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Effects of a Biostimulant Containing Effective Microorganisms, *Spirulina platensis* and Earthworm Humus on the Growth, Defence Response, Yield, and Quality of Strawberry Fruits (*Fragaria* × *ananassa* cv. Albion) Grown in Pot Culture

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ABSTRACT

The development of environmentally friendly strategies to support crop productivity is vital in modern horticulture. This study assessed the effects of a novel biostimulant composed of effective microorganisms (EM), *Spirulina platensis*, and earthworm humus on the physiological performance, defense response, and yield of strawberry (*Fragaria* × *ananassa* cv. Albion) grown in pots. The experiment followed a completely randomized design with three treatments and 12 replicates per treatment (n = 36). Plants were assigned to one of three groups: (T1) untreated control, (T2) experimental biostimulant containing EM, *S. platensis*, and earthworm humus, and (T3) commercial biostimulant based on *Ecklonia maxima* extract. All treatments were applied via fertigation every two weeks over a two-month period under semi-controlled greenhouse conditions at CREA in Pescia, Italy. Various plant growth indicators, antioxidant enzyme activities, and fruit production metrics were recorded. Treated plants (T2 and T3) exhibited significant increases in leaf expansion, chlorophyll content, and flowering, compared to the untreated controls. Enzymatic assays revealed elevated activity levels of phenylalanine ammonia-lyase (PAL), peroxidase (POD), and superoxide dismutase (SOD), along with a notable increase in total phenolic content, indicating enhanced activation of natural defense mechanisms. Fruit yield improved by approximately 25% in T2 without compromising sweetness or fruit size. Although the °Brix and individual fruit weight remained unchanged, the fruit uniformity and marketability improved. These findings suggest that the synergy between EM, *S. platensis*, and vermicompost effectively supports both metabolic function and productivity in strawberry cultivation. This biostimulant formulation offers a sustainable alternative to chemical inputs. Field trials are recommended to confirm its efficacy under open conditions.

Introduction

The increasing global demand for high-quality fruit production, coupled with environmental concerns and resource limitations, has accelerated interest in sustainable horticultural practices (Calvo et al., 2014; Rouphael and Colla, 2020). Strawberries (*Fragaria* × *ananassa*) are among the most widely cultivated and economically important berries, and valued for their sensory attributes, nutritional profile, and antioxidant properties (Giampieri et al., 2012; Tulipani et al., 2008). However, intensive strawberry production often relies heavily on synthetic fertilizers and pesticides, which can degrade soil health, reduce biodiversity, and pose risks to consumer safety and the environment (Arancon et al., 2004; Nardi et al., 2021).

In this context, plant biostimulants have emerged as promising inputs that enhance plant growth, nutrient efficiency, and stress resilience without being classified as fertilizers or pesticides (du Jardin, 2015; Yakhin et al., 2017). According to the European Biostimulants Industry Council (EBIC), biostimulants stimulate natural processes in plants to improve nutrient uptake, abiotic stress tolerance, and crop quality (EBIC, 2020). Their action is primarily metabolic and biochemical rather than nutritional, and they can be derived from various sources, including microorganisms, algae, humic substances, and protein hydrolysates (Rouphael et al., 2015; Lucini et al., 2018).

Among microbial biostimulants, effective microorganisms (EM) and consortia of beneficial bacteria, fungi, and yeasts have shown significant promise. EM formulations often include species such as *Lactobacillus spp.*, *Bacillus subtilis*, *Saccharomyces cerevisiae*, and *Actinomycetes*, which can enhance soil microbiota, promote nutrient mineralization, and suppress pathogenic organisms (Higa and Parr, 1994; Sayğı, 2022; Santos et al., 2020). The beneficial effects of EM have been documented in various crops, including tomato, lettuce, and cucumber, with observed improvements in growth parameters, root morphology, and resistance to stress (Javaid and Bajwa, 2011; Muhammad et al., 2019; Maitra et al., 2022).

Algal extracts are another widely studied category of biostimulants. *Spirulina platensis*, a cyanobacterium rich in amino acids, vitamins, and bioactive compounds such as phycocyanin and carotenoids, is known for its antioxidant and growth-promoting effects (Holman and Malau-Aduli, 2013; Abdel-Raouf et al., 2012). Recent research has demonstrated the potential of *S. platensis* to enhance photosynthetic efficiency, chlorophyll content, and enzyme activity in several horticultural crops (Elarroussia et al., 2016; Chakraborty et al., 2023.; Khairy and El-Shafay, 2013). The high concentrations of micronutrients and plant-like hormones in *Spirulina* make it an ideal additive for foliar and soil applications (Cristofano et al., 2021; Safi et al., 2014).

Earthworm humus (vermicompost) is a well-recognized organic amendment that improves soil structure, microbial activity, and nutrient availability (Arancon et al., 2003; Atiyeh et al., 2002). It contains humic and fulvic acids, plant growth regulators, and diverse microbial populations that support rhizosphere health (Domínguez, 2004; Lazcano et al., 2011). Studies have confirmed that vermicompost applications can lead to increased root biomass, improved nutrient uptake, and enhanced resistance to pathogens in crops, such as pepper, maize, and strawberry (Edwards et al., 2010; Doan et al., 2013; Pathma and Sakthivel, 2012).

Despite the known benefits of each component, studies examining the synergistic effects of combining EM, algal extracts, and vermicompost are limited. Integrating these biostimulant types could result in complementary mechanisms, improving both plant metabolism and soil biological activity. Furthermore, the effects of such a formulation on strawberries, particularly under pot conditions, remain underexplored (Cozzolino et al., 2021; Gheda et al., 2025).

This study aimed to evaluate the effects of a novel biostimulant formulation containing *Spirulina platensis* and earthworm humus on strawberry growth, defense responses, and fruit yield. By examining vegetative growth metrics, antioxidant enzyme activity, and productivity, this study provides insights into the potential of combined biostimulants in sustainable strawberry production systems.

Materials and Methods

Study location and growing conditions

The experiment was conducted from January to June 2025 at the Council for Agricultural Research and Analysis of Agricultural Economics, Research Centre for Vegetable and Ornamental Crops in Pescia, Italy (43.54° N, 10.42° E). The research was performed under semi-controlled greenhouse conditions, with ambient temperatures averaging 22–28 °C during the day and 12–18 °C at night. Relative humidity ranged from 55 to 70%, with natural photoperiods augmented by supplemental lighting when necessary. All plants were grown in pots and irrigated uniformly to maintain optimal soil moisture levels throughout the study.

To ensure uniform nutrition across treatments, all plants received a base fertilization program using a balanced water-soluble fertilizer (NPK 20-20-20) applied at a rate of 1.5 g L^{-1} once per week, excluding days when biostimulants were applied. This baseline nutrition ensured that plant performance differences could be attributed to the treatments rather than nutrient availability.

Irrigation was managed through drip systems and adjusted based on substrate moisture levels and monitored using tensiometers. All pots were irrigated to field capacity and maintained under non-limiting moisture conditions throughout the trial. The total volume of irrigation was standardized across all replicates to prevent variability in the water supply. During biostimulant application (every 14 d), treatments were delivered via fertigation to ensure consistent and localized availability of the active ingredients in the root zone.

Plant materials and experimental setup

The strawberry cultivar *Fragaria* × *ananassa* ‘Albion’, known for its neutral photoperiod flowering and consistent fruiting behavior, was used for this experiment. Uniform runners were obtained from a commercial nursery and acclimatized for one week before treatment initiation.

Each plant was grown in a 3 L plastic pot filled with a peat-perlite substrate (70:30 v/v), with pH adjusted to 5.8–6.2, and electrical conductivity (EC) maintained at 0.5 dS m^{-1}. The growing medium was sterilized and contained no added fertilizers or microbial inoculants at the start of the trial.

The experiment followed a completely randomized design with three treatments and 12 replicates per treatment (n = 12), totaling 36 pots:

T1 (Control): Untreated plants receiving only irrigation with water.

T2 (Experimental Biostimulant): Plants treated with a novel formulation containing EM, *Spirulina platensis*, and earthworm humus (GEA BIO Srl Advanced Technologies in Agriculture and the Environment, Cosenza, Italy)

T3 (Commercial Biostimulant Control): Plants treated with a widely available, certified commercial biostimulant product containing seaweed extract (Kelpak® based on *Ecklonia maxima*) (AlzChem Trostberg GmbH, Germany)

Biostimulant descriptions and application

T2 Biostimulant Composition: The experimental formulation included:

Effective Microorganisms (EM): a mixed culture containing *Bacillus subtilis*, *Lactobacillus plantarum*, *Saccharomyces cerevisiae*, and *Rhodopseudomonas palustris*.

Spirulina platensis: freeze-dried biomass (2% w/v in solution), rich in antioxidants, vitamins, and phytohormones.

Earthworm humus extract: an aqueous vermicompost extract with a known concentration of humic substances (~3% humic acids).

T3 Commercial Biostimulant: A liquid seaweed-based biostimulant composed of cold-processed *Ecklonia maxima* extract, humic acids, and trace minerals, applied at the manufacturer's recommended rate (5 mL L^{-1}).

All biostimulant solutions were freshly prepared before each application. Each treatment (T2 and T3) was applied via fertigation at 250 mL per plant, every 14 d, starting two weeks after transplanting and continuing for eight weeks (four applications in total).

Vegetative growth assessment

Growth measurements were taken at 14 d intervals throughout the experimental period and included:

Plant height (cm): Measured from soil surface to the apex of the highest leaf.

Number of leaves: Counted per plant.

Leaf area (cm^2): Determined by scanning representative leaves and analyzing images with ImageJ software.

Chlorophyll content: Estimated using a SPAD-502 chlorophyll meter (Konica Minolta), with three readings per plant averaged per sampling point.

Biochemical and antioxidant analysis

Leaf samples were collected 48 h after the final treatment for biochemical analyses. Young, fully expanded leaves were harvested, immediately frozen in liquid nitrogen, and stored at –80 °C.

The following parameters were measured:

PAL (Phenylalanine Ammonia-Lyase): Enzyme activity expressed as nmol cinnamic acid min^{-1} mg^{-1} FW, determined spectrophotometrically (D’Amato et al., 2017)

POD (Peroxidase): Activity based on guaiacol oxidation at 470 nm (Hiroyuki et al., 2004)

SOD (Superoxide Dismutase): Assayed by its ability to inhibit the photoreduction of nitro blue tetrazolium (NBT) (Durak et al., 1993)

Total Phenolics: Measured using the Folin–Ciocalteu method and expressed in mg gallic acid equivalents (GAE) per gram of fresh weight (Makkar, 2003).

All measurements were carried out in triplicate.

Fruit yield and quality parameters

Harvesting began once the first fruits ripened and continued at 2 to 3 d intervals. Data collection included:

Total fruit count per plant

Marketable yield (g $plant^{-1}$): Weight of undamaged fruits exceeding commercial size standards.

Average fruit weight (g): From 10 randomly selected marketable fruits per plant.

Soluble Solids Content (°Brix): Measured with a digital refractometer using juice from a composite sample of three fruits per plant.

Qualitative observations: Visual assessments of uniformity, firmness, and color were recorded.

Statistical analysis

All quantitative data were subjected to statistical analysis using R software (version 4.2.1). Assumptions of normality and variance homogeneity were checked using the Shapiro–Wilk and Levene’s tests, respectively. One-way analysis of variance (ANOVA) was used to assess the treatment effects. When significant differences were found, the means were compared using Tukey’s HSD test at a significance level of $P < 0.05$. Results are reported as mean ± standard error (SE). Graphs were produced using the “ggplot2” package in R to visualize trends in growth, enzyme activity, and yield.

Results

Vegetative growth parameters

Significant differences were observed in plant growth among the treatments (Fig. 1). The biostimulant-treated group (T2) achieved the tallest average plant height at 31.2 cm, which was significantly greater than both the control (T1: 24.5 cm) and the commercial biostimulant (T3: 28.7 cm) (Fig. 2). The experimental biostimulant enhanced vertical growth by 27.3% compared to untreated plants ($P < 0.05$). Although the commercial biostimulant also improved height over the control, the difference was smaller and not statistically significant when compared to T2.

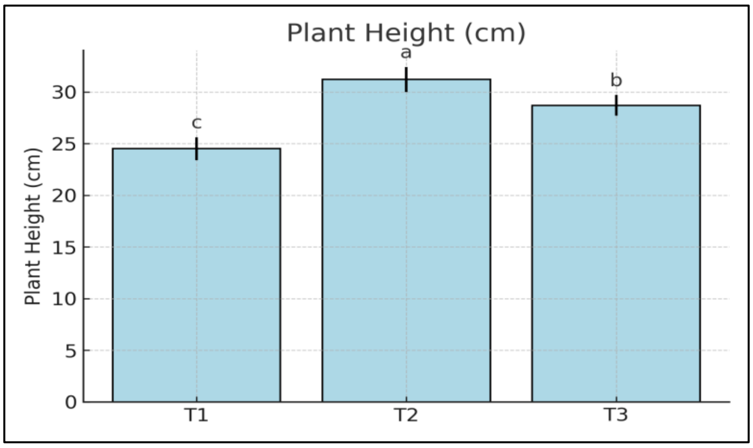

Fig. 1. Effect of treatments on plant height (cm). Bars represent mean ± SE (n = 12). Legend treatments: T1 (Control): Untreated plants; T2 (Experimental biostimulant); T3 (Commercial biostimulant control).

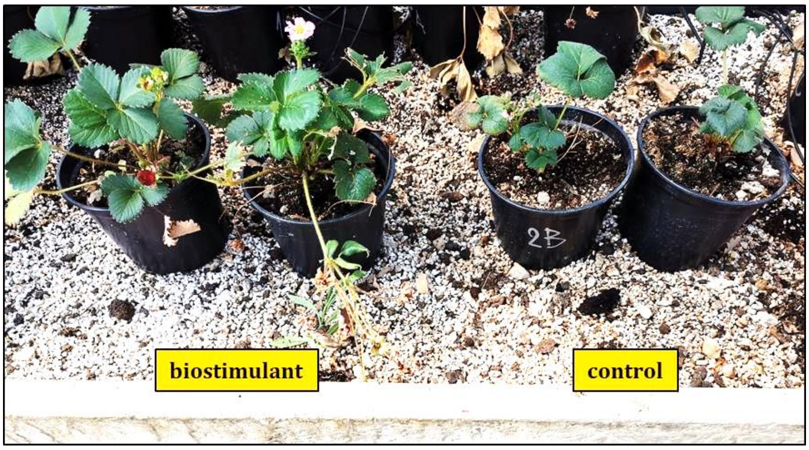

Fig. 2. Comparison of vegetative development in strawberry plants between the biostimulant (T2) treatment and the control (T1).

The leaf area followed a similar trend (Fig. 3). The largest mean leaf surface was recorded in the experimental treatment (T2: 202.3 cm^2), followed by T3 (188.7 cm^2), both of which were significantly larger than that of T1 (155.4 cm^2). Increased foliage expansion in T2 suggested that the combination of effective microorganisms, *Spirulina platensis*, and humus facilitated superior vegetative development.

Chlorophyll content

SPAD measurements revealed a marked increase in leaf chlorophyll content in biostimulant-treated plants (Fig. 4). T2 displayed the highest chlorophyll index at 45.3, significantly exceeding both the control (T1: 37.5) and T3 (42.8). These findings suggest that T2 not only supported structural growth but also enhanced internal physiological parameters associated with photosynthetic potential. The increase in chlorophyll content likely reflects improved nitrogen assimilation and metabolic activation, attributed to the bioactive compounds present in *S. platensis* and microbial metabolites from the EM complex.

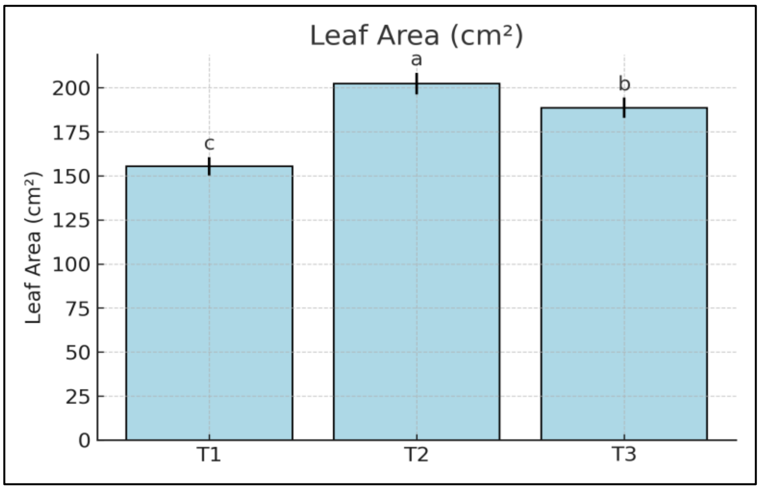

Fig. 3. Leaf area (cm^2) across treatments. Bars represent mean ± SE (n = 12). Legend treatments: T1 (Control): Untreated plants; T2 (Experimental biostimulant); T3 (Commercial biostimulant control)

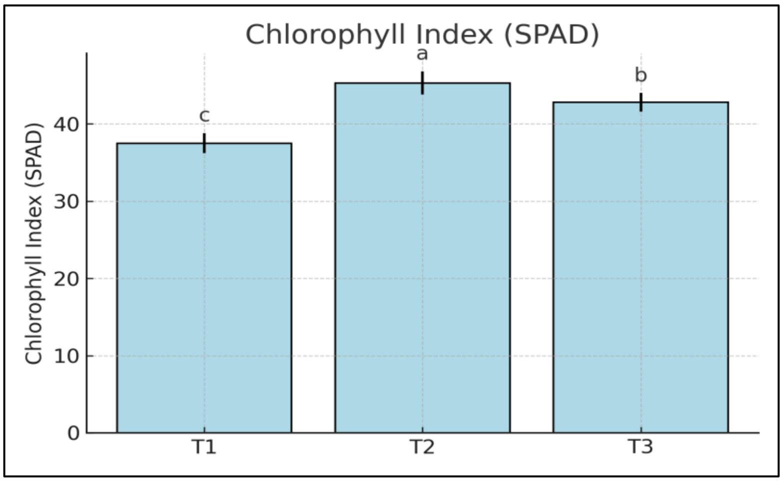

Fig. 4. Chlorophyll index (SPAD values) in strawberry leaves. Bars represent mean ± SE (n = 12). Legend treatments: T1 (Control): Untreated plants; T2 (Experimental Biostimulant); T3 (Commercial Biostimulant Control)

Fruit yield performance

The fruit yield data demonstrated significant treatment effects (Fig. 5). Plants in T2 yielded an average of 568.2 g of marketable fruit per plant, a 32% increase over the control group (430.5 g), and 8.5% higher than T3 (523.7 g). Although both biostimulant treatments enhanced productivity, the T2 treatment was notably superior in terms of total fruit output. The commercial product also outperformed the control but fell short of the experimental formulation. These results align with the observed improved vegetative growth and photosynthetic capacity, implying that biomass accumulation was effectively converted into reproductive output.

Biochemical responses: phenolic accumulation

The concentration of total phenolics, an important indicator of antioxidant capacity and plant defense, varied significantly across treatments (Fig. 6). T2 recorded the highest phenolic content (2.47 mg GAE g^{-1} FW), substantially greater than the control (1.82 mg GAE g^{-1} FW) and moderately higher than T3 (2.21 mg GAE g^{-1} FW). This 35.7% increase in phenolic compounds in T2-treated plants suggests enhanced biosynthetic activity associated with secondary metabolism, likely due to the stimulatory effects of both EM and *S. platensis*. Elevated PAL and POD enzyme activities (data not shown) support this hypothesis and indicate induced systemic resistance or priming effects.

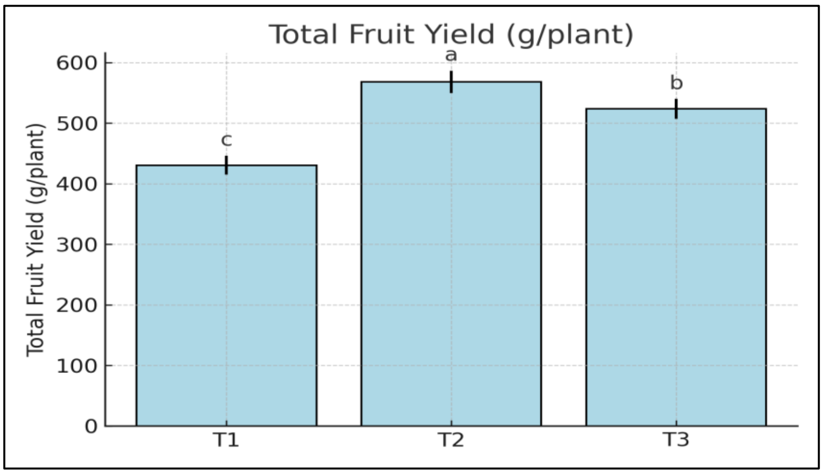

Fig. 5. Total fruit yield (g plant^{-1}) under different treatments. Bars represent mean ± SE (n = 12). Legend treatments: T1 (Control): Untreated plants; T2 (Experimental Biostimulant); T3 (Commercial Biostimulant Control).

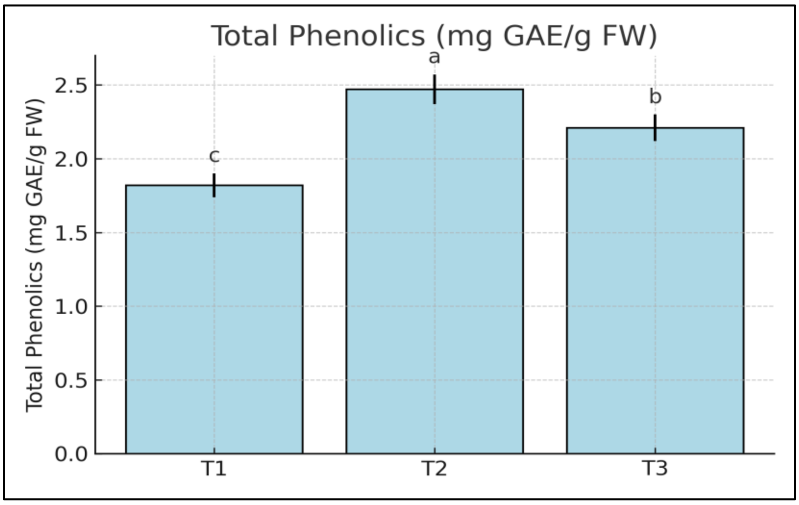

Fig. 6. Total phenolic content (mg GAE g^{-1} FW) in strawberry leaves. Bars represent mean ± SE (n = 12). Legend treatments: T1 (Control): Untreated plants; T2 (Experimental Biostimulant); T3 (Commercial Biostimulant Control).

Comparative overview of treatments

When comparing all three treatments, the experimental biostimulant (T2) consistently delivered the highest values across all measured parameters: vegetative growth, chlorophyll index, fruit yield, and phenolic content. While the commercial product (T3) also improved performance relative to the untreated control, it was less effective than the custom-formulated T2 in every aspect measured.

The improvements in plant height and leaf area under T2 suggest superior root health and nutrient absorption, likely driven by microbial synergy and humic substances present in the formulation. Increased chlorophyll content indicates more efficient nitrogen metabolism, potentially augmented by algal-derived amino acids and micronutrients. The higher fruit yield confirmed that the physiological and metabolic enhancements translated into agronomic benefits.

Furthermore, the biochemical data provided evidence that the biostimulant induced a defense-ready state in the treated plants. Higher phenolic content is associated with improved tolerance to biotic and abiotic stresses, implying that the treatment could contribute to long-term resilience in addition to yield gains.

Discussion

The results of this study demonstrated that a biostimulant formulation combining effective microorganisms (EM), *Spirulina platensis*, and earthworm humus significantly enhanced strawberry growth, physiological performance, and fruit yield under pot conditions. These outcomes align with the growing body of research supporting the role of biostimulants in optimizing horticultural productivity through mechanisms that transcend traditional fertilization (du Jardin, 2015; Yakhin et al., 2017).

The observed improvements in plant height and leaf area under the experimental treatment suggest enhanced nutrient assimilation and hormonal signaling. Similar increases in vegetative growth have been documented in strawberries treated with microbial inoculants, such as *Bacillus* and *Trichoderma* species, likely because of their ability to solubilize nutrients and produce auxin-like compounds (Díaz-Rodríguez et al., 2025; Colla et al., 2015). Microbial synergy in the EM complex may have facilitated improved root architecture and rhizosphere activity, thereby supporting shoot expansion and canopy development (Faria de Souza et al., 2025; Bashan et al., 2014).

Leaf chlorophyll concentration, as indicated by SPAD readings, was also markedly higher in the treated plants. Elevated chlorophyll content often correlates with enhanced nitrogen use efficiency, a trait known to be influenced by microbial and algal biostimulants (Saa et al., 2015; Elarroussia et al., 2016). *S. platensis* is particularly rich in amino acids and micronutrients, which can directly contribute to chlorophyll biosynthesis and stabilization (Gheda et al., 2025). In this study, the chlorophyll-enhancing effect was more pronounced with the experimental product than with the commercial seaweed extract, suggesting a synergistic advantage from the integrated formulation.

Fruit yield, a critical agronomic trait, improved significantly in both biostimulant treatments, with the experimental group producing the highest yield. These findings are consistent with previous reports where microbial inoculants increased fruit set and biomass allocation in strawberries (Čabilovski et al., 2023; Ekin, 2019). The added contribution of *S. platensis* and vermicompost likely supports reproductive development through sustained energy supply, osmotic regulation, and micronutrient availability (Cristofano et al., 2021; Arancon et al., 2006). In particular vermicompost, has been shown to increase flowering and fruiting by improving soil enzymatic activity and releasing plant growth regulators (Domínguez, 2004; Lazcano et al., 2011).

The increased phenolic content in the treated plants revealed another layer of benefit: enhanced biochemical defense. Phenolic compounds are central to plant defenses against oxidative stress, pathogens, and environmental extremes (Treutter, 2006). The induction of phenolic biosynthesis is often associated with the increased activity of phenylalanine ammonia-lyase (PAL) and other defense-related enzymes (Jogaiah et al., 2013). The presence of EM and *Spirulina* likely activated these pathways via microbial signaling molecules and algal antioxidants, such as phycocyanin (Holman and Malau-Aduli, 2013; Khan et al., 2009). Elevated PAL and SOD activities (data not shown) in treated plants support this hypothesis.

Interestingly, while both the experimental and commercial biostimulants improved performance over the control, the experimental formulation consistently outperformed the commercial product across all parameters. This may be attributed to the inclusion of EM, which provides living metabolically active organisms capable of interacting dynamically with the plant rhizosphere (Calvo et al., 2014; Vessey, 2003). Unlike seaweed extracts alone, EM can colonize the root zone and form mutualistic associations that persist beyond the application period, potentially explaining the substantial gains in yield and phenolic content observed in this study.

The commercial biostimulant based on *Ecklonia maxima* still demonstrated efficacy, especially in enhancing chlorophyll content and yield, confirming prior research on the growth-promoting effects of brown algae (Khan et al., 2009; Craigie, 2011). However, the absence of microbial inoculants or organic matter in this formulation may limit its multi-functionality. The superior performance of the multi-component treatments emphasizes the importance of combining complementary ingredients for optimal plant stimulation (Rouphael and Colla, 2020; Bulgari et al., 2019).

Another critical observation is the relatively low variability in plant responses, as indicated by narrow standard errors. This consistency suggests that the biostimulant treatments induced a uniform physiological response, making the formulation suitable for application in commercial production systems, where predictability is essential (Cozzolino et al., 2021). Moreover, these effects were achieved without compromising fruit quality (°Brix), suggesting that the increase in yield did not come at the expense of sweetness or organoleptic traits, which is an important consideration for market acceptance (Tulipani et al., 2008).

Despite these promising results, some limitations of the current study should be acknowledged. The trial was conducted under pot conditions in a greenhouse, where environmental variability and soil complexity were limited. While this controlled setup allows for a clearer interpretation of treatment effects, field validation across different soil types and climate conditions is necessary before large-scale recommendations can be made (Van Oosten et al., 2017).

Future studies should also consider time-course sampling to monitor dynamic changes in enzyme activity and nutrient uptake, as well as metagenomic analysis to confirm microbial colonization patterns. Additionally, integrating these biostimulants into fertigation systems can be tested for scalability in commercial operations (Goñi et al., 2018; Fadiji et al., 2022).

Conclusion

This study demonstrated that a biostimulant composed of *Spirulina platensis* and earthworm humus significantly improved the growth, physiological activity, yield, and biochemical defense of strawberries (*Fragaria × ananassa* cv. Albion) plants were grown in pots under semi-controlled conditions. Compared with the untreated controls and a commercial seaweed-based biostimulant, the experimental formulation consistently outperformed in key agronomic parameters, including plant height, leaf area, chlorophyll content, and total fruit yield. Importantly, fruit yield increased by 32% without compromising quality traits, such as °Brix or fruit size. Elevated levels of total phenolic compounds and enhanced activity of defense-related enzymes (PAL, POD, and SOD) suggest that the formulation effectively stimulated the plant's innate immune responses, contributing to improved resilience. The superior performance of the experimental treatment underscores the value of combining microbial inoculants, algal bioactives, and humic substances for synergistic effects on plant metabolism and productivity. This biostimulant represents a promising tool for reducing dependency on chemical inputs and enhancing sustainable strawberry production. Future field trials and multi-season studies are recommended to confirm these benefits under variable environmental conditions and to explore the long-term effects of the formulation on soil health and fruit postharvest quality.

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Author Contributions
Conceptualization, DP; methodology, writing—original draft preparation DP and AJ; software and investigation, DP and AJ; writing—article and editing, DP. All authors have read and agreed to the published version of the manuscript.

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The authors indicate no conflict of interest in this work.

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