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Effect of Environment and Hoagland Solution on Vegetative, Reproductive Traits and Mucilage Content of Italian Basil (*Ocimum basilicum* var. *Genovese*)

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ABSTRACT

The cultivation of medicinal plants is expanding worldwide, with many species playing a vital role in the pharmaceutical industry. Italian basil (*Ocimum basilicum* L. var. Genovese) is an annual aromatic plant from the Lamiaceae family. This study aimed to evaluate the effects of Hoagland nutrient solution on vegetative and reproductive traits of Italian basil. Two levels of Hoagland solution (50% and 100%) and two growing environments (open field and greenhouse conditions) were tested in separate factorial experiments arranged in a completely randomized design with three replications (two plants per replication). Results indicated that the open field environment combined with 50% Hoagland solution significantly enhanced vegetative growth and yield of Italian basil. Specifically, branch number and leaf count per plant (10.33 and 45.5 respectively), plant height and internode length (25.67 cm and 3.17 cm), fresh and dry leaf weight (15.3 g and 5.38 g), fresh and dry stem weight (8.06 g and 6.7 g), fresh and dry aerial biomass (32.91 g and 18.64 g), fresh and dry root weight (21.48 g and 6.32 g), aerial-to-root DW ratio (2.96), total fresh and dry biomass (54.39 g and 24.96 g), and leaf-to-stem and total plant weight ratios (0.8 and 0.21) were all significantly affected at $P \leq 0.01$. Photosynthetic pigments including chlorophyll a, chlorophyll b, total chlorophyll, and carotenoids were significantly influenced, with values of 3.228, 0.622, 3.85, and 0.187 mg/g, respectively. Reproductive traits such as fresh and dry inflorescence weight (9.47 g and 7.228 g), number of inflorescences per plant (27), 1000-seed weight (1.6 g), seed yield per plant (2.03 g), and seed number per plant (1356.5) were also significantly enhanced ($P \leq 0.01$). Overall, the field-grown plants treated with 50% Hoagland solution exhibited the highest positive effect on most vegetative and reproductive traits.

Keywords: Active substances, Development, Fertilization, Growth, Flowering, Medicinal plants

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INTRODUCTION

Currently, approximately one-third of medicines consumed by humans are plant-based [1]. The pharmaceutical industry's demand for raw materials and the need to conserve natural plant resources have intensified research on the cultivation and processing of medicinal and aromatic plants [2, 3]. Although medicinal and aromatic plants occupy a small portion of agricultural land compared to staple crops, rising concerns about drug sensitivities, resistance to synthetic compounds and risk of exposure to some toxins have renewed interest in plant-based remedies [4].

Iran's plateau, with diverse climatic and geographic conditions, harbors a rich flora of approximately 8,000 species, many of which are medicinal. Beyond their economic value, medicinal plants are well-suited for organic cultivation. Environmental factors such as soil physical and chemical properties, nutrient availability, and soil pH significantly affect medicinal plants' growth and secondary metabolite content [4, 5]. Thus, nutrient management should consider plant growth and bioactive compounds' quality, as inappropriate fertilization can increase yield but reduce secondary metabolite content or alter its composition detrimentally [6, 7].

Basil serves as an important medicinal plant, rich in essential oils, and encompasses many distinct species [7]. Italian basil (*Ocimum basilicum* L.) is an annual, herbaceous, aromatic plant that reaches 30–60 cm in height. Its leaves, stems, calyx, and flowers are covered with various structures, including glandular and non-glandular trichomes, which serve as sites for essential oil accumulation. Leaves contain the highest essential oil content, making them the most economically valuable part of the plant. Medicinal plants produced several types of secondary metabolites and one of the important one is essential oils which have different properties and have been used as carminatives, lactation enhancers, cardiotonic agents, appetite stimulants, and antispasmodics [8]. Numerous researchers have identified the type and composition of the soil (Nutrient or fertilizers) as a crucial factor influencing the composition of secondary metabolites [9, 10]. As noted by Hornok, the addition of three key nutrients to the soil (nitrogen, phosphorus, and potassium) has typically resulted in enhanced oil yields [11]. However, the individual application of these nutrients produced varying outcomes regarding both the yield and the composition of the oils. Furthermore, other studies have indicated that, unlike the yield, the composition of the essential oil remained unaffected by the nitrogen concentration present in the soil.

One of the most profound studies regarding the direct and indirect impacts of environmental factors on the secondary metabolites of medicinal plants was conducted by Bernath and Tetenyi [9]. They provided a comprehensive explanation of the phenomenon and demonstrated that soil nutrients have an indirect influence on secondary metabolites. They cited significant examples, particularly in alkaloidal medicinal plants, and clarified that while nitrogen is a primary nutrient for alkaloid structure, an increase in nitrogen does not directly influence the alkaloid content in two distinct Solanum species. Fertilizers vary in composition, containing macroelements, microelements, or both. Maintaining an appropriate balance between macro- and micronutrients in the growing medium enhances plant growth and resistance to pests and diseases [12]. A proper nutrient solution must consider plant-specific requirements and growth stages, while also accounting for environmental factors like light and temperature, which can increase or decrease nutrient demand [13].

Research regarding the nutrition and fertilization of medicinal plants has demonstrated that both micronutrients and macronutrients indirectly influence growth and secondary metabolites [6]. Zinc nutrition in basil has shown that zinc plays a critical role in protecting plants against reactive oxygen species [14].

This study aims to provide a precise scientific evaluation of the effects of nutrient solutions like Hoagland solution on basil growth, reproductive traits, and seed production. Basil seeds, rich in mucilage, are also used to enhance immunity, reduce cancer risk, regulate blood pressure, and lower cholesterol [15]. Therefore, this study investigates the combined effects of growth environment (open field vs. greenhouse conditions) and Hoagland nutrient solution on vegetative and reproductive traits of basil in two separate experiments.

MATERIALS AND METHODS

This study was conducted during spring and summer 2021 in the research greenhouses of the Faculty of Agriculture, Ferdowsi University of Mashhad. The greenhouse is situated at a latitude of 36 °30' and a longitude of 59 °52', with an elevation of 1097 meters above sea level. The temperature within the greenhouse ranged from 28 to 32 °C, while the relative humidity fluctuated between 75 and 80%. To evaluate the effects of Hoagland nutrient solution (Table 1) and different growing environments (open field and greenhouse conditions) on vegetative and reproductive traits, including flowering and seed production of Italian basil, two separate experiments were performed. Both experiments were designed as factorial trials in a completely randomized block design with three replications, each replication consisting of two plants.

Table 1 Nutrient composition of the Hoagland solution used in the experiment

Compounds	Content (g/l)
KNO_3	0.6
$Ca(NO_3)_2$	1.05
$NH_4H_2PO_4$	0.23
$MgSO_4.7H_2O$	0.24
KCl	0.074
H_3BO_3	0.061
$MnSO_4.H_2O$	0.169
$ZnSO_4.7H_2O$	0.287
$CuSO_4.5H_2O$	0.249
H_2MoO_4	0.161
Fe-EDTA	0.346

Fig. 1 Mass planting of Italian basil in seedling trays (a) and pots in fields (b) and greenhouses (c)

Basil seeds were sown in seedling trays in mid-June to prepare seedlings (Fig. 1). Germination began approximately one week after sowing, and after about six weeks (early August), seedlings were transplanted into pots in both greenhouse and field environments. Hoagland nutrient solution was applied at two levels (50% and 100%) in both greenhouse and field conditions (Fig. 1). The potting medium consisted of leaf mold, garden soil, and sand in a 1:1:1 ratio. In addition to Hoagland solution, all treated and control pots received an N-P-K solution (20:20:20) as basal fertilization. Pots were irrigated daily along with the nutrient solution, with 1.5 liters of water applied per five pots.

Vegetative Traits Measurements

Leaf Number per Plant

The number of leaves per plant was manually counted and recorded weekly, starting two weeks after transplanting.

Plant Height

Plant height was measured weekly from the base of the stem to the base of the inflorescence using a ruler and recorded in centimeters (cm).

Internode Length

Internode length was measured weekly using a digital vernier caliper and expressed in centimeters.

Stem Diameter

Stem diameter was measured at three positions (upper, middle, and lower stem) using a digital vernier caliper. The average of these three values was calculated and reported in millimeters (mm).

Number of Lateral Shoots

The number of lateral branches per plant was counted weekly and used as an indicator of plant architectural development.

Fresh and Dry Weight of Leaves, Stems, Inflorescences, and Roots

Fresh weights of individual plant organs (leaves, stems, inflorescences, and roots) were measured immediately after harvest using a digital balance (AND, accuracy 0.01 g). Root samples were gently extracted, washed, and left to drain on an inclined surface before weighing. For dry weight measurements, all samples were oven-dried at 75 °C for 48 hours and then reweighed.

Fresh and Dry Weight of Shoots

Aerial parts were separated by cutting the plant at the crown level. Leaves and stems were separated and weighed individually for both fresh and dry weights. Drying was conducted in an oven at 75 °C for 48 hours.

Total Fresh and Dry Weight

Total Weight was calculated as:

Total Fresh Weight = Fresh Weight of shoots + Fresh Weight of Roots

Total Dry Weight = Dry Weight of shoots + Dry Weight of Roots

Leaf to Total dry Weight Ratio (LWR)

This ratio was calculated using the following formula:

LWR = Leaf Dry Weight / Total Dry Weight

Leaf-to-Stem Dry Weight Ratio

This ratio was calculated by dividing the dry weight of leaves by the dry weight of stems, and it was considered a key indicator of biomass allocation strategy.

Reproductive traits Measurements

Number of Inflorescences per Plant

Inflorescences were counted weekly per plant from the onset of flowering to the end of the reproductive stage.

Number of Seeds per Plant

At the seed maturation stage, each plant was harvested at the crown level. The total number of seeds per plant was manually counted and recorded.

Thousand-Seed Weight (TSW)

One hundred seeds were randomly selected from each plant and weighed using a precision digital balance (±0.01 g). The TSW was calculated using the following formula:

TSW = (Weight of 100 Seeds × 1000) / 100

Extraction and measurement of mucilage

To determine the swelling index, one gram of seeds from each treatment was placed in a 50 ml graduated cylinder, and the volume was adjusted to 50 ml with distilled water. During the first hour of incubation, the contents of the cylinder were gently shaken every 10 minutes to ensure uniform seed settlement. After 3 hours, the volume occupied by the swollen seeds was measured and recorded in milliliters. This procedure was carried out using the standard volumetric measurement technique [16].

Biochemical traits Measurements

Photosynthetic Pigments

Photosynthetic pigments (chlorophyll a, chlorophyll b, total chlorophyll, and carotenoids) were extracted using methanol. The extract was diluted (1:5 ratio) and absorbance was measured using a spectrophotometer at wavelengths of 666 nm (chlorophyll a), 653 nm (chlorophyll b), and 470 nm (carotenoids). Concentrations were calculated using the formulas provided by [17].

Chlorophyll a = $(15.65 \times A_{666}) - (7.340 \times A_{563})$

Chlorophyll b = $(27.05 \times A_{653}) - (11.21 \times A_{666})$

Carotenoides = $(1000 \times A_{470} - 2.860 \times$ chlorophyll a-129.2×chlorophyll b/245

Total chlorophyll =chlorophyll a + chlorophyll b

Data Analysis

Statistical analyses were performed using Minitab software (version 17). Mean comparisons of main effects were conducted using Tukey's test at 1% and 5% probability levels. Graphical presentations were prepared using Microsoft Excel (2013).

RESULTS

Vegetative Traits

Analysis of variance (ANOVA) indicated that both Hoagland nutrient solution and environmental conditions (greenhouse vs. open field) significantly influenced most vegetative traits of Italian basil (*Ocimum basilicum* L.) (Tables 2 and 3).

According to Table 2, Hoagland solution had a highly significant effect ($P \leq 0.01$) on stem, shoot, leaf, root and total FW, leaf DW. Environmental factors also significantly affected shoot, leaf, root and total FW, leaf DW (Table 2). Shoot/ Root Ratio, Leaf/Stem Ratio, Stem Diameter, Number of leaves per plant, Height, Internode length, Number of lateral branches significantly affected by Hoagland solution and environment (Table 3).

Stem FW and DW

Based on the comparison of means, in the case of stem fresh weight, 50% Hoagland solution treatments in both greenhouse (8.267 g) and field (8.063 g) had the highest values, while the lowest values were for the control treatment in the field (7.22 g) and greenhouse (6.457 g). In the case of stem dry weight, 50%

Hoagland treatment in the field (6.703 g) and greenhouse (6.116 g) showed the highest values, respectively, and the lowest values were for the control treatment in the field (5.067 g) and greenhouse (4.859 g). In general, the results indicated a significant superiority of 50% Hoagland treatment over the control treatment in both cultivation environments on basil stem yield (Table 4).

Shoot FW and DW

The results showed that the highest amount of fresh shoot weight (32.912 g) was related to the treatment of 50% Hoagland's solution application in the field. In contrast, the lowest amount of this trait (25.159 g) was observed in the control plants cultivated in thegreenhouse. This difference of 753.7 g indicates the positive and simultaneous effect of nutritional treatment and favorable environmental conditions on the vegetative growth of the plant. Also, a similar pattern was observed in the case of dry shoot weight. So that the highest dry weight (18.645 g) belonged to the 50% Hoagland's treatment in the field, while the lowest value (12.858 g) was recorded for the control plants in the greenhouse environment

Table 2 Analysis of variance (mean square) of the effect of environmental factors and Hoagland nutrient solution on FW and DW of Italian basil (*Ocimum basilicum* L.)

S.O.V	Df	Stem		Shoot		Leaf		Root		Total	
		FW	DW	FW	DW	FW	DW	FW	DW	FW	DW
Hoagland	2	289.5 **	446.8 **	73.70 **	678.34 **	169.9 **	784.3 **	305.58 **	92.8 **	248.887 **	78.158 **
Environment	1	583 ns	435.1 **	889.85 **	798.43 **	623.23 **	114.13 **	467.29 **	323.8 **	224.32 **	609.76 **
Hoagland × Environment	2	707.0*	108.0*	55.2 **	614.3 *	72.1 *	45.1 *	699.2 *	729.0 *	919.6 *	151.4 *
Error	30	189.0	297.0	458.0	779.0	392.0	293.0	562.0	211.0	1.364	1.145
% CV	–	9.6	13.7	9.2	16.1	9.6	24.1	12.1	19.6	10.1	13.8

* and ** indicate significant effect at 5% and 1% probability levels, respectively

Table 3 Analysis of variance (mean square) of the effect of environmental factors and Hoagland nutrient solution on vegetative traits of Italian basil (*Ocimum basilicum* L.)

S.O.V	Df	Shoot/ Root Ratio	Leaf/Stem Ratio	Leaf/Total Ratio	Stem Diameter	Number of leaves per plant	Height	Internode length	Number of lateral branches
Hoagland	2	4414.0 *	1039.0 **	183.001 **	861.8 **	221.083 **	298.083 **	694.1 **	24.778 **
Environment	1	2426.0 ns	3646.0 **	694.0 **	694.4 **	36.667 **	188.1 **	694.4 **	30.25 **
Hoagland × Environment	2	4983.0 **	214.0 **	95.0 **	194.0 ns	194.13 *	694.22 *	861.0 *	1 **
Error	30	0.0821	0.037	0.000133	1.83	361.3	456.4	239.0	172.0
% CV	–	11.8	19.6	11.3	16.2	16.6	9.8	20.1	20.9

*, ** and ns indicate significance at 5% and 1% probability levels, and non-significance, respectively

Table 4 Interaction effects of different concentrations of Hoagland and medium on FW and DW of Italian basil

Treatments		Stem		Shoot		Leaf		Root		Total	
		FW	DW	FW	DW	FW	DW	FW	DW	FW	DW
Open Field	Control	7.22 c	5.07 c	27.21 c	14.21 cd	13.02 bc	3.68 b	16.27 c	4.5 b	43.81 c	18.72 d
	50%	8.06 ab	6.70 a	32.91 a	18.65 a	15.30 a	5.38 a	21.48 a	6.3 a	54.39 a	24.97 a
	100%	7.56 abc	5.15 c	30.28 b	15.92 b	13.93 b	4.73 a	19.16 b	5.7 a	49.10 b	21.61 b
Greenhouse	Control	6.46 d	4.86 cd	25.16 d	12.86 d	11.66 dc	3.02 b	15.56 c	3.9 b	40.50 d	16.78 e
	50%	8.27 a	6.12 b	29.12 b	15.20 bc	12.83 b	3.39b	19.16 b	5.5 a	48.12 b	20.74 bc
	100%	7.35 bc	4.75 d	26.85 c	15.46 cd	12.90 c	3.76 b	16.77 c	4.2 b	43.71 c	19.02 cd

This difference of 787.5 g was not only statistically significant, but also physiologically indicates the clear superiority of this treatment in increasing the accumulation of dry matter. Overall, the findings indicate that the 50% Hoagland treatment in the open field was identified as the most efficient treatment in improving the vegetative growth indices of Italian basil (Table 4).

Root FW and DW

The highest root fresh weight (21.48 g) was observed in Hoagland 50% and the field environment. The lowest root fresh weight was observed in the control plant and the greenhouse environment (15.557 g). The highest root dry weight (21.48 g) was observed in Hoagland 50% and the open environment. The lowest root dry weight was observed in the control plant and the greenhouse environment (3.923 g) (Table 4).

Leaf FW and DW

The comparison of the means indicated that the 50% Hoagland solution treatment in the open field was recognized as the best treatment, with the highest fresh weight (15.303 g) and the highest dry weight (5.38 g). In contrast, the lowest values of these traits belonged to the control plants in both the open field and the greenhouse. Also, plants fed with 100% Hoagland in the field and 50% Hoagland in the greenhouse showed average values (Table 4).

Total Fresh and DW

The highest total fresh weight was (3.54 g) in Hoagland 50% and field environment. The lowest total fresh weight was (40.495 g) in control plants in greenhouse. The highest total fresh weight was (3.54 g) in Hoagland 50% and field. The lowest total fresh weight was (40.495 g) in control plants in greenhouse environment (Table 4).

Shoot-to-Root DW Ratio

The results of the comparison of the average fresh weight of the leaves are shown in Fig 2. 100% Hoagland solution in the greenhouse had the highest ratio of shoot to root dry weight of

3.421. The lowest ratio of shoot to root dry weight of basil was 2.757 for the plants treated with 50% Hoagland in the (Fig. 2).

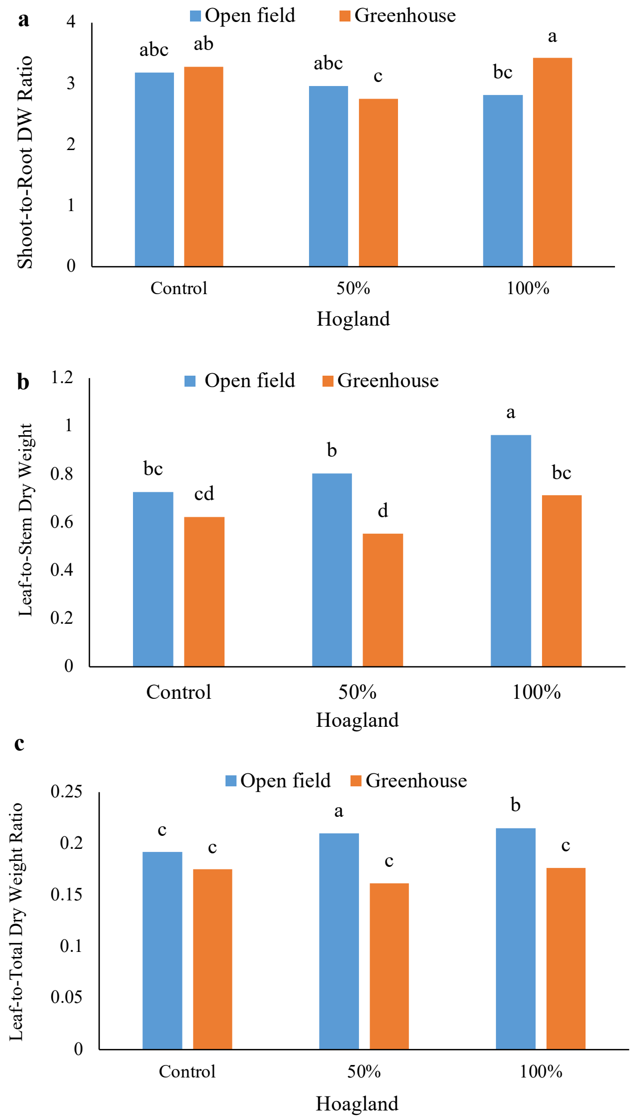

Fig. 2 Interaction effects of different concentrations of Hoagland and culture medium on shoot-to-root dw ratio (a), leaf-to-stem dw ratio (b) and leaf-to-total dw ratio (c) of Italian basil.

Leaf-to-Stem DW Ratio

According to the results of the comparison of means (Fig. 2), a significant interaction between Hoagland fertilizer treatments and environmental conditions on leaf weight ratio was observed. Accordingly, the highest leaf weight ratio (0.963) was recorded in the combined treatment of 100% Hoagland and field environment, while the lowest value of this index (0.555) was recorded in the treatment of 50% Hoagland in the greenhouse environment. This significant difference of 0.408 units indicates that the plant response to different nutritional levels is strongly influenced by environmental conditions. It seems that the combination of high concentration of nutrients (100% Hoagland) with field environmental conditions (including higher light intensity, better drainage, and other microclimatic factors) has led to the preferential allocation of photosynthetic materials to the leaf section, resulting in a significant increase in leaf weight ratio (Fig. 2).

Leaf-to-Total DW Ratio

Results showed significant effects ($P \leq 0.01$) of Hoagland solution and environment on the leaf-to-total DW ratio, with the interaction also being significant at $P \leq 0.01$. The highest ratio (0.21) was recorded under 50% Hoagland in the field, while the lowest (0.175) was observed in greenhouse control plants (Fig. 2).

Stem Diameter

Based on the results of the comparison of means, the effect of different treatments on basil stem diameter was evaluated. The highest stem diameter (6.333 mm) was observed in the combined treatment of 50% Hoagland solution and field environment, which showed a significant superiority over the other treatments. In contrast, the lowest stem diameter values were recorded in the control plants of both environments, so that 4.666 mm was measured in the field environment and 4 mm in the greenhouse. However, the statistical comparison between these two control treatments did not show a significant difference (Fig. 3).

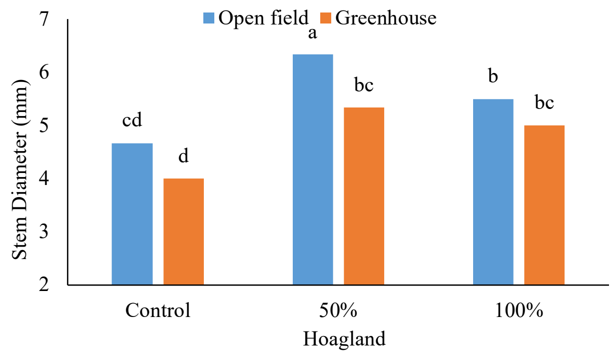

Fig. 3 Interaction effects of different concentrations of Hoagland and culture medium on stem diameter of Italian basil.

Number of Leaf per Plant

Based on the results of the comparison of means (Fig 4), the effect of different treatments on the number of leaves per basil plant was significant. The highest number of leaves per plant (45.5) was recorded in the combined treatment of cultivation in the field environment with the application of 50% Hoagland solution. In contrast, the lowest number of leaves (27.333) was recorded in the control plants in the greenhouse environment. This difference of 18.167 leaves between the two treatments indicates the significant effect of the optimal interaction between the cultivation environment and nutrition on leaf production in basil plants (Fig. 4).

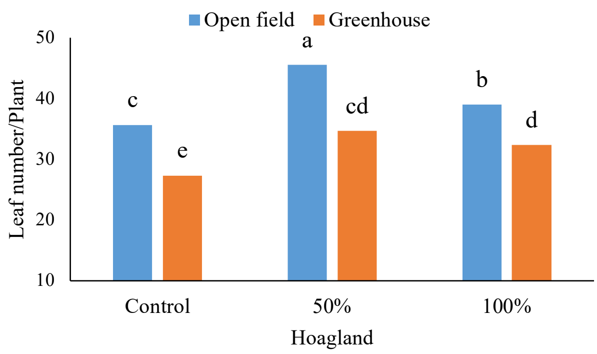

Fig. 4 Interaction effects of different concentrations of Hoagland and culture medium on leaf number of Italian basil.

Plant Height

The tallest plants (53 cm) were observed under 50% Hoagland solution in the greenhouse. The shortest heights were recorded for control plants in the field and greenhouse (44.667 cm and 41.167 cm, respectively). Intermediate heights were observed under 50% Hoagland solution in the field (52.667 cm), 100% Hoagland in the greenhouse (49.667 cm), and 100% Hoagland in the field (47.833 cm) (Fig. 5).

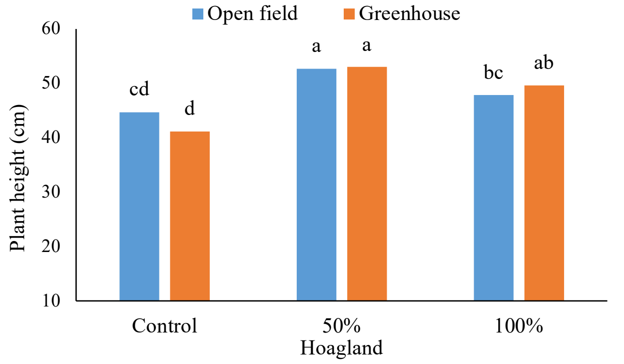

Fig. 5 Interaction effects of different concentrations of Hoagland and culture medium on plant height of Italian basil.

Internode Length

Based on the results of the comparison of means, the effect of different treatments on the length of internodes in basil plants was significant. The highest internode length (4.5 cm) was recorded in the combined greenhouse cultivation treatment with the application of 50% Hoagland solution. In contrast, the lowest internode length (3.333 cm) was related to the control plants in the field environment. This difference of 1.167 cm between the two treatments indicates a significant effect of the interaction between the cultivation conditions and nutrition on the length of internodes (Fig. 6).

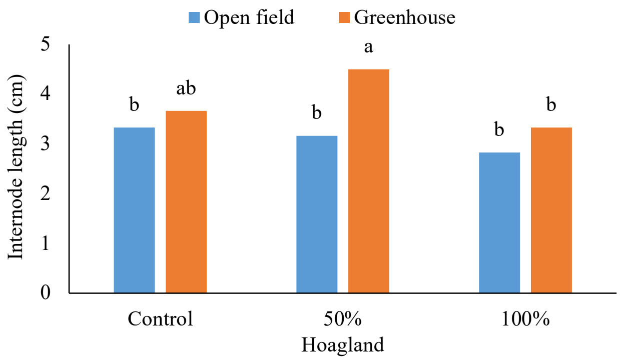

Fig. 6 Interaction effects of different concentrations of Hoagland and culture medium on internode length of Italian basil.

Number of Lateral Shoots

Based on the results of the comparison of means (Fig. 7), the interaction effect of the cultivation conditions and Hoagland solution treatments on the number of lateral shoots in Italian basil was significant. The highest number of lateral shoots (10.333) was recorded in the combined cultivation treatment in the field with the application of 50% Hoagland solution. In contrast, the lowest number of lateral shoots (5.5) was recorded in the control plants in the greenhouse. This difference of 4.833 lateral shoots indicates the significant effect of the optimal interaction between the cultivation medium and nutrition on the production and development of number of lateral shoots in Italian basil (Fig. 7).

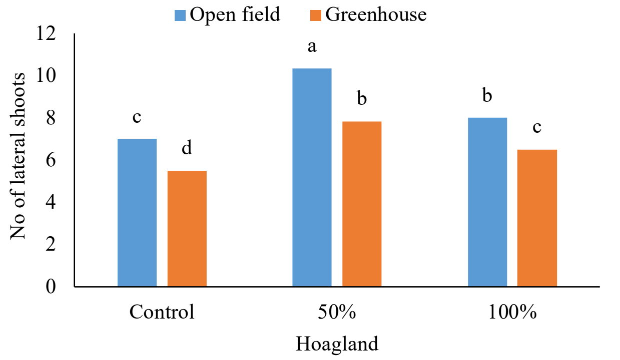

Fig. 7 Interaction effects of different concentrations of Hoagland and culture medium on the number of lateral shoots of Italian basil.

Reproductive Traits

Analysis of variance for reproductive traits (Table 5) revealed that Hoagland nutrient solution had a highly significant effect ($P \leq 0.01$) on seed number per plant, seed weight per plant, 1000-seed weight, number of inflorescences, and fresh and dry inflorescence weight. Environmental conditions also significantly influenced all reproductive traits except for 1000-seed weight. Interaction effects between nutrient solution and environment were significant for most reproductive traits, indicating that optimal nutrient supply in the field environment maximized reproductive performance.

Inflorescence FW and DW

The highest fresh inflorescence weight (473.9 g) was observed under 50% Hoagland in the field, followed by 100% Hoagland in the field (452.8 g) and 50% Hoagland in the greenhouse (313.8 g). The lowest FWs occurred in control plants under field (338.7 g) and greenhouse (325.8 g) conditions. Dry inflorescence weights followed similar trends (Fig. 8).

Inflorescence Number per Plant

Based on the results of the comparison of means (Fig. 9), the interaction effect of the cultivation medium and nutrition treatments on the number of inflorescences in basil plants was significant. The highest number of inflorescences (27 per plant) was recorded in the combined cultivation treatment in the field with the application of 50% Hoagland solution. In contrast, the lowest number of inflorescences (14.667 per plant) was related to the control plants in the greenhouse. This difference of 12.12 flowers indicates the significant effect of the optimal interaction between the cultivation medium and nutrition on the production of reproductive organs in basil plants (Fig. 9).

Table 5 Analysis of variance (mean square) of the effect of environmental factor and feeding with Hoagland nutrient solution on reproductive traits of Italian basil (*Ocimum basilicum* L.)

S.O.V	Df	Inflorescence FW	Inflorescence DW	Inflorescence Number	1000-Seed Weight	Seed Weight per Plant	Seed Number per Plant
Hoagland	2	10.032 **	10.17 **	285.444 **	1.6 **	2.165 **	517716 **
Environment	1	9.207 **	10.59 **	66.694 **	1.07 **	0.498 **	221057 **
Hoagland × Environment	2	0.589 *	0.752 *	0.023 **	0.066 *	0.294 *	29413 *
Error	30	136.2	15.0	0.928	0.042	0.0199	8844
% CV	–	12.5	18.1	21.2	6.2	20.9	20.9

* and ** indicate significant effect at 5% and 1% probability levels, respectively

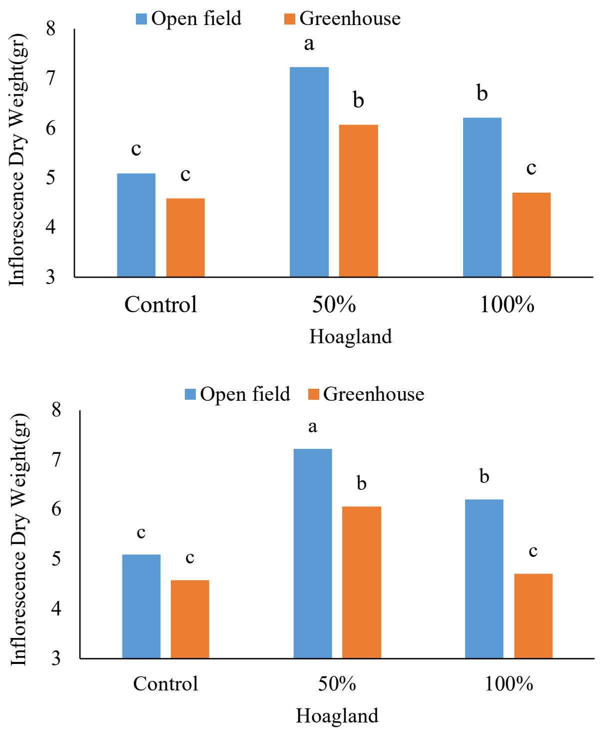

Fig. 8 Interaction effects of different concentrations of Hoagland and culture medium on inflorescence FW and DW of Italian basil

Thousand-Seed Weight

The highest weight (1.597 g) was recorded under 50% Hoagland in the field, while the lowest (1.348 g) was observed in greenhouse control plants. Intermediate values were obtained under 100% Hoagland in the field (1.498 g) and greenhouse (1.495 g) (Fig. 10).

Seed Weight per Plant

Based on the results of the comparison of means (Fig 11), the effect of different treatments on the weight of 1000 seeds of basil was significant. The highest weight of 1000 seeds (1.597 g) were recorded in the combined treatment of cultivation in the field environment with the application of 50% Hoagland solution. In contrast, the lowest weight of 1000 seeds (1.348 g) were related to the control plants in the greenhouse environment. This difference of 0.249 g indicates a significant effect of the interaction between the cultivation medium and nutrition on the distribution of dry matter and seed development. The results of the other treatments showed that 100% Hoagland solution in both the field environment (1.498 g) and the greenhouse (1.495 g) showed an average performance that, although superior to the control treatment, did not reach the level of the 50% Hoagland treatment in the field environment (Fig. 11).

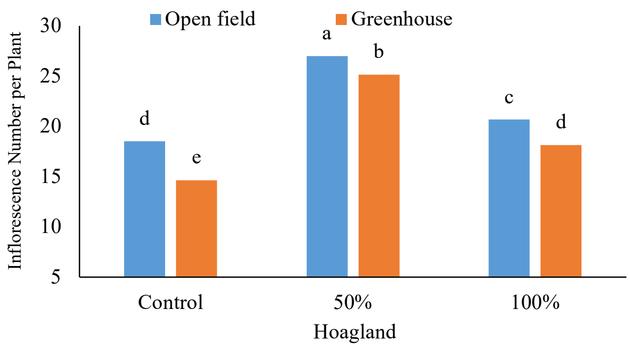

Fig. 9 Interaction effects of different concentrations of Hoagland and culture medium on inflorescence number per plant of Italian basil.

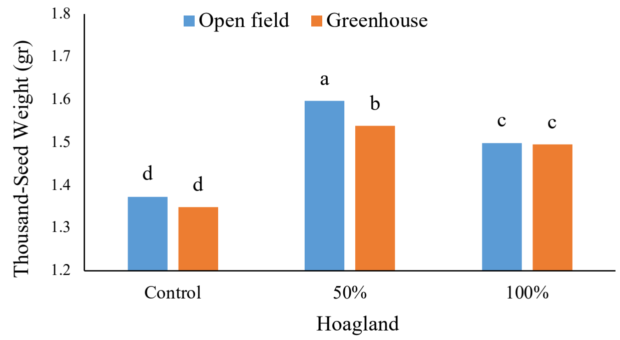

Fig. 10 Interaction effects of different concentrations of Hoagland and culture medium on thousand-seed weight of Italian basil

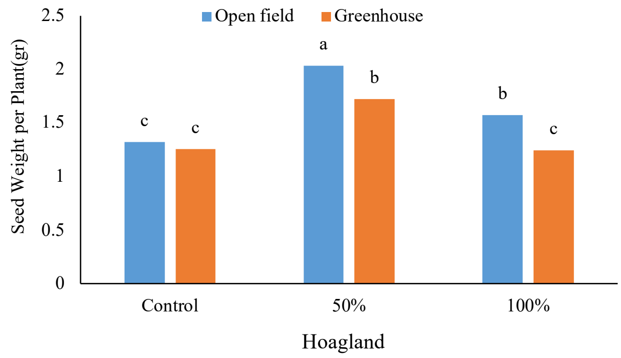

Fig. 11 Interaction effects of different concentrations of Hoagland and culture medium on thousand-seed weight of Italian basil.

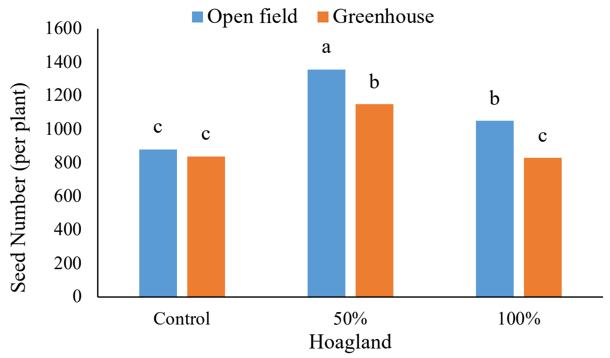

Fig. 12 Interaction effects of different concentrations of Hoagland and culture medium on seed number of Italian basil.

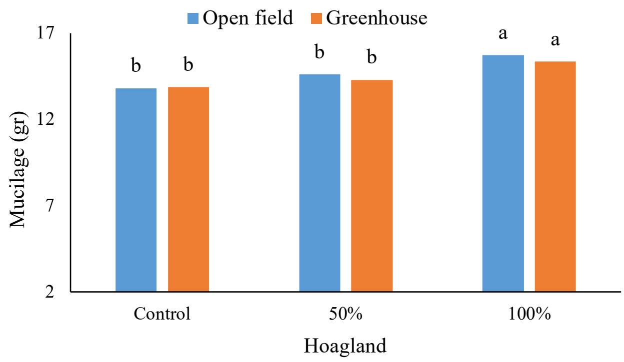

Fig. 13 Interaction effects of different concentrations of Hoagland and culture medium on mucilage of Italian basil.

Seed Number

Mean comparison showed that the highest number of seeds per plant (5,356 seeds) was observed in plants treated with 50% Hoagland solution under open-field conditions, while the lowest number of seeds per plant (837 seeds) was recorded in the control plants grown in the greenhouse. The results demonstrated that the interaction between environment and Hoagland fertilizer significantly affected the growth characteristics of Italian basil. In this study, evaluating the effects of the two factors-environment and Hoagland nutrient solution-on reproductive traits of Italian basil revealed that both factors had a significant relationship with enhanced performance of the examined traits (Fig. 12).

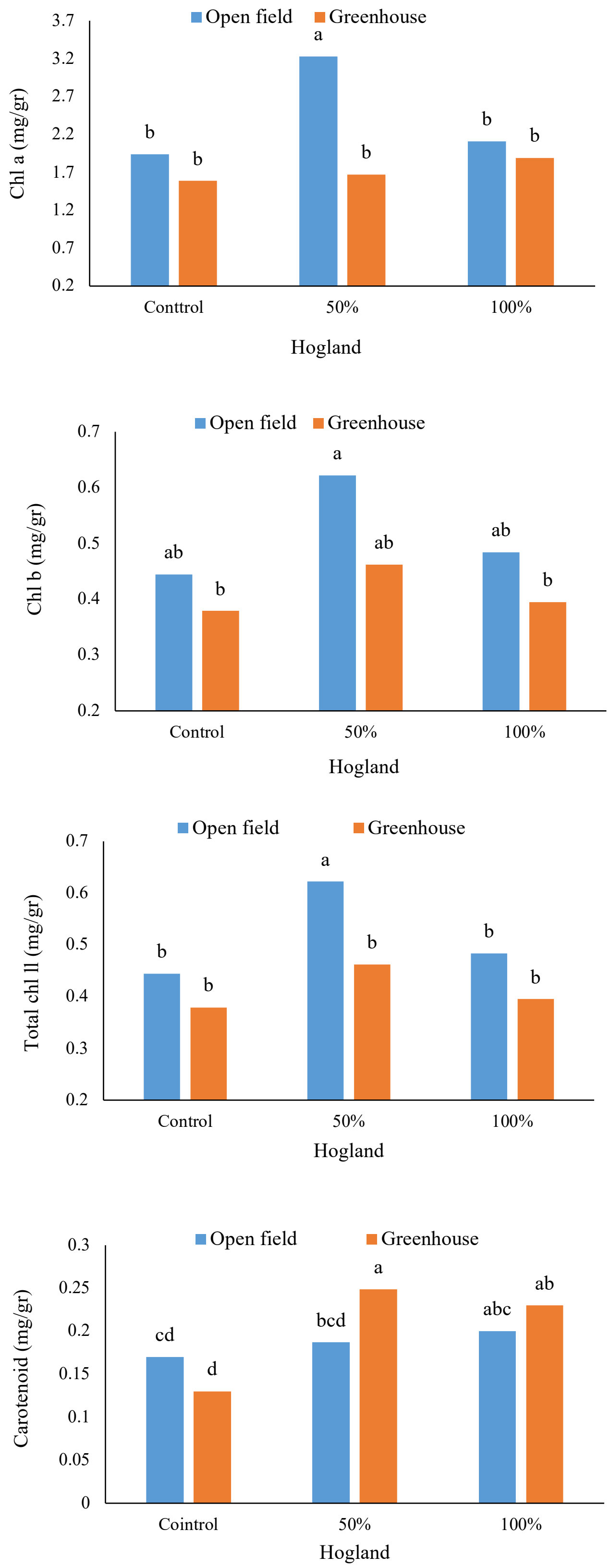

Fig. 14 Interaction effects of different concentrations of Hoagland and culture medium on a, b, total and carotenoids of Italian basil.

Mucilage

The mucilage analysis results revealed that both nutrient solution concentration and growing conditions significantly affected the basil mucilage content. Overall, mucilage content increased with higher Hoagland solution concentrations from control to 100% in both growing environments. The highest mucilage content (15.72%) was obtained from the 100% Hoagland treatment under open field conditions, while the lowest value (13.8%) was observed in the control treatment of the same environment. Although open-field cultivation maintained its advantage across all nutritional levels, this superiority became more pronounced at higher nutrient concentrations. These findings indicate that despite the positive effect of increased nutrient levels, field environmental conditions - potentially due to factors such as higher light intensity and mild environmental stresses - may serve as a stronger stimulator for mucilage synthesis in basil than nutritional status alone (Fig. 13).

Biochemical Traits

Results showed that photosynthetic pigments, including chlorophyll a, chlorophyll b, total chlorophyll, and carotenoids, were significantly affected by Hoagland nutrient solution, environment, and their interactions. Specifically, chlorophyll a and total chlorophyll contents were highly significantly enhanced ($P \leq 0.01$) by nutrient application, while carotenoids were also positively influenced. These results suggest that optimized nutrient supply enhances photosynthetic capacity, contributing to improved vegetative growth and biomass accumulation (Table 6).

Photosynthetic Pigments

Mean comparisons (Fig. 14) showed that chlorophyll a was highest under 50% Hoagland in the field (3.228 mg/g FW), followed by 100% Hoagland in the field (2.11 mg/g FW), and lowest in greenhouse control plants (1.587 mg/g FW). Chlorophyll b and total chlorophyll showed similar trends, with the highest values under 50% Hoagland in the field (0.622 mg/g FW for Chl b; 3.85 mg/g FW for total chlorophyll) and the lowest in greenhouse control plants. Carotenoids were highest under 50% Hoagland in the greenhouse (0.249 mg/g FW) and lowest in greenhouse control plants (0.153 mg/g FW).

DISCUSSION

The overall pattern of the results of this study showed that feeding with half-concentrated Hoagland solution (50%) - especially under field conditions - was superior in most vegetative traits (fresh and dry weight of organs, stem diameter, number of leaves, internode length and number of lateral branches) and reproductive behaviors (number of inflorescences, fresh and dry weight of inflorescences, thousand-seed weight and number of seeds), and the main effects of "nutrient solution" and "culture medium" were both significant and in many cases interactive. This pattern arises from two defensible physiological/ecophysiological mechanisms: (1) the sensitivity of basil yield to daily light integration (DLI) and the open field microclimate compared to the greenhouse, and (2) the existence of a nutritional optimum at medium concentrations that prevents osmotic/ionic stress caused by higher concentrations. Controlled evidence has shown that increasing DLI regularly increases fresh and dry mass, branching, and the vegetative growth index (SPAD) of basil, and its effect on growth is more important than changes in nutrient solution concentration; therefore, the superiority of field treatments in the present study is consistent with higher light and natural temperature fluctuations [18].

Table 6 Analysis of variance (mean square) of the effect of environmental factor and feeding with Hoagland nutrient solution on biochemical traits of Italian basil (*Ocimum basilicum* L.)

S.O.V	Df	Chl a	Chl b	Total Chlorophyll	Carotenoids
Hoagland	2	1.793 **	0.037 ns	2.345 **	0.012 **
Environment	1	2.215 **	0.028 ns	5.096 **	0.004 *
Hoagland × Environment	2	2.318 **	0.078 *	1.856 **	0.005 **
Error	30	0.241	0.015	0.311	0.001
% CV	–	3.42	3.13	3.19	2.13

*, ** and ns indicate significance at 5% and 1% probability levels, and non-significance, respectively

The finding that 50% Hoagland was more effective than 100% for many traits is consistent with the principles of plant nutrition. At concentrations above the required level, the total salinity/conductivity of the solution increases [18], the osmotic potential of the rhizosphere decreases, and the development or uptake of water and nutrients, especially in soil culture, is limited; a consequence that manifests itself as stunted growth of vegetative and root organs [19, 20]. Consistent with this logic, in many conditions, solutions with an EC close to 1 dS m^{-1} (e.g. ½ Hoagland) provide optimal growth performance and further increases in concentration do not provide an advantage [19].

In addition, in leafy crops and microgreens, it has been repeatedly observed that half the Hoagland concentration gives maximum biological performance and excessive increases in concentration reduce yield or at least reduce economic benefit; In a study of basil microgreens, 50% Hoagland produced the highest yield (≈3.07 kg m^{-2}), with higher concentrations resulting in reduced yield. This pattern can be explained by the "diminishing returns" of fertilizer use [21]. Other studies have also reported that electrical conductivity higher than 1 deciS/m resulting from increased concentrations has no significant positive effect on basil growth parameters [18, 20, 22].

On the other hand, the present study showed that reducing the Hoagland concentration to less than 50% will also reduce growth, which is consistent with the studies of Fallovo *et al.* [2009] and Rouphael *et al*. [2012], who have shown that very low values of electrical conductivity resulting from low concentrations of nutrients can reduce the growth rate of various plants [23, 24].

In the present study, many traits were better in the field than in the greenhouse; a difference that could be related to higher light, air turbulence, and mild mechanical/thermal stresses of the open field, ultimately leading to higher photosynthesis, carbon allocation to vegetative organs, and mechanical strength (stem diameter). Controlled studies have shown that increasing daily light integrals increases growth, branching, and even greenness index, and in some cultivars, also affects internode length and height; also, daily light integrals plays a more decisive role in basil flowering than day length [18]. A two-year field study in Turkey (comparing open field with shaded kiwi orchard) reported that the highest fresh and dry yields as well as the highest essential oil percentage were obtained for green basil under open field conditions; confirming the role of higher radiation and reduced shade in improving aroma quality/intensity [25].

On the other hand, direct comparisons of field/greenhouse systems have shown that the pattern of trait dominance can vary depending on the trait and production objective (biomass, essential oil, phenolic compounds); recent findings have also reported significant differences in secondary compounds and biomass between field and greenhouse, confirming the importance of environmental conditions [26].

The increase in stem diameter, leaf number and lateral branches in the 50% Hoagland field treatment indicates an optimal combination of nutrient availability and higher light, which stimulates branching and expands the photosynthetic surface area. Photo-morphological studies of basil show that higher light intensity increases fresh mass, dry matter and sometimes branching [27]. The changes in the leaf/stem and leaf/whole ratios in basil can be mainly explained by two mechanisms: light and osmosis: outdoors, due to higher irradiance and a more favorable ratio of red to far-infrared light, the shade avoidance response is not activated, stem elongation is reduced, and as a result, the leaf to stem ratio increases [27- 29].

On the other hand, increasing the concentration of the nutrient solution beyond the requirement increases the electrical conductivity (EC) and limits the expansion of the rhizosphere and leaf growth by reducing the osmotic potential; therefore, solutions with a median EC close to ~1 dS m^{-1} (e.g. ½ Hoagland) usually increase the leaf-to-total ratio, but higher concentrations have no advantage [19, 20]. At the level of biomass allocation pattern, meta-analyses also show that with improved light, the leaf share increases and the stem share decreases, which is consistent with the maximization of leaf/stem ratios in open-field treatments and the maximization of leaf/whole in intermediate nutrition [30]. In contrast, the maximum "shoot/root (dry weight)" ratio in 100% greenhouse Hoagland follows the principles of source-reservoir allocation: when nitrogen/dissolved ions are more abundant, carbon investment is skewed toward shoots and the root ratio is reduced; in relative nutrient deficiency or lower light, plants generally show higher root/shoot ratios [31].

The significant increase in chlorophyll a and total chlorophyll with mineral nutrition in this study is consistent with the known relationship between nitrogen nutrition and green leaf pigmentation; greenhouse studies of basil have shown that SPAD/atLEAF instrument readings are positively and strongly correlated with nitrogen concentration and fresh mass, so improving nitrogen status can lead to increased chlorophyll and ultimately increased biomass [32]. In the case of carotenoids, the patterns of increase are not only influenced by nutrition, but also by the environment and "mild stresses"; in basil and other plants, salinity/osmotic stress as well as changes in light intensity can cause a greater accumulation of carotenoids as an antioxidant shield, which explains the extrapolation of the patterns observed in some greenhouse treatments [33].

The increase in fresh/dry weight of inflorescences, number of inflorescences and number of seeds in the 50% Hoagland of the field indicates that the moderate nutritional optimum, together with the higher daily light integral in the field conditions, enhanced both the formation and differentiation of reproductive organs and supported the capacity for seed filling. Recent studies have confirmed that daily light integral plays a stronger role than photoperiod in accelerating flowering/reducing nodes below the first open flower; therefore, it is expected that reproductive indices will improve in the field with higher cumulative light [34]. On the other hand, nitrogen nutrition can increase inflorescence number, although its effect on other aspects of flowering may be limited; this is consistent with the observation of improved inflorescence number in nitrogen nutrition treatments [35]. From a source-sink physiology perspective, balanced nutrition allows for carbon allocation to the grain reservoir without disrupting field transport and grain filling capacity due to high salinity/osmosis (at higher concentrations) [36]. These outcomes are further supported by shifts in biomass allocation patterns (e.g., increased leaf-to-stem and leaf-to-total dry weight ratios) and pigment content (notably chlorophyll a), which collectively demonstrate that moderate nutrient input coupled with high light availability not only supports vegetative growth but also enhances reproductive efficiency. These findings reinforce the concept of diminishing returns at high nutrient concentrations and highlight the ecological and agronomic value of optimizing rather than maximizing fertilization, particularly in light-responsive species such as basil.

CONCLUSION

The findings of this study clearly demonstrate that the application of 50% Hoagland nutrient solution, particularly under open-field conditions, significantly enhances the vegetative and reproductive performance of Italian basil (*Ocimum basilicum* L.). This treatment resulted in superior biomass accumulation (fresh and dry weight of all organs), improved morphological traits (stem diameter, leaf number, branching, and plant height), and enhanced reproductive outcomes (number and weight of inflorescences, seed number, and thousand-seed weight) compared to both the control and full-strength treatments. The results suggest that moderate nutrient availability—combined with favorable environmental conditions such as higher light intensity and field microclimate—provides a more efficient balance between growth, resource use, and physiological adaptation. Moreover, the superiority of 50% Hoagland over 100% in many traits highlights the importance of avoiding overfertilization, which may impose osmotic stress without delivering proportional gains. Overall, these findings emphasize that optimizing rather than maximizing fertilization, particularly under high-light environments, is a more sustainable and effective strategy for basil cultivation.

Consent for Publication

Not Applicable.

Data Availability

The data supporting the findings of this study are available from the corresponding author upon reasonable request.

Conflict of Interests

The authors have not declared any conflict of interest.

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Authors' contributions

F.A: Investigation, Methodology, Data curation; M. Sm: Data curation, Formal analysis, Writing-original draft; M.A: Supervision, Conceptualization, Project administration P.F: Data curation, Formal analysis; M.N: Writing-review and editing, Data Analysis.

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