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Impact of Honey Soil Supplementation on Growth and Antioxidant Activity in Basil (*Ocimum basilicum* L.) Plants

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Abstract: This study explores the potential of honey as a natural soil amendment to enhance plant growth and biochemical resilience in *Ocimum basilicum* L. Honey solutions at varying concentrations (2.5%, 5%, and 10%) were applied to evaluate their effects on growth parameters, biomass accumulation, and antioxidant activity. The results revealed that lower honey concentrations (2.5%) had a minimal impact on plant height, while higher concentrations (5% and 10%; −42% and −43%, respectively) exhibited inhibitory effects, suggesting a dose-dependent response. The leaf count remained stable across treatments, indicating a consistent morphological outcome. The biomass analysis highlighted variability in the plant biomasses, reflecting the influence of honey concentrations on plant energy allocation. Despite unchanged chlorophyll and ascorbic acid levels, significant enhancements in antioxidant compounds and activity were observed, particularly at lower concentrations (antioxidant activity at 2.5% and 5%; +26% and +30%, respectively), underlining the role of honey in bolstering the antioxidant defense system. These findings demonstrate honey's dual role as a growth modulator and antioxidant enhancer, emphasizing its relevance in sustainable agricultural practices. This research contributes to the development of eco-friendly strategies for improving crop performance and resilience through the application of naturally derived biostimulants.

Keywords: antioxidant activity; crop growth; honey; natural biostimulant; *Ocimum basilicum*

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1. Introduction

In a context where environmental sustainability and human health are increasingly at the center of global concerns, the search for alternative agricultural methods that respect the ecosystem becomes imperative. The excessive use of chemical fertilizers in conventional agriculture has harmful repercussions on soil quality, biodiversity and human health [1]. Thus, it is crucial to explore natural and sustainable alternatives to increase the yield and the quality of food, preserving the environment and the health of consumers.

One promising solution in sustainable agriculture involves the use of biostimulants, which contain organic substances or microorganisms that, when applied to plants or soil,

can enhance plant growth [2], improve nutrient uptake [3], increase resilience to abiotic stress (such as drought, salinity, nutrient deficiency, and extreme temperatures) [4–7], and promote overall plant health [8]. Unlike synthetic chemicals, which can lead to soil degradation [9], loss of biodiversity [10], and negative impacts on human health [11], biostimulants offer a sustainable alternative that aligns with ecological principles and circular economy practices [12]. A biostimulant is defined as "*any substance or microorganism applied to plants with the aim to enhance nutrition efficiency, abiotic stress tolerance and/or crop quality traits, regardless of its nutrients content*" [12]. Biostimulants, such as natural extracts, microbial inoculants, and protein hydrolysates, have the potential to replace certain chemical fertilizers, growth regulators, and soil conditioners by enhancing nutrient uptake, improving plant resilience, and stimulating natural metabolic processes. Examples of biostimulants replacing chemical products can already be observed in specific agricultural practices. For instance, seaweed extracts are increasingly used as natural growth promoters in horticulture, reducing reliance on synthetic growth regulators. Similarly, microbial inoculants have successfully replaced certain chemical nitrogen fertilizers by enhancing soil microbiota and facilitating nitrogen fixation. However, the widespread replacement of synthetic chemicals with biostimulants has not yet occurred for several reasons. First, the efficacy of biostimulants can be context-dependent, varying with plant species, soil type, and environmental conditions, which can limit their adoption. Second, synthetic chemicals often provide immediate and predictable results, whereas biostimulants may act more gradually and require more tailored application strategies. Finally, regulatory frameworks, cost considerations, and limited awareness among farmers further delay the transition.

Biostimulants include a variety of organic compounds, such as humic and fulvic acids [13], seaweed extracts [5,14,15], microbial inoculants [16,17], protein hydrolysates [6,18], and even certain natural by-products like honey [19], which can act as a biostimulant due to its complex composition of sugars, enzymes, amino acids, vitamins, and minerals that enhance microbial activity in the soil, promote nutrient availability, and improve plant resilience to environmental stress. These substances act through various mechanisms, such as enhancing the efficiency of the plant's metabolic processes [8], stimulating beneficial microbial activity in the soil [20], and activating the plant's defense systems against pests and diseases [18,21]. As a result, they contribute to healthier, more resilient crops and can even improve the nutritional and nutraceutical quality of the food produced [22].

Honey, a natural product with a complex composition, including sugars, vitamins, phenolic compounds, and enzymes, has recently emerged as a potential biostimulant in sustainable agriculture [23–25]. Its well-documented antioxidant [26], antibacterial [27], and anti-inflammatory [28] properties have led to increased interest in exploring honey's applications beyond human consumption, particularly in plant health and productivity. While biostimulants are clearly defined under frameworks such as the EU Fertilizing Products Regulation [29], honey's use in agriculture is often unregulated or falls outside of formal classifications. This gap highlights the need for further research to explore its application and establish clear regulatory pathways, particularly in countries like Algeria and Italy, where sustainable agriculture is a growing priority. Honey has the potential to improve soil microbial activity, increase nutrient availability, and protect plants from abiotic and biotic stressors, thus enhancing crop yields in a sustainable manner. Several studies have highlighted the antioxidant [23–25], antibacterial [27], and anti-inflammatory [28] properties of honey, underscoring its potential as a natural biostimulant. These bioactive properties make honey a promising candidate for use in agriculture to stimulate plant resilience to environmental stress and the nutraceutical quality of plants, enhancing its value beyond nutritional applications.

Basil (*Ocimum basilicum* L., var. 'Riviera Ligure') is a significant aromatic herb that plays a pivotal role in various global cuisines due to its distinctive aromatic profile and flavor. As a member of the Lamiaceae family, basil is characterized by its broad range of volatile compounds, including linalool, eugenol, and estragole, which contribute to its unique sensory qualities [30]. These compounds not only enhance the taste and aroma of culinary dishes but also have been investigated for their potential therapeutic properties. In traditional and modern culinary practices, basil is utilized in a multitude of preparations, such as sauces, soups, salads, and marinades. Its diverse applications extend beyond the kitchen, where it is also employed in medicinal and cosmetic products, attributed to its purported anti-inflammatory, antimicrobial, and antioxidant effects [31].

The aim of this work is to assess the effectiveness of soil enrichment with different concentrations of honey (2.5%, 5%, and 10%) on the growth of basil, evaluating honey's effects on biometric, photosynthetic, and biochemical plant features.

2. Materials and Methods

2.1. Experimental Design

Ocimum basilicum seeds were imbibed in distilled water for 2 h, sown between layers of wet paper in a vertical tray, and allowed to germinate for 1 week at 24 °C in the dark [32]. Subsequently, homogenous seedlings of ca. 3 cm were transplanted into plastic pots (3 seedlings/pot) containing 80 g of a commercial growing medium (VigorPlant Italia srl; 43% moisture content, 92% porosity, 5.30 ± 0.03 pH, 1.12 ± 0.01 mS cm^{-1} electrical conductivity, and 56.9 ± 2.7 meq 100 $g_{DW}{}^{-1}$ cation exchange activity).

Lavender honey was selected for its richness in bioactive compounds, such as flavonoids, phenolic acids, and essential oils, which are known for their antioxidant, antibacterial, and anti-inflammatory properties [33], their mild flavor, and their regional availability, including Provence in France, Liguria and Tuscany in Italy, and parts of Spain. In addition to these well-known areas, North African countries like Algeria are also important producers of lavender honey [34]. The climate in these regions is particularly suited for lavender growth, resulting in high-quality honey that is prized for both its flavor and medicinal properties. The chemical composition of lavender honey includes concentrations of sugars, primarily fructose (0.071 ± 0.001 mg g^{-1}), glucose (0.018 ± 0.001 mg g^{-1}), sucrose (0.005 ± 0.001 mg g^{-1}), and pectin (0.452 ± 0.004 mg g^{-1}). It also contains polyphenols (0.08 ± 0.01 mg/g), flavonoids (0.02 ± 0.005 mg/g), and hydroxymethylfurfural (HMF) (185 ± 2 mg kg^{-1}).

For the honey treatment groups (biological replicates per each treatment = 6), consisting of concentrations of 0% (control), 2.5%, 5%, and 10% (*v*/*v*), honey was added to the soil once a week instead of standard irrigation. The characteristics of each honey concentration are reported in Table 1. The electrical conductivity (EC) of all honey solutions was determined using a conductivity meter after the preparation of each concentration. The hydroxymethylfurfural (HMF) content was determined by dissolving 5 g of honey in 25 mL of distilled water, and then treated with Carrez solutions I and II. Subsequently, the mixture was filtered, and two aliquots were prepared: one with distilled water and the other with sodium bisulfite. The absorbance was measured at 284 nm and 336 nm using a UV-Vis spectrophotometer, and finally the *HMF* content was calculated using the following equation [35]:

$$HMF\ (mg/kg) = ((A284 - A336) \times 149.7 \times 5)/M$$

where M is the mass in grams of the honey.

Table 1. Characteristics of honey treatments. EC: Electrical Conductivity (μS cm^{-1}), HMF Hydroxymethylfurfural (mg kg^{-1}). CTRL: control 0% honey solution; H 2.5%: 2.5% honey solution; H 5%: 5% honey solution; and H 10%: 10% honey solution.

Honey Solutions	pH	EC	HMF
CTRL	4.50 ± 0.03	80 ± 3	—
H 2.5%	4.51 ± 0.03	114 ± 2	18.0 ± 0.5
H 5%	4.48 ± 0.02	198 ± 2	18.0 ± 0.4
H 10%	4.46 ± 0.05	434 ± 2	18.0 ± 0.5

Following transplantation, plants were cultivated in a climate-controlled chamber under conditions of a 16/8 h photoperiod, temperatures of 25/20 °C, a photosynthetically active radiation (PAR) light intensity of 250 μmol m^{-2} s^{-1}, and a relative humidity of 70%. To minimize potential edge effects in the environmental chamber, the pots were arranged using a randomized block design. Each block contained all treatment groups, and the positions of the pots within the chamber were rotated weekly to ensure uniform exposure to light and environmental conditions. This arrangement was implemented to reduce any variability associated with positional effects within the chamber. The environmental chamber was equipped with SANSI BR30 40W LED (Sansi Co., Shanghai, China) grow lights, which provide a full spectrum of light optimized for plant growth. The lights emit a combination of wavelengths, with a red-to-blue light ratio designed to enhance photosynthesis and growth. According to the manufacturer specifications, the spectrum covers both red (approximately 660 nm) and blue (approximately 450 nm) wavelengths, ensuring a balanced light quality suitable for plant development. Throughout this period, pots were maintained at 60% of their water holding activity (WHC) daily, which was determined using the gravimetric method. Initially, the soil was saturated with water and then allowed to drain until field capacity was reached. The WHC was calculated as a percentage of the water retained at this point relative to the dry soil weight. Regular weighing of the pots was conducted, and water was added as necessary to maintain the desired WHC throughout the experiment. The experiment lasted 10 weeks.

2.2. Biometric Parameters

Upon harvest, the height of each plant was measured considering the distance from the soil surface to the apex of the plant. Concurrently, the number of leaves on each plant was counted. Furthermore, the shoot fresh weight was determined using a precision balance. Subsequently, to maintain sample integrity, all samples were promptly stored at −20 °C until further analysis.

2.3. Photosynthetic Parameters

Photosynthetic parameters (content of total chlorophyll, chlorophyll *a*, chlorophyll *b*, and carotenoids) were determined following the methodology reported by Fedeli et al. [36]. Samples (50 mg) were homogenized in 80% methanol, and subsequently were first put in an ice bath in the dark for 30 min, and afterwards were centrifuged at 3500 rpm for 20 min. The resulting supernatant was collected and subjected to spectrophotometric analysis (470, 653, and 666 nm) using a UV-Vis spectrophotometer (8453, Agilent, Santa Clara, CA, USA). The concentration of each component was calculated using the following equations:

$$Chla\ (mg/g) = 15.65 \times A666 - 7.34 \times A653$$
$$Chlb\ (mg/g) = 27.05 \times A653 - 11.21 \times A666$$
$$Total\ Chlorophyll\ content\ (mg/g) = Chla + Chlb$$
$$Carotenoids\ (mg/g) = 2451000 \times A470 - 2.86 \times Chla - 129.2 \times Chlb$$

2.4. Antioxidant Compounds

2.4.1. Vitamin C

The quantification of the content of vitamin C was conducted following the method reported by Fedeli et al. [37]. A total of 200 mg of plant tissue was homogenized with 0.8 mL of 10% (*w/v*) trichloroacetic acid (TCA) using an ULTRA-TURRAX® (T 10 basic, Werke GmbH & Co. KG, Staufen, Germany). The resulting homogenates were filtered through gauze and chilled in an ice bath before centrifugation at 3000 rpm for 5 min. A total of 0.4 mL of the extract was mixed with 0.2 mL of 0.2 M Folin–Ciocalteu reagent (Carlo Erba, Cornaredo, Italy). The absorbance was then measured at 760 nm using a UV-Vis spectrophotometer (8453, Agilent, Santa Clara, CA, USA). The concentration of vitamin C was determined using a calibration curve established using 0.05–0.2 mL of pure ascorbic acid (BioXtra, ≥99.0%, crystalline).

2.4.2. Polyphenols, Flavonoids, and Total Antioxidant Power

To assess the antioxidant compounds in basil shoots, an extract was prepared following the method described by Azarnejad et al. [38]. Basil shoots were dried in a dry, dark environment at 30 °C for 24 h using a ventilation oven. Subsequently, 1 g of the dried plant material was mixed with 10 mL of 80% methanol, homogenized for 30 min, and then incubated in the dark at 4 °C for 48 h. The resulting solution was filtered using a Whatman filter n.1 to obtain the extract necessary for quantifying total polyphenols, flavonoids, and the total antioxidant power.

The content of total polyphenols was determined using the method reported by Fedeli et al. [39]. A total of 125 µL of the extract was mixed with 2 mL of dH_2O and 125 µL of Folin–Ciocalteu reagent. After thorough mixing for 3 min, 1.25 mL of 7% Na_2CO_3 and 1 mL of dH_2O were added. The mixture was incubated in the dark for 90 min, and the absorbance was measured at 760 nm using a UV-Vis spectrophotometer (8453, Agilent, Santa Clara, CA, USA). The results were expressed as mg of gallic acid equivalent per g of dry weight (mgGAE/gDW) utilizing a standard curve (5–300 µg mL^{-1}) prepared with pure gallic acid (98%, Thermo Fisher Scientific Inc., Rodano, Milano, Italy).

The content of total flavonoids was determined using the method reported by Lamaro et al. [40]. A total of 10 µL of the extract was combined with 200 µL of dH_2O. Subsequently, 75 µL of 5% $NaNO_2$ was added, followed by a 5 min incubation of the sample in the dark. Afterwards, 75 µL of 10% anhydrous $AlCl_3$ was added, and the mixture was shaken and further incubated for 5 min in the dark. Afterwards, 500 µL of 1N NaOH was added, thoroughly mixed, and then incubated for 15 min in the dark. The absorbance was measured at 415 nm, and the results were expressed as mg of quercetin equivalent per g of dry weight (mgQE/gDW) using a standard curve (12.5–150 µg mL^{-1}) prepared with quercetin (≥95%, Merck KGaA, Darmstadt, Germany).

The total antioxidant activity was determined by evaluating the DPPH radical scavenging activity, following the method reported by Fedeli et al. [41], with minor modifications. A total of 100 mL of the extract was mixed with 1 mL of DPPH methanolic solution (obtained by dissolving 3.9 mg DPPH in 100 mL of 80% (*v/v*) methanol). The control used for this test was the DPPH methanolic solution and the blank was 80% methanol. After incubation for 1 h in the dark, the absorbance was measured at 517 nm, and the inhibition percentage was calculated according to the following formula:

$$DPPH(\%) = [(AC - AE) / AC] \times 100$$

where *AC* is the absorbance of the control and AE is the absorbance of the extract.

2.5. Statistical Analysis

The data approached a normal distribution (Shapiro–Wilk test, $p > 0.05$) and were expressed as means ± standard error. A one-way ANOVA was run to test for differences ($p < 0.05$) in honey treatments, followed by Tukey's post hoc test to check for differences ($p < 0.05$) among treatments. The statistical analysis was carried out using the software Statistica 7.2.1 [42].

3. Results

The honey treatment resulted in a distinctive pattern in the biometric parameters of basil plants (Table 2), with H2.5 not being different from the control, and higher honey concentrations (H5 and H10) determining a statistically significant decrease in both plant height and fresh weight; no effect was observed for the number of leaves.

Table 2. Effect of different honey concentrations on biometric parameters of basil plants. CTRL: control, plants treated with 0% honey solution; H 2.5%: plants treated with 2.5% honey solution; H 5%: plants treated with 5% honey solution; and H 10%: plants treated with 10% honey solution. Different letters indicate statistically significant ($p < 0.05$) differences between treatments.

Treatment	Height (cm)	Nr. of Leaves	Fresh Weight (g)
CTRL	9.8 ± 1.0 ^{a}	4.2 ± 0.4	0.7 ± 0.1 ^{a}
H 2.5%	8.8 ± 1.6 ^{ab}	4.3 ± 0.8	0.5 ± 0.2 ^{ab}
H 5%	8.0 ± 0.8 ^{bc}	3.8 ± 0.4	0.4 ± 0.1 ^{b}
H 10%	6.8 ± 2.0 ^{c}	4.2 ± 1.0	0.4 ± 0.2 ^{b}

The honey treatment did not show any effect on the photosynthetic parameters of basil plants (Figure 1), irrespective of a trend in decreasing chlorophyll *a* and carotenoids and increasing chlorophyll b for H 10%. Effects were also not detected for the content of vitamin C (Figure 2).

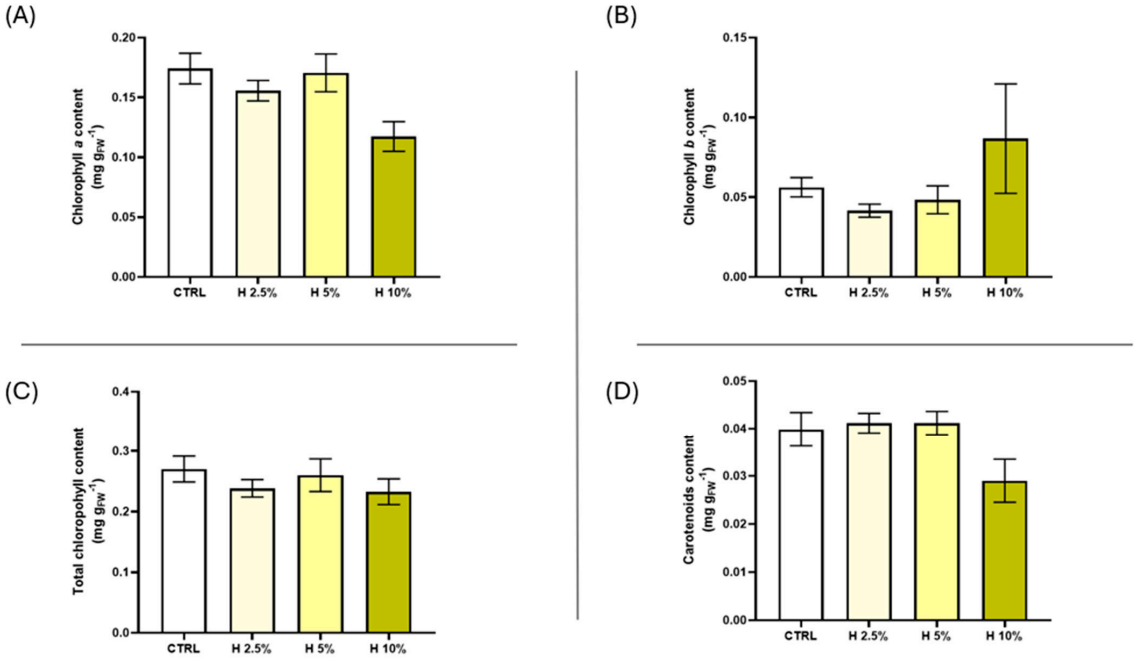

Figure 1. Content of chlorophyll *a* (**A**), chlorophyll *b* (**B**), total chlorophyll (**C**), and carotenoids (**D**) in basil plants. CTRL: control, plants treated with 0% honey solution; H 2.5%: plants treated with 2.5% honey solution; H 5%: plants treated with 5% honey solution; and H 10%: plants treated with 10% honey solution.

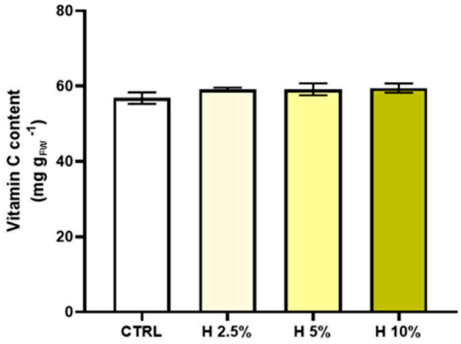

Figure 2. Vitamin C content of basil plants. CTRL: control, plants treated with 0% honey solution; H 2.5%: plants treated with 2.5% honey solution; H 5%: plants treated with 5% honey solution; H 10%: plants treated with 10% honey solution.

The treatment of basil plants with honey solutions caused an increase in both total phenolic compounds and flavonoids at all concentrations, parallel to the honey amount (Figure 3).

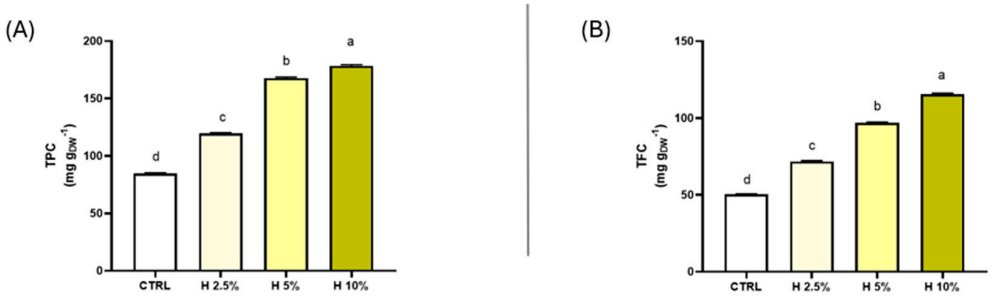

Figure 3. Total Phenolic Content (TPC) (**A**) and Total Flavonoid Content (TFC) (**B**) in basil plants with different honey treatments. CTRL: control, plants treated with 0% honey solution; H 2.5%: plants treated with 2.5% honey solution; H 5%: plants treated with 5% honey solution; and H 10%: plants treated with 10% honey solution. Different letters indicate statistically significant ($p < 0.05$) differences between treatments.

The total antioxidant activity in the basil plants treated with solutions containing various concentrations of honey showed an hormetic effect of honey (Figure 4), with intermediate concentrations (H 2.5% and H 5%) being statistically increased, and no effect for the higher (H 10%) honey concentration.

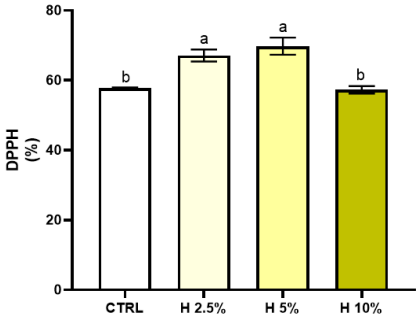

Figure 4. Total antioxidant power (DPPH) of basil plants treated with different honey concentrations. CTRL: control, plants treated with 0% honey solution; H 2.5%: plants treated with 2.5% honey solution; H 5%: plants treated with 5% honey solution; and H 10%: plants treated with 10% honey solution. Different letters indicate statistically significant ($p < 0.05$) differences between treatments.

4. Discussion

The observed results between honey supplementation and the effects on basil plants could have been influenced by various mechanisms, e.g., the presence of antibacterial properties in honey [43], leading to an impact on the soil microbiome, potentially influencing plant growth [44], but these issues have not been addressed experimentally. In fact, the antimicrobial activity of honey, which is attributed to the presence of hydrogen peroxide, methylglyoxal, and other bioactive compounds, could potentially alter the balance of beneficial and pathogenic microorganisms in the rhizosphere. Such alterations in microbial communities can affect nutrient availability, plant hormone production, and disease suppression, all of which are crucial for plant health and growth [45]. Nevertheless, the bioactive molecules of honey are known to exert a beneficial effect on plants and improve their ability to face adverse environmental conditions, acting on their primary or secondary metabolism [46].

In our study, the application of honey to the soil positively influenced the content of phenolic compounds in basil plants. Honey is rich in a wide array of bioactive compounds, including phenolic compounds and flavonoids, which can be readily available for plant uptake, determining a high phenolic and flavonoid content in basil plants [47,48]. Additionally, these compounds may also contribute to the synthesis of phenolic compounds within plant tissues. Moreover, honey solutions may stimulate phenolic and flavonoid biosynthesis pathways, triggering an upregulation in the production of phenolic compounds as a part of the plant's defense response against environmental stress [49].

Honey can regulate the antioxidant defense systems of plants, leading to an increase or decrease in endogenous antioxidant production or activity [49]. In our study, lower concentrations of honey (2.5% and 5%) resulted in an enhanced antioxidant potential in basil plants compared to a higher concentration (10%), suggesting that higher concentrations of honey may induce stress in plants, which is likely due to osmotic effects, reducing the overall antioxidant potential. Furthermore, certain components of honey can become toxic to plants at elevated concentrations, further decreasing the antioxidant activity [50].

While there is a notable lack of studies specifically investigating the effects of honey on basil plants, existing research on the use of honey as a biostimulant for other cultivated plants provides valuable insights. The application of honey has been shown to improve various aspects of plant growth, yield, and quality, which can be relevant to understanding its potential effects on basil.

For instance, Sabir and Tas [51] demonstrated that foliar application of honey significantly enhanced the biochemical characteristics and yield in lettuce. This improvement was attributed to honey's rich nutrient profile and bioactive compounds, which are known to boost antioxidant activity. Similarly, El-Gioushy et al. [52] reported that a honey solution increased growth, yield, and fruit quality in tomato plants. These findings suggest that honey's beneficial effects on plant health and productivity are likely due to its diverse range of nutrients and bioactive substances.

In another study, El-Gioushy et al. [53] observed significant improvements in growth, yield, and quality in cabbage plants when honey was used as a biostimulant. The increase in vitamins and minerals in the cabbage leaves supports the idea that honey can positively affect plant nutritional quality. Moreover, Ahmed and Gad [54] found that honeybee extracts enhanced growth and fruit quality in strawberries, leading to improved vegetative growth and increased levels of phenolic compounds and antioxidants.

5. Conclusions

This study investigated the effectiveness of soil supplementation with solutions at different honey concentrations (2.5%, 5%, and 10%) on basil plants, evaluating the effects on

biometric, photosynthetic, and biochemical features. No effects were observed for biometric and photosynthetic parameters, as well as for the content of vitamin C, while the total phenolic and flavonoid contents increased along with increasing honey concentrations. The total antioxidant activity showed a hormetic effect.

Overall, this work underscores the potential of honey as a plant biostimulant in sustainable agriculture, emphasizing the importance of optimal concentrations to maximize the benefits, while avoiding adverse impacts.

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Conflicts of Interest: The authors declare no conflicts of interest.

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