First record of Cladosporium oxysporum isolated of tomato plant roots and control it using some factors of induce systemic resistance

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Abstract:

This study aimed *Chaetomium* sp. were evaluated in control of the pathogens. The results showed that presence of the fungi: *Cladosporum oxysporum*, *Fusarium* spp. in the infected roots of tomato plants. The results of the pathogenicity test displayed that the fungus *Cladosporium oxysporum* reduced the germination rate of cabbage seeds to 35.55% and *Fusarium* spp to 42.22 % compared with the control (cabbage seeds only) that was 93%. Further identification of *C. oxysporum* fungus based on its molecular characteristics confirmed this fungus as first record causing root rot disease of tomato plant in Iraq. Also, the biological agents *T. harzianum* and *Chaetomium* sp. achieved high inhibition percentage against *C oxysporum*. Additionally, the results showed that the treatment of the biological factor *Chaetomium* spp was the best in reduction of the root rot disease severity (8.33 %), followed by the treatment of the *T. harzianum* with (16.66%) while the treatments of *T.harzianum* and were 25% for each compared to the control (*C.oxysporum* alone) that was 75%. All treatments demonstrated a significant increase of soft and dry weight of the root and root length compare with *C.oxysporum* only treatment .

Keywords : *Cladosporium oxysporum* , *Chaetomium* sp., *Trichoderma harzianum* , to isolate and diagnose the causal agents of tomato root rot disease and assess its pathogenicity using plate method. The efficiency of some induction factors salsilic acid (SA) and the fungi *Trichoderma harzianum* and, salicylic acid , tomato plant.

التسجيل الاول للفطر *Cladosporium oxysporum* المعزول من جذور نباتات الطماطة و مقاومة بأستخدام بعض العوامل المستحثة للمقاومة الجهازية.

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المستخلص:

هدفت الدراسة الى عزل وتشخيص مسببات مرض تعفن جذور الطماطة والكشف عن عزلاته الممرضه باستعمال طريقة الاطباق مع تقيم كفاءة عامل الاستحثاث حامض السالسليك والفطرين الاحيائيين

T.harzianum و .sp *Chaetomium* في مقاومة المسبب المرضي. اظهرت نتائج العزل وجود الفطرين و *Fusarium* sp. *Cladosporium oxysporum* في جذور نباتات الطماطة المصابة. وبينت نتائج اختبار المقدرة الامراضية تفوق عزلة الفطر *Cladosporium oxysporium* في خفض نسبة انبات بذور اللهانة الى 35.55 % اما الفطر spp *Fusarium* فقد بلغت نسبة انبات البذور فيه 42.22 % قياسا بمعاملة المقارنة التي كانت نسبة الانبات فيها 93% تشخيص ادق بالاعتماد على الخصائص الجزيئية للفطر *Cladosporium oxysporium* اكد التشخيص المبدئي لهذا الفطر ويعتبر هذا التسجيل هو الاول لهذا الفطر كمسبب لمرض تعفن جذور نباتات الطماطة في العراق. كما حقق الفطريين الاحيائيين*T.harzianum* و.sp *Chaetomium* مقدرة تضادية عالية ضد الفطر *C oxysporium* في الوسط الزرعي PDA وبينت نتائج تجربة الاصص البلاستيكية ان معاملة العامل الاحيائي *Chaetomium* spp حققت اعلى نسبة خفض في شدة اصابة المجموع الجذري اذ بلغت 8.33 % تلتها معاملة استخدام الفطر الاحيائي *T.harzianum* مع حامض السالسليك رشاً والتي بلغت شدة الاصابة فيها16.66% ثم معاملة الفطر الاحيائي *T. harzianum* وحامض السالسليك كلا على حده والتي كانت شدة الاصابة فيها 25% قياسا بمعاملة الفطر *C.oxysporium*لوحدهُ التي بلغت شدة الاصابة فيها75%. واظهرت جميع المعاملات زيادة ملحوظة في مؤشرات النمو المدروسة المتمثلة بالوزن الطري والجاف للمجموع الجذري وطول النبات قياساً بمعاملة المقارنة الفطر الممرض *C. oxysporium* بمفرده.

Introduction:

Tomato (*Solanum lycopersicum* L.) is a summer vegetable crop belonging to the *Solanaceae* family, It is produced in most of the Iraqi provinces and most of the countries due to its importance in the consumption as fresh or processed as a part of many food products (16). The original place of the tomato plant is thought to be in South America and transferred by European colonizers to their countries in the sixteenth century, and then it cultivated worldwide in cluding some of Arab countries such as Syria, Egypt and Iraq in the twentieth century (21 and 9). The tomato fruit contains several nutrients, including calcium, phosphorus, potassium and iron. Many vitamins are also included such as A, B3, B2, B6, C, E, folic acid and citric acid (19). Due to these reasons and others, the production of tomatoes has been increased worldwide and in many provinces of Iraq through different ways such as protected cultivation and open fields methods (10). However, the tomato crop is subjected to many pathogens causing various diseases, including fungal diseases such as leaf diseases, early blight and late blight, as well as root rot diseases and others (3 and 18). Recently, biocontrol has achieved good results in controlling of several pathogens as additional to be safer to the environment compared with chemical pesticide usage that negatively affected on the environment and organisms (14).

Materials and Methods:

Isolation and identification of pathogenic fungi

Diseased tomato plants were collected randomly from tomato fields in Aoun district in Karbala province. The roots were washed gently with tap water for cleaning any suspended soil and compost and cut into small pieces (0.5-1 cm). These root pieces were then sterilized with 1% sodium hypochlorite for 3 minutes and washed in sterile distilled water for 2 minutes to remove the effects of sterilization. Subsequently, the sterilized root pieces were dried using sterilized filter paper and transferred to 9 cm diameter Petri dishes containing potato dextrose agar (PDA) medium that was autoclaved at 121 ° C and 1.5 kg /cm^2 pressure for 15 minutes and added the antibiotic tetracycline (200 mg /L). All dished were incubated at 25 ± 1 ° C for 3 days, then the fungal colonies were purified in PDA medium using hyphal tip method. The fungi associated with symptomatic root were initial identified based on their morphological features. Furthermore, molecular identification was carried out by extraction the genomic DNA from the pure fungal mycelia using a DNeasyPlant Mini Kit following the manufacturer's instructions. The universal primers ITS1 and ITS4 (22) were applied to amplify the entire internal transcribed spacer (ITS) region of ribosomal DNA (rDNA). The PCR product was then sequenced at Macrogen company in South Korea. The sequence obtained was deposited in the Genbank database and compared with other sequences using Basic Local Alignment Search Tool (BLAST) analysis.

Pathogenicity assessment

The pathogenicity of *Fusarium* sp. and *Cladosporium oxysporum* fungi was evaluated by plate method (7) that is briefly including Petri Dishes 9 cm diameter containing 15-20 ml of water agar medium. This water agar medium was prepared by mixing 20 g of agar in a liter of distilled water and sterilized for 15-min by autoclave with the same conditions mentioned previously. After solidification of media, the center of dishes was inoculated with a disc 0.5 cm in diameter that was taken from the edges of the pure 5 days old colonies of both fungi. Other dishes containing the same medium were not inoculated and used as a control treatment. All inoculated and non-inoculated dishes were incubated at 25 ± 1°C for three days. Seeds of local cabbage were sterilized with 1% sodium hypochlorite for two min and washed with distiller water for three times and then placed circularly near the edge of the inoculated and non-inoculated dishes with 15 seed / plate. Three dishes were used for each fungal isolate in addition to the comparative treatment. The dishes were placed in the incubator at 1±25°C. The results were then taken after 7 days by calculating the percentage of seed germination according to the following equation:

$$\text{The percentage of germination} = \frac{\text{number of seeds germinated}}{\text{total number of seeds}} \times 100$$

Evaluation of the antagonistic ability of *Trichoderma harzianum* and sp. against the fungus *C.oxysporum*

Double culture technology was applied to assess the antagonistic ability of *T.harzianum* and *Chaetomium*.sp . using PDA containing petri dishes taking a disk 0.5cm in diameter from the edges of the colony of the fungus *C. oxysporum* placed in the center of one half of the dish while the center of the other half of the dish was inoculated with 0.5 cm disc taken from the edges of the colony of the biological agents *T. harzianum* and *Chaetomium* sp. The comparative treatment was contained *C. oxysporum*, *Chaetomium* sp. and *T.harzianum* only. All inoculated plates were incubated at 25 ± 1°C until the fungal growth the control treatment were reached the edge of the dish. The antagonism of biological factors was estimated according to the five-standard criteria (6) as following:

1 –The growth of biological agent covers the entire area of the dish without allowing *C. oxysporum* fungus to grow.

2 – The growth of biological agent covers two-thirds of the area of the dish and the growth of *C. oxysporum* fungus covers the remaining one-third.

3 - The growth of biological agent covers half of the dish and the growth of *C. oxysporum* fungus covering the other half with no buffer zone between the colonies.

4 – The growth of biological agent covers one-thirds of the area of the dish and the growth of *C. oxysporum* fungus covers the remaining two-thirds.

5 - Non-growth of biological agent and the growth of *C. oxysporum* covers the entire area of the dish.

The effective biological agent is considered when its antagonism has a degree of 2 or less with the pathogenic fungus *C. oxysporum*.

Evaluation of the two biological agents *T.harzianum* and *Chaetomium* .sp. and salsilic acid in protection of tomato plant of root rot disease caused by *C. oxysporum* in plastic house

This experiment was conducted in a plastic house at the Agriculture College of University of Karbala in the autumn season of 2017. The inoculum of the pathogenic fungus *C. oxysporum* was prepared by adding small pieces of pure growth culture of *C. oxysporum* (1 plate -1 kg) to the compost that was autoclaved twice for one hour.. The inoculated compost was then placed in the incubator under 25±1°C for a week (20) and then distributed in a plastic container 100 g and . it and was planted with tomato plants and complemented the following treatments:

1 - The pathogen *C. oxysporum* only.

2. *T.harzianum* + *C. oxysporum*

3. *Chaetomium* spp + *C. oxysporum*

4- *T.harzianum* + SA spray + *C. oxysporum*

5. SA spray + *C. oxysporum*

6- Tomato plants only

7- *T.harzianum* only.

8. *Chaetomium* spp only.

9. *T. harzianum* + SA spray.

10 – SA spray only .

The experiment was carried out using the completely randomized design and three replicates for each treatment. The inoculum of *T.harzianum* and *C.* sp. loaded on millet seeds by 1% (weight: weight) (13) before planting plants. Salicylic acid spray was sprayed at 1.0 mmol and 100 ml per pot.Tomato plants in comparison treatment were planted autoclaved non-inoculated compost. The results were collected after a month of planting. Soft and dry weight of tomato root and root length were measured and the severity of root rot disease was estimated by following the severity scale:

0 = roots intact
1 = presence of ulceration and discoloration of more than 1-25% on the roots
2 = presence of ulceration and discoloration of more than 25-50% on the roots
3 = rot and discoloration of more than 50-75% on the roots
4 = rot and discoloration of more than 75-100% on the roots

The percentage of severity of injury was calculated according to the formula mentioned in (17) that is:

$$\% \text{ the severity} = \frac{(\text{number of plants in degree } 0 \times 0) + .+ (\text{number of plants in degree } 4 \times 4)}{\text{number of plants studied x } 4} \text{ x100}$$

Results and Discussion:

Two fungi *Fusarium* sp. *Cladosporium* sp. were isolated of diseased roots of Tomato plants and identified based on their morphological features (15). The PCR(Figure1) result showed the possibility of PCR amplification of the ITS region of *Cladosporium* sp the PCR products were sequenced and deposited in genebank database at the national center for biotechnology information (NCBI) with accession number MF511908. The BLAST analysis showed >99% identity with several known sequences from *C.oxysporum* species. Revising references related to pathogens causing root rot and damping - off diseases of tomato plants in Iraq has been confirmed that no report of - *C. oxysporum* fungus causing root rot disease on tomato plant thus this is the first record of this fungus in Iraq.

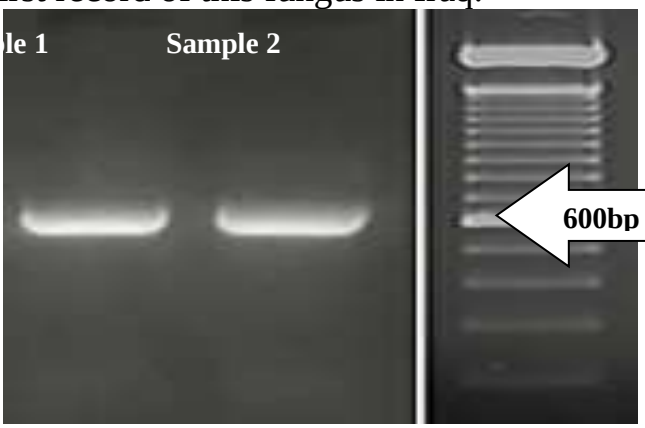

Figure 1: Gel electrophoresis of the ITS region amplification of *C. oxysporum*

The pathogenicity assessment

The results showed table (1) that both fungi *C.oxysporum* and *Fusarium* sp. isolated achieved a significant reduction (35. 55% and 42.22%, respectively) in the percentage of germination of cabbage seeds compared with the control treatment that recorded 93%. The *C. oxysporum* fungus was isolated for the first time from the roots of the tomato plants as well as high pathogenicity displayed, it was used in subsequent experiments.

Table1: The test pathogenicity of fungi by using seeds cabbage

Isolate	Germination (%)
C.oxysporum	35.55
Fusarium sp.	42.22
Control	93
LSD 0.05	1.99

Evaluation of the antagonistic ability of the bioagent *T.harzianum* and *Chaetomium* sp. against the fungus *C.oxysporum*

The results of this test showed that both biological agents achieved high antagonism ability against the fungal pathogen *C. oxysporum* occupied the level (2) according to the specific scale (6) used in this study. These results were in greement with many studies related to the bioprotective *T. harzianum* and *Chaetomium* sp. in inhibiting growth of various pathogens on the PDA media (2, 12).

Evaluation of the two biological agents *T.harzianum* and *Chaetomium* . sp. and salsilic acid in protection of tomato plant of root rot disease caused by *C. oxysporum* in plastic house

This study examined the effect of two biological factors *T.harzianum* and *Chaetomium.* sp. and the salicylic acid in reducing tomato root rot diseased caused by the *C. oxysporum* fungus. The results shown in Table (2) displayed the positive effect of *Chaetomium* sp. in reducing the disease to 8.33% and increase the soft and dry weight roots (0.62 and 0.08 g) and the root length (15.66 cm) followed by the treatment of *T. harzianum* and salicylic acid where the severity of root infection was 16.66% and soft and dry weight of roots reached 0.37 and 0.07 g while root length was16 cm. On the other hand the treatments of *T. harzianum* and salicylic acid alone reduced the disease severity to 25% whereas the soft and dry weight were 0.38 and 0.06 g, respectively, 0.61 and 0.08 g, respectively and the root length recorded 15 and 17 cm, respectively. However, the control treatment (the fungal pathogen *C. oxysporum* alone) was with severity reached 75%,soft and dry weight 0.21 and 0.03 g respectively and root length 12 cm. In contrast, the control treatment (without the addition of fungal pathogen) was with disease severity 0.00% and soft, dry weight and length of tomato roots were relatively normal.

This result is due to the high inhibitory capacity of the *C.oxysporum* and its secretion of many enzymes, including and phenol oxidase, which play a key role in causing the infection on the host plant by breaking down the structural content of the plant cells

(1). *Chaetomium* sp. reduced the severity of the roots disease and this result was in agreement with other studies (12). This confirm the efficiency of this biological fungus that is due to stimulate and strengthen the growth of the plant becoming resistant to pathogens.

Salicylic acid effectiveness coincide with results of several previous studies that proved its role in control of plant many pathogens (4 and, 11). This is due to the role of salicylic acid in the stimulation and activation of the biochemical defenses in the plant, which increases the effectiveness of enzymes related to resistance in the plant such as glucanase and chitinase enzyme and increase production of proteins related to disease that raise the resistance of the plant (8). The efficacy of the bioagent *T. harzianum* in reduction of the disease severity and increase of the root growth parameters is due to possess many mechanisms that affect the pathogenic fungi such as the secretion of antibiotics and some enzymes that analysis the walls of fungal cells such as protease and B-1,3- glucanas (5).

Table (2): Effect of add fungi *T. harzianum* , and *Chaetomium* spp and salicylic acid in reducing injury and root fungus *C.oxysporum* increased some growth indicators in tomato plants.

*Treatments	The severity %	Root length (cm)	Root weight (g)	
			weight soft	dry weight
C.O only	75%	12	0.21	0.03
T.h + C.o	25%	15	0.38	0.06
C.h + C.o	8.33%	15.66	0.62	0.08
SA Spray + C.o	25%	17	0.61	0.08
T.h+SA spray+C.o	16.66%	16	0.37	0.07
plants tomato	00%	21	0.70	0.10
T.h only	00%	21.5	0.92	0.12
C.h only	00%	24.33	1.01	0.11
SA spray only	00%	25	0.78	0.11
T.h+ SA spray	00%	22	0.71	0.10
L.S.D 0.05	1.20	1.70	0.03	0.01

*** Each number in the table represents a rate of three replicates**

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