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Virulence test of Rhizoctonia solani and Macrophomina phaseolina in cucumber root rot disease

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ABSTRACT

The effect of treatments on leaf area, the Salcylic + Tricoderma treatment gave the highest leaf area of 206.20 cm2, and the Bacllis subtulas treatment gave the lowest leaf area of 147.07 cm2 compared to the contaminated comparison, which gave a leaf area of 113.46 cm2 compared to the healthy comparison, which gave a leaf area of 218.70 cm2. As for the effect of treatments on plant length, the healthy comparison treatment gave the highest plant length of 186.16 cm, followed by the Salcylic + Tricoderma treatment, which gave a plant length of 178.00 cm compared to the contaminated comparison treatment, which gave the lowest plant length of 115.50. As for the effect of treatments and fungi on the fresh green weight, the healthy Control treatment gave the highest fresh green weight of 976 g, followed by the Salcylic + Tricoderma treatment, which gave a fresh green weight of 930 g compared to With the control treatment, the pollutant gave the lowest fresh green weight of 576.33 g.

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1. INTRODUCTION

Cucumber crop Cucumis sativus L. is a vegetable crop that belongs to the Cucurbitaceae family. Cucumber is grown for its fruits, which are consumed fresh and used in pickling. Its fruits have several medical benefits, including maintaining the freshness of human skin, alleviating nervous disorders, purifying the body from toxins, and acting as a headache reliever and thirst quencher. Cucumber fruits also help balance blood pressure [1]. Its fruits contain a high percentage of water, estimated at about 95%, and proteins, carbohydrates, calcium, phosphorus, iron, potassium, and vitamins [2]. This crop is grown in all Iraqi governorates. The economic importance of cucumber crop increases because it is planted in the spring and autumn seasons and is characterized by its rapid growth and the consumer demand for it increases continuously and the loss rate in it is low, so the interest in this crop has a good economic result and competes with the low production compared to other vegetable crops [3] The total production in Iraq amounted to 40,775 tons for the year 2020 with a total cultivation area of 13,718 dunums Central Statistical Organization (2020).

2. Materials and methods

Field survey

The field survey was conducted on cucumber fields for open cultivation for a period between 20-26 days of the sixth month of 2023 during the flowering and early contract stages in which disease symptoms appear in four agricultural areas, namely (Al-Jam'iyah, Al-Hardaniyah, Juwaizrat, Al-Aberiyah) affiliated with the Nahrain district / Al-Duluiyah district located south of Samarra. Three fields were selected in each area included in the field survey and two varieties of the cucumber crop were identified, namely (Akro, Local), through a questionnaire for farmers about the type of cultivated variety. Three homogeneous agricultural lines were identified in the area of each

agricultural field, and 100 plants were identified in each line and the number of diseased plants on which disease symptoms were observed, such as yellowing, partial wilting and plant death.

Field experiments

A: Preparing the experimental land

Gourd seeds were planted on 5/5/2024 in a fallow land that had not been cultivated for seven years and were deeply plowed, modified and smoothed after preparing the land by making a 3m-wide ridge between one ridge and another. Then the drip network was distributed along the lines and its efficiency was checked. Then a commercial formalin solution was sprayed at a concentration of 37% and at a dilution rate of 1 formalin: 9 water using a backpack sprayer and the ridge was covered directly with a polyethylene cover. Then the irrigation system was operated for three hours as a calibration ball and to homogenize the distribution of sterilization vapors in the soil pores for 15 days. Then the cover was lifted to get rid of the formalin vapor. The field was divided into eight treatments and each treatment was repeated into three replicates, so the field became composed of 24 treatments or experimental units, the length of each replicate was 25 m and the length of each treatment was 3 m. Cucumber seeds were planted in holes with a distance of 40 cm between each hole and three seeds were planted in each hole, at a rate of 36 seeds for each experimental unit, leaving sufficient space between one seed and another for the purpose of plant growth in a horizontal manner. After that, the treatments were distributed and marked.

B- **Experimental design:**

The experiment was designed according to a complete randomized block design.

Characteristics studied.

A: Infection rate: The infection rate was estimated according to the following equation:

Field infection percentage = (infected plants number)/(examined plants total number) ×100%

B- Infection coefficient

A pathological guide was adopted to calculate the infection index (Disease index) according to what was published by (Mckinney, 1923) based on a scale (0 - 5) for the infection coefficient:

Infection coefficient severity % = ((5×5 degree of plants number +⋯+0×0 degree of plants number)/(5×examined plants total number) ×100

Isolation and phenotypic diagnosis of the pathogens of cucumber root rot: Samples were collected from the stem of cucumber plants and the crown area during the summer agricultural season of 2023. Symptoms of the disease were shown, which were gradual yellowing, especially on the leaves of old plants, and some of them were stiff, and the roots rotted and turned brown from the fields of the Nahrain district. The pathogen was isolated according to the steps indicated by {4}. The samples were placed in polyethylene bags and transferred to the plant pathology laboratory. Parts of the root samples were washed with running water for 15 minutes and cut into small pieces of 0.5 cm in length and sterilized by immersion for 2 minutes in a solution containing 1.5% sodium hypochlorite, which was taken from a commercial preparation (Chlorex 6%) for 2 minutes. The pieces were washed with distilled water and dried by placing them on sterile filter paper. The pieces were distributed on 9 cm diameter Petri dishes containing sterile culture medium Pottatz Dextrose Agar (PDA) which was prepared by dissolving 39 gm PDA in powder form in one liter of distilled water and placing it on a magnetic shaker and then gradually dissolving it with stirring and sterilizing it in an autoclave at a temperature of 121 °C (1.5 kg/cm3) for 20 minutes with the addition of the antibiotic Amoxicillin 200 mg/liter. After sterilization when it reached the laboratory temperature, every four pieces were planted on Potato Dextrose Agar (PDA) medium produced by the Indian company in 9 cm diameter Petri dishes. The dishes were incubated in the incubator at a temperature of 1 ± 25 °C for 7 days. After that, the dishes were examined and the fungal isolates growing on the PDA medium were purified. After the fungal isolates grew, they were coded with semantic keys. The growing isolates were given abbreviated names of letters, the first letter of which indicates the name of the region from which they were collected, and the second letter indicates the part of the plant from which they were isolated. After the second incubation period, the isolates were morphologically divided according to the color and texture of the fungal colony and were divided on this basis into 25 isolates and then diagnosed based on the morphological characteristics of the colony shape and the reproductive structures that can be seen under microscopic examination at a magnification of X10 and were diagnosed to the level of genus and species.

Estimation of the total plant yield.

The total yield was estimated by harvesting each treatment separately and weighing it using a regular balance according to the yield of each plant and each replicate and in a cumulative manner.

Estimation of the activity of the peroxidase enzyme.
The method of Hammer Schmidt and others (1982) was adopted in testing the activity of the peroxidase enzyme, which was expressed as units/mg of protein.
Estimation of the activity of the polyphenol oxidase enzyme.
The amount of the polyphenol oxidase enzyme was estimated according to Mayer and others (1965) by mixing (0.1) ml of the enzyme filtrate with (2.5) ml of Chatecol solution and for each treatment separately and using a spectrophotometer the absorbance of the reaction mixture was measured at a wavelength of (470) nm, and the amount of the polyphenol oxidase enzyme was estimated using the same method of estimating the peroxidase enzyme.

Statistical analysis.
The experiment was carried out according to a complete randomized block design (RCBD), with three replicates and two factors for distributing the treatments. The means were compared according to Duncan's multiple range test, and the results were analyzed according to the SAS program (Al-Rawi and Abdul Aziz, 2000).

Results and discussion

- Effect of treatments and fungi on the leaf area of cucumber plant (cm2)

Through the results of the statistical analysis in Table (8), it was shown that there were significant differences between the treatments, as the healthy Control treatment gave the highest leaf area of 218.70 cm2. It was followed by the Salcylic + Tricoderma treatment, which gave a leaf area of 206.20 cm2, compared to the contaminated Control treatment, which gave the lowest leaf area of 113.46 cm2.

As for the interaction of treatments with the effect of fungi, the healthy Control treatment of the fungus M. phaseolina was superior, which gave the highest leaf area of 221.29 cm2, followed by the Salicylic + Tricode treatment of the fungus M. phaseolina with an average leaf area of 211.73 cm2 compared to the infected Control treatment of the fungus R. solani, which gave the lowest leaf area of 109.27 cm2.
As for the rate of fungal effect, the fungus M. phaseolina outperformed with a leaf area of 168.26 cm2 compared to the fungus R. solani, which gave an area of 163.73 cm2. Table No. (8) shows the effect of treatments and fungi on the leaf area of cucumber plant (cm2)

Effect rate of treatments Microphomni Rhizoctonia Treatments
168. 26 a 163.73 b Fungal effect rate
* Each number represents the average of three replicates

Table 1: Effect of treatments and fungi on plant length (cm)

Treatments	Rhizoctonia	Microphomni	Effect rate of treatments
Tricoderma	153.55 gf	156.82 f	155.18 d
Bacllus Subtilis	149.77 g	144.37 h	147.07 e
salicylic	149.98 g	149.70 g	149.84 e
salicylic +Tricode	200.67 c	211.73 b	206.20 b
salicylic + Bacllus	173.37 e	187.65 d	180.51 c
Fostel	157.15 f	156.90 f	157..03 d
Control Pollutant	109.27 j	117.66 i	113.46 f
Control Healthy	216.12 b	221.29 a	218.70 a
Mushroom Effect Rate	163.73 b	168. 26 a	

The results of the statistical analysis in Table (9) showed significant differences between treatments, as the healthy Control treatment gave the highest plant length of 186.16 cm. It was followed by the Salsilk + Tricode treatment, which gave a plant length of 178.00 cm, compared to the contaminated Control treatment, which gave the lowest length of 115.50 cm.
As for the interaction of treatments with the effect of fungi, the healthy Control treatment of the fungus M. phaseolina outperformed, giving the highest plant length of 187.00 cm, followed by the Salcylic Tricoderma treatment of the fungus M. phaseolina with an average plant length of 175.33 cm compared to the infected Control treatment of the fungus R. solani, which gave the lowest leaf area of 114.33 cm.
As for the rate of fungal effect, there is no significant difference between the two fungi, as the fungus M. phaseolina gave a plant length of 157.79 cm, while the fungus R. solani gave an area of 155.73 cm. It is noted that the reason

for the increase in plant length in the Salcylic + Tricoderma treatment can be attributed to several reasons, including competition for food with the pathogen or the secretion of harmful substances by the pathogen, in addition to urging the plant to resist the pathogen (Cook, 1983). The reason for the increase in plant length may be due to the production of the fungus of biological resistance, plant growth regulators, such as some compounds that are similar in their mechanism of action to auxins, in addition to the production of many plant hormones that have a stimulating effect on plant elongation. The reason for the increase in the weight of the dry vegetative group may be due to the production of biological resistance factors known as Plant Growth Promoter, meaning growth stimulating factors, in addition to their interference with the production of some plant hormones, and this is consistent with the results of Windham, 1986; Bjorkman et al., 1998; Motaher et al., in addition to the fact that the effect of the fungicide leads to curbing and limiting the damage caused by the pathogen.

Table 2: Shows the effect of treatments and fungi on plant height (cm)

Treatments	Rhizoctonia	Microphomni	Effect rate of treatments
Tricoderma	152.66 d	149.66 ed	151.16 d
Bacllus Subtilis	146.00 ed	143.00 ed	144.50 e
salicylic	132.66 g	137.33 gf	135.00 e
salicylic +Tricode	180.66 ab	175.33 b	178.00 b
salicylic + Bacllus	167.00 c	164.33 c	165.50 c
Fostel	164.33 c	165.33 c	164. 83 c
Control Pollutant	114.33 h	116.66 h	115.50 g
Control Healthy	185.33 a	187.00 a	186.16 a
Mushroom Effect Rate	155.37 a	157.79 a	

The effect of treatments and fungi on the fresh green weight of the cucumber plant (g)

From the results of Table (10), it was shown that there were significant differences between the treatments, as the healthy Control treatment gave the highest fresh green weight of 976 g. It was followed by the Salcylic + Tricoderma treatment, which gave an average fresh green weight of 930 g, compared to the contaminated Control treatment, which gave the lowest fresh green weight of 576.33 g. As for the interaction between treatments with the effect of the fungus, the healthy control treatment of the two fungi M. phaseolina was superior, which gave a fresh green weight of 980.67 g, followed by the +Salsilk Tricode treatment of the fungus M. phaseolina with an average fresh green weight of 932.33 g compared to the infected control treatment of the fungus R. solani, which gave the lowest fresh green weight of 577.67 g.

As for the effect rate of the fungus, the fungus M. phaseolina was superior with a fresh green weight of 799.75 g compared to the fungus R. solani, which gave a fresh green weight of 769.87 g.

Table (3) shows the effect of treatments and fungi on the fresh green weight of the cucumber plant (g).

Treatments	Rhizoctonia	Microphomni	Effect rate of treatments
Tricoderma	748.00 d	758 d	753.33 c
Bacllus Subtilis	674.33 e	695.00 de	684.66
salicylic	613.67 e	646.00 e	629.83 d
salicylic +Tricode	928.00 b	932.33 b	930.33 a
salicylic + Bacllus	926.67 b	916.00 b	921.33 abq
Fostel	785 cd	889 c	837.00 b
Control Pollutant	577.67 f	580.67 f	576.33 e
Control Healthy	972.33 a	980.67 a	976 a
Mushroom Effect Rate	769.87 b	799.75 a	

CONCLUSION

1- The effect of the fungi Rhizoctonia and Macrophomina in their pathogenicity to the cucumber plant and a significant effect on the germination rate and reducing the loss in growth characteristics, and the effect increased with the interaction of the two fungi together.

2- The effect of the filtrate of the fungi Rhizoctonia and Macrophomina on the germination rate for both varieties, and the effect of the filtrate varied with the variation of the fungus and concentration.

3- The effect of the fungi Rhizoctonia and Macrophomina in a significant reduction in growth and yield characteristics, and the effect varied with the variation of the variety and the pathogenic fungus.

4- The effect of the filtrate of the two biological control agents Bacillus subtillies and Trichoderma harzianum in inhibiting the growth of the fungal colony in the laboratory, and a significant effect was achieved for both of them in reducing the percentage and severity of infection.

5- The significant superiority of the induction factors when interacting together compared to their use alone in reducing the percentage and severity of infection and loss in growth and yield characteristics

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