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The effect of biological factors on Fusarium oxysporum inhibition causing Fusarium wilt disease on cowpea (_Vigna unguiculata_)

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Abstract

This study was conducted for the agricultural season 2023-2024 with the aim of evaluating the efficiency of bio agent of *Bacillus cereus*, *Trichoderma Longibrachiatum* and *Saccharomyces cerevisiae* on Cowpea plants infected with Fusarium wilt disease in pots. Three isolates R2, R3 and R7 of *Fusarium oxysporum* were isolated from the roots of Cowpea plants that showed symptoms of wilt. The isolated fungi were diagnosed morphologically and partially. The results of molecular diagnosis of the fungi showed 100% matching of the isolates. Pathogenicity tests were conducted on cowpea seeds in the laboratory using Petri dishes containing W.A medium as a pathogen for cowpea plants, as the germination percentage of cowpea seeds for all isolates reached 76.66, 66.66 and 63.66% compared to the control treatment which reached 100%. The results of the pot experiment showed that there were no significant differences in the infection severity by the pathogenic fungus in the three isolates, as R2 reached 82.00, R3 82.00 and R7 87.33% respectively compared to the control treatment which reached 0.00%. The results of the pot experiment indicated the effect of bio agent in increasing some plant growth indicators and reducing the infection severity which reached 0.00% in the T. Longibrachiatum, B. cereus and S. cerevisiae treatments. It was also noted from the results that the highest germination rate of cowpea seeds was in the yeast S. cerevisiae treatment, which reached 96.00% compared to the control treatment, which reached 100%. The results of chlorophyll estimation indicated that the treatment of the biological fungus T. Longibrachiatum and the yeast S. cerevisiae was similar to the control treatment, which reached 0.82 mg/g^{-1}.

I. Introduction

The cowpea crop (*Vigna unguiculata* L. Walp) is one of the crops of the legume family Leguminosae, which is grown in Africa and Asia and is consumed in many parts of the world as a high-quality source of vegetable protein. Cowpea has gained attention from consumers and researchers due to its health benefits, including its health properties as it is a good anti-inflammatory, anti-diabetic, anti-cancer, anti-hyperlipidemia and anti-hypertensive (Jayathilake et al., 2018). The cowpea plant is characterized by its high productivity and stability in production, as well as its high ability to withstand environmental stresses such as drought. Cowpea is considered a safe and always available food in most regions, in addition to its low price compared to other sources of protein. This plant is also one of the main sources of calories for a large segment of the world's population, as its seeds contain approximately 53-66% of carbohydrates, most of which are in the form of starch, and contain a high percentage of amylose (Da Silva, 2018). The cowpea plant is important in fixing up to 80% of nitrogen in the soil, which reduces the demand for nitrogen fertilizers and their cost in general. It is also considered a main crop as a vegetative cover. In addition, cowpea seeds are used for other purposes such as animal feed and for various therapeutic purposes, as it is used as an antipyretic and diuretic, and to treat liver and spleen problems, intestinal spasms, and leukemia, where it is used as a decoction or soup (Abebe et al., 2022). This plant faces major challenges due to fungal diseases such as Fusarium wilt. The Fusarium fungus was first discovered by the scientist Link in 1809 and is one of the most important fungi that cause plant diseases, causing significant losses to crops all over the world. This fungus is widespread in Basra Governorate/Iraq, where it ranked fifth among the ten most important fungi that cause plant diseases. The genus Fusarium contains less than 200 species (Abdel-Azeem et al., 2019). Several methods have been used to combat Fusarium wilt, including the use of pesticides, and despite their effectiveness, they pose risks to the health of workers and consumers, in addition to polluting the

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environment and being expensive (Schreinemachers et al., 2016). Recently, several alternative methods have been used, including microbial biocontrol agents (BCAs), which are characterized by being effective, environmentally friendly, sustainable, and inexpensive, as they have a number of mechanisms such as parasitism, antibiosis, and stimulating host resistance (Purohit et al., 2023).

II. Materials and methods

Isolation and identification of F.oxysporum fungi isolates morphologically and molecularly from the roots of cowpea plants

Cornflower plant samples showing symptoms of wilting were collected from cowpea fields in northern Basra province, which included Al-Nashwa, Ezz El-Din Salim and Al-Madinah for the agricultural season 2023. The samples were placed in nylon bags and brought to the Plant Pathology Laboratory in the Department of Plant Protection - College of Agriculture - University of Basra. The samples were washed well with running water to remove the suspended dirt, then left to dry. The roots were then cut into small pieces of 0.5 cm, then sterilized with a 10% sodium hypochlorite (NaOCl) solution for two minutes, then washed with sterile water for two minutes, then dried on Whatman No. 1 filter papers. They were then transferred to 9 cm Petri dishes containing sterile PDA medium with the antibiotic Chloramphenicol added at an average of 250 mg/L to prevent bacterial growth. 4-5 pieces were planted in each dish and incubated at 25±2°C for 6 days (Agrios, 2005). The growth around the pieces was examined, and the purification process was repeated several times on the PDA medium, then examined for the purpose of identifying the fungus according to the taxonomic keys (Leslie and Summerell, 2006).

Molecular identification of fungi associated with Fusarium wilt disease of cowpea

Fungal cultures of each pathogenic isolate grown on PDA medium at 7 days of age at 25±2°C were used. The surface layer of the fungi was scraped off, frozen rapidly using liquid nitrogen and ground with a mortar and pestle to a fine powder. Up to 25 mg of this powder was then transferred to an Eppendorf 1.5 tube for DNA extraction using the gSYNCTM DNA Extraction Kit following the steps mentioned according to the manufacturer's instructions (Fayyadh et al., 2024). Electrophoresis was then performed to detect DNA and after migration was completed, the gel was examined by Gel Documentation to observe DNA bands (Sambrook et al., 1989). Special primers were used for diagnosis (Table 1) and the polymerase chain reaction (PCR) mixture was prepared in a 200 µl Eppendorf tube and the final volume of the components was 25 µl (Table 2). The mixture was then placed in a small centrifuge (snip) to mix the components, then the mixture was placed in the PCR device and the special program was set (Table 3) (Abd-Elsalam et al., 2003). After confirming the PCR product amplification process by electrophoresis, 20 µl of the amplification product for each isolate was sent to the Korean company Macrogen to determine the sequences of the nitrogenous bases in the genes used and then match them with the National Center for Biotechnology Information (NCBI) and register them (Manea et al., 2023)

Table (1) Sequences of primers used in PCR technology.

Primers temperature	Primers name	Sequence of primers	No.
64.5	ITS1	F:TCCGTAGGTGAACCTGCGG	1
70.8	ITS4	R:TCCTCCGCTTATTGATATGC	2

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Table (2) Quantities of materials used in PCR technique in microliters.

Final size	Nuclease-free water	DNA template	Primers	Master Mix	Chemical
25	6.5	5	)1forward + reveres(	12.5	Volume

Table (3) DNA amplification program.

Number of cycles	Time (minutes(	temperature	Stages
1	5:00	94	Initial Denting
35	0:30	94	Denting
	0:45	58	Adhesion
	2:00	72	Elongation
1	7:00	72	Final Elongation

Then, the molecular identification of the obtained and confirmed sequences was processed through Chromas 2.6.5 3 program, and multiple alignment of each sequence sample was performed separately with the NCBI database using BLAST program (Al-Saad et al., 2018). Then, the processed sequences of the selected isolates were submitted to NCBI for registration.

Pathogenicity testing of F.oxysporum isolates using cowpea seeds.

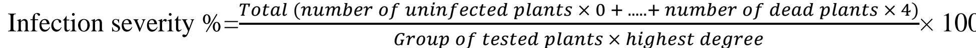
The pathogenicity of three isolates of F.oxysporum isolated from cowpea roots was tested. Petri dishes with a diameter of 9 cm were used, containing 15-20 ml of W.A (Water Agar) medium with 250 mg/L of Chloramphenicol added to it. The dishes were inoculated by taking one disk with a diameter of 0.5 cm from a 7-day-old F.oxysporum fungus colony in the center of the dish. The dishes were incubated at a temperature of 25±2°C for 3-4 days. After ensuring that fungal growth had occurred, the Cowpea seeds were distributed at an average of 10 seeds/dish in a circular manner at a distance of 1 cm from the edge of the dish after they were sterilized with a solution of sodium hypochlorite at a concentration of 10% for two minutes, washed with sterile water and dried on filter paper. The experiment was carried out with 3 replicates for each treatment, leaving the control treatment without adding the pathogenic fungus. The percentage of germination was recorded after the end of the incubation period (Kaiser, 1992) according to the following equation.

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

Pathogenicity testing of F.oxysporum isolates using Cowpea seeds in pots.

The pathogenicity of all F.oxysporum isolates was tested using a mixture of soil and peat moss at a ratio of 1:2, 2 kg/pot. The soil was sterilized in an autoclave at 121°C and 15 lb/in² pressure for 1 hour and was re-sterilized the next day. After that, the soil was contaminated with F.oxysporum isolates carried on millet seeds at an average of 1% (w/w) by mixing it with the soil (Jones et al., 1984). Nine pots were prepared with the control treatment (without adding the pathogenic fungus). The soil of the pots was moistened for three days, after which it was planted with Cowpea seeds (sterilized with a 10% sodium hypochlorite solution for two minutes, washed with sterile water and dried on filter paper) at an average of 10 seeds per pot, then watered carefully. After 60 days, the severity of the infection was calculated according to the Lia et al. (2018) scale according to the following equation:

Then the Mickenny equation was applied)) 1923 mentioned by Al-Waily in ((2004 as follows:



$$\text{Infection severity \%} = \frac{Total\ (number\ of\ uninfected\ plants \times 0 + ...+ number\ of\ dead\ plants \times 4)}{Group\ of\ tested\ plants \times highest\ degree} \times 100$$

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Preparation of the pathogenic fungus inoculum and bio agent for the pot experiment

The inoculum of the pathogenic fungus F. oxysporum isolates previously isolated and subjected to pathogenicity tests, including R2, R3, R7, was prepared, while the other factors were obtained It was obtained from previous studies such as the fungus T. longibrachiatum obtained from Al-Abbad (2020) using the method of Dewan (1989) and the bacteria B. cereus obtained from the study of Musa (2024) and prepared according to the method of Bakker et al. (1986) and the yeast S. cerevisiae obtained from the study of Thari (2024) and the yeast suspension was prepared according to Issa and Saleh (2012). Evaluation of the effectiveness of bio agent (B. cereus, S. cerevisiae, T. longibrachiatum) and the pathogenic fungus F.oxysporum and their interaction on some plant growth indicators in pots. The pot experiment was carried out in the fields of the College of Agriculture/University of Basra for the agricultural season 2023-2024 (Figure 1). The mixture of mixed soil and peat moss brought from Basra nurseries was used after the mixed soil was sterilized with an autoclave at a temperature of 121°C and a pressure of 15 pounds/inch2 for an hour. The sterilization process was repeated the next day and then left to cool. After that, peat moss was added to it at a mixing ratio of 1:2 (soil: peat moss). The sterilized soil was distributed in equal quantities in 3 kg plastic pots. The T. longibrachiatum bio-factor inoculum loaded on millet seeds was added to the treatments at an average of 1% weight/weight for each pot and to all treatments that required addition. Also, the S. cerevisiae yeast suspension inoculum grown on NYDB (Nutrient Yeast Dextrose Broth) was added at an average of 50 ml/kg and diluted to 2*108 colony-forming units for each pot in all treatments that required the addition of the yeast inoculum. This suspension was prepared in two 2-liter beakers, each liter containing NYDB medium sterilized in an autoclave at a temperature of 121°C and a pressure of 15 pounds/inch² for 20 minutes. After the medium was left to cool, the two beakers were inoculated with S. cerevisiae yeast and incubated at a temperature of 25±2°C for 48 hours (Eissa et al., 2012). The B. cereus bacteria vaccine was added in an amount of 100 ml and at a dilution of 1*10 to all pots that required its addition. The vaccine was prepared after multiplying the isolate on the liquid culture medium N.B Nutrient Broth. After sterilizing the flasks with a capacity of 250 ml in an autoclave at a temperature of 121°C and a pressure of 15 pounds/inch² for 15 minutes, they were left to cool and then inoculated with the B. cereus bacteria isolates growing on the N.A medium. After mixing the flasks well, they were incubated at a temperature of 37°C for 24-48 hours (Bakker et al., 1986; Fayyad and Awad, 2021). After mixing well with the soil, and after three days of watering the soil, it was inoculated with the fungus F. oxysporum carried on millet at an average of 1% weight/weight for each pot (Dewan, 1989). It was then watered for three days and the seeds were planted. Sterilized Cowpeas at an average of 10 seeds/ac. The experiment was carried out with three replicates, leaving an uncontaminated treatment as a control treatment. After germination, the germination percentage was calculated according to the following equation:

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

The experiment included the following treatments:

1. Control
2. *T.Longibrachiatum*
3. *B. cereus*
4. *S. cerevisiae*
5. Fungicide
6. R3
7. R2
8. R7
9. R3+*T.Longibrachiatum*
10. R2 +*T.Longibrachiatum*
11. R7+*T.Longibrachiatum*
12. R3 +*S. cerevisiae*

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13. R2 +*S. cerevisiae*
14. R7 +*S. cerevisiae*
15. R2 +*B. cereus*
16. R3 +*B. cereus*
17. R7 +*B. cereus*
18. R2+*T.Longibrachiatum +S. cerevisiae*
19. R3+*T.Longibrachiatum +S. cerevisiae*
20. R7+*T.Longibrachiatum +S. cerevisiae*
21. R2+*T.Longibrachiatum +B. cereus*
22. R3 +*B. cereus+ T.Longibrachiatum*
23. R7 *B. cereus+ T.Longibrachiatum*
24. R2 +*S. cerevisiae +B. cereus*
25. R3 +*S. cerevisiae +B. cereus*
26. R7 +*S. cerevisiae +B. cereus*
27. R2 +Fungicide
28. R3+Fungicide
29. R7+Fungicide
30. *T.Longibrachiatum +S. cerevisiae +B. cereus*
31. *S. cerevisiae +B. cereus*
32. *B. cereus+ T.Longibrachiatum*
33. *T.Longibrachiatum +S. cerevisiae*
34. R3+*T.Longibrachiatum +S. cerevisiae + B. cereus*
35. R2+*T.Longibrachiatum +S. cerevisiae +cereus .B*
36. R7+*T.Longibrachiatum +S. cerevisiae +cereus .B*

The studied traits

The severity of the infection was calculated using the five-degree pathological index for fusarium wilt of cowpea, after the appearance of wilt symptoms on the plants, according to the scale of Lia et al. (2018).

Grade 0 = healthy plant

1= less than 1-25% of leaves are yellowed and wilted

2= 26-50% of leaves are yellowed and wilted

3= 51-75% of leaves are yellowed and wilted

4= 75-100% of leaves are yellowed and wilted

5= dead plant

Then the Mickenny equation (1923) mentioned in Al-Waily (2004) was applied as follows:

$$\text{Infection severity } \% = \frac{Total\ (number\ of\ uninfected\ plants \times 0 + \ldots + number\ of\ dead\ plants \times 4)}{Group\ of\ tested\ plants \times highest\ degree} \times 100$$

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Measurement of chlorophyll in Cowpea leaves

Chlorophyll was estimated for each treatment by taking three leaves randomly (3 replicates for each treatment) where 1 gm of fresh, tender leaves was weighed and placed in a ceramic mortar, then 10 ml of 80% diluted acetone for chlorophyll extraction, then the sample was finely ground and placed in a sterile tube and centrifuged at 3000 rpm for 10 minutes. Then the filtrate was taken and placed in a glass cell in the Spectrophotometer at wavelengths 633 and 645 (Porra, 2002). The reading was taken from the device according to the following equation:

Total Chlorophyll=[8.02× D (645)+20.2×D(663)(V/W× 1000)× 100] = D Optical Density

(663)D= Optical absorption reading at wavelength 663 nm

(645)D= Optical absorption reading at wavelength 645 nm

V= Final volume of extract (ml)

W= Leaf tissue weight (g)

Figure (1) Evaluation of the effectiveness of bio agent (B. cereus, S. cerevisiae, T. longibrachiatum) for Cowpea plants infected with wilt in pots.

Statistical analysis

All laboratory experiments were carried out using a completely randomized design (C.R.D), while pot experiments were carried out using a completely randomized block design (R.C.B.D). The averages were compared using the least significant difference (LSD) test. S D. Under the probability level of 0.01 for laboratory experiments and 0.05 for pot experiments (Al-Rawi and Khalaf, 1980) using the statistical programs SPSS and Genstat.

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III. Results and discussion

Phytomorphic and molecular diagnosis of fungi pathogenic to the root system

The preliminary diagnosis results showed the presence of the fungus F.oxysporum in all samples that were diagnosed phenotypically (Figure 2, 3 and 4) where it was noted that the fungal colonies were characterized by their pink color for the Ezz El-Din Salim isolate and red to purple for the Madinah isolate and creamy white to light pink for the Nashwa isolate and the back of the colony was characterized by its light purple to purple color. Also, the isolates were represented by the presence of aerial mycelium and differences in the shapes of the spores in their numbers. The presence of Chlamydospore was also noted in the dishes in the form of chains chians or single or double and was either intermediate or terminal depending on the classification criteria of Booth (1971) and Pitt and Hocking (1997).

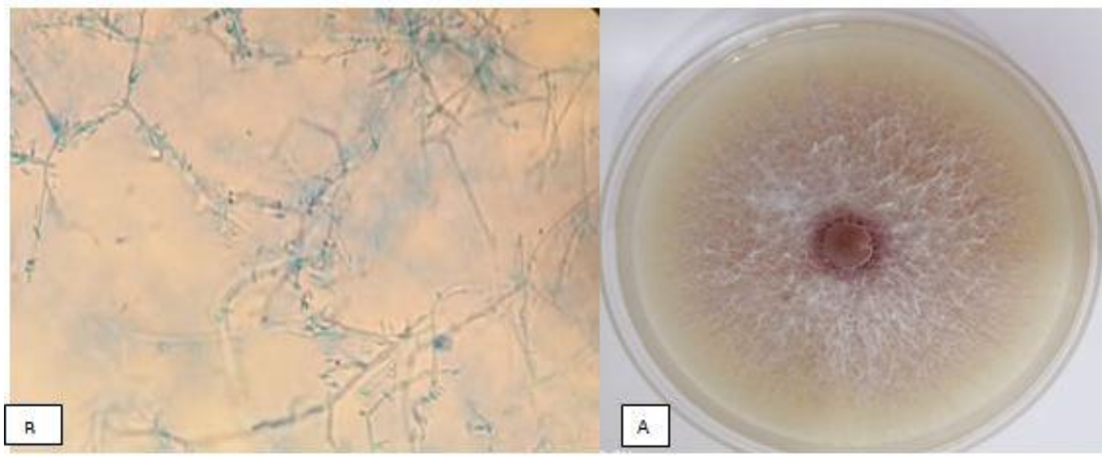

Figure 2: Izz El-Din Salim isolate A = F.oxysporum colony

B = F.oxysporum mycelium and spores

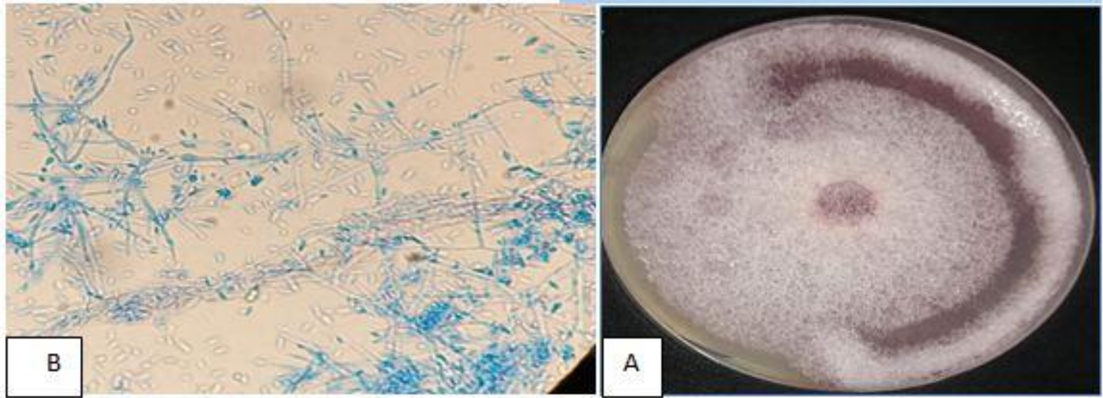

Image 3: City isolate A: = F.oxysporum colony

B= F.oxysporum mycelium and spores

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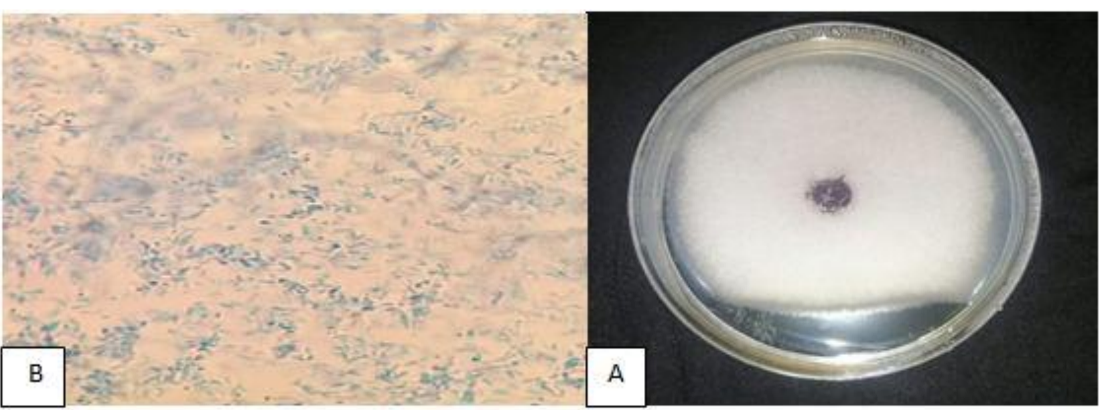

Image 4: Ecstasy isolate A = F.oxysporum colony

B= F.oxysporum mycelium and spores

The results of the morphological diagnosis of pathogenic fungi were consistent with the results of the molecular diagnosis based on the ITS region. The isolates R2, R3 and R7 were registered at the National Center for Biotechnology Information (NCBI). The isolate R2 F.oxysporum was registered under accession number PQ310663.1 and matched the isolate registered under accession number MN871804.1 with a 100% match rate. The isolate R3 F.oxysporum was registered under accession number PQ310664.1 and matched the isolate OR884165.1 with a 100% match rate. The isolate R7 F.oxysporum, registered under accession number PQ310665.1, matched the isolate registered under accession number PP898068.1 with a 100% match rate. These results are consistent with other studies indicating that the fungus F.oxysporum causes wilt disease on cowpea plants, as both do Amaral et al. (2022) and Hao et al. explained. (2024) and Shahiba et al. (2024) that the fungus F.oxysporum is a major cause of Fusarium Wilt disease on cowpea plants in most countries of the world and it causes great losses to this crop.

Testing the pathogenicity of F.oxysporum isolates using cowpea seeds.

The results of the pathogenicity test, Table (4), showed that the pathogenic fungus F.oxysporum reduced the percentage of germination of cowpea seeds if the isolate R7 reached 76.66%, which is significantly different from the control treatment of 100%, as the isolate R3 recorded 66.66%, which is not significantly different from the isolate R7, while the isolate R3 recorded 63.66%, which is significantly different from the isolate R7. These results agreed with the study of Jassim (2017), which showed that the pathogenic fungus F.oxysporum recorded the highest inhibition rate, which reached 85%. This fungus has the ability to produce enzymes that decompose pectin and cellulose, which cause seeds to rot and prevent germination (Hussein, 2019), in addition to the fungus producing toxic substances, including Lycomarasmine and Fusaric acid, which affect the xylem vessels and affect cellular respiration by combining iron with oxidase enzymes in the respiration process (Fayyad et al., 2018). Al-Waily (2004) explained that the F. oxysporum fungus isolate is highly pathogenic and causes seed rot. Radhi et al. (2010) indicated that the F. oxysporum fungus caused a reduction in seed germination to 54.66% compared to the control treatment, which reached 100%. Manea (2022) also indicated that Fusarium spp. fungus isolates had a negative effect on the germination of date palm seeds, which reached 53.33 and 56.67%, while the control treatment without the fungus reached 93.33%.

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Table (4) Effect of the pathogenic fungus F.oxysporum on the germination percentage of Cowpea seeds in dishes.

Germination percentage(%)	treatments
63.66	F.oxysporum isolate R2
66.66	F.oxysporum isolate R3
76.66	F.oxysporum isolate R7
100	Comparison without pathogenic fungus
10.0	L.S.D at 0.01 level

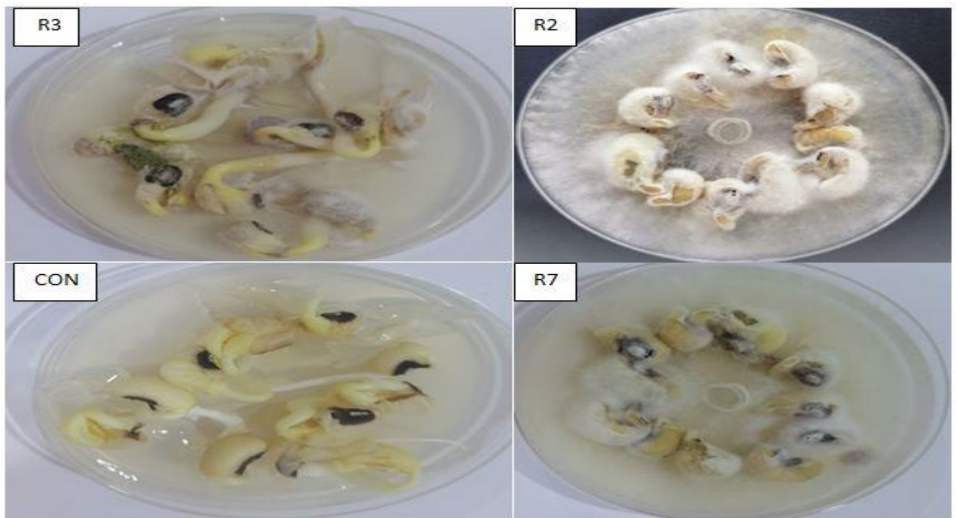

Figure (5) Effect of pathogenic fungal isolates F.oxysporum on the germination percentage of Cowpea seeds on aqua agar medium (WA).

R2 = F.oxysporum Madinah isolate, R3 = F.oxysporum Ezz El-Din Salim isolate, R7 = F.oxysporum El-Nashwa isolate, CON = Control

Testing the pathogenicity of F.oxysporum isolates using Cowpea seeds in pots

The results (Table 5) showed that all pathogenic fungal isolates caused infection in plants, which was represented by the appearance of symptoms of wilting of the plant and yellowing of the leaves. The fungi were re-isolated from the infected plants on PDA medium under laboratory conditions according to Koch's hypothesis. The F.oxysporum R7 isolate was superior in the infection severity, reaching 87.33%, F.oxysporum R2 reached 84.00%, and F.oxysporum R3 reached 82.00%. There were no significant differences between the isolates. These results agreed with Dhari (2011), where the infection severity by F.oxysporum reached 50% on watermelon plants. These results also agreed

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with Abbas (2022), where the infection severity by F.oxysporum on cucumber plants in pots reached 62.10%. This is due to the ability of the fungus F.oxysporum to attack the root system through fungal hyphae that settle in the brown spaces of the cells and the fungal hyphae continue to grow and lead to the closure of the transport vessels (Rahman et al., 2021). The fungus Fusarium spp. is also characterized by its ability to produce enzymes such as cellulase and protease (da Rosa-Garzon et al., 2019). Al-Waily (2004) also explained that the fungus F.oxysporum isolate has a high pathogenicity and has the ability to cause complete wilting of the plant.

Table (5) Pathogenicity of F.oxysporum isolates using Cowpea seeds in pots

%severity of injury	treatments
84.00	F.oxysporum isolate R2
82.00	F.oxysporum isolate R3
87.33	F.oxysporum isolate R7
0.0	Comparison without pathogenic fungus
5.767	L.S.D at 0.05 level

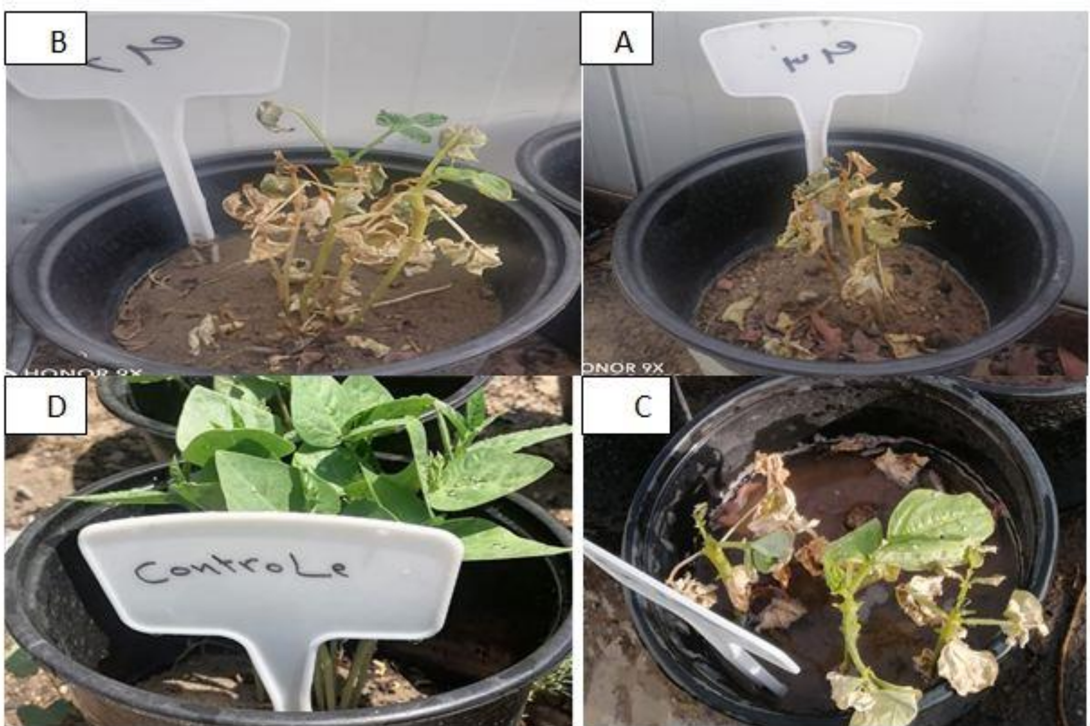

Figure (5) Effect of pathogenic fungal isolates F.oxysporum on using Cowpea seeds in pots.

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A = F.oxysporum R7 m3 Nashwa isolate, B = F.oxysporum R2 m2 Madinah isolate, C = F.oxysporum R3 m1 Ezz El-Din Salim isolate, D = Control

Evaluation of the effectiveness of bio agent (B. cereus, S. cerevisiae, T. longibrachiatum) on Cowpea plants infected with wilt in pots.

The results (Table 5) showed that F.oxysporum isolates affected the germination of Cowpea seeds in pots and increased the infection severity and caused a decrease in the percentage of chlorophyll compared to the control treatment. The results recorded the highest germination percentage of Cowpea seeds in pots treated with yeast S. cerevisiae, which reached 96%, and the lowest germination percentage was recorded for the treatment with T. longibrachiatum and B. cereus, which reached 90%, respectively, compared to the control factor, which reached 100%. The results also showed that the TB treatment was the best combination in the pot experiment, as it recorded the highest germination percentage, which reached 86.7%, compared to the control factor (100%). The results also showed that the nurse treatments R3, R2, R7 led to a reduction in the germination percentage to 10, 20 and 30%, respectively, compared to the control factor, which reached 100%. While TR7 treatment was the best interaction treatment between the pathogenic fungus and the bio agent, reaching 96.7% compared to the pathogenic factor R7, which reached 10%, while the R3T and SR3 treatments recorded 93.33 and 96.7% respectively, compared to the pathogenic fungus treatments, which reached 30%. The germination percentage in the rest of the treatments was close. Spadaro et al. (2002) indicated the possibility of the yeast S.cerevisiae in competing with pathogens for nutrients due to its ability to reproduce and grow rapidly. Bourbos et al. (1997) also indicated that the fungus T. Longibrachiatum has a high ability to suppress pathogenic fungi in the soil due to its mechanisms represented by antagonism, parasitism and competition. As EI-Meleigi et al. (2007) proved, when treating wheat seeds with Bacillus spp. bacteria, this led to a reduction in the incidence of wheat root rot disease caused by the fungus F.garmineaum. The results (Table 5) also showed that the highest amount of chlorophyll in the leaves of the Cowpea plant in the biological factor treatments was the T. Longibrachiatum and S.cerevisiae treatment, which reached 0.83 $mg.g^{-1}$ compared to the control treatment, which reached 0.82 $mg.g^{-1}$. As for the interaction treatments between the pathogenic fungus and the bio agent used in the pot experiment, the TSBR2 treatment recorded 0.4388 $mg.g^{1}$ compared to the R2 pathogen treatments, which reached 0.10 $mg.g^{-1}$. The results also showed that the TSB treatment is the best combination, as it recorded 0.3415 $mg.g^{-1}$ compared to the control treatment, in which the chlorophyll content reached 0.83 $mg.g^{-1}$. The results also showed that the pathogenic fungus treatments R3, R2, and R7 led to a reduction in chlorophyll by 0.071 and 0.1%. and 0.1207 $mg.g^{-1}$ respectively compared to the control treatment (0.83 $mg.g^{-1}$), while the rest of the treatments had similar ratios. This result agreed with Attia et al. (2024) who indicated a significant decrease in the amount of chlorophyll in pepper seedlings infected with F. oxysporum, and the percentage of chlorophyll in tomato leaves infected with Fusarium wilt F. oxysporum decreased (Attia et al., 2022). While Al-aamel et al. (2023) indicated that the interaction between Trichoderma and the fungus Fusarium oxysporum led to an increase in the chlorophyll content in pepper seedlings infected with Fusarium wilt, as the addition of the fungus Trichoderma caused an increase in the chlorophyll content as a result of the increased activity of enzymes and thus increased the absorption of nutrients that support the metabolic process, which leads to an increase in the transpiration process and thus increases the phenolic compounds, which leads to an increase in the chlorophyll content.

The results (Table 5) also showed that the use of bio agent led to a significant reduction in the infection severity, as it recorded 0.00% for bio agent, and this percentage is similar to the control treatment (0.00%). The infection severity for the combinations used in the experiment SB, TB, and TSB recorded similar percentages in the infection severity, as it reached 0.67%, while TS recorded the lowest percentage, which reached 2%. As for the factors interacting with the pathogenic fungus, they led to a reduction in the infection severity, as the treatments R3 T and R2T recorded an infection severity of 0.00%. As for R3 TS, it recorded 16.67%. The results of Table 5 also showed that there were no significant differences between the pathogenic fungal isolates, as the infection severity of cowpea plants with the pathogenic fungus increased, as the infection severity with R3 reached 84%, R2 82%, and R7 387.3%, respectively. This study was consistent with Dhari (2011) that bacteria and biological fungi have the ability to reduce the infection

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severity of watermelon plants infected with Fusarium wilt disease F. oxysporum. Elad et al. (1983) also indicated that the fungus Trichoderma spp. has a role in reducing the infection severity. Bora (2000) indicated that bacteria used as a biological agent have the ability to reduce the infection severity with F. oxysporum. The bacteria cereus. B. cereus produces extracellular enzymes and lipopeptides that stimulate systemic resistance (Kulkova et al., 2023). The high effectiveness of T. Longibrachiatum treatment is due to the increased availability of nutrients in the soil environment of the Cowpea plant, which improves plant growth indicators in pots (Liu et al., 2023). T. Longibrachiatum also stimulates a number of primary and secondary metabolite pathways, thus increasing plant growth and activating defenses against various diseases (Abdurrahman et al., 2016). S. cerevisiae yeast is also characterized by its ability to increase the activity of the enzyme Chitinase and Glucanase and stimulate systemic resistance, thus affecting plant growth levels and thus increasing plant growth indicators. It also produces compounds (toxins) that inhibit the metabolic activity of fungal growth (Kowalska et al., 2022).

Table (5) effect of bio agent on the germination rate, infection severity, and chlorophyll percentage in pots

Chlorophyll (mg.g⁻¹.fresh weight(	Infection severity	Germination percentage (pots(	treatments	Chlorophyll (mg.g⁻¹.fresh weight(	Infection severity	Germination percentage (pots(	treatments
0.3375	0.67	63.3	S+B	0.83	0.00	100	control
0.2397	0.67	96.7	R3+S	0.83	0.00	90.00	T
0.3080	0.67	26.7	R2+S	0.83	0.00	96.00	S
0.3408	2.00	46.7	R7+S	0.28	0.00	90.00	B
0.2021	4.00	66.7	R3+S+B	0.071	84.00	20.00	R3
0.2657	3.33	66.7	R2+S+B	0.100	82.00	0030.	R2
0.4129	2.67	63.3	R7+S+B	0.1207	87.33	10.00	R7
0.1316	2.67	73.3	R3+B	0.2506	0.00	93.33	R3+T
0.1466	0.67	83.3	R2+B	0.2582	0.00	86.7	R2+T
0.3267	1.33	73.3	R7+B	0.2802	0.67	96.7	R7+T
0.2761	0.00	50.0	Fungicide	0.3547	4.00	56.7	R3+T+B
0.2430	4.00	43.3	R3+Fungicide	0.3157	2.00	73.3	R2+T+B
0.4956	6.67	66.6	R2+Fungicide	0.1326	4.67	80.00	R7+T+B

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0.4956	**4.00**	**70.0**	**R7+Fungicide**	**0.2459**	**9.33**	**63.33**	**R3+T+B+S**
0.3415	**0.67**	**73.3**	**T+S+B**	**0.4388**	**12.00**	**50.00**	**R2+T+B+S**
0.3371	**0.67**	**86.7**	**T+B**	**0.3191**	**2.67**	**36.66**	**R7+T+S+B**
0.2755	**2.00**	**50.0**	**T+S**	**0.1599**	**16.67**	**23.33**	**R3+T+S**
0.2325	**16.67**	**73.3**	**R7+T+S**	**0.1600**	**14.00**	**86.7**	**R2+T+S**

*Each treatment represents three replicates

R.L.S.D for treatments Chlorophyll percentage = 0.05243

R.L.S.D for treatments Infection severity = 4.907

R.L.S.D for treatments Germination percentage = 11.402

Fusarium oxysporum fungus isolate R3 = Ezz El-Din Salim

Fusarium oxysporum fungus isolate R2 = Al-Madinah

Fusarium oxysporum fungus isolate R7 = Al-Nashwa

T = Fungus longibrachiatum T.

S = Yeast S. cerevisiae

B = Bacteria B. cereus

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