Effect of Chitosan on Some Fungi Associated with Melon Stem Blight Disease in Basrah Governorate, Iraq

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Abstract

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This study was conducted during the period 2023-2024, and aimed to isolate and identify pathogens associated with melon stem blight disease, both morphologically and molecularly. Additionally, the research involved testing the pathogenicity of these pathogens and evaluating the in vitro effect of chitosan on them. The pathogenicity results showed that *Macrophomina phaseolina* and *Fusarium equiseti were* the most pathogenic in a pots experiment, and it was significantly superior to others in relation to seed germination rate, which reached 53.33 and 63.33%, respectively, and damping off, which reached 44.33 and 37.33%, respectively. The antagonistic ability of chitosan at concentrations of 2, 3, and 5% showed that the concentration of 5% completely inhibited all fungal species (100%), and the *Rhizoctonia solani* was the most affected fungus by chitosan as the inhibition rate reached 100.00%, when the two concentrations of 2 and 3% were used, whereas the fungus *M. phaseolina* was less affected by chitosan, and inhibition reached 55.66% at the 2% concentration.

Keywords: Chitosan, melon, stem blight disease.

Introduction

Melon, *Cucumis melo* L. belongs to the Cucurbitaceae family, and is an important source of nutrients such as carbohydrates, fiber, vitamins, and minerals (Yavuz *et al.,* 2021). The melon plant is susceptible to various diseases, such as stem blight caused by *Didymella bryoniae* and wilt caused by *Fusarium oxysporum* f. sp. *melonis*, which lead to significant losses in crop yield and quality (Garampalli *et al.,* 2016). Al-Khazraji & Ismaeel (2021) demonstrated comparable pathogenicity of *F. oxysporum* f. sp. *melonis* and *Macrophomina phaseolina* in causing melon plant wilt. Additionally, the soil-borne fungus *Rhizoctonia solani* causes damping-off and root rot disease in melons (Boughalleb *et al.,* 2018). Various methods, including chemical control, have been used to manage melon diseases. However, pesticides have adverse effects on the environment, humans, and non-target organisms (Kasumbwe *et al.,* 2014). Consequently, there has been a shift towards using alternative compounds, such as chitosan. Chitosan, a compound commonly found in nature and a component of chitin in crustaceans, insects, and fungi, enhances the strength of plant cell walls. It is usually obtained as a white to light red powder, dissolves in organic acids, and is insoluble in water (Xing *et al.,* 2015). Chitosan can improve plant defense mechanisms by stimulating photochemistry and enzymes related to photosynthesis when used as a spray, coating, or by immersing fruits and vegetables for a period of time (Betchem *et al.,* 2019; Li *et al.,* 2020).

The mechanism of action of chitosan involves the breakdown of the fungal cell membrane (Kheiri *et al.,* 2017). Chitosan is a non-toxic compound that does not cause environmental pollution (Aranaz *et al.,* 2021). Given the lack of specialized studies on stem blight disease in melon in Basrah Governorate, and the limited research on alternative compounds for chemical pesticides.

This study aimed to isolate and identify the pathogens causing stem blight disease in melon and to evaluate the efficacy of chitosan in controlling these pathogens as an alternative method to chemical control.

Materials and Methods

Isolation of fungi associated with melon stem blight disease

Stem samples of melon plants infected with stem blight disease were collected. The affected parts were washed with tap water for a minute and then cut using a sterile scalpel into small pieces of 0.5 cm long, and then sterilized with a 10% sodium hypochlorite solution (NaOCl) for two minutes, then washed with sterile distilled water to remove traces of the sodium hypochlorite solution, then dried on sterile filter paper and placed in sterile Petri dishes (9 cm) containing previously prepared sterile potato dextrose agar amended with 250 mg/L of the antibiotic chloramphenicol. Five pieces of infected plant stems were then placed per plate, and incubated at a temperature of 25±2°C for four days. The fungal colonies around the isolated pieces were then examined and they were purified on new plates containing the same PDA medium (Manea *et al.,* 2023).

Morphological identification

The isolated fungi were phenotypically identified by using the morphological features such as, the colony texture, the color, size and color of conidia. The morphological identification were done according to previously described keys (Crous *et al.,* 2006; El-Shafey *et al.,* 2019; Leslie & Summerell, 2006). Following identification, the frequency of

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the isolated fungi was calculated by using the following equation:

$$\text{Frequency (\%)} = \frac{\text{number of fungal isolates}}{\text{total number of fungal isolates}} \times 100$$

Molecular identification

DNA extraction was done using a Geneaid gSYNC^{TM} China kit following a protocol described earlier (Manea *et al.,* 2021). The ITS1-ITS4 marker was employed for molecular identification using the primers pair: F:TCCGTAGGTGAACCTGCGG and R:TCCTCCGCTTATTGATATGC. The PCR product was sequenced by Macrogen Company (Korea), and processed by Chromas 2.6.5 software (Al-Saad *et al.,* 2018). Multiple alignment utilizing the National Center for Biotechnology Information (NCBI) database was performed using BLAST software. The identified isolates were submitted to NCBI for registration.

Assessing pathogenicity of fungi associated with melon stem blight disease *in vitro*

Petri dishes, 9 cm in diameter, filled with water agar were used. The plate center was inoculated with a 0.5 cm diameter disk of a colony of each fungus isolated seven days earlier, and then incubated for three days at a temperature of 25±2°C. Red radish seeds, previously sterilized with a 1% sodium hypochlorite solution, were placed at a rate of 20 seeds per dish distributed in the outskirts of the colony in a circular manner, three dishes for each isolate, leaving three non-inoculated dishes as a control. The plates were incubated at a temperature of 25±2°C for seven days, then the germination rate (%) was calculated at the end of the incubation period, as follows (Alghazali, 2022):

$$\text{Germination rate (\%)} = \frac{\text{no. of germinated seeds}}{\text{total number of seeds}} \times 100$$

In addition, the damping-off rate (%) was calculated according to the following formula:

$$\text{Damping off rate (\%)} = \frac{\text{no. of dead seedlings}}{\text{total number of seeds}} \times 100$$

Pathogenicity pot experiment of fungi associated with melon stem blight disease

a mixture of soil and peat moss was used in a ratio of 1:3 w/w. The soil was sterilized twice using an autoclave at a temperature of 121°C and a pressure of 1.5 kg/cm for an hour. The fungal inoculum of soil-pathogenic fungi was then added at a ratio of 1% (w/w) in 1 kg plastic pots and mixed well. Pots were moistened with water for two days before planting. Three pots were planted for each pathogen treatment at a rate of ten seeds of local muskmelon, leaving three pots without inoculation as a control treatment, and the pots were irrigated carefully. Symptoms were observed 7, 10, 14 and 21 days after planting and seed germination rate was calculated.

Four weeks later, the number of dead seedlings was calculated according to the previous formula.

Evaluation of chitosan efficiency on fungal pathogens growth

Chitosan was obtained from HIMEDIA Company and prepared by dissolving 20 g of normal chitosan in 50 ml of concentrated acetic acid (92%), the solution was blended with a magnetic stirrer for 1 hour to dissolve the chitosan, and the volume was completed to 1000 ml sterile distilled water and mixed well as the base stock solution. Three concentrations 2, 3, and 5% in sterile potato dextrose agar (PDA), were prepared and thoroughly mixed, and then poured into 9 cm sterile dishes and left to solidify. Plates were then inoculated with pathogenic fungi discs (0.5 cm in diameter). Three replicates were prepared for each fungal isolate, leaving three plates non-inoculated as a control. All dishes were incubated at a temperature of 25±2 °C for seven days. The fungal growth inhibition rate (%) was measured according to the following formula:

$$\text{Inhibition rate \%} = \frac{\text{treated colony diameter-control colony diameter}}{\text{control colony diameter}} \times 100$$

Results and Discussion

Isolating fungi from Melon stem Blight disease in the studied regions

Results obtained showed the possibility of isolating different fungi associated with the melon stem blight disease (Table 1). In general, the fungus *Fusarium* sp. occurred at the highest frequency among fungi isolated from melon stem blight disease samples, where the frequency of isolates at the areas Abu Al-Khasib, Al-Hartha, and Almidaina reached 67, 63 and 63%, respectively. The frequency of fungus *Rhizoctonia solani* came as second at Abu Sukhair and Al-Hartha regions and reached 34 and 33%, respectively. Such a result is in agreement with previous findings (Sarica & Karaca, 2024), for example, *F. oxysporum, R. solani* and *M. phaseolina* were the most common fungi isolated from the soil planted with muskmelon plants, and *F. equiseti, F. semitectum, F. solani and F. verticillioides* were the other *Fusarium* species isolated from the same soil. *R. solani* was previously reported from Italy, USA and Brasil, as an important agent causing root rot and wilting on melons (Andrade *et al.,* 2005). This could be due to continued plantation of local varieties in the same area over many years resulting in the increase of pathogens and the non-use of specialized fungicides. This has increased the pathogen inoculum density and thus raised the infection rate. This conclusion is consistent with a previous study (Al-Samar, 2003), in which repeated cultivation of the same variety was often found associated with an increase in the infection severity with the same pathogen.

Table 1. Frequency of fungi associated with melon stem blight disease in the studied region.

Fungi	Frequency of fungi in the studied regions (%)				
	Abu Sukhair	**Al-Hartha**	**Abu Al-Khaseeb**	**Almidaina**	**Alluhais**
Fusarium	47	63.0	67.0	63	34.0
Rhizoctonia	34	33.0	19.0	20	24.0
Aspergillus	3	2.0	2.0	2	2.0
Rhizopus	-	-	4.0	5	1.0
Alternaria	2	1.0	3.0	2	5.0
Nigrospora	1	0.2	0.9		-
Colletotrichum	4	-	1.0	-	1.0
Cladosporium	1	0.2	-	3	6.0
Ulocladium	1	0.2	-	-	18.0
Mucor	-	-	-	3	-
Scaytalidium	-	-	-	-	0.7
Bipolaris	-	-	-	-	0.3
Macrophomina	2	0.4	4.0	2	8.0
Chaetomium	5	-	-	-	-
Total	**100**	**100.0**	**100.0**	**100**	**100.0**

Phenotypic and molecular identification of fungi

The results of molecular identification were in agreement with the phenotypic identification (Figure 1 and Table 2). Phenotypic identification of isolates was dependent on the morphological characteristics of both colonies and spores on the PDA medium. The results identified three species of *Fusarium*, in addition to *Rhizoctonia solani*, *Ceratobasidium* sp., and *Macrophomina phaseolina*. Colony shape of *Fusarium incarnatum* on the PDA medium produced aerial mycelia that were initially off-white and then turned to light orange. The mycelium of *Fusarium equiseti* was initially white, then turned to pale brown then to dark brown with age (Leslie & Summerell, 2006). *Fusarium brachygibbosum* colony was white with abundant aerial mycelium, creating red pigmentation on PDA (Tirado-Ramirez *et al.,* 2021). *Rhizoctonia solani* produced white to deep brown mycelium on PDA medium and was often recognized by branched at an almost right angle with a narrow restriction near the branch area with a transverse barrier (Hussein, 2019). The colonies color of *Ceratobasidium* sp. was cottony white, delicate, and radial in appearance, turned to light brown with age. Slight constriction of the fungal hyphae is observed near the septum in the branching point of the hypha (Bhuiyan *et al.,* 2021). *M. phaseolina* was characterized by hyaline mycelium with thin walls to light brown or dark brown mycelium with septa. Branches from the main hyphae were generally formed at the right angle on parent hyphae with constriction at the point of origin. (Crous *et al.,* 2006).

Molecular identification was based on the amplification of the ITS1-ITS4 gene region of rDNA, and all isolates were registered in National Center for Biotechnology Information (NCBI). The fungus *F. brachygibbosum* was registered under the accession number PP504877.1, and this isolate was 100% identical with the isolate registered in NCBI with the accession number MN498031.1. This is consistent with what has been reported by Khamas (2021) who mentioned that the fungus *F. brachygibbosum* is one of the main causes of wilt disease in tomatoes.

The fungus *F. incarnatum* was registered in the NCBI under accession number PP504876.1, and this isolate was 100% identical to a reference isolate registered under accession number OR467185.1. (Wonglom & Sunpapao, 2020). The fungus *Fusarium equiseti* was registered under the accession number PP524761.1, and this isolate was very similar to the reference isolate MK733980.1 (99.18%), which is in agreement with what has been reported by Ismail *et al.* (2021). The fungus *Macrophomina phaseolina* was registered under the accession number PP524762.1, and was identical (100.00%) with the reference isolate MT032391.1 (de Assis Melo *et al.,* 2021). *Ceratobasidium* sp. was registered under the accession number PP524763.1 and was 100.00% identical to the reference isolate HQ270171.1. (De Holanda Almeida *et al.,* 2024). *Rhizoctonia solani* isolate from Iraq was also registered under the accession number PQ606660.1, with high similarity (99.63%) with the reference isolate under the accession number KX118362.1 (Porto *et al.,* 2020).

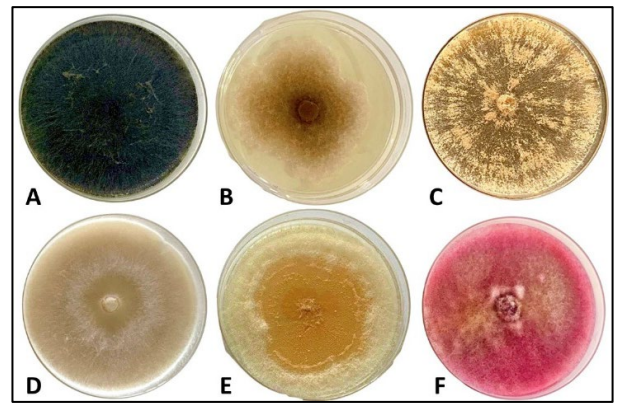


Figure 1. The colonies of fungi associated with melon stem blight disease grown on PDA medium, (A) *Macrophomina phaseolina,* (B) *Fusarium equiseti,* (C) *Rhizoctonia solani,* (D) *Ceratobasidium* sp.*,* (E) *Fusarium incarratum. and* (F) *Fusarium brachygibbosum.*

Table 2. Molecular identification of fungal pathogens from Iraq registered in the National Center for Biotechnology Information (NCBI).

Scientific name	Accession number	Accession number of matching reference strains	Percent similarity (%)	Query cover (%)
Fusarium brachygibbosum	PP504877.1	MN498031.1	100.00	99
Rhizoctonia solani	PQ606660.1	KX118362.1	99.63	100
Fusarium incarnatum	PP504876.1	OR467185.1	100.00	100
Ceratobasidium sp.	PP524763.1	HQ270171.1	100.00	100
Macrophomina phaseolina	PP524762.1	MT032391.1	100.00	100
Fusarium equiseti	PP524761.1	MK733980.1	99.18	100

Pathogenicity experiment in pots

The results of the pathogenicity experiment showed that all tested isolates achieved a significant decrease in the germination rate of melon seeds (Table 3, Figure 2). The lowest germination rate was achieved by *M. phaseolina* (53.33%), compared to 4% in the control treatment. As reported earlier by Khan (2007), *M. phaseolina* invades the host very rapidly, establishing itself in the host within 24 to 48 h, at the seedling infection stage. The high pathogenicity of *M. phaseolina* may be due to the production of several potent phytotoxins, including botryodiplodin, phaseolinone and some isolates may produce other unidentified phytotoxin(s) responsible for virulence (Abbas *et al.,* 2019). The results obtained also showed that the highest damping off rate was achieved by the two isolates of the fungus *R. solani* and *M. phaseolina*, reaching 45.00 and 44.43%, respectively, compared to 80% for the control treatment. Several studies conducted earlier have shown the importance of these pathogens in infecting melon plants, causing root rot and decline in the shoot system (Beas-Fernández *et al.,* 2006).

Fusarium species have also shown a high ability to reduce seed germination rate and increase damping off rate. This decrease is attributed to the nature of the substances produced by *Fusarium* species, including toxins and degrading enzymes, which kill seeds and prevent them from germinating (Stack *et al.,* 2017). These results are in agreement with has been reported by Sarica & Karaca (2024) who indicated that *R. solani, M. phaseolina* and *Fusarium* spp. are important pathogens of the Cucurbitaceae family and cause stem rot of melon in Türkiye.

The results obtained by this experiment also agreed with what has been reported by Al-Lamy (2012), who showed that the fungus *R. solani* was more pathogenic on muskmelon plants, and the damping off rate was 35.5%, whereas the seed germination rate was 56%. The effect of the fungus *Rhizoctonia solani* is attributed to its ability to secrete cell wall degrading enzymes (CWDE) such as cellobiose dehydrogenase, β-xylanase, β-glucanase, β-glucosidase, cutinase, and glucosyl transferase that play an important role in necrotrophy (Ghosh *et al.,* 2018). In addition, some phytotoxins [3-methylthiopropionic acid, 3-methylthioacrylic acid] can then be secreted to complement the infection arsenal (Mat Razali *et al.,* 2021).

Table 3. The effect of pathogenic fungi on the seed germination rate and damping off rate of melon plant in pots.

Fungi	Seed germination rate (%)	Damping off rate (%)
Fusarium brachygibbosum	60.00	38.33
Rhizoctonia solani	60.00	45.00
Fusarium incarnatum	60.00	38.66
Ceratobasidium sp.	60.00	38.33
Macrophomina phaseolina	53.33	44.33
Fusarium equiseti	63.33	37.33
Control	80.00	4.00
$LSD_{0.01}$	18.37	24.26

Evaluation of chitosan efficacy on pathogenic fungi growth *in vitro*

Results obtained (Figure 3 and Table 4) showed that the 5% chitosan concentration completely inhibited the growth of all fungi by 100%, with *R. solani* completely inhibited at the three concentrations used: 2%, 3%, and 5%. The fungus *M. phaseolina* was the least affected by inhibition, as the inhibition rate reached 55.66 and 70.66 at 2 and 3% concentrations, respectively. The inhibitory susceptibility of chitosan arises from its ability to disrupt the activity of essential fungal enzymes and proteins. This disruption is mediated by an increase in the permeability of the fungal cellular membrane, attributed to the interaction between the positively charged chitosan and the negatively charged fungal membrane. Consequently, DNA synthesis is altered, leading to the inhibition of protein enzyme production and a deficiency in essential nutrients necessary for fungal growth (Younes & Rinaudo, 2015). This study coincided with previous studies, where Al-Ramahi (2021) pointed out that addition of ordinary chitosan to the PDA medium with concentrations of 250, 500, 1000, 2000, 3000, 4000, 5000 and 6000 ppm inhibited the growth of *Aspergillus flavus* fungus on the PDA medium with inhibition rates of 28.23, 31.37, 32.54, 45.09, 49.02 , 61.17, 64.70 and 82.35%, respectively, as compared with the control treatment have shown the highest inhibitory effect when using the 6000 ppm concentration.

Table 4. Effect of different concentrations of chitosan in inhibiting the fungi grown in the PDA medium.

Fungi	Inhibition rate (%) of pathogenic fungi at conc. (%)		
	2	3	5
Fusarium brachygibbosum	70.00	98.00	100.0
Rhizoctonia solani	100.00	100.00	100.0
Fusarium incarnatum	71.00	89.00	100.0
Ceratobasidium sp.	96.66	93.00	100.0
Macrophomina phaseolina	55.66	70.66	100.0
Fusarium equiseti	77.60	84.66	100.0
$LSD_{0.01}$	12.34	7.482	0.0



Figure 2. Pathogenicity of isolated fungi on melon in pots, (A) *Macrophomina phaseolina,* (B) *Fusarium equiseti,* (C) *Rhizoctonia solani,* (D) *Ceratobasidium* sp., (E) *Fusarium incarratum,* (F) *Fusarium brachygibbosum,* (G) control.

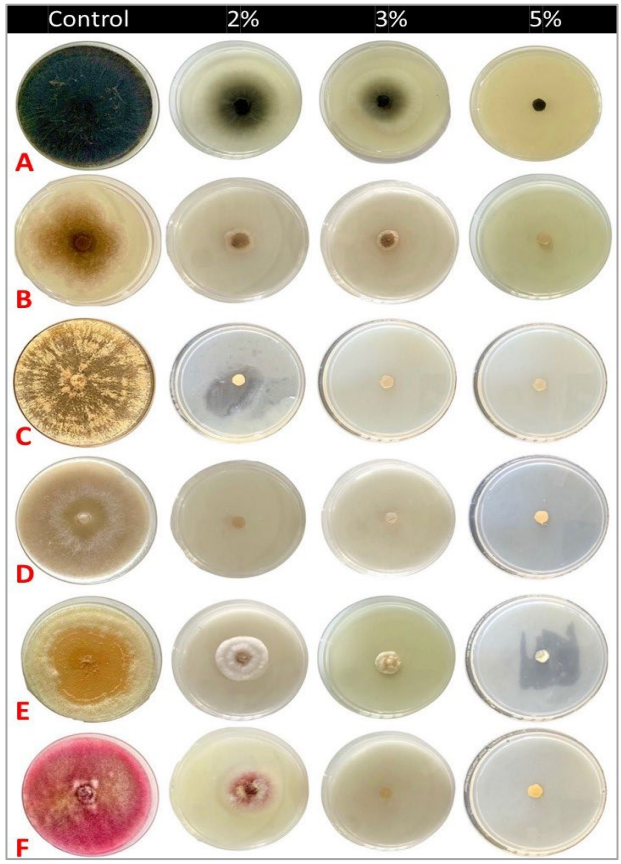


Figure 3. Effect of chitosan concentrations on pathogenic fungi isolated from Melon stem blight disease.

It can be concluded from this study that melon stem blight disease was associated with several fungi such as *Macrophomina phaseolina, Fusarium equiseti, Rhizoctonia solani, Ceratobasidium* sp., *Fusarium incarratum*, and *Fusarium brachygibbosum*. All these fungi showed high pathogenicity on melon plant in pot experiments. The high pathogenicity of these pathogens may be due to the production of several potent phytotoxins, such as botryodiplodin and cell wall degrading enzymes (CWDE) such as cellobiose dehydrogenase, β-xylanase, β-glucanase, β-glucosidase, cutinase, and glucosyl transferase, which kill seeds and prevent them from germinating. The use of chitosan has a high inhibitory effect for all the fungi tested in Petri dishes. The inhibitory effect increased with the increase of concentration used. Chitosan inhibits fungal growth by disrupting essential enzyme and protein synthesis and increase cellular membrane permeability. Such interactions alters DNA synthesis, leading to reduced protein production and nutrient deficiency.

الملخص

محمد، إيمان جبار وعلاء مانع. 2026. تأثير الكيتوسان (Chitosan) على بعض الفطور المرافقة لمرض لفحة ساق البطيخ في محافظة البصرة، العراق. مجلة وقاية النبات العربية، 44(3):342–349. https://doi.org/10.22268/AJPP-001414

أجريت الدراسة في مختبر الأمراض النباتية، قسم وقاية النبات، كلية الزراعة، جامعة البصرة، خلال الفترة 2023–2024 بهدف عزل وتشخيص المسببات المرضية المرافقة لمرض لفحة ساق البطيخ مظهرياً وجزيئياً واختبار قدرتها الإمراضية وتأثير فعالية الكيتوسان على الفطور مختبرياً. أظهرت نتائج القدرة الإمراضية أن

العزلتين *Macrophomina phaseolina* و *Fusarium equesti* هما الأكثر إمراضية، إذ بلغت نسبة تثبيط إنبات بذور الفجل 96.66 و 100.00%، على التوالي، في حين بلغت نسبة الإنبات في بذور الشاهد 3.33 و 0.00%، على التوالي. أما في تجربة الأصص، تفوقت العزلتان *M. phaseolina* و *F. equesti* معنوياً عن غيرهما بنسبة إنبات بلغت 53.33 و 63.33%، على التوالي، ونسبة موت بادرات بلغت 44.33 و 37.33%، على التوالي. أظهرت نتائج القدرة التضادية للكيتوسان للتراكيز 2، 3 و5% غ/لبتر تفوق التركيز 5% بتحقيقه نسبة تثبيط عالية لجميع العزلات الفطرية، حيث بلغت نسبة التثبيط 100.00%، وحقق الفطر *Rhizoctonia solani* نسبة تثبيط بلغت 100.00 للتركيزين 2 و 3%، في حين كان الفطر *M. phaseolina* هو الأقل تأثراً بالتثبيط حيث بلغت نسبة التثبيط 55.66% عند استخدام التركيز 2%.

كلمات مفتاحية: الكيتوسان، البطيخ، مرض لفحة الساق.

عناوين الباحثين: إيمان جبار محمد* وعلاء مانع، قسم وقاية النبات، كلية الزراعة، جامعة البصرة، العراق. *البريد الإلكتروني للباحث المراسل: pgs.eman.jabar@uobasrah.edu.iq

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