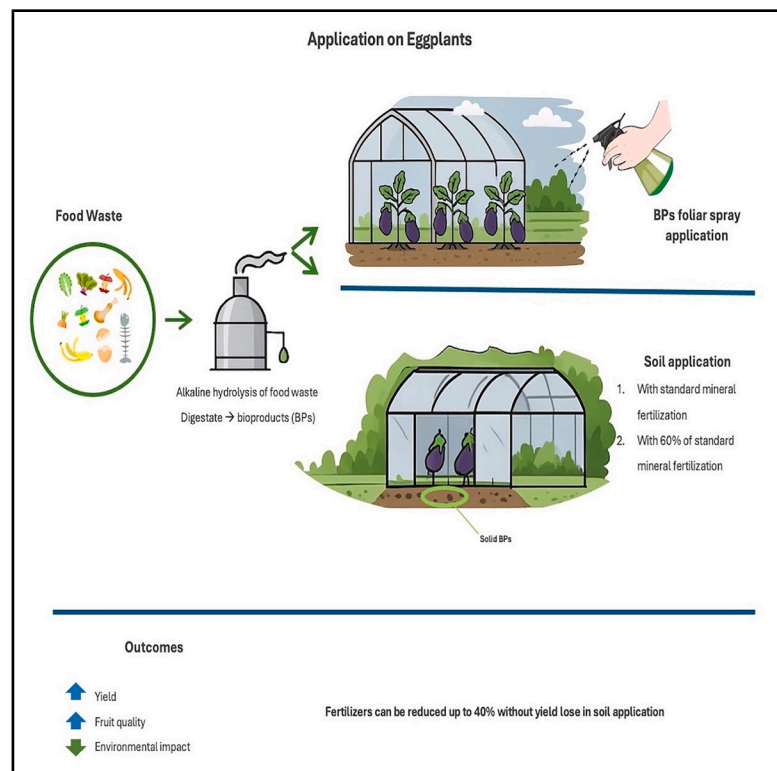


Sustainable eggplant cultivation: Distinct effects of foliar and root applications of alkaline-extracted bioproducts from digestate

Graphical abstract



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In brief

Plant Biology; Plants; Horticulture

Highlights

- Bioproducts (BPs) from food waste anaerobic digestate improve eggplant yield
- Innovative method tested in greenhouse: foliar and soil application
- Soil BP sustains fruit yield and quality with 40% less mineral fertilizers
- Eco-friendly strategy for sustainable eggplant cultivation within a circular economy



Article

Sustainable eggplant cultivation: Distinct effects of foliar and root applications of alkaline-extracted bioproducts from digestate

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SUMMARY

Increasing crop yields and quality, and reducing the doses of mineral fertilizers are two main concerns to counteract the environmental negative effects. The present study reports the use of bioproducts (BPs) extracted by alkaline hydrolysis from food waste anaerobic digestate in the cultivation of eggplants in a cold greenhouse, aiming to reduce chemical inputs in agricultural practices. Two application methods, root and foliar, were compared. The effect of solid BPs was also evaluated, reducing by 40% the regular fertilization used for the common production of eggplants in Sicily. The results suggested that BPs allow reducing up to 40% mineral fertilization, without critically affecting the plant performance, fruit yield, and even ameliorating the quality of the fruits. These findings suggest that BPs can act as sustainable biostimulants providing evidence for integrating bioproducts into circular bioeconomy strategies, reducing fertilizer dependency, and contributing to environmentally friendly agriculture.

INTRODUCTION

Among the many societal concerns today, two significant issues stand out. First, the depletion of fossil sources used for producing fuels and chemicals. Second, the increasing production of waste has a potentially negative impact on the environment. One approach to mitigate the above-mentioned issues is to utilize biowastes as a renewable feedstock, as an alternative to fossil sources. Achieving this objective is challenging, as it relies heavily on biomass availability, collection, and sustainable processing. Previous studies¹ have shown that waste biomass may be valorized as an alternative feedstock to produce multifunctional value-added bioproducts (BPs) for use in the chemical industry, agriculture, and waste treatment sectors.

Specific issues in the agriculture sector arise from current agricultural practices. These enhance plant and crop production using mostly mineral fertilizers and synthetic agrochemicals.² To this end, mineral fertilizers are usually applied in soil at doses higher than plant requirements, determining an excess fertilizer accumulation in soil, and possible leaching of nutrients in groundwater, causing eutrophication, as they contain the key N, P, K plant nutrients.³ Moreover, nitrogen fertilizer can be lost to the environment through ammonia volatilization, leakage, nitrification, and denitrification after being applied to the soil, which not only misses a lot of resources, but also causes enormous economic losses and environmental pollution.^{4,5} Also, high nitrate levels in soil can reach the food chain and, conse-

quently, affect human and animal health, as well as determine a negative impact on several crops.^{6,7} In addition to mitigating the environmental impact, reducing the consumption of mineral fertilizers has relevant economic effects. This is particularly true in countries relying on high mineral fertilizers import, such as the European Union, which is largely dependent on imports for most mineral fertilisers.⁸ The reduction of mineral fertilizer applied doses in European agriculture and/or substitution with locally produced eco-friendlier agrochemicals is expected to lower dependence on imports and consequently attain socio-economic benefits. Yet, achieving this objective requires facing social controversy.

Most current R&D work for biomass valorization as renewable feedstock has focused on processing plants and crops specifically cultivated for non-food production. This raises social concerns due to the exploitation of agricultural land for the production of non-food energy and chemical crops.⁹ On the contrary, the utilization of biowastes as feedstock for the production of energy and chemicals is expected to meet unanimous social consent, since it prospects the double benefit of saving land for the production of food plants and, at the same time, alleviates the widespread concern arising from waste disposal by landfilling. Whereas biowaste materials are produced from different sources, including the agriculture and agro-industrial sectors, municipal biowaste (MBW) represents the most readily available and sustainable, potentially renewable feedstock. This latter may be used for the production of innovative agrochemicals and to



develop an eco-friendly agriculture industry,¹⁰ as well as to improve current MBW plant treatment and turn them into eco-friendly bio-refineries, producing fuel and the multifunctional value-added bioproducts (BPs) for use in the chemical industry, agriculture, and waste treatment sectors.¹

Previous work has demonstrated the BPs properties and performance as biostimulants and anti-pathogen agents in the cultivation of a wide variety of plants, including several ornamentals (euphorbia, lantana, murraya, hibiscus) and vegetable species (tomato, red pepper, radish, spinach, maize, bean, wheat, tobacco, lettuce and oilseed rape).¹¹ Biostimulants are defined as substances or microorganisms that, when applied to plants or soil, enhance plant growth and development by improving nutrient uptake, the tolerance to abiotic stress, or crop quality, without directly providing nutrients as fertilizers.¹² The BPs applied at doses lower than those of regular mineral N P K fertilizer allow increasing crop production by several orders of magnitude, while reducing nutrients leaching through the soil.¹³ For example, in the studies on the cultivation of ornamental plants^{14–18} and beans,¹⁹ the plants grown on the substrate containing added BPs exhibited 1 to 3 orders of magnitude higher biomass growth, crop production, water use efficiency, leaves' chlorophyll content, leaf gas exchange, N assimilation, enzymes and soluble protein content in leaves and roots, compared to the plants grown on substrate containing peat and Leonardite. All trials consistently indicated a plausible auxin-like effect by BPs, as already seen for several other humic molecules. For instance, Scaglia et al.²⁰ demonstrated that humic acids (HAs) obtained from vermicompost exhibited auxin-like activity in *in vitro* assays on lettuce seeds, enhancing the development of IAA activity in seedlings. Furthermore, other studies available in the literature reported an IAA-like activity for various humic-like substances.^{21,22} As regards the influence of BPs in relation to nutrient uptake, Fragalà et al.¹³ showed how their application in lettuce cultivation can provide a remarkable reduction of conventional mineral fertilization, optimizing the use of fertilizers. A similar indication was confirmed in the cultivation of tomato, pepper, maize, tobacco, and spinach.¹¹ Other studies proved that BPs cause no toxic effect on animals²³ or plants^{24,25} and have no negative environmental impact on air and water.¹⁰ Consistently with these findings, as reported by Fragalà et al.,¹³ the BPs used in our study contain low levels of heavy metals (Zn, Cu, Cd, Pb, and Hg), ensuring the absence of risks or limitations in the short-term. Moreover, Fragalà et al.¹⁰ also tested these BPs on the germination process of different crop species to assess their potential phytotoxicity, and no adverse effects were observed. Other studies about BPs showed their properties as plant protection agents, adjuvants to reduce eutrophication effects due to mineral fertilizers leaching through soil into waters and, at the same time, to increase food safety.^{10,13,26–28} All these studies confirmed that the BP performance was equal to or better than commercial products claimed to have the above effects.

From a biochemical point of view, the MBW-derived BPs are mixtures of complex biomolecules with molecular weights ranging from 5 to over 750 kDa.¹ They are made by long aliphatic and aromatic C chains, substituted by acid and basic functional groups of different strengths, bonding and/or complexing sev-

eral mineral elements. These chemical features are inherited from the pristine water insoluble biowastes.

The MBW-derived BPs have never been tested before for the cultivation of eggplants. Eggplant (*Solanum melongena esculentum*), also known as aubergine, is a tender perennial plant of the nightshade family (Solanaceae). Its leaves are large, ovate, and slightly lobed. The shape and color of fruits depend on the variety.²⁹ The importance of eggplants in the economy of the agricultural sector is well-known, especially in Sicily.³⁰ Eggplants are essential components of everyday dishes, and are a common element of the Mediterranean diet, characterizing a lifestyle and culture proven to contribute to better health and quality of life.³¹ They constitute the fifth most economically important solanaceous crop after potato, tomato, pepper, and tobacco.³² In 2014, the global production of eggplant was around 50 Mt/yr, with a net value >10 billion US\$/yr.³² In 2022 eggplant consumption increased to an estimated global value of >59 Mt/yr, representing Italy the 8th world country for eggplant production, according to FAOSTAT.³³

As further support for the implementation of BP-based practices for the development of eco-friendly and more sustainable modern agriculture, the present experimental study focuses on the use of BPs in eggplant cultivation. The aim of the study was to evaluate the biostimulant effect of BPs by assessing the yield and quality of fruits, as well as the morpho-biometric parameters and biochemical responses of eggplant. Two different application methods were considered: root drenching and foliar spraying. In the case of the root application, as biostimulants increase nutrient uptake, the potential reduction of standard mineral fertilization was also investigated. As a practical application, this study focuses on eggplant cultivation under standard agronomic conditions in Sicily, i.e., greenhouse conditions. As regards the root application, a 40% reduction in mineral fertilization was evaluated. This case study provides a concrete context to assess the effectiveness of BP application under real agricultural conditions.

RESULTS

Morpho-biometric parameters

During the experimental trials, in order to follow and evaluate the growth performance of eggplants, only non-destructive methods were applied, and the results are reported in [Figure 1](#) and in [Table S1](#). Treatments and legend are reported in the [Table 1](#) included in [STAR Methods](#) section.

The canopy cover was followed until 39 DAT ([Figure 1A](#)), and the treatment BPs 100%MF showed a significantly higher value than the control, both at 14 and 39 DAT. As regards the spray treatment, unfortunately, no observation may be made as the measurement was performed before the first foliar application ([Figure 1A](#)). Concerning canopy height ([Figure 1B](#)), vigor plant ([Figure 1C](#)), and N-test ([Figure 1D](#)), no significant differences were observed among all treatments and the control.

Overall, as reported in [Table 2](#), all the morpho-biometric parameters of eggplant, measured at the end of the experimental period (6 months), have shown no significant differences among all treatments and the control, except for the leaf FW in the treatment BPs spray. In this latter, the leaf FW resulted to be the

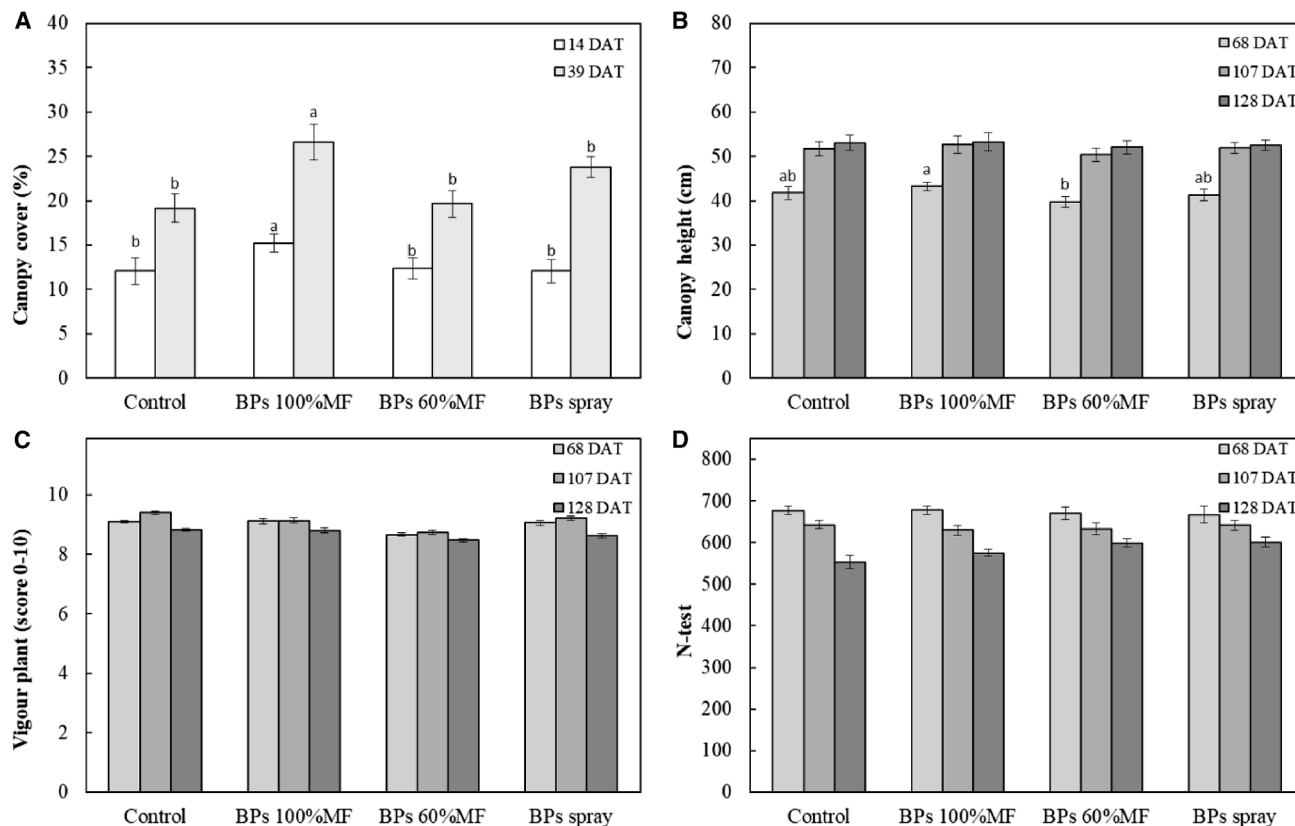


Figure 1. Morpho-biometric parameters measured during experimental trials

(A–C) Canopy cover at 14 and 39 DAT; (B) Canopy height at 68, 107, and 128 DAT; (C) Vigor plant at 68, 107, and 128 DAT; (D) N-test at 68, 107, and 128 DAT. Error bars indicate standard deviation (\pm SD). Values at the same DAT followed by different letters are significantly different ($p < 0.05$); the absence of letters indicates no significant differences.

highest one in both respects to the control (around 20%) and the soil treatments (around 18%).

Yield and quality parameters of eggplants' fruits

Fruits were harvested during 6 separate picking events, and yield parameters (weight of marketable fruits, % of not marketable fruits, number of marketable fruits per plant, medium weight for

Table 1. Legend of treatments performed on eggplants

Legend	Treatment	Amount BPs	Application (number)
Control	Application of standard mineral fertilization (MF)	–	–
BPs 100%MF	Soil treatment before transplant with 150 kg/ha BPs +100% MF	150 kg/ha	1
BPs 60%MF	Soil treatment before transplant with 150 kg/ha BPs +60% MF	150 kg/ha	1
BPs spray	Foliar spray application starting from flowering +100% MF	50 mg/L	3

fruit, dry matter, and ash for fruit) are reported in Table 3. As regards the marketable fruit weight, no significant differences among the treatments with BPs by soil application (BPs 100%MF and BPs 60%MF) and control were detected; on the contrary, a strong reduction (around 40%) with respect to the control was recorded in the weight of fruits from plants treated by foliar spray application. Moreover, the percentage of non-marketable fruits in BPs 100%MF (7.8%) was significantly lower than the control (13.2%). The positive effect of BPs 100%MF was also observed as regards the number of marketable fruits per plant, resulting in higher than the control (around 26%). On the contrary, a negative effect of the treatment BPs spray was recorded in the number of marketable fruits (around 38%) and in the weight per fruit (around 9%). Finally, no significant differences for ashes were reported.

As regards the quality, when averaged for each harvesting event, eggplant's fruits revealed no significant differences in the firmness values, with respect to the control plants (Table 3). Interestingly, only the fruits subjected to the treatment BPs 60%MF showed a significantly lower degree of browning (DB) (around 31%) and color difference (CD) (around 30%), with respect to the control.

Finally, no significant differences in terms of firmness values, with respect to the control plants (Table 3) were observed.

Table 2. Morpho-biometric parameters of eggplant at the end of the experimental period

Treatment	Leaf FW (g)	Leaf DW (%)	Root Surface (%)	Root vigor (score 0–10)
Control	2401.33 ± 88.33b	18 ± 0.15	51.19 ± 2.01	9.20 ± 0.5
BPs 100%MF	2450.83 ± 90.93b	21 ± 0.22	48.99 ± 3.05	9.10 ± 0.6
BPs 60%MF	2420.00 ± 79.75b	20 ± 0.31	47.26 ± 2.30	9.35 ± 0.3
BPs spray	2880.36 ± 85.93a	17 ± 0.10	40.12 ± 3.18	9.50 ± 0.4

All data are expressed as mean ± standard error of the mean (SEM). Different letters within each column indicate significant differences according to Tukey's protected LSD test ($p < 0.05$); ± indicates the standard error mean. The absence of letters indicates no significant differences.

Pigments and total protein content

Figure 2 A and Table S2 show the contents of chlorophylls and carotenoids in leaves, measured at the end of the experimental trials. BPs spray induced the greatest values in all pigment concentrations. In particular, spray treatment determined an increase of around 20% for Ch-a, 23% for Ch-b, and 24% for C, with respect to the control. On the contrary, the treatment BPs 60%MF showed values always similar to those recorded in the control.

The total protein content in eggplants' tissues subjected to all BP treatments was always significantly similar to the control (Figure 2B).

Enzymatic activities related to nitrogen metabolism in eggplants' leaves

The nitrate reductase (NR) activities (Figure 3A, Table S3) in eggplants' leaves have shown a significant increase under the treatment BPs 100%MF, recording a higher value with respect to the control of around 57%. Other treatments showed values similar among them and compared to the control.

Similarly, glutamine synthetase (GS) activities (Figure 3B, Table S3) measured in eggplants' leaves showed a similar trend

to the NR, although the increase in BPs 100% treatment, with respect to the control % is lower (51%) than that observed for NR activity. The other treatments recorded values always similar to the control.

Finally, the glutamate synthase (GOGAT) activities (Figures 3C, Table S3) showed different performance with respect to previous enzymatic activities. Indeed, the highest activity was recorded with BPs 100%MF, which showed a significant increase of around 62% with respect to the control, followed by the treatment BPs 60%MF, which reached a significantly higher value (around 40%) than that recorded in the control. Finally, the BPs spray treatment showed a similar value to the control.

Soil enzymatic activity

Fluorescein diacetate hydrolysis, measured in soil at the end of the experiment, is reported in Figure 4 and Table S4. FDA activity showed no significant differences among all treatments and the control. Before the analysis, the soil was characterized and the results are reported in the Table 4 included in STAR Methods section.

DISCUSSION

Eggplant is considered a very important crop in the traditional Mediterranean diet and can be cultivated in open fields and in greenhouses. The eggplant fruits are rich in water, fibers, and essential minerals such as phosphorus, potassium, sulfur, calcium, iron, zinc and, copper, as well as important vitamins, including A, B1, B2, and B5, and C.³⁴ Therefore, support is required for the production and consumption of eggplant fruits, a highly beneficial food, promoting a trend toward the use of natural biostimulants. These substances enhance nutrient uptake efficiency in plants, reducing the need for excessive chemical fertilizers, which may have negative impacts on the environment, as well as on human and animal health.

In this context, the application of BPs as a biostimulant to support eggplant cultivation may represent a useful and eco-friendly strategy to increase yield and reduce the use of chemical inputs. Indeed, the biostimulant effect of BPs derived from

Table 3. Yield and quality parameters of eggplants' fruits at the end of the experimental period

Parameters	Control	BPs 100%MF	BPs 60%MF	BPs spray
Marketable fruits (kg/plant)	10.92 ± 2.47a	12.70 ± 2.10a	9.83 ± 1.28a	6.56 ± 1.17b
Not marketable fruits (%)	13.20 ± 3.50a	7.80 ± 3.70b	12.50 ± 2.89a	14.60 ± 3.57a
Marketable fruits (N°/plant)	23.60 ± 5.59b	29.80 ± 3.96a	19.60 ± 3.21b	14.50 ± 4.50c
Weight per fruit (g)	462.4 ± 5.81a	456.28 ± 9.90a	447.75 ± 7.12a	421.56 ± 8.01b
Dry matter fruits (%)	7.24 ± 0.54	7.87 ± 0.67	7.34 ± 0.52	7.45 ± 0.54
Ash fruits (%)	7.64 ± 0.74	7.05 ± 0.70	7.39 ± 1.02	7.36 ± 0.69
Firmness (kg/cm2)	6.50 ± 0.47	6.60 ± 0.10	5.90 ± 0.32	6.60 ± 0.27
DW ₀	23.73 ± 4.32b	23.26 ± 3.66b	26.51 ± 5.32a	24.72 ± 4.42b
DW ₃₀	35.75 ± 3.74b	33.99 ± 2.86b	34.75 ± 3.89b	37.98 ± 5.06a
Degree of browning (DB)	12.02 ± 4.62a	10.73 ± 3.91a	8.24 ± 4.41b	13.26 ± 6.74a
Color difference (CD)	12.18 ± 4.67a	11.03 ± 4.11a	8.47 ± 4.46b	13.58 ± 6.65 a

All data are expressed as mean ± standard error. Different letters within each column indicate significant differences according to Tukey's protected LSD test ($p < 0.05$); ± indicates the standard error mean. The absence of letters indicates no significant differences.

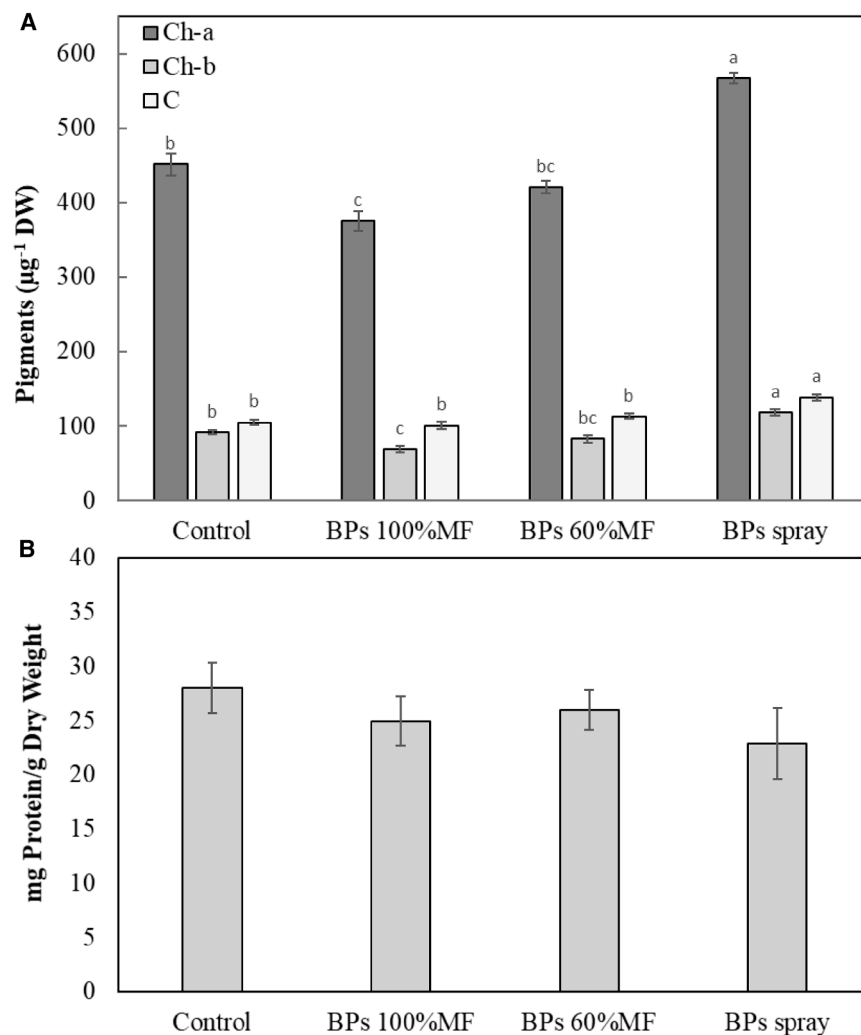


Figure 2. Pigments and proteins in eggplant leaves

(A and B) Chlorophylls-a, chlorophylls-b, and carotenoids and (B) total protein content in eggplants. Error bars indicate standard deviation (\pm SD). Values followed by different letters are significantly different ($p < 0.05$). The absence of letters indicates no significant differences.

plication of BPs on lettuce at concentrations ranging from 10 to 100 mg/L was able to enhance lettuce growth by stimulating chlorophyll accumulation and nitrogen metabolism. Notably, the effect of spray application of BPs was evident in this study on the fresh weight of lettuce, a horticultural crop in which the edible portion is the vegetative tissue.

The effects on eggplants were assessed through both soil application, using the highest dose of BPs (150 kg/ha), that had previously shown the greatest enhancement in lettuce fresh weight,¹³ and foliar spray application, using a medium concentration (50 mg/L) of BPs in solution previously demonstrated to be effective on lettuce.³⁵ In order to evaluate potential reductions in fertilizer inputs, a treatment with a 40% reduction in the regular mineral fertilization used for eggplant production was also tested. The daily air temperature and relative humidity levels in the greenhouse are reported in the Figure 5 included in STAR Methods section.

The morpho-biometric parameters measured during cultivation showed no

different pristine biowaste feedstocks (digestate from the anaerobic fermentation of unsorted food wastes, compost of private gardening and public park trimming residues and digestate, public park trimming residues and sewage sludge, exhausted tomato plants at the end of the crop harvesting season), applied to the soil in solid form, was proven to exert a biostimulant effect on different plant species such as tomato and pepper,¹¹ lettuce,^{10,13} bean.¹⁹

Fragalà et al.¹³ have shown that the application to the soil of solid BPs at concentrations of 50 and 150 kg/ha (this latter concentration was chosen for the present work) contributed to improving lettuce production in pots, by reducing the consumption of mineral fertilizers during lettuce cultivation and by reducing environmental impact due to the mineral nutrients leaching. In particular, the best performances, both in lettuce growth and reduced nitrate leaching, were obtained by adding 150 kg/ha BPs to the soil, allowing a 40% reduction in mineral fertilization used for lettuce cultivation.¹³

As regards the foliar spray application of BPs in water solution form, Fragalà et al.,³⁵ have shown, for the first time, that the ap-

plification of BPs to the soil allows an increase in canopy cover at the beginning of the plant growth. Although BP's 60%MF achieved a canopy cover value similar to that of the control, no comparison with the spray treatment can be performed, as the first foliar application was performed after 39 DAT. As regards the morpho-biometric parameters evaluated on the plant at the end of the experimental trials, surprisingly, the best performance, mainly regarding the fresh weight of eggplant leaves, was obtained in the treatment BPs spray, reaching an increase in fresh

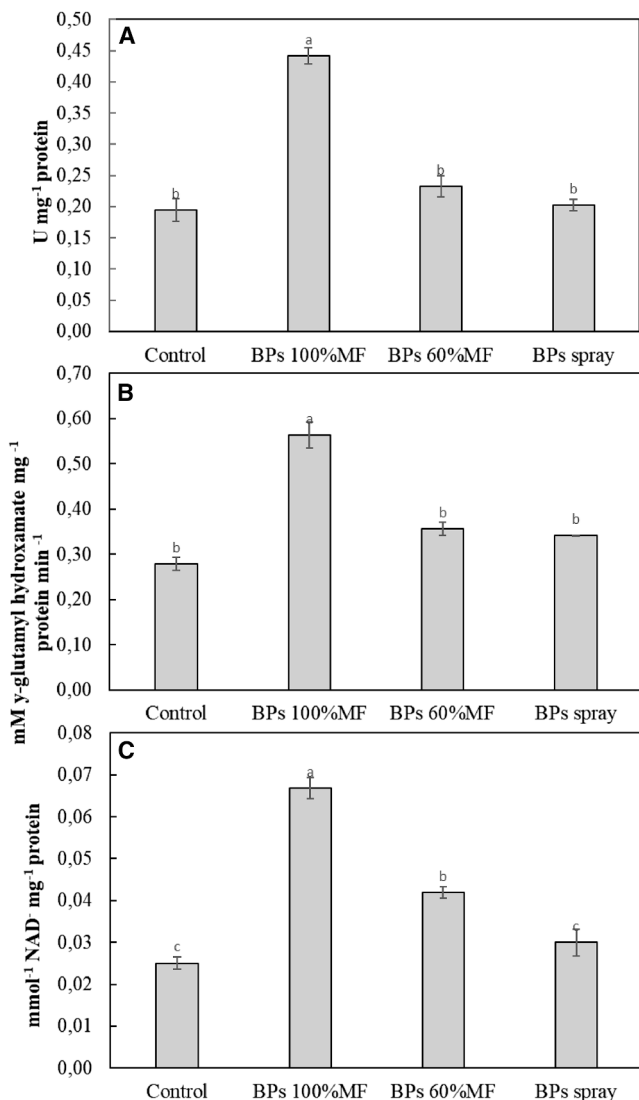


Figure 3. Enzymatic activities related to nitrogen metabolism in eggplants' leaves

(A–C) Nitrate reductase, (B) glutamine synthetase, and (C) glutamate synthase. Error bars indicate standard deviation (\pm SD). Values followed by different letters are significantly different ($p < 0.05$).

weight of around 20% respect to the control (Table 2). Noteworthy, the treatment BPs 60%MF reached morpho-biometric parameters, both during cultivation and at the end of cultivation, always to the control, suggesting that the foliar part of the plant has a benefit from the application to the soil of BPs even though the mineral fertilization input was reduced by 40%. These results are peculiar with respect to previous studies on the effect of BPs on other crops.^{11,13} Indeed, comparing our results with the cultivation of lettuce in Fragalà et al.,¹³ the same application of BPs to the soil at the same concentration of 150 kg/ha in the presence of regular fertilization induced an increase in the fresh weight of around 24% with respect to the control, which in eggplants' leaves was not recorded. On the contrary, the spray treatment

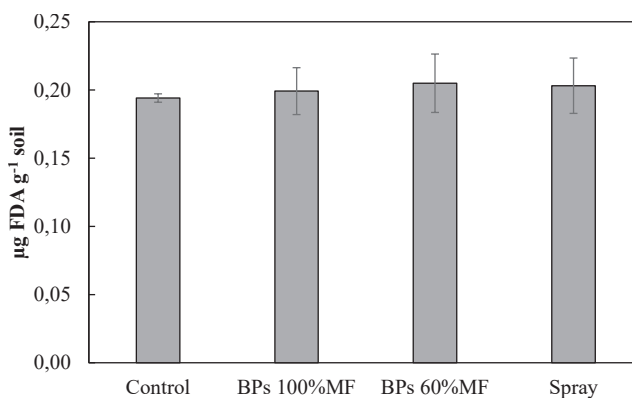


Figure 4. Fluorescein diacetate hydrolysis activity

Fluorescein diacetate hydrolysis activity was expressed in μ g FDA per g of soil. Error bars indicate standard deviation (\pm SD). The absence of letters indicates no significant differences.

of lettuce with BPs 10 ppm on lettuce, reported in Fragalà et al.,¹³ raised a similar effect to eggplants' leaves, inducing an increase of 28% respect to the control.

But what about the yield and quality of eggplants' fruits? Surprisingly, despite the BPs spray having produced a better plant growth performance (Table 2), the best results in terms of yield (Table 3) were observed with the application of BPs to the soil. In particular, the treatment with BPs 100%MF showed, with respect to the control, a significant increase in the number of fruits of around 26%, which maintains even the same weight per fruit and the same cumulative marketable fruit weight of the control plants. Moreover, a significant reduction as regards the non-marketable fruits was also observed (Table 3), and all the quality parameters of eggplants' fruits were not affected by the higher production (Table 3). Although, these results are very positive, they are not comparable with those obtained with lettuce, in which BPs act directly on the edible part of the crops, but neither with pepper,³⁷ which recorded a significant increase of yield in term of total crop (kg/m^2), due prevalently at an increased weight of each fruit, after application of 140 kg/ha of BPs from compost of private gardening and public park trimming residues and digestate. Unfortunately, no comparison between eggplant and pepper can be performed regarding the marketable fruits and number of fruits, which are not reported in Sortino et al.³⁷ A hypothesis of the differences in terms of yield may be related to the different kinds of fruit and consistency, as well as the contribution to the total weight of unmarketable fruits. In the case of eggplant, the weight of each fruit was always within the standard size of eggplant's fruit,³⁸ allowing a greater number of marketable fruits, leading therefore to a higher economic profit during commercialization.

A different behavior was observed in the treatment BPs spray, thus inducing a reduction in all yield parameters, except for non-marketable fruits (Table 3). An explanation may be related to the evidence that the spray treatment enhanced only the vegetative part of the plant (Table 2), as also observed in lettuce,³⁵ but negatively affected the yield of fruits. This apparent inconsistency may be attributed to physiological aspects,

Table 4. Characterization of the soil used in the experimental trials

	Electrical Conductivity (mS/cm)	Organic Carbon (%)	C.E.C (cmols+)/ kg	N (g/ kg)	P (mg/ kg)	K (mg/ kg)	
pH	7.80	513	1.07	7.59	1.06	10	42

particularly related to nutrient partitioning and hormonal regulation. Foliar spray application, while readily absorbed by leaves, may alter the source-sink dynamics by promoting vegetative sinks at the expense of reproductive organs.³⁹ Hence, the enhanced shoot growth could result in a greater demand for assimilates, reducing their allocation to developing fruits.⁴⁰ Moreover, certain nutrients, especially those with limited phloem mobility, may not be efficiently translocated from leaves to fruits, leading to localized accumulation in leaves and reduced fruit development.⁴¹ In addition, foliar formulations may contain hormone-like substances that can influence endogenous hormonal imbalances, and modification in the ratios of auxins, cytokinins, gibberellins, and abscisic acid may determine vegetative growth or even induce premature fruit drop, depending on the timing and concentration of application.⁴²

Interestingly, all the yield parameters showed no significant differences with respect to the control, when the mineral fertilization was reduced (Table 3), suggesting a possible reduction of 40% mineral fertilization in view of a yield maintained at the same level as control plants, in which 100%MF was routinely applied. Moreover, in BPs 60%MF, the quality of fruits seems to be greatly improved (Table 3). The selection for a reduced degree of browning in commercial varieties has probably resulted in the indirect selection of materials with lower concentrations of phenolics.⁴³ Both traits (degree of browning and color difference) are obtained from the same color parameters (L^* , a^* , and b^*), and they are

easy to calculate and provide complementary information. Therefore, to make the best treatment, it is advisable to use both parameters simultaneously for a better evaluation of the change in color.⁴³ When eggplant pulp is sliced, the polyphenol oxidase enzymes are released, which, in the presence of oxygen, oxidizes phenolics and polymerizes o-quinones, resulting in brown pigments.⁴⁴ Interestingly, the fruits subjected to the treatment BPs 60%MF showed a significantly lower degree of browning and color difference respect to the control. Our study found that the application of biostimulant and the simultaneous reduction of mineral fertilization effectively reduced the browning potential of the fruit, hence reducing the oxidative potential of the fruit pulp. Indeed, as previously reported, biostimulants interact with plant secondary metabolism and enzymes involved in defense against oxidative stress.⁴⁵ As the consumers and the industry prefer varieties with a luminous white color,⁴³ our results suggest that the treatment BPs 60%MF is more suitable for consumer preferences.

According to the positive effect on the morpho-biometric traits of the plant (Table 2), BPs spray increased chlorophylls and carotenoids in the leaves with respect to the control (Figure 2), whereas a reduction of the pigments was recorded for the soil treatment of BPs. In particular, in BP spray treatment, chlorophyll-a showed a 25% increase with respect to the control (Figure 2). These results are similar to those obtained on lettuce in Fragalà et al.¹³ with the spray application of BPs; however, they are different from results obtained in Fragalà et al.³⁵ on lettuce using the solid BPs applied to soil, where a rather constant chlorophyll content was recorded during the lettuce cultivation. This effect is probably due to the foliar spray application, which positively affects the chlorophyll content of plants, but it is not always correlated with the fruit production.⁴⁶ Indeed, BPs spray efficacy indicated the increment in the plant growth condition of eggplant in greenhouse conditions; however, it reduced the eggplant fruit yield production (Table 3).

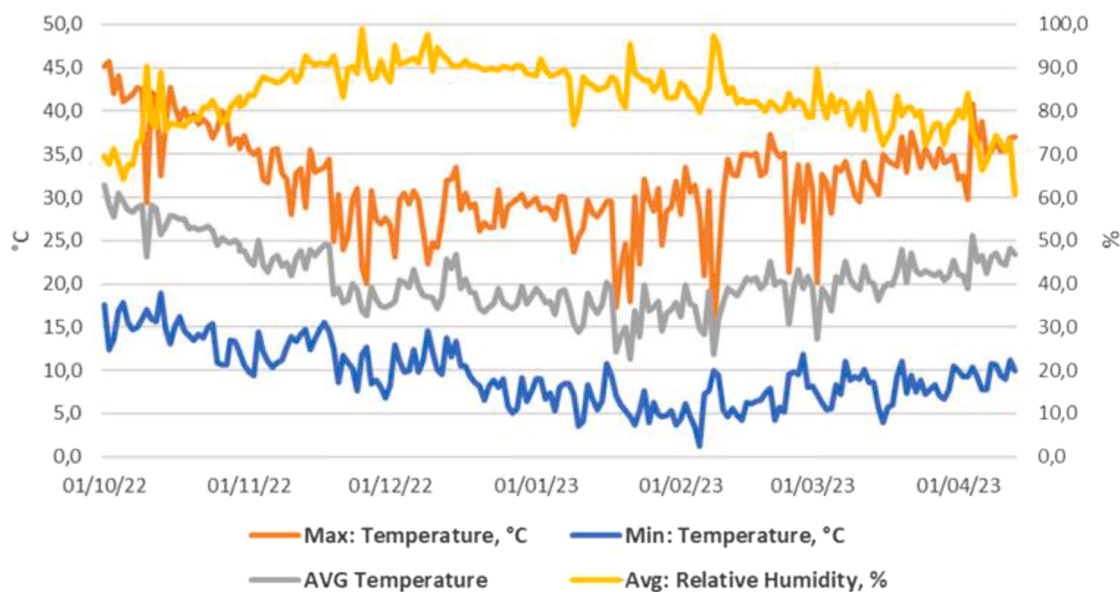


Figure 5. Climatic data recorded throughout the trial by a data logger installed in the greenhouse

Overall, these results suggest that the spray application, may be a better option with respect to soil application to increase the plant fresh weight, as also shown for lettuce in Fragalà et al.³⁵ In fact, among application methods, foliar spray treatment represents an advantage in field conditions above all on broad leafy vegetables, as it increases fresh and dry biomass, leaf area, and enhances chlorophyll biosynthesis, determining an improved yield performance of the edible part of the crop.^{47,48} However, the edible part of eggplants is the fruit, and great importance should be given to the fruit production; therefore, in this case, the soil application of BPs may be more suitable. In particular, the treatment BPs 60%MF allows for reducing the mineral fertilization of the soil, reaching an optimal yield comparable to the regular practice (control), and furthermore increases the quality of the fruit, reducing the oxidative processes on the fruits (Table 3).

Regarding the nitrogen primary metabolism, the N assimilation pathway may be linked to the incorporation of nitrate within the cells, and the cytosolic enzyme NR may be considered to be the limiting factor for the growth and development of the vegetative part of the plants.⁴⁹ The other enzymes GS and GOGAT play a crucial role through ammonium incorporation into organic compounds.^{50,51} Although the protein content in leaves was constant in all the treatments (Figure 2), the results showed that all the enzymatic activities related to the N metabolism are strongly enhanced by the treatment BPs 100%MF (Figure 3), recording with respect to the control an increase of 127%, 102% and 167% for NR, GS, and GOGAT, respectively. On the contrary, the treatment BPs 60%MF showed values of activities similar to the control for NR and GS, but a higher value of around 67% for GOGAT, suggesting a favored assimilation of the ammonium into the amino acid skeleton of glutamate.^{50,51} A hypothesis may be linked to the activation of nitrogen uptake by a hormone-like stimulation. Indeed, Scaglia et al.²⁰ demonstrated that the application to the soil of humic acids obtained from vermicompost, at concentrations in the range of 100–6000 mg carbon L⁻¹, can exert biostimulating effects due to the presence of auxin-like molecules.

Finally, BPs spray recorded values of activities always comparable to the control, which may be compatible with a greater growth of the vegetative part of the plant, but with a reduction of yield. Effectively, different processes contributing to yield components are rather independent of different other factors influence each other, and that may lead to a substantially different effect from plant growth and production.⁵² Thus, because different factors are involved and they are continuously changing, crop responses cannot be easily predicted. However, the abovementioned changes in metabolism, which may determine these discrepancies, are not completely understood, so it is rather difficult to investigate these mechanisms at the level of metabolic processes.⁵² In addition to plant responses, it is also important to consider the impact of BPs on soil biochemical fertility. Various soil enzymes play a pivotal role in key soil processes and can be used to assess the effects of different products on soil health and fertility.^{53–55} In this context, exploring sustainable ways to enhance microbial activity becomes particularly relevant. The FDA assay is a useful method for estimating overall microbial activity in soil,⁵⁶ and our results showed that BPs treatments did not neg-

atively affect native soil microbial populations (Figure 4), suggesting a neutral impact on indigenous microbiota.

Finally, the economic aspect of fertilization strategies deserves careful consideration. In this study, conventional NPK fertilization was used, with a market cost ranging from 0.70€ to 1.10€ per kg, resulting in an average fertilization expense of around €400 per hectare. Integrating BPs into the fertilization plan, a 40% reduction in mineral fertilizer usage was achieved, leading to a cost of €240 of NPK consumption per hectare. Moreover, according to Montoneri et al.,^{1,11} the production cost of BPs was estimated at around €390 per ton of dry matter. This means that the application of 150 kg/ha of BPs has a cost of around €59 per hectare. Consequently, combining BPs with 60% of conventional mineral fertilization resulted in a final cost of around €300 per hectare for eggplant cultivation, leading to an evident economic saving of 25% per hectare. These results taken together suggest that the best performance on the yield of eggplants may be reached by the application of BPs to the soil. Interestingly, for a better sustainable practice, the use of BPs in eggplant cultivation guarantees the same yield obtained using the regular fertilization of the soil, saving 40% of mineral fertilizer consumption. This suggests a clear economic benefit for farmers in addition to the already demonstrated agronomic and environmental advantages of using a sustainable practice.

Conclusion

The use of biostimulants in agricultural practices may nowadays be considered an encouraging and promising treatment, as it reduces the environmental impact and potentially increases yields in crop production. Our results provide insights into the application of BPs in agriculture as biostimulants. Although the foliar spray application of BPs increased the growth of the vegetative part of the plants, the yield of fruits was negatively affected. On the contrary, the use of solid BPs directly into the soil may allow us to reach the same yield as regular cultivation, using only 60% of mineral fertilizers, thus reducing the cost of chemical fertilizers and avoiding pollution in the environment. Although the mechanism by which the simultaneous reduction of mineral fertilization and the application of BPs decreases the browning potential of the fruit, and consequently reduces the oxidative potential of the fruit pulp, requires further investigation, this finding may be highly relevant for agricultural practices in eggplant cultivation.

Therefore, the application of solid BPs may be considered an ecofriendly strategy for supporting the reduction of mineral fertilization during eggplant cultivation, in the current context of a circular economy. Together with the previously published BPs studies on food and ornamental plant species, the data obtained in the reported eggplant case study offer further support for the implementation of BPs-based practices for the development of eco-friendly, more sustainable modern agriculture.

Limitations of the study

A more in-depth investigation into the effects of BPs under long-term applications may be carried on, taking into account also the soil health aspects, putative limitations, or accumulation of eventual residual compounds, future multi-season field trials, in order to allow a more comprehensive evaluation of their integration into broader agroecosystem models.

RESOURCE AVAILABILITY**Lead contact**

Requests for further information and resources should be directed to and will be fulfilled by the lead contact, Andrea Baglieri (abaglie@unict.it).

Materials availability

This study did not generate new unique reagents.

Data and code availability

- Data: This study did not generate or analyze datasets. All results are presented within the article.
- Code: No custom code was used in this study.
- Other: This article does not report additional resources.

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AUTHOR CONTRIBUTIONS

Conceptualization, I.P. and A.B.; methodology, C.C., I.C., F.F., E.L.B., R.S., and E.S.; investigation, C.C., I.C., F.F., E.L.B., R.S., and E.S.; writing – original draft, E.S.; writing—review and editing, I.P. and A.B.; data curation, E.M. and E.P.; funding acquisition, I.P. and A.B.; resources, I.P. and A.B.; supervision, I.P. and A.B.

DECLARATION OF INTERESTS

The authors declare no competing interests.

STAR★METHODS

Detailed methods are provided in the online version of this paper and include the following:

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SUPPLEMENTAL INFORMATION

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STAR★METHODS

KEY RESOURCES TABLE

REAGENT or RESOURCE	SOURCE	IDENTIFIER
Biological samples		
Eggplant variety Dulciana F1	Local nursery, Vittoria (Ragusa, Italy)	N/A
Soil	Vittoria (Ragusa, Italy)	N/A
Chemicals, peptides, and recombinant proteins		
Acetone	Sigma-Aldrich	179124
Mannitol	Sigma-Aldrich	BP1007
Sucrose	Sigma-Aldrich	S8501
Ethylene glycol-bis(2-aminoethylether)-N,N,N',N'-tetraacetic acid (EGTA)	Sigma-Aldrich	E4378
Cysteine	Sigma-Aldrich	861677
HEPES	Sigma-Aldrich	H3375
Potassium hydroxide (KOH)	Sigma-Aldrich	757551
Ammonium sulfate ((NH ₄) ₂ SO ₄)	Sigma-Aldrich	A4418
Potassium phosphate monobasic (KH ₂ PO ₄)	Sigma-Aldrich	795488
Potassium nitrate (KNO ₃)	Sigma-Aldrich	221295
Sodium nitrite (NaNO ₂)	Sigma-Aldrich	237213
Imidazole	Sigma-Aldrich	I2399
Hydrochloric acid (HCl)	Sigma-Aldrich	258148
Hydroxylamine	Sigma-Aldrich	255580
Sodium arsenate dibasic heptahydrate (Na ₂ HAsO ₄ · 7H ₂ O)	Sigma-Aldrich	A6756
Manganese dichloride (MnCl ₂)	Sigma-Aldrich	328146
Adenosine 5'-diphosphate (ADP)	Sigma-Aldrich	A2754
Glutamine	Sigma-Aldrich	1294808
Ferric chloride hexahydrate (FeCl ₃ 6H ₂ O)	Sigma-Aldrich	F2877
Trichloroacetic acid	Sigma-Aldrich	T6399
γ-glutamyl hydroxamate	Sigma-Aldrich	G2253
Sodium hydroxide (NaOH)	Sigma-Aldrich	221465
α-ketoglutaric acid	Sigma-Aldrich	75 890
β-Nicotinamide adenine dinucleotide (NADH)	Sigma-Aldrich	481913
Disodium ethylenediaminetetraacetate (Na ₂ EDTA)	Sigma-Aldrich	E4884
Sodium phosphate	Sigma-Aldrich	S9763
Fluorescein diacetate	Sigma-Aldrich	F7378
Fluorescein	Sigma-Aldrich	46955
Bradford reagent	Sigma-Aldrich	B6916
Software and algorithms		
Canopeo	Patrignani and Ochsner, 2015 ⁵⁷	https://canopeoapp.com/
Statistical software	IBM SPSS 30	https://software.unict.it/Spss29.aspx
Other		
Soluble bio-products (BPs)	Montoneri et al. (2020) ⁵⁸	N/A
Raw material was Municipal Biowaste	ACEA Pinerolese Industriale S.p.A. (Pinerolo, Turin, Italy)	N/A
Solid ternary fertilizer NPK	HidroFert, (Barletta, Italy)	N/A

EXPERIMENTAL MODEL AND STUDY PARTICIPANT DETAILS

The soluble bio-products (BPs) were prepared from the solid anaerobic digestate, as described by Montoneri et al.⁵⁸ The raw material was Municipal Biowaste provided by the ACEA Pinerolese Industriale S.p.A. (Pinerolo, Turin, Italy) waste treatment plant. The residual digestate was hydrolysed in water at pH 13 and 60°C, and then subjected to sedimentation, followed by centrifugation and ultrafiltration through a 5 kDa cutoff polysulphone, respectively, in order to separate the water-soluble hydrolysate phase from the insoluble residue and from the excess alkali reagent. Finally, the membrane retentate was dried at 60°C and the recovered product was used to perform the eggplants cultivation trials.¹⁰ The nutritional status of BPs is reported in [Tables S5](#) and [S6](#).

The eggplant variety used for the experimental trials was Dulciana F1.

METHOD DETAILS

Site and experimental condition

The agricultural trials were performed in a cold greenhouse located in Vittoria (Ragusa, Italy) (36°56'40.50"N - 14°23'41.61"E), from November 2022 to April 2023. The trials were conducted alongside the regular practises of farmers for eggplant cultivation.⁵⁹ In order to record the climatic data, a data logger was installed in the greenhouse, and the daily air temperature and relative humidity levels are reported in [Figure 5](#).

The substrate was a sandy soil. Before the analysis, it was air dried and sieved at 2 mm. Then, it was characterized for pH, electrical conductivity (E.C.), organic carbon, Cation Exchange Capacity (C.E.C), total nitrogen, phosphorus, and potassium, according to the official procedures described in Puglisi et al.⁶⁰ The results of the soil characterization are reported in [Table 4](#).

The eggplant cultivation was carried out with a randomized complete block (RCB) experimental design, with a plant rate of 14.280 plants/ha, with a row spacing of 0.7 x 1.2 m, according to the locally farmers' practices. The mineral fertilization (MF) consisted of a solid ternary fertilizer NPK made up of NH₄NO₃, KH₂PO₄, and KNO₃, and according to the conventional routine farm practice, MF 100% corresponds to the application of 116.60 kg/ha NH₄NO₃, 162.32 kg/ha KH₂PO₄, and 138.60 kg/ha KNO₃.

The BPs treatments were performed directly in the soil along the plant rows, using 150 kg/ha of BPs mixed with the soil at either 100% MF or 60%MF. The 60% MF was chosen based on previous results obtained by Fragalà et al.¹³ in lettuce, who demonstrated that reducing the MF of 40% and applying 150 kg/ha of BPs allowed to obtain a comparable yield to those obtained in standard conditions with 100% MF. The foliar spraying was performed applying three independent and consecutive treatments with a timing of 15-day intervals, using two hollow cone nozzles, with a BP solution concentration of 50 mg/L in water. The concentration was chosen on the basis of previous results shown by Fragalà et al.,³⁵ who demonstrated that the spray application of 10 and 100 mg/L BPs had a biostimulant effect on lettuce; hence in our study we chose an average of the two concentrations corresponding to 50 mg/L. The first foliar spray application was performed at the first stage of eggplant flowering (40 days after transplanting (DAT)), as suggested by Haggag et al.⁶¹ for the best phenological phase in which to apply spray biostimulant on eggplant. The uniformity of application among replicates was ensured by spraying the same amount of solution for each plant. All the treatments are listed in [Table 1](#). Control plants were regularly grown using 100% MF. For each treatment 3 replicates were performed.

Soil N, P, K contents in the different treatments were calculated based on the BP application and MF composition.

Morpho-biometric parameters

During the experimental trials the health of eggplants was monitored in the field using non-destructive method measurements. The canopy growth at 14 DAT and 39 DAT was evaluated using the app Canopeo (<https://canopeoapp.com/>), by estimating of the leaf area covering the soil surface, recording it at the height of 1 m.⁶² The plant height was measured with a flexible ruler in the eggplant, monthly from January to March (68 DAT, 107 DAT and 128 DAT). The plant vigour (68 DAT, 107 DAT and 128 DAT), was visually assessed on a 0-10 index scale, where 0=dead plants and 10= the optimal plant conditions in the field.⁶³ The nitrogen chlorophyll content of leaves, related to the nitrogen status of the plant at 68 DAT, 107 DAT and 128 DAT, was measured, using in field condition a portable N-Tester (Konica, Minolta, Japan), as average of three different points of the last expanded leaf of 10 eggplants for each treatment and replica.¹³

At the end of the experimental trials (6 months), the plants were manually harvested, to avoid leaf damage to the leaves, and the leaves were flash-frozen with liquid nitrogen, and stored at -80°C for further enzymatic analysis. Eggplant leaves were weighed in order to obtain the fresh weight leaf (FW). Dry weights (DW) were obtained by placing leaves in a drying oven (Thermo scientific Heratherm) at 105°C until constant weight was reached and was expressed as a percentage of dry matter. Then allowed to cool for 2 hrs inside a closed bell jar and finally the dry weights were calculated.

Finally, the root growth was evaluated as the percentage of root area using the app Canopeo,⁵⁷ and the root vigour was evaluated, according a 1-10 index scale, where 1 = no roots and 10 = optimal root conditions in the field.⁶⁴

All measurements were performed on 5 plants per treatment and replicate.

Yield and quality parameters of eggplant fruits

During cultivation, eggplant fruits were collected at 7 different stages, weighed and immediately processed for quality analysis.

The yield was evaluated by weighing the harvestable fruits at each fruit-picking event. The cumulative yield was calculated and reported as kg per plant, separating the unmarketable fruits, representing the undersized or unripe eggplants, currently considered unsuitable for human consumption.⁶⁵ Moreover, the average number of fruits per plant and the average weight per fruit were recorded.

The dry matter of fruits was obtained by placing whole fruits in a drying oven (Thermo Scientific-Heratherm) at 105°C until a constant weight was reached, then allowed to cool inside a closed bell jar for 2 h, and finally the dry weights were obtained, and the percent of dry matter was reported. The ashes were determined at 505°C in a muffle (Falc, Italy) and expressed as a percentage of ash with respect to dry weight. All measurements were performed on 3 fruits for harvesting event, treatment and replicate.

Regarding the quality, the fruit flesh colour was measured by using a Minolta Chroma CR-300 colourimeter (Minolta Corporation, Ltd., Osaka, Japan), fitted with an 8 mm diameter aperture and expressed in the “CIELAB1976 colour coordinates”.⁶⁶

Eggplant fruits were cut transversely at the midpoint between the blossom and stem ends, and measurements were made immediately after being cut (0 minutes) and 30 minutes later in the central part. To produce clean cuts, a straight edge plastic knife was used. Colour space had been divided into a three-dimensional (L, a and b) so that L (lightness; 0 black and 100 white); a (red to green); and b (blue to yellow). The distance of pure white (DW) was measured as Euclidean distance between the colour coordinates to the pure white coordinates ($L^* = 100$ $a^* = 0$ $b^* = 0$) using the following formula⁴³:

$$DW = [(100 - L^*)^2 + a^{*2} + b^{*2}]^{0.5}$$

The difference between DW at 30 min (DW_{30}) and at 0 min after the fruit was cut (DW_0) was used to measure the degree of browning (DB) suffered by the fruit.⁴³

The colour differences (CD) were measured as a Euclidean distance between the colour coordinates at 0 and 30 min and calculated by using the following formula⁶⁶:

$$CD = [(L^*_{30} - L^*_0) + (a^*_{30} - a^*_0)^2 + (b^*_{30} - b^*_0)^2]^{0.5}$$

Both measures provide distinct and complementary information regarding the evolution of the fruit colour.

Chlorophyll and carotenoid contents

Chlorophylls and carotenoids in eggplant leaves were photometrically determined according to the method described by Sumanta et al.⁶⁷ To sum up, leaf tissue samples (0.5 g) were homogenized using 10 mL 80% acetone as the extraction solvent. Samples were centrifuged at 10,000 rpm for 15 min at 4°C, then an aliquot of supernatant (0.5 mL) was mixed with 4.5 mL of extraction solvent. Chlorophyll and carotenoid contents were measured at three wavelengths, 470, 646.8, and 663.2, and the relative amount of chlorophyll-a (Ch-a), chlorophyll-b (Ch-b), and carotenoids (C) were calculated as follows and expressed as mg g^{-1} leaf dry weight (DW):

$$\text{Ch-a} = 12.25 A_{663.2} - 2.79 A_{646.8}$$

$$\text{Ch-b} = 21.5 A_{646.8} - 5.1 A_{663.2}$$

$$C = (1000 A_{470} - 1.82 \text{ Ch-a} - 85.02 \text{ Ch-b})/198$$

Total protein extraction

Extraction of total proteins and enzymes from leaves and roots of eggplant was performed as described in La Bella et al.⁶⁸ Briefly, samples of frozen eggplant leaves were ground with an extraction buffer containing 220 mM mannitol, 70 mM sucrose, 1 mM EGTA, 10 mM cysteine, and 5 mM HEPES–KOH pH 7.5, at a ratio of 1:1.25 w/v. The homogenate was then filtered through three layers of cheesecloth and centrifuged at 13,000 rpm for 30 min at 4°C. The resulting supernatant was recovered, and the total proteins were precipitated with solid $(\text{NH}_4)_2\text{SO}_4$ at 55% of saturation. Total protein content, expressed as mg protein g^{-1} DW, was quantified according to Bradford⁶⁹ (1976), using bovine serum albumin as standard.

All measurements were performed in triplicate for treatment and replicates.

Enzymatic activities related to nitrogen metabolism in eggplants' leaves

Each enzymatic activity was assayed using an aliquot of the total protein extract, obtained as previously described, containing crude enzymes.

Nitrate reductase (NR) activity was measured according to the method described by Kaiser and Huber.⁷⁰ Briefly, a solution containing 100 mM KH_2PO_4 and 100 mM KNO_3 was incubated at 28°C for 15 min with an appropriate amount of enzyme extract. The mixture was then centrifuged at 500 rpm for 5 min, the supernatant was recovered, and the activity was measured spectrophotometrically at 540 nm (Jasco V-530 UV–vis spectrophotometer, Tokyo, Japan), using as standard a calibration curve of NaNO_2 . Activity was expressed as Unit mg^{-1} protein.

Glutamine synthetase (GS) was assayed according to Canovas et al.⁷¹ In brief, the assay mixture contained 90 mM imidazole–HCl (pH 7.0), 60 mM hydroxylamine (neutralized), 20 mM $\text{Na}_2\text{HAsO}_4 \cdot 7\text{H}_2\text{O}$, 3 mM MnCl_2 , 0.4 mM ADP, 120 mM glutamine, and the

suitable amount of enzyme extract. The enzymatic reaction was incubated at 37°C for 15 min, then a mixture (at a ratio 1:1:1) of 10% (w/v) FeCl₃ 6H₂O in 0.2 M HCl, 24% (w/v) trichloroacetic acid, and 50% (w/v) HCl was added. The activity was determined spectrophotometrically at 540 nm, using a standard curve of γ -glutamyl hydroxamate, and was expressed as mM glutamyl hydroxamate mg⁻¹ protein min⁻¹.

Glutamate synthase (GOGAT) activity was assayed as described by Avila et al.⁷² (1987). Briefly, the assay mixture, containing 25 mM HEPES–NaOH (pH 7.5), 2 mM L-glutamine, 1 mM α -ketoglutaric acid, 0.1 mM NADH, 1 mM Na₂EDTA, and the suitable amount of enzyme extract, was measured spectrophotometrically (Jasco V-530 UV–vis spectrophotometer, Tokyo, Japan), by following NADH oxidation at 340 nm and using a molar extinction coefficient of 6220 L mol⁻¹ cm⁻¹. GOGAT activity was expressed as mmol NAD min⁻¹, mg⁻¹protein.

Soil enzymatic activity

Soil analysis was performed at the end of the experimental trial. In detail, fluorescein diacetate hydrolysis activity (FDA) was assayed according to Green et al.⁷³ Soil samples (1 g) were suspended in 60 mM sodium phosphate buffer, pH 7.6 and reaction started by adding 4.9 mM fluorescein diacetate as the substrate. Samples were incubated for 3 h at 37°C, and then the reaction was stopped by adding 2 mL of acetone. After centrifugation and supernatant filtration, the absorbance was measured spectrophotometrically at 490 nm and the fluorescein concentration hydrolysed during the reaction was calculated from a fluorescein standard calibration curve.

QUANTIFICATION AND STATISTICAL ANALYSIS

Data were analysed by one-way ANOVA ($p < 0.05$) followed by Tukey's test for multiple comparison procedures using a statistical software (IBM SPSS 30) to investigate the effect of each treatment on eggplant. Values used for analyses were the means of all replicates. As regard fruits, the values used for statistical analysis were the means obtained at each harvesting events for replicates and for each treatment.