits number (n°)	Fruits weight (g)
CTRL	37.56 b	243.57 c	132.54 d	24.21 d	26.48 c
WH	38.12 ab	262.19 b	144.45 c	32.46 c	31.43 b
BL	37.40 b	258.07 b	156.05 b	34.42 b	30.41 b
BR	38.82 a	285.05 a	165.02 a	39.61 a	36.39 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 ($P = 0.05$).Legend: (CTRL): control; (WH): 21 % ammoniacal nitrogen; (BR): Ecoat; (BL): 21 % ammoniacal nitrogen, SO3 57.7 % with nitritification inhibitor dcd (dicyandiamide) and DMPP 3,4 Dimethylpyrazole-phosphate

Table.2 Evaluation of zeolites on agronomic characters of *Polygala myrtifolia*

Groups	Plant height (cm)	Vegetative weight (g)	Roots weight (g)	Flowers number (n°)	Flowers life (days)
CTRL	40.90 c	367.57 c	244.17 d	34.86 d	6.20 c
WH	43.31 b	371.56 b	252.00 c	42.11 b	7.80 b
BL	43.33 b	370.39 b	256.25 b	39.23 c	8.60 a
BR	46.62 a	377.52 a	262.07 a	48.00 a	9.20 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 ($P = 0.05$).Legend: (CTRL): control; (WH): 21 % ammoniacal nitrogen; (BR): Ecoat; (BL): 21 % ammoniacal nitrogen, SO3 57.7 % with nitritification inhibitor dcd (dicyandiamide) and DMPP 3,4 Dimethylpyrazole-phosphate

Table.3 Evaluation of the mineral and microbial content of the cultivation substrate of strawberry cv Candonga®Sabrosa

Groups	pH	Microbial Count (cfu/g)	N total (mg/kg)	P (mg/kg)	K (mg/kg)
CTRL	7.1	2.3×10^{2} c	0.81 c	24.36 d	90.33 d
WH	6.7	3.4×10^{2} c	1.27 a	27.61 b	98.90 b
BL	6.8	2.2×10^{3} b	0.94 b	26.15 c	92.43 c
BR	6.5	3.5×10^{4} a	1.33 a	28.41 a	100.24 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 ($P = 0.05$).Legend: (CTRL): control; (WH): 21 % ammoniacal nitrogen; (BR): Ecoat; (BL): 21 % ammoniacal nitrogen, SO3 57.7 % with nitritification inhibitor dcd (dicyandiamide) and DMPP 3,4 Dimethylpyrazole-phosphate

Table.4 Evaluation of the mineral and microbial content of the cultivation substrate of *Polygala myrtifolia*

Groups	pH	Microbial count (cfu/g)	N total mg/kg	P mg/kg	K mg/kg
CTRL	7.1	2.6×10^2 c	0.76 d	25.33 d	89.05 d
WH	6.7	4.6×10^2 c	1.27 b	27.54 b	95.04 b
BL	6.8	2.3×10^3 b	0.90 c	26.57 c	92.25 c
BR	6.5	3.2×10^4 a	1.56 a	29.46 a	102.53 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).Legend: (CTRL): control; (WH): 21 % ammoniacal nitrogen; (BR): Ecoat; (BL): 21 % ammoniacal nitrogen, SO3 57.7 % with nitritification inhibitor dcd (dicyandiamide) and DMPP 3,4 Dimethylpyrazole-phosphate

Fig.1 Detail of the fertiliser granules used in the experiment, (WH): 21 % ammoniacal nitrogen; (BR): Ecoat; (BL): 21 % ammoniacal nitrogen, SO_3 57.7 % with nitritification inhibitor dcd (dicyandiamide) and DMPP 3,4 dimethylpyrazole-phosphate

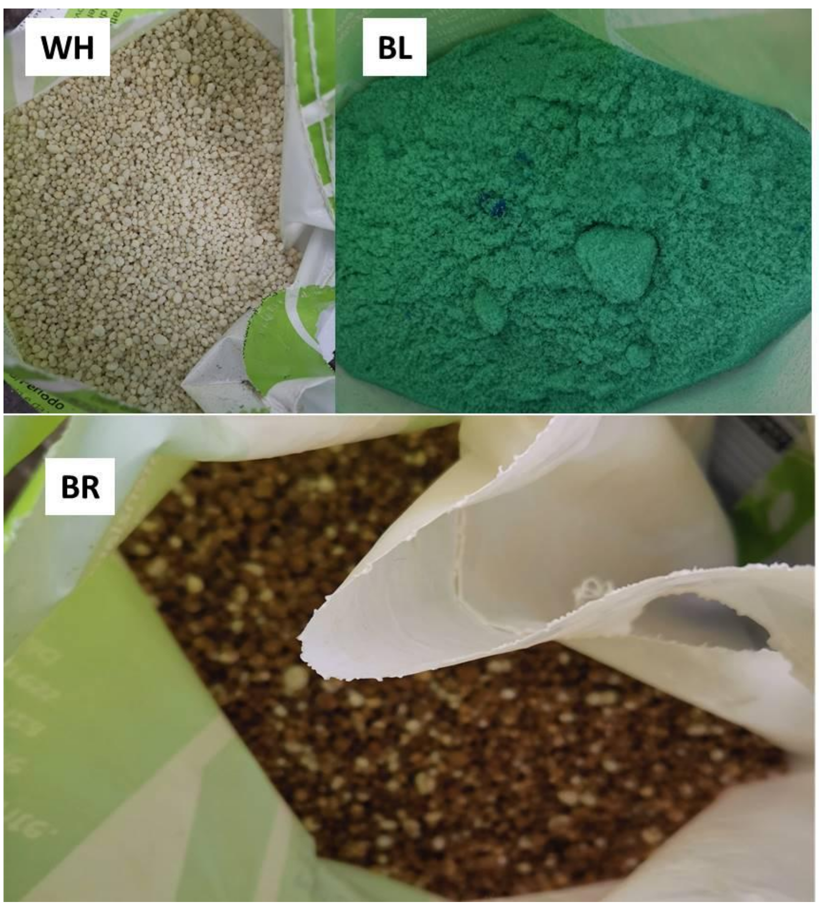


Fig.2 Detail of the fertiliser granules used in the experiment, (WH): 21 % ammoniacal nitrogen; (BR): Ecoat; (BL): 21 % ammoniacal nitrogen, SO3 57.7 % with nitrification inhibitor dcd (dicyandiamide) and DMPP 3,4 Dimethylpyrazole-phosphate

Fig.3 Comparison of different zeolite treatments in strawberry (A) and *Polygala myrtifolia* (B). Effect of BR treatment: Ecoat on strawberry (C) and *Polygala myrtifolia* (D)

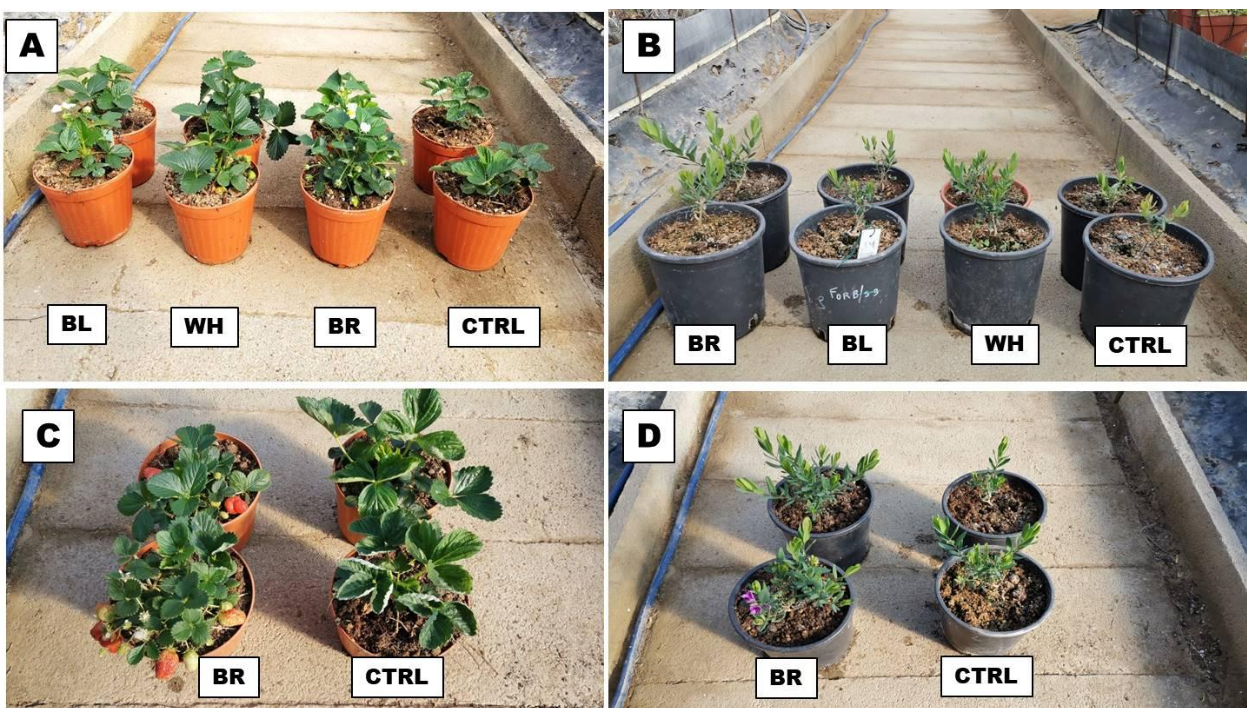
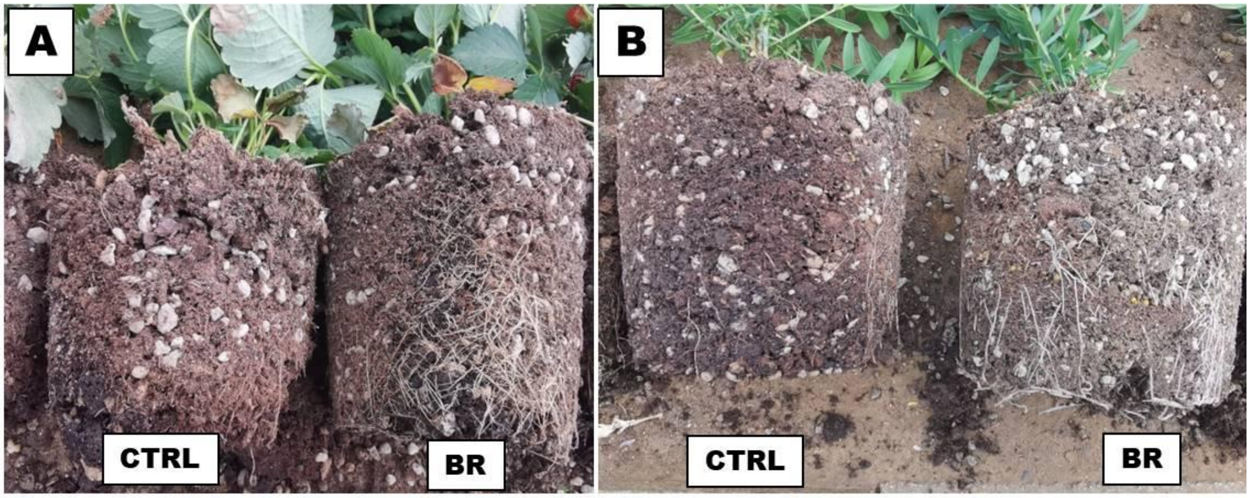


Fig.4 Effect of BR treatment (Ecoat) on roots growth of strawberry (A) and *Polygala myrtifolia* (B)



In this experiment, it was particularly noticeable that substrates enriched with zeolite, in particular Ecoat with encapsulated zeolite, resulted in a significant increase in all agronomic parameters analysed (plant height, vegetative and root weight, fruits number and weight, flowers number and duration) in strawberry and *Polygala myrtifolia*. These aspects have already been confirmed in other trials on vegetable and ornamental plants, where zeolite improved the fertilising capacity of the substrate and plant protection. The trial also showed that mineral-loaded zeolite can act as a slow-release fertiliser, influencing the pH of the substrate and the microbial component. A number of articles published on this subject show that an increase in the soil microbial population is characteristic of soils amended with zeolitic minerals, which influence their fertility (Prisa, 2020a). Other work (Leggo *et al.*, 2001). shed light on the interaction between the ion exchange properties of the mineral zeolite and the humic component of the organic material, which cause high ion mobility in the soil solution for organic vegetable production.

Zeolite is able to retain water and fertilizer, which reduces leaching into the groundwater and allows optimization of plant use. This is of particular importance when water with high salt concentrations is used (chabazite captures sodium and releases potassium or even in the case of nitrate problems, as zeolite regenerates in the presence of nitrogen) (Mackown and Tucker, 1985). The interesting aspect found in this study was that the microbial presence improves the cation exchange characteristics of zeolite. The bacteria probably accelerate the passage of nutrients captured by zeolite to the plant through acidification of the substrate, which solubilises the minerals and makes them more available to the plant. This mechanism can occur naturally over a long period of time in the substrate and in the field with indigenous microorganisms normally present, but it can be accelerated if particularly performing microbial colonies were added to zeolite products (Prisa 2020b). Zeolites can act as a "home" for microorganisms, as is normally the case in nature with clays. In fact, under water stress conditions, microorganisms take refuge inside the clay particles until the environmental conditions are suitable again to colonize the soil. In particular, the direct contact of zeolites with the root surface stimulates the production of mucilaginous substances (mucigel) by carrying out a lubricating action able to favour the absorption by the root of minerals and water(Prisa, 2020b; Ferguson *et al.*, 1987).. Evidence shows that zeolites, once inserted into growing substrates, can lead to an improvement in the physiological aspects of plants, in particular the net photosynthesis rate and chlorophyll content. A plant that photosynthesizes more has more energy reserves available for different metabolic functions. As shown in some experiments, the use of zeolite does not cause changes in the immobilisation of C-N by the biomass. The nitrogen inserted with the loaded zeolite is immediately available to the microbial biomass, which causes a bacterial-dominated environment (low C/N). Microorganisms immediately feed on the nitrogen present and influence plant growth (Prisa, 2020c).

Research has shown that the use of loaded zeolite can significantly improve the agronomic and production quality of strawberry and *Polygala myrtifolia* plants. In addition, the application of these aluminosilicates in substrates can influence the pH and the microbial component that is essential for the cultivation and defence of plants. The Ecoat product that performed best in the trial can play the role of both a nitrogen-based fertiliser and, thanks to its organic matrix, of stimulating microbial development in the substrate in which the plants are grown.

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