Pak. J. Bot., 57(5): DOI: http://dx.doi.org/10.30848/PJB2025-5(15)

CHRYSANTHEMUM INDICUM GROWTH AND RESISTANCE TO *FUSARIUM OXYSPORUM* ENHANCED BY THE APPLICATION OF BASIL AS SOIL AMENDMENT

MOHAMMED A. AL-YAFRASI[1], FAHED A. ALMANA[1], OMAR A. BASUDAN [2], MAHMOUD HOSNI EL_KOMY[3], ANWAR H. SHARAFADDIN[3] AND HOSAM O. ELANSARY[1*]

[1]Department of Plant Production, College of Food and Agricultural Sciences, King Saud University, P.O. Box 2455, Riyadh 11451, Saudi Arabia.
[2]Department of Pharmacognosy, College of Pharmacy, King Saud University, P. O. Box 2457, Riyadh 11451, Saudi Arabia
[3]Department of Plant Protection, College of Food and Agricultural Sciences, King Saud University, P.O. Box 2455, Riyadh 11451, Saudi Arabia
**Corresponding author's helansary@ksu.edu.sa*

Abstract

Chrysantheumum spp. is considered as one of major ornamental and medicinal plants in the Mediterranean and middle east regions. The fungal infections by *Fusarium oxysporum* are important biotic stress that limit the production of this crop. Therefore, the current study investigates the application of 5 % (w:w) dried *Ocimum basilicum* L (basil) as soil amendment to *F. oxysporum* ARF1 or ARF2)-infected *C. indicum* plants. The fungal strains ARF1 and ARF2 were isolated from local infected plants and identified by sequencing of *ITS* rDNA and the translation elongation factor 1 alpha (*tef1α*) genes. The essential oil GC/MS analysis in the soil amended with basil showed a reduction of monoterpenes (e.g. linalool, geraniol and cineol) and sesquiterpenes and an increase in sesquiterpenes (e.g. caryophyllene). The visual disease assessment of *F. oxysporum* ARF1 or ARF2*)* in *C. indicum* plants grown in the amended soil had significant lower disease index compared to those with non-amended soil. Plants inoculated with *F. oxysporum* ARF1 or ARF2*)* and grown in the amended soil showed higher plant height, branch number, fresh and dry weights, root length, flowers per plant, total chlorophyll, photosynthetic and transpiration rates, and leaf stomatal conductance, compared to plants infected by fusarium and grown in non-amended soil. The positive effects of the application of basil as soil amendment on the fungal infections by *F. oxysporum* ARF1 or ARF2*)* in *C. indicum* are attributed to multi-mechanisms, including biotic stress tolerance and physiological regulation. This study demonstrated the novel application of basil leaves as soil amendment as biotic stress ameliorant in fusarium infected *C. indicum* plants.

Key words: *Chrysanthemum indicum*; Resistance; Essential oil; Soil amendment; *Fusarium oxysporum*.

Introduction

The *chrysanthemum* is an ornamental and medicinal plant, commonly known as mum, and belonging to the Asteraceae family. The genus contains 41 species (including *Chrysanthemum indicum*) native to East Asia and North-eastern Europe and represent one of the main cut-flowers produced worldwide (Kang *et al*., 2012; Kumar *et al*., 2023). Previous investigations showed medicinal properties of *C. indicum* as well as other species have antioxidant, antimicrobial, anticancer, anti-inflammatory properties attributed to dozens of active ingredients reflecting great pharmaceutical potential (Sun *et al*., 2019; Zhang *et al*., 2024). The plant is vulnerable to different diseases caused by pathogens such as bacteria, fungi, and viruses which influence the nursery production of this important horticultural crop and cause major losses (Song *et al*., 2022).

One of the important soil-borne pathogens influencing the production of *Chrysanthemum* is *Fusarium* wilt, which is caused by *F. oxysporum* that invades the roots, blocks the vascular bundles, obstructs nutrient uptake and end with yellowing and plant death (Da Silva & Kulus, 2014; Khan *et al*., 2021; Eisa *et al*., 2022). The genus was identified in Chrysanthemum in several countries, including China, Vietnam, and India using the sequencing the *ITS*, *Tef-1α*, *RPB1*, and *RPB2* regions. The control of such fungal diseases is sophisticated and includes the removal or destruction of infected plants, which cause major losses. The regular control will depend on a mixture of factors, including the host crop, the environment, and the pathogen itself. Developing novel tools and strategies for fungal disease control in horticultural crops that contains (Alpha-Lipoic Acid) ALA is critical for the development of agricultural production and industries (Chae, 2016).

Basil plants have a long history as traditional medicinal plants (Patil *et al*., 2024). *Ocimum basilicum* L. belongs to the family *Lamiaceae* and naturally occurs in Saudi Arabia and most Middle Eastern countries. The fresh/dried plants are mainly used as herbal medicine for the treatment of indigestion, menstrual pain, coughs, asthma, fever, and headaches. The fresh leaves are used in soft drinks and as garnishes for salads in some countries. The essential oil (EO) shows great diversity and is used in the pharmaceutical, cosmetic, and food industries due to strong antimicrobial activity against several microorganisms (Poonkodi, 2016, Zagoto *et al*., 2021).

The current investigation explores the possible use of dried basil herb as soil amendment to improve the growth of *Chrysanthemum indicum* L. and control *Fusarium oxysporum* infections. For current study the basil herb was used for soil amendment because of its pharmacological action and also it has potential to fix the nitrogen from the environment. The morphological and physiological performances of the treated plants were investigated. A novel approach was studied by using basil dried herbs as a biotic stress ameliorant. The mechanisms were identified in these activities and are discussed here.

Material and Methods

Preparation of *Ocimum basilicum* L. as soil amendment: *Ocimum basilicum* L (basil) plantlets (10 cm) were obtained from commercial nurseries. The plants were identified and vouchered at King Saud University. The plants were grown in greenhouse conditions. The mean growing temperatures were 15.2°C at night and 24.3°C during the day, relative humidity ranged between 67% and 81%, and photosynthetic active radiation was 900 W m^{-2} at 12:00 p.m. Water of the plants was conducted every 2 d until drop-off. After 8 weeks, the whole vegetative parts (shoots and leaves), were harvested. The plant material was ground, then air-dried until the moisture content reached 6%–7% in dark conditions. A mixture of 2 sand:1 peat: 1 clay with defined physiochemical properties as shown in (Table 1) were prepared. The Kjeldahl method was used to determine the nitrogen composition (Nelson, 1972), while phosphorus was determined by the phosphomolybdic acid method (Neal *et al*., 2000). Organic carbon was determined following (Emamgholizadeh *et al*., 2018). Fe, Cu, Mn, and Zn were determined by atomic absorption spectrophotometry (AASVario 6, Germany). The basil plant material was mixed with the sandy soil at rate of 5% (w:w, plant material:soil). The soil mixture (including basil vegetative parts) was transferred into 2 L pots in a glasshouse. Basil plant material decomposition was initiated to minimize the C/N ratio, by employing temperatures ranging from 18 to 28°C. Untreated pots with soil-aromatic basil mixture were considered as controls. For each soil mixture 60 pots were used. The pots were irrigated weekly and stored in a greenhouse until the plant material used as an amendment decomposed. To follow the decomposition of the plant material, the C/N ratio was used as decomposition indicator. Soil samples (10 g) were taken from the soil mixes at 0, 15, 30, 60 and 90 days and the organic carbon (organic matter) composition was determined (Emamgholizadeh *et al*., 2018). The total nitrogen composition was determined by the Kjeldahl method (Nelson,1972). Experiments were conducted during the years 2022 and 2023.

Table 1. Physiochemical properties of soils used.

Composition	Soil	LSD *P=0.05*
pH	8.1	0.10
EC (mmho cm^{-1})	0.978	0.30
Water holding capacity (%)	44.3	2.1
Soil organic carbon (SOC, %)	0.53	0.01
Nitrogen (N, g ha^{-1})	185 x 10^3	3.2
Phosphorus (P, g ha^{-1})	17.9 x 10^3	1.3
Potassium (K, g ha^{-1})	195.3 x 10^3	3.1
Iron (Fe, mg kg^{-1})	4.5	0.1
Copper (Cu, mg kg^{-1})	0.42	0.04
Zink (Zn, mg kg^{-1})	5.0	0.05
Manganese (Mn, mg kg^{-1}))	2.7	0.02

Volatile constitutes in amended soil: The volatile oil composition of the soil (2 kg) for each treatment as well as controls was evaluated after 0, 15, 30 d by hydro distillation using a Clevenger-type apparatus. The oil was dried over anhydrous sodium sulphate, filtered, and kept at 4°C under dark conditions (Elansary *et al*., 2016). Agilent GC 7890A combined with a triple axis detector 5975 C single quadrupole mass spectrometer were used for GC-MS analysis. The chromatographic column was an Agilent HP 5MS column (30 m × 0.25 mm × 0.25 µm film thickness), with high-purity helium as the gas carrier, at a flow rate of 1.2 mL/min. The injector temperature was 250°C and it was equipped with a split less injector at 20:1. The source temperature of MS was set at 230°C and the Quad temperature was at 150°C .The oven temperature was initially at 40°C (held for 1 min), then was increased to 150°C at 5°C min^{-1} (held for 1 min), then increased further to 280°C at 5°C min^{-1} for 1 min. The injection volume was 1 µl and the scan range was set at 40 to 600 mass ranges at 70 eV electron energy and the solvent delay of 4 minutes. The total time required for analyzing a single sample was 51 minutes. The retention times and indices of *n*-alkanes (C_{10}–C_{36}) were used for the identification of detected compounds along with computer matching in the NIST mass spectra ver. 2.0 and WILEY libraries. Literature references were also used for accurate identification and comparison (Adams, 2007).

***Chrysanthemum indicum* L. plant material:** Young *Chrysanthemum indicum L* plantlets containing 1-2 buds (5 cm height) were obtained from local nurseries in two successive seasons (January 2022 and 2023). The plants were identified and vouchered at King Saud University. The plants were maintained in a greenhouse until starting experiments. The mean growing temperatures were 15.2°C at night and 25.3°C during the day, relative humidity ranged between 67% and 81%, and photosynthetic active radiation was 900 W m^{-2} at 12:00 p.m. Watering of the plants was conducted every 2 d until drop-off.

Fungal strains, inoculum preparation, and soil infestation

Isolation, purification, and preservation of *Fusarium*: Root samples were collected from *Chrysanthemum* plant tissues showing wilt disease symptoms, including yellowing, vascular discoloration, and wilt. The samples were collected from Riyadh Saudi Arabia. The chrysanthemum root samples were washed with tap water, then rinsed with distilled water, and cut into 0.5 cm^3 pieces. The root pieces were surface sterilized using 1% Sodium Hypochlorite at 2–3 min, next rising with 70% Ethanol at 30 s., then placed onto sterilized filter paper to dry. The sterilized root pieces were placed onto Petri dishes containing Difco potato dextrose agar (PDA) (4 pieces per plate) amended with 100 µg mL^{-1} streptomycin sulfate. The plates were incubated for 7 days at 25°C. Small pieces of PDA from the edge of *Fusarium* colony were cut, transfer to the center of PDA plates (Adams, 2007) and the colonies were incubated at 25°C for 5 – 7 days. Fusarium strains were single-spored, and the purified colonies were transferred to PDA slants and incubated at 25°C for seven days. Then, the strains were kept for long term preservation at -80°C in 15% glycerol solution in two ml sterilized cryogenic vials (Thermo Fisher Scientific, Rochester, NY, USA). The *Fusarium* strains morphological

characterization were studied, pure strains were grown on PDA and Spezieller Nahrstoffarmer agar (SNA) cultures for 7–8 days at 25°C, after that, the *Fusarium* strains were morphologically examined (like a color colony and pigmentation on PDA), also microscopically (like macro- and microconidia presents/absence and chlamydospores, moreover conidiophore), which guide to the identified to species level according to Leslie and Summerell.

DNA extraction: Fungal mycelia were ground using a mortar and pestle under liquid nitrogen and then transferred to 1.5 ml Eppendorf tubes. The extraction of genomic DNA was done using the CTAB method. The quality and quantity of DNA were evaluated using a 1% agarose gel stained with 1 µg/mL acridine orange. The DNA samples were diluted to achieve a final 20 ng^{-1}µL concentration (Chi *et al*., 2009).

PCR and phylogenetic studies: Two regions were amplified and sequenced: the ITS rDNA and the translation elongation factor 1 alpha (*tef1α*) genes. For the ITS rDNA region, primers ITS4 (5′-TCCTCCGCTTATTGATATGC-3′) and ITS5 (5′-GGAA GTAAAAGTCGTAACAAGG-3′) were used. For the *tef1α* gene, primers EF1 (5′-ATGGGTAAGGARGACAAGAC-3′) and EF2 (5′-GGARGTACCAGTSATCATGTT-3′) were employed (Adams, 2007). The PCR reaction contained 1 ng/µL genomic DNA, 2X Promiga master mix × PCR buffer (Bioline US Inc., Taunton, Massachusetts, USA), 1.5 mM MgCl2, 0.2 mM dNTPs, 0.25 µM of each primer, and 1.0 unit Taq DNA polymerase (Bioline) in a 30 µL reaction volume. The PCR program was: one cycle at 94°C for3 min, 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, and a terminal incubation at 72°C for 5 min. All the PCR amplifications were carried out in a TC-412 thermocycler (Techne, Cambridge, United Kingdom). The amplification products were electrophoretically separated on 1.5 % agarose gel in 0.5× TBE buffer stained with 1 µg/mL acridine orange. HyperLadder IV molecular weight marker (BioLine) was used alongside the PCR products. The gels were visualized under UV and photographed. PCR amplicons were cleaned and sequenced at the University of Kentucky Advanced Genetic Technologies Center. The DNA sequences were edited and aligned using BioEdit software (http://www.mbio.ncsu.edu/BioEdit/bioedit.html).

Two Fusarium strains, ARF1 and ARF2, were identified using the MycoBank database (https://fusarium.mycobank.org). The identification was based on comparing molecular characteristics. The accession numbers for these strains were obtained from the NCBI database. Based on the available taxonomic information, the strains were then categorized within the *F. oxysporum* species complex.

Fungal inoculation and *C. indicum L* cultivation: Young *C. indicum L* plants mentioned in section 2.3 were removed from the pots gently then washed using sterile water. The roots of *C. indicum* L were submerged in spore suspensions of either *F. oxysporum* ARF1 or ARF2 (based on the treatment) for ten minutes at 65 rpm on a rotary shaker. The plants were then moved into the pots indicated in Section 1 and filled with the amended soil according to (Table 2). The plants were maintained in warm temperatures (25 ± 1°C) and high relative humidity (80%, using mist system) in the greenhouse for the first 2 days of inoculation. Non-inoculated plants were considered as controls. These controls were transplanted to the pots of section 1 as well.

Disease assessment: The plants were examined for infection by examining their roots and crowns for any discoloration of the vascular system and possible pathogen isolation on Komada's medium. The plants were classified as healthy when disease symptoms were absent and the pathogen was not isolated (Adams, 2007). Over an infection-time course from 0 to 50 days post-inoculation (d.p.i.), disease severity was rated using a standard morphological 0–5 scale according to the percentage of damaged leaf area (0 = no visible disease damage, 1 = < 5% damaged leaf area, 2 = ≤ 5-20% damaged leaf area, 3 = 20-40% damaged leaf area, 4 = 40-60% damaged leaf area, and 5 = >60% damaged leaf area).

Morphological measurements of *C. indicum*: After 8 weeks, *C. indicum* leaf area (cm^2) per plant, plant height (cm), number of branches per plant, the fresh (FW, g) and dry weights (DW, g), root length, root fresh (FW, g) and dry weight (DW, g) and number of flowers per plant were obtained. The dry weight was determined by preliminary drying at room temperature (20°C), then dried in the oven at 30°C until reaching constant weight following.

Physiological measurements of *C. indicum*: Total chlorophyll was determined in leaves following (Kim and Wolyn 2014). Net photosynthetic rate (*A*), transpiration rate (*E*), and stomatal conductance (g_s) were determined using portable gas exchange equipment (ADC LCi, Bioscientific Ltd., Hoddesdon, UK) in the morning (10:00 a.m.) in mature leaves at the end of the experiment (Kim & Wolyn, 2014). Experiments were repeated twice in triplicate.

Table 2. Treatments applied and their appreciations

Treatment abbreviation	Treatments
C	non inoculated *Chrysanthemum indicum* L. plants (Control)
Cbasil	soil mixture including Basil vegetative parts
Cbasil + ARF1	soil mixture including Basil vegetative parts + *F. oxysporum* ARF1 inoculation
Cbasil + ARF2	soil mixture including Basil vegetative parts + *F. oxysporum* ARF1 inoculation
ARF1	soil mixture + *F. oxysporum* ARF1 inoculation
ARF2	soil mixture + *F. oxysporum* ARF2 inoculation

Statistical analysis

A randomized complete block design was used with 14 pots (replicates) for each one of the five treatments (Table 2). There was 1 treatment as controls (control non-amended soil + non-inoculated *C. indicum* plants). Four Treatments were used, including concentrations of basil plant material as soil amendment (5%) and/or inoculations with fungi (*F. oxysporum ARF1 or ARF2)* as shown in Table 2. The entire experiment was repeated twice in the years 2022-2023. The data for the two successive years were combined for the analysis of variance and no significant differences were found between them. The means were averaged over the two successive experiments. At a significance threshold of $p<0.05$, mean treatment differences were detected using the least significant differences (LSD) techniques. The results of the Bartlett's test for homogeneity of variances, which showed that the data were not heterogeneous, supported the combined analysis of the data. All analyses were performed using Statistica (ver. 7.06, Statsoft Inc).

Results

Soil Characteristics

Carbon/Nitrogen ratio: The C/N ratio changed during the incubation period of 0, 15, 30, 60 and 90 days in soil samples treated with basil plant material are shown in (Fig. 1). A gradual downtrend in the C/N ratio with the progress of the experiment was detected during the first 30 days of incubation. The stable value was achieved after 30 days from.

Essential oil composition: There was a downtrend in the essential oil percentage in the treated soil with the progress of the experiment (Table 3). Over time, the percentage of oil yields dropped, and after 60 days, it had dropped by 98%.

The qualitative and quantitative compositions of the essential oil in the distilled material of 5% basil amended soil during the experiment are shown (Table 4 and Fig. 2).

The GC/MS analysis of the essential oil showed significant differences in the major volatiles during experiment (Table 4). The major essential oil constitutes were linalool (41.32%), cis-Geraniol (11%), and Isomethyleugenol (10.5%). The proportion of specific essential oil constitutes including linalool (41.32%), cis-Geraniol (11%), and Isomethyleugenol (10.5%). was reduced overtime. Linalool is the major essential oil constitute, accounting for 41.32% of the initial basil oil was decreased to 70% by the end of the experiment. Other main constitutes such as cis-Geraniol (11%), and Isomethyleugenol (10.5%) followed similar patterns.

Identification of the two chrysanthemum *F. oxysporum* strains: The identification of the two chrysanthemum *Fusarium* strains using the MycoBank database and the NCBI accession numbers confirmed that both strains belong to the *Fusarium oxysporum* species complex (Table 5).

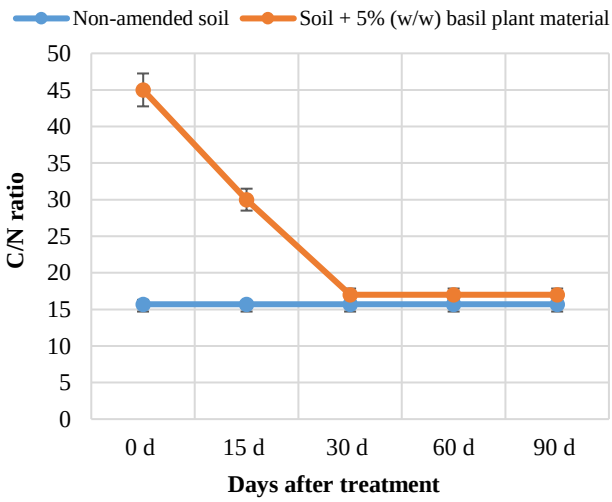

Fig. 1. The C/N ratio changes in soil sample taken at different sampling times (0, 15, 30, 60 and 90 days) after the incorporation of 5 % (w:w) basil plant material.

Table 3. Essential oil ratio at 0, 15, 30, 60, and 90 days.

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 plant material	173.49 ± 9.2a	30.91± 2.4 b	5.52 ± 0.31c	2.17 ± 0.21d	0.71 ± 0.03e

*Different letters within a row indicate significant differences at $p<0.05$

Table 4. Major volatiles found in basil plant material

S. No.				Concentration				
1.	Main essential oil components	RI*	LRI**	0d	15d	30d	60d	90d
2.	1,8-Cineole	1040	1040	0.62 ± 0.07	0.31 ± 0.03	0.08 ± 0.01	0.0 ± 0.01	0.0 ± 0.0
3.	Linaool	1117	1117	41.32 ± 0.2	33.35 ± 0.1	21.52 ± 0.1	14.15 ± 0.1	12.13 ± 0.01
4.	cis-Geraniol	1252	1253	11.00 ± 0.9	8.51 ± 0.1	6.03 ± 0.1	4.00 ± 0.1	2.29 ± 0.01
5.	Geranyl acetate	1377	1377	3.30 ± 0.07	2.61 ± 0.1	1.02 ± 0.1	0.13 ± 0.1	0.10 ± 0.01
6.	Elemene	1379	1380	2.99 ± 0.1	2.5 ± 0.9	1.7 ± 0.1	1.2 ± 0.3	0.93 ± 0.02
7.	Isomethyleugenol	1397	1398	10.50 ± 0.1	8.72 ± 0.1	7.71 ± 0.1	5.64 ± 0.1	3.36 ± 0.03
8.	trans-α-Bergamotene	1434	1434	4.42 ± 0.1	3.42 ± 0.3	3.33 ± 0.7	2.21 ± 0.1	1.41 ± 0.07
9.	Caryophyllene	1443	1443	1.52 ± 0.01	0.99 ± 0.1	0.80 ± 0.7	0.62 ± 0.3	0.17 ± 0.05
10.	α-Muurolol	1642	1642	6.36 ± 0.1	5.09 ± 0.1	4.10 ± 0.7	2.02 ± 0.3	1.17 ± 0.05

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

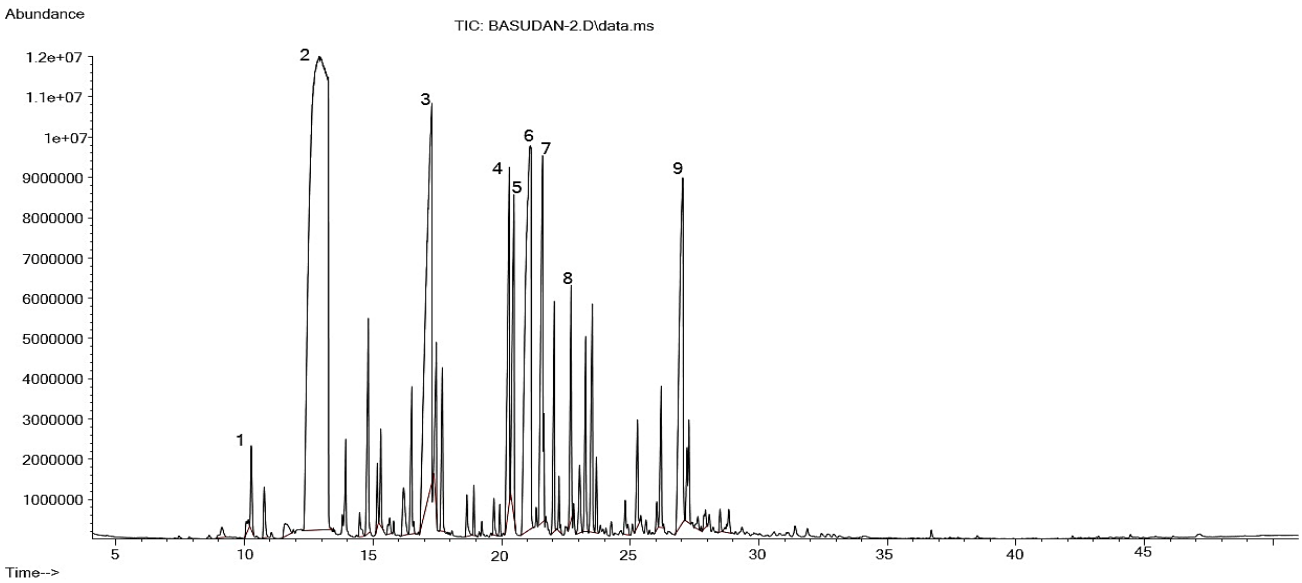

Fig. 2. GC-MS chromatogram showing the major essential oil constitutes were: 1: 1,8 Cineol, 2: linalool, 3: cis-Geraniol, 4: Geranyl acetate, 5: Elemene, 6: Isomethyleugenol, 7: trans-α-Bergamotene, and 8: Caryophyllene , 9: α-Muurolol.

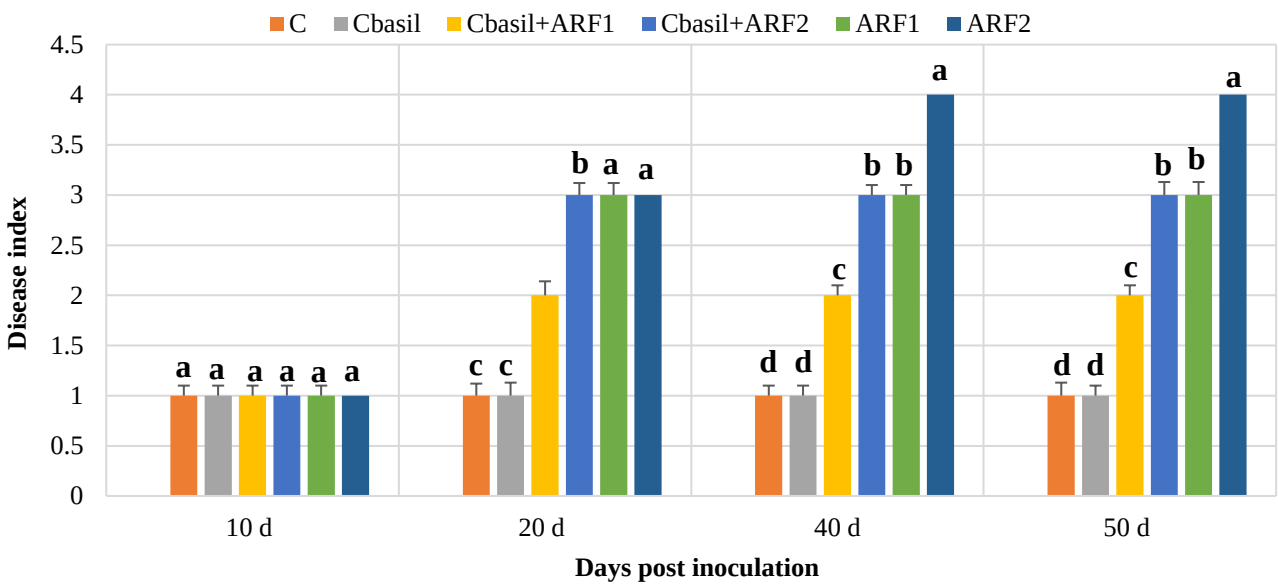

Fig. 3. Visual disease assessment over an infection-time course from 0 to 50 days' post-inoculation (d.p.i.) with *F. oxysporum ARF1 or ARF2* in *C. indicum* plants grown in soil amended with 5 % (w/w) of basil plant material. Disease index was determined according to the percentage of damaged leaf area (0 = no visible disease damage, 1 = < 5% damaged leaf area, 2 = ≤ 5-20% damaged leaf area, 3 = 20-40% damaged leaf area, 4 = 40-60% damaged leaf area, and 5 = >60% damaged leaf area). Data are means and SEs of the disease index calculated from five replications. Different letters among treatments indicate significant differences at $p \leq 0.05$. C: Control, Cbasil: soil mixture including basil vegetative parts, Cbasil+ARF1: soil mixture including Basil vegetative parts + *F. oxysporum* ARF1, Cbasil+ARF2: soil mixture including Basil vegetative parts + *F. oxysporum* ARF2, ARF1: soil mixture + *F. oxysporum* ARF1, ARF2: soil mixture + *F. oxysporum* ARF1.

Table 5. *Fusarium* strains recovered from chrysanthemum-growing in Riyadh Saudi Arabia.

No.	Strain ID	Strain accession number	Species ID	Accession number of similar strains	Size bp	Similarity	Gene
1.	ARF1	PP819611	*Fusarium oxysporum* species complex	EF453091	540	100%	ITS-rDNA
2.	ARF2	PP819612	*Fusarium oxysporum* species complex	EF453158	540	99.8%	ITS-rDNA
3.	ARF1	BankIt2866081	*Fusarium oxysporum* species complex	FJ985363	100%	686	*tef1α*
4.	ARF2	BankIt2866082	*Fusarium oxysporum* species complex	AY527620	98.47 %	698	*tef1α*

Disease assessment: The visual disease assessment over an infection-time course from 0 to 50 days' post-inoculation (d.p.i.) with *F. oxysporum* ARF1 or ARF2 in *C. indicum* plants is shown in (Fig. 3). At 20-50 d.p.i. The visual assessment revealed that treatments of Soil + 5% (w/w) basil plant material + inoculated *C. indicum* with *F. oxysporum ARF1 or ARF2* (Cbasil ARF1 and ARF2) had significant lower disease index in *C. indicum* plants compared to those with non-amended soil subjected and to *F. oxysporum* ARF1 or ARF2 infection (ARF1 and ARF2). Further, with the progress of the experiment (20-50 d), the amended soil treatments performed better than ARF1 and ARF2 and kept this significant difference. In Cbasil ARF1, the visual assessment revealed that treatments of Soil with 5% (w/w) basil plant material had significantly lower disease index in C. *inducum* plants compared to those Cbasil ARF2, indicating higher pathogenicity of ARF2.

Vegetative growth: The morphological effects of basil soil amendment and/or *F. oxysporum* inoculations on Chrysanthemum are shown in (Figs. 4 &5). Plants infected with *F. oxysporum* (ARF1 and ARF2) produced shorter plants, lower branch number, lower fresh and dry weights in the vegetative part and roots, shorter roots and lower number of flowers per plant compared to non-infected control (C). The application of basil vegetative parts as soil amendment at 5% significantly increased plant height, branch number, and fresh and dry weights, root length, flowers per plant compared to infected plants with *F. oxysporum* (ARF1 and ARF2) as well as non-infected control (C). Plants infected with ARF1 showed higher plants, increased branch number and higher fresh and dry weights compared to ARF2 inoculated plants grown in non-/amended soil.

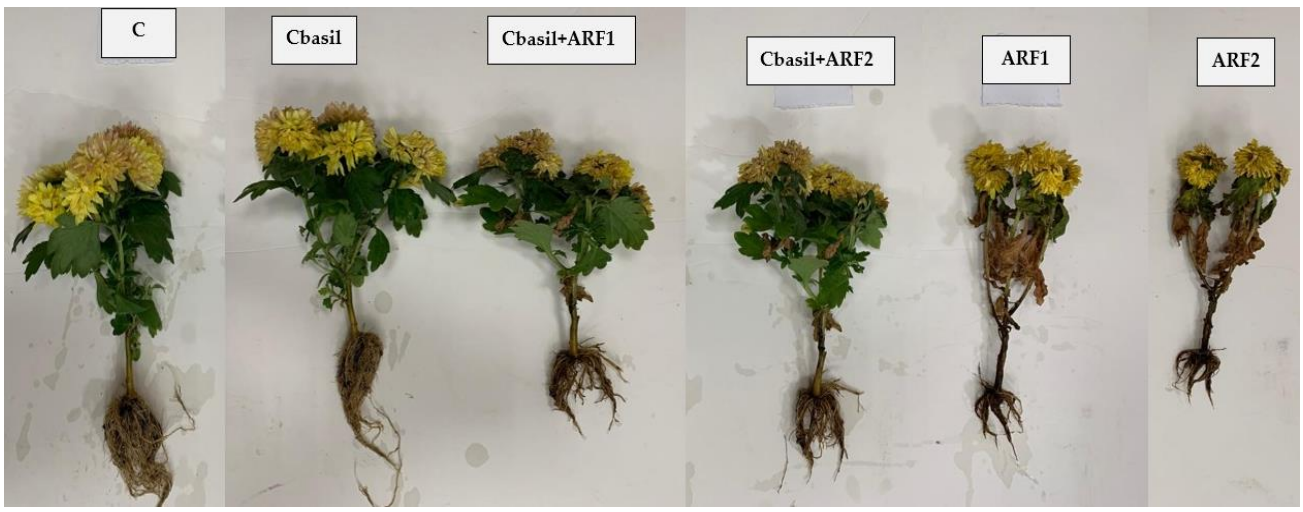

Fig. 4. *Chrysanthemum* morphological performance following application of basil plant material as soil amendment (5% of soil mixture, w:w) and *F. oxysporum* (ARF1 and ARF2) inoculations. C: Control, Cbasil: soil mixture including basil vegetative parts, Cbasil+ARF1: soil mixture including Basil vegetative parts + *F. oxysporum* ARF1, Cbasil+ARF2: soil mixture including Basil vegetative parts + *F. oxysporum* ARF2, ARF1: soil mixture + *F. oxysporum* ARF1, ARF2: soil mixture + *F. oxysporum* ARF1.

Table 6. Antifungal activity of the essential oils of basil species using agar disc diffusion method.

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

Gas exchange measures and chlorophyll content: The gas exchange parameters and chlorophyll content were determined in the leaves at the end of the experiment in plants treated with basil as soil amendment (5% of soil mixture, w:w) and *F. oxysporum (*ARF1 / ARF2*)* inoculations (Fig. 6). Plants infected with *F. oxysporum* (ARF1 and ARF2) showed lower photosynthetic and transpiration rates, stomatal conductance and chlorophyll content compared to non-infected control (C). The application of basil leaves as soil amendment significantly increased the photosynthetic and transpiration rates, the conductance and chlorophylls compared to control C. Plants treated with soil amendment and inoculated with *F. oxysporum* (ARF1) showed higher photosynthetic and transpiration rates, conductance, and chlorophylls compared to *F. oxysporum* (ARF2) inoculated plants. The best gas exchange performance by means of higher photosynthetic and transpiration rates and the conductance was achieved using basil soil amendments in chrysanthemum plants.

***In vitro* antifungal assay:** The antifungal activities of leaves essentials oils of basil, using agar disc diffusion method is shown in (Table 6). The MIC of basil essential oil was 0.125 µg/mL for both fungi tested, but the inhibition zone was larger in *F. oxysporum* ARF1 compared (23.73 mm) to *F. oxysporum* ARF2 (20.0 mm). The antifungal activity of basil essential oil showed significant higher antifungal activity against *F. oxysporum* ARF1 compared to *F. oxysporum* ARF2.

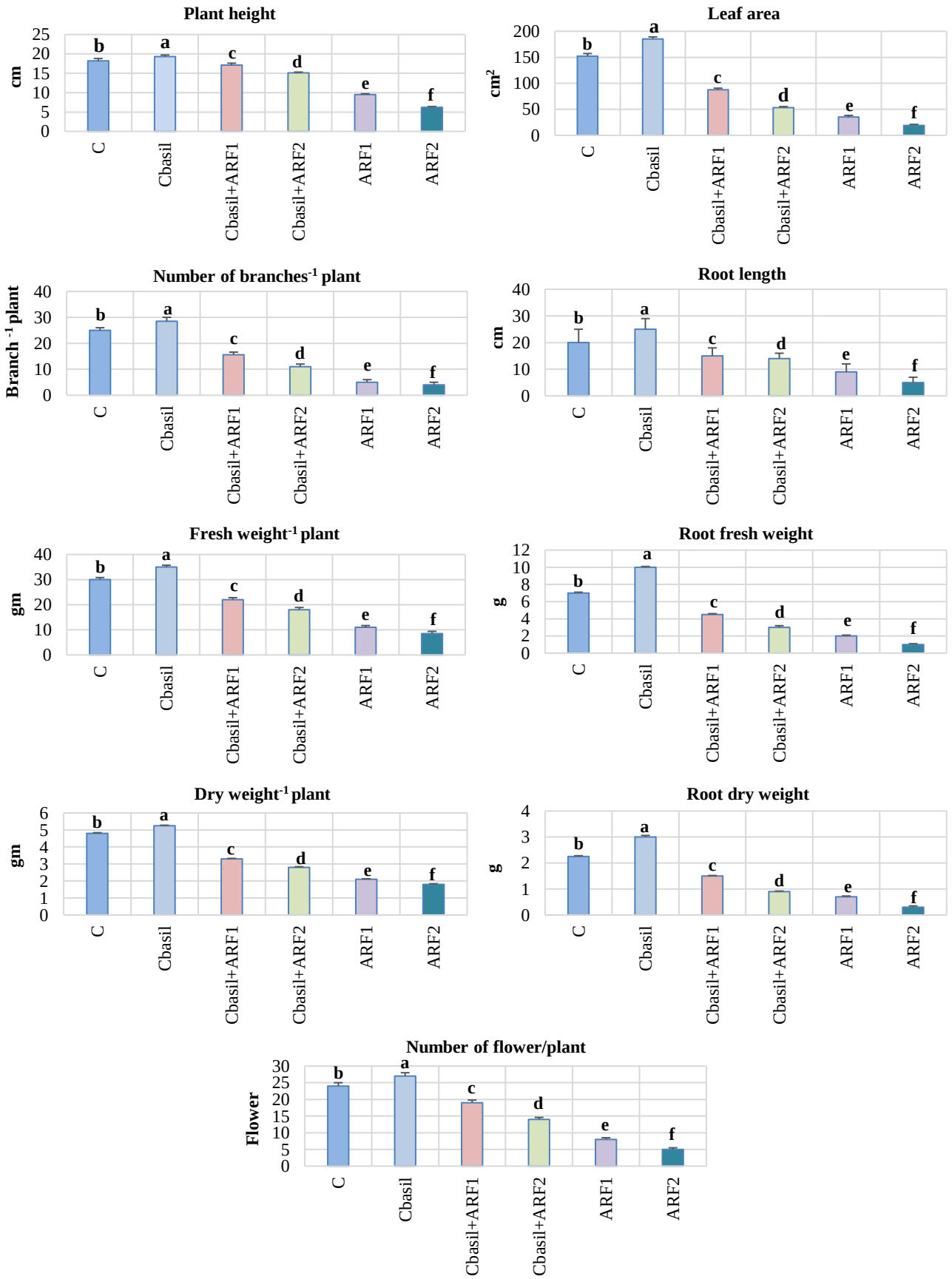

Fig. 5. *Chrysanthemum* mean morphological performance following application of basil plant material as soil amendment (5% of soil mixture, w:w) and *F. oxysporum* (ARF1 and ARF2) inoculations. Different letters among treatment indicate significant differences at $p \leq 0.05$. C: control, C: Control, Cbasil: soil mixture including basil vegetative parts, Cbasil+ARF1: soil mixture including Basil vegetative parts + *F. oxysporum* ARF1, Cbasil+ARF2: soil mixture including Basil vegetative parts + *F. oxysporum* ARF2, ARF1: soil mixture + *F. oxysporum* ARF1, ARF2: soil mixture + *F. oxysporum* ARF1.

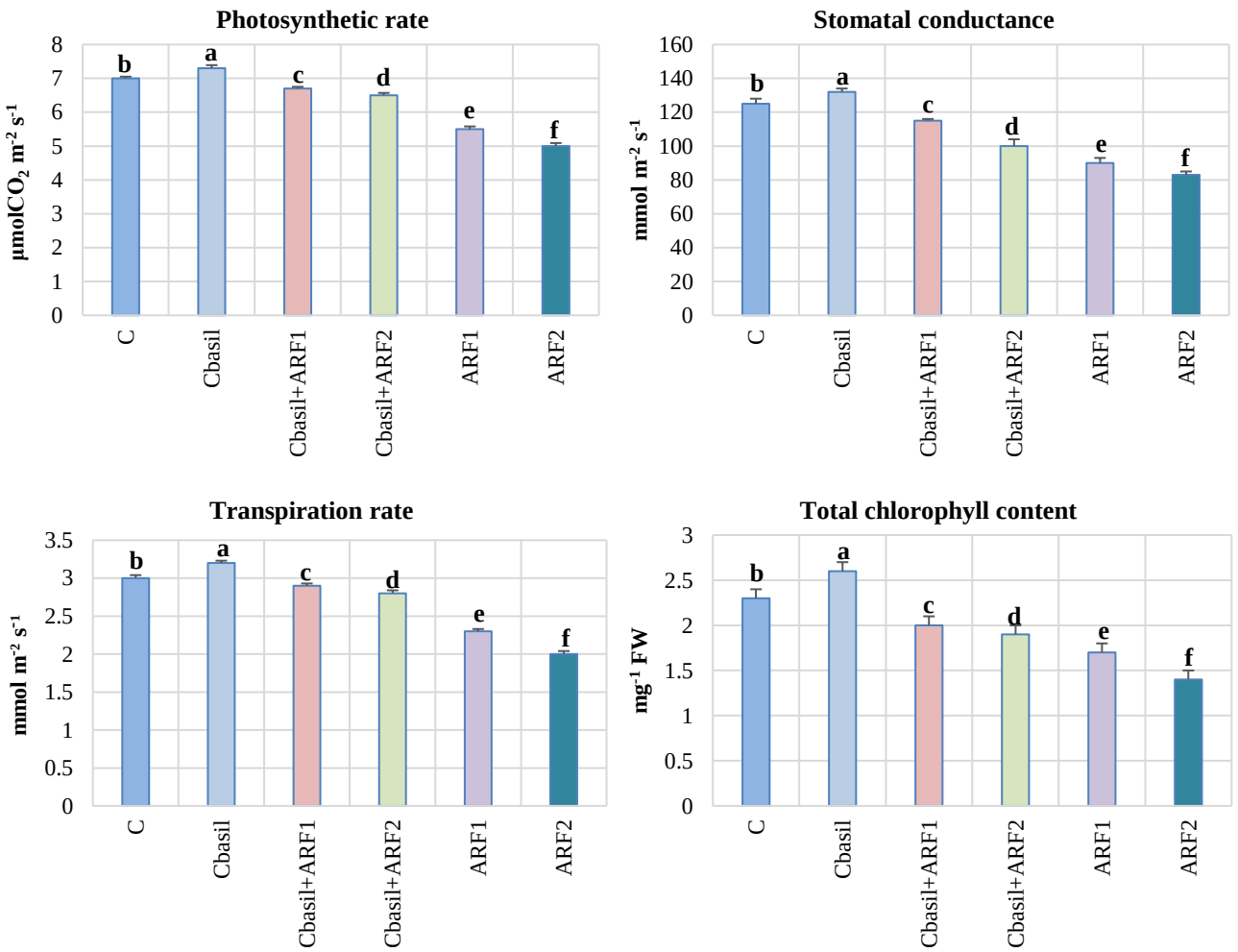

Fig. 6. Mean values of *Chrysanthemum* photosynthetic rate (P_n, µmolCO_2 m^{-2} s^{-1}), transpiration rate (E, mmol m^{-2} s^{-1}), stomatal conductance (g_s, mmol m^{-2} s^{-1}), and total chlorophyll composition (mg^{-1}FW) following application of basil plant material as soil amendment and *F. oxysporum* (ARF1 / ARF2) inoculations. Different letters among treatment indicate significant differences at $p \leq 0.05$.C: control, C: Control, Cbasil: soil mixture including basil vegetative parts, Cbasil+ARF1: soil mixture including Basil vegetative parts + *F. oxysporum* ARF1, Cbasil+ARF2: soil mixture including Basil vegetative parts + *F. oxysporum* ARF2, ARF1: soil mixture + *F. oxysporum* ARF1, ARF2: soil mixture + *F. oxysporum* ARF1.

Discussion

According to earlier research, the C/N ratio variations in soil samples collected throughout the course of this study were a reliable predictor of compost maturity. After 30 days, the C/N ratio in the soil modified with basil leaves, which is consistent with earlier studies on different plants. When basil plant material was added to the soil, the essential oil ratio of the amended soil increased significantly in comparison to the control (Frabboni *et al*., 2011).

The gradual reduction of essential oil in the soil had been reported in previous investigation (Sullivan, 2001). The essential oil analysis in the soil amended showed significant differences in the major volatiles obtained in samplings throughout different days of treatment from 0-90 d. The major essential oil constitutes were linalool (41.32%), cis-Geraniol (11%), and Isomethyleugenol (10.5%). Linalool as major compound as well as other main constitutes were previously reported in basil in other countries (Sullivan, 2001). The proportion of specific essential oil constitutes including linalool (41.32%), cis-Geraniol (11%), and Isomethyleugenol (10.5%) was reduced overtime. The reduction of monoterpenes in the soil environment and the and the stability of sesquiterpenes had been reported when treating soils with essential oils of mint and oregano (Sullivan, 2001). They reported that L-Menthol which was the major essential oil constitute, accounting for 62-63 % of the initial peppermint oil was decreased to 2.17-2.33% by the end of the experiment. In the current study, we found similar pattern as found in Linalool (from 41.32% to 12.13%).

The visual disease assessment *F. oxysporum ARF1 or ARF2* infections in Chrysanthemum plants over a time course from 0 to 50 days' post-inoculation in soil amended with basil plant material revealed that plants treated with basil plant material had significant lower disease index compared to control. The effect of compost as antifungal material could be attributed to several mechanisms including competition, antibiosis, induction of resistance in the treated plant (St. Martin & Ramsubhag, 2015). Previous reports showed that the use of compost as soil amendment may reduce the severity of plant pathogens such as the *Fusarium* spp., *Rhizoctonia*

spp., and *Pytheium* (Pascual *et al*., 2000; Van Elsas *et al*., 2007). However, few reports found that the use of aromatic plants could control the infection and reduce the severity of fungal diseases. For example, A study reported that local basil from Greece compost may increase the organic carbon mineralization, as well as nitrogen, phosphorus and potassium compositions (Kadoglidou *et al*., 2020). The antifungal activity found in the current study is attributed mainly to the major constitutes of the essential oil of basil including linalool (41.32%), cis-Geraniol (11%), and Isomethyleugenol (10.5%). An *In vitro* experiment showed that Linalool (the main constitute of the essential oil in this study) has strong antifungal activities against different fungi (Elansary *et al*., 2020; Karpiński, 2020). The antifungal activity of linalool is attributed to binding to ergosterol and forming a complex, thus preventing monoterpenes from binding to ergosterol and blocking the formation of fungal cell membrane in *Candida albicans* (Dias *et al*., 2017).

The application of basil leaves as soil amendment significantly increased most morphological parameters including plant height, branch number, and fresh and dry weights, root length, flowers per plant compared to infected plants with *F. oxysporum* (*ARF1* or *ARF2*) as well as non-infected control. These vegetative increases were associated with reduced disease index *F. oxysporum* (ARF1 or ARF2) infections in Chrysanthemum plants, which explain such increases in the vegetative growth of basil leaves treated plants. Previous investigation on tomato revealed increases in the vegetative and productive parameters in plants treated with comparable aromatic plants such as mint leaves as soil amendment Kadoglidou (Kadoglidou *et al*., 2020; Li *et al*., 2022).

The application of basil leaves as soil amendment significantly increased the physiological performance of *Chrysanthemum* plants such as increased chlorophyll compositions, photosynthetic and transpiration rates, and stomatal conductance in treated plants compared to control. This may indicate that the application of basil as soil amendment might reduce the negative effects of *F. oxysporum* on chlorophyll compositions in infected plants. The application of soil amendment such as lime and bisphosphonates was associated with improved photosynthetic rates in *Salix* sp. in previous investigation (Kadoglidou *et al*., 2020). The increase in the photosynthetic ratio is explained by the increases in the stomatal conductance and transpiration rates. This association between the photosynthetic ratio and the stomatal conductance and transpiration rates is well documented in previous investigations (Elansary & Yessoufou, 2015; Al-Ghamdi & Elansary, 2018; Elansary & Zin El-Abedin, 2019). The increases of the chlorophyll compositions, photosynthetic and transpiration rates, and stomatal conductance in plants infected with fusarium and grown in amended soil compared to control is considered physiological regulation and biotic stress tolerance mechanism. In the current investigation, we showed the novel finding that basil leaves as soil amendment alleviate *F. oxysporum* as biotic stress conditions in *Chrysanthemum* plants by multi-mechanisms, including stress tolerance and physiological regulation.

Conclusion

The current study reports a novel application of basil vegetative parts as soil amendment to control the fungal infections of *F. oxysporum* (ARF1 or ARF2) in *C. indicum* plants. In basil amended soil, there were gradual downtrends in the C/N ratio with the progress of the experiment until reaching comparable values after 30 days. The stable values were achieved in 60 days. The essential oil GC/MS analysis in the soil amended with basil showed reduction of monoterpenes including Linalool and others. The visual disease assessment of *F. oxysporum in C. indicum* plants grown in the amended soil had a significantly lower disease index compared to those with non-amended soil. The application of basil as soil amendment significantly increased most morphological parameters including plant height, branch number, and fresh and dry weights, root length, flowers per plant compared to infected plants with *F. oxysporum* (*ARF1* or *ARF2*) as well as non-infected control. Plants inoculated with *F. oxysporum* (*ARF1* or *ARF2*) and grown in the basil amended soil showed higher chlorophyll, photosynthetic and transpiration rates, and leaf stomatal conductance. The effects of basil soil amendment on the fungal infections by *F. oxysporum* (*ARF1* or *ARF2*) in *C. indicum* is attributed to several mechanisms, including stress tolerance and physiological regulation.

Funding: Researchers Supporting Project No. RSP2025R118, King Saud University.

Acknowledgements

The authors extend their deep appreciation to Researchers Supporting Project No. RSP2025R118, King Saud University, Riyadh, Saudi Arabia.

References

Adams, R.P. 2007. Identification of essential oil compounds by gas chromatography/mass spectrometry. *Allured pub Corp, Carol Stream*, IL.

Al-Ghamdi, A.A. and H.O. Elansary. 2018. Synergetic effects of 5-aminolevulinic acid and Ascophyllum nodosum seaweed extracts on Asparagus phenolics and stress related genes under saline irrigation. *Plant Physiol. Biochem.*, 129: 273-284.

Chae, S.C. 2016. An up-to-date review of phytochemicals and biological activities in *Chrysanthemum* spp. *Biosci. Biotechnol. Res.*, 13(2): 615-623.

Chi, M.H., S.Y. Park and Y.H. Lee 2009. A quick and safe method for fungal DNA extraction. *Plant Pathol. J.*, 25(1): 108-111.

Da Silva, J.A.T. and D. Kulus. 2014. Chrysanthemum biotechnology: discoveries from the recent literature. *Folia Hort.*, 26(2): 67-77.

Dias, I., E. Trajano, R. Castro, G. Ferreira, H. Medeiros and D. Gomes. 2017. Antifungal activity of linalool in cases of *Candida* spp. isolated from individuals with oral candidiasis. *Braz. J. Biol*., 78: 368-374.

Eisa, E.A., A. Tilly-Mándy, P. Honfi, A.Y. Shala and M.A. Gururani. 2022. Chrysanthemum: A comprehensive review on recent developments on *In vitro* regeneration. *Biology*, 11(12): 1774.

Elansary, H.O. and K. Yessoufou. 2015. Growth regulators and mowing heights enhance the morphological and physiological performance of *Seaspray turfgrass* during drought conditions. *Acta Physiologiae Plantarum*., 37(11): 1-11.

Elansary, H.O. and N.A. Ashmawy. 2013. Essential oils of mint between benefits and hazards. *J. Essential Oil Bearing Plants*, 16(4): 429-438.

Elansary, H.O. and T.K. Zin El-Abedin. 2019. Omeprazole alleviates water stress in peppermint and modulates the expression of menthol biosynthesis genes. *Plant Physiol. Biochem.*, 139: 578-586.

Elansary, H.O., A. Szopa, P. Kubica, H. Ekiert, M. Klimek-Szczykutowicz, D.O. El-Ansary and E.A. Mahmoud. 2020. Polyphenol profile and antimicrobial and cytotoxic activities of natural *Mentha piperita* and *Mentha longifolia* populations in Northern Saudi Arabia. *Processes*, 8(4): 479.

Elansary, H.O., K. Yessoufou, S. Shokralla, E.A. Mahmoud and K. Skaicka-Wozniak. 2016. Enhancing mint and basil oil composition and antibacterial activity using seaweed extracts. *Ind. Crops Prod.*, 92: 50-56.

Emamgholizadeh, S., F. Esmaeilbeiki, M. Babak, D. zarehaghi, E. Maroufpoor and H. Rezaei. 2018. Estimation of the organic carbon content by the pattern recognition method. *Comm. Soil Scu. Plan.*, 49(17): 2143-2154.

Frabboni, L., G. da Simone and V. Russo. 2011. The influence of different nitrogen treatments on the growth and yield of basil (*Ocimum basilicum* L.). *J. Chem. Chem. Engin.*, 5(9):799-803.

Hao, D.C., Y. Song, P. Xiao, Y. Zhong, P. Wu and L. Xu. 2022. The genus chrysanthemum: phylogeny, biodiversity, phytometabolites, and chemodiversity. *Front. Plant Sci*., 13: 973197.

Kadoglidou, K., P. Chatzopoulou, E. Maloupa, A. Kalaitzidis, S. Ghoghoberidze and D. Katsantonis. 2020. Mentha and oregano soil amendment induces enhancement of tomato tolerance against soilborne diseases, Yield and Quality. *Agronomy*, 10(3): 406.

Kang, M., E. Heuvelink, S.M. Carvalho and P. De Reffye. 2012. A virtual plant that responds to the environment like a real one: the case for chrysanthemum. *New Phytol*., 195(2): 384-395.

Karpiński, T.M. 2020. Essential Oils of Lamiaceae Family Plants as Antifungals. *Biomolecules*, 10(1): 103.

Khan, A.U., M.A.R. Choudhury, A.U. Khan, S. Khanal and A.R.M. Maukeeb. 2021. Chrysanthemum production in bangladesh: significance the insect pests and diseases management: A review. *J. Multidiscip. Appl. Nat. Sci.*, 1(1): 25-35.

Kim, J. and D.J. Wolyn. 2014. Freezing tolerance assessment for seedlings of three asparagus cultivars grown under controlled conditions. *Can. J. Plant Sci*., 95(3): 495-504.

Li, X., Q. Wang, H. Li, X. Wang, R. Zhang, X. Yang, Q. Jiang and Q. Shi. 2022. Revealing the mechanisms for linalool antifungal activity against *Fusarium oxysporum* and its efficient control of fusarium wilt in tomato plants. *Int. J. Mol. Sci.*, 24(1): 458.

Neal, C., M. Neal and H. Wickham. 2000. Phosphate measurement in natural waters: two examples of analytical problems associated with silica interference using phosphomolybdic acid methodologies. *Sci Total Environ.*, 251(2000): 511-522.

Nelson, D.W. and L.E. Sommers. 1972. A simple digestion procedure for estimation of total nitrogen in soils and sediments. *American Society of Agronomy, Crop Science Society of America, and Soil Science Society of America*, 1(4): 423-425.

Pascual, J., T. Hernández, C. Garcia, F.A.A.M. Leij and J. Lynch. 2000. Long-term suppression of *Pythium ultimum* in arid soil using fresh and composted municipal wastes. *Biol. Fertility Soils*, 30: 478-484.

Patil, S., A. Kulkarni, P. Jadhav, R. Yadav, M. Pagariya, T. Saha, T.A. Shabeer, D. Sarode, A. Sharma and K. Prasad. 2024. Potential assessment of Chrysanthemum flowers from various cultivars as sources of natural antioxidants and bioactive compounds. *Gen. Resour. Crop Evol.*, 1-19.

Poonkodi, K. 2016. Chemical composition of essential oil of *Ocimum basilicum* L. (Basil) and its biological activities-an overview. *J. Crit. Rev*., 3(3): 56-62.

Shahrajabian, M., W. Sun, P. Zandi and Q. Cheng. 2019. A review of Chrysanthemum, the eastern queen in traditional Chinese medicine with healing power in modern pharmaceutical sciences. *Appl. Ecol. Environ. Res.*, 17(6): 13355-13365.

Sharma, N., M. Kumar, N. Kumari, S. Puri, N. Rais, S. Natta, S. Dhumal, N. Navamaniraj, D. Chandran and P. Mohankumar. 2023. Phytochemicals, therapeutic benefits and applications of chrysanthemum flower: A review. *Heliyon*, 9(10): 20232.

St. Martin, C.C.G. 2014. Potential of compost tea for suppressing plant diseases. *CABI Reviews*, 1-38.

Sullivan, D.M. and R.O. Miller. 2001. Compost quality attributes, measurements, and variability, *Lewis Publishers: Boca Raton, FL, USA.*

Van Elsas, J.D. and J. Postma. 2007. Suppression of soil-borne phytopathogens by compost. *Waste Management Series, Elsevier*, 8: 201-214.

Zagoto, M., G.F.E. Cardia, E.M.T. da Rocha, K.S.M. Mourão, V. Janeiro, R.K.N. Cuman, A. A. Pinto, R.L. Contiero and P.S.L. de Freitas. 2021. Biological activities of basil essential oil: a review of the current evidence. *Res. Soc. Develop.*, 10(12): e363101220409-e363101220409.

Zhang, Z.J., W.J. Hu, A.Q. Yu, L.H. Wu, D.Q. Yang, H.X. Kuang and M. Wang. 2024. Review of polysaccharides from *Chrysanthemum morifolium* Ramat.: Extraction, purification, structural characteristics, health benefits, structural-activity relationships and applications. *Int. J. Biol. Macromol.*, 134919.

(Received for publication 11 September 2024)