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NATURAL ACIDIFIER APPLICATION WITH EFFECTS ON SOIL MICROBIAL ACTIVITY IN SWISS CHARD, TUSCANY BLACK CABBAGE AND *TETRAGONIA TETRAGONIOIDES* CULTIVATION

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ABSTRACT

Research goal: *This research aimed to evaluate the effects of a natural product with acidifying activity on seed germination and growth of Swiss chard (Beta vulgaris subsp. vulgaris), Tuscany black cabbage (Brassica oleracea var. acephala) and Tetragonia tetragonioides plants and whether there were any interactions or interferences with soil microbial activity and fertility parameters.*

Materials and Methods: *The experiments, which started in January 2022, were conducted in the greenhouses of CREA-OF in Pescia (Pt), Tuscany, Italy (43°54'N 10°41'E). The experimental groups were: 1) group control in peat, irrigated with water and substrate previously fertilized; 2) group control in compost, irrigated with water and substrate previously fertilized; 3) group with organic acidifiers (organic acidifying product by Fertalis SRL) in peat irrigated with water and substrate previously fertilized; 4) group with organic acidifiers in compost irrigated with water and substrate previously fertilized.*

Results and Discussion: *The trial significantly improved the agronomic parameters analyzed on Swiss chard, Tuscany black cabbage and Tetragonia tetragonioides plants treated with an organic acidifier. In particular, there was an increase in plant height, vegetative and roots weight, seeds germination and average germination time. In addition, the experiment showed that in plants treated with the organic acidifier, there was a lowering of the pH and a significant increase in substrate microfauna.*

Conclusions: *According to the results obtained in this above-ground experiment, applying a natural acidifier in peat- or compost-based substrate can be beneficial for increasing plant productivity and quality and positively influence the microbiology of the soil. In the experiment, a reduction in the pH of the substrate was found in the theses treated with the acidifier. At the same time, a significant increase in the agronomic quality of the plants (in terms of productivity and growth) was observed. Furthermore, in these theses, it was evident how the microbial presence increased, which positively affected the substrate structure and fertilizer uptake.*

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

INTRODUCTION

The different types of soils worldwide influence the kinds of plants that grow there because the minerals present generally influence them. The stresses that plants have to overcome can be chemical (e.g. soil acidity and alkalinity, sodicity and salinity, element deficiencies and toxicity, presence or absence of organic matter) and physical (e.g. texture, moisture, temperature, bulk density) [1]. These factors can precisely influence the type and severity of stress plants face, and these issues have been addressed in numerous research works [2][3].

The difference between acid and alkaline soils is generally associated with the amount of rainfall compared to evapotranspiration. When rainfall exceeds evapotranspiration in most years, soils are leached, and acid soils are formed [4][5]. On the other hand, if evapotranspiration exceeds precipitation, soils are, in most cases, neutral or alkaline. The general differences between acid and alkaline soils are the boundaries between forests and grasslands; neutral to alkaline soils are mainly found in suburban, semi-arid and arid climates; in these soils, one can find high concentrations of soluble salts such as K and Na and contain sufficient amounts of Ca in the form of $CaCO_3$ to be defined as calcareous. Calcareous soils make up about 30% of the earth's surface, and $CaCO_3$ levels in these soils vary greatly (from 5 to 95%). Magnesium in calcareous soils can be high and interact with other elements causing deficiencies [6][7]. Alkaline soils generally have abundant 2:1 layer clay minerals (montmorillonite) and are well distributed throughout the world, often presenting mineral deficiency problems. Toxicity in plants is often caused by carbonate ions and high hydroxyl and borate ions concentrations. The most severe problems in plant growth are generally caused by indirect actions, such as insolubilisation of iron, copper and manganese, which causes chlorosis of the leaves [8]. The activity of microorganisms can also be impaired by high alkalinity, which has negative consequences on all soil chemical, physical, and nutritional characteristics. Compact soil also hinders root development and generates a reduction in the presence of oxygen, which is indispensable for microbial and root growth [9]. Most crops show intolerance symptoms at ESP (Degree of Exchange Complex Saturation) values between 10 and 15, while they stop growing entirely at values above 50. Most pome and stone fruits, nuts and beans are considered very sensitive, while cotton, alfalfa, barley, tomato and beet tolerate ESP values up to 60. On the other hand, spontaneous vegetation can tolerate higher values, although fewer species [10][11].

Soil Alkalinity: Environmentally Friendly Regulation Methods

The first principle for correcting alkaline soils is to decompose sodium carbonate and remove exchangeable sodium through correctors. The most commonly used compound is gypsum, its relatively low cost and effectiveness. The correction generates a lowering of the pH, as sodium sulphate is a neutral salt [12]. At the same time, the other components give the soil a pH similar to that of calcareous soils, not exceeding 8.5. Replacing sodium with calcium on colloids increases the plants' ability to absorb minerals and improves the structural condition of the soil. In alkaline soils, the application of acidifying or pH-correcting fertilisers such as superphosphate is recommended [13][14]. When adding corrective fertilisers, water must be added to the solution to facilitate the leaching of the salts formed and those that may already be present in the soil. The leaching of alkaline soils is a complicated 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 [15]. It is possible to utilise these soils through improvement works with hydraulic and agronomic measures and the use of chemical correctives. Plants can live in conditions of salt stress, as confirmed by wild species that can survive in conditions of high salinity and alkalinity, such as marine environments. Therefore, research is directed towards the development and creation of new resistant plant species and the application of new methods and products with a low environmental impact that can reduce pH and increase the microbiological activity of the soil [16].

OBJECTIVES

This research aimed to evaluate the effects of a natural product with acidifying activity on seed germination and growth of Swiss chard (*Beta vulgaris subsp. vulgaris*), Tuscany black cabbage (*Brassica oleracea var. acephala*) and *Tetragonia tetragonioides* plants and whether there were any interactions or interferences with soil microbial activity and fertility parameters.

MATERIAL AND METHODS

The experiments, started in January 2022, were conducted in the greenhouses of CREA-OF in Pescia (Pt), Tuscany, Italy (43°54′N 10°41′E) on Swiss chard *(Beta vulgaris subsp. vulgaris)* (Figure 1A), Tuscany black cabbage (*Brassica oleracea var. acephala*) (Figure 1B), *Tetragonia tetragonioides* (Figure 1C). 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 (1.5 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 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 7, 2022, number of seeds germinated (100 seeds), average germination time, plants height, vegetative and roots weight, 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 [17];
- 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 [17];

- Analysis equipment: IP67 PHmeter HI99 series – Hanna instruments; Combined test kit for soil analysis - HI3896 - Hanna instruments; microbial diversity of culturable cells [17].

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 significantly improved the agronomic parameters analysed on Swiss chard, Tuscany black cabbage and Tetragonia tetragonioides plants treated with an organic acidifier. In particular, there was an increase in plant height, vegetative and roots weight, seeds germination and average germination time. In addition, the experiment showed that in plants treated with the organic acidifier, there was a lowering of the pH and a significant increase in substrate microfauna.

In (Table 1), there was a significant increase in plant height in Swiss chard with 6.28 cm in (ACPE) compared to 5.34 cm in (CTPE), 5.17 cm in (ACCO) and 4.86 cm in (CTCO). In terms of vegetative weight, (ACPE) was the best with 14.72 g, followed by (CTPE) with 12.47 g, (ACCO) 11.32 g and (CTCO) with 10.32 g (Figure 1). The same trend for the roots, where (ACPE) showed a weight of 9.42 g, (CTPE) 8.39 g and finally (ACCO) and (CTCO) with 8.30 g and 7.62 g, respectively. In terms of seed germination, (ACPE) was the best thesis with 85.60 seeds, followed by (CTPE) and (ACCO) with 76.20 and 71.80 respectively, and finally (CTCO) with 68.40 seeds. The thesis (ACPE) was also the one where a reduction in the average germination time was found, with 10.60 days, together with (CTPE) 13.80 days, compared to 14.80 days of (ACCO) and 16.00 days (CTCO). The trial also showed a reduction in pH in the organic acid-treated theses, 5.60 (ACPE) and 6.45 (CTPE), compared to 7.39 (ACCO) and 8.52 (CTCO). There was also a significant increase in substrate microbiology in the organic acid-treated theses, 1.17×10^3 (ACCO) and 4.85×10^2 (CTCO), compared to 3.63×10^2 (ACPE) and 2.75×10^2 (CTPE).

In (Table 2), in Tuscany black cabbage, there was a significant increase in plant height in (ACPE) with 13.78 cm compared to 12.49 cm in (CTPE), 10.41 cm in (ACCO) and 9.66 cm in (CTCO). In terms of vegetative weight, (ACPE) was the best with 24.73 g, followed by (CTPE) with 22.68 g, (ACCO) 21.53 g and (CTCO) with 20.57 g (Figure 2). The same trend for roots, where (ACPE) showed a weight of 16.52 g, (ACCO) and (CTPE) 14.92 g and 14.79 g respectively, and finally (CTCO) with 13.59 g. In terms of seed germination, (ACPE) was the best thesis with 86.20 seeds, followed by (ACCO) with 77.20 and (CTPE) with 76.60, and finally (CTCO) with 72.20 seeds. The thesis (CTPE) was also the one where a reduction in the average germination time was found, with 10.80 days, together with (ACCO) 11.60 days, compared to 13.20 days of (CTCO) and 13.00 days (ACPE). The test again showed a reduction in pH in the theses treated with an organic acid, 5.87 (ACPE) and 6.44 (CTPE), compared to 7.46 (ACCO) and 8.29 (CTCO). There was also a significant increase in substrate microbiology in the organic acid-treated theses, 1.36×10^3 (ACCO) and 5.31×10^2 (CTCO), compared to 3.79×10^2 (ACPE) and 2.89×10^2 (CTPE).

In (Table 3), in Tetragonia tetragonioides, there was a significant increase in plant height in (ACPE) with 18.43 cm compared to 15.89 cm in (CTPE), 15.23 cm in (ACCO) and 13.34 cm in (CTCO).

In terms of vegetative weight, (ACPE) was the best with 32.87 g, followed by (CTPE) with 30.10 g, (ACCO) 28.82 g and (CTCO) with 27.64 g (Figure 3). The same trend for roots, where (ACPE) showed a weight of 27.27 g, (CTPE) 24.38 g and (ACCO) 22.67 g, and finally (CTCO) with 21.49 g (Figure 4). In terms of seed germination, (ACPE) was the best thesis with 90.20 seeds, followed by (CTPE) with 80.40 and (ACCO) with 77.40, and finally (CTCO) with 71.40 seeds. The (ACCO) thesis was also the one where a reduction in the average germination time was found, with 12.20 days, together with (CTPE) with 12.40 days, compared to 13.20 days of (ACPE) and 13.80 days (CTCO). The test again showed a reduction in pH in the organic acid-treated theses, 5.73 (ACPE) and 6.60 (CTPE), compared to 6.82 (ACCO) and 7.40 (CTCO). There was also a significant increase in substrate microbiology in the organic acid-treated theses, 1.23×10^3 (ACCO) and 5.79×10^2 (CTCO), compared to 4.15×10^2 (ACPE) and 3.29×10^2 (CTPE).

Microbial Biodiversity Effects on Soil Acidity

In conjunction with the increase in world population, the effects of climate change have led to the need to increase the productivity of agricultural land on all continents [18]. This has led to a depletion of soils; current agricultural practices, therefore, aim more at sustainable land management and the maintenance of soil fertility [19]. One of the significant problems of soils and thus a relative reduction in plant productivity is the lack of organic matter and available nitrogen, especially in arid soils and where water resources are minimal. Increased pH and high calcium content in the soil cause the unavailability of certain essential mineral elements for plants, such as phosphorus [20]. At the same time, potassium and magnesium can be antagonistic. The unavailability of zinc and iron can also obviously limit agricultural production. Most of the world's farmers try to overcome these problems by using inorganic fertilisers to maintain or increase soil fertility [21]. However, using these products leads to water and environmental pollution problems due to the leaching of fertilisers into groundwater. The excessive use of fertilisers has led to an accumulation of heavy metals in the soil and groundwater, so it is necessary to evaluate alternative strategies that can improve soil fertility and, at the same time, reduce synthetic chemicals [22]. One of the possible solutions could be the application of organic substances to the soil, which can improve fertility, and microbic biodiversity, reduce soil pH to increase the availability of mineral elements and increase agricultural productivity [23][24]. It is well known that soil microbial activity, highlighted in various experiments in horticulture and floriculture, can lead to a significant increase in water and nutrient uptake by roots, increase resistance to biotic and abiotic stresses, and increase plant productivity and quality. In this experiment, the positive effect of an organic acidifier, used above ground on peat and compost-based substrate, on reducing pH and increasing the presence and activity of microbes in the rhizosphere was shown [25][26]. The trial also showed how a natural acidifying product could significantly improve the agronomic parameters of the plants, probably related to the microbial activity in the substrates [27]. The plants that grew the most were those grown in substrates where the microbial presence was most significant, which also positively influenced a lowering of the pH by facilitating the probable uptake of nutrients by the roots [28][29][30]. Particular attention must be paid to product dilutions, as an excessive amount in the germination phase can disproportionately increase microbial colonisation, causing seed death [31][32]. Another aspect noted related to the increased microbial presence following the acidifier treatment is the reduction in compaction and the increase in substrate porosity, which increases root habitability and improves plant-soil interactions [33][34]. All these results observed in this and a previous experiment obtained in above-ground conditions are translatable to field crops, where these problems are more pronounced [35]. The presence of a rich telluric microbial biodiversity improves the fertility and structural properties of the soil. It brings clear benefits to agricultural production regarding product quality and resistance to physiopathology [36][37].

Table 1 - Evaluation of organic acidifier on agronomic and substrate characters on Swiss chard (*Beta vulgaris subsp. vulgaris*)

Groups	Plant height (cm)	Vegetative weight (g)	Roots weight (g)	Germinated seeds (n°)	Average germination time (days)	pH	Microbial count (cfu/g)
CTPE	5,34 b	12,47 b	8,39 b	76,20 b	13,80 c	6,45 c	2,75 x 10^2 d
CTCO	4,86 c	10,32 d	7,62 c	68,40 d	16,00 a	16,00 a	4,85 x 10^2 b
ACPE	6,28 a	14,72 a	9,42 a	85,60 a	11,60 d	5,60 d	3,63 x 10^2 c
ACCO	5,17 b	11,32 c	8,30 b	71,80 c	14,80 b	7,39 b	1,17 x 10^3 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: (CTPE): control in peat; (CTCO): control in compost; (ACPE): organic acidifiers in peat; (ACCO): organic acidifiers in green compost

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Table 2 - Evaluation of organic acidifier on agronomic and substrate characters on Tuscany black cabbage (*Brassica oleracea var. acephala*)

Groups	Plant height (cm)	Vegetative weight (g)	Roots weight (g)	Germinated seeds (n°)	Average germination time (days)	pH	Microbial count (cfu/g)
CTPE	12,49 b	22,68 b	14,79 b	76,60 c	10,80 b	6,44 c	2,89 x 10^2 d
CTCO	9,66 d	20,57 d	13,59 c	72,20 c	13,20 a	8,29 a	5,31 x 10^2 b
ACPE	13,78 a	24,73 a	16,52 a	86,20 a	13,00 a	5,87 d	3,79 x 10^2 c
ACCO	10,41 c	21,53 c	14,92 b	77,20 b	11,60 b	7,46 b	1,36 x 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 *Tetragonia tetragonioides*

Groups	Plant height (cm)	Vegetative weight (g)	Roots weight (g)	Germinated seeds (n°)	Average germination time (days)	pH	Microbial count (cfu/g)
CTPE	15,89 b	30,10 b	24,38 b	80,40 b	12,40 b	6,60 c	3,29 x 10^2 d
CTCO	13,34 d	27,64 d	21,49 d	71,40 c	13,80 a	7,40 a	5,79 x 10^2 b
ACPE	18,43 a	32,87 a	27,27 a	90,20 a	13,20 a	5,73 d	4,15 x 10^2 c
ACCO	15,23 c	28,82 c	22,67 c	77,40 b	12,20 b	6,82 b	1,23 x 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

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Figure 1 - Comparison between control in peat (CTPE) and treatment with organic acidifiers in peat (ACPE) on growth of Swiss chard (*Beta vulgaris subsp. vulgaris*)

Figure 2 - Comparison between control in peat (CTPE) and treatment with organic acidifiers in peat (ACPE) on vegetative growth of Tuscany black cabbage (*Brassica oleracea var. acephala*)

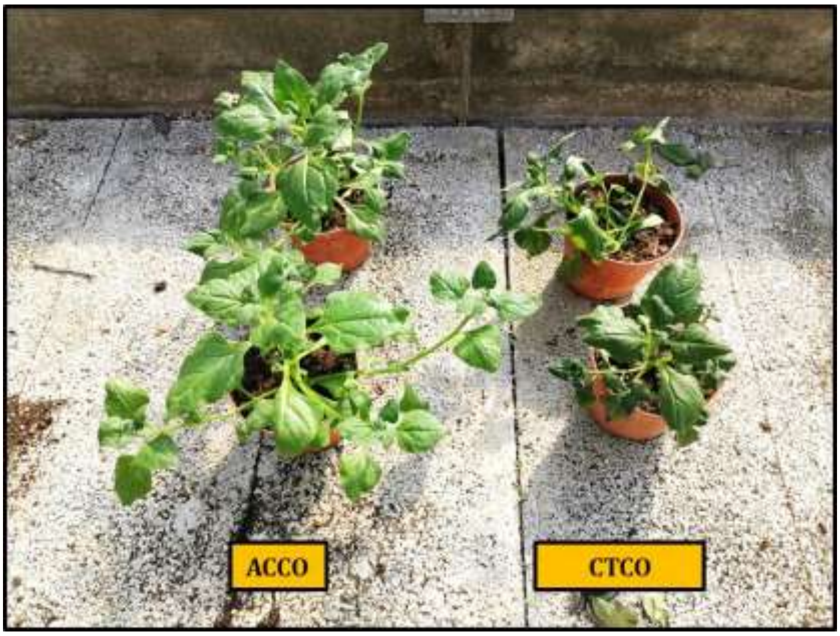

Figure 3 - Comparison between control in green compost (CTCO) and treatment with organic acidifiers in green compost (ACCO) on vegetative growth of *Tetragonia tetragonioides*

Figure 3 - Comparison between control in green compost (CTCO) and treatment with organic acidifiers in green compost (ACCO) on roots growth of *Tetragonia tetragonioides*

CONCLUSION

According to the results obtained in this above-ground experiment, applying a natural acidifier in peat- or compost-based substrate can be beneficial for increasing plant productivity and quality and positively influence the microbiology of the soil. In the experiment, a reduction in the pH of the substrate was found in the theses treated with the acidifier. At the same time, a significant increase in the agronomic quality of the plants (in terms of productivity and growth) was observed. In these theses, it was evident how the microbial presence increased, which positively affected the substrate structure and fertiliser uptake. Organic acidulants can, therefore, not only be helpful in lowering the pH of soils and substrates but can improve microbial activity with repercussions on plant quality. Microorganisms not only regulate the uptake of nutrients and water by the roots but are also crucial in increasing plant resistance to biotic and abiotic stresses. These are aspects that should not be underestimated, especially today, when climate change, lack of water resources for irrigation and alkaline soils are problems that need immediate resolution.

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

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