



SURVEY ON INCIDENCE OF DAMPING- OFF AND ROOT ROT/WILT DISEASES OF CORIANDER (*Coriandrum sativum* L.) IN MINIA GOVERNORATE

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ABSTRACT

Coriander seedling damping-off and root rot/wilt diseases considers as the most destructive diseases causes serious economic loss on coriander production worldwide, particularly in tropical and subtropical regions. *Fusarium solani*, *Fusarium oxysporum* f.sp. *cumini*, *Rhizoctonia solani*, *Phoma* sp, *Pythium aphanidermatum*, *Macrophomina phaseolina*, *Alternaria* sp. and *Sclerotinia sclerotiorum* were reported as the most fungi associated with these diseases. In this study, a survey has been conducted to report the incidence of coriander root rot/ wilt diseases in five different districts belongs to Minia Governorate. The survey was performed when plants were at physiological maturity stage of the growth, indicated that this diseases are prevalent in all areas under this study. Our results show that the disease incidence ranged between 7 and 45%, and disease severity was 5-32%. Twenty-nine isolates of fungi, belong to nine different species were isolated from naturally infected plants. All isolated fungi were able to infect coriander (Balady cv.) plants, with different degrees of incidence and severity. Among the 29 isolates, three isolates, which caused the highest DI,% and DS,%, were identified as *Fusarium oxysporum*, *Rhizoctonia solani* and *Macrophomina phaseolina* based on their morphological, cultural characters, and molecular analysis of the internal transcribed spacer (ITS) regions. In addition, the identified pathogens were able to cause seedling damping - off and root rot/wilt on *caraway*, *cumin*, *dill*, *fennel* and *parsley*, whereas *carrot* showed more resistant. Furthermore, physiological studies demonstrated that PDA, corn meal dextrose, malt dextrose agar, coriander dextrose agar and Nutrient dextrose agar were the most favorable media for these fungal isolates. The fungi can grow in a wide temperature and relative humidity ranged between 15-35°C and 50-100% RH, with optimum temperature of 20-30°C, and 84 to 100% RH, depending on the fungus under study.

keywords: Coriander, damping-off, root rot/wilt, host range, media, temperature, RH.

INTRODUCTION

Coriander (*Coriandrum sativum* L.) is an annual growing important glabrous, aromatic, herbaceous crop belonging to the family *Apiaceae* (*Umbellifera*). It is native to the Mediterranean and Middle Eastern regions and has been known in ancient Egypt and Asian countries for thousands of years. Coriander has been known of its medical uses, and the essential oil of seeds has carminative, antiseptic, bactericidal and fungicidal effects and muscle relaxant (Singh *et al.*, 2007; Laribi *et al.*, 2015).

Several pathogens attacking coriander, and causing many diseases such as leaf blight, anthracnose, powdery mildew, rust and leaf spots, seed rot and grain mold diseases. (Rajan, 1990), vascular wilt caused by *Fusarium oxysporium* f. sp. *coriander* (Narula and Joshi (1963) and Srivastava (1972), basal stem and charcoal rot (*M. phaseiocola*), seedling root rot, root rot (*Rhizctonia solani*), seedling blight, stem gall (Das, 1971), bacterial blight, soft rot, reniform and root knot nematodes, phyllody and viruses diseases (Khare *et al.*, 2017). Among those, the wilting, damping-off, and root rot of coriander considers as of the most serious diseases affecting coriander plants at all of its ages from seedling to adult plants (Singh *et al.*, 2007). Many pathogens have been reported to causes these diseases including *Fusarium oxysporum*, *F. moniliforme*, *F. solani*, *Macrophomina phaseolina*, *Rhizoctonia solani*, *Pythium* sp., *Alternaria alternata* and *Sclerotinia sclerotiorum* were isolated from coriander plants showed damping-off, root rot and wilt symptoms collected from Qalubiya and Sharkiya governorates, Egypt (Nada *et al.*, 2014). The symptomatic plants collected from

Qalubiya and Sharkiya governorates, Egypt showed that *R. solani* presented the highest frequency of the total isolates (19.1%) followed by *S. sclerotiorum* (13.5%), *F. oxysporum* (13.2%), but *A. alternata* was the least frequented one (5.7%) (Nada *et al.*, 2014).

Our study aims were to conduct a wide survey at coriander cultivations area in Minia governorate to detect, isolate and identify the pathogen(s) associated with damping-off and root rot/wilt diseases, and to study the susceptibility of some others members of the family *Apiaceae* to the infection with those pathogens, and at last to study some environmental factors that may influence those pathogens growth *in vitro*.

MATERIALS AND METHODS

1- Survey of root rot/wilt of coriander in El-Minia Governorate, Egypt

A survey was carried out to record the incidence and severity of coriander root rot/wilt disease during the period from 17 December 2018 to 6 February 2019 in five major coriander growing districts of northern, middle, and southern Minia Governorate, viz., Maghagha (Tanbadi), Beni Mazar (Ashroubah, Om Assas, Al Qays and Sandafa), Minia (Izbat Silim Basha), Malawi (Darwah and Om Nakhla) and Deir Mawas (Nazlet Badraman and Bani Haram) districts. Plants were at physiological maturity stage of the growth and the data pertaining to survey work is presented in **Table (1)**.

The disease incidence and severity for coriander root rot/wilted plants was reported three times during the season in three different periods. The time for each observation was recorded (**Table 2**).

In each field, 100 plants were randomly selected at five different locations (four corners of the field and one at the field center). Diseases incidence and severity were recorded and calculated by using the following formula for disease incidence.

Disease incidence, % =

$$\frac{\text{Number of infected plants}}{\text{Total number of plants}} \times 100$$

Whereas, the disease severity of root rot/wilt was assessed according to **Jiménez-Fernández et al. (2013)** using a rating scale of 0 to 4 on the basis of root discoloration or leaf yellowing as follows: The roots of coriander plants were washed and divided into five categories according to the percentage of all roots with lesions typical of root rot; zero (0), trace to 10% (1), >10% and ≤30% (2), >30% and ≤60% (3), and >60% (4).

Disease severity,% =

$$\frac{\text{Rating No.} \times \text{No. of plants in the rating}}{\text{Total no. of plants} \times \text{highest rating}} \times 100$$

2-Sampling, isolation, and identification:

Natural field-wilted coriander plants were collected from surveyed fields and used for isolation of the pathogen(s) associated with disease symptoms. Rotted portions of coriander seedlings and wilted plants were surface sterilized by dipping in mercuric chloride solution (0.1%) for 2 minutes and washed several times in sterilized distilled water, then were cut into small pieces about 2 - 5 mm long, and transferred into petri dishes containing a 15 ml sterilized potato dextrose agar (PDA) medium supplemented with

penicillin (40 IU plate⁻¹). The inoculated plates were incubated at 25±2°C for 5-7 days, then the developed fungal growth was sub-cultured on PDA medium, and purified by using hyphal tip, or single spore techniques (**Leyronas et al., 2012; Zhang et al., 2013**). Purified isolates were kept in test tubes (10 ml) containing PDA slants at 4°C as stock cultures for further studies.

The isolated fungi were identified depending on the basis of their morphological and microscopically characteristics according to **Gilman (1957), Booth (1971), Barnett and Hunter (1972) and Ismail et al. (2015)** and the highest three pathogenic isolates (one from each genera) were sent to the Molecular Biology Research Unit, Assiut University for DNA extraction using Patho-gene-spin DNA/RNA extraction kit provided by Intron Biotechnology Company, Korea, for identification of the internal transcribed spacer (ITS) regions.

Frequency of fungi isolated from diseased coriander samples was made according to the following equation:

Frequency, % =

$$\frac{\text{Number of each fungus isolates}}{\text{Total Number of isolates}} \times 100$$

2-1Molecular identification of fungal isolates:

Three fungal isolates *Fusarium oxysporum* (FOC1), *Macrophomina phaseolina* (MPC2) and *Rhizctonia solani* (RSC4), which belong to different genera, were chosen for molecular identification upon their high virulence. The fungal isolates were grown in sterile Petri plates containing autoclaved potato dextrose agar (PDA) medium and incubated for 7 days at 25 ±2°C (**Pitt and Hocking,**

2009). Cultures were sent to the Molecular Biology Research Unit, Assiut University for DNA extraction using Patho-gene-spin DNA/RNA extraction kit provided by Intron Biotechnology Company, Korea. Fungal DNA samples were then sent to SolGent Company, Daejeon, South Korea for polymerase chain reaction (PCR) amplification and sequencing of the internal transcribed spacer (ITS) regions. To amplify the (ITS) regions, PCR using the universal primers ITS1 (5'-TCCGTAGGTGAACCT GCGG-3') (forward) and ITS4 (5'- TCC TCC GCT TAT TGA TAT GC -3') (reverse), was performed (White *et al.*, 1990). The obtained sequences were analyzed using Basic Local Alignment Search Tool (BLAST) from the National Center of Biotechnology Information (NCBI) website. Phylogenetic analysis of sequences was done with the help of MegAlign (DNA Star) software version 5.05.

3-Pathogenicity Test

Pathogenicity test for all isolated fungi, 29 isolates, was carried out under greenhouse conditions at the Experimental Farm of the Plant Pathology Department, Faculty of Agriculture, Minia University, using seeds of coriander (Balady cv.) which obtained from Department of Horticulture, National Research Center, Giza, Egypt.

Soil and clay pots (25 cm-in diam.) sterilization was carried out 15 days before soil sowing by autoclaving the soil for one hour at 121°C (Devash *et al.*, 1980) and dipping the pots in 5% formalin solution for 5 minutes, then soil and pots were aerated for 14 days before being used. Inoculum of each fungal isolate was

prepared, separately. Disks (5mm) of five days old cultures of each fungal isolate were grown on sterilized barley grains (150gm grains +200 ml water / 500-ml Erlenmeyer flask). Inoculated flasks were kept at 25±2°C for two weeks then were used for soil infestation. Soil infestation was applied, 7 days before planting, by thoroughly mixing 2% of the inoculum: soil (W:W), representing a barley culture of single fungus with the soil in the pot. The infested soil was irrigated daily till planting.

Three replicates (pots), each was sown with ten surface disinfected coriander (Balady cv.) 30 days later the plants were thinned to only 5 plants/pot. Sterilized and non-inoculated barley medium was used as a control treatment. Pots were watered when necessary, and plants were daily observed for disease symptoms.

Re-isolation was carried out from diseased seedlings and wilted plants to satisfy Koch's postulates.

3-1-Disease assessment:

The pre- and post-emergence damping off was calculated, 2 and 4 weeks after sowing, respectively, whereas the severity of wilt/root rot diseases was rated 60 days post sowing using the indexing method as described above.

Based on the results of pathogenicity test, isolates of *Fusarium oxysporum* (FOC1= AM-11^(*)), *Rhizctonia solani* (RSC4= AM-13) and *Macrophomina phaseolina* (MPC2= AM-3), that have the highest virulent, were chosen to carry out the laboratory experiments.

(*) This code is mentioned in the tree of molecular PCR identification.

4- Susceptibility of different *Apiaceae* plants to coriander seedlings damping-off and root rot/wilt pathogens:

Pathogenicity test of *F. oxysporum* (FOC1), *R. solani* (RSC4) and *M. phaseolina* (MPC2) against six members of the family *Apiaceae*, viz. **caraway** (*Carum carvi* L.), **carrot** (*Daucus carota* L), **cumin** (*Cuminum cyminum* L), **dill** (*Anethum graveolens* L), **fennel** (*Foeniculum vulgare* Mill.) and **parsley** (*Petroselinum crispum* (Mill.) Fuss) was estimated. The experiment was conducted as described above except that with this experiment the coriander seeds with the seed of each tested crop separately. Also, the same disease estimate equations were used to calculate the percentages of pre-, post-emergence damping-off and wilt/root rot diseases.

5-Laboratory experiments

5-1-Physiological studies:

The fungi used in these investigations were those that proved to be highly pathogenic to seedlings and plants of coriander. In the following tests, fungal linear growth was measured as a criterion for evaluation the effect of treatment. Except of the media experiment, all experiments were performed on PDA. Media were sterilized by autoclaving, poured into 9cm diameter glass Petri plates, and a 5–mm disc, cut with a sterile cork-borer from the advancing margins of the cultures were used for inoculation. One Petri plate represents one replicate, and each treatment was represented by three replicates for each isolate. The inoculated plates were incubated at $25\pm 2^{\circ}\text{C}$. The linear growth of fungal isolates on different test was measured when the growth in plates of any treatment was completed.

5-1-1- Effect of different media on linear mycelial growth of the tested fungi:

Seven different natural or artificial media, i.e., potato dextrose agar, corn meal dextrose, malt dextrose agar, coriander dextrose agar, Nutrient dextrose agar, Martin's, Richard's and Czapek's) were used in this experiment to evaluate the growth performance of *F. oxysporum* (FOC1), *M. phaseolina* (MPC2) and *R. solani* (RSC4) on each media.

5-1-2- Effect of temperature on linear growth of the tested fungi:

Seven temperature degrees, 10, 15, 20, 25, 30, 35 and 40°C have been chosen to study the impact of temperature on the linear growth of *F. oxysporum* (FOC1), *R. solani* (RSC4) and *M. phaseolina* (MPC2), coriander inducing root rot/wilt disease.

5-1-3- Effect of relative humidity on linear growth of the tested fungi:

To study the effect of relative humidity (RH) on fungal linear growth, the method described by **Solmon (1951)** was used. Petri dishes containing PDA medium were inoculated with 5 mm fungal discs and turned upside down. Ten milliliters of appropriate concentrations of KOH or NaCl were poured into the lid of each dish to give relative atmospheric humidity levels of 14.5, 50, 65, 75, 80, 84 and 100%. Three replicates were applied for each humidity level. The linear growth of each fungus was measured, 4- 7 days after incubation at $25\pm 2^{\circ}\text{C}$.

Statistical analysis

Data of all treatments were arranged and presented as mean from three replicates. The experimental designs of all experiments were completely randomized. Data were statistically analyzed for significance in Statistix (8th edition,

Analytical Software, USA, **Steel et al., 1997**) using analysis of variance (ANOVA). Significance between means was compared by Duncan's multiple range test at $p < 0.05$ probability according to the method of **Gomez and Gomez (1984)**.

RESULTS

1-Survey for the Incidence and severity of coriander wilt in Minia Governorate, Egypt:

Observations in many fields of coriander (*Coriandrum sativum* L.) in Minia districts during winter 2018 - 2019 season showed several symptoms due to root rot and wilt infections.

The survey revealed the prevalence of coriander root rot/wilt in all locations under this study. The disease incidence ranged between 7 and 45%, whereas disease severity (DS,%) ranged between 5 and 32% in different villages of the districts surveyed. The highest disease incidence (DI,%) and severity (DS,%) (45 and 32%, respectively) were recorded in fields of Sandafa and Darwah, respectively, whereas the least incidence and severity (7 and 5%, respectively) was recorded at Ashroubah village in Beni Mazar district.

The highest disease incidence (37.5%) and severity (31%), at the third observation, were recorded in Malawi district (**Table 1**), followed by Beni Mazar, Maghagha and Deir Mawas for disease incidence (35.5- 36.97%). Whereas, the lowest disease incidence (29.3%) and severity (14.4%), in the same period, were recorded in Minia and Beni Mazar districts, respectively. Generally, the average disease incidence (36.32%) and severity (20.72%) were recorded at the

third observation (from 14 Jan. 2021 to 6 Feb. 2021).

Coriander root rot/wilt was severe in Mallawi district compared to other districts, this could be because of favorable environmental conditions and initial inoculum prevailed in this district might have helped in the rapid development of the disease in winter.

2-Isolation and identification of fungi associated with infected coriander root rotted/ wilted plants

Twenty-nine isolates of different fungi, belongs to nine different species (Table 2), were isolated. The most dominant genus was *Fusarium* which presented the highest frequency (48.28%), followed by *Rhizoctonia solani* (20.69%) and *Macrophomina phasiolina* (17.24%) and 13.80% of each *Alternaria* and *Mucor* sp. (Table 3).

The highest frequency of isolated fungi was recorded with samples from Ezbat Silim Basha (Minia district, 44.80%), followed by Tanbidi village (Maghaghah district, 34.50 %) while the lowest rate of isolated fungi was associated with samples collected from Dayer Mawas district (20.70%) (Table 3). *Macrophomina phasiolina* was isolated from all three districts under study, while *Rhizoctonia solani* was isolated from Minia and Dayer Mawas districts only. Also, *F. oxysporum* and *F. solani* were isolated from Dayr Mawas and Maghaghah. *Fusarium moniliform*, *F. equiseti* and *Mucor* sp was isolated only from Maghaghah. *Alternaria alternata* was isolated from samples collected from Dayr Mawas (Tables 2 and 3).

3-Pathogenicity test:

Pathogenicity test was carried out during winter season of 2019 using seeds

of coriander Balady cv. at the farm of the Plant Pathology Department, Faculty of Agriculture, Minia University.

Data in Table (4) could be summarized at the following points:

1- Pre- emergence damping off:

Data showed that *Rhizoctonia solani* induced the highest percentages of pre-emergence damping off (32.5%), followed by *Fusarium* spp. (28.45%) and then *M. phaseolina* (26.33%). While, *Alternaria alternata* and *Mucor* sp. induced the lowest percentages of pre-emergence damping off (7.5 and 5%, respectively). Isolates RSC4 and RSC1 of *R. solani* caused the highest percentages of pre-emergence damping – off (41.67 and 40.0%, respectively), followed by *Fusarium semitectum* isolate FSC2, and *F. equiseti* isolate FEC1 causing 38.33%. *Rhizoctonia solania* (isolates RSC2 and RSC3), *Macrophomina phaseolina* (MPC2, MPhC4, MPhC5), and *F. semitectum* (isolates FSC1 and FSC5) caused 28.33 – 33.33% pre-emergence damping-off, while, *Fusarium oxysporum* isolates caused the lowest percentages of pre-emergence damping- off (13.33 – 20.0%).

2- Post-emergence damping off:

Data also indicated that the highest percentages of post- emergence damping off induced by *M. phaseolina* (35.00%) followed by *R. solani* (27.50) and *Fusarium* spp. (22.97%). Isolates MPC2, MPhC1 and MPhC3 induced the highest percentages of post emergence damping off (40.0 and 38.33 % respectively), followed by *R. solani* (isolates RSC5, RSC1 and RSC2) caused 36.67 and 30.0%, respectively. *Fusarium solani* (isolate FsoC2) and *F. semitectum* (isolate FSC3) caused 36.67 and 33.33% post emergence damping off, respectively. Few number of plants were post- emerging

damped off due to infection with *F. oxysporum* (8.33-13.33%).

2- Coriander wilt:

Data presented in Table (4), clearly showed that *Fusarium* spp. caused the highest wilt incidence and severity (41.43 and 27.98%, respectively), followed by *M. phaseolina* (33.33 and 14.67%) and then *R. solani* causing 24.44 and 11.39%, respectively. The highest disease incidence (80%) and disease severity (53.33%) induced by *F. oxysporum* (isolate FOC1) which consider the highest aggressive isolate inducing wilt for coriander plants under Minia conditions. Isolates can be categorized depending on their ability to induce wilt for coriander plants to 3 categories, a) highly pathogenic isolates, causing between 60 – 40% DI% and 35 – 16.67% DS, including isolates FOC2, FOC3, FSC1, FSC3, FSoC1, FSoC2, FEC1, FMC1 (From genus *Fusarium*), RSC4 (*R. solani*), and isolates MPC2 and MPhC3 which belonging to Genus *Macrophomina*. b) moderate pathogenic, inducing between 39 – 30% DI%, and between 13 -25% Ds,%, including isolates FSC2, FSC5, FsoC3, RSC5 and MPhC1) and C): weakly pathogenic isolates, FSC4, FMC2, RSC1, RSC2, RSC6, MPhC4, MPhC5, AaC1 and AaC2., which induced between 29% to more than 10% DI and 5 - 24% DS.

Based on the results of pathogenicity test, isolates of *Fusarium oxysporum* (FOC1), *Rhizctonia solani* (RSC4) and *Macrophomina phaseolina* (MPC2), that have the highest virulent, were chosen to carry out the laboratory experiments. The resulting ITS (541, 696 and 568-bp for FOC1, RSC4 and MPC2, respectively), and sequences of isolates FOC1, RSC4 and MPC2 were deposited in GenBank (accession nos. OP107938, OP108814 and OP108816, respectively). According to the morphological and molecular characters,

the isolates FOC1, RSC4 and MPC2 were identified as *F. oxysporum*, *R. solani* and *M. phaseolina*, respectively (Figure 1).

4- Susceptibility of hosts belongs to family Apiaceae to coriander seedling damping-off and root rot/wilt inducing pathogens:

Six plant species belonging to family *Apiaceae*, viz. **caraway** (*Carum carvi*), **carrot** (*Daucus carota*), **cumin** (*Cuminum cyminum*), **dill** (*Anethum graveolens*), **fennel** (*Foeniculum vulgare*) and parsley (*Petroselinum crispum*) were tested for their reaction to seedling damping-off and root rot/wilt inducing fungi isolated from naturally infected coriander plants.

Data in Figures (2 and 3) show that *M. phaseolina* (isolate MPC2), *F. oxysporum* (isolate FOC1) and *R. solani* (isolate RSC4), which isolated from natural infected coriander plants, were able to attack all tested plant species with different degrees of infection. No significant differences in cumin, dill and fennel seedling damping-off were recorded. Cumin is the most susceptible host to seedling damping-off (39.72%), followed by dill (36.67%) and fennel (35.86). While parsley (30.42%, DI) and caraway (26.67%) were moderate. Carrot seedlings were the most resistant to damping off presented 14.59% infection. *Rhizoctonia solani* was the aggressive pathogen causing 55.93% seedlings damping-off. But, no significant differences were showed between *M. phaseolina* (35.19%) and *F. oxysporum* (30.83%). Data presented also that the highest seedling infection was induced by *R. solani* in fennel (70.0%) and cumin (67.22%). Whereas cumin followed by dill were the most susceptible to *M. phaseolina* (causing 57.78% and 49.44% respectively). Parsley was more susceptible to *F. oxysporum* (46.67%).

Also, no significant differences were recorded between dill, cumin and fennel (35.56, 33.89 and 32.22%, respectively). The lowest seedling damping-off was recorded in carrot, 8.33% either with *M. phaseolina* or *F. oxysporum*, and 41.67% with *R. solani*.

The percentages of wilted plants and severity were recorded in Figures (4, 5, 6 and 7). Data showed that *F. oxysporum* induced the highest wilt incidence and severity (34.44 and 15.83%, respectively), followed by *R. solani* and *M. phaseolina*. The highest disease incidence and severity was induced on cumin (33.34 and 16.25%, respectively, with no significant differences were showed on dill and fennel plants (28.33 and 26.67%, for DI% and 15.0 and 12.08% for DS%). Disease incidence and severity on caraway, parsley were ranged between 18.34 and 15.0 % DI and 6-5% DS, respectively, whereas, carrot showed the least incidence 15% and severity 3.75%. Dill followed by cumin were the most susceptible hosts to infection with *F. oxysporum* (53.33, 46.67 DI and 30.0, 23.33 DS, respectively). No significant differences were showed when cumin plants were infected with *F. oxysporum*, *M. phaseolina* or *R. solani* (46.67 – 40.0% DI and 18.33 – 23.33% DS).

Laboratory experiments:

5-Physiological studies:

In these studies, the effect of various media, temperature, and relative humidity on the growth of *Fusarium oxysporum* (isolate FOC1), *Rhizoctonia solani* (isolate RSC4) and *Macrophomina phaseolina* (isolate MPC2), the causal pathogens of coriander seedling damping-off and wilt, was tested in the laboratory

5-1-1- Effects of different media on fungal growth:

The effect of 8 media on the fungal growth (in mm) of the most pathogenic fungi (i.e. *F. oxysporum*, *R. solani* and *M. phasiolina*) was estimated, 7 days after inoculation. Results presented in **(Figure 8)** showed that the natural media were the best for growth of all tested isolates (the fungal growth was ranged between 90.6 – 100%. Whereas, the artificial media were differed in their encouragement fungal growth of the tested fungi. Nutrient glucose medium was the best artificial media for *F. oxysporum* and *R. solani*. Czapek's and Martin's media were the most favorable for *Rhizoctonia solani*. Whereas, Richard's medium was inappropriate for growth of all tested three fungi. Except of *R. solani* on Martin' media, the poorest growth of tested fungi (15 – 33.8 mm) occurred on Richard's and Martin' media.

5-1-2- Effect of temperature on fungal growth:

Fungal linear growth (in mm) of *Fusarium oxysporum*, *Macrophomina phasiolina* and *Rhizoctonia solani*, the most pathogenic fungi to seedling damping off and wilt of coriander, was estimated, 7 days after culturing in PDA medium. The obtained results in **(Figure 9)** showed that all 3 fungi tested grew in a wide range of temperature (15 - 35°C). The optimum temperature for growth of *F. oxysporum* was 30-35 °C, *M. phasiolina* was 30°C, and 25 - 30°C for *Rhizoctonia solani*. Moderate growth (40- 70 mm) for *F. oxysporum* and *M. phasiolina* was obtained on medium at 20 -25 °C, and at 20 and 35 °C for *R. solani*. Also, poor mycelial growth was obtained on medium at 15°C. All tested fungi failed to grow at 5 and 40 °C.

5-1-3- Effect of relative humidity (RH,%) on mycelial growth:

The effect of atmospheric humidity on the linear growth of *F. oxysporum*, *M. phasiolina* and *R. solani*, 14.5, 50, 65, 75, 80, 84 and 100% of relative humidity (RH), was tested. Data in **(Figure 10)** show that all the tested fungi could grow in wide range of RH, ranged between 50 and 100 % RH. Increasing value of the atmospheric humidity (RH, %) from 50 to 100% gradually increased the linear growth of any of the tested pathogens. The maximum growth of the tested fungi was obtained at 84 and 100% RH. However, differences between the obtained values of all tested fungal growth at 50 and 75% levels of RH were statistically insignificant at 0.05 level. It is, also, clear that all three tested fungi failed to grow at 14.5% relative humidity.

DISCUSSION

Coriander (*Coriandrum sativum* L.) is an annual growing, glabrous, aromatic herbaceous crop, belonging to the family *Apiaceae* (*Umbellifera*), native to the Mediterranean and Middle Eastern regions. All parts of the coriander plant are edible, but the fresh leaves and the dried seeds are most commonly used.

Damping off and wilt are the major constraints in the production of coriander. Damping-off caused by several soil borne fungi is dangerous threatening disease affecting coriander plants in Egypt and presumably in many other countries over the world.

During 2018-2019 season, damping off, yellowing, and wilt symptoms were observed on coriander plants cultivated in some private fields belonging to Minia Governorate. A field survey was conducted to gather some information about the incidence and severity seedling damping off and root rot from coriander

growing districts of El-Minia Governorate. This study revealed the prevalence of coriander root rot/wilt in all districts (Dayr Mawas, Mallawi, Minia, Beni Mazar and Maghagha) under study. Coriander damping-off and root rot incidence (DI,%) ranged between 4 and 30%, whereas disease severity (DS,%) ranged between 1 and 18.25% in different villages of the districts surveyed. The highest DI, % and DS, % (30 and 18.25%, respectively) were recorded in Tanbadi (Maghagha district), whereas the least ones (4 and 1%, respectively) were recorded in Ashroubah village; Beni Mazar district. The highest average for either disease incidence or severity of 23.8 and 13.67 were recorded at the third observation (which carried out from 14 Jan. 2019 to 6 Feb. 2019).

The highest disease incidence (30%) and severity (18.25%), at the third observation were recorded in Maghagha district, followed by Dayr Mawas (24 and 14.75), and between 22.0 -23.24DI% and 11.43- 14.5 DS% for Minia, Mallawi and Beni Mazar, in the same period. Coriander root rot/wilt was severe in Maghagha district compared to other districts, this could be due to favorable environmental conditions and initial inoculum prevailed in this district might have helped in the rapid development of the disease in winter. These results were similar to that obtained by **Nada et al. (2014)**, who found that seedling damping off, root rot and wilt causes highly losses on coriander yield in Qalubiya and Sharkiya Governorates, Egypt. Also, **Zaky (1998)** mentioned that the percentages of wilt and root rot of coriander ranged between 17.9 – 40% in natural infected fields in Beni Suef, Minia and Assiut Governorates, Egypt, at 1994 and 1995 seasons. The authoress reported that the highest disease percentage was in Minia Governorte than

the other ones. The losses in seed and foliar yields reduced by 5 - 60% as a result of wilt infection caused by *Fusarium* spp. (**Prasad and Patel, 1963** and **Manoranjitham et al., 2003**).

Six isolates of *Rhizoctonia solani*, five isolates of each *Fusarium semitectum* and *Macrophomina phaseolina*, three isolates of each *F. solani*, and *Fusarium oxysporum*, two isolates of either *Fusarium moniliforme*, *Alternaria alternata* or *Mucor* sp. and one isolate of *Fusarium equiseti* were isolated from naturally infected seedlings and wilted coriander plants which were collected from the different location of survey. The most dominant genus was *Fusarium* which presented the highest frequency (48.28%), followed by *R. solani* (20.69%) and *M. phasiolina* (17.24%) whereas *Alternaria* and *Mucor* sp. represented 6.90%. The highest frequency of fungi (44.80%) was isolated from Minia district, followed by (34.50%) from Maghagha district, while the lowest frequency of fungi (20.70%) was isolated from Dayr Mawas district. *Macrophomina phasiolina* was isolated from all three districts under this study, while *R. solani* was isolated from Minia and Dayr Mawas districts. Also, *F. oxysporum* and *F. solani* were isolated from Dayr Mawas and Maghagha. Whereas, *F. moniliforme*, *F. equiseti* and *Mucor* sp. were isolated only from Maghagha but *Alternaria alternata* was isolated from samples collected from Dayr Mawas. These results are similar to that obtained by several researchers. **Nada et al. (2014)** isolated *A. alternata*, *F. oxysporum*, *F. moniliforme*, *F. solani*, *Pythium* sp., *M. phaseolina*, *R. solani* and *S. sclerotiorum* from rotted roots and wilted coriander plants in Qalubiya and Sharkiya governorates, Egypt. **Zaky (1998)** reported *F. oxysporum*, *F. solani*.

R. solani, *Sclerotinia sclerotiorum*, *Drechslera* sp., *Alternaria* sp., and *Phytophthora* sp. are the pathogens of coriander root rot/ wilt diseases. **Khare et al., (2017)** mentioned more than eighteen diseases attacked coriander plants. *Rhizoctonia solani* was reported as coriander damping-off pathogen (**Anonymous, 2018**). Also, wilt caused by *F. oxysporum* f. sp. *corianderii* was recorded in India (**Srivastava, 1972**), in Argentina (**Madia et al., 1999**), in California (**Koike and Gordon, 2005**) and in Italy by **Gilardi et al. (2019)**. Coriander root rot caused by *F. solani* was reported by **Bhaliya and Jadeja (2014)**. Charcoal rot has been reported from Bulgaria (**Rodeva et al., 2010**) caused by *M. phaseolina* and then was reported caused by *R. bataticola* (Taub) Butle (**Khare et al., 2017**). *Pythium aphanidermatum* was isolated from several coriander growing regions of India (**Ashwathi et al., 2017**).

Pathogenicity test proved the ability of tested fungi to infect coriander seedlings causing damping off and root rot. *Rhizoctonia solani* (isolates RSC4 and RSC1) induced the highest percentages of pre-emergence damping-off (41.67 and 40.0%, respectively) followed by *Fusarium semitictum* (isolate FSC2), and *F. equiseti* (isolate FEC1) causing 38.33%. Moderate infection (28.33-33.33%) was caused by *Rhizoctonia solania* (isolates RSC2 and RSC3), *Macrophomina phaseiolina* (MPhC2, MPhC4, MPhC5), and *F. semitictum* (isolates FSC1 and FSC5). The lowest pre-immersion damping-off was recorded by *F. oxysporum*. *Macrophomina phaseiolina* induced (35.0%) the highest percentages of post immersion damping off, followed by *R. solani* (27.50) and then *Fusarium* spp. (**22.97%**).

The highest wilt incidence and severity were caused by *Fusarium* spp., followed by *M. phaseiolina* and then *R. solani*. Isolate FOC1 of *Fusarium oxysporum*, which consider the highest aggressive isolate, induced the highest coriander wilt incidence (80%) and severity (53.33%) under Minia conditions. Species of these soil borne fungi can live saprophytically or parasitically. Their parasitic stage often depends on external factors. When conditions are favorable for the fungus but less for the host, these fungi can be pathogenic and caused pre-, and post-emergence damping-off of seeds and seedlings, and rotting of roots, stems and fruits. Young or watery tissues are preferentially affected. Infection takes place when spores or sclerotia produce germ tubes (**Spencer and Cooper, 1967**) or hyphal elements form appressoria and penetrate the plant by means of infection pegs (**Miller et al., 1966**).

Macrophomina phaseiolina (isolate MPC2), *F. oxysporum* (isolate FOC1) and *R. solani* (isolate RSC4), which isolated from natural infected coriander plants, were able to attack all tested plant species belonging to family *Apiaceae*, viz. **caraway, carrot, cumin, dill, fennel** and parsley, under El-Minia conditions, with different degrees of infection. No significant differences between cumin, dill and fennel seedling damping-off were recorded. Cumin is the most susceptible host, but Carrot seedlings were the most resistant to seedling damping-off, followed by dill and fennel, whereas, parsley and caraway were moderate. *Rhizoctonia solani* was the aggressive pathogen causing 55.93% seedlings damping-off. As well as *F. oxysporum* induced the highest wilt incidence and severity, followed by *R. solani* and *M. phaseiolina*. The highest wilt incidence and severity was induced on cumin, but no

significant differences were showed on dill and fennel plants. Carrot showed the least incidence and severity to wilt infection. These results are in agreement with that obtained by **Zaky (1998)** who found that seven hosts belonging to Family *Apiaceae*, i.e. **fennel, anise, caraway, celery, dill, cumin and parsley**, were highly susceptible to infection with root rot/wilt disease

This study revealed that corn meal, malt, coriander extract, and PDA were the best for growth of all tested isolates, Whereas, Nutrient glucose medium (NGA) was the best artificial media for both *F. oxysporum* and *R. solani*. Czapek's and Martin's media were the most favorable for *Rhizoctonia solani* but Richard's medium was inappropriate for growth of all tested three fungi. All three tested isolates grew in a wide range of temperature (15-35°C). The optimum temperature (25-35°C) was recorded for *Rhizoctonia solani*, *M. phaseolina* and *F. oxysporum* growth in ascending order. Whereas, moderate growth for the three isolates tested was obtained at 20 and 25°C. Also, poor mycelial growth was obtained at 15°C. Increasing value of the atmospheric RH from 50-100% gradually increased the linear growth of any of the tested pathogens *in vitro*. The maximum growth of the tested fungi was obtained at 84 and 100% RH, whereas their failed to grow at 14.5% relative humidity. Our results are in close agreement with those obtained by several researchers, **Chinoko and Naqvi (1989)** reported that environmental elements, such as relative humidity (RH) and temperature, were play an important role and found to have a positive correlation and a significant impact on fungal infectiousness. The majority of the fungi studied had their optimum development temperatures between 25 and 30°C, whereas, with

temperatures exceeding 40°C resulting in poor growth and, in some cases, death (**Sharma and Razak, 2003**). The maximum radial growth and sporulation of *Fusarium oxysporum* f. sp. *ciceri* and *F. oxysporum* f. sp. *cubense* were 25 and 30 °C and 20-25°C for *F. oxysporum* f. sp. *melon*, after 7 days of inoculation, but were drastically reduced below 15°C and above 35°C, with negligible growth or sporulation at 5 or 40°C. (**Farooq et al.; 2005, Naik et al., 2010, Nath et al., 2017; and Bhavya et al., 2019**). *Fusarium solani* and *F. tricinctum*, the seedling damping off and root rot pathogens of soybean, were grown *in vitro* at temperature ranged between 8.5–34.3°C and 3.1–32.2°C, respectively, with optimum temperature 29.3 and 24.4°C, respectively (**Yan and Nelson, 2020**). *Fusarium oxysporum* f. sp. *ceciri* showed best growth and sporulation on chickpea seed-meal agar and Czapek dox agar, while *M. phaseolina* was obtained the maximum mycelial growth and sclerotial formation on PDA medium followed by Richard's medium (**Jha and Dubey; 2000, Farooq et al., 2005 and Parmar et al., 2018**). The optimum temperature for *M. phaseolina* growth and sclerotial development was found to be between 25 and 35 °C (**Parmar et al., 2018**). This fungus can cause significant production losses in crops like coriander, soybean and sorghum at 30–35°C and soil moisture below 60% (**Kaur et al., 2012**). In 2017, **Khare et al.** reported that the favoured soil moisture (60 -70%) and optimum temperature (24- 27°C) were the best for development of coriander wilt caused by *Fusarium oxysporum* f. sp. *coriander*.

Table (1): Survey of coriander root rot/wilt in different districts of Minia Governorate, at three periods of vegetable season

District	Village	First observation ⁽¹⁾		Second observation		third observation	
		Wilt incidence	Wilt severity	Wilt incidence	Wilt severity	Wilt incidence	Wilt severity
Dair Mawas	Nazlet Badraman	29 ⁽²⁾	19%	33%	24%	36%	26%
	Bani Haram	28%	20%	28%	20%	35%	27%
	mean	28.50%	19.50%	30.50%	22.00	35.50	26.50
Mallawi	Darwah	25%	17%	28	26	40	32
	Om Nakhla	28%	20%	30	23	35	30
	mean	26.50%	18.30%	29%	24.50%	37.50	31.00
Minia	Izbat Silim Basha	22%	13%	28	16	29.3	17.3
Beni Mazar	Ashroubah	7%	5%	15	8.3	36.6	11
	Om Assas	10%	7%	26	11.6	38.3	15
	Al Qays	11%	9%	20	10.6	28	13.3
	Sandafa	17%	10%	25	13.3	45	18.3
	mean	10.95	7.73	21.50	10.95	36.97	14.40
Maghagha	Tanbadi	20%	11%	29.3	13.3	40.0	17.3
	Mean	20	13	26.23	16.61	36.32	20.72

¹⁾1st observation in Deir Mawas, Mallawi and Minia districts was done at 17 Dec.2018 in Beni Mazar was carried out at 24 Dec.2020 and 9 January 2019 in Maghagha and then interval 15 days between each second and third observation in the same district.

⁽²⁾ Each reading is an average of 100 samples

Table (2): Frequency of isolated fungi obtained from diseased plants collected from different locations of Minia Governorates

Fungi	Code of isolate	No. of isolates	Locality of sample		Frequency, %
			District	Village	
<i>F. solani</i>	FSC7, FSC12	2	Maghaghah	Tanbidi	6.90
<i>F. moniliforme</i>	FMC5	2	Maghaghah	Tanbidi	6.90
<i>F. equiseti</i>	FEC8	1	Maghaghah	Tanbidi	3.45
<i>F. oxysporum</i>	FOC9- FOC 10	2	Maghaghah	Tanbidi	6.90
<i>M. phaseolina</i>	MPC18	1	Maghaghah	Tanbidi	3.45
<i>Mucor sp.</i>	MSC1	2	Maghaghah	Tanbidi	6.90
<i>R. solani</i>	RSC10 - RSC14	5	Minia	Ezbat Silim Basha	17.24
<i>F. semitictum</i>	FSC1-FSC5	5	Minia	Ezbat Silim Basha	17.24
<i>M. phaseolina</i>	MPC1 & MPC3	3	Minia	Ezbat Silim Basha	10.32
<i>F. oxysporum</i>	FOC3	1	Dayer Mawas	Nazlat Badraman	3.45
<i>F. solani</i>	FSC8- FSC 13	1	Dayr Mawas	Nazlat Badraman	3.45
<i>A. alternata</i>	AAC1	2	Dayr Mawas	Nazlat Badraman	6.90
<i>M. phaseolina</i>	MPC2	1	Dayr Mawas	Nazlat Badraman	3.45
<i>R. solani</i>	RS C15	1	Dayr Mawas	Bani Haram	3.45

Table (3): The percent of different genera attacking coriander plants according to their locations

Location (District)	Percent of different genera				Total Percent of isolated fungi
	<i>Fusarium</i>	<i>Rhizoctonia</i>	<i>Macrophomina</i>	Others	
Maghaghah	24.15	0.0	3.45	6.9	34.50
Minia	17.24	17.24	10.32		44.80
Dayr Mawas	6.9	3.43	3.43	6.9	20.70
Total	48.28	20.69	17.24	13.80	100.0

Table (4): Pathogenicity test, after 15, 30 days for pre- and post- emergence damping off and 60 days after seed sowing for wilting symptoms

Fungi	Code of isolate	Seedling damping off ⁽¹⁾			Wilt ⁽²⁾	
		Pri-emergence %	Post-emergence %	Suvirival seedling %	DI %	DS %
<i>F. oxysporum</i> (9)	FOC1	20.00 ⁽³⁾	11.67	68.33	80.00	53.33
<i>F. oxysporum</i>	FOC2	15.00	13.33	71.67	60.00	35.00
<i>F. oxysporum</i>	FOC3	13.33	8.33	78.33	40.00	30.00
<i>F. semitictum</i>	FSC1	28.33	25.00	46.67	46.67	30.00
<i>F. semitictum</i>	FSC2	38.33	16.67	45.00	33.33	21.67
<i>F. semitictum</i>	FSC3	23.33	33.33	43.33	40.00	23.33
<i>F. semitictum</i>	FSC4	28.33	23.33	48.33	26.67	20.00
<i>F. semitictum</i>	FSC5	30.00	26.67	43.33	33.33	25.00
<i>F. solani</i>	FSOC1	31.67	28.33	40.00	46.67	28.33
<i>F. solani</i>	FSOC2	26.67	36.67	36.67	40.00	25.00
<i>F. solani</i>	FSOC3	28.33	28.33	43.33	33.33	33.33
<i>F. equiseti</i>	FEC1	38.33	25.00	36.67	40.00	30.00
<i>F. moniliforme</i>	FMC1	23.43	18.33	40.00	40.00	23.33
<i>F. moniliforme</i>	FMC2	26.67	26.67	46.67	20.00	13.33
	average	28.45	22.97		41.43	27.98
<i>R. solani</i>	RSC1	40.00	30.00	30.00	13.33	5.00
<i>R. solani</i>	RSC2	30.00	30.00	40.00	13.33	5.00
<i>R. solani</i>	RSC3	30.00	21.67	48.33	20.00	10.00
<i>R. solani</i> (10)	RSC4	41.67	28.33	30.00	46.67	25.00
<i>R. solani</i>	RSC5	25.00	36.67	38.33	26.67	13.33
<i>R. solani</i>	RSC6	28.33	31.67	40.00	26.67	10.00
	average	32.5	27.50		24.44	11.39
<i>M. phaseolina</i>	MPhC1	21.67	38.33	40.00	33.33	15.00
<i>M. phaseolina</i>	MPC2	30.00	40.00	30.00	40.00	18.33
<i>M. phaseolina</i>	MPhC3	18.33	38.33	43.33	40.00	16.67
<i>M. phaseolina</i>	MPhC4	28.33	30.00	41.67	26.67	15.00
<i>M. phaseolina</i>	MPhC5	33.33	28.33	38.34	26.67	8.33
	average	26.33	35.00		33.33	14.67
<i>A. alternata</i>	AaC1	6.67	5.00	88.33	20.00	6.67
<i>A. alternata</i>	AaC2	8.33	10.00	81.67	20.00	10.00
	average	7.5	7.50		20.00	8.33
<i>Mucor sp.</i>	MusC1	5.00	5.00	90.00	0.00	0.00
<i>Mucor sp.</i>	MusC2	5.00	5.00	90.00	0.00	0.00
	average	5.00	5.00		0.00	

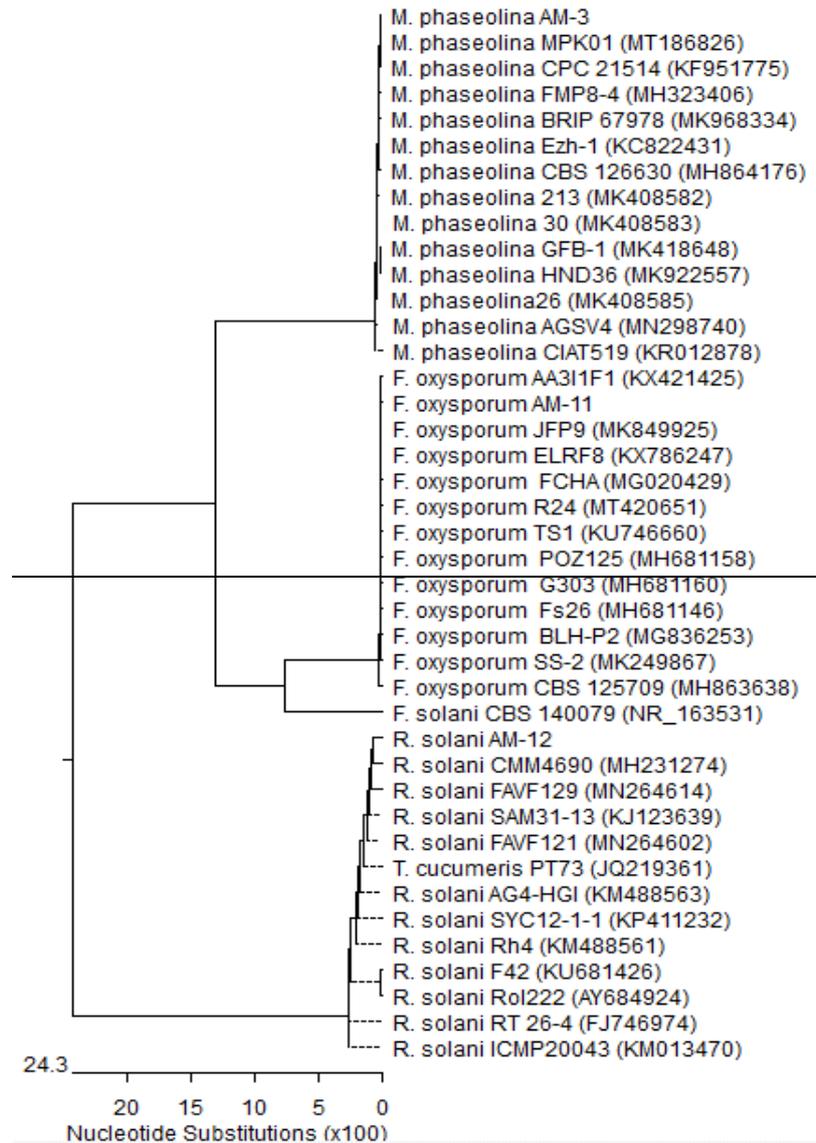


Fig. (1): Phylogenetic tree based on ITS region of rDNA of fungal strains isolated in the current study (AM-3; MPC2, AM-11; FOC1; and AM-12, RSC4) aligned with sequences of related strains accessed from the Genbank. The phylogenetic tree is comprised of two major branches. Strains of *Rhizoctonia solani* (*Basidiomycetes*) are shown in the lower branch. The upper branch contains two subclades of *Ascomycetes*; one for strains of *Macrophomina phaseolina* and the second for strains of *Fusarium oxysporum*.

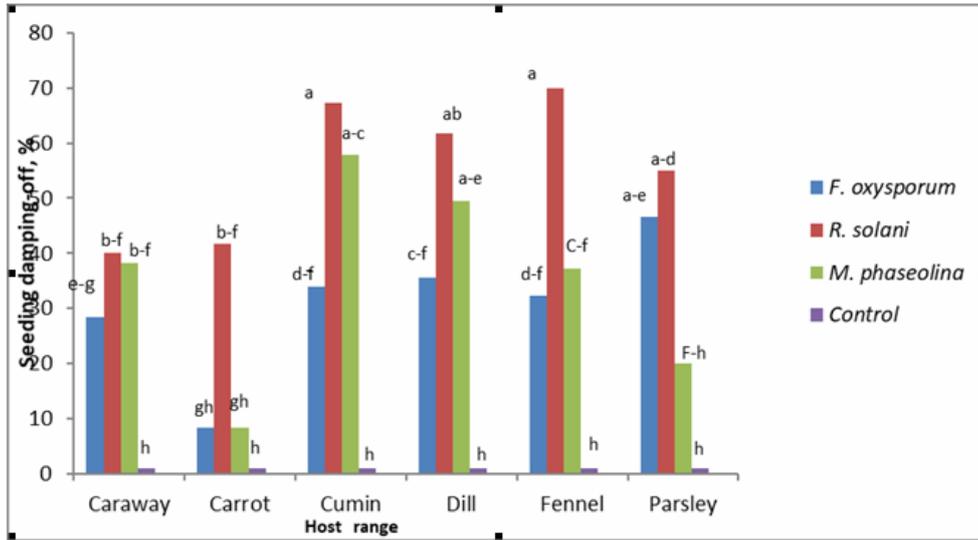


Fig. (2):Susceptibility of different hosts belonging to family *Apiaceae* to damping-off incidence, 30 days after sowing, at 2020-2021 winter season.

Data presented the average of three replicates each contains 3 plants.

Data with the same letter(s) within a column are not significantly different according to Duncan's a new multiple range test

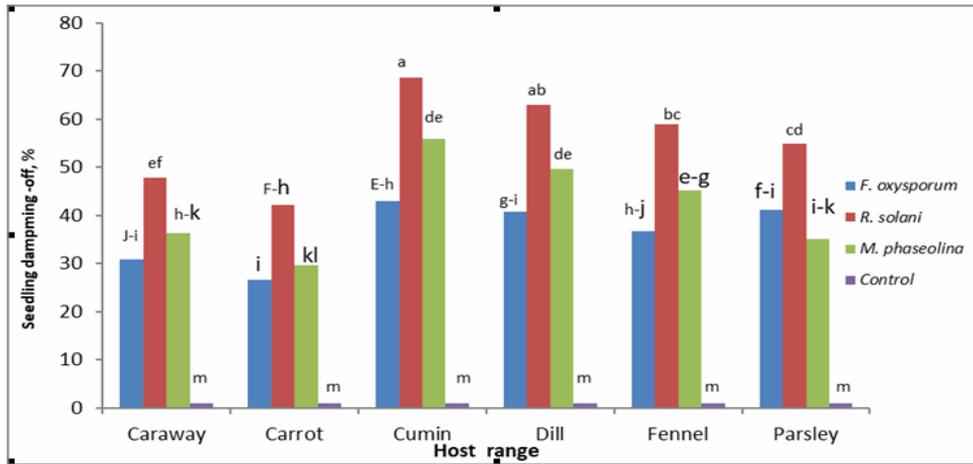


Fig. (3):Susceptibility of different hosts belonging to family *Apiaceae* to damping-off incidence, 30 days after sowing, at 2021- 2022 winter season

Data with the same letter(s) within a column are not significantly different according to Duncan's a new multiple range test

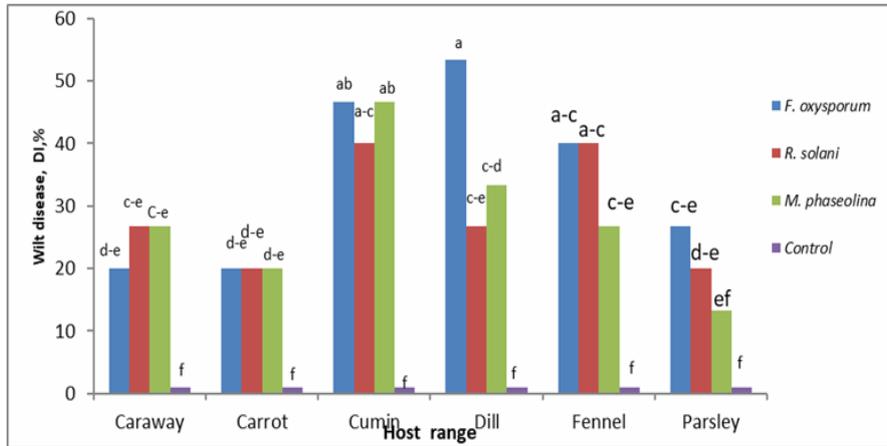


Fig.(4): Susceptibility of different hosts belonging to family *Apiaceae* to wilt incidence (DI,%) , 60 days after sowing at 2020/ 2021 Season

Data presented the average of three replicates each contain 10 plants
Data with the same letter(s) within a column are not significantly different according to Duncan's a new multiple range test

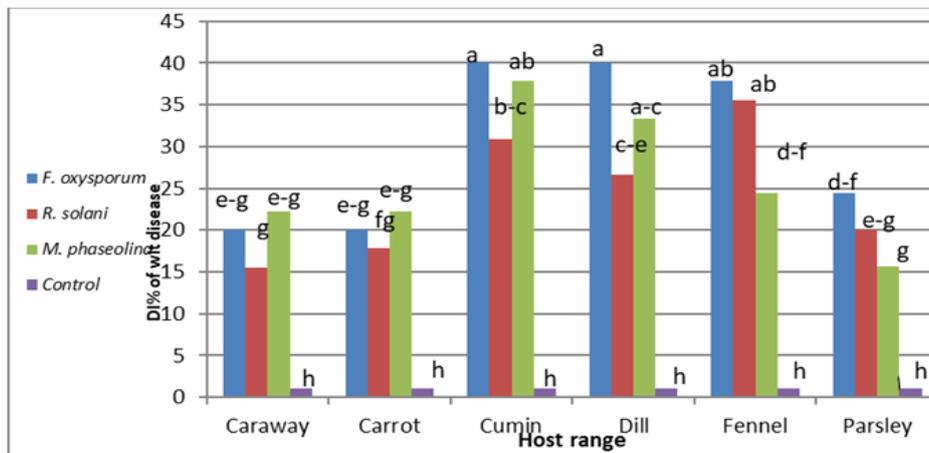


Fig. (5):Susceptibility of different hosts belonging to family *Apiaceae* to wilt incidence (DI,%) , 60 days after sowing at 2021/ 2022 season

Data presented the average of three replicates each contains 5 plants
Data with the same letter(s) within a column are not significantly different according to Duncan's a new multiple range test.

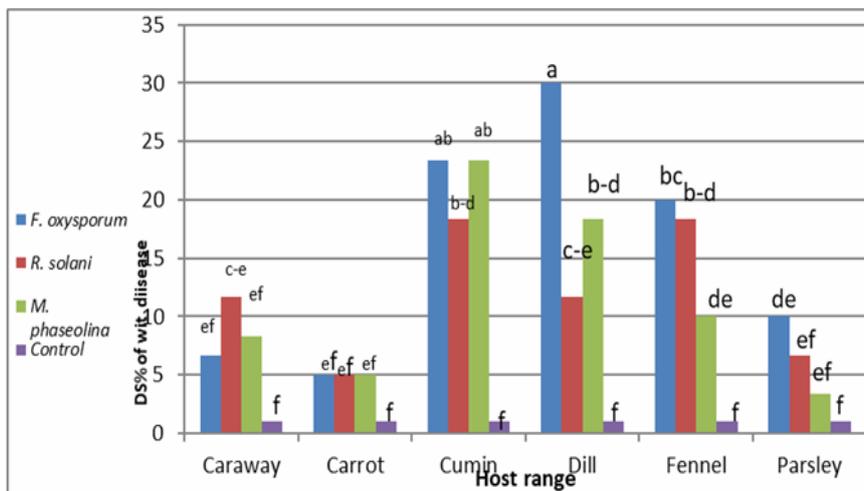


Fig. (6): Susceptibility of different hosts belonging to family *Apiaceae* to wilt severity (DS,%), 60 days after sowing, at the 1st season.

Data presented the average of three replicates each contains 5 plants

Data with the same letter(s) within a column are not significantly different according to Duncan's a new multiple range test

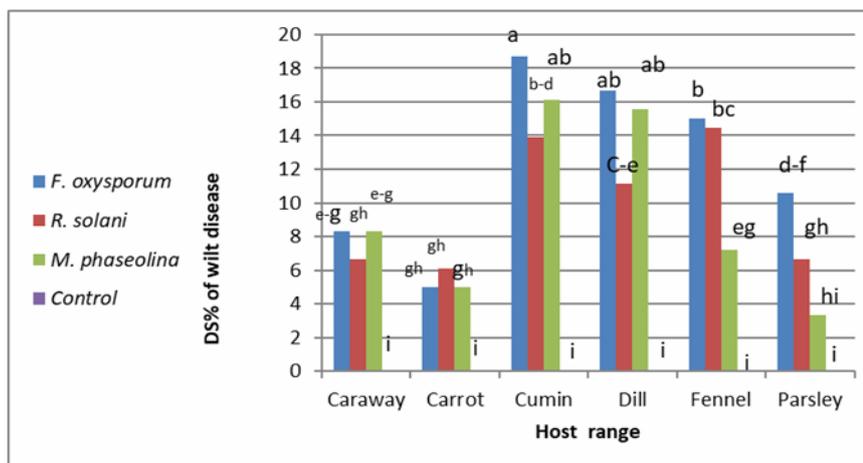


Fig. (7): Susceptibility of different hosts belonging to family *Apiaceae* to wilt severity (DS,%), 60 days after sowing, at the 2nd season.

Data presented the average of three replicates each contains 3 transplants

Data with the same letter(s) within a column are not significantly different according to Duncan's a new multiple range test.

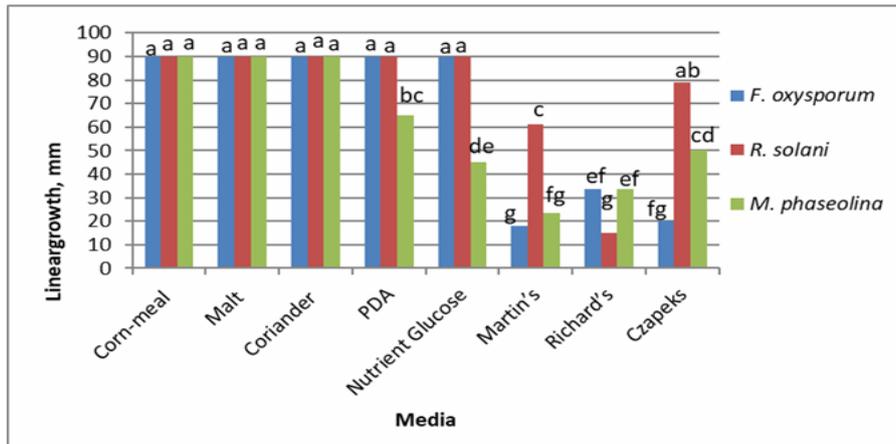


Fig. (8): Effect of different media on the growth (in mm) of three fungi causing coriander seedling damping-off and wilt, 7 days after inoculation.

Each reading presented an average of 3 replicates.

Data with the same letter(s) within a column are not significantly different according to Duncan's a new multiple range test

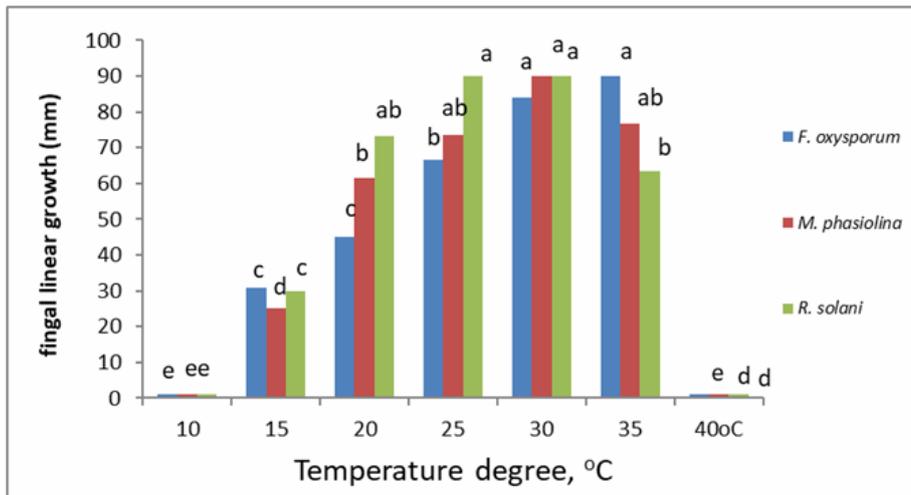


Fig. (9): Effect of different degrees of temperature on linear growth (in mm) of tested pathogens.

⁽¹⁾Each value is represented average of three replicates

Data with the same letter(s) within a column are not significantly different according to Duncan's a new multiple range test

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حصر أمراض موت البادرات وعفن الجذور والذبول في نباتات الكزبرة في محافظة المنيا

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يعتبر أمراض موت البادرات وعفن الجذور والذبول في الكزبرة من الأمراض التي تسبب فقدا كبيرا في المحصول خاصة في المناطق الإستوائية وتحت الاستوائية. وقد سجلت مسببات مرضية عديدة من قاطنات التربة كمسببات لهذه الأمراض. وقد أجرى حصر لهذه الأمراض في خمس مراكز تتبع محافظة المنيا، تبين منها انتشار هذه الأمراض في جميع هذه المراكز ولكن بدرجات مختلفة من نسبة وشدة الإصابة حيث تراوحت نسبة الإصابة بالذبول وعفن الجذور بين ٧ - ٤٥% وشدة الإصابة بين ٥ - ٣٢%. وقد تم عزل ٢٩ عزلة من الفطريات التي تتبع خمس أنواع فطرية مختلفة من العينات المصابة التي تم جمعها من مناطق الحصر. وقد ثبت قدرتها على احداث نفس الأعراض على الكزبرة صنف بلدى ولكن بدرجات متباينه. وقد تم اختيار أشد ثلاث عزلات في احداث أعراض موت البادرات وعفن الجذور والذبول ليتم تشخيصها باستخدام اختبار PCR بالإضافة إلى اختبارات الشكل المورفولوجى والخصائص المزرعية وثبت أنها الفطريات فيوزاريوم اكسيسورم، ريوكتونيا سولانى وماكروفومينا فاصولينا. وبينت التجارب قدرة هذه الفطريات على إصابة جميع النباتات المختبرة، وهى الكراوية، الكمون، الشبت، الشمر، والبقدونس والجزر، إلا أن الجزر كان أشدها مقاومة. بينت الدراسات الفسيولوجية لهذه الفطريات أن أفضل نمو لها حدث على بيئات مستخلص البطاطس، مستخلص دقيق الذرة ومستخلص الشعير وبيئة مستخلص بذور الكزبرة وبيئة دكستروز الاجار المغذى NDA، كما أن النتائج بينت أن هذه الفطريات تنموى مجال واسع من درجات الحرارة وال22رطوبة النسبية، تتراوح بين ١٥ - ٣٥ م و ٥٠-١٠٠% رطوبة نسبية، مع درجة حرارة مثلى تراوحت بين ٢٠-٣٠م ودرجة مثلى من الرطوبة النسبية تراوحت بين ٨٤ - ١٠٠%.