Egyptian Journal of Phytopathology, Vol. 51, No. 1, pp 122-141 (2023)
DOI 10.21608/ejp.2023.210512.1092

ORIGINAL PAPER

Characterization and Control of Root Rot and Wilt Fungi of Geranium (*Pelargonium graveolens* L.) with Special Reference to First Record for *Fusarium proliferatum* as Wilt Pathogen on Geranium in Egypt

Abdel-Rahman, T.F.M.; Abdel-Wahed, G.A. and Hassanin, M.M.H.

Received: 12 May 2023 / **Accepted**: 29 June 2023 / **Published online**: 30 June 2023.

ABSTRACT

Geranium root rot and wilt are the most destructive geranium diseases, limiting global production. During the present study, yielded fungal isolates belonging to four fungal species were recovered from diseased geranium (*Pelargonium graveolens* L.) plants showing root rot and wilt symptoms collected from nurseries and fields in Qaliobia and Behera governorates during 2021. Four fungal species were identified as *Fusarium proliferatum*, *Fusarium solani*, *Macrophomina phaseolina*, and *Rhizoctonia solani* with frequencies of 40.78, 17.48, 22.33, and 19.42, respectively. Pathogenicity tests showed that all the tested fungi were pathogenic on geranium to different degrees. However, *Fusarium proliferatum* showed only wilt symptoms recognized with yellowing of leaves, discoloration of the internal vascular tissues, and no rot symptoms were developed on the plants. According to the available literature, the present study suggests that this is the first report of *F. proliferatum* as a wilt pathogen of geranium in Egypt. Moreover, control studies were performed *in vitro* and under greenhouse and field conditions to control root rot and wilt fungi of geranium. The *in vitro* studies showed that the three tested essential oils, *i.e.*, marjoram, peppermint, and thyme, completely (100%) inhibited the growth of the tested fungi *in vitro* at 4000, 5000, and 4000 ppm, respectively. Also, the greenhouse experiment showed that dipping healthy rooted cuttings in the tested essential oils at the above-mentioned concentrations before planting with another treatment as soil drench at 30 days after planting significantly decreased disease severity percentages (DS%) of root rot and wilt of geranium by 89.68, 71.07, and 92.20%, for the three tested essential oils, respectively. This is compared to 75.37, and 61.61, for the check treatments with Plant Guard (4 mL/l) biocide, Rhizo-N (3 g/l) biocide, and Occidor 50% SC (2g/l) fungicide, respectively, and compared to 89.42% for Occidor 50% SC (2g/l) fungicide. Meanwhile, a similar trend was obtained in fields naturally infested with the root rot and wilt fungi of geranium in Beheira and Qaliobia governorates during 2022/2023 season. Treatments by dipping the rooted cuttings before planting and then treatment at 30 days after planting as soil drench in marjoram, peppermint, and thyme essential oils, significantly, decreased DS% by 49.50, 34.55, and 55.87 %, significantly. This is compared to 43.49, 49.23, and 55.87% for Plant (4 mL/l), and Rhizo-N (3 g/l) and Occidor 50% SC (2g/l) fungicide, respectively. Furthermore, all these essential oils treatments under field conditions showed significant improvement effects in growth characteristics (plant height, number of branches, fresh weight) and the oil productivity of geranium grown in field naturally infested with root rot and wilt fungi.

Keywords: Geranium, *Pelargonium graveolens*, *Fusarium proliferatum*, root rot and wilt, essential oils, biocides

***Correspondence: Abdel-Rahman, T.F.M**
E-mail: taghreed.fekry@arc.sci.eg
Taghreed F.M. Abdel-Rahman
https://orcid.org/0000-0003-3668-7337
Gomaa A. Abdel-Wahed
https://orcid.org/0000-0001-5539-8827
Mahmoud M.H. Hassanin
https://orcid.org/0000-0002-3127-0551
Plant Pathology Research Institute, Agricultural Research Center, 12619, Giza, Egypt.

INTRODUCTION

Geranium (*Pelargonium graveolens* L.) family: Geraniaceae (Saraswathi *et al*., 2011) is one of the most famous greenhouses potted and bedding plants. It is regarded as among the most important medicinal aromatic crops in Egypt and throughout the world (Adolf, 2016), which is mainly grown to produce high-value essential oil (Narnoliya *et al*., 2019). Egypt is ranked the 2nd largest producer and exporter of geranium oil after China and is the second highest quality globally (Riahi *et al*., 2020, El-Shafey *et al*., 2021). Because of its high content of essential oils, phenolics and flavonoids, the plants are currently used in medicinal, pharmaceutical, food, flavor, perfumery, cosmetics, and chemical industries (Okla *et al*., 2022). It is also used in folk medicine for its anti-bacterial, antifungal, inflammatory, oxidant, diabetic and insecticidal properties. It can also be used as a plant compound for bioremediation against heavy metal contamination (Mazeed *et al*., 2022). In addition, it is used as an ornamental plant due to its ornamental value (Csorba *et al*., 2020, and

Salachna 2022). There are several sites for geranium production in Egypt, the best of which is in Shoubra El-Khima and El-Qanater El-Khaireya of Qaliobia governorate, El-Sadat city, and then Beni-Suef governorate (El-Shafey *et al*., 2021) and Sinai (Abdel-Hamid *et al*., 2022).

However, many soil-borne fungal diseases attack geranium plants (Adolf, 2016). Some of them are more problematic in greenhouse production than when plants are grown in fields (Rosa and Moorman, 2016) which leads to huge economic losses in geranium oil yield and quality, moreover, it also reduces the production of flowering and ornamental plants. Eventually, the entire crop may be destroyed (Ghazi *et al*., 2018). In Egypt, root rot and wilt diseases are the most devastating diseases on geranium. Several fungal species were reported to be associated with these diseases in Egypt including *Fusarium* spp., *Rhizoctonia solani*, *Macrophomina phaseolina* (El-Gamal, 1995 and Dewidar *et al.*, 2019) *F. solani* (Haggag and Abdel-Latif, 2001), *Macrophomina phaseolina* (Ghazi *et al*., 2018), and *Pythium ultimum* (Adolf, 2016). Also, *Fusarium proliferatum* was reported as root rot fungal species on geranium in Egypt (Adolf, 2016). Meanwhile, symptoms of root rot and wilt, and yellowing of geranium are increasing year after year in Egypt in nurseries and fields. Although fungicides can effectively manage root rot and wilt infections, they have a harmful impact on the environment and human health. As stated by Lahlali *et al*. (2022) that extensive, long-term use of fungicides may lead to the development of some groups of fungal pathogens for fungicide resistance. Likewise, Gikas *et al*. (2022) mentioned that overall, extensive fungicide implementation has various detrimental environmental impacts it cannot be disregarded.

Accordingly, innovative solutions are urgently needed as an environmentally safe alternative for the development of fungicides with alternative anti-fungal agents (Nazir *et al*., 2022) such as bio-based biocides (Sarhan 2020, and Lahlali *et al*., 2022), and several essential oil emulsions (Dhaouadi *et al*., 2018, Sarhan, 2020, and Santos *et al*., 2022).

Therefore, the current study's objectives were: 1) isolation and identification of fungal species associated with root rot and wilt of geranium in two of the major geranium cultivation regions in Egypt, 2) evaluation of the pathogenic potentiality of the recovered fungal species, and 3) the efficacy of certain eco-friendly essential oils, compared to biological control and fungicide Occidor, as alternative control methods to control root rot and wilt diseases on geranium and their possible effect in plant quality and yield of oil, under greenhouse and field conditions.

MATERIALS AND METHODS

Laboratory and greenhouse trials were carried out in the "Plant Pathology Research Institute, Ornamental, Medicinal, and Aromatic Plant Diseases Research Department, in both Giza and Alexandria, Agricultural Research Center (ARC)", However, the field trials were carried out at the "Experimental Farm of Agricultural Research Stations, Beheira, and Qaliobia governorates", (fields with a previous history of root rot, and wilt diseases on pelargonium).

1. Isolation and identification of fungi associated with root rot and wilt of geranium:

Naturally infected geranium mature plants displaying symptoms of root rot and wilt were collected from nurseries and different fields located in "Beheira and Qaliobia governorates", during 2021, for isolation of the root rot and wilt associated fungi. The collected samples were washed with running tap water. Then, cut into small fragments contain diseased tissues along with a healthy portion, thoroughly washed with sterilized distilled water, then surface sterilized for 3 min. in 1% (v/v) sodium hypochlorite solution, rinsed several times in sterilized distilled water, and dried between two layers of sterilized filter paper. The plant segments were placed on PDA in Petri plates supplemented with 1g/L streptomycin sulphate, then incubated at 26 ±1 °C under dark conditions and examined periodically for one week (Dhingra and Sinclair 1995, Ahmed *et al*., 2017, and Reyad *et al*., 2022). The isolated fungi were purified using hyphal tip and/or single-spore methods (Brown, 1924 and Johnston and Booth, 1983). Fungi were identified depending on their morphological and cultural characteristics according to the descriptions of Nelson *et al*. (1983) and Leslie *et al*. (2006) for *Fusarium* spp., for *Rhizoctonia* spp. and *Macrophomina* sp., Dhingra and Sinclair (1978); Moubasher (1993) and Mahmoud and Budak (2011) for all isolated fungi. Identifications were also confirmed, and photographed, at Assiut University, Moubasher Mycological Center (AUMMC). Samsung M51 Cell Phone was used for capturing images of the fungal colony grown on CYA medium for 7 days at 25 Celsius. Microscopic images: Wet preparations of fungal hyphae and spores were stained with lactophenol cotton blue and

examined using Axiostar plus trinocular research microscope. Images were taken with a digital camera (PowerShot G6, 7.1 megapixels) made in Japan. Images of stained hyphae, conidiophores, and conidia were taken either at 400× or at 1000× magnification. Frequencies of fungal species were calculated as follows:

$$\text{Percentage frequency of fungus} = \frac{\text{No. of fungal isolates colonies for each fungus colony}}{\text{Total No. of fungal isolates colonies for all fungi}} \times 100$$

Then, purified cultures were transferred into PDA slants and kept at a low temperature (5°C) for further studies and every 6-8 weeks, the isolates were subculture onto a new medium (Ranjbar *et al*., 2022).

2. Pathogenicity tests:

Pathogenicity trial was conducted under greenhouse conditions during 2022 for the recovered fungal species *i.e*., *F. proliferatum*, *F. solani*, *Macrophomina phaseolina*, and *Rhizoctonia solani* according to Abdel-Monaim and Atwa (2019).

2.1. Preparation of fungal inoculum:

Inocula of the isolated fungi were prepared individually on potato dextrose agar medium and incubated at 26±1 °C (Pitt and Hocking, 2009). Then, 5 discs of each fungus with a diameter of 5 mm were carefully individually transferred from the mycelial margins of these colonies onto pure sterilized sorghum grains medium (100g corn + 50g fine washed sand + 100 ml. distilled water) in 500 mL sterilized glass bottles. Then, incubated for two weeks at 26±1 °C. until each fungus completely colonized the sorghum medium.

2.2. Pots preparation, inoculation and cultivation:

Pots made of plastic with a thirty Centimeter diameter were sterilized with Clorox (sodium hypochlorite at 3 %) and used. Then, they were inverted and allowed to dry for two days before transplanting. The sterilized pots were filled with sterilized soil (6 kg/pot) of a blend of sand, peat moss, and clay (at 1:1:1 w: w: w). Sand and clay were sterilized by autoclaving at 121°C for 30 minutes, then left to dry for two days before use**.**

Soil infestation was separately conducted with each of the above-isolated fungi inocula was applied in pots at the rate of three percent of the soil weight (3 % w/w) (Mahdy and Mahmoud, 2022). Then, they were thoroughly mixed with the soil up to a depth of 10 cm. (Degani *et al*., 2022), followed by irrigation directly and then every two days regularly for a week before transplanting, to ensure the even distribution of the inoculated fungi in the soil and enhance their colonization.

Ostensibly healthy well-recognized geranium-rooted terminal cuttings (*Pelargonium graveolens* L.) were obtained from a well-known commercial nursery in El-Qanater El-Khaireya, Qaliobia governorate, washed with tap water, and then with sterile distilled water before transplanting. Each pot was transplanted with five geranium-rooted terminal cuttings and treatments were replicated 3 times (5 geranium-rooted cuttings /pot, 3 replicate pots). Three pots without fungus were transplanted to serve as the control. Every pot was placed in a randomized block design. Kept were in the greenhouse (27± 2°C, 11±2 h photoperiod, and 61-63% relative humidity) for 90 days.

2.3. Disease assessment:

After 60 days from transplanting, percentages of disease incidence were recorded according to Ahmed *et al*. (2017). Estimation was carried out by recording the percentage of plants infected with root rot/wilt compared to the total number of plants examined. Also, at the end of the trial (90 days after transplanting), plants were uprooted of the plastic pots and roots were gently washed with tap water. Then, plants were rated for wilt evaluation using a 0-4 disease scale according to Abo-Elyousr and Mohamed (2009) as follows: 0= healthy with no symptoms, 1= ≤ 25% of plant leaves are yellow with dark brown vascular root bundle, 2= ≥26 - ≤ 50% of plant leaves are yellow with dark brown vascular root bundle, 3= ≥51 - ≤ 75% of plant leaves are yellow with dark brown vascular root bundle, 4= ≥76 - ≤ 100% of plant leaves are yellow with dark brown vascular root bundle. Also, a 0-4 disease scale was used based on Abebe *et al*. (2016) for root rot evaluation as follows: 0= Healthy roots with no visible rot symptoms, 1= ≤ 25% of root surface rotted, 2= ≥ 26 - ≤ 50% of root surface rotted, 3= ≥ 51 - ≤ 75% of root surface rotted, 4= ≥76 - ≤ 100% of root surface rotted. Then, disease severity of root rot and/or wilt was counted as determined by the following formula (Farag *et al*., 2018):

$$\text{Disease severity (\%)} = \frac{[\sum (n \times c)]}{(N \times C)} \times 100$$

Whereas:

n = Count of infected plants

c = Category countr

N = Total count of examined plants

C = The highest category count of infections.

Also, the fungal pathogenicity was confirmed, through the successful re-isolation of the fungal pathogens from artificially infected roots, and basal stems and roots wilted geranium plants, fulfilling its causal relationship to the disease according to Koch's postulates.

3. Molecular characterization of the fungal species associated with root rot and wilt of geranium:

Two of the most aggressive isolates of those associated with geranium root rot and/or wilt diseases were cultured on potato sucrose agar (PSA) medium and incubated at 26±1 ºC for 5 days to obtain pure cultures (Pitt and Hocking, 2009). DNA extraction was performed from these isolates at the "Molecular Biology Research Unit, Assiut University" using a Patho-gene-spin DNA/RNA extraction kit "Intron Biotechnology Company, Korea" utilizing a rapid mini-preparation method (Edel *et al.*, 2021). Molecular characterization was done with the help of "SolGent Company, Daejeon, South Korea" via Polymerase Chain Reaction (PCR) and sequencing. The Internal Transcript Spacer (ITS) Region of the rRNA gene for samples 3 and F8 were amplification using the universal forward primer ITS1 (5' - TCCGTAGGTGAACCTGCGG - 3') and reverse primer ITS4 (5'- TCCTCCGCTTATTGATATGC -3') were incorporated into the reaction mixture. The same primers and ddNTPs were added to the reaction mixture during the sequencing of the purified PCR product (White *et al*., 1990). The obtained sequences compared to those in GenBank were analyzed using "The Basic Local Alignment Search Tool (BLAST)" from "the National Center of Biotechnology Information (NCBI)" website. The obtained sequences were uploaded to GenBank. Mega Align (DNA Star) software version 5.05 was used to analyze sequences and create phylogenetic trees.

4. The *in vitro* antifungal effect of certain essential oils on growth of root rot and/or wilt fungi of geranium:

This trial was carried out to study the effect of three essential oils (peppermint, marjoram, and thyme) on the radial growth of pathogenic fungi causing root rot and/or wilt diseases of geranium compared with two biocides (Plant Guard, and Rhizo-N), and the Occidor 50%SC fungicide. Sources and concentrations tested are shown in Table (1).

Table (1): Essential oils, biocides, fungicide, their commercial names, composition, concentrations tested, and sources

Commercial name	Composition and Concentration
	Essential oils*
Marjoram	*Majorana hortensis L.* (1000, 2000, 3000, 4000, & 5000 ppm)
Peppermint	*Mentha piperita* L. (1000, 2000, 3000, 4000, & 5000 ppm)
Thyme	*Thymus vulgaris* L. (1000, 2000, 3000, 4000, & 5000 ppm)
	Biocides**
Plant Guard	*Trichoderma harzianum*, 30×10^6 CFU/mL (4 mL/l)
Rhizo-N	*Bacillus subtilis*, 30×10^6 Cells /g (3 g/l)
	Fungicide***
Occidor 50% SC	Common name: Carbendazim (2 g/l) Chemical Composition: methyl benzimidazol-2-ylcarbamate

Source of both * Medicinal and Aromatic Plants Res. Department, Horticultural Res. Inst., Agric. Res. Center (ARC), Giza, Egypt; **(Bayou-Tech for biopsies and pesticides) and ***(Agriphar S.A., Belgium)

Essential oils were kept at 4ºC until use under aseptic conditions. Essential oils emulsion stock solutions were prepared as 10 milliliters of each essential oil and 5 milliliters of Tween 80, a non-ionic surfactant (obtained from Egypt EL-Nasr Pharmaceutic AL. Chemicals Co. Egypt). Then, 85 mL of sterile distilled water were added to reach the last mix of each to 100 ml. To fully incorporate and aid in spreading, the essential oils individually were stirred for 30 minutes using a magnetic stirrer (Perczak *et al*., 2019; Tripathi *et al*., 2021, and Proto *et al*.,2022).

In test tubes, five serial dilutions of each of the three essential oils were prepared at 1000, 2000, 3000, 4000, and 5000 ppm, v/v concentrations (Table 1.) to test their efficacy on the tested fungi as described by Abbas *et al*. (2022). A proper amount of each essential oil emulsion was added to the medium separately to have the required concentration, and well

mixed before pouring. A control (unamended) was prepared with the addition of 0.5 mL sterile distilled water and 0.1% Tween 80 without the addition of the essential oils. To avoid bacterial contamination, the antibiotic chloramphenicol (0.1 mg/l) was added to the sterilized (PDA) flasks. Approximately 20 mL of amended PDA and/or control (unamended) were poured into each Petri dish independently under the aseptic conditions.

After solidification of the medium, all plates were inoculated centrally and individually with each fungal species (*F. proliferatum, F. solani, Macrophomina phaseolina*, and *Rhizoctonia solani*). This is conducted using an agar disc 5 mm diameter mycelial disc taken from the margins of the actively growing mycelium edge of 7-day-old PDA cultures of each isolate. Three replicate plates were prepared for each isolate. Then at 26±1ºC, plates were incubated in the dark. When mycelial growth on the control plates (unamended), completely colonized the dish, Diameter of colony radial growth of fungal mycelium was recorded (Tripathi *et al*., 2021, and Derbalah *et al*., 2022). Fungal growth inhibition percentages were calculated according to El-Shahir *et al*. (2022), as follows:

$$\text{Inhibition \%} = \frac{\text{The radial growth of the fungus in control - The radial growth of the fungus in treatment}}{\text{The radial growth of the fungus in control}} \times 100$$

5. Effect of certain essential oils, compared to certain biocides and fungicide, on root rot and wilt of geranium under greenhouse conditions:

This trial was conducted under greenhouse conditions during 2022 using the minimum concentrations of the tested oils (Marjoram, 4000 ppm, Peppermint, 5000 ppm, and Thyme, 4000 ppm) that inhibited the *in vitro* growth of all fungi tested. The fungal inoculum was prepared, and soil infestation was carried out with the tested fungi as mentioned before under pathogenicity test. Then, one week after soil infestation, fifteen rooted cuttings were treated by dipping (for 20 mins.) in the tested essential oils and were transplanted (five cutting/pot), and three replicate pots were used for each treatment. This was compared with sterile distilled water and Tween 80 (0.05%) as control. Also, uniform rooted cuttings were dipped in Biocides, Plant Guard (4 mL/l), and Rhizo-N (3 g/l), and the systemic fungicide, Occidor 50% SC (2g/l), at the recommended concentrations. Then, 30 days after planting the same treatments were repeated as soil drench (Serag El-Din *et al*., 2020).

Eventually, 90 days after transplanting, percentages of disease severity were recorded as described under pathogenicity test. Additionally, the percentage of reduction was determined using the following formula (El-Sersawy *et al*., 2022):

$$\text{Reduction (\%)} = \frac{\text{Diseases severity \% of control - Diseases severity \% of treatment}}{\text{Diseases severity \% of control}} \times 100$$

6. Effect of certain essential oils, compared to certain biocides and fungicide, to control root rot and wilt of geranium in field naturally infested with the plant pathogenic fungi:

This trial was conducted in fields, with previous history of being infested with root rot and wilt fungi of geranium during sampling, at two locations in the Agricultural Research Stations in Beheira and Qaliobia governorates during 2022/2023. Efficiency of the tested essential oils and their effect on the geranium-rooted cuttings, were compared to the biocides, Plant Guard (4 mL/l), and Rhizo-N (3 g/l), and fungicide, Occidor 50%SC (2 g/l) and with sterile distilled water and Tween 80 (0.05%) as the control. The same rates applied in the greenhouse experiment were used. Also, uniform rooted cuttings were dipped in each treatment individually before planting then treatment at 30 days after planting same treatments were applied as soil drench to control the natural infection of root rot and/or wilt in naturally infested fields.

All agricultural operations were conducted in accordance with the recommendations of the Egyptian Ministry of Agriculture. The first week of November 2023, cultured rooted cuttings of geranium cv. *Pelargonium graveolens* L. were transplanted in hills 30 cm apart on both sides. Three replicates were created from the prepared soil of each treatment. They were used in the completely randomized design of the experiment. The plot (300 cm in length and 90 cm in width) was separated by a guard distance of 50 cm between replicates, and 100 cm between treatments. Each section is comprised of 3 rows, in each row, ten rooted cuttings of geranium were transplanted. Ninety days after transplanting in the naturally infested soil, the same disease estimations recorded before in the greenhouse were also conducted.

7. Effect of certain essential oils, compared to certain biocides and fungicide, on growth characteristics of geranium

growing in field naturally infested with root rot and wilt fungi of geranium:

After 90 days from transplanting in each field of the two locations, 5 plants/replicate, were randomly selected to determine the different vegetative growth characteristics in terms of plant height (cm), number of branches/plant, and plant fresh weight according to Ahmed *et al*. (2022). Also, at harvest stage, fresh geranium leaves (200 g/ replicate /treatment) were evaluated for the volatile essential oil content by the hydro-distillation method for 3 hours using a Clevenger device according to Hassanpour *et al*. (2014) at the "Medicinal and Aromatic Plants Res. Dept. Laboratory, Hort. Res. Ins., ARC" Giza.

Statistical analysis:

The software program "Statistix" was used to do the analysis, and means comparisons were carried out with the 5% LSD test, according to (Snedecor and Cochran, 1989).

RESULTS

1- Isolation and identification of fungi associated with root rot and wilt of geranium:

Tests of isolation were conducted from naturally infected geranium (*Pelargonium graveolens* L.) showing root rot and/or wilt symptoms collected from different nurseries and fields in Qaliobia and Beheira governorates during 2021 yielded fungal isolates belonging to four fungal species, *Fusarium proliferatum* (Matsushima) Nirenberg, *F. solani* (Mart) Sacc., *Macrophomina phaseolina* (Tassi) Goid, and *Rhizoctonia solani* Kühn (Fig. 1 and Table 2). Meanwhile, data presented in Table (2) demonstrate that *F. proliferatum* was the most prevalent fungus (40.78%) followed by, *M. phaseolina* (22.33%) and, *R. solani* (19.42%), whereas *F. solani* showed the lowest frequency, being 17.48%. Also, it was found that *F. proliferatum* possessed certain unique morphological traits that were seen in the isolates under light microscope (Fig.2).

2. Pathogenicity tests:

Pathogenicity testing was carried out in a greenhouse, showed that all tested isolates of the recovered fungal species were pathogenic on geranium as they incited root rot and/or wilt diseases in different degrees (Fig. 3). However, data in Table (3) show that the highest percentages of disease incidence were recorded with *F. proliferatum* (80.00%), followed by *M. phaseolina* (33.33%). Also, the highest disease severity values (%) were recorded for *F. proliferatum* and *M. phaseolina*, being 91.11, and 58.33%, respectively. On the contrary, *F. solani* and *R. solani* exhibited the least pathogenic fungi with disease incidence values of 20% for each, and retained the lowest disease severity (20.83, and 37.50 %, respectively). It was also recorded that *F. proliferatum* incited only leaf yellowing and wilt with internal vascular discoloration and no root rot symptoms were noticed (Fig.3). Successful re-isolation of the fungi from the artificially inoculated plants confirmed Koch's hypotheses.

3- Molecular characterization of two fungal species associated with root rot and wilt of geranium:

Based on pathogenicity tests, the two most pathogenic fungal species were analyzed at the molecular level and confirmed (Fig. 4). *Fusarium proliferatum* AUMC15577 (arrowed) was aligned with closely related strains accessed from the GenBank. This strain showed (99.63-99.82%) identity and (99-100%) coverage with several strains of the same species, Nine of *Fusarium* spp. are included in the tree as an outgroup strain (Fig. 4). Also, *Macrophomina phaseolina* AUMC15578 (arrowed) was matched with closely related strains accessed from GenBank. This strain displayed (99.65-100%) identity and (99-100%) coverage by many strains of a single species (Fig. 5).

Table (2): Fungi associated with root rot and wilt of geranium in samples conducted from different nurseries and fields in Beheira and Qaliobia governorates during 2021.

Isolated fungi	Frequency (%)
Fusarium proliferatum (Matsushima) Nirenberg	40.78
Fusarium solani (Mart) Sacc.	17.48
Macrophomina phaseolina (Tassi) Goid	22.33
*Rhizoctonia solani (*Kühn)	19.42
Total	100

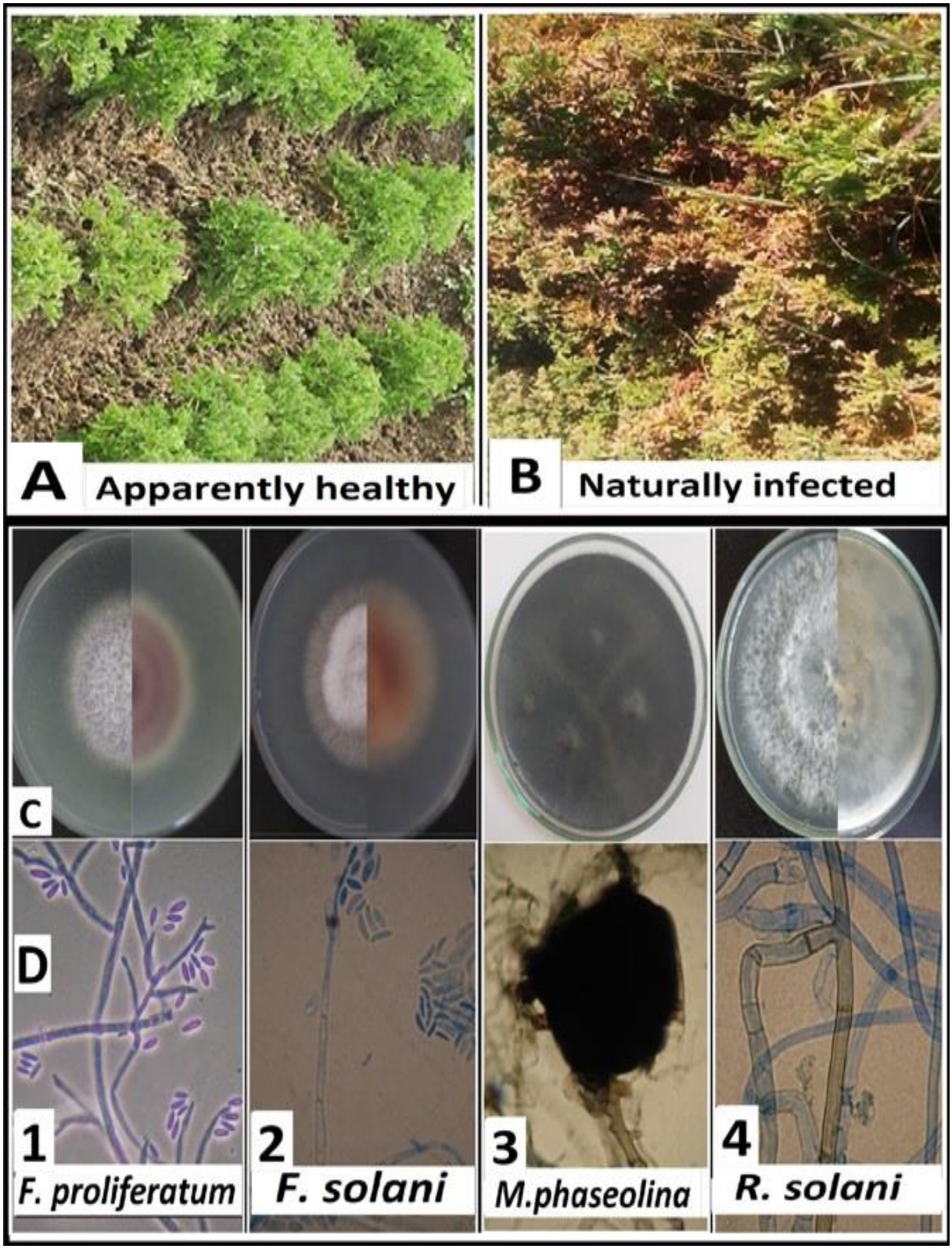

Figure (1): Symptoms, Morphological and cultural characteristics, of fungi recovered from geranium plants, showing root rot and wilt symptoms, collected from nurseries and different fields in different areas in Beheira and Qaliobia governorates, during 2021 (A) Apparently healthy geranium plants. (B) Geranium plants naturally infected with root rot and wilt fungi. (C) fungi colony culture. (D) Microscopic examination for conidia (1&2), *M. phaseolina* pycnidium (3), and mycelium of *R. solani* (4)

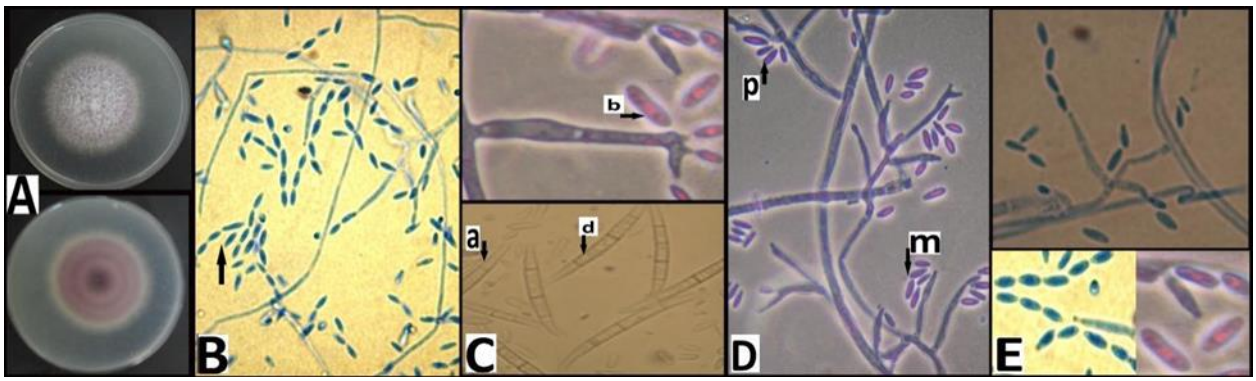

Figure (2): Morphological characteristics of *Fusarium proliferatum* isolated from geranium (*Pelargonium graveolens* L.). (A) Fungal colony of *F. proliferatum* above and reverse on potato dextrose agar (PDA) medium incubated at 26 ±1 ˚C. and has a diameter of 5 cm after 4 days of growth and appears as white villous colonies with light purple pigmentation. (B) (Microconidia are club-shaped with a flattened base and no septate with diameters 2×10–15 µm in size. Conidia are in situ on false heads, chains, hyaline, and smooth, as indicated by arrows. (C) Conidiophores are cylindrical, often proliferated, branched, or unbranched, with monophialides (m), and polyphialides (p). False heads containing microconidia number of 8–16 robust sickle-shaped conidia with no septa. (D) Macroconidia are slender, almost straight tips slender sickle-shaped sub falcate to needlelike at two ends were observed. It usually has one to four septate, hyaline, and smooth. One septum (1s) was 2×26 µm, two septum (2s), and 3 septa (3s) were 2×48 µm, are found in pale orange sporodochia.

Table (3): Pathogenicity of fungal species recovered from geranium plants showed root rot and wilt symptoms collected from different nurseries and fields in Beheira and Qaliobia governorates, 90 days after transplanting under greenhouse conditions

Fungi	Symptoms	Disease incidence (%)	Disease severity (%)
Control (without fungus)	-	00.00c	00.00d
Fusarium proliferatum	Wilt	80.00a	91.11a
F. solani	Root rot	20.00bc	20.83cd
Macrophomina phaseolina	Root rot	33.33b	58.33b
Rhizoctonia solani	Root rot	20.00bc	37.50bc
L.S.D. at 5%		29.71	32.35

Values are an average of 15 potted geranium/ treatment, values in each column followed by a different letter(s) are significantly different at 0.05 of probability

Figure (3): Symptoms of wilt developed by *Fusarium proliferatum* on geranium-rooted terminal cuttings (*Pelargonium graveolens* L.) in the pathogenicity test under greenhouse conditions. (A) Control (Healthy plants). (B) Wilt represented by yellowing of leaves, brown discoloration, collapse and defoliation of the diseased plant, and (C) vascular discoloration

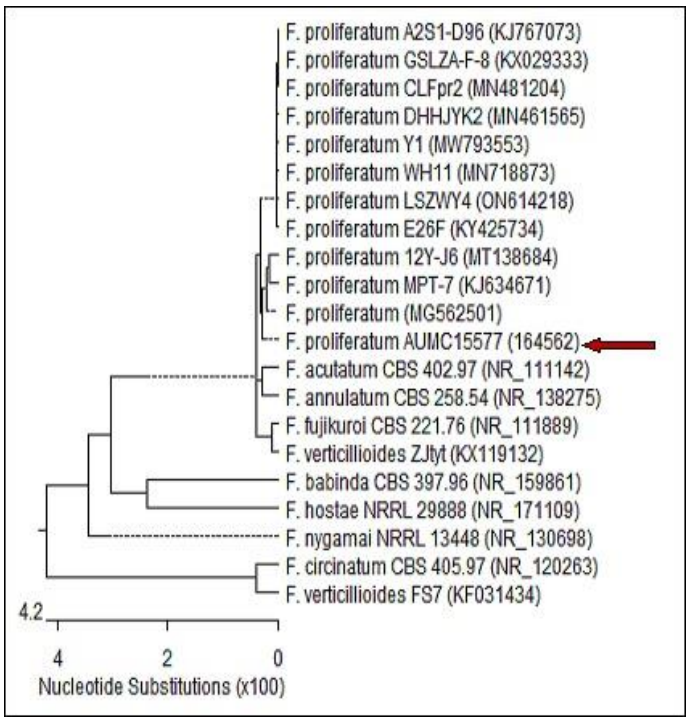

Figure (4): Phylogenetic tree constructed using ITS-rDNA sequences (543 letters), presenting 13 known *Fusarium proliferatum* strains obtained from GenBank database, including that isolated in the present study from *Pelargonium graveolens* diseased plant [*F. proliferatum* AUMC15577 GenBank: OP164562 indicated with a red arrow]. Phylogenetic distances were calculated using the e-neighbor-joining method. Nine of *Fusarium* spp. are included in the tree as an outgroup strain

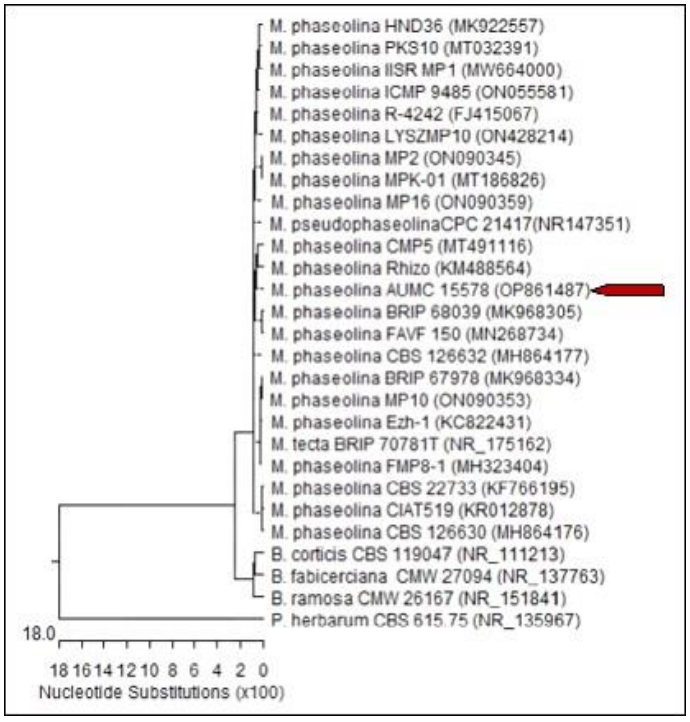

Figure (5): Phylogenetic tree constructed using ITS-rDNA sequences (565 letters), presenting 23 known *Macrophomina phaseolina* strains obtained from GenBank database, including that isolated in the present study from *Pelargonium graveolens* diseased plant [*M. phaseolina* strain AUMC15578, OP861487, indicated with a red arrow. Phylogenetic distances were calculated using the e-neighbor-joining method. *Phoma herbarum* is included in the tree as an outgroup strain. B.= *Botryosphaeria,* M. = *Macrophomina,* and P. =Phoma

4. The *in vitro* antifungal effect of certain essential oils on growth of root rot and/or wilt fungi of geranium:

Data in Table (4) exhibit that, the three examined essential oils (peppermint, marjoram, and thyme) showed significant reduction in the radial growth of all the examined isolates of the root rot and/wilt fungi (*Fusarium proliferatum*, *F. solani*, *M. phaseolina*, and *Rhizoctonia solani*) and this effect was increased with increasing concentrations (1000-5000 ppm), compared with untreated control. However, Thyme and marjoram essential oils showed the highest reduction effect and completely (100%) inhibited radial growth of all fungi at 4000 ppm, while at dosage of 5000 ppm, peppermint essential oil totally prevented the radial growth of all fungi.

Table (4): The *in vitro* inhibition of Marjoram, Peppermint, and Thyme essential oils on root rot and wilt fungi of geranium on PDA medium, 7 days after inoculation and incubation in the dark at 26±1 °C

Treatment	Conc. ppm	Fungi Inhibition %				Mean
		Fusarium proliferatum	*Fusarium solani*	*Macrophomina phaseolina*	*Rhizoctonia solani*	
Control	(0.0)	00.00i	00.00j	00.00k	00.00k	0.00
Marjoram	1000	69.26f	62.96g	50.37i	59.26h	60.46
	2000	78.15d	70.74f	69.26f	72.59f	72.69
	3000	91.11b	88.52c	85.93c	88.15c	88.43
	4000	100.0a	100.0a	100.0a	100.0a	100.0
	5000	100.0a	100.0a	100.0a	100.0a	100.0
Peppermint	1000	41.11h	42.59i	25.93j	38.52j	37.04
	2000	48.52g	54.81h	51.11i	54.07i	52.13
	3000	74.44e	77.78e	74.81e	77.41d	76.11
	4000	92.59b	94.44b	89.63b	91.11b	91.94
	5000	100.0a	100.0a	100.0a	100.0a	100.0
Thyme	1000	79.26d	69.63f	55.93h	65.19g	67.50
	2000	86.30c	83.70d	59.63g	74.44e	76.02
	3000	100.0a	100.0a	82.59d	88.52c	92.78
	4000	100.0a	100.0a	100.0a	100.0a	100.0
	5000	100.0a	100.0a	100.0a	100.0a	100.0
LSD.at 5%		1.563	1.394	1.564	1.603	-

Values are average of 9 amended PDA plates /treatment, values in every single column are significantly different with 0.05 probability if it is followed by a different letter or letters

5. Effect of certain essential oils, compared to certain biocides and fungicide, on root rot and wilt fungi of geranium under greenhouse conditions:

In a greenhouse trial the three tested essential oils were tested for their efficacy to control root rot and/or wilt fungi of geranium. This was conducted by using the most effective concentrations that completely (%) inhibited all isolates radial growth *in vitro* and against the most aggressive isolate in each of the root rot and/or wilt fungal species revealed in the pathogenicity experiment.

Data in Table (5) show that thyme (4000 ppm) being most effective and showed the highest mean reduction of root rot and/or wilt disease severity (DS) value of 92.2% which was not significantly different from Occidor 50% SC (2 g/l) which showed reduction of 89.42%, and marjoram (4000 ppm) with 89.68% DS reduction. This was followed by Plant Guard (4 mL/l), peppermint (5000 ppm), Rhizo-N (3 g/l) with DS reductions of 75.37, 71.07, and 61.61%, respectively (Table 5).

6. Effect of certain essential oils, compared to certain biocides and fungicide, to control root rot and wilt of geranium in field naturally infested with the associated fungi:

Findings shown in Table (6) show that the three quizzed emulsions of essential oils exhibited significant reduction in disease severity percentages against the natural infection of plants, compared to biocides, fungicide Occidor 50% SC, and untreated control plant treatments. The essential oils treatments significantly reduced percentages disease severity of root rot and wilt in both locations (Beheira and Qaliobia governorates) after 60 days of treatments in a trend very

clause to the greenhouse experiment but with much lower inhibition effect values. Thyme (4000ppm) was also the most effective and showed root rot and/or wilt DS mean reduction over the two locations of 55.87% which was not significantly different from Occidor 50% SC (2 g/l) and marjoram (4000 ppm). This was followed by Rhizo-N (3 g/l), Plant Guard (4 mL/l), and peppermint (5000 ppm), with DS mean reductions of 49.23%, 43.49%, and 34.55%, respectively (Table 6).

7. Effect of certain essential oils, compared to certain biocides and fungicide, on growth characteristics of geranium growing in field naturally infested with root rot and wilt fungi of geranium:

According to information in Table (7) and Fig. (6), all the tested essential oil emulsions significantly improved all the tested growth parameters of plants, *i.e.*, plant height, No. of branches, and plant fresh weight, in both locations of the experiment (Beheira and Qaliobia, governorates) compared to untreated control. However, it was evident that Thyme essential oil treatment was the most effective compared to the other essential oil treatments and the tested biocides and fungicide Occidor 50% SC at the tested concentrations over the two governorates.

Also, data illustrated in Fig. (7) show that all treatments markedly increased the percentage of essential oil content in geranium plants compared to the control. The three tested oil treatments exhibited a better performance than the Plant Guard, and Rhizo-N biocides and the Occidor fungicide with the highest effect was for Thyme essential oil treatment over the two governorates.

DISCUSSION

In Egypt, geraniums (*Pelargonium graveolens* L.) infected with root rot and wilt complex, caused by soil-borne fungal species, are regarded as serious and destructive fungal diseases (Ghazi *et al*., 2018, Serag El-Din *et al*., 2020, and Reyad *et al.*, 2022). Results in current investigation displayed that four fungal species were associated with root rot and wilt in the surveyed regions. These fungal species were *F. proliferatum*, *F. solani, Macrophomina phaseolina*, and *Rhizoctonia solani* and were recovered in frequencies of 40.78%, 17.48%, 22.33%, and 19.42 %, respectively, where *F. proliferatum* was the most dominant. Also, the pathogenicity trials displayed that all the tested isolates representing the recovered fungal species were pathogenic to geranium plants causing typical symptoms of root rot and/or wilt in different degrees. Meanwhile, *F. proliferatum* was the most virulent pathogen and showed high percentages of disease incidence and disease severity of 80.00% and 91.11%, respectively. This was followed by *M. phaseolina* with 33.33% and 58.33% for both disease parameters, respectively. However, *Rhizoctonia solani* and *F. solani* showed lower disease severity being 37.5% and 20.83%, respectively, with 20% disease incidence for each. These results are in harmony with the findings of Mohamed *et al*., (2012), Adolf (2016), Ghazi *et al*. (2018), Dewidar *et al.* (2019), Serag El-Din *et al*. (2020), Avan (2021), and Reyad *et al*. (2022).

However, it was recorded here in that *Fusarium proliferatum* isolates only showed wilt symptomes on the inoculated geranium plants with yellowing of leaves, discoloration of the internal vascular tissues and no rot symptoms were noticed which indicat that *Fusarium proliferatum* is a wilt pathogen on geranium. According to the available literature, the present study suggests that this is the first report of *F. proliferatum* causing wilting of geranium (*Pelargonium graveolens* L.) in Egypt. Thesis is consistent with reports in other part of the world (Canada and California, and Khuzestan Province) by Punja (2020), and Ghaedi *et al*., 2021 on other hosts (*Cannabis sativa* L., and date palm). Also, several investigators reported *Fusarium proliferatum* as a cause of wilt in other hosts and other countries by Bhale, *et al*. (2012) on *Rumex acetosa* L. in Maharashtra, India, Kim *et al.* (2016) on Safflower in Korea, Namsi *et al*., (2021) on Date Palm (*Phoenix dactylifera*) in Tunisia, and Mishra *et al*. (2022) on wild accessions of pigeonpea in India. This is despite that Mohamed *et al*., (2012); Adolf (2016), and Elshahawy, (2017) in Egypt previously indicated *Fusarium proliferatum* as a root rot pathogen on geranium in their work. Meanwhile, the outcomes of the morphological characterization were supported by molecular analysis data and identification of the two most frequent fungal species identified as *Fusarium proliferatum* AUMC15577, and *Macrophomina phaseolina* AUMC15578, aligned with closely related strains accessed from GenBank. (Sarhan, 2020, and Reyad *et al.*, 2022).

Table (5): Effect of treatment with Marjoram, Peppermint, and Thyme essential oils, compared to Plant Guard and Rhizo-N biocides and the Occidor fungicide, on percentage of disease severity of root rot and wilt fungi of geranium under greenhouse conditions during 2022 season

Treatment	Conc.	Disease Severity %				Mean Disease Severity %	Mean Reduction %
		Fusarium proliferatum	*Fusarium solani*	*Macrophomina phaseolina*	*Rhizoctonia solani*		
Control	-	82.92	25.00	75.00a	33.33a	54.06A	-
		Essential oils					
Marjoram	4000ppm	25.00	00.00	08.33	00.00	8.330	89.68
Peppermint	5000 ppm	29.17	08.33	16.67	08.33	15.63	71.07
Thyme	4000 ppm	16.67	00.00	08.33	00.00	06.25	92.20
		Biocides					
Plant Guard	4 mL/l	33.33	00.00	25.00	08.33	16.67	75.37
Rhizo-N	3 g/l	37.50	08.33	37.50	08.33	22.92	61.61
		Fungicide					
Occidor	2 g/l	16.67	00.00	16.67	00.00	8.33	89.42
Overall mean		34.47	5.95	26.79	8.33	19.28	68.48
LSD at 5%							
			Fungi (F) 7.230				
			Treatment (T) 9.564				
			F × T 19.127				

Table (6): Effect of treatments with Marjoram, Peppermint, and Thyme essential oils, compared to Plant Guard and Rhizo-N biocides and the Occidor fungicide on severity of root rot and wilt developed on geranium grown in fields naturally infested with the associated fungi in two governorates during the 2022/2023 growing season, 90 days after transplantation

Treatments	Conc.	Disease severity % Governorates		Mean	Reduction % Governorates		Mean
		Beheira	Qaliobia		Beheira	Qaliobia	
Control	-	54.84	58.60	56.72A	-	-	-
		Essential oils					
Marjoram	4000 ppm	27.69	29.60	28.65	49.51	49.48	49.50
Peppermint	5000 ppm	36.96	37.21	37.00	32.61	36.50	34.55
Thyme	4000 ppm	25.00	25.00	25.00	54.41	57.33	55.87
		Biocides					
Plant Guard	4 mL/l	32.78	31.20	31.99	40.23	46.75	43.49
Rhizo-N	3 g/l	27.72	29.88	28.80	49.44	49.02	49.23
		Fungicide					
Occidor	2 g/l	25.00	25.00	25.00	54.41	57.33	55.87
Overall mean		32.86	33.79	29.41	40.09	42.34	41.22
LSD at 5%							
Treatment (T)		4.179			3.962		
Location (L)		2.234			7.412		
T × L		5.910			10.482		

Table (7): Effect of treatment with Marjoram, Peppermint, and Thyme essential oils, compared to Plant Guard and Rhizo-N biocides and the Occidor fungicide, on growth characteristics of geranium growing in field naturally infested with root rot and wilt fungi of geranium in two governorates during the 2022/2023 growing season

Treatment	Plant height (cm)		Mean	Number of branches /Plant		Mean	Fresh weight (g)/plant		Mean
	Beheira	Qaliobia		Beheira	Qaliobia		Beheira	Qaliobia	
Control	51.30	56.50	53.90	12	11	12	1158.5	1018.5	1088.5
	Essential oils								
Marjoram	67.50	67.66	67.58	34	14	24A	1846.6	1966.8	1906.7
Peppermint	66.50	74.66	70.58	33	13	23B	1732.4	1822.5	1777.4
Thyme	68.83	79.16	73.99	34	15	25	1850.2	1980.8	1915.5
	Biocides								
Plant Guard	67.33	69.66	68.49	32	13	22	1638.4	1558.5	1598.5
Rhizo-N	67.50	70.76	69.13	34	13	23	1706.4	1608.3	1657.4
	Fungicide								
Occidor	67.22	72.30	69.76	32	13	23	1632.1	1850.9	1741.5
LSD at 5%									
Treatment (T)	1.842			1.300			46.975		
Location (L)	0.985			0.695			25.109		
T × L	2.606			1.838			66.433		

Concentrations: Marjoram, Peppermint, and Thyme (4000, 5000, and 4000 ppm respectively), Plant Guard (4 mL/l), and Rhizo-N (3 g/l), and Occidor (2 g/l)

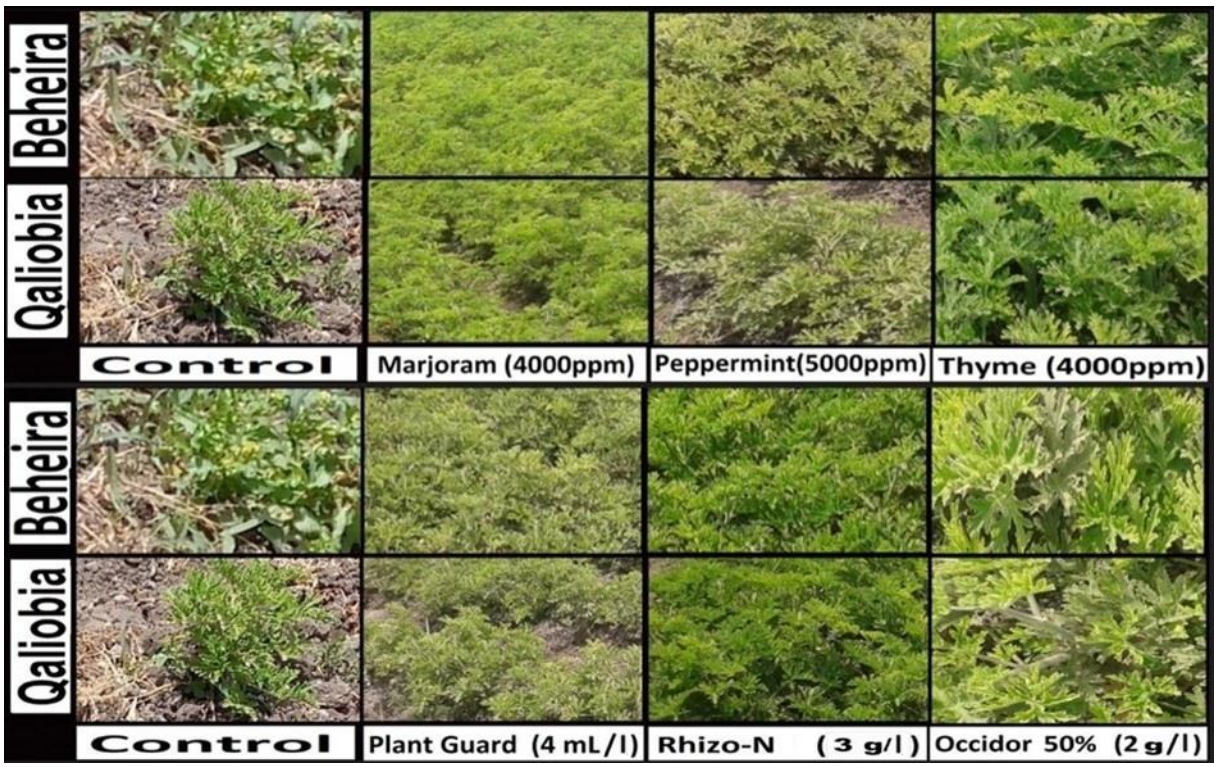

Figure (6): Effect of treatments with Marjoram, Peppermint, and Thyme essential oils compared to Plant Guard, and Rhizo-N, biocides and Occidor fungicide on root rot and wilt on geranium plants grown in field naturally infested with root rot and wilt fungi of geranium, during the 2022/2023 growing season

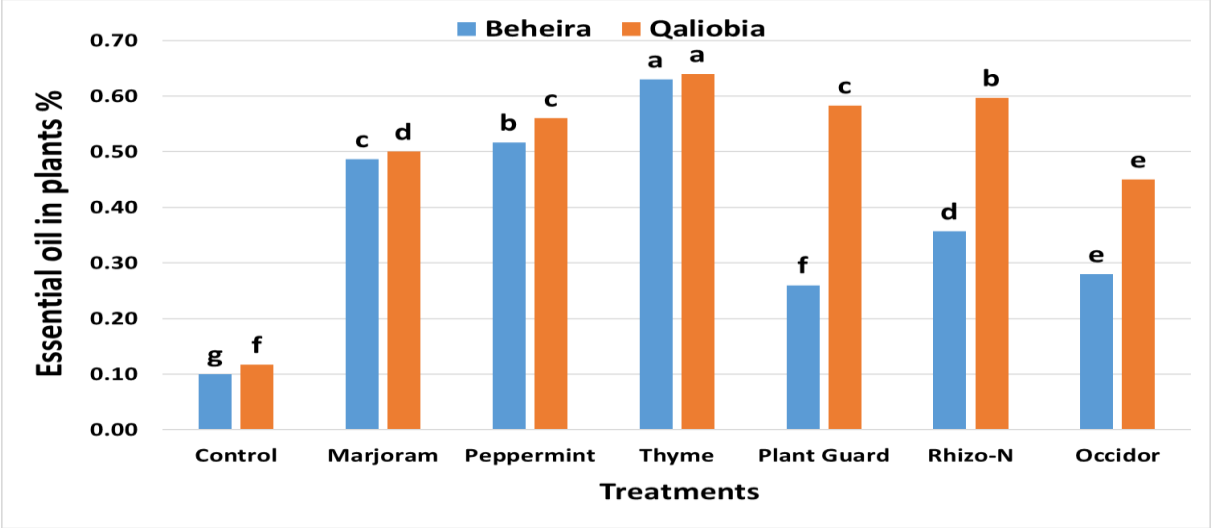

Figure (7): Effect of treatments with Marjoram (4000 ppm), Peppermint (5000 ppm), and Thyme (4000 ppm) essential oils compared to Plant Guard (4 mL/l), and Rhizo-N (3 g/l) biocides and Occidor fungicide (2 g/l), on essential oil content in geranium plants grown in fields naturally infested with root rot and wilt fungi of geranium in two governorates during the 2022/2023 growing season

The *in vitro*, use of essential oils, namely marjoram, peppermint, and thyme at concentrations of 4000, 5000, and 4000 ppm, respectively completely (100%) inhibited fungal growth of the tested root rot and wilt fungi of geranium, *i.e*., *F. proliferatum*, *F. solani*, *M. phaseolina*, and *R. solani*. Numerous researchers reported similar outcomes (Mokhtar *et al.*, 2011; Dhaouadi *et al*., 2018, and Sarhan, 2020). Meanwhile, the results obtained under greenhouse conditions supported the *in vitro* results. Root rot and wilt disease severity, developed on geranium rooted cutting by pot soil infestation with

aggressive isolates of root rot and wilt fungi of geranium, significantly decreased compared to the untreated control. Thyme essential oil (4000 ppm) was the most effective and showed the highest mean reduction of root rot and/or wilt disease severity (DS) value of 92.2% which was even not significantly different from the fungicide Occidor 50%SC (2 g/l) which showed reduction of 89.42%, and not significantly different from marjoram essential oil (4000 ppm) with 89.68% DS reduction. However, this was followed by Plant Guard (4 mL/l), and Rhizo-N (3 g/l) biocides with DS reductions of 75.37, 71.07, and 61.61%, respectively.

The evidence that some of the essential oils including thyme, peppermint, and marjoram have antifungal abilities against some phytopathogenic fungi, were frequently reported (Chang *et al*., 2021; Mani-López *et al*., 2021 and Ghavam *et al*., 2022). They pointed out that essential oils have the potential to perform anti-fungal functions due to their antifungal compounds, *i.e.,* ketones, phenolics, alcohols, terpenes, aldehydes, and additional chemicals and antimicrobial substances, that can partially or totally stop or delay the progression of fungal infection processes and disease. They may be able to protect seedlings from infections that cause root rot and wilt after transplantation.

On the other hand, in a trial in two different governorates, Beheira and Qaliobia, in fields naturally infested with root rot and wilt fungi of geranium. The essential oils treatments significantly decreased the DS (%) of root rot and wilt in both locations (Beheira and Qaliobia governorates) after 90 days of treatments in a trend very clause to the greenhouse experiment but with much lower inhibition values. Thyme (4000ppm) was also the most effective and showed DS mean reductions root of 55.87% over the two governorates which was not significantly different from the fungicide Occidor 50%SC (2 g/l) and marjoram (4000 ppm). This was followed by Rhizo-N (3 g/l), and Plant Guard (4 mL/l) biocides with DS mean reductions of 49.23%, and 43.49%, respectively. These outcomes are consistent with Imarah, (2005) and Talha, (2011) who indicated that such essential oils may stimulate some defense mechanisms such as the oxidative enzymes in the treated geranium plants. Also, this may be explained in view that Thymus plants (*Thymus vulgaris* L.) are the source of thyme essential oil, and thymol, which is a volatile monoterpenoid phenol that has great effect on fungus control as reported by Chang *et al*. (2021), and Ghavam *et al*. (2022). Meanwhile, other investigators (Zengin, and Baysal, 2015 and Ghavam *et al*., 2022) showed that the lipoprotein cytoplasmic membrane of such soil fungi is damaged as a result of thyme oil that penetrating chitin of the cell wall of the fungus, which allows cytoplasm to escape. Meanwhile, the present study revealed that treatments with such essential oil to geranium improved characteristics of plant growth' *e.g*., plant height, No. of branches, and plant fresh weight compared to the untreated control. As plant becoming more vigorous and healthier this could help plant to be more tolerant and resist the fungal infection. These findings are consistent with other investigators (Imarah, 2005; Talha, 2011, and Saltos-Rezabala *et al.,* 2022).

CONCLUSIONS

According to the available literature, this is the first report of *Fusarium proliferatum* causing wilting of geranium (*Pelargonium graveolens* L.) in Egypt. The importance of medicinal aromatic crops, and their cultivation require environmentally friendly alternative materials to control soil-borne diseases, *i.e.,* root rot and/or wilt. The study provides insights for the development of new phytosanitary products based on essential oils (Marjoram, Peppermint, and Thyme), compared with biocides (Plant Guard (4 mL/l), and Rhizo-N (3 g/l),) to help and/or reduce environmental burdens on agriculture and achieve organic agriculture. We recommend dipping geranium root cuttings for 30 minutes before transplanting in the soil by one of the treatments *i.e.*, Thyme, Marjoram essential oils (4000 ppm), or Plant Guard (4 mL/l) biocide, respectively.

CONFLICTS OF INTEREST

The author(s) declare no conflict of interest.

REFERENCES

Abbas, A.M.; Novak, S.J.; Fictor, M.; Mostafa, Y.S.; Alamri, S.A.; Alrumman, S.A.; Taher, M.A.; Hashem, M. and Khalaphallah, R. 2022. Initial *in vitro* assessment of the antifungal activity of aqueous extracts from three invasive plant species. Agriculture, 12(8): 1152.

Abdel-Hamid, M.S.; Atta, H.M.; Abo El-Ela, H.K.; El-Ghamry, A.A. and Fouda, A. 2022. Isolation and characterization of geranium (*Pelargonium graveolens*) bacterial endophytes. Egypt. J. Appl. Sci., 37(1-2): 31-57.

Abdel-Monaim, M. and Atwa, M.A.M. 2019. Induction of systemic resistance against charcoal-rot of cowpea caused by *Macrophomina phaseolina* using some inducer resistance chemicals. Egypt. J. Phytopathol., 47(1): 145-174.

Adolf, K.M. 2016. Root rot of Geranium transplants and its biological control. Int. J. Agric. Technol., 12(5): 899-914.

Abebe, A.; Wai, K.; Siddique, M.; Mo, H.; Yoo, H.; Jegal, Y.; Byeon, S.; Jang, K.; Hwang, S. and Kim, B. 2016. Evaluation of Phytophthora Root Rot- and Bacterial Wilt-resistant inbred lines and their crosses for use as rootstocks in pepper (*Capsicum annuum* L.). Hortic. Environ. Biotechnol. 57(6):598-605.

Abo-Elyousr, K.A. and Mohamed, H.M. 2009. Biological control of Fusarium wilt in tomato by plant growth-promoting yeasts and rhizobacteria. Plant Pathol. J., 25(2): 199 - 204.

Ahmed, H.Z.; Armanious, H.A.; Abdel Moneim, M.L. and Galal, A. 2017. Control of root rots and wilt diseases of Marjoram by using some mineral compounds and antioxidants. Minia J. Agric. Res. and Develop., 37(2): 141-156.

Ahmed, M.F.A.; Ali., A.A.M. and Shahin, S.I. 2022. Efficiency of some organic

matter on suppression of soil-borne diseases of tomato plants under organic agriculture system. Egypt. J. Phytopathol., 50(2): 124-137.

Avan, M. 2021. Important fungal diseases in medicinal and aromatic plants and their control. Turkish Journal of Agricultural Engineering Research (TURKAGER), 2(1): 239-259.

Bhale, U.N.; Chatage, V.S. and Ambuse, M.G. 2012. First report of *Fusarium proliferatum* inciting wilt of *Rumex acetosa* L. in Maharashtra, India. J Plant Pathol Microbiol, 3(1):116.

Brown, W. 1924. Two mycelial methods. II. A method of isolation single strains on fungi by cutting out a hyphal tip. Ann. Bot., 38: 402-404.

Chang, Y.; Harmon, P.F.; Treadwell, D.D.; Carrillo, D.; Sarkhosh, A. and Brecht, J.K. 2021. Biocontrol potential of essential oils in organic horticulture systems: from farm to fork. Front. Nutr., (8): 805138.

Csorba, A.B.; Pánczél, T.; Bandi, A.; Nyárádi, I.I. and Bálint, J. 2020. The effect of different fungicides and bactericides on rooting of pelargonium cuttings. DRC Sustainable Future, 1(2): 155-160.

Degani, O.; Becher, P. and Gordani, A. 2022. Pathogenic interactions between *Macrophomina phaseolina* and *Magnaporthiopsis maydis* in mutually infected cotton sprouts. Agriculture, 12: 255.

Derbalah, A.; Shebl, A.M.; Elgobashy, S.F.; Ahmad, A.A.; Ramadan, N.E.; Behiry, S.I.; Abdelkhalek, A.; Saleem, M.H.; Al-Askar, A.A.K. and Elsharkawy, M.M. 2022. Resistance induction and direct antifungal activity of some monoterpenes against *Rhizoctonia solani*, the causal of root rot in common Bean. Life, 12(7): 1040.

Dewidar, A.A.; Kenawy, A.G.M. and Ghebrial, E.W.R. 2019. Influence of different garlic treatments on controlling basal stem rot, root rot and infection by broomrape in geranium plants. Egypt. J. Phytopathol 47(1): 347-366.

Dhaouadi, S.; Rouissi, W.; Mougou-Hamdane, A.; Hannachi, I. and Nasraoui, B. 2018. Antifungal activity of essential oils of *Origanum majorana* and *Lavender angustifolia* against Fusarium wilt and root rot disease of melon plants. Tunisian Journal of Plant Protection, 13 (1): 39-55.

Dhingra, O.D. and Sinclair, J.B. 1978. Biology and pathology of *Macrophomina phaseolina*. Australas. Plant Pathol., 7: 25.

Dhingra, O.D. and Sinclair, J.B. 1995. Basic Plant Pathology Methods. CRC Press Inc., Florida, USA. 448 pp.

Edel, V.; Christian, S.; Gautheron, N.; Recorbet, G. and Alabouvette, C. 2021. Genetic diversity of *Fusarium oxysporum* populations isolated from different soils in France. FEMS Microbiol. Ecol., (36): 61-71.

El-Gamal, N.G. 1995. Relationship between rhizospheric microflora and fungi causing some root diseases of geranium plants. M.Sc. Thesis, Fac. of Agric., Ain Shams Univ., Cairo, Egypt. 85 pp.

El-Sersawy, M.M.; Atta, H.M.; Abd El-Gawad, A.M.; El-Ghamry, A.A. and Hassan, S.E. 2022. Potential of some plant growth-promoting rhizobacterial strains as biocontrol agents against fusarium wilt disease in cucumber. Egypt. J. of Appl. Sci., 37(1-2):1-30.

Elshahawy, E.; Saied, N.M. and Morsy, A.A. 2017. *Fusarium proliferatum* the main cause of clover rot during storage, reduces clove germination and causes wilt of established garlic plants. Journal of Plant Pathology. 99 (1): 85-93.

El-Shafey, N.M.; Marzouk, M.A.; Yasser, M.M.; Shaban, S.A.; Beemster, G.T.S. and Abd Elgawad, H. 2021. Harnessing endophytic

fungi for enhancing growth, tolerance, and quality of rose-scented Geranium (*Pelargonium graveolens* (L'Hér) Thunb.) plants under Cadmium stress: A Biochemical Study. J. Fungi, 7(12): 1039.

El-Shahir, A.A.; El-Wakil, D.A.; Abdel Latef, A.A.H. and Youssef, N.H. 2022. Bioactive compounds and antifungal activity of leaves and fruits methanolic extracts of *Ziziphus spina-christi* L. Plants, 11: 746.

Farag, M.F.; Ghebrial, E.W. and Zawam, H.S. 2018. Efficacy of some medicinal and aromatic plants combined with different biocides against *Fusarium oxysporum* f.sp. *lycopersici-Meloidogyne incognita* Disease Complex in Tomato. Egypt. J. Phytopathol., 46(2): 85-106

Ghaedi, Z.; Kocheili, M.L. and Farrokhi-Nejad, R. 2021. Report of *Fusarium proliferatum* causing leaf wilt disease of date palm in Khuzestan Province. Plant Prot., 43(3):21-36.

Ghavam, M.; Bacchetta, G.; Castangia, I. and Manca, M.L. 2022. Evaluation of the composition and antimicrobial activities of essential oils from four species of Lamiaceae Martinov native to Iran. Scientific Reports, 12: 17044.

Ghazi, A.A.; Attia, E.A. and Rashed, N.M. 2018. Management of charcoal rot (*Macrophomina phaseolina*) infection in geranium (*Pelargonium graveolens* L.) using biocontrol agents and essential oils. Env. Biodiv. Soil Security, (2): 131-142.

Gikas, G.D.; Parlakidis, P.; Mavropoulos, T. and Vryzas, Z. 2022. Particularities of fungicides and factors affecting their fate and removal efficacy: A review. Sustainability, (14): 4056.

Haggag, W.M. and Abdel-Latif, F.M. 2001. Interaction between vasicular arbuscular mycorrhizae and antagonistic biocontrol microorganisms on controlling root rot disease incidence of geranium plants. J. Biol. Sci., 1(12): 1147-1153.

Hassanpour, H.; Khavari-Nejad, R.A.; Niknam, V.; Razavi, F.; Najafi, K. 2014. Effect of penconazole and drought stress on the essential oil composition and gene expression of *Mentha pulegium* L. (Lamiaceae) at flowering stage. Acta Physiol. Plant, 36: 1167–1175.

Imarah, D.A.A. 2005. Integrated management of some root rot diseases of *Pelargonium graveolens* L. Ph. D. Thesis, Fac. Agric., Cairo Univ., Giza, Egypt. 209 pp.

Johnston, A. and Booth., C. 1983. Plant Pathologist's Pocket Book; Oxford and IBH Pub. Co.: Calcutta, India. 136 pp.

Kim, S.G.; H-C.; Ko., O-S.; Hur, B.P.; Luitel, J-H.; Rhee, M-S.; Yoon, H-J.; Baek, K-Y.R. and Sung, J.S. 2016. First report of fusarium wilt caused by *Fusarium proliferatum* on Safflower. Research in Plant Disease, 22 (2):111-115.

Lahlali, R.; Ezrari, S.; Radouane, N.; Kenfaoui, J.; Esmaeel, Q.; El Hamss, H.; Belabess, Z.and, E.; Barka, A. 2022. Biological control of plant pathogens: a Global perspective. Microorganisms, 10(3): 596.

Leslie, J.F. and Summerell, B.A. and Bullock, S. 2006. The Fusarium Laboratory Manual. 1st ed. Wiley-Blackwell Publishing. 416 pp.

Mahdy, R.E. and Mahmoud, A.F. 2022. Disease severity and correlation study for eight sesame cultivars undergoing Fusarium wilt and charcoal rot resistance screening. SVU International Journal of Agricultural Sciences, 4(2): 84-95.

Mahmoud A. and Budak, H. 2011. First report of charcoal rot caused by *Macrophomina phaseolina* in sunflower in Turkey. Plant Disease, 95(2): 223.

Mani-López, E.; Cortés-Zavaleta, O. and López-Malo, A. 2021. A review of the methods used to determine the target site or the mechanism of action of essential oils and their components against fungi. SN Applied Sciences, 3:44.

Mazeed, A.; Maurya, P.; Kumar, D.; Yadav, S.S. and Suryavanshi, P. 2022. Efficient nutrient management for rose scented geranium (*Pelargonium graveolens* L′ Herit ex Ait). J. Appl. Res. Med. Aromat, 31: 100409.

Mishra, R. K.; Mishra, M.; Bohra, A.; Naik-SJ, S. and Kumar, K. 2022. First report of *Fusarium proliferatum* causing wilt and root rot disease on wild accessions of pigeonpea from India. J. Plant Pathol., 104: 40.

Mohamed E.F.; El-Refaeil, M.I.; Hilal, A.A. and Abdel-Wahed, A.G. 2012 Using of tissue culture to decrease the deterioration of productivity of *Pelargonium graveolens* plant in Egypt. Researcher, 4(6):25-35.

Mokhtar, M.; Abdel-Kader, M.; El-Mougy, N.S. and Lashin, S.M. 2011. Essential oils and *Trichoderma harzianum* as an integrated control measure against Faba Bean root rot pathogens. J. Plant Prot. Res., 51(3): 307-313.

Moubasher, A.H. 1993. Soil fungi in Qatar and Other Arab Countries. Center for Scientific and Applied Research, Doha, Qatar. 568 pp.

Namsi, A.; Rabaaoui, A.; Masiello, M.; Moretti, A.; Othmani, A.; Gargouri, S.; Gdoura, R. and Werbrouck, S.P.O. 2021. First report of leaf wilt caused by *Fusarium proliferatum* and *F.*

brachygibbosum on Date Palm (*Phoenix dactylifera*) in Tunisia. Plant Disease, 105: 1217.

Narnoliya, L.K.; Jadaun, J.S. and Singh, S.P. 2019. The phytochemical composition, biological effects and biotechnological approaches to the production of high-value essential oil from Geranium. Essential Oil Research, 327-352.

Nazir, N.; Badri, Z.A.; Bhat, N.A.; Bhat, F.A.; Sultan, P.; Bhat, T.A.; Rather, M.A. and Sakina, A. 2022. Effect of the combination of biological, chemical control and agronomic technique in integrated management of pea root rot and its productivity. Sci. Rep., (12): 11348.

Nelson, P.E.; Toussoun, T.A. and Marasas, W.F.O. 1983. *Fusarium* species an illustrated manual for identification', University Park: The Pennsylvania State University Press, University Park and London. 193 pp.

Okla, M.K.; Rubnawaz, S.; Dawoud, T.M.; Al-Amri, S.; El-Tayeb, M.A.; Abdel-Maksoud, M.A.; Akhtar, N.; Zrig, A.; AbdElgayed, G. and AbdElgawad, H.A. 2022. Laser light treatment improves the mineral composition, essential oil production and antimicrobial activity of mycorrhizal treated *Pelargonium graveolens*. Molecules, 27(6): 1752, 13pp.

Perczak, A.; Gwiazdowska, D.; Marchwińska, K.; Juś, K.; Gwiazdowski, R. and Waśkiewicz, A. 2019. Antifungal activity of selected essential oils against *Fusarium culmorum* and *F. graminearum* and their secondary metabolites in wheat seeds. Arch. Microbiol., 201: 1085–1097.

Pitt, J.I. and Hocking, A.D. 2009. Fungi and food spoilage. Springer Nature Switzerland AG. Part of Springer Nature. 520 pp.

Proto, M.R.; Biondi, E.; Baldo, D.; Levoni, M.; Filippini, G.; Modesto, M.; Vito, M. D.; Bugli, F.; Ratti, C.; Minardi, P. and Mattarelli, P. 2022. Essential oils and hydrolates: potential tools for defense against bacterial plant pathogens. Microorganisms, 10,702.

Punja, Z.K. 2020. First report of *Fusarium proliferatum* causing crown and stem rot, and pith necrosis, in cannabis (*Cannabis sativa* L., Marijuana) plants, Can. J. Plant Pathol., 43(2): 236-255.

Ranjbar, A.; Ramezanian, A.; Shekarforoush, S.; Niakousari, M. and Eshghi, S. 2022. Antifungal activity of thymol against the main fungi causing pomegranate fruit rot by suppressing the activity of cell wall degrading enzymes. LWT - Food Science and Technology, 161: 113303.

Reyad, N.E.A.; Elsayed, T.R.; Naguib, D.M.; Azoz, S.N. 2022. Biocontrol of root rot in geranium with antimycotic rhizobateria. Rhizosphere, 24: 100607.

Riahi, L.; Cherif, H.; Miladi, S.; Neifar, M.; Bejaoui, B.; Chouchane, H.; Masmoudi, A.S. and Cherif, A. 2020. Use of plant growth promoting bacteria as an efficient biotechnological tool to enhance the biomass and secondary metabolites production of the industrial crop *Pelargonium graveolens* L 'Hér. under semi-controlled conditions. Industrial Crops and Products, 154: 112721.

Rosa, C and Moorman, G. W. 2016. Diseases of Geranium. Handbook of Part III Florists' Crops Flowers, 941-973. Part of the Handbook of Plant Disease Management Diseases: Potted book series (HPDM) McGovern R.J., W.H. Elmer (eds.), Handbook of Florists' Crops Diseases, Handbook of Plant Disease Management 1365 pp.

Salachna, P. 2022. Trends in ornamental plant production. Horticulturae, 8: 413.

Saltos-Rezabala, L.A.; Silveira, P.R.D.; Tavares, D.G.; Moreira, S.I.; Magalhães, T.A.; Botelho, D.M.D.S.; Alves, E. 2022. Thyme essential oil reduces disease severity and induces resistance against *Alternaria linariae* in Tomato Plants. Horticulturae, 8: 919.

Santos, M.I.S.; Marques, C.; Mota, J.; Pedroso, L. and Lima, A. 2022. Applications of essential oils as antibacterial agents in minimally processed fruits and vegetables-A Review. Microorganisms, 10: 760.

Saraswathi, J.; Venkatesh, K.; Baburao, N.; Hilal, M.H. and Rani, A.R. 2011. Phytopharm-acological importance of *Pelargonium* species. J. Med. Plant Res., 5(13): 2587-2598.

Sarhan, E.A.D. 2020. Effectiveness of certain biocides and essential oils in controlling damping-off and root-rot diseases of Soybean (*Glycine max* (L.) Merr.). Journal of Plant Protection and Pathology, Mansoura Univ., 11(2): 79-87.

Serag El-Din, W.M.; Hassanin, M.M.H. and Halawa, A.E.A. 2020. Using alternative fungicides and fertilizers for management root rot and wilt diseases and their effect on productivity of rosemary plant. Egypt. J. Hort. 47 (2):161-180.

Snedecor, G.W. and Cochran, W. 1989. Statistical Methods, Eighth edition, Iowa State University Press, USA. 524 pp.

Talha, G.A.A. 2011. Using of tissue culture and fungicide alternatives in control of root rot

and wilt fungal diseases of pelargonium (*Pelargonium graveolens* L.). Ph. D. Thesis, Fac. Agric., Fayoum Univ., Egypt. 243 pp.

Tripathi, P.; Yami, H. and Shukla, A. 2021. Determination of antifungal activity against phytopathogenic fungi by essential oils extracted from some medicinal plants. Biopestic. Int.17(2): 163-171.

White, T.J.; Bruns, T.; Lee, S. and Taylor, J. 1990. Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. In: PCR Protocols: A Guide to Methods and Applications (Innis MA, Gelfand DH, Sninsky JJ & White TJ eds), pp. 315-322. Academic Press, San Diego.

Zengin, H. and Baysal, A.H. 2015. Antioxidant and antimicrobial activities of thyme and clove essential oils and application in minced beef. J. of Food Processing and Preservation, 39: 1261-1271.