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The effectiveness of the Fungus *Beauveria bassiana* in reducing the infection of okra plant *Abelmosechus eseulentus* with fungal root diseases.

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Abstract

This study was conducted in the laboratories of the College of Agriculture - University of Basra in the years 2020 and 2021, in which the fungus *B.bassiana* was used. To combat the root fungus that infects okra, the results showed the isolation of many pathogenic fungi, and these fungi showed a significant difference in the effect on seed germination and seedling fall. The entomopathogenic fungus *B.bassiana* has a high ability to colonize roots and promote plant growth, such as plant height, fresh weight, and plant dry weight. The results of field surveys of four areas in Basra governorate showed, namely Shatt Al-Arab, Abu Al-Khasib, Al-Qurnah and Al-Madina. The highest incidence and severity of okra root rot disease was in Al-Madinah region, where the infection rate was 37% and the infection intensity was 67.33%, followed by Abu Al-Khasib district, where the infection rate was 24% and the infection severity was 52.89%. This disease is caused by the fungi *Rhizoctonia solani*, *Fusarium solani* and *Macrophomina phaseolina*. Pathogenicity test results showed that F. solani gave the highest mortality rate for seedlings, followed by *R. solani* and *M.phaseolina* efficiently inhibiting the growth of pathogenic fungi. In the greenhouse experiment test, the experiment showed that the highest length of the vegetative group was in the biological treatment of fungi,
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Introduction

The okra plant, *Abelmosechus eseulentus* L., belongs to the family Malvaceae and is an important summer vegetable crop in the warm regions of the world (Oyelade et al., 2003 and Andras et al., 2005 and Saifullah and Rabbani, 2009). It is believed that its original homeland is Africa, specifically Ethiopia (Getachew, 2001, Simmone et al., 2004, Dandena, 2010, Sathish, and Eswar, 2013), and this crop is the most well-known and used species of the family Malvaceae (Naveed et al., 2009).

Okra plant is infected with many pathogens, especially root diseases caused by the fungi *Macrophomina phaseolina*, *Rhizoctonia solani* and *Fusarium* spp. *Phytophthora* spp. It infects a wide range of plants, causing seed damage, rotting and seedling death before and after emergence, and infects plants in the crown area, stems, leaves, buds and fruits close to the soil surface (Kapadiya et al., 2014), due to the losses caused by plant infection with pathogens and the occurrence of mutations in resistance to pesticides and their direct impact on the health of Humans and animals, as well as the environmental imbalance as a result of the excessive and indiscriminate use of pesticides (Al-Adel, 2006; Matrood and Rhouma, 2021a). Insect pathogenic fungi have been important natural death factors for arthropods for more than 100 years to date,

and they are effective as biological protection agents for insect and other pests and an increasing number of recent studies show that entomopathogenic fungi can play important roles such as, endogenous fungi in plants, plant growth stimulants, Antibodies against plant diseases, and root zone colonizers (Vega et al., 2009; Matrood and Rhouma, 2021b).

Materials and working methods:

Field survey

A field survey was conducted to estimate the percentage of root diseases affecting okra plants during the year 2019-2020. The survey included Shatt Al-Arab District, Abu Al-Khasib District, Qurna District, and Al-Madina District. The survey method relied on selecting a specific area in the mentioned areas, then 100 plants were selected from each. area and randomly, as the number of total plants (infected and healthy) was calculated in each area, then the percentage of infection was calculated according to the following equationthe severity of the injury was also calculated according to the five-point scale, as follows:

Class Description

Zero does not exist.

1discoloration of part of the roots in brown.

2 Color the entire roots brown.

3 Root discoloration and plant yellowing.

4 Complete death of the plant.

Then Mickenny's equation (1923) given in Expelled (2015) was applied.

% Severity of injury =

$$\frac{Total\ (No.of\ uninfected\ plants\ x\ 0 + \cdots + No.\ of\ dead\ plants\ x\ 4)}{Total\ plants\ examined\ x\ highest\ score} \times 100$$

Isolation and identification of the pathogens that infect the roots of the okra plant

Okra plants were collected from different areas in Basra Governorate, which included the areas (Shatt Al-Arab, Al-Qurnah District, and Al-Madina). The infected plant roots were washed with running water to remove the mud stuck to the roots, then left in the laboratory to dry, then cut into small pieces of 1-0.5 cm, then sterilized. Surface with commercial sodium hypochlorite NaOCl solution at a concentration of 10% for about two minutes, then the pieces were lifted from the solution and washed with distilled water to remove the effects of sterilization, the pieces were dried by sterile filter paper (Whatman No. (Potato Dextrose Agar) sterilized and supplemented with the antibiotic Cloramphenicol 250 mg/L at a rate of 4 pieces per plate and 3 replications. Then the dishes were incubated in the incubator for 4 days at a temperature of 25 ± 2 °C. After the appearance of the fungal colonies, they were purified on the same pathogen. The plant had both according to its phenotypic and microscopic characteristics, based on the characteristics mentioned in Parameter and Whitny (1970) and Lessilkie and Summurel (2006). 2-8 o'clock until use (Matrood et al., 2021a; Matrood and Rhouma, 2021c).

Pathogenicity test of *F.solani, R. solani* and *M.phaseolina*

The pathogenicity of *R. solani, F. solani* and *M. Phaseolina* was tested. according to the method of Bolkan and Butler (1974) by inoculating 9 mm diameter Petri dishes containing 15-20 ml of Water & Agar medium with a 0.5 cm drop disc from a colony of developing fungus aged for five days on PDA culture medium, then the dishes were incubated at room temperature. 25 ± 2 C. For three days after that, local okra seeds were sown superficially sterilized with 10% sodium hypochlorite solution, in a circular motion near the edge of the plate, at a rate of three replications, with 7 seeds per plate, leaving the control treatment containing only seeds. Then the dishes were incubated at a temperature of 25 ± 2 C. The results were recorded after 7 days of planting by calculating the percentage of germination as in the following equation:

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

Effect of different concentrations of *B. bassiana* filtrate and sterilized filtrate on the growth of pathogenic fungi *F. solani, R. solani* and *M.phaseolin*.

The liquid medium was prepared Broth Potato Scrose, consisting of 200 gm of potato extract and 10 gm of scrose/liter of distilled water, distributed in 250 ml conical flasks at a rate of 200 ml/ beaker. Sterilize the food media with a steam sterilizer at a temperature of 121°C and a pressure of 15 pounds/in2 for 20 minutes. The flasks were cooled and each of them inoculated with five 0.5 cm diameter discs each of PDA culture medium on which *B. bssiana* was grown at five days of age. Then the flasks were incubated at a temperature of 25 ± 2 for 14 days, taking into account that the contents of the flasks were shaken every 2-3 days. Fungi cultures were filtered during No.1 Whatman filter paper, after which the filtrate was sterilized through a fine filter (Millipore 0.20µm) the filtrate of the bio-fungi was added to the sterilized PDA food media before solidification at a ratio of 10, 20, 30 ml, taking into account the adjustment of the akar ratio before sterilizing the medium and with three replications. As for the comparison treatment It included adding sterile distilled water to the medium in the same proportions as the filtrate. The nutrient media containing and not containing filtrates were poured into sterile Petri dishes with a diameter of 9 cm. After hardening, the media was inoculated with 0.5 cm diameter discs of media containing the five-day-old pathogens *F. solani*, *R. solani* and *M. phaseolina* at the center of each. A plate was then incubated in the incubator at a temperature of 25 ± 2 ° C. The rate of fungal growth was measured by taking the average of two perpendicular diameters passing through the center of the plate after the growth of the fungus in the comparison treatment reached the edge of the plate and the percentage of inhibition was calculated according to the following equation

$$\% \text{ of inhibition} = \frac{Fungal\ growth\ rate\ in\ comparison - fungal\ growth\ rate\ in\ the\ treatment}{Fungal\ growth\ rate\ in\ comparison} \times 100$$

The effect of the biological resistance fungus *B.bassiana* in reducing the fungi of roots that infect the okra plant and some growth characteristics

The field experiment was carried out in the fields of / College of Agriculture / University of Basra on 1/15/2020 in the greenhouse and its dimensions were 8 m x 22 m. The land was prepared and divided into 5 meadows, each 20 m long, and the distance between each meadow is 1.25 m, with a total of 8 experimental units. Each experimental unit contains 10 seeds, knowing that the distance between the holes is 40 cm and the height of the mead is 40 cm. The biological fungus *B. bassiana* and the pathogenic fungi *F.solani*, *R. solani* and *M. phaseolina* grown on millet seeds were added to millet seeds. 10 gm of millet grown on the above fungi were applied separately, and the irrigation continued. After that, the holes were planted with okra seeds, a local variety, at a rate of 10 seeds per hole. The percentage of germination, fresh and dry weight of the vegetative and root groups of the plant, as well as the height of the plant and the severity of infestation were calculated.

Results and discussion

Field survey of okra root rot diseases

Through Table No. (1), the results of the field survey of the different areas of okra cultivation showed the presence of a prevalence of seedling death and root rot of okra plants in all areas covered by the survey, with varying rates of infection ranging between 24% - 37% and the severity of infection from 43.65 - 67.33%. The highest infection rate was in the city areas, reaching 37%. The reason for the increase in the infection rate in these areas may be due to the increase in the number of plants in them, as well as the high percentage of humidity, the method of cultivation and the quality of water.

Table (1) Rate and severity of infection

Scanning areas	Injury rate	Injury severity
Shatt Al Arab District	31%	48.26
Abi Al-Khasib District	24%	52.89
Kurna District	33%	43.65
Almdaina district	37%	67.33
L.S.D	7.71	5.97

Picture No. (1) represents the pathological evidence of okra rotrot

Zero plant healthy, 1 discoloration of part of the roots in brown, 2 discoloration of the entire roots in brown and. 3discoloration of roots and yellowing of the plant.

Pathogenicity test of the pathogenic fungi *F. solani*, *R.solani* and *M. phaseolina* in germination of okra seeds in dishes.

The results of the experiment in Table No. (2) showed that the pathogenicity of the fungi isolated from the roots of the okra plant and tested led to a reduction in the percentage of germination in okra seeds, where the percentage of germination was 61.33, 55.89 and 45.33% for the fungi *M.phaseolina*, *R. solani* and *F.solani* on respectively, with a significant difference from the control treatment, which amounted to 90.54%. The reason for the decrease in the percentage of germination in the treatment of *F. solani* fungus is due to the secretion of many enzymes that degrade the walls of plant cells, which help them to penetrate the wall of the host, and these enzymes are Protease, Cellulose Chitinase, and Polygalcturinase, where These enzymes have a major role in biotrophic parasitism (Vidhyaskaran, 1997). In addition, the ability of F. solani to produce many metabolic compounds and toxins that have a major role in the events of infection such as Fusaric acid, Jaranicin, Polypeptidetoxin and Anhydrofusarubin, which The fungus helps decompose the plant host tissue and then feed on it, this condition is called necrotrophic parasitism (Baker et al., 1981). Alassaadi and Alwan, 2012 and Al-Hasnawi, 2017). As for the fungus R. solani, the reason for the decrease in germination may be due to the ability of the fungus to secrete some enzymes that degrade cell walls and destroy the middle plate, which leads to the death of seedlings and rotting of seeds such as Cellulase enzyme, Pectinase and Polygalacturoasse (Ogoshi et al., 1996). Or, the reason for killing the seeds may be due to the fungus secreting the enzyme amylase, which breaks down the stored starch for the fungus to benefit from, thus depriving the seed embryo of the nutrients needed to produce the energy necessary for seed germination and thus killing the cells (Mahmoud et al., 2007; Matrood and Rhouma, 2021a). This result agrees with several studies on the ability of the fungus *R. solani* to reduce the percentage of seed germination (Saleh and Al-Maarij, 2016; Al-Hasnawi, 2017; Matrood et al., 2021b). As for the fungus *M.phaseolina*, the reason for the decrease in the percentage of germination is due to the ability of the fungus to produce toxic substances that have a role in the disease events of the plant, which are transmitted vascularly and cause what is called necrosis in the plant (Ramezani et al., 2008; Matrood et al., 2021b; Matrood and Rhouma, 2021d).

Table (2) Effect of the pathogenic fungi *F.solani, R.solani* and *M. phaseolina* on the percentage of germination in okra seeds. in the dishes

Treatments	% for germination
F.solani	45.33
R.solani	55.89
M. phaseolina	61.33
Control	90.54
L.S.D0.01	11.82

Effect of B. bassiana filtrate concentrations in inhibiting the growth of pathogenic fungi

The results of the experiment showed Figure No. (1) the effect of the biofiltrate of *B. bassiana* on the pathogens of root diseases that infect the pamaya plant because it contains enzymes, antibiotics and chemicals that have a significant effect on the inhibition process. In this experiment, concentrations of 10%, 20% and 30% were used, and all of these concentrations affected the fungal growth of the pathogenic fungi *R.solani, F. solani* and *M. Phaseolina* Perhaps the reason that led to the inhibition of the growth of plant pathogenic fungi is the ability of the biological fungus *B. bassiana* to produce antibiotics that have the greatest role in inhibiting the growth of pathogenic fungi. This is consistent with previous studies conducted by (Jaber, 2018) that the effect of the biofiltrate of fungi in inhibiting the growth of many pathogens in PDA culture media may be the transformation of some non-toxic or low-toxic compounds to more toxic compounds.

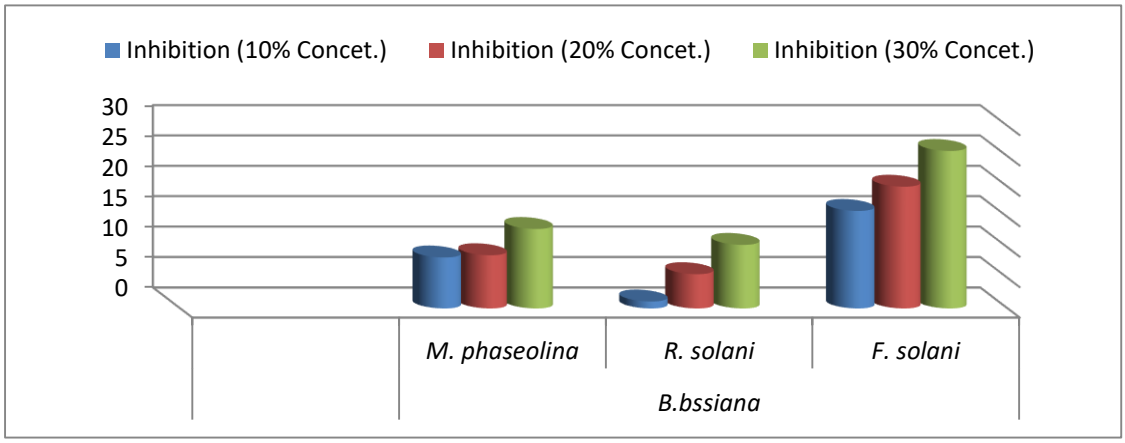

Figure No. (1) The effect of *B. bssiana* filtrate on the diameters of pathogenic fungi colonies and on the percentage of their growth inhibition.

Effect of the biological fungus *B. bassiana* on root pathogenic fungi and some field growth indicators.

The results of the experiment showed in Table No. (3) the increase in plant heights in the biological fungus treatment, as the plants height reached 83.30 cm for treatment B, with significant differences compared to the control treatment, which reached 65.30 cm, and the pathogenic fungi *F. solani, R.solani* and *M. phaselina* treatments, as the height of the plants reached The plant has 56.00, 47.50 and 51.70 cm, respectively. The addition of the biological fungus *B. bassiana* with the treatments of the pathogenic fungi increased the plant height. The reason may be due to the additional supply of nitrogen through the mycelium of the fungus *B. bassiana* to the roots of the plants (Behie et al. 2012 ; Behie and Bidochka, 2014), and increase the activity of plant hormones production (Raad et al., 2019). Jaber (2018) stated. The colonization of the fungus by *B. bassiana* to the roots of plants had the effect of increasing the length of the plant and lateral branches and increasing the buds compared to the non-colonized plants. The reason for the increase in the soft weight of the vegetative group and the

root system may also be due to the biological factors that encourage plants to exploit nutrients and improve physiological characteristics and photosynthesis, and the reason for this is due to the readiness of the elements to plants by the action of biological factors, which helps to absorb them easily and their role in increasing the branches in Roots through their production of growth regulators. The fungus *B. bassiana* used in this study is able to colonize plants, previous studies reported the colonization of *B. bassiana* in other plant species with different pollination methods (Quesada-Moraga et al., 2009; Akutse et al., 2013; Matrood and Rhouma, 2021d). All previous studies reporting a positive plant growth-promoting effect by *B. bassiana* fungi against plant pathogens and these results are in agreement with (Jaber and Enkerli, 2016 and Lopez and Sword, 2015). It was also clear from the same table that the highest fresh weight of the shoot of okra in treatment B was 416.80 g, which achieved significant differences with the control treatment, where the fresh weight of the shoot was 320.40 g. The results also showed the superiority of the biological fungus treatment B On all treatments of pathogenic fungi and the interaction between them and biological fungi. The same table showed the superiority of treatment B over the control treatment in the fresh weight of the root group, as the average fresh weight of the root group was 33.24 g and the control treatment was 29.41 g, but without significant differences with the control treatment. It was noted from the same table that the dry weight of the vegetative group did not achieve any significant differences with the control treatment, which weighed 29.41, but treatment *B. bassiana* was superior to the group of treatments, as it led to an increase in the dry weight of the vegetative group, where the dry weight of the vegetative group reached 98.80 g. As for the dry weight of the root total, treatment *B. bassiana* outperformed, with significant differences, whose weight reached 21.97 gm, than the control treatment, where the weight reached 16.98. Also, treatment *B. bassiana* outperformed all treatments with significant significant differences from the rest of the treatments. It was observed through the statistical analysis that plant pathogenic fungi reduced the studied traits in this table. The reason is attributed to the ability of pathogenic fungi to negatively affect plants and destroy the root system. This study demonstrates the ability of the biological fungus *B. bassiana* to colonize roots, and promote plant growth, for example, plant height, fresh weight, dry weight of shoot and root system.

Table No. (3) Effect of the biological fungi *B. bassiana* and the pathogenic fungi *F. solani*, *R. solani* and *M. phaselina* and the interior between them on plant height and fresh and dry weight of the vegetative and root systems of okra in the field

Treatments	plant length/cm	vegetative total fresh weight/g	dry weight/g	radical sum fresh weight/g	dry weight/g
Co	65.30	320.4	74.3	29.41	16.98
B. bassiana	83.30	416.8	98.8	33.24	21.97
F. solani	55.00	197.50	40.40	11.48	12.02
R.solani	47.50	224.80	52.40	9.58	12.56
M. phaselina	51.70	227.60	55.60	16.00	12.63
B. bassiana+ F. solani	70.00	327.30	73.50	25.18	15.37
B. bassiana+R.solani	70.00	253.90	58.70	29.02	14.91
B. bassiana+ M. phaselina	71.70	272.30	58.70	25.99	16.52
L.S.D	14.23	25.73	31.33	5.66	2.34

Effect of biological fungi on the severity of infection with the pathogenic fungi of *F. solani*, *R. solani* and *M. phaselina* of okra and the interaction between them:

The results of Table No. (4) showed that the pathogenic root fungi *F. solani*, *R. solani* and *M. phaselina* led to an increase in the severity of infection in okra, where the infection severity was 44.76, 53.61 and 55.88%. Straight. The results of the same table also showed that the addition of *B. bassiana* fungus reduced the severity of infection and reached 35.02, 41.54 and 37.67% compared to the pathogenic fungi treatments, and the highest average severity of infection was 55.88% for the treatment of the disease. Pathogenic fungi *R. solani*. Infection with pathogenic fungi: Studies have confirmed the ability of fungi to infect the roots of the host and cause damage and blockage of transport vessels, thus affecting the rise of water and nutrients and disrupting the vital functions of plants such as photosynthesis and respiration. As for the reason for the decrease in the severity of infection with treatments in which plant pathogenic fungi and *B. bassiana* interact, the reason may be due to the possible mechanisms of the fungus activity in biological control against pathogens, and these mechanisms are competition for space and nutrients, and/or induced systemic resistance ISR may be Possible mechanism of biological control activity Evidence for the ISR includes a reduction in disease symptoms on the part of the plant remote from the area in which the catalyst is active, that could be the cause of the reduced severity of infection (Griffin et al., 2006; Griffin, 2007; Matrood and Rhouma, 2021a). Colonization also induces to produce lignin and other cell wall sediments in response to mechanical defense. This also may prevent or limit infection by causing disease through plant pathogens.

Table No. (4) The effect of biological fungi on the percentage of infection severity

Treatments	% of germination before emergence	% of germination after emergence	% severity of injury
Co	26.67	26.70	9.65
B. bassiana	0.00	0.00	0.00
F. solani	40.00	20.00	44.76
R.solani	40.00	16.70	53.61
M. phaselina	50.00	13.30	55.86
B. bassiana+ F. solani	43.33	10.00	33.02
B. bassiana+R.solani	56.67	6.70	41.54
B. bassiana+ M. phaselina	33.33	16.70	37.76
L.S.D	8.82	12.56	8.56

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