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NATURAL ACIDIFIER IMPROVES PLANT GROWTH CHARACTERISTICS AND MICROBIOLOGY IN POTTED ORNAMENTAL PLANTS

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ABSTRACT

Research goal: *The aim of this research was to evaluate a natural product with acidifying activity on plant growth and the effects on rhizosphere microfauna in ornamental succulent plants.*

Materials and Methods: *The experiments, started in July 2021, were conducted in the greenhouses of CREA-OF in Pescia (Pt), Tuscany, Italy (43°54'N 10°41'E) on Tacinga imoneana, Euphorbia submammillaris, Marlothistella uniondalensis and Cotyledon ladismithiensis. The experimental groups were: i) group control in peat, irrigated with water and substrate previously fertilized; ii) group control in compost, irrigated with water and substrate previously fertilized; iii) group with organic acidifiers (organic acidifying product by Fertalis srl) in peat irrigated with water and substrate previously fertilized; iv) group with organic acidifiers in compost irrigated with water and substrate previously fertilized.*

Results and Discussion: *The trial showed a significant improvement in the agronomic parameters analysed on Tacinga imoneana, Euphorbia submammillaris, Marlothistella uniondalensis and Cotyledon ladismithiensis plants treated with an organic acidifier. In particular, there was an increase in plant height, vegetative and roots weight, flowers number and life. In addition, the experiment showed that in theses treated with the organic acidifier, there was a pH lowering and a significant increase in the substrate microfauna.*

Conclusions: *According to the results obtained in this study, the application of a natural acidifier in peat-based substrate or compost can be useful for increasing plant productivity and soil microbiology.*

KEY-WORDS: *sustainable agriculture, beneficial microorganisms, plant interactions, succulent plants, substrate microbiology, organic acidifier, alkaline soil*

INTRODUCTION

Alkaline soils, characterised by high amounts of exchangeable sodium and a pH above 8.5, contain sodium carbonate and sodium bicarbonate to a greater or lesser extent. The formation of these salts can be explained in various ways, each of which has its own significance and importance in the different conditions encountered during cultivation: i) in the presence of moist soils with specific bacteria and alterable organic matter, sodium sulphate is reduced to sulphide, which in the presence of water and carbon dioxide, is transformed into carbonate; ii) certain plants that accumulate cations, especially sodium, can contribute to the alkalinisation of the soil [1]. The formation of alkaline carbonates occurs from their residues following mineralisation. Some chemical fertilisers tend to raise the pH of the soil, but their action does not lead to decisive effects, given the relatively small quantities that are commonly used [2]. Through irrigation, large quantities of salts can reach the soil, which, depending on their composition, can contribute decisively to the alkalinisation of the soil [3]. The properties of alkaline soils vary considerably depending on the environmental situation and their stage of evolution. The basic characteristics that distinguish them are: a low electrical conductivity, a high percentage of exchangeable sodium and the presence of sodium carbonate and bicarbonate [4]. One of the most important factors on which the mineral nutrition of plants in alkaline soils depends is the high percentage of exchangeable sodium, which affects the amount of other cations, both exchangeable and in solution. Calcium, because of its strong affinity for colloid surfaces and the fact that it precipitates easily as a carbonate, is sometimes present in soil solutions in such low concentrations that it cannot fully satisfy the physiological needs of plants; the uptake of magnesium and potassium is also hampered by high amounts of sodium [5]. The presence of the carbonate ion, of high concentrations of hydroxyl and sometimes borate ions, exerts a specific toxic action on crops. The most serious drawbacks for plant growth are to a large extent indirect actions: high alkalinity causes first of all the complete insolubilisation of iron, copper and manganese; iron deficiency is manifested by chlorosis of the leaves, a phenomenon that is also commonly observed in calcareous soils. Microbial activity is greatly depressed by high alkalinity, with adverse consequences on all the chemical, physical and nutritional characteristics of the soil [6]. The great compactness of the soil, as well as hindering the development of the root system, causes hydromorphic conditions, resulting in a lack of oxygen necessary for microbial and root respiration. Most crops show symptoms of intolerance at ESP (Degree of Sodium Saturation of the Exchange Complex) values between 10 and 15, while they stop growing completely at values above 50. Most pome and stone fruit, walnut and beans are considered very sensitive, while cotton, alfalfa, barley, tomato and beet tolerate ESP values up to 60. Spontaneous vegetation can tolerate even higher values, but is very sparse, both in number of species and in vegetative development, and in extreme cases may be absent altogether [7,8].

Regulating Methods for the alkalinity soils

The basic principle underlying the correction of alkaline soils is the decomposition of sodium carbonate and the removal of exchangeable sodium, through the use of correctives. A number of compounds are suitable for this purpose. The most frequently used compound is gypsum, firstly because of its relatively low cost and also because, despite its poor solubility, it has proved to be very effective [9]. The correction leads to a considerable lowering of the pH, since sodium sulphate is a practically neutral salt and the other compounds give the soil a pH similar to that of calcareous soils, i.e. no higher than 8.5. The replacement of sodium by calcium on the colloids leads to a considerable improvement in the nutrient capacity and structural condition of the soil. Sulphur compounds with a low oxidation number can also be used successfully to remove sodium and correct alkalinity [10]. The use of sulphur and other compounds that are oxidised by microorganisms to sulphuric acid. In cultivated soils undergoing alkalinisation, the use of acidifying fertilisers or fertilisers with corrective action, such as superphosphate, which contains high amounts of calcium sulphate, is recommended [11]. After the addition of the corrective fertilisers, water must be added to bring the fertilisers into solution, to allow the chemical reactions to take place, and also to facilitate the leaching of the salts formed and any salts already present in the soil. The leaching of alkaline soils is a very difficult problem, due to their low permeability and the frequent presence of surface water tables, which hinder the removal of drainage water. In many areas of the earth's surface there are large expanses of saline and alkaline soils in which crops cannot grow or give low yields [12]. Until now, the use of these soils has been based on improving them by means of hydraulic and agronomic measures and the use of chemical correctives, i.e. following the concept of adapting the soil to the plants [13]. In fact, plant life is not incompatible with salinity, and this is confirmed by the observation of spontaneous plants in saline and alkaline soils, and those in the marine environment. Starting from this principle, research is directed towards the creation of new plant varieties and the search for new methods and products with a low environmental impact that can reduce the pH and improve the microbial activity of soils [14].

OBJECTIVES

The aim of this research was to evaluate a natural product with acidifying activity on plant growth and the effects on rhizosphere microfauna in ornamental succulent plants.

Figure 1 - Particulars of *Tacinga imoneana* (A), *Cotyledon ladismithiensis* (B), *Marlothistella uniondalensis* (C) and *Euphorbia submammillaris* (D) flowers

MATERIAL AND METHODS

The experiments, started in July 2021, were conducted in the greenhouses of CREA-OF in Pescia (Pt), Tuscany, Italy (43°54′N 10°41′E) on *Tacinga imoneana* (Figure 1A), *Euphorbia submammillaris* (Figure 1B), *Marlothistella uniondalensis* (Figure 1C) and *Cotyledon ladismithiensis* (Figure 1D). The plants were placed in ø 12 cm pots; 30 plants per thesis, divided into 3 replicas of 10 plants each. All plants 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 in peat (**CTPE**) (peat 50% + pumice 50%, pH 6,5), irrigated with water and substrate previously fertilized;
- Group control in compost (**CTCO**) (green compost 50% + pumice 50%, pH 8), irrigated with water and substrate previously fertilized;
- Group with organic acidifiers (Organic acidifying product by Fertalis srl) in peat (**ACPE**) (peat 50% + pumice 50%) irrigated with water and substrate previously fertilized, dilution product 1% in water, treatment every 15 days (20 ml per plant);
- Group with organic acidifiers in compost (**ACCO**) (green compost 50% + pumice 50%) irrigated with water and substrate previously fertilized, dilution 1% in water, treatment every 15 days (20 ml per plant);

The plants were watered 2 times a week and grown for 7 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 February 7, 2022, plants height, vegetative and roots weight, flowers number and life, substrate microbial count, pH of substrate were analyzed.

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 [15];
- 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 [15];
- Analysis equipment: IP67 PHmeter HI99 series – Hanna instruments; Combined test kit for soil analysis - HI3896 - Hanna instruments; microbial diversity of culturable cells [15].

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 showed a significant improvement in the agronomic parameters analysed on plants of *Tacinga imoneana*, *Euphorbia submammillaris*, *Marlothistella uniondalensis*, *Cotyledon ladismithiensis* treated with an organic acidifier. In particular, there was an increase in plant height, vegetative and roots weight, flowers number and life. In addition, the experiment showed that in theses treated with the organic acidifier, there was a pH lowering and a significant increase in the substrate microfauna.

In (Table 1), in *Marlothistella uniondalensis* there was a significant increase in plant height in (ACPE) with 7.41 cm compared to 6.72 cm in (CTPE), 6.06 cm in (CTCO) and 5.79 cm in (CTCO). Regarding vegetative weight, the (ACPE) thesis was the best with 59.05 g, followed by (CTPE) with 56.73 g, (ACCO) 54.67 g and (CTCO) with 52.84 g. The same trend for roots where (ACPE) showed a weight of 48.06 g, (CTPE) 45.01 g and finally (ACCO) and (CTCO) with 43.95 g and 43.02 g respectively. In terms of flowers number, (ACPE) was the best thesis with 30.40, followed by (CTPE) and (ACCO) with 23.20 and 23.00 respectively, finally (CTCO) with 20.60 (Figure 2). Thesis (ACPE) was also the one where flowers lasted the longest with 4.20 days, compared to (CTPE) 3.20 and (ACCO) 2.40 and (CTCO) 2.20. The trial also showed a reduction in pH in the organic acid treated theses, 5.78 (ACPE) and 7.50 (ACCO), compared to 6.52 (CTPE) and 8.04 (CTCO). There was also a significant increase in substrate microbiology in the organic acid treated theses, 1.27 x 10^3 (ACCO) and 8.33 x 10^2 (ACPE), compared to 5.23 x 10^2 (CTCO) and 2.62 x 10^2 (CTPE).

In (Table 2), in *Cotyledon ladismithiensis* there was a significant increase in plant height in (ACPE) with 27.65 cm compared to 24.08 cm in (CTPE) and 23.49 cm in (ACCO) and 21.24 cm in (CTCO). Regarding vegetative weight, the (ACPE) thesis was the best with 59.06 g, followed by (CTPE) with 55.93 g, (ACCO) 54.44 g and (CTCO) with 52.80 g (Figure 3A). The same trend for roots where (ACPE) showed a weight of 47.52 g, (CTPE) 43.67 g and finally (ACCO) with 42.94 g and (CTCO) with 39.86 g. In terms of flowers number, (ACPE) was the best thesis with 6.00, followed by (CTPE) with 4.60 and (ACCO) with 3.80, finally (CTCO) with 2.80 (Figure 3B). In *Cotyledon ladismithiensis*, the thesis (ACPE) was also the one where flowers lasted the longest with 5.60 days, compared to 4.80 in (CTPE) and 4.20 in (ACCO) respectively and finally (CTCO) 3.40. There was also a reduction in pH in the organic acid treated theses, 5.88 (ACPE) and 7.04 (ACCO), compared to 6.54 (CTPE) and 8.04 (CTCO). The number of microbial colonies in the substrates treated with organic acid also increased, namely 1.40 x 10^3 (ACCO) and 8.85 x 10^2 (ACPE), compared with 3.80 x 10^2 (CTCO) and 3.13 x 10^2 (CTPE).

In (Table 3), in *Tacinga imoneana* there was a significant increase in plant height in (ACPE) with 36.98 cm compared to 32.88 cm in (CTPE) and 32.35 cm in (ACCO) and 30.48 cm (CTCO). Regarding vegetative weight, the (ACPE) thesis was the best with 48.86 g, followed by (CTPE) with 45.61 g, (ACCO) 44.09 g and (CTCO) with 43.25 g (Figure). The same trend for roots where (ACPE) showed a weight of 39.86 g, (CTPE) 38.81 g and finally (ACCO) with 37.58 g and (CTCO) with 35.77 g (Figure 4). In terms of flowers number, (ACPE) was the best thesis with 12.60, followed by (CTPE) with 9.40 and (ACCO) with 7.20, finally (CTCO) with 5.80. In *Tacinga imoneana*, the thesis (ACPE) was also the one in which flowers lasted the longest with 6.80 days, compared to 5.20 in (CTPE) and 5.00 in (ACCO) respectively and finally (CTCO) 4.20. As with the other plant species, *Tacinga imoneana* also showed a reduction in pH in the theses treated with organic acid, 5.76 (ACPE) and 7.06 (ACCO), compared with 6.54 (CTPE) and 8.02 (CTCO). The number of microbial colonies in the substrates treated with organic acid also increased, 1.48 x 10^3 (ACCO) and 7.98 x 10^2 (ACPE), compared with 5.56 x 10^2 (CTCO) and 4.68 x 10^2 (CTPE).

In (Table 4), in *Euphorbia submammillaris* there was a significant increase in plant height in (ACPE) with 37.06 cm compared to 34.95 cm in (CTPE) and 33.29 cm in (ACCO) and 32.73 cm in (CTCO). Regarding vegetative weight, the (ACPE) thesis was the best with 45.79 g, followed by (CTPE) with 42.14 g, (ACCO) 41.85 g and (CTCO) with 40.71 g (Figure 5). The same trend for roots where (ACPE) showed a weight of 39.27 g, (CTPE) 36.06 g and finally (ACCO) with 34.77 g and (CTCO) with 33.21 g. In terms of flowers number, (ACPE) was the best thesis with 33.60, followed by (CTPE) with 26.60 and (ACCO) with 25.40, finally (CTCO) with 24.20. In *Euphorbia submammillaris*, the thesis (ACPE) was also the one where flowers lasted the longest with 4.40 days, compared to 3.20 in (CTPE) and (ACCO) respectively and finally (CTCO) 2.60. As for the other plant species, *Euphorbia submammillaris* also showed a significant reduction in pH in the theses treated with organic acid, 5.78 (ACPE) and 7.04 (ACCO), compared to 6.58 (CTPE) and 8.10 (CTCO). The number of microbial colonies in the substrates treated with organic acid also increased, 1.34 x 10^3 (ACCO) and 6.69 x 10^2 (ACPE), compared with 5.89 x 10^2 (CTCO) and 5.20 x 10^2 (CTPE).

Soil Acidity and Micro-Organisms

The increase in world population has made it necessary to improve the productivity of agricultural land throughout the world. To this end, increasing, protecting and maintaining soil fertility is one of the most important management practices concerning sustainability. Alkaline soils generally tend to lack organic matter and available nitrogen, especially in arid and semi-arid areas [16]. The high pH and increased calcium content of the soil makes phosphorus insoluble, while potassium and magnesium can be antagonistic. Zinc and iron deficiency can also limit agricultural production. Worldwide, most farmers tend to use inorganic fertilisers to try to maintain or increase soil fertility. However, these production techniques are not sustainable due to the problems of groundwater pollution caused by incorrect fertilisation[17]. The uncontrolled use of fertilisers has also led to the accumulation of heavy metals in soils. It is therefore necessary to evaluate suitable strategies to improve soil fertility and reduce the use of excessive synthetic chemicals [18,19]. One of the possible solutions, for example, would be the possible use of products of organic origin capable of improving the state of the soil, reducing the pH and favouring the development of microbial consortia that can improve plant growth and defence [15]. It is well known that microbial activity in the soil, as already shown in various experiments in horticulture and floriculture, can positively influence the uptake of water and nutrients by the roots and increase resistance to various biotic and abiotic stresses [20,21,22]. In this experiment, in particular, the positive effect of an organic acidifier, used above ground on a peat and compost-based substrate, on reducing pH and increasing the presence and activity of microbes in the rhizosphere was highlighted. The trial also showed how a natural acidifying product can bring about a significant improvement in the agronomic parameters of the plants, probably linked to the microbial activity in the substrates. The plants that grew the most were those grown in substrates where the microbial presence was greatest. It was also interesting to note that plants treated with the organic acidifier had a greater number of flowers and an increase in flower life, which was undoubtedly linked to a greater availability of nutrients absorbed by the roots and linked to the activity of the microfauna. Another aspect highlighted was the reduction in compaction and increased porosity of the substrate, which increased the habitability of the roots and improved plant-soil interactions. All this generates stability in the agro-ecosystem, since a rich and structured soil biodiversity favours regular plant activity even under conditions of environmental stress [23].

Table 1 - Evaluation of organic acidifier on agronomic and substrate characters on *Marlothistella uniondalensis*

Groups	Plant height (cm)	Vegetative weight (g)	Roots weight (g)	Flowers number (n°)	Flowers life (days)	pH	Microbial count (cfu/g)
CTPE	6,72 b	56,73 b	45,01 b	23,20 b	3,20 b	6,52 c	2,62 x 10^2 d
	5,79 c	52,84 d	43,02 c	20,60 c	2,20 c	8,04 a	5,23 x 10^2 c
CTCO		59,05 a	48,06 a	30,40 a	4,20 a	5,78 d	8,33 x 10^2 b
ACPE	7,41 a	54,67 c	43,95 c	23,00 b	2,40 c	7,50 b	1,27 x 10^3 a
ACCO							
	6,06 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: (CTPE): control in peat; (CTCO): control in compost; (ACPE): organic acidifiers in peat; (ACCO): organic acidifiers in green compost

Table 2 - Evaluation of organic acidifier on agronomic and substrate characters on *Cotyledon ladismithiensis*

Groups	Plant height (cm)	Vegetative weight (g)	Roots weight (g)	Flowers number (n°)	Flowers life (days)	pH	Microbial count (cfu/g)
CTPE	24,08 b	55,93 b	43,67 b	4,60 b	4,80 b	6,54 c	$3,13 \times 10^2$ d
CTCO	21,24 c	52,80 d	39,86 d	2,80 d	3,40 c	8,04 a	$3,80 \times 10^2$ c
ACPE	27,65 a	59,06 a	47,52 a	6,00 a	5,60 a	5,88 d	$8,85 \times 10^2$ b
ACCO	23,49 b	54,44 c	42,94 c	3,80 c	4,20 b	7,04 b	$1,40 \times 10^3$ a
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: (CTPE): control in peat; (CTCO): control in compost; (ACPE): organic acidifiers in peat; (ACCO): organic acidifiers in green compost

Table 3 - Evaluation of organic acidifier on agronomic and substrate characters on *Tacinga imoneana*

Groups	Plant height (cm)	Vegetative weight (g)	Roots weight (g)	Flowers number (n°)	Flowers life (days)	pH	Microbial count (cfu/g)
CTPE	32,88 b	45,61 b	38,81 b	9,40 b	5,20 b	6,54 c	$4,68 \times 10^2$ d
CTCO	30,48 c	43,25 c	35,77 d	5,80 d	4,20 c	8,02 a	$5,56 \times 10^2$ c
ACPE	36,98 a	48,86 a	39,86 a	12,60 a	6,80 a	5,76 d	$7,98 \times 10^2$ b
ACCO	32,35 b	44,09 c	37,58 c	7,20 c	5,00 b	7,06 b	$1,48 \times 10^3$ a
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: (CTPE): control in peat; (CTCO): control in compost; (ACPE): organic acidifiers in peat; (ACCO): organic acidifiers in green compost

Table 4 - Evaluation of organic acidifier on agronomic and substrate characters on *Euphorbia submammillaris*

Groups	Plant height (cm)	Vegetative weight (g)	Roots weight (g)	Flowers number (n°)	Flowers life (days)	pH	Microbial count (cfu/g)
CTPE	34,95 b	42,14 b	36,06 b	26,60 b	3,20 b	6,58 c	$5,20 \times 10^2$ d
CTCO	32,73 d	40,71 c	33,21 d	24,20 d	2,60 b	8,10 a	$5,89 \times 10^2$ c
ACPE	37,06 a	45,79 a	39,27 a	33,60 a	4,40 a	5,78 d	$6,69 \times 10^2$ b
ACCO	33,29 c	41,85 b	34,77 c	25,40 c	3,20 b	7,04 b	$1,34 \times 10^3$ a
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: (CTPE): control in peat; (CTCO): control in compost; (ACPE): organic acidifiers in peat; (ACCO): organic acidifiers in green compost

Figure 2 - Comparison between control in peat (CTPE) and treatment with organic acidifiers in peat (ACPE) on growth and flowering of *Marlothistella uniondalensis*

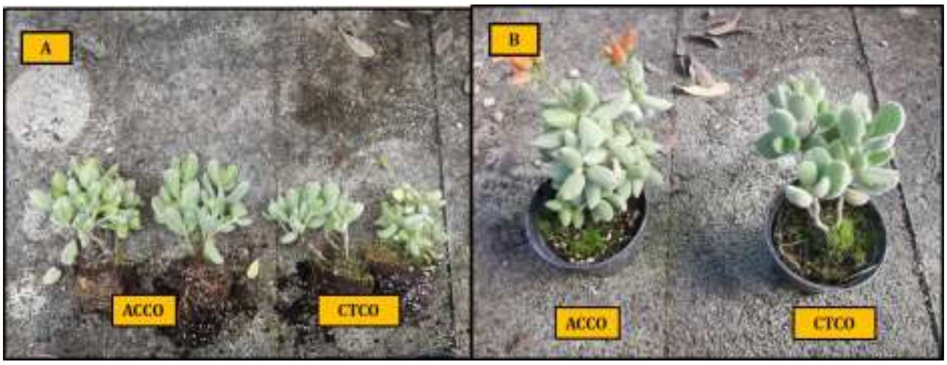

Figure 3 - Comparison between control in green compost (CTCO) and treatment with organic acidifiers in green compost (ACCO) on growth (A) and flowering (B) of *Cotyledon ladismithiensis*

Figure 4 - Comparison between control in peat (CTPE) and treatment with organic acidifiers in peat (ACPE) on vegetative and roots growth of *Tacinga imoneana*

Figure 5 - Comparison between control in green compost (CTCO) and treatment with organic acidifiers in green compost (ACCO) on vegetative and roots growth of *Euphorbia submammillaris*

CONCLUSION

According to the results obtained in this study, the application of a natural acidifier in peat-based substrate or compost can be useful for increasing plant productivity and soil microbiology. The substrate pH decreased and promoted increased plant growth and flowering. All treatments with organic acidifier resulted in increased vegetative and root growth of the plants, probably because they influence the organic carbon content of the substrate and the development of beneficial microbial consortia. The organic acidifier can therefore be useful not only to lower the pH of soils and substrates, but can improve microbial activity with repercussions on plant quality. Micro-organisms not only regulate the uptake of nutrients and water by the roots, but are also crucial in increasing the plants' resistance to biotic and abiotic stresses. A further trial is currently underway to evaluate the organic acidifier on the germination and cultivation of potted vegetable plants.

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Conflicts of interest
The authors declarethat they have no conflict of interest

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