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CARNATION VEGETATIVE AND FLOWERING PERFORMANCE IN BASIL-TREATED SOIL AND *FUSARIUM OXYSPORUM* RESISTANCE

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Abstract

Carnation (*Dianthus caryophyllus*) is a key ornamental species widely cultivated across the Mediterranean and Middle East. This plant is an economically valuable decorative horticultural element worldwide, recognized for its esthetic, cultural, and medicinal importance. However, *Fusarium oxysporum*, a soil-borne fungal pathogen responsible for severe wilt diseases, frequently hinders its commercial production. The present study evaluated the influence of 5% (w/w) soil incorporation of dried *Ocimum basilicum* L. (basil) leaves on the performance of carnation plants challenged with *F. oxysporum* isolates ARF1 and ARF2. Both fungal strains were obtained from locally diseased plants and molecularly characterized by sequencing the rDNA internal transcribed spacer (ITS) region and the translation elongation factor 1-alpha (tef1α) gene. Gas chromatography–mass spectrometry (GC–MS) profiling of essential oils from the basil-amended soil indicated compositional shifts, including reductions in certain monoterpenes (e.g., linalool, geraniol, and cineole) and some sesquiterpenes, along with elevated levels of others such as caryophyllene. Carnation plants grown in basil-amended soil exhibited markedly lower wilt severity than those cultivated in unamended, infected soil. Furthermore, basil amendment substantially improved plant growth and physiological traits—such as plant height, branch proliferation, fresh and dry biomass, root elongation, flower production, chlorophyll content, photosynthetic rate, transpiration, and stomatal conductance. These findings suggest that soil enrichment with dried basil leaves mitigates *F. oxysporum* infection through multiple mechanisms, including enhanced physiological resilience, enhanced gas exchange parameters, and modulation of soil biochemical interactions. Overall, the results highlight the potential of *O. basilicum* leaf residues as a sustainable, plant-based soil amendment for reducing Fusarium wilt and improving overall vigor in carnation cultivation.

Key words: Dianthus caryophyllus; Carnation; Basil; Soil amendment; *Fusarium oxysporum*

Introduction

Carnations (*Dianthus caryophyllus*) are among the most widely cultivated and economically valuable decorative horticultural plants worldwide, recognized for its esthetic, cultural, and medicinal importance (Whealy, 1992). This species belongs to the family *Caryophyllaceae* and is native to the Mediterranean region of southern Europe (Fassou, 2022). Although inherently a herbaceous perennial, carnation is commonly grown as an annual in commercial horticultural systems. Its characteristic fragrance is primarily attributed to the presence of eugenol, benzyl benzoate, and methyl benzoate (Wang *et al*., 2025). Prior research has demonstrated that carnations and their related species possess various medicinal attributes, such as antioxidant, insecticidal, antimicrobial, anticancer, and anti-inflammatory effects, alongside traditional applications in folklore for treating wounds and gastrointestinal ailments in China, Japan, and Korea (Chandra *et al*., 2016).These effects are associated with numerous phytochemical constituents, underscoring the plant's significant pharmaceutical potential (Chandra *et al*., 2016; Survase *et al*., 2024). Despite its economic importance, carnation production is often limited by various diseases caused by bacterial, fungal, and viral pathogens, which adversely affect nursery and field performance, leading to considerable economic losses (Wolcan *et al*., 2016). To address these challenges, breeding programs aim to develop cultivars with enhanced pathogen resistance, greater color diversity, and improved postharvest longevity (Mekapogu *et al*., 2023).

Fusarium wilt, caused by *Fusarium oxysporum*, is one of the major soil-borne diseases affecting carnation cultivation. The pathogen penetrates the root system, colonizes the vascular tissues, and disrupts nutrient and water transport, ultimately leading to chlorosis, wilting, leaf blight, vascular discoloration, and plant death (Filgueira-Duarte *et al*., 2024). The presence of F. oxysporum infecting horticultural crops has been confirmed in several countries, such as Saudia Arabia, Egypt, the United States, and India, through molecular identification using ITS, TEF-1α, RPB1, and RPB2 gene sequencing (Cañizares *et al*., 2015; Filgueira-Duarte *et al*., 2024; Al-Yafrasi *et al*., 2025). Managing this pathogen remains challenging and typically requires an integrated approach that combines chemical, cultural, and biological control strategies.

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Ocimum basilicum L. (basil), a Lamiaceae family member, has long been recognized as a valuable medicinal and aromatic herb (Patil *et al*., 2024). The species naturally occurs in Saudi Arabia and is extensively cultivated across the Middle East. Traditionally, both the fresh and dried forms of basil have been used in herbal remedies to relieve digestive disturbances, menstrual pain, respiratory ailments such as cough and asthma, fever, and headaches. Fresh basil leaves are widely used as a flavoring component in beverages and as a culinary garnish for salads in several cultures.

This study investigates the potential application of basil plants as a soil supplement to enhance carnation growth and prevent *F. oxysporum* infections. Basil plants are known to fix nitrogen from the environment; hence, the herb was used as a soil amendment in the current investigation. The morphological and physiological characteristics of the treated plants were examined. The use of dried basil as a biotic stress alleviator was investigated as a unique method in carnation production. These activities were used to identify the mechanisms, which are detailed here.

Materials and Methods

Plant materials: *Ocimum basilicum* L (basil) plantlets (10 cm) were procured from commercial nurseries to be used as soil amendment. Plants were identified at King Saud University. The plants were grown in a greenhouse. At 12:00 p.m., the photosynthetic active radiation was 900 W m-2, relative humidity varied between 67% and 81%, and the average growth temperature was 15.2°C at night and 24.3°C during the day. Up until drop-off, the plants were watered every two days. The entire vegetative portion, including the leaves and shoots, was collected after 8 weeks. After being ground, the plant material was air-dried in a dark environment until the moisture level reached 6%–7%. Two sands, one peat, and one clay were combined to create a mixture with the specific physicochemical characteristics listed in Table 1.

Mineral composition: Phosphorus was determined using the phosphomolybdic acid method (Neal *et al*., 2000), and nitrogen composition was assessed using the Kjeldahl method (Nelson & Sommers, 1972). The organic carbon content was determined following the procedure described by Emamgholizadeh *et al*., (2018). The elemental concentrations of Fe, Cu, Mn, and Zn were quantified using atomic absorption spectrophotometry (AAS Vario 6, Germany). Dried basil plant material was incorporated into sandy soil at a 5% (w/w; plant material-to-soil) ratio. The resulting mixture, containing the vegetative portions of basil, was transferred into 2-L pots and stored in a glasshouse. The basil residues were decomposed under controlled temperatures ranging from 18°C to 28°C to promote a reduction in the C/N ratio.

The control treatments consisted of unamended pots containing only the soil–basil mixture without additional processing (unamended soil). Each soil treatment was replicated 3 times and totalling sixty pots for the complete experiment. The pots were irrigated weekly and kept under greenhouse conditions until the basil material had fully decomposed. The decomposition progress was monitored through changes in the C/N ratio. Soil samples (10 g each) were collected at 0, 15, 30, 60, and 90 days to determine organic carbon levels following the method described by Emamgholizadeh *et al*., (2018). The total nitrogen content was analyzed using the Kjeldahl method (Nelson & Sommers, 1972). The experimental trials were conducted during the growing seasons of 2022 and 2023.

Table 1. Soil properties.

Composition	Unit	Soil	LSD *P=0.05*
pH		8.0	0.09
EC	(mmho cm^{-1})	0.968	0.20
Water-holding capacity of soil	%	43.5	2.0
organic carbon	(SOC, %)	0.52	0.03
N	g ha^{-1}	184 x 10^3	3.1
P	g ha^{-1}	17.7 x 10^3	1.2
K	g ha^{-1}	194.1 x 10^3	3.2
Fe	mg kg^{-1}	4.4	0.1
Cu	mg kg^{-1}	0.41	0.02
Zn	mg kg^{-1}	5.1	0.03
Mn	mg kg^{-1}	2.6	0.01

Analyses of essential oils: In improved soil, the volatile components include three treatments: After 0, 15, and 30 days. The soil's volatile oil content (2 kg) for each treatment and control was assessed by hydro distillation with a Clevenger-style device. The oil was filtered, dried over anhydrous sodium sulfate, and stored at 4°C in a dark environment (Elansary *et al*., 2016). An Agilent GC 7890A single quadrupole mass spectrometer with a triple axis detector (5975 C) was utilized for GC-MS analysis. High-purity helium was used as the carrier gas in an Agilent HP-5MS capillary column (30 m × 0.25 mm i.d., 0.25 µm film thickness) operated at a constant flow rate of 1.2 mL min^{-1}. The injector was maintained at 250°C and used in splitless mode with a split ratio of 20:1. The mass spectrometer interface (Quad) and ion source were set at 150°C and 230°C, respectively. The oven temperature program began at 40°C (held for 1 min), increased to 150°C at a rate of 5°C min^{-1} (held for 1 min), and was then ramped to 280°C at the same rate and held for an additional minute. A 1 µL sample was injected, and data acquisition was performed over a scan range of 40–600 m/z, using an electron impact energy of 70 eV and a solvent delay of 4 min.

The total analysis time per sample was approximately 51 min. Compound identification was achieved by comparing the retention times and calculated retention indices of n-alkanes (C_{10}–C_{36}) with those of reference standards, supported by mass spectral matching using the National Institute of Standards and Technology (NIST) (version 2.0) and WILEY libraries, as well as comparison with published literature (Adams, 2007).

Carnation plants: In two consecutive seasons (January 2022 and 2023), young carnation plantlets with one to two buds (5 cm in height) were procured from nearby nurseries. King Saud University identified and vetted the plants. The plants were kept in a greenhouse before the tests began. The photosynthetic active radiation was 900 W m-2 at 12:00 p.m., the relative humidity varied between 67% and 81%, and the average growth temperature was 15.1°C at night and 25.2°C during the day. Up until drop-off, the plants were watered every two days.

Fungal strains, inoculum preparation, and soil infestation

Isolation, purification, and preservation of fusarium strains: Root samples were collected from carnation plants in Riyadh, Saudi Arabia, exhibiting typical symptoms of wilt disease, including vascular darkening, yellowing, and wilting. The roots were washed thoroughly with tap water, rinsed with distilled water, and cut into approximately 0.5 cm^3 segments. Surface sterilization was performed by immersing the pieces in 1% sodium hypochlorite for 2–3 min, followed by a 30-s treatment with 70% ethanol, after which the root segments were air-dried on sterile filter paper. Sterilized pieces (four per plate) were then placed on Difco potato dextrose agar (PDA) plates supplemented with 100 μg mL^{-1} streptomycin sulfate and incubated at 25°C for 7 days. Emerging Fusarium colonies were subcultured by transferring small agar blocks from the colony margins to fresh PDA plates and incubating for an additional 5–7 days at 25 °C to obtain pure cultures (Adams, 2007). Single-spore isolates were maintained on PDA slants at 25°C for 7 days and subsequently stored in 2 mL cryogenic vials containing 15% glycerol at −80°C for long-term preservation (Thermo Fisher Scientific, Rochester, NY, USA). Pure Fusarium isolates were grown on both PDA and Spezieller Nährstoffarmer Agar (SNA) for 7–8 days at 25°C for morphological characterization. Colony features (including color and pigmentation on PDA) were recorded, and structures such as macroconidia, microconidia, chlamydospores, and conidiophores were observed by microscopic examination. Species-level identification was performed according to Leslie and Summerell's criteria (Anon., 2006).

DNA extraction, PCR amplification, and sequencing: Fungal mycelia were ground in liquid nitrogen using a mortar and pestle and transferred into 1.5 mL Eppendorf tubes for genomic DNA extraction, which was performed using the CTAB method. DNA quality and quantity were assessed on a 1% agarose gel stained with 1 μg/mL acridine orange. Subsequently, the samples were diluted to a final concentration of 20 ng/μL for downstream applications (Chi *et al*., 2009).

For polymerase chain reaction (PCR) amplification and phylogenetic analysis, the internal transcribed spacer (ITS) region of rDNA and the translation elongation factor 1-alpha (tef1α) gene were targeted. The primers ITS4 (5′-TCCTCCGCTTATTGATATGC-3′) and ITS5 (5′-GGAA GTAAAAGTCGTAACAAGG-3′) were used to amplify the ITS region, while EF1 (5′-ATGGGTAAGGARGACAAGAC-3′) and EF2 (5′-GGARGTACCAGTSATCATGTT-3′) were employed for tef1α (Adams, 2007). Each 30 μL PCR reaction contained 1.0 unit of Taq DNA polymerase (Bioline), 1.5 mM $MgCl_2$, 0.2 mM dNTPs, 0.25 μM of each primer, 1 ng/μL genomic DNA, and 2× Promega master mix × PCR buffer (Bioline US Inc., Taunton, MA, USA). The thermal cycling program consisted of an initial denaturation at 94°C for 3 min, followed by 30 cycles of 94°C for 1 min, 55°C for 1 min (ITS rDNA) or 56°C for 1 min (tef1α), and 72°C for 1 min, with a final extension at 72°C for 5 min. PCR amplification was performed using a TC-412 thermocycler (Techne, Cambridge, UK).

The amplification products were resolved on a 1.5% agarose gel in 0.5× TBE buffer containing 1 μg/mL acridine orange, alongside the HyperLadder IV molecular weight marker (BioLine). The gels were visualized and photographed under UV light. Purification and sequencing of polymerase chain reaction (PCR) amplicons were performed at the University of Kentucky Advanced Genetic Technologies Center. DNA sequence alignment and editing were performed using BioEdit (http://www.mbio.ncsu.edu/BioEdit/bioedit.html). The MycoBank database (https://fusarium.mycobank.org) was used to identify two Fusarium strains, ARF1 and ARF2. A comparison of molecular traits served as the basis for the identification of a novel gene. The accession numbers for these strains were provided by the NCBI database. The strains were then classified within the *F. oxysporum* species complex using available taxonomic data.

Carnation cultivation and fungal inoculation: Seedlings described in Section 2.3 were gently removed from their containers and rinsed with sterile water. Carnation roots were submerged in spore suspensions of *F. oxysporum* ARF1 or ARF2 for 10 min with continuous agitation at 65 rpm using a rotary shaker, according to the specific treatment. Plants were transplanted into the pots described in Section 1, which had been pre-filled with the prepared soil as detailed in Table 2, following inoculation.

During the initial 48 h post-inoculation, the plants were maintained in a greenhouse environment with elevated relative humidity (approximately 80%, achieved via a misting system) and a controlled temperature of 25 ± 1°C. Non-inoculated plants, serving as experimental controls, were transferred to the same pots as described in Section 1.

Table 2. Treatments applied and their appreciations.

Treatment abbreviation	Treatments
C	Untreated control carnation plants
Cbasil	soil amended with basil
Cbasil+ARF1	soil amended with Basil + *F. oxysporum* ARF1 inocullation
Cbasil+ARF2	soil amended with Basil + *F. oxysporum* ARF1 inoculation
ARF1	soil + *F. oxysporum* ARF1 inoculation
ARF2	soil + *F. oxysporum* ARF2 inoculation

Disease assessment: The roots and crowns of carnation plants were examined for vascular discoloration to evaluate infection, and potential pathogens were isolated using Komada's medium. Plants showing no disease symptoms and from which the pathogen could not be recovered were classified as healthy (Adams, 2007). Disease severity was quantified using a standard 0–5 scale based on the proportion of leaf area affected, monitored over a 50-day period post-inoculation (d.p.i.): 0 = no visible symptoms, 1 = <5% leaf area affected, 2 = 5%–20%, 3 = 20%–40%, 4 = 40%–60%, and 5 = >60% leaf area affected. Several morphological parameters were recorded at 8 weeks post-inoculation, including root length, fresh weight (FW, g) and dry weight (DW, g) of roots, number of flowers per plant, plant height (cm), number of branches per plant, and leaf area per plant (cm^2). The dry weight was determined by first air-drying the samples at room temperature (20°C) and subsequently in an oven at 30°C until a constant weight was achieved.

Carnation physiological measurements: Total chlorophyll in leaves was measured using the methodology described by Kim and Wolyn (2014). The net photosynthetic rate (A), transpiration rate (E), and stomatal conductance (gs) of mature leaves were measured at the conclusion of the experiment at 10:00 a.m. using portable gas exchange equipment (ADC LCi, Bioscientific Ltd., Hoddesdon, UK) (Kim & Wolyn 2014). Three duplicate experiments were conducted.

Statistical analysis

The experiment was arranged in a randomized complete block design, with each of the five treatments replicated across 14 pots (Table 2). Non-inoculated carnation plants grown in non-amended soil served as the control. The remaining four treatments consisted of plants inoculated with *F. oxysporum* isolates ARF1 or ARF2 with or without the addition of 5% (w/w) basil plant material as a soil amendment, as detailed in Table 2.

This study was conducted over two consecutive seasons (2022–2023). Data from both years were combined for analysis, as no significant differences were detected between the two trials. The mean values from the two experiments were averaged for statistical evaluation. Differences among treatment means were determined using the least significant difference (LSD) test at a significance level of $p<0.05$. Bartlett's test confirmed the homogeneity of variances across treatments, thereby validating the results of the combined analysis. Statistical analyses were performed using Statistica version 7.06 (Statsoft Inc.).

Results

Essential oil composition: As the trial progressed, the percentage of essential oils in the treated soil decreased (Table 3). The percentage of oil output decreased over time, falling by 98% after 60 days.

Table 4 displays the qualitative and quantitative compositions of the essential oil in the distilled material of the 5% basil-amended soil used in the experiment. Significant variations in the main volatiles during the trial were revealed by GC/MS analysis of the essential oil (Table 4). Linalool (41.32%), cis-geraniol (11%), and isomethyleugenol (10.5%) were the main essential oil constituents. The percentage of certain essential oils, such as linalool (41.32%), cis-geraniol (11%), and isomethyleugenol (10.5%), decreased over time. By the end of the experiment, the concentration of linalool, the main essential oil component, had dropped from 41.32% of the original basil oil to 70%. Similar trends were observed for other major constituents, including isomethyleugenol (10.5%) and cis-geraniol (11%).

Identification of *F. oxysporum* strains: The two carnation Fusarium strains were identified using the NCBI accession codes and the MycoBank database, confirming that they are both members of the *F. oxysporum* species complex. They matched those published by our research group ARF1 (Accession No. PP819611) and ARF2 (Accession No. PP819612) for ITS-Rdna and ARF1 (Accession No. BankIt2866081) and ARF2 (Accession No. BankIt2866082) for *tef1 α* (Al-Yafrasi *et al*., 2025).

Disease evaluation: The visual disease evaluation of carnation plants from 0 to 50 days after d.p.i is shown in Fig. 1. Inoculation with F. oxysporum ARF1 or ARF2. The visual evaluation at 20–50 days post-infection showed that treatments of Soil + 5% (w/w) basil plant material + inoculated Carnation with F. oxysporum ARF1 or ARF2 (Cbasil ARF1 and ARF2) had significantly lower disease indices in Carnation plants than those with non-amended soil that had been infected with *F. oxysporum* ARF1 or ARF2 (ARF1 and ARF2). Additionally, as the experiment progressed (20–50 days), the improved soil treatments outperformed ARF1 and ARF2 and maintained this notable difference.

The visual evaluation of Cbasil ARF1 showed that treatments of soil containing 5% (w/w) basil plant material had a considerably lower disease index in carnation plants than Cbasil ARF2, suggesting that ARF2 is more pathogenic.

Table 3. Soil essential oil composition at 0, 15, 30, 60, and 90 d.

Treatment	Essential oil (µL 100^{-1} g of soil)				
	0d	15d	30d	60d	90d
C	0.00d*	0.00d	0.00d	0.00d	0.00d
5% basil soil amendment	172.32 ± 4.1a	31.83 ± 1.5 b	6.41 ± 0.25c	3.23 ± 0.32d	0.82 ± 0.05e

*Significant differences at $p<0.05$ is indicated by different letters within the same row

Table 4. The main constituents of basil oil.

	Main components of essential oils	RI*	LRI**	Concentration %				
				0d	15d	30d	60d	90d
1.	1,8-Cineole	1040	1040	0.61 ± 0.06	0.32 ± 0.01	0.09 ± 0.01	0.0 ± 0.00	0.0 ± 0.0
2.	Linaool	1117	1117	41.21 ± 0.1	33.42 ± 0.2	21.42 ± 0.1	14.13 ± 0.1	12.15 ± 0.01
3.	cis-Geraniol	1252	1253	11.02 ± 0.8	8.41 ± 0.1	6.13 ± 0.1	4.03 ± 0.1	2.25 ± 0.01
4.	Geranyl acetate	1377	1377	3.29 ± 0.05	2.62 ± 0.1	1.11 ± 0.1	0.14 ± 0.1	0.11 ± 0.01
5.	Elemene	1379	1380	3.00 ± 0.1	2.51 ± 0.1	1.72 ± 0.1	1.19 ± 0.3	0.94 ± 0.01
6.	Isomethyleugenol	1397	1398	10.60 ± 0.1	8.70 ± 0.1	7.73 ± 0.1	5.61 ± 0.1	3.35 ± 0.02
7.	trans-α-Bergamotene	1434	1434	4.43 ± 0.1	3.41 ± 0.2	3.34 ± 0.2	2.24 ± 0.1	1.42 ± 0.05
8.	Caryophyllene	1443	1443	1.51 ± 0.01	0.89 ± 0.1	0.81 ± 0.05	0.65 ± 0.08	0.15 ± 0.03
9.	α-Muurolol	1642	1642	6.35 ± 0.1	5.07 ± 0.1	4.12 ± 0.02	2.03 ± 0.1	1.15 ± 0.03

*Retention index. ** Literature retention index (Elansary & Ashmawy, 2013)

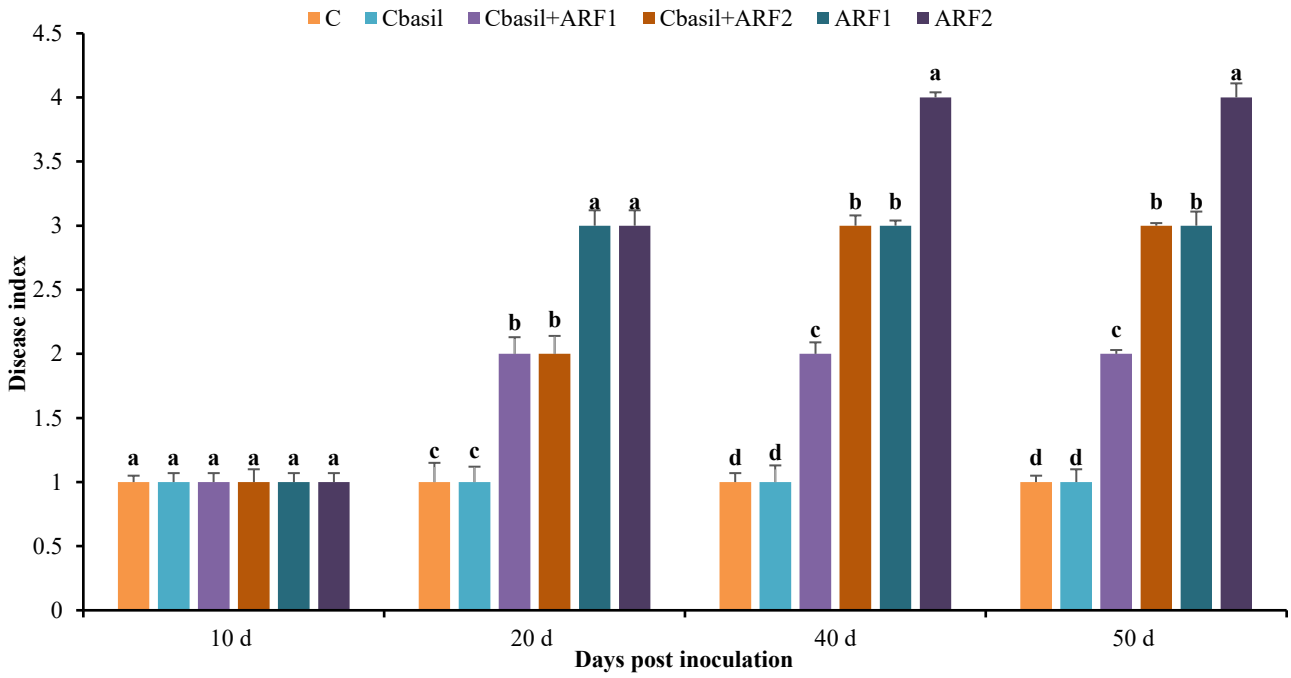

Fig. 1. Visual disease assessment of carnation plants grown in soil supplemented with 5% (w/w) basil plant material during an infection-time course with *F. oxysporum* ARF1 or ARF2 from 0 to 50 days post-inoculation (d.p.i.) In order to calculate the disease index, the percentage of damaged leaf area was calculated as follows: 0 indicates no visible disease damage, 1 indicates less than 5% damage, 2 indicates between 5% and 20% damage, 3 indicates 20%–40% damage, 4 indicates 40%–60% damage, and 5 indicates more than 60% damage. Five replications were used to calculate the means and SEs of the illness index. Significant changes at $p \leq 0.05$ are indicated by different letters among treatments.

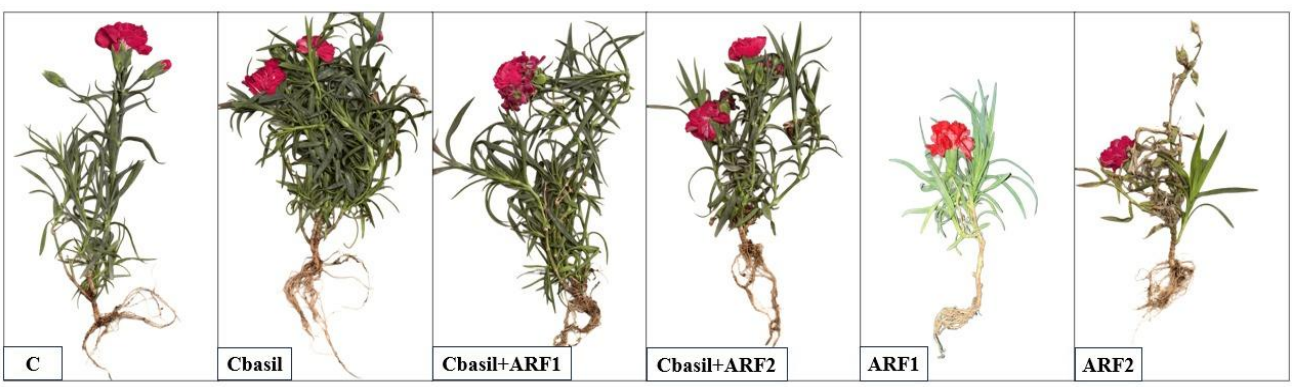

Fig. 2. Morphological performance of carnations after *F. oxysporum* (ARF1 and ARF2) inoculations and the addition of basil plant material as a soil supplement (5% of the soil mixture, w:w).

Figures 2 and 3 show the effects of *F. oxysporum* inoculation and basil soil amendment on carnation morphology. Plants infected with *F. oxysporum* (ARF1 and ARF2) exhibited reduced vegetative growth compared with the non-inoculated control (C), including shorter plant height, fewer branches, decreased fresh and dry biomass of both shoots and roots, shorter root length, and fewer flowers per plant. The addition of 5% basil vegetative material to the soil significantly improved these parameters in infected plants, resulting in increased plant height, branch number, shoot and root biomass, root length, and flower production relative to both the infected treatments without amendment and the non-infected control. ARF1-inoculated plants generally performed better than ARF2-inoculated plants, showing higher plant numbers, more branches, and greater fresh and dry weights in both amended and nonamended soils.

Gas exchange measurements and chlorophyll content: At the conclusion of the experiment, the gas exchange parameters and chlorophyll content of the leaves of plants treated with *F. oxysporum* (ARF1/ARF2) inoculations and basil as a soil supplement (5% of soil mixture, w:w) were measured (Fig. 4). Compared with the non-infected control (C), plants infected with *F. oxysporum* (ARF1 and ARF2) exhibited reduced photosynthetic and transpiration rates, stomatal conductance, and chlorophyll content. Compared with control, the addition of basil leaves as a soil amendment considerably increased the photosynthetic and transpiration rates, conductance, and chlorophyll content. Compared with plants infected with *F. oxysporum* (ARF2), carnation plants treated with soil amendment and inoculated with ARF1 exhibited increased photosynthetic and transpiration rates, conductance, and chlorophyll content. The use of basil soil amendments in carnation plants produced the best gas exchange performance through increased photosynthetic and transpiration rates as well as conductance.

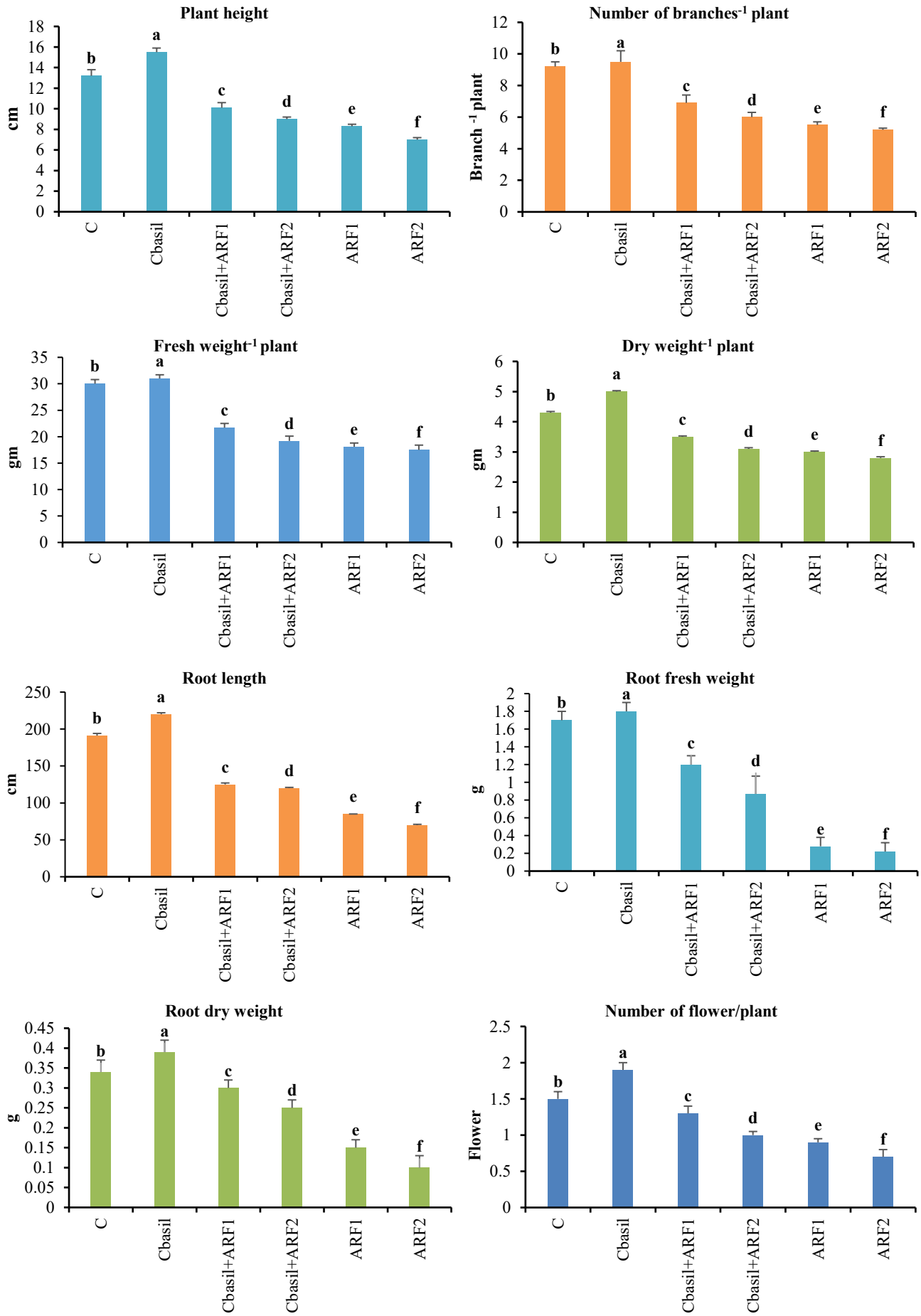

Fig. 3. Mean morphological performance of carnations after inoculation with *F. oxysporum* (ARF1 and ARF2) and addition of basil plant material as a soil supplement (5% of the soil mixture, w:w). Significant changes at $p \leq 0.05$ are indicated by different letters among treatments.

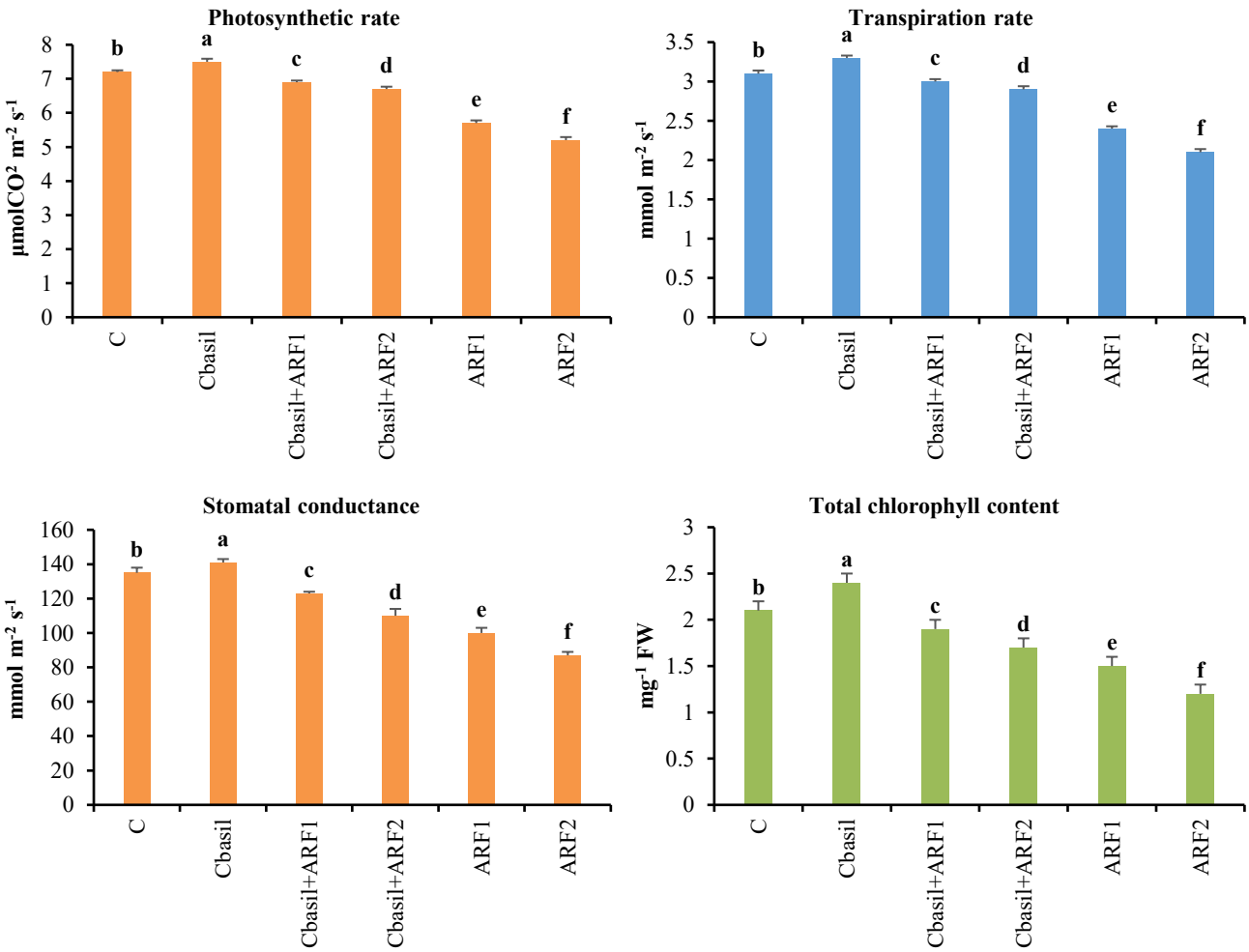

Fig. 4. Mean values of gas exchange parameters and chlorophyll content. After applying basil plant material as a soil amendment and *F. oxysporum* (ARF1 / ARF2) inoculations, the mean values of the carnation photosynthetic rate (Pn, µmol CO^2 m^{-2} s^{-1}), transpiration rate (E, mmol m^{-2} s^{-1}), stomatal conductance (gs, mmol m-2 s^{-1}), and total chlorophyll content (mg-1FW) were determined. Significant changes at p≤0.05 are indicated by different letters among treatments.

Table 5. Agar disc diffusion method showing the antifungal action of the essential oils of basil.

Fungi	Inhibition zone (mm)	MIC (µg/mL)	Negative control (mm)	Positive control (Fluconazole 0.5 mg/Ml)
F.oxysporum ARF1	23.73a	0.125	-	0.5
F. oxysporum ARF2	20.06b	0.125	-	0.5

***In vitro* antifungal assay:** The antifungal activity of basil leaf EOs was evaluated using the agar disc diffusion method (Table 5). The MIC for both *F. oxysporum* isolates was determined to be 0.125 µg/mL. However, the inhibition zone was larger for ARF1 (23.73 mm) than for ARF2 (20.0 mm), indicating that basil essential oil exhibited stronger antifungal effects against *F. oxysporum* ARF1 than ARF2.

Discussion

Integrating compost into soil helps diminish the occurrence and intensity of plant diseases induced by pathogens such as Pythium, Rhizoctonia, and Fusarium spp. (Pascual *et al*., 2000; Van Elsas & Postma, 2007). Conversely, a restricted number of research have examined the application of aromatic herbs in the management of fungal infections and the reduction of illness severity. Composted local basil from Greece is said to accelerate organic carbon mineralization and increase the availability of key nutrients such as potassium, phosphorus, and nitrogen (Kadoglidou *et al*., 2020).

The antifungal activity observed in this study is largely attributed to the following major constituents of basil essential oil: linalool (41.32%), cis-geraniol (11%), and isomethyleugenol (10.5%). Linalool—the predominant component—has been shown to exhibit strong antifungal effects against a range of fungal species in vitro (Karpiński, 2020). Linalool's antifungal action mechanism is thought to involve its interaction with ergosterol, forming a complex that disrupts the synthesis of fungal cell membranes, thereby inhibiting growth, as demonstrated in *Candida albicans* (Dias *et al*., 2017).

The addition of basil leaves as a soil amendment markedly enhanced several vegetative traits in carnation plants, including plant height, branch number, and fresh and dry biomass of shoots and roots. The observed

improvements in vegetative growth were associated with a lower disease index of *F. oxysporum* (ARF1 or ARF2) in carnation plants, suggesting that the basil leaf amendment helped mitigate pathogen impact and thereby promoted vegetative development. Similar findings have been reported in tomato, where soil amendments with aromatic plants, such as mint leaves, enhanced both vegetative growth and productivity (Kadoglidou *et al*., 2020; Li *et al*., 2022).

Previous studies have reported that EOs persist in soil but degrade slowly over time Kadoglidou *et al*., 2020; Li *et al*., 2022). Analysis of the essential oils in the amended soil across the 0–90 day period revealed considerable fluctuations in the concentrations of major volatile compounds. Linalool (41.32%), cis-geraniol (11%), and isomethyleugenol (10.5%) were identified as the predominant constituents. These findings are consistent with those of earlier reports documenting the presence of linalool and other principal components in basil from different regions (Sullivan & Miller, 2001; Mulugeta *et al*., 2023).

Previous research has recorded the gradual deterioration of essential oils in soil (Kadoglidou *et al*., 2020; Li *et al*., 2022). The examination of the essential oil composition in the supplemented soil for a duration of 0–90 days demonstrated significant fluctuations in the predominant volatile chemicals.Predominant constituents were linalool (41.32%), cis-geraniol (11%), and isomethyleugenol (10.5%). Similar profiles, particularly high levels of linalool, have been reported in basil from other regions (Sullivan & Miller, 2001).Evaluation of *F. oxysporum* ARF1 and ARF2 infections in chrysanthemum plants grown in soil amended with basil plant material over the 0–50 day post-inoculation period revealed that treated plants exhibited a substantially lower disease index compared with the untreated control. The antifungal effect of the basil amendment may be attributed to multiple mechanisms, including microbial competition, the production of inhibitory compounds (antibiosis), and the activation of plant defense responses (St Martin, 2015).

The incorporation of basil leaves as a soil amendment markedly improved the physiological performance of carnation plants. Amended plants displayed increased chlorophyll content, higher photosynthetic and transpiration rates, and enhanced stomatal conductance compared with the control. These findings suggest that basil amendments may mitigate the negative effects of *F. oxysporum* on chlorophyll levels in infected plants. Similar improvements in photosynthetic activity have been reported in *Salix* sp. following soil amendments with lime and bisphosphonates (Kadoglidou *et al*., 2020).

The observed increases in transpiration and stomatal conductance likely contributed to the enhanced photosynthesis rate. Previous studies have highlighted a strong relationship between photosynthesis and both transpiration and stomatal conductance (Elansary & El-Abedin, 2019; Al-Ghamdi & Elansary, 2018; Sato *et al*., 2024). These improvements in chlorophyll content, photosynthetic efficiency, and gas exchange in *F. oxysporum*-infected plants grown in amended soil are thought to result from enhanced physiological regulation and increased tolerance to biotic stress. This study demonstrates that using basil leaves as a soil amendment can alleviate Fusarium-induced stress in carnation plants through physiological regulation and stress tolerance enhancement.

Another important aspect is the potential role of basil-derived compounds in inducing plant defense responses. Secondary metabolites from *O. basilicum* may act as elicitors, triggering systemic resistance pathways in carnation plants. This could involve the activation of defense-related enzymes, accumulation of phenolic compounds, and strengthening of cell walls, all of which contribute to increased resistance against pathogen invasion. Such induced resistance mechanisms have been widely reported in plants treated with organic amendments and natural extracts, providing an additional layer of protection beyond direct antimicrobial effects.

Collectively, the findings of this study highlight a multifaceted mode of action of basil leaf residues in mitigating *F. oxysporum* infection. The combined effects of direct antifungal activity, improved soil microbial balance, enhanced nutrient availability, and induced plant defense responses contribute to the overall improvement in plant health and productivity. These results support the growing interest in sustainable and eco-friendly disease management strategies in ornamental horticulture and emphasize the potential of plant-based amendments as viable alternatives to chemical control methods.

Conclusion

This study highlights a novel approach for mitigating *F. oxysporum* (ARF1 or ARF2) infections in carnation plants using the vegetative portions of basil as a soil amendment. Throughout the experiment, the C/N ratio in basil-amended soil gradually declined, reaching comparable levels after 30 days and stabilizing by 60 days. The GC/MS analysis of the essential oils in the amended soil indicated reductions in linalool and other monoterpenes. Visual disease assessments revealed a substantially lower disease index in carnations grown in basil-amended soil than in those grown in non-amended soil. Moreover, the addition of basil significantly enhanced several morphological traits, including plant height, branch number, shoot and root fresh and dry biomass, root length, and flower production, relative to both infected and non-infected control plants. Physiological parameters were also improved; chlorophyll content, photosynthetic and transpiration rates, and stomatal conductance were higher in basil-treated plants following *F. oxysporum* inoculation. The beneficial effects of basil soil amendment on carnation plants are likely mediated through multiple mechanisms, including enhanced stress tolerance and physiological regulation.

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