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Isolation and Identification of Pathogens Associated with Root Rot and Wilt Disease on Pepper and Testing Their Pathogenicity

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ABSTRACT

This study was conducted in the Department of Plant Protection Research, Agricultural Research Office with the aim of isolating and identifying the pathogens associated with the disease of wilt and rotting of the roots and bases of the stems of pepper plants and identifying them phenotypically and testing their pathogenicity in vitro and in pots. The results of isolation and identification showed the presence of 50 isolates of fungi associated with pepper plants return to eight genera that varied in appearance according to the areas from which they were collected, namely Babylon, Baghdad and Karbala provinces and pathogenic fungi showed that all the tested fungi led to a significant reduction in the percentage of germination of pepper seeds compared to the control treatment and the results of testing the effect of 16 pathogenic isolates in pepper seedlings in pots showed that all treatments had a high percentage of severity of infection with disease of rotting the roots and bases of pepper stems and the *Monosporascus eutypoides* isolates was the highest from other fungal isolates.

Key words: *Capsicum anuum L.*, fungi,*Monosporascus eutypoides*, soil-born fungi.

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INTRODUCTION

Sweet pepper *Capsicum anuum L*. belong to the plants of the Solanaceae family, which is one of the important seasonal crops in the world and Iraq, it's grown in different areas in open and protected fields, as well as, the sweet peppers contain antioxidants, phenolic compounds and vitamin A, C and E which are important in human food (Howard, 2000). During the period of its growth, this crop is infected with many insect pests such as aphids, thrips, and other insect pests (Jawad, 2020), as well as, exposed to many pathogens, especially those that attack the seeds, vegetative and root system, which cause diseases of seed and root rot or the death of seedlings and wilting of the plant and thus lead to the death of the entire plant, including fungal pathogens *Alternaria spp.*, *Fusarium spp.*, *Macrophomina phaseolina*, *Phytophthora infestans*, *Pythium aphanidermatium* and *Rhizoctonia solani* (Crosby, 2008; Hantoosh and Suhail, 2016; Oljira and Berta, 2020). An important pathogen is the fungus *Monosporascus eutypoides*, which belongs to the Ascomycota division, Xylariales order, and Diatrypaceae family. It causes root rot and wilt disease on various crops, including cucumber, trees, squash, watermelon, and strawberry (Castro et al.,2020); (Najem and Kareem, 2018; Salem et al., 2013; Zhang et al., 2025). Symptoms of root rot disease caused by the fungus *Fusarium spp., M. eutypoides* and *R. solani* appear in the form of poor growth, wilting and yellowing of the vegetative system, rotting of the root system and its brown color extends to the crown area. Fusarmic wilt disease is one of the most important diseases that affect the pepper plant and it is caused by the fungus *F. oxysporum f. sp. capcisi*, which attacks the roots and carrier vessels, causing it Wilting, curvature and death of leaf petiole (Al-aamel and Al-maliky, 2023; Al-Morad and Al-Khafagi, 2011; Velarde-Felix et al., 2018). A

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number of researchers have pointed out that fungal pathogens are endemic in the soil such as *Fusarium spp.*, *M. phaseolina*, *M. eu-typoides*, *P. aphanidermatium*, *R. solani* and *Sclerotinia sclerotiarum* infect a number of plants and cause a number of diseases (Almohammedy and Al-maliky, 2020; Ndiaye, 2007). Several control methods have been used to reduce the risk of this disease, including chemical control and the use of fungicides such as Beltanol, Benomyl, Blitox 50, Captan, Carbendazim and Topsin-M, which have shown high effectiveness in combating this disease (Attri and Sharma, 2019; Bashir et al., 2018; Dholu et al., 2022). Due to the danger of fungal chemical pesticides harmful to humans and polluting the environment and the dam-age of their residues to the agricultural yield, researchers have been prompted to find alter-native methods of control. Alternative methods used include agricultural, physical, mechanical, biological control, resistant varieties and integrated management approach to control this disease, and the use of normal and nanoparticles compounds and all contributed to reducing the damage caused by these pathogens of this disease and reducing its losses (Yasir and Almaliky, 2023). The importance of the rot roots and wilt disease on pepper in Iraq and the economic losses caused by it, whether in open or protected agriculture and in order to reach effective and applicable methods in the fight against this disease in a way that suits the expansion of pepper plant cultivation and work to increase production, preserve the environment and achieve the goals of sustainable development, so this study aimed to isolate and identify the pathogens causing the root rot and wilt dis-ease on pepper and experiment its pathogenicity on pepper seeds in the vitro and seedlings in pots.

MATERIALS AND METHODS

Sample Collection: It was 150 samples were collected from pepper seedlings and plants that showed symptoms of root rot and wilt disease on pepper roots represented by root rot and dark brown color, the presence of ulcers in the crown area, rotting and yellowing of plants and their wilting (Figure 1) from some fields and nurseries in the governorates of Babylon (Jebalah and Musayyib), Baghdad (Rashidiya and Yusufiya) and Karbala (Ibrahimia and Abbas Shrine) for the agricultural season 2023-2024, as the samples were placed in polyethylene bags and recorded on them the place and date of collection, then transferred to the lab and kept in the refrigerator at Temperature 4 °C for the purpose of isolation and identification on the next day.



Figure 1. Symptoms of root rot and wilt on pepper

Isolation and Identification of Pathogens

The isolation process was conducted in the lab of the Plant Pathology Division, Plant Protection Research Department, Agricultural Research Office, as the vegetative part of the samples was cut and part of the stem and root system were retained, the samples were washed with running water from the tap for the purpose of getting rid of the dust stuck to them and other impurities, the roots and the crown area were cut into small pieces ranging from 0.5-1 cm and sterilized superficially with sodium hypochlorite solution (1% free chlorine) for two minutes, which was prepared

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according to the method of (O'Neill, 2009) according to the following equation

Volume of stock solution = Final volume × (Final concentration) / (Stock concentration) ……. (1)

Washed with sterile distilled water to get rid of chlorin and dried on Whatman No-1 sterile filter paper, each five pieces were planted in a 9 cm diameter petri dish containing a sterile Potato Dextrose Agar (PDA) culture media in Autoclave at a temperature of 121 °C and a pressure of 1.5 $kg.cm^{-2}$ for 15 minutes after which add the antibiotic Tetracycline at a concentration of 250 $mg.L^{-1}$ to prevent bacterial growth, the dishes were placed in the incubator at a temperature of 25± 2 °C for three days, after which the fungi that appeared were purified by taking a swab from the edge of the colony for each fungus and replanted in petri dishes containing sterile PDA culture media and incubated in the incubator for five days. The morphology identification of fungi was based on the phenotypic characteristics of the colony, its color, the microscopic characteristics of the structures, phorid and spores formed by the fungus were identified by using approved taxonomic keys according to (Hafizi et al., 2013; Leslie and Summerell, 2008; Ndiaye, 2007; Sneh et al., 2013; Stalpers and Anderson, 1996). The isolated fungi were kept in small vials with a tight cover contain a sterile and oblique PDA for use in subsequent experiments.

Pathogenesis Experiencing in Vitro.

The isolates obtained from the isolation process will be tested for pathogenicity according to (Bolkan and Butler, 1974), the center of each petri dish containing the sterile PDA culture media and the antibiotic is added to it with a disc diameter of 0.5 cm taken from the edge of a fungal colony at the age of five days for each isolation of fungal isolates separately, the dishes were incubated in the incubator for three days at a temperature of 25 ± 2 °C, the pepper's seeds (California Wonder variety from Top Harvest company, Holand) which sterilized with a solution of sodium hypochlorite 1% free chlorine (prepared according to the previous method) for two minutes after washing with sterile distilled water and dried with foil sterile blotter ring, planted in a circular manner near the edge of the dish at the rate of 25 seeds per dish, each treatment was repeated four replicates with a control treatment without fungi and incubated in the incubator at a temperature of 25 ± 2 °C, after seven days the percentage of germination was calculated according to the following equation:

The percentage of seeds germination = (Germinated seeds) / (Total seeds) × 100 …… (2)

Pathogenesis Experiencing in Pots: This experiment was carried out in the wooden canopy of the Department of Plant Protection Research, Agricultural Research Office, as California Wonder variety of pepper seedlings were planted in pots of 2 kg containing a mixture of soil and sterile peat moss in a ratio of 2:1 by five seedlings per pot and three replicates for each treatment and after seven days the fungal inoculum for 16 isolated was added to mantras on sterile millet seeds, each individually, which showed high efficiency in reducing germinating (as in the previous test) with a control treatment planted pepper seedlings without adding any fungus, the experiment was designed according to a completely randomized design (CRD) and after 30 days the percentage of the severity of infection was estimated using the Coefficient of Disease Index of five degrees according to (Sun et al., 2015) after some modifications to it

0 = healthy plant, 1 = roots are light brown with the stem base intact, 2 = roots are dark brown and the base of the stem begins to be discolored, 3 = roots and stem base are dark brown, 4 = roots rot and entire stem base (plant death).

The percentage of severity of infection was calculated as the equation follows:

% PSI = ((NSG 0×0) +...+ (NSG 4×4)) / (TNSE ×4) × 100 …… (3)

PSI = The Percentage of Severity of Infection

NSG = Number of Seedlings of Grade

TNSE = Total Number of Seedlings Examine

RESULTS AND DISCUSSION

Isolation and Identification

The results of isolation and identification of the pathogens associated with the roots and crown area of the infected pepper plants, which were collected from the governorates of Babylon (Jebalah, Musayyib), Baghdad

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(Rashidiya, Yusufiya) and Karbala (Ibrahimia, Abbas Shrine) through microscopic examination of fungal growth growing on plant plots, showed the presence of 50 isolates belonging to eight genera of fungi, namely 12 isolates of *Fusarium spp.*, 8 isolates of *Macrophomina phaseolina*, 10 isolates of *Monosporascus eutypoides*, 9 isolates of *Rhizoctonia solani* and three isolates each of *Sclerotinia sclerotiarum*, *Alternaria spp*. and *Aspergillus spp*. and two isolates of *Penicillium spp*. (Table 1).

Table 1. Fungi associated with infected pepper roots, their locations and date of collection.

Isolation	Sample Code	Place of collection	Date of collection
Fusarium spp.	BFu1	Babylon / Musayyib	2024/03/08
Fusarium spp.	BFu2	Babylon / Jebalah	
Fusarium spp.	BFu3		
Fusarium spp.	BFu4		
Fusarium spp.	BFu5		
Fusarium spp.	BFu6		
Fusarium spp.	BFu7		
Fusarium spp.	YFu8	Baghdad / Rashidiya	2024/03/07
Fusarium spp.	YFu9	Baghdad / Youssoufia	
Fusarium spp.	YFu10		
Fusarium spp.	KFu11	Karbala / Abbasid Shrine	2024/03/09
Fusarium spp.	KFu12		
Macrophomina phaseolina	BMa1	Babylon / Musayyib	2024/03/08
Macrophomina phaseolina	BMa2	Babylon / Jebalah	
Macrophomina phaseolina	BMa3		
Macrophomina phaseolina	YMa4	Baghdad / Youssoufia	2024/03/07
Macrophomina phaseolina	YMa5		
Macrophomina phaseolina	KMa6	Karbala / Ibrahimia	2024/03/09
Macrophomina phaseolina	KMa7	Karbala / Abbasid Shrine	
Macrophomina phaseolina	KMa8		
Monosporascus eutypoides	BMe1	Babylon / Musayyib	
Monosporascus eutypoides	BMe2		
Monosporascus eutypoides	BMe3	Babylon / Jebalah	
Monosporascus eutypoides	BMe4		
Monosporascus eutypoides	YMe5	Baghdad / Youssoufia	
Monosporascus eutypoides	YMe6		
Monosporascus eutypoides	YMe7		
Monosporascus eutypoides	YMe8	Baghdad / Rashidiya	
Monosporascus eutypoides	KMe9		
Monosporascus eutypoides	KMe10	Karbala / Abbasid Shrine	
Rhizoctonia solani	BRh1	Babylon / Musayyib	2024/03/08
Rhizoctonia solani	BRh2	Babylon / Jebalah	
Rhizoctonia solani	YRh3	Baghdad / Youssoufia	2024/03/07
Rhizoctonia solani	YRh4		
Rhizoctonia solani	YRh5	Baghdad / Rashidiya	
Rhizoctonia solani	KRh6	Karbala / Ibrahimia	2024/03/09
Rhizoctonia solani	KRh7	Karbala / Abbasid Shrine	
Rhizoctonia solani	KRh8		
Rhizoctonia solani	KRh9		
Alternaria spp.	BAl1	Babylon / Jebalah	2024/03/08
Alternaria spp.	BAl2	Baghdad / Youssoufia	2024/03/07
Alternaria spp.	BAl3	Karbala / Abbasid Shrine	2024/03/09
Scleractinia seclerotiarum	YSc1	Baghdad / Youssoufia	2024/03/07
Scleractinia seclerotiarum	YSc2	Baghdad / Rashidiya	
Scleractinia seclerotiarum	YSc3	Karbala / Abbasid Shrine	2024/03/09
Aspergillus flavus	BAf	Babylon / Jebalah	2024/03/08
Aspergillus niger	BAn1	Babylon / Musayyib	2024/03/08
Aspergillus niger	BAn2	Karbala / Ibrahimia	2024/03/09
Penicillium spp.	BP1	Babylon / Jebalah	2024/03/08
Penicillium spp.	BP2	Karbala / Abbasid Shrine	09/03/2024

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The presence of *Aspergillus spp*. and *Penicillium spp*. may be due to their high intrusive ability on plants and the penetration of their fungal filaments into the tissues of the host that has been infected with the fungus causing this condition. These results are consistent with the findings of Al-aamel and Al-maliky (2023) the presence and spread of pepper-pathogenic soil fungi in the governorate of Baghdad where they found through the study, the most important fungi causing root rot disease and wilting of pepper plants are *F. oxysporum*, several researches have indicated the isolation of these fungi *F. oxysporum*, *F. solani*, *M. phaseolina*, *M. eutypoides*, *R. solani* and *S. sclerotium* from different plants and soil which caused root rot disease of a number of plants (Abdullah and Al-Juboory, 2020; Castro et al., 2020).

Pathogenesis Experiencing in Vitro

The results of the pathogenesis test of isolated fungi showed the variation of the isolates of the fungi in their pathogenicity on pepper seeds (Table 2),

Table 2. Laboratory Pathogenesis Test of Fungi Associated with the Roots of Pepper Plants on Pepper

Isolation	Symbol of isolation	% of germination
Fusarium spp.	BFu1	39
Fusarium spp.	BFu2	52
Fusarium spp.	BFu3	15
Fusarium spp.	BFu4	0.0
Fusarium spp.	BFu5	1.0
Fusarium spp.	BFu6	0.0
Fusarium spp.	YFu7	51
Fusarium spp.	YFu8	53
Fusarium spp.	YFu9	13
Fusarium spp.	KFu10	58
Fusarium spp.	KFu11	14
Fusarium spp.	KFu12	29
M. phaseolina	BMa1	13
M. phaseolina	BMa2	11
M. phaseolina	YMa3	16
M. phaseolina	YMa4	23
M. phaseolina	YMa5	13
M. phaseolina	Kma6	13
M. phaseolina	Kma7	10
M. phaseolina	Kma8	14
M. eutypoides	BMe1	0.0
M. eutypoides	BMe2	10
M. eutypoides	BMe3	2.0
M. eutypoides	BMe4	6.0
M. eutypoides	YMe5	4.0
M. eutypoides	YMe6	0.0
M. eutypoides	YMe7	35
M. eutypoides	YMe8	0.0
M. eutypoides	KMe9	0.0
M. eutypoides	KMe10	0.0
R. solani	KRh1	33
R. solani	KRh2	27
R. solani	KRh3	29
R. solani	KRh4	18
R. solani	KRh5	14
R. solani	KRh6	29
R. solani	KRh7	11
R. solani	KRh8	20
R. solani	KRh9	18
S. seclerotiarum	YSc1	59
S. seclerotiarum	YSc2	46
S. seclerotiarum	YSc3	55
Alternaria spp.	BAl1	81
Alternaria spp.	BAl2	77
Alternaria spp.	BAl3	69
Control without fungi		96
L.S.D(0.05)		3.482

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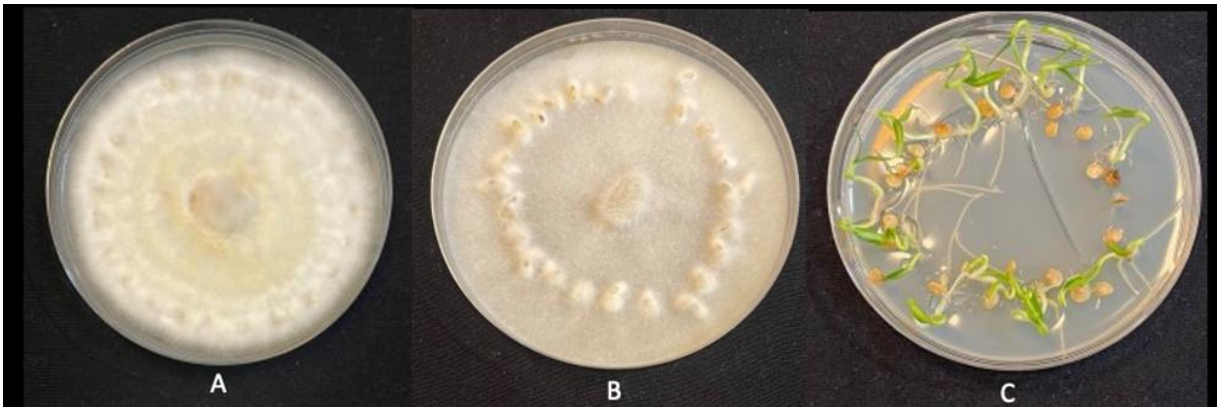

Figure 2. The effect of isolates of pathogenic fungi on the germination of pepper seeds in vitro. A: *Fusarium spp.*, B: *M. eutypoides,* C: Without fungi (control).

as all the tested fungi caused a significant reduction in the percentage of germination of pepper seeds compared to the control treatment, in which was 96% and the isolates of *Fusarium spp*. (BFu4, KFu6) and *M. eutypoides* (BMe1, YMe6, YMe8, KMe9, KMe10) were the highest pathogenicity which prevented pepper seeds from germinating and the percentage of germination in its treatments was 0% and did not differ significantly from the *Fusarium spp*. isolates (BFu5) and *M. eutypoides* (BMe3) in reducing the percentage of germination, reaching 1 and 2%, respectively, followed by isolates YMe5 and BMe4, reaching 4 and 6%, respectively (Figure 2), while other isolates achieved a significant reduction in pepper seed germination rates, which ranged in their treatments between 6-81%. These results are consistent with what Al-aamel and Al-maliky (2023) found that most of the tested fungi are *F. oxysporum f. sp. capsici* which causes pepper root rot, caused a significant reduction in seed germination rates on the PDF culture media, as the percentage of germination of pepper seeds reached 15%. Also, in previous study conducted in the vitro, it was found that the fungus *Monosporascus spp.* had a significant effect in reducing the percentage of germination of watermelon seeds.

These differences in the pathogenesis of the tested fungi in reducing the percentage of germination of pepper plant seeds may be due to environmental and genetic differences in the compositions of fungal isolates and may be attributed to the effect of fungal isolates to toxic secondary metabolic secretions that lead to the killing of seed embryos and to the ability to produce decomposers that are responsible for the occurrence of rotting in the seeds and thus lead to non-germination and these enzymes Cellulase, Chitinase and Ligninase, which have an important role in the events of infection (Abbas et al., 2017; Chen et al., 2016).

Pathogenesis Experiencing in Pots

The results of the test showed the effect of 16 isolates of pathogenic fungi on pepper seedlings in pots under the conditions of woody straws showed a difference in the symptoms that appeared on the roots between light brown to dark brown discoloration and rotting, ulceration and shortening of the stem bases or the death of the entire plant (Figure 3) as well as, the difference in isolates in their pathogenicity, as the severity of infection of pathogenic isolates ranged between 36.67-55%, with a significant difference from the control treatment, as it reached 0%, that the isolation of the fungus isolate *M. eutypoides* (YMe6) it outperformed the rest of the isolates in the severity of infection, which amounted to 55%, but did not differ significantly from the other isolates BMe1, BMe3, BMe8 and the fungus isolates *Fusarium spp.* (BFu4, BFu6 and YFu9), as the infectivity ranged between 50–52.33% has differed significantly isolates other fungi among themselves in terms of the infectivity of pepper plant has recorded isolation fungus *M. phaseolina* (BMa5) less infectivity as it reached 36.67% while the infectivity in the treatment of control 0% (Table 3).

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Table 3. Severity of infection of some fungus isolates on pepper seedlings

Sample Name	Sample Code	Infectivity %
Fusarium spp.	BFu4	51.67
Fusarium spp.	BFu5	46.67
Fusarium spp.	BFu6	51.33
Fusarium spp.	YFu9	50.00
Fusarium spp.	YFu11	45.00
M. phaseolina	BMa2	45.00
M. phaseolina	BMa5	36.67
M. phaseolina	BMa7	38.33
M. eutypoides	BMe1	52.33
M. eutypoides	BMe2	48.33
M. eutypoides	BMe3	50.00
M. eutypoides	BMe6	55.00
M. eutypoides	YMe8	50.00
M. eutypoides	YMe10	45.33
R. solani	KRh5	43.67
R. solani	KRh7	46.67
Control without fungi		00.00
L.S.D(0.05)		5.196



Figure 3. Pathological evidence of the severity of infection in fungus isolates on pepper plant 0 = healthy plant, 1 = roots are light brown with the stem base remaining intact, 2 = roots are dark brown and stem discoloration begins, 3 = roots and stem base are dark brown, 4 = roots rot and entire stem base (plant death).

These results are consistent with the findings of (Al-aamel and Al-maliky, 2023; Zhang et al., 2025) that pathogenic fungi cause a significant reduction in seed germination and cause rotting of the roots and crown of the pepper plant. The difference in fungal isolates in their ability to produce secondary metabolites, which are toxic substances, as well as the number of enzymes that act as decomposers for the cell walls of the plant host and help in the penetration process, including Cellulase, Chitinase, Protease and Polygalcturinase, which play an important role in the events of infection may led to differences in the severity of infection (Abbas et al., 2017; Chen et al., 2016). Which increases their risk is these fungi have a wide family range and because they inhabit the soil, the pathological symptoms appear on the vegetative system after they destroy the root system.

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CONCLUSIONS

All the 50 fungal isolates showed inhibition to pepper seeds germination in the pathogenicity test with significant differences among them. Their treatments had 0-81% germination compared to 96% in the control treatment. the isolates of Fusarium spp. (BFu4, KFu6) and M. eutypoides (BMe1, YMe6, YMe8, KMe9, KMe10) excelled with its pathogenicity with 0% seed germination. As well as, in the infectivity test for 16 isolates on pepper seedling showed that all treatments showed a high per-centage of severity of infection of the disease of rotting the roots and bases of pepper stems.

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CONFLICT OF INTEREST

The authors declare that they have no conflicts of interest.

AUTHOR/S DECLARATION

Originality of Figures and Tables: We confirm that all Figures and Tables presented in this manuscript are original and were prepared by the authors. No Figures or images belonging to other authors or sources have been reproduced without the required permission.

Ethical Approval Statement: The authors confirm that this study did not involve human participants, human subjects, or personal data. Therefore, ethical approval for human research was not required.

Ethical Clearance and Animal Welfare: This study did not involve animals or animal experimentation. Therefore, ethical clearance and animal welfare approval were not applicable.

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AUTHOR'S CONTRIBUTION STATEMENT

The first author conceived and designed the study, conducted the experiments, collected and analyzed the data, and prepared the manuscript. The second author contributed to the experimental work, data interpretation, and revision of the manuscript. Both authors reviewed and approved the final version of the manuscript.

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عزل وتشخيص المسببات المرضية المرافقة لمرض تعفن جذور وذبول الفلفل واختبار مقدرتها الامراضية

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المستخلص

أجريت هذه الدراسة في دائرة البحوث الزراعية / قسم بحوث وقاية المزروعات بهدف عزل وتشخيص المسببات المرضية المرافقة لمرض ذبول وتعفن جذور وقواعد السيقان لنباتات الفلفل وتشخيصها مظهريا واختبار مقدرتها الامراضية في المختبر وفي الاصص. بينت نتائج العزل والتشخيص وجود 50 عزلة من الفطريات المرافقة لنباتات الفلفل تعود الى ثماني اجناس تباينت بالظهور باختلاف المناطق التي جمعت منها وهي محافظة بابل، بغداد وكربلاء وان جميع الفطريات المختبرة أدت الى خفض معنوي في النسبة المئوية لإنبات بذور الفلفل قياسا بمعاملة المقارنة وبينت نتائج اختبار تأثير 16 عزلة ممرضة في شتلات الفلفل في الاصص ان جميع المعاملات أظهرت ارتفاع النسبة المئوية لشدة الإصابة بمرض تعفن جذور وقواعد سيقان الفلفل وتفوقت عزلات الفطر Monosporascus eutypoides على باقي عزلات الفطريات.

الكلمات المفتاحية: *Capsicum anuum L.*، الفطريات، *Monosporascus eutypoides*، فطريات التربة.