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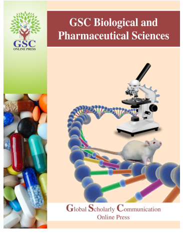

(RESEARCH ARTICLE)

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Biostimulant based on liquid earthworm humus for improvement quality of basil (*Ocimum basilicum* L.)

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Abstract

In this study, the possibility of using a biostimulant based on liquid earthworm humus to improve the growth and quality of basil plants (*Ocimum basilicum L.*) was evaluated. The 3 experimental groups in cultivation were: i) group without biostimulant (CTRL), irrigated with water and substrate previously fertilized; ii) group with 1% liquid earthworm humus (ON1) and fertilised substrate; iii) group with 2% liquid earthworm humus (ON2) and fertilised substrate. The test showed a significant increase in agronomic parameters and in the percentage increase in the main constituents of basil plants treated with liquid earthworm humus. The biostimulant derived from earthworm humus combines nutritional and microbial characteristics that make it a versatile product. The varied microbial component present within the biostimulant product is very important, as it can improve both the characteristics of the soil once used and determine greater control against pathogenic microorganisms in the treated areas near the plants.

Keywords: Vegetables; Plant growth; Microorganisms; Rhizosphere; Earthworms

1. Introduction

Basil is a horticultural species of ancient traditions very widespread both in Italy and in the rest of Europe, appreciated for its aromatic characteristics both in the kitchen and in the industry of essences and very often as an ornamental plant, with a dual purpose, on balconies and in gardens. The essence is extracted from the plants harvested at the beginning of flowering, dried and stored in a dry place in the shade. In Italy the most extensive crops are found in Liguria both in the open air and in the greenhouse, especially in Albenga, as it is a significant trade even during the winter period [1]. Extensive crops are also found in southern Italy where it is widely used in the tomato processing industry. It is an annual herbaceous species belonging to the family of the Lamiaceae; it has taproot and a quadrangular stem ramified up to the base, the fruit is an achene used as seed. In its places of origin (Asia and Tropical Africa), it is mainly utilized for the extraction of the essence and for this purpose it is cultivated in Jamaica, in Reunion Island and also in the central and southern areas of Russia [2].

Earthworm humus is a fine-sized, peat-like soil improver material, microbiologically stable and active, with a low C/N ratio, high porosity and high water retention capacity, containing many nutrients for the plants, which forms are easily available for their nutrition. This material results from the interactions between earthworms (Lumbricidae) and microorganisms which occur during the degradation of the organic substance and is composed mainly of faecal residues (casts) of earthworms which build the starting organic matrix for their needs metabolics. It is a compound very rich in organic substance characterized by high rates of this has a very positive effect on the availability of nutrients for the plants, particularly with regard to ammonia and nitrate forms [3].

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Earthworm humus is an accelerated process of bio-oxidation and stabilization of organic matter, promoted by the interaction between earthworms and microorganisms. It is an eco-sustainable process that does not require high technological levels for its process and can also be used for the treatment of organic residues from separate collection. It is a complex process both at a biological and ecological level because, although earthworms are the primary factor of the process (from which it takes its name), the presence of complex interactions between organic matter, microorganisms, earthworms and other soil organisms (meso and macrofauna) are the basis of the process of fragmentation, bio-oxidation and stabilization of organic matter [3,4].

Specific microbial groups respond differently to the environment of the digestive tract and there are selective effects on the presence and/or abundance of microorganisms during the entire passage of the organic substance through the digestive system of the observed earthworm species (*E. andrei, E. fetida, Eudrillus eugeniae* etc.). As an example, several bacteria are activated during the passage through the intestine while others remain inactive or, others, are digested by the earthworm (thus decreasing its population density). These changes can alter the decomposition phase of the organic substance as the microbial biomass is strongly influenced by the casts released from the earthworm. Since the microbial population of the casts has, in general, a greater population density and a difference in specific composition, it is more than appropriate to think that the inoculation of these communities in the fresh organic matter can go to promote changes similar to those that occur when earthworms are actively present in the soil.

The number of species recognized so far is enormous; according to Reynolds [5], 7254 species of *Oligochaeta* have been recognized and about half (3627) of these species are earthworms, with an increase of about 68 new species each year. For most of these species, the description of the genus and species are the only information available and, for most of these, little is known about their life cycle, distribution, ecology, ethology.

Earthworm humus has a great potential especially used for the formulation of potted substrates [6]. Researchers evaluated the effect of different sources of N (manure, ammonium nitrate and earthworm humus) and obtained higher yields on *Raphanus sativus L.* and *Capsicum annum L.* with earthworm [6]. Earthworms influence the structure of the compost by forming macropores, which allow oxygen to enter, and also increase the stability of the humus and its ability to retain water [7]. On the occasion of the passage through the worms, not only organic matter but also the mineral components that serve as food are subject to the following to digestive enzymes and a grinding process [8]. A improved grass grow tharound worm waste indicates increased availability of plant nutrients, with perhaps nitrogen being the mineral that is subject to the greatest influence [6]. Within its digestive tract, soil material under goes transformations, with decomposition of organic matter and availability of nutrients for plants.

Earthworm humus significantly stimulates the growth of a number of plant species including several horticultural [9] crops such as peppers [10], garlic [11], aubergines [12], strawberries [13], sweet corn [14] and beans [15]. In addition, there were positive results on some aromatic and medicinal plants [16], cereals such as sorghum and rice [17], tree crops such as banana and papaya [18], and on ornamental plants such as geranium [19], marigold [20], petunia [21], chrysanthemum [22] and poinsettia [23]. Positive effects have also been found on forest species such as *Acacia sp.*, *Eucalyptus sp*. and *Pinus sp.* [4].

In this study, the possibility of using a biostimulant based on liquid earthworm humus to improve the growth and quality of basil plants (*Ocimum basilicum L.*) was evaluated.

2. Material and methods

2.1. Greenhouse experiment and growing conditions

The experiments, which started in early March 2019, were conducted in the CREA-OF greenhouses in Pescia (Pt), Tuscany, Italy (43°54′N 10°41′E) on basil plants (*Ocimum basilicum L.*). The plants were placed in pots ø 12 cm; 90 plants for experimental thesis, divided into 3 replicas of 30 plants each. All plants were fertilized with a controlled release fertilizer (3 kg m^{-3} Osmocote Pro® 6 months with 190 g/kg N, 39 g/kg P, 83 g/kg K) mixed with the culture medium before transplanting. The 3 experimental groups in cultivation were:

- group without biostimulant based on liquid earthworm humus (CTRL), irrigated with water and substrate previously fertilized;
- group with 1% liquid earthworm humus (ON1) and fertilised substrate;
- group with 2% liquid earthworm humus (ON2) and fertilised substrate.

For the product based on liquid earthworm humus, ONUS CLT® by Centro Lombricoltura Toscano of San Giuliano Terme (PI) was used. Treatments were carried out every 15 days during the entire cultivation cycle. The plants were watered 4 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 25 September 2019, plant height, number of leaves, vegetative and root weight, number of branches and weight of inflorescences were recorded. Samples from the essential oil obtained were analysed using liquid gas chromatography HPLC-10 (RECIRC CHILLER 115VAC 1700W). The chromatogram obtained and the GC analysis report for each sample were analysed to calculate the percentage of the main components of the volatile oil (α-pinene, Β-pinene, limonene, linalool, borneol).

2.2. 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

3.1. Plant growth

The test showed a significant increase in the agronomic parameters analysed in plants treated with biostimulant based on liquid earthworm humus.

In fact, all plants treated with (Onus CLT®) showed a significant increase in plant height, leaves number, vegetative and roots weight, branches number and inflorescences weight. In basil, the plants height was 74.5 cm (ON2), compared to 68.44 cm (ON1) and 56.33 cm (CTRL). For the leaves number, 64.67 (ON2), 57.83 (ON1) and 44.91 (CTRL) were found. There was a significant increase in vegetative biomass in (ON2) 57.96 and 54.23 g (ON1), compared to 45.72 g in the untreated control. The same trend applies to root weight 52.89 g in (ON2), 44.19 g in (ON1) and 35.73 g (CTRL). Theses with Onus then showed a significant increase in the ramifications number, 3.79 (ON2) versus 3.66 (ON1) and 2.88 in the untreated control. The inflorescences weight also showed an increase in treatment with (ON2) 3.18 g, against 3.11 g of (ON1) and 1.56 g of the control.

Table 1 Evaluation of the effect of biostimulant based on liquid earthworm humus on basil plants

Groups	Plant height (cm)	Leaves number (n°)	Vegetative weight (g)	Root weight (g)	Branches number (n°)	Inflorescences weight (g)
CTRL	56.33 ^{a}	44.91 ^{a}	45.72 ^{a}	35.73 ^{a}	2.88 ^{a}	1.56 ^{a}
ON1	68.44 ^{b}	57.83 ^{b}	54.23 ^{b}	44.19 ^{b}	3.66 ^{b}	3.11 ^{b}
ON2	74.51 ^{c}	64.67 ^{c}	57.96 ^{c}	52.89 ^{c}	3.79 ^{b}	3.18 ^{b}
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 ($P = 0.05$)

The test also showed a percentage increase in the main constituents of basil plants, in the experimental theses treated with Onus. In particular for α-pinene, 0.43 (ON2), 0.42 (ON1) and 0.30 in the untreated control. For Β-pinene, 1.93 (ON2), 1.94 (ON1) and 1.88 in the control. In limonene 8.48 (ON2), 8.56 (ON1), 7.33 in the untreated control. Same trend for linalool 49.33 (ON2), 49.45 (ON1) and 46.78 (CTRL) and borneol, 13.67 (ON2), 13.90 (ON2) and 13,44 in the untreated control. For these two metabolites, therefore, the 1% dosage of Onus resulted in a higher increase than the 2% dosage.

Table 2 Evaluation of the effect of biostimulant based on liquid earthworm humus on main constituents percentage of basil plants

Groups	α-pinene	B-pinene	limonene	linalool	borneol
CTRL	0.30	1.88	7.33	46.78	13.44
ON1	0.42	1.94	8.56	49.45	13.90
ON2	0.43	1.93	8.48	49.33	13.67
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 ($P = 0.05$).

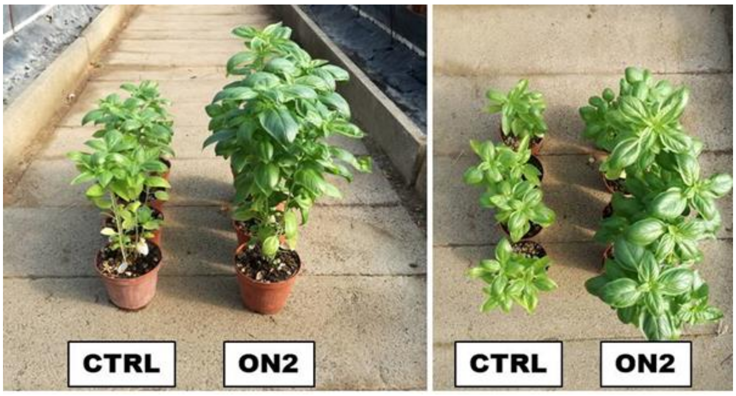

Figure 1 Effect of biostimulant based on liquid earthworm humus on growth of basil.
Legend: (CTRL) control; (ON2) Onus 2%.

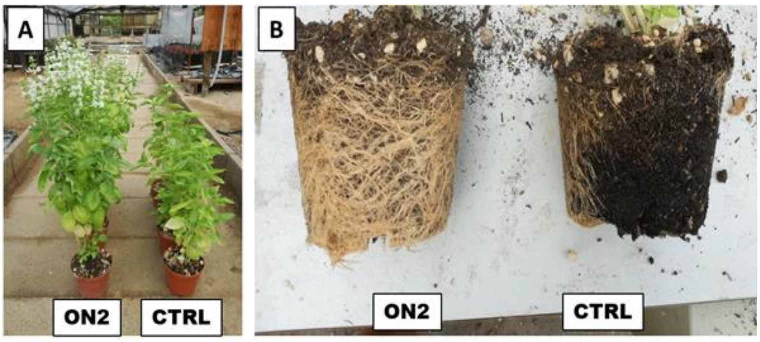

Figure 2 Effect of biostimulant based on liquid earthworm humus on inflorescences production (A) and roots growth (B). Legend: (ON2) Onus 2%; (CTRL) control.

4. Discussion

The use of liquid earthworm humus can stimulate flowering, increase plant and root biomass and plant production and growth. Liquid earthworm humus may also increase the nutritional quality of some crops as was the case in this test on basil, where there is a significant improvement in the development of treated plants and the production of metabolites. However, there are also studies that report that the use of earthworm humus can decrease the growth of plants or even cause their death. The variability of earthworm humus effects may depend on the culture system in which it is incorporated, which strongly depends on the basic organic matrix, earthworm species used, production processes and age [7,8]. It constitutes a reserve of macro and micronutrients for plants. Although many of these nutrients are present in inorganic form and, therefore, readily available to plants, most of them are gradually released through the mineralization of organic matter [24]. However, unlike chemical fertilizers, the amount of nutrients available can vary greatly depending on the starting matrix, the process time and the maturity of the humus of the row [25]. Several studies show that earthworms can be important agents that can influence the production of PGR of microorganisms through their stimulation and promotion of their activity in organic matter. Liquid earthworm humus may also have biocontrol effects on fungal organisms, in particular extracts of earthworm humus have reduced the sporulation of *Phytopthora cryptogea*, *Botrytis cinerea, Sclerotinia sclerotium, Corticium rolfsii, Rhizoctonia solani* and *Fusarium oxysporum* [26,27]. The addition of earthworm humus in the germination substrates of tomato seeds reduces the infection caused by *Fusarium lycopersici* and *Phytopthora nicotianae* [28]. Earthworm humus has a microbial community different from the organic matrix from which it was created, with a lower level of activity and biomass, but with a greater metabolic diversity. This microbiologically active substrate can have important effects on the microbial properties of the soil by influencing plant growth.

5. Conclusion

The test showed that the use of a liquid biostimulant obtained from earthworm humus can significantly improve the growth and production of nutraceutical metabolites in basil. The application of a liquid product can facilitate the grower's use compared to the solid product and the amount of field intervention is also significantly reduced. The biostimulant derived from earthworm humus combines nutritional and microbial characteristics that make it a multi-purpose product. The varied microbial component is very important, as it can improve both the characteristics of the soil where it is inoculated, and the properties of biocontrol against pathogenic microorganisms.

Compliance with ethical standards

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Disclosure of conflict of interest

The author declares no conflict of interest.

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