



(RESEARCH ARTICLE)



Application of Biohumus at different substrate replacement rates in the germination and cultivation of *Zea mays*

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GSC Advanced Research and Reviews, 2023, 15(03), 193–200

Publication history: Received on 05 May 2023; revised on 13 June 2023; accepted on 15 June 2023

Article DOI: <https://doi.org/10.30574/gscarr.2023.15.3.0237>

Abstract

Research objective: This research aims to evaluate the biostimulant potential of biohumus in different substrate replacement rates on the growth of *Zea mays* seedlings. This is to increase knowledge of the use of biohumus in plant cultivation, which could be used in the formulation of new growing media and organic fertilisers.

Materials and Methods: The experiments, which began in January 2023, were conducted in the CREA-OF greenhouses in Pescia (Pt), on seedlings of *Zea mays*. The experimental groups were: group control, irrigated with water and substrate previously fertilized; group with 10% biohumus, irrigated and fertilized substrate; group with 20% biohumus, irrigated and fertilized substrate; group with 30% biohumus, irrigated and fertilized substrate; group with 40% biohumus, irrigated and fertilized substrate; group with 50% biohumus, irrigated and fertilized substrate; group with 60% biohumus, irrigated and fertilized substrate; group with 70%, irrigated and fertilized substrate; group with 100% biohumus, irrigated and fertilized substrate. On May 18, 2023, plant height, vegetative weight, roots volume and length, the number of microorganisms and pH in the substrate were determined.

Results and Discussion: The experiment showed that the use of biohumus enriched with microorganisms can significantly improved the vegetative and root growth of sown *Zea mays* seedlings. In general, a significant increase in plant height, vegetative and root weight, and root length was observed from 20% substitution of biohumus in the substrate. The theses that show a particularly clear improvement for all agronomic parameters analysed are those with 50% and 60% biohumus, peat substitution. Plants that received biohumus grew better than those that did not, and cereal plants grew better than those that were grown in plain soil. The nutrient content of biohumus stimulates the growth of the following crops: tomatoes, peppers, garlic, sweet corn, aubergines, bananas. Among other things, it stimulates the growth of chrysanthemum, marigold, geranium, petunia, and poinsettia flowers, as well as acacia and eucalyptus. Due to its macro- and micronutrient content, biohumus has a positive effect on plants (leaf area, root volume, root branching), and improves the soil's biological functions. Plants that received biohumus grew better than those that did not, and cereal plants grew better than those that were grown in plain soil. The growth of barley and cereals improved on a maize farm after biohumus was applied. There was a significant improvement in soil porosity on a maize farmland. Among the benefits of using biohumus as a fertiliser are: it eliminates harmful insects, reduces the infestation of harmful insects, reduces pathogen infestation of plants, fertilizes the soil, and improves the soil structure as a soil conditioner.

Conclusions: In order to maintain soil health and fertility, farmers need a sustainable alternative that is economically viable and productive. As part of ecological agriculture, the protection of food, agricultural and human ecosystems is emphasized, along with the improvement of soil fertility and the development of secondary income for farmers. The use of biohumus provides the best answer to ecological agriculture, which is synonymous with 'sustainable agriculture'. This article provides a basis for further research

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Keywords: Biohumus; Microorganisms; Plant growth; Biofertilizers; Rhizosphere

1. Introduction

Biofertilizers, which can enhance plant growth and productivity in a wide spectrum, have become increasingly sought after in recent years due to growing organic agriculture demand. Composting is a biochemical and microbiological process in which organic matter is hydrolyzed to produce a stable and disinfected humus, which can be produced from both household wastes and various organic wastes. In addition to increasing soil and crop productivity, biofertilizers contain living microorganisms that fix nitrogen, solubilize phosphorus, or mobilize nutrients [1,2]. As bacteria break down organic substrates, they produce water, carbon dioxide, and heat by utilizing carbon, nitrogen, and oxygen as energy sources, enriching soil with compost in the process [3]. As an additional soil additive, compost contains biologically stable significant humus. Compared to chemical fertilizers (NPK), fertilization with beneficial microorganisms increased tomato plant growth and decreased tomato defenses against insect pests [4,5]. Furthermore, microbes enhance plant growth through various direct and indirect promotion mechanisms, including the production of enzymes and the solubilization of potassium, zinc, and phosphorus during the application of microbial fertilizers. In a study [6], soil microorganisms produce an enzyme that decreases ethylene levels and allows plants to resist various environmental stresses. An inoculation of basil plants under water stress with three bacterial species (*Bacillus lentus*, *Azospirillum brasilens*, *Pseudomonades* sp.) improved growth, significantly improved CAT enzyme activity (GPX and APX activity) and chlorophyll content in leaves [7]. As well as under natural conditions, plant-associated microbes can help plants grow. An author suggests that plants growing in arid or semi-arid areas may be able to tolerate drought better if they are inoculated with native beneficial microorganisms [8]. Using beneficial microorganisms to convert agricultural waste into biofertilizer can optimize resource use and generate income. Using a well established fertilizer dose in soil fertilization practices is recommended in this context [9] for a beneficial response from plants. Organic fertilizers, compost, or manure are also excellent options, which are capable of gradually releasing nutrients and do not threaten the environment with contamination [10]. Organic fertilizers improve soil organic C, cation exchange capacity, and microbiological activity, according to [11], thus improving soil health and properties and promoting agroecosystem sustainability. Furthermore, organic fertilizers may relieve plants from stress caused by aridity (low fertility) and increase their photosynthetic capacity [12,13]. However, such fertilizers have the disadvantage of a lag period, which is necessary for soil microorganisms to mineralize nutrients to forms accessible to trees. Bio humus is not just a soil fertilizer; it also acts as a matrix that temporarily holds soil minerals and makes them available to plants gradually [14]. It also plays a nutritive role by improving soil structure. Optimal nutrition can be achieved through the use of a combination of chemical, organic and microorganism based fertilizers [15,16].

1.1. Research Objectives

This research aims to evaluate the biostimulant potential of biohumus in different substrate replacement rates on the growth of *Zea mays* seedlings.



Figure 1 Details of the experiment at CREA-OF

2. Material and methods

The experiments, which began in January 2023, were conducted in the CREA-OF greenhouses in Pescia (Pt), Tuscany, Italy (43°54'N 10°41'E) on seedlings of *Zea mays*. Plants were placed in 54-hole containers, 6 plants per 3 replications, for a total of 18 seedlings per experimental thesis.

All plants were fertilised with a controlled-release fertiliser (2 kg m⁻³ Osmocote Pro®, 9-12 months with 190 g/kg N, 39 g/kg P, 83 g/kg K) mixed into the growing medium before sowing. The experimental groups were:

- group control (CTR) (peat 100%) irrigated with water and substrate previously fertilized;
- group with 10% biohumus (peat 90% + biohumus 10%) (BIO10) irrigated and fertilised substrate;
- group with 20% biohumus (peat 80% + biohumus 20%) (BIO20) irrigated and fertilized substrate;
- group with 30% biohumus (peat 70% + biohumus 30%) (BIO30) irrigated and fertilized substrate;
- group with 40% biohumus (peat 60% + biohumus 40%) (BIO40) irrigated and fertilized substrate;
- group with 50% biohumus (peat 50% + biohumus 50%) (BIO50) irrigated and fertilized substrate;
- group with 60% biohumus (peat 40% + biohumus 60%) (BIO60) irrigated and fertilized substrate;
- group with 70% biohumus (peat 30% + biohumus 70%) (BIO70) irrigated and fertilized substrate;
- group with 100% biohumus (biohumus 100%) (BIO100) irrigated and fertilized substrate;

The plants were watered one time a day and grown for five months. Then, 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 leaching fraction.

The characteristics of the product were: pH 6.5; Dry matter % 43.71; Organic matter % 63.37; Total nitrogen % 1.05; Total phosphorus % 0.43; Total potassium % 0.87; Total sodium % 0.07; Total calcium % 1.90; Total magnesium % 0.51; Total sulphur % 0.59; Cadmium mg/Kg 0.21; Arsenic mg/Kg 0.50; Chromium mg/Kg 23.0; Nickel mg/Kg 5.57; Lead mg/Kg 7.70; Copper mg/Kg 42.1; Zinc mg/Kg 106; Mercury < 0.02; Organic carbon % 38.39.

On May 18, 2023, plant height, vegetative weight, roots volume and length, the number of microorganisms and pH in the substrate were determined.

2.1. Analysis methods

- Microbial count: directly determining total microbial count by microscopy cells contained in a known sample volume using counting chambers (Thoma chamber). The surface of the slide is etched with a grid of squares, with the area of each square known. Determination of viable microbial load after serial decimal dilutions, spatula seeding (1 ml) and plate counting after incubation [17];

2.2. Statistics

The experiment was carried out in a randomized complete block design. Collected data were analyzed 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 the LSD multiple-range tests ($P = 0.05$). Statistics and graphics were supported by the programs Costat (version 6.451) and Excel (Office 2010).

3. Results

The experiment showed that the use of biohumus enriched with microorganisms can significantly improved the vegetative and root growth of sown *Zea mays* seedlings. In general, a significant increase in plant height, vegetative and root weight, and root length was observed from 20% substitution of biohumus in the substrate (Figure 2). The theses that show a particularly clear improvement for all agronomic parameters analysed are those with 50% and 60% biohumus, peat substitution (Table 1). There was also a significant increase in the microbial colonisation of the substrate as the biohumus content in the substrate is increased. The results on the agronomic parameters appear to increase significantly up to 60% substitution, after which the results for 70% and 100% drop conspicuously (Figure 3 and Figure 4). The pH of the substrate drops significantly as the biohumus content in the substrate is increased. No plant failures were observed.

Table 1 Evaluation of the use of Biohumus on the vegetative and root biomass of *Zea Mays*

Groups	Plant height (cm)	Substrate total Bacteria (Log CFU/g soil)	Vegetative weight (g)	Roots volume (cm ³)	Roots length (cm)	pH
CTR	23.47 g	1.82 h	16.21 fg	8.35 h	3.30 g	7.31 a
BIO10	23.47 g	2.19 g	16.32 f	8.54 g	4.17 f	7.24 a
BIO20	24.30 f	2.86 f	17.72 e	9.54 e	5.07 e	7.10 b
BIO30	25.54 d	3.36 e	18.35 d	10.52 d	5.98 d	7.02 bc
BIO40	25.98 c	3.88 d	19.29 c	11.38 c	7.07 b	6.96 c
BIO50	28.93 b	4.06 c	24.73 b	14.79 b	10.14 a	6.86 d
BIO60	29.64 a	4.36 b	25.29 a	15.38 a	10.16 a	6.78 de
BIO70	24.82 e	4.41 b	16.27 f	8.75 f	6.19 c	6.74 e
BIO100	23.17 g	5.11 a	15.92 g	7.98 i	5.95 d	6.54 f
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: (CTR) control; (BIO10) 10% biohumus; (BIO20) 20% biohumus; (BIO30) 30% biohumus; (BIO40) 40% biohumus; (BIO50) 50% biohumus; (BIO60) 60% biohumus; (BIO70) 70% biohumus; (BIO100) 100% biohumus

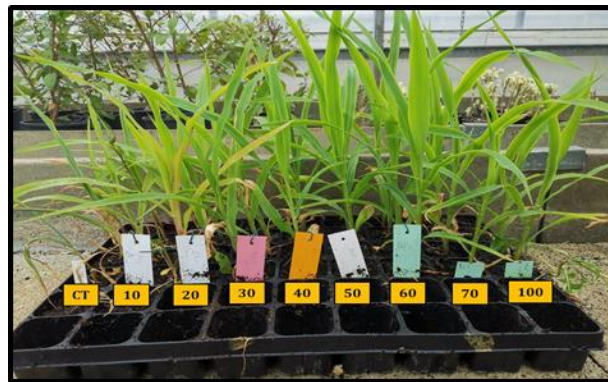


Figure 2 Comparison of different experimental theses additivated with a different percentage of biohumus in growing medium



Figure 3 Effect of different biohumus content in growing medium on root growth



Figure 4 Effect on root growth between 100% peat (CT) and 100% biohumus (100)

4. Discussion

The nutrient content of biohumus stimulates the growth of the following crops: tomatoes, peppers, garlic, sweet corn, aubergines, bananas [18,19,20,21]. Among other things, it stimulates the growth of chrysanthemum, marigold, geranium, petunia, and poinsettia flowers, as well as acacia and eucalyptus [22]. Due to its macro- and micronutrient content, biohumus has a positive effect on plants (leaf area, root volume, root branching), and improves the soil's biological functions [23,24]. Plants that received biohumus grew better than those that did not, and cereal plants grew better than those that were grown in plain soil [25,26,27]. The growth of barley and cereals improved on a maize farm after biohumus was applied. There was a significant improvement in soil porosity on a maize farmland [28,29]. Among the benefits of using biohumus as a fertiliser are: it eliminates harmful insects, reduces the infestation of harmful insects, reduces pathogen infestation of plants, fertilizes the soil, and improves the soil structure as a soil conditioner [30,31]. In this trial, the use of biohumus improved the vegetative and root growth of *Zea mays* plants and increased the content of microorganisms in the cultivation substrate, which is very interesting, especially if the product is supplied to polluted areas that are poor in microbial biodiversity. Biohumus also significantly influenced the pH of the substrate, probably making nutrients in the soil more available. As soil particles form clusters, humus provides channels for air passage and improves water retention, while the humic acid in humus provides binding sites for plant nutrients such as calcium, iron, potassium, phosphorous and sulfur. Humic acid in biohumus stimulates plant growth even in small quantities by storing nutrients in a form that is readily accessible to the plants. Humic acid in biohumus stimulates plant growth even in small quantities [32]. There are four basic ways that humic acids are essential to plants: a) they help plants obtain nutrients from the soil; b) they dissolve unresolved minerals so that organic matter can be used; c) they stimulate root growth; and d) they help plants deal with stress. In addition to helping chemical fertilisers work better, humus is thought to inhibit plant-damaging pathogens, fungi, nematodes, and bacteria [33,34,35,36]. Plant diseases such as root rot can be combated by biohumus. In addition to improving plant health and utilizing soil moisture more efficiently, humus also increases water permeability and water retention capacity. Biohumus also provides agronomic benefits, such as suppressing soil-borne diseases and removing salinity from the soil [37]. A study found that in soils amended with biohumus, root disease was reduced by 82% to 18% in tomato and 98% to 26% in capsicum. In biohumus, the beneficial microorganisms are ten to twenty times more active than in other organic matter or soil [38]. Biohumus stimulate beneficial soil microbes such as nitrogen-fixing bacteria, actinomycetes, and mycorrhizal fungi. As a result of the humus content, biohumus also has "high porosity", "aeration", "drainage", and "water-holding capacity" compared to conventional compost. Biohumus appears to have more "induced hormonal activities" in plants than conventional compost due to its high levels of nutrients, humic acids, and humates. Despite the fact that plants are already receiving 'optimal nutrition', the use of biohumus further stimulates their growth. By simply converting mineral nutrients into plant-available forms, it has consistently improved seed germination, seedling growth, and productivity. We used a plant bioassay method to test the growth-promoting activity of biohumus [39,40]. Maize (*Zea mays*) seedlings were measured 48 hours after immersion in vermicompost water and normal water. The marked difference between the two groups indicates that biohumus contains plant growth-promoting hormones. Aside from making plants grow faster and stronger, biohumus also stimulates seed germination in various plant species, including green gram, tomato plants, petunias and pine trees [41]. As well as stimulating shoot and root growth, biohumus is beneficial to vegetative growth. Seeding morphology is altered, including increased leaf area and root branching; flowering is also stimulated, with an increase in the number and biomass of flowers produced as well as fruit yield [42]. Certain antibiotics and actinomycetes in biohumus enhance plant biological resistance to pests and diseases. When biohumus was used in agriculture, chemical pesticides were reduced by more than 75% [43,44].

5. Conclusion

In order to maintain soil health and fertility, farmers need a sustainable alternative that is economically viable and productive. A new concept is 'ecological farming', which by definition is different from 'organic farming', which primarily focuses on producing chemical-free food. As part of ecological agriculture, the protection of food, agricultural and human ecosystems is emphasized, along with the improvement of soil fertility and the development of secondary income for farmers. It has also been endorsed by the United Nations. The use of biohumus provides the best answer to ecological agriculture, which is synonymous with 'sustainable agriculture'. As a result, the environment is the greatest beneficiary at present of this system. This article provides a basis for further research.

Compliance with ethical standards

Acknowledgments

The research is part of the project HUMEORT: Study and evaluation of the stimulating activity of humus in the cultivation of horticultural plants. We would like to thank the company Sanasoil Biotech for their cooperation and for providing the products for the research, in particular Dr. Donatas Andrikis.

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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