The effect of some plant extracts on the infection of okra plant *Abelmoschus esculentus* with root rot disease

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Abstract

The study aimed to know the best treatment to control the fungi that cause okra root rot by using aqueous extract of the leaves of eucalyptus, Bamber (*Cordia myxa*) and oleander plants, with finding the best concentrations to inhibit the growth of pathogenic fungi. The results showed the presence of three pathogenic fungi R. solani, F. oxysporum and M. phaseolina, and the most prominent of them were R. solani in the areas from which samples were collected in Basrah province.The results showed that the fungus R. solani had a clear effect in reducing the percentage of germination of Cabbage seeds, which amounted to 32.7%, with a significant difference from the treatment of F. oxysporum and M. phaseolina, which amounted to 55.0 and 57.7%, respectively, while it reached 63.93% in the control treatment.The results of the pot experiment showed that the fungus R.solani reduced the germination rate of okra seeds, reaching 57.7%, while it was 58.2 and 64.4% in the two fungi F. oxysporum and M. phaseolina treatments, with a significant difference from the 90% control treatment.The fungi also caused the death of seedlings, where the percentage reached 28.6% for R.solani and F. oxysporum and 19.0% in M. phaseolina treatment with a significant difference from 0% control treatment.The aqueous extracts affected the percentage of pathogenic fungi growth in the laboratory. The aqueous extract of the leaves of the eucalyptus plant gave the highest percentage of inhibition of the growth of the fungus R. solani, the percentage was 43.11%, with a significant difference from the extracts of the leaves of the plant Bamber (Cordia myxa) and oleander , which amounted to 39.62 and 29.68%, respectively.The effect of the extracts increases with the increase of the concentration, where the aqueous extract of eucalyptus at a concentration of 40% gave the highest percentage of inhibition which amounted to 73.89%, with a significant difference from the extracts of Bamber (Cordia myxa) and oleander .

Keywords: plant extracts, okra plant, Abelmoschus esculentus, root rot disease
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Introduction

Okra (*Abelmoschus esculentus*) is a summer vegetable crop belonging to the family Malvaceae. It is native to Africa and has spread to the eastern Mediterranean, India, the Arabian Peninsula, America, Europe and other countries. It is one of the most desired vegetables in Iraq that needs a warm growing season to grow Wanted et al, 1989). It is considered one of the plants of high nutritional value, where its fresh fruits contain a high

percentage of water and many important nutrients such as carbohydrates, calcium, phosphorous, iron, sodium and magnesium (Gemede et al., 2015).The okra plant is infected with many agricultural pests such as insects such as aphids, cutworms, and various fungal diseases that have increased with protected agriculture, and the most important diseases are powdery mildew, wilt and root rot, as well as snake worms, Root rot disease is considered one of the common diseases that cause great losses caused by many fungi in the soil, including Rhizoctonia solani, and one of the symptoms of this disease is the rotting of the roots and the bases of the stems that are close to the surface of the soil and thus leads to weak plants and thus wilt and plant death. The plant, which caused losses in the crop (Jassim 2012). The fight against these causes is one of the difficulties facing workers in this field due to its ability to remain in the form of static structures for a long time and its ability to restore dead organic matter when the host is not available (Agrios, 2007).Several methods have been used to combat soil fungi, including chemical control, which is the most common method because it is easy to use and has a quick effect on pathogens, but its negative effects are due to repeated use and the emergence of resistance in addition to its danger to humans and animals and environmental pollution (Taylor et al., 2002),Many strategies have been used to control diseases caused by fungi such as Rhizoctonia solani and Fusarium oxysporum, such as soil sterilization, planting resistant varieties, and following crop rotations, but they did not achieve effective control of these pathogens (El-mohamedy et al., 2011).Recently, plant extracts have been used as an important part in the biological control of plant pathogens, and several attempts have been made to use them in the control of plant pathogenic fungi such as Macrophomina phaseolina and Fusarium. oxysporum and R. solani (Al-Rubaie, 2008). This is due to its abundance and being of low toxicity compared to chemical pesticides, as well as being easy to decompose (Harish et al., 2004),The leaves, flowers, and roots of many plants contain chemical compounds that have a toxic effect on agricultural pests (insect, bacterial and fungi), such as the inhibitory effect of the ethanolic extract of Nerium oleander on four types of important plant pathogenic fungi R. solani, F. oxysporum and A. alternate. The extract has a highly effective effect on inhibiting F. solani and F. solani species. oxysporum (Hadizadeh et al., 2009 .).The eucalyptus plant is also one of the fast-growing trees and is used for medicinal and therapeutic purposes (Mubark, 2015), as its leaves contain phenolic compounds such as sterols, suponins, tainnins, flavonoids and triterpeoids (Shayoub et al., 2015).Al-Khair and Al-Obaidi (2020) also referred to the study of the effect of two types of alcoholic and aqueous extracts of eucalyptus leaves on the growth of isolates of M. phaseolina, R. solani and F. solani, as the alcoholic extract was excelled in inhibiting the growth of isolated fungi except for R. solani, which was only significantly inhibited by the aqueous extract of eucalyptus leaves. The Bamber (Cordia myxa) plant is one of the plants grown in central and southern Iraq, native to Burma and used to combat pathogens in soil (Chacravarty), (1976).Where between Sarhan and Al-Shibli, 2003)) in a study of the effect of three concentrations of 0.5, 0.10 and 0.15% for each extract of Bamber (Cordia myxa) (cold aqueous extract, hot aqueous extract and alcoholic extract) on radial growth, dry weight and spore germination percentage of a number of plant pathogenic fungi Fusarium solani And Alternaria alternate, Rhizoctonia solani, Helminthosporium sativum and Rhizopus stolonifer, where the hot aqueous extract recorded a remarkable effect, followed by the cold aqueous extract, while the alcoholic extract did not have any significant effects.In view of the importance of the okra plant for being one of the vegetables with high nutritional and marketing value, the study included isolating and diagnosing the fungal pathogens and finding the best treatment to combat the pathogenic fungus by using aqueous extract of the leaves of eucalyptus, Bamber and oleander plants with finding the best concentrations to inhibit the growth of pathogenic fungi.

Materials and methods:-

Isolation and identification of pathogens

The pathogenic fungi F. oxysporum, M. phaseolina and solani.R were isolated from the roots of the okra plant. Samples of the roots of the okra plant

that showed symptoms of yellowing and wilting were collected from different regions of Basrah province, including Abu Al-Khasib, Al-Bahadria, Al-Sahel, Al-Qurna District, Al-Sharsh and Shatt Al-Arab region Al-Hawtah was placed in marked plastic bags and brought to the laboratory. The roots were washed with running water for half an hour to get rid of the mud stuck to them, then they were cut to a length of (1 cm) the cuts were superficially sterilized with a solution of 10% sodium hypochlorite (Naocl) solution at a concentration of 6% of the commercial preparation for 2-3 minutes, then It was washed with sterile distilled water to remove the remnants of the sterilization solution for 2 minutes and left on filter paper for the purpose of drying. They were cultured in Petri dishes with a diameter of 9 cm containing sterile PDA medium supplemented with the antibiotic Chloramphenicol in 3 replicates at 250 mg/L. The dishes were incubated in the incubator at 25±2°C for 4 days. Then the isolates of fungi were purified by transferring parts of the tip of the fungal hyphae of the colony of pathogenic fungi using a sterile needle to Petri dishes containing sterile PDA media, and the plates were incubated in the incubator under the same temperature for 4 days for the fungi isolated after adding lactophenol dye by Prof. Dr. Yahya Ashour Saleh according to the taxonomic keys contained in (Parameter and Whitney, 1970), the fungal isolates were kept on the PDA medium at a tilt in the refrigerator until completion of the experiments.

Testing the effect of the pathogenic fungi *F. oxysporum*, *M. phaseolina and.*R *solani* using Cabbage seeds on the aquatic aquarium culture media:-

The germination rate of the seeds of Cabbage was tested treated with isolates of the pathogenic fungi R.solani, F. oxysporum and M.phaseolina according to the method of Bolkan and Butler, 1974) The nutrient medium was prepared Water Agar after sterilization with the Autoclave steam device and the antibiotic was added to it Chloramphenicol and shaken well and then poured into Petri dish with a diameter of 9 cm was then left to harden and then the center of each dish was inoculated with a disk of fungi(0.5) cm diameter from a colony of fungi grown on PDA medium at the age of 7 days, then the dishes were incubated at a temperature of 25±2°C for a period of 3 days. After this period, the broccoli seeds were superficially sterilized with a solution of sodium hypochlorite at a concentration of 10% of the commercial solution 6% and in a circular motion near the edge of the dish and at a rate of three replications and at a rate of 10 seeds for each dish, leaving a control treatment containing broccoli seeds and without any pathogenic fungi. The dishes were incubated at a temperature of 25±2°C, and the results were recorded after (7 days) of planting by calculating the percentage of germination as in the following equation:

germination percentage$=\frac{(number\ of\ germinated\ seeds)}{(total\ number\ of\ seeds)} \times 100$

Pathogenicity test of the isolated fungi R.solani, F.oxysporum and M.phaseolina on potted okra plants:-

The pathogenicity of isolates of the pathogenic fungi R.solani, F.oxysporum and M.phaseolina was tested, using agricultural soil sterilized with formalin solution (1 part formalin: 50 parts water),The solution was used at a ratio of 3 L/m3 (Poultry, 1975). The soil was placed in plastic bags and closed well for 3 days. Then it was exposed to the air under the sun for 3 days in order to volatilize formalin. A quantity of peat moss was sterilized in the same way as in sterilizing the soil, mixing the peat moss with the soil in a ratio of 1:3 (soil: peat moss).Then the fungal pollen grown on millet seeds was added to it at a ratio of 1% w/w and the fungal pollen was mixed well with the sterilized soil using cellophane bags to ensure the homogeneity of the vaccine with the sterilized soil. By 5 seeds per pot and an average of three replicates, leaving three replicates as a comparison treatment.After the appearance of the symptoms of wilting and yellowing, the percentage of germination and the percentage of seedling death were calculated after 10 and 20 days of planting.

Germination%
$=\frac{(number\ of\ germinated\ seeds)}{(total\ number\ of\ seeds)} \times 100$

seedling death%$=\frac{Number\ of\ dead\ seedling}{Total\ number\ of\ seedlings} \times 100$

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Preparation of the aqueous extract of the leaves of plants used in the experiment

Metspula et al. (2001) used the method of preparing the aqueous extracts of the plants used in the study, which are the leaves of eucalyptus,Bamber (Cordia myxa) and oleander . 20 gm of dry plant powder was taken. Placed in glass flasks of 500 ml, each flask containing 200 ml of distilled water, then mixed the dry plant material in a Panasonic electric mixer for 15 minutes, then left for 30 minutes, then filtered with a dull cloth to separate the large plankton,Then centrifugation was conducted at 3000 cycles for 30 minutes, then the filtrate was taken and filtered through Whatman No.1 filter paper and sterilized using Millipore microfilters of 0.45 µm diameter. An appropriate amount of each plant extract was taken and the filtrate was used as a base solution. It was stored in the refrigerator at 4°C until use.

The effect of the aqueous extract of the tested plants on the growth of the pathogenic fungus R. solani

The medium of the PDA culture medium was prepared and sterilized by an Autoclave device at a temperature of 121 °C and a pressure of 15 pounds / inch for 20 minutes. After decreasing the temperature, the aqueous extract was added to the glass beaker containing the food medium at concentrations of 10, 20, 30 and 40% for each extract, then the beaker was shaken for the purpose of homogenization of the extract with The culture medium, then poured into Petri dish 9 cm in diameter, The plate was inoculated upon solidification of the nutrient medium with a 0.5 cm diameter disc from the growing medium containing the pathogenic R. solani fungus and with three replications for each concentration of the extract. Then the plates were incubated at a temperature of 25 ± 2 °C for a period of 7 days. According to the growth rate, the average of two perpendicular diameters was taken for the growth of the fungal colony from the bottom plate, and the percentage of inhibition was calculated according to the following equation:

Percentage of inhibition

$$= \frac{(\text{The growth rate of the fungus in the treatment} - \text{the growth rate of}}{(\text{The growth rate of the fungus in the treatm}} \times 100$$

Results and Discussion

Isolation and diagnosis

The results of isolation and diagnosis Table (1) for okra plants infected with root rot and plant death showed the presence of three pathogenic fungi, *F.oxysporum*, *R.solani* and *M.phaseolina*, and the most prominent ones were in the areas from which samples were collected in Basra Governorate, namely, Abu Al-Khasib, Hamdan, Al-Sahel and Al-Houta Shatt areas. Arabs, Bahadriya and Qurna. Its phenotypic and microscopic characteristics were consistent with what was mentioned by (Watnabe and Shiyome, 1975) and (Sinclair, 1982, Leslie and Summurel, 2006).

Table (1) shows the pathogenic fungi isolated from the roots of okra plants grown in different areas of Basrah province.

No.	Region	pathogenic fungi
1	Abi Al-Khasib (Hamdan)	***M. phaseolina*** ***R. solani***
2	alsaahil region	***R. solani*** ***M. phaseolina*** ***F.oxysporum***

3	Al-Houta	***R. solani*** ***M. phaseolina***
4	Al-Qurna / Shark region	***R. solani*** ***M. phaseolina*** ***F.oxysporum***
5	Al-Bahadria	***R. solani*** ***F.oxysporum***

Pathogenicity of the pathogenic fungi F.oxysporum, R.solani and M.phaseolina in germination percentage:-

Effect of pathogenic fungi on germination rate on Cabbage seeds:

The results of this experiment showed Table (2) that the fungi isolated R.solani, M.phaseolina and F.oxysporum caused a reduction in the germination rate of Cabbage seeds, but the effect of the pathogenic fungus R.solani in reducing the percentage of seed germination was greater than the effect of other fungi The germination rate reached 32.7% compared to the fungi F.oxysporum and M.phaseolina, which amounted to 55.0% and 57.5%, respectively, compared to the control treatment, which had a germination rate of 63.93%.This is due to the ability of the pathogenic fungus R. solani degrading enzymes such as Polygalacturonase, Cellulases and Pectinases, as these enzymes work on decomposing plant cell walls, as well as destroying the middle lamina (Ravjit et al., 1999). (Weinhold and Sinsclair, 1996),

Or, the reason may be due to the fungus' invasion of the seed coat and growth between the starch granules and the secretion of enzymes Amylases that lead to killing cells and turning the seeds into a dark brown color (Mahmoud et al., 2007). The reason is attributed to the mycotoxins that the fungus produces that inhibit seed germination and its ability to secrete kinase-degrading enzymes in the host cell wall, and this is consistent with what was found by Gordon et al, 1989).As for the fungus M.phaseolina, the results agreed with (Hassan et al., 2015), as the pathogenic fungus attacks the seeds of many plant families and leads to preventing them from germination and rotting because it secretes toxic compounds that lead to the killing of embryos, as the fungus secretes many enzymes that degrade cellulose and protein, which in turn It causes seed rot and infection.

Table (2) Effect of pathogenic fungi on the percentage of germination of Cabbage seeds

germination%	**Tretments**
32.7	***R.solani***
55.0	***F. oxysporum***
57.7	***M.phaseolina***
63.93	**Control**
27.35	**(0.01)L.S.D****

*Each number represents an average of three replicates

Testing the pathogenicity of the pathogenic fungi R.solani, M.phaseolina and F.oxysporum in the percentage of germination and seedling death of okra plants in pots:

The results of the study showed in Table (3) that the pathogenic fungi R.solani, F.oxysporum and phaseolina.M had a clear effect in reducing the percentage of germination of okra seeds, where the germination percentage in the treatment of the fungus R.solani was 57.7%, while it was 58.2% and 64.4% In the two treatments of F.oxysporum and M.phaseolina, respectively, with a significant difference from the control treatment, which amounted to 90.0%,The results also showed the ability of these pathogenic fungi to infect seedlings after germination, where the percentage of seedling

death in the treatment of the pathogenic fungi R.solani and F.oxysporum at an average of 28.6%, while it reached 19.0% in the treatment of M.phaseolina with a significant difference from the control treatment. 0%,These results agreed with previous studies regarding the ability of the fungus R.solani to reduce the percentage of germination of seeds of many crops such as okra, sesame, cotton and tomato (Fayyad and Al-Atabi, 2018), because the fungi R.solani and F.oxysporum cause a decrease in the germination rate of plant seeds. Al-Haidari okra, (2007) because the fungus R.solani secretes enzymes that degrade plant cell walls: Proteinate, Cellulase and Pectinases and secrete toxins that kill the protoplasm of plant cells. (Kawchuk, 2002).The reason for the appearance of symptoms of infection on seedlings infected with F.oxysporum fungus, where the plant wilt with yellowing leaves was observed. The reason may be due to the fungus entering the plant tissue by penetrating the cell walls due to its ability to produce enzymes degrading chitinase, cellulose and pectin that break down cell walls (Booth, 1971).Also, M.phaseolina has the ability to produce vasodilating toxic substances that break down cellulose and pectin, causing rotting of seeds and death of seedlings (Mahato et al., 1987).

Table (3) Pathogenicity of the pathogenic fungi R.solani, M.phaseolina and F.oxysporum on germination of okra seedlings and seedling death

fungi	**seedling death%**	**germination%**
R.solani	28.6	57.7
F.oxysporum	28.6	58.2
M.phaseolina	19.0	64.4
Control	0	90.0
L.S.D*	22.6	15.11

Effect of aqueous extracts on the growth of pathogenic R. solani . fungus

The results of this experiment showed Table (4) that the type of extract had a significant effect on inhibiting the growth of pathogenic fungi, where the eucalyptus extract gave the highest percentage of inhibiting the growth of the pathogenic fungus R. solani with a rate of 43.11%, with a significant difference from the aqueous extracts of Bamber (Cordia myxa) and oleander , with a percentage of 39.62 and 29.68%, respectively.The results also showed that the effect of the aqueous extracts of all studied plants increases with the increase in the concentration of the extract, as the percentage rate of inhibition reached 19.75, 25.68, 43.08 and 61.06% for the 10, 20, 30 and 40% concentrations, respectively.Also, the aqueous extract of eucalyptus at a concentration of 40% gave the highest percentage of inhibiting the growth of the pathogenic fungus R. solani, which was 73.89%, with a significant difference from the extracts of Bamber (Cordia myxa) and oleander . Previous studies indicated that the aqueous extract of eucalyptus is effective in reducing the growth rate of pathogenic fungi R.solani and F.solani (Al-Hamiri, 2016).He also due (Al-Khair and Al-Obaidi, 2020) the superiority of eucalyptus leaf extract and its effect on root rot fungi of pine seedlings, because eucalyptus leaves contain Triterpeoids, sterols and flavonoids, which have an effective role in inhibiting the growth of pathogenic fungi.

Table (4) Effect of aqueous extracts of some plants on the growth of the pathogenic fungus Rhizoctonia solani in the laboratory

Effect average of extract type	**fungus growth inhibition%**				**extract**
	Concentrations used				
	%40	**%30**	**%20**	**% 10**	

39.62	54.99	54.44	29.63	19.44	***Cordia myxa***
43.11	73.89	41.48	32.03	24.07	***Eucalyptus***
29.68	54.29	33.33	15.37	15.74	***Nerium oleander***
	61.06	43.08	25.68	19.75	**Average effect of concentrations**
The least significant difference (L.S.D) test at the level of significance 0.01					
Interaction		**concentrations**		**extract**	
8.68		5.01		4.34	

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