Evaluation of the efficiency of some bio-control elements and plant cultivar in resisting the death and wilt disease of eggplant plants caused by the fungus *Verticillium nubilum* in Babylon / Iraq

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Abstract

Isolation and identification results showed that different isolates were obtained from different regions of the pathogenic fungus *Verticillium* spp. The results showed that there was a high antagonistic ability between the pathogenic fungus *V.nubilum* and the elements of the biocontrol , compared to the control treatment, which amounted to 0.0%, as the treatment of *Bacillus subtilis* gave the highest inhibition rate against the pathogenic fungus, which amounted to 96.75%, followed by the treatment of *Trichoderma harzianum*. It reached 91.07%, and Beltanol treatment gave an inhibition rate of 100%. The results of the greenhouse also showed the effect of the used biotreatments on the inhibitory effect of the pathogenic fungus *Verticillium nubilum*, significantly, as it provided good protection for some varieties of eggplant plants used in the experiment from disease, which led to a significant reduction in the percentage of infection severity.The treatment of integration of the biofungus *T. harzianum* and the bacteria *B.subtilis* excelled in reducing the severity of infection with the pathogenic fungus of the three selected eggplant cultivars (Barcelona, Samara, and Zumurud), which amounted to 5.55, 8.33, and 11.11, respectively.Compared with the treatment of the pathogenic fungus *V. nubilum* alone, in which the infection intensity was 83.33, 91.66, and 94.44, respectively.The results of the field experiment confirmed the results of the greenhouse experiment, as the addition of biocontrol factors represented by the fungus *Trichoderma harzianum* and the bacterium *Bacillus subtilis* had a significant antagonistic effect against the pathogenic fungus *V. nubilum*, The biointegration treatment of *T. harzianum* and bacteria *B.subtilis* excelled in reducing the infection severity of the selected cultivars (Barcelona, Samara, Zumurud), reaching 2.77, 5.55, and 8.33, respectively, compared to the control treatment (the plant with the pathogenic fungus), which amounted to 77.77, 80.55. , 83.33 respectively ,This was positively reflected in the rate of vegetative and root growth length, fresh and dry weight of the plant and root for the canopy experiment and the field experiment. The Barcelona cultivar also gave the best results in terms of resistance to the pathogen and plant growth criteria.

introduction:-

The eggplant plant (*Solaum melongena* L.) is considered one of the important vegetable crops for human health because it contains a high percentage of minerals and vitamins. It has been classified in terms of nutritional value as the third largest crop after tomatoes and potatoes of the Solanaceae family [16,17]. Eggplant has been known since ancient times, when it was growing in India and China, which are considered its origin country, and it is cultivated in temperate and hot regions of the world [29]. The eggplant plant in open fields and greenhouses is affected by many viral, fungal, and bacterial pathogens that reduce the yield qualitatively and quantitatively and cause severe damage to it.The wilting diseases caused by some fungi are among the most important and widespread discovered diseases, and the correlation of the amount of loss resulting from the infections is largely related to the density of the pathogenic fungus pollen present in the soil, the sensitive host, and the planting date, in addition to environmental factors [46]. The genus *Verticillium* spp. Plant diseases that cause billions of dollars annually in damage to a cultivar of agricultural crops around the world, where *verticillium* species are transmitted through the soil and cause *verticillium* wilt, which causes great losses in agricultural crops,

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and which is difficult and costly to control in the presence of a suitable plant host. The remaining species of the genus *Verticillium* spp. In the soil for years small stone bodies that are resistant to inappropriate environmental conditions and that survive until the host and suitable conditions are available [43]. Attention has been directed in recent periods towards interest and work in biocontrol factor through the use of microorganisms that are non-pathogenic to plants and that work to inhibit pathogenic organisms in the soil without affecting the remaining aggregates of microorganisms, where these organisms have different mechanisms used in bio control. These mechanisms direct the parasitism of pathogens and competition for nutrients as well as the production of secondary metabolites such as antibiotics and mycotoxins [49]. Among those organisms that have been used in bio control are *Trichodirma* spp. For its ability to improve the growth and development of roots and their resistance to different environmental conditions and increase crop productivity and nutrient uptake [45].

Materials and methods:

1- Isolation of the pathogenic fungus. *Verticillium* spp.:

Samples of eggplant plants showing signs of infection were collected in Babylon province, where the infected and healthy plants were randomly tested, located within the intersection of the diameters of each site. Some of the infected plants were uprooted and placed in polyethylene bags and transported to the laboratory. The roots were washed with running water for 10 minutes to remove the dust suspended in them, and small pieces were taken and sterilized with sodium hypochlorite solution (2% free chlorine) for 2-3 minutes, then transferred to sterile distilled water for two minutes and washed well, placed on sterile filter paper inside the hood to dry it of excess water, Infected root parts were transferred by sterilized forceps and planted in 4 plant pieces in Petri dishes with a diameter of 9 cm containing 15-20 ml of Potato Dextrose Agar (PDA) to which the antibiotic Tetracycline was added at a concentration of 250 mg/l. After sterilizing it with an autoclave at a temperature of 121°C and a pressure of 1.5 kg/cm2 for a period of 15-20 minutes, then the dishes were incubated in the incubator at a temperature of 25±1°C for 4 days, after which an examination was conducted for the growing fungal colonies by taking small parts from each colony and Colonies of *Verticillium* spp. were purified. The growths in the dishes are transferred to new dishes containing culture medium (PDA).The dishes were incubated at a temperature of 25±1°C for 7 days. It was kept in the refrigerator until use, taking into account its continuous renewal to preserve the presence of the fungus colony.

2- Diagnosis of the pathogenic fungus: the person of the fungus based on the following traits:

A- Visual and microscopic traits:

The dishes were cultivated at 25 1 °C for 10-15 days after a 0.5 cm diameter disc was transplanted from the periphery of the new colony of pure pathogenic fungal isolates to the centre of a Petri dish with PDA culture media.

Once the incubation period was through, the fungus was recognised at the species level based on the nature of the mycelium, the properties of the fungal colony, and the structures it develops, as well as the authorised taxonomic keys [52].

B- Molecular diagnosis of the fungus *Verticillium* spp. using the Polymerase Chain Rection (PCR) technique:

DNA extraction of fungi for the pathogenic fungus:

The extraction experiment was coundecte in the laboratory of the DNA Glow Company for Molecular Research, where the genetic

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material (DNA) of the fungus to be diagnosed (*Verticillium* spp.) was isolated. After growing the fungus in a Potato Dextrose Agar in a 9-cm diameter petri- dish , Zymo research, an American firm, produced the (ZR) Fungal/Yeast/Bacterial (DNA) MiniPrep^{TM} kit. . and followed the recommendations of the private company that supplied the standard kit in extracting the DNA of the fungus isolates.

Polymerase Chain Reaction technique Primer

The (ITS1) and (ITS4) primers manufactured by Integrated DNA technology were used to perform the amplification chain reaction (PCR). The dried primers were dissolved in standard water (free of DNA-degrading enzyme) according to the manufacturer's instructions to give a final concentration of 100 picomoles/microliter and prepare a stock solution. To make the daily working solution, dilute the stock solution to 10 picomoles/microliter**.**

Prepare the master mix

The reaction mixture was made in a 25 microliter final volume.

The ingredients of the reaction mixture were stirred for several seconds, then the tube was placed in a thermocycler, according to the instructions of the Korean business, Intron Biotechnology. The samples' DNA amplification reaction.

Electrophoresis on an agarose gel for nucleic acid

Electrophoresis was performed to detect the result of the PCR reaction while in the presence of a standard DNA bundle size characterization. The acarose gel was prepared according to [48]. by adding 1.5 gm of acarose in 100 ml buffer solution (Tris - Borate EDTA TE) (its concentration is 1.5%). Heat to boiling point, then leave to cool until reaching 45-50°C. Pour the gel into the gel hardening trough carefully to avoid the formation of bubbles, then immerse the comb in the trough to make holes in the gel and leave the gel to solidify. Gently lift the comb and immerse the gel in a TBE buffer after it has fully solidified in the electrolytic relay basin.PCR products were injected into the wells of the immersed gel plate at 5 µl per well in addition to the standard Ladder solution.It was electrocuted at 700 volts/hour (for 21 hours). The stained DNA pieces were tested with a gel document device under UV rays.

Determine the sequence of nitrogenous bases (DNA Sequencing)

To identify the sequence of the nitrogenous bases of the DNA replication segments in the isolates' amplification products, the amplification products were given to the Macrogen firm in order to determine the nucleotide sequence of the fungus' recombinant DNA. Using eggplant seeds and the culture medium (water agar), isolates of the pathogenic fungus *Verticillium* spp were tested for pathogenicity:

According to [15]. method, the pathogenicity of four isolates of *Verticillium* spp. was tested by inoculating nine-centimeter-diameter petri dishes, 15-20 cm 3 of culture medium, and water agar to which the Tetracycline, an antibiotic, was added at a rate of 250 mg/L after being sterilised in an autoclave for 20 minutes at 121 °C and 1.5 kg/cm2. When it was 10 days old, a cork puncture in the area of a fungal colony destroyed it. In the centre of the plate, the disc was put. Four dishes were given to each group. On the medium W.A., a container for the control treatment was left behind. The plates were not put in the incubator for four days at a temperature of 25 ° C till after that. After that, 25 seeds per dish of local eggplants were planted (their germination percentage having already been checked) and superficially sterilised with Apply 2% free chlorine sodium hypochlorite solution in a circular motion along the dish's edge for two minutes. In addition to the pathogenic fungus-free control treatment, each

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isolate received four dishes.. The dishes were incubated for 15 days at a temperature of 25 ° C after the eggplant seeds were placed in the incubator, and then the percentage was measured.

3- Pathogenicity test:

Detection of pathogenic isolation with eggplant seeds:

The pathogenesis of four isolates of the fungus *Verticillium* spp. as stated in the method of [15]. inoculation of petri dishes diameter 9 cm containing 15-20 cm3 of the acr culture medium and water agar)) added to the antibiotic Tetracycline at the rate of 250 mg / l after sterilization with a closed device under a temperature of 121 ° C and a pressure of 1.5 kg / cm2 for 20 minutes with a tablet diameter of 5 mm taken by a cork piercer from near the edges of the mushroom colony at the age of 10 days Tablet placement In the center of a petri dish containing the medium W.A. only. Then the dishes were placed in the incubator under a temperature of 25±1 ° C for 4 days, then the local eggplant seeds were planted non-dusty (their germination rate was previously tested) and superficially sterilized with a solution of sodium hypochlorate (2% free chlorine) for two minutes by 25 seeds / dish in a circular manner near the edge of the dish and at approximately equal distances, 4 dishes were used for each isolation in addition to the comparison treatment without a pathogenic mushroom The dishes were incubated after planting eggplant seeds In the incubator under a temperature of 25±1 °C for 15 days, the percentage of seed germination was calculated according to the following formula:

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

4- Preparation of *Bacillus subtilis* suspension:

Bacillus subtilis was obtained from the pathology laboratory of Department of biological control techniques, Al-Mussaib Technical . Prepare a beaker with a capacity of 250 ml containing the Nutrient Broth culture medium and sterilize it with an autoclave for a period of (15-20 minutes). After cooling the medium, inoculate with the Loop from a 24-hour-old bacterial colony growing on the Nutrient Agar culture medium. It was incubated at a temperature of 25±1°C for 48 hours [5]. The isolates were kept in the refrigerator at a temperature of 4 degrees for subsequent studies and experiments.

5- Method for isolating fungi from soil:

Random samples were taken from the soil of the rhizosphere of the corn crop in Babylon provainc, where these plants, from which the bio fungus sample was taken, were characterized by good and distinct vegetative growth. The soil was left in the laboratory to air-dry for 24 hours, and it was sieved with a 1 mm sieve, and a series of dilutions of soil samples were prepared. Transfer 1 ml of the fifth dilution to sterilised Petri dishes with a 9 cm diameter. Sterile P.D.A nutrient medium was 200 milligrammes per litre (mg/L) of the antibiotic Tetracycline was also added. with three replicates for each dilution and each soil sample. The dishes were moved with a capillary movement to ensure the distribution of the sample and its homogeneity with the P.D.A food medium, and the dishes were incubated in the incubator at a temperature of 25 ± 1 C for 4 days. After that, fungal isolates were purified by transferring portions of the fungal colony's margins to Petri dishes containing sterile water using a sterile loop. P.D.A food medium. The plates were incubated in the incubator at a temperature of

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25±1°C for 5 days, then the fungal isolates were identified by following the approved taxonomic keys [20]. The isolated fungal isolates were used to test the antagonistic ability against the pathogenic fungus, and one of the biofungal isolates was selected as measured on a scale Bell et al [14]. consisting of five degrees as follows:

1. The entire surface of the dish is covered in the hostile fungi.

2. Two-thirds of the dish's surface is covered in fungus.

3. The dish is split in half by pathogenic and antagonistic fungus.

4. The pathogenic fungus takes up two thirds of the dish's surface, while the antagonistic fungus occupies the remaining third.

5. The entire dish is covered in the dangerous fungus.

6- Testing the antagonistic ability of the fungus *Trichoderma harzianum* and *B. subtilis* in the inhibition of the pathogenic fungus *V. nubilum* on PDA culture medium:

The antagonistic susceptibility *of B.subtillus* against the pathogenic fungus *V. nubilum* was tested on N.A culture medium by dilution method (10^{-1}-10^{-8}) from a 72-hour-old bacterial suspension, where 0.5 ml was transferred to 9 cm Petri dishes containing Nutrient Agar. With moving the plate with a propeller movement to distribute the suspension homogeneously by four dishes as replicates for each dilution (10^{-4}-10^{-8}), A disk with a diameter of 5 mm of fungus culture at the age of 7 days was placed in the middle of the dishes for each dilution. 4 dishes were used for fungus without adding bacteria as a comparison. The dishes were incubated at a temperature of 25 ± 1 C for 15 days [22]. The growth rate of the pathogenic fungus and the percentage of inhibition was calculated according to the following equation [38].

inhibit fungal

$$\textbf{growth\%} = \frac{\textit{Mean diameter of the control colony - the mean diameter of the treatment colony}}{\textit{Average colony diameter of control treatment}} \times \mathbf{100}$$

The highest effective dilution in inhibiting the fungus was the eighth dilution (10^{-8}).

The antagonistic ability of the fungus *T. harzianum* isolated from the roots of the maize plant against the pathogenic fungus *V.nubilum* isolated from the roots of the infected eggplant plants was also tested by adopting the double culture technique in Petri dishes containing the PDA culture medium, as the medium was sterilized with a steam sterilizer. (Al-Musaqdah) for 15-20 minutes,Then pour the medium into sterilized Petri dishes with a diameter of 9 cm. After the medium hardens, divide each Petri dish into two equal parts. Inoculate the center of the first half of the dish with a fungal disk taken by means of a cork puncture with a diameter of 0.5 cm from the growing food medium on which the pathogenic fungus *V.nubilum* (V4) is aged. 7 days, while the center of the other part of the plate was inoculated with a 0.5 cm disc from the edges of the T. harzianum colony at the age of 7 days. Each treatment was repeated four times, as was the control treatment , by inoculating the center of the first section of the plate with the pathogenic fungus [24,3]. The dishes were incubated at a temperature of 25 ± 1 ° C, and after the growth of the pathogenic fungus in the control treatment reached the edge of the dish, the percentage of fungal growth inhibition was calculated according to the formula of [38]. mentioned above.

7- Evaluation of the efficiency of biological factors represented by the fungus *T.harzianum*, bacteria *B.subtillus*, selected plant varieties (Barcelona, Samara and Zumurud) and the pesticide Beltanol in the severity of infection with the pathogenic fungus *V. nubilum* and some growth parameters of eggplant in the plastic house and under the conditions of greenhouse

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A test of the pathogenicity of the isolate of the pathogenic fungus *V. nubilum* was carried out on eggplant seedlings under greenhouse conditions at the Technical College - Musayyib for the year 2022. In this experiment, a mixture of mixed soil and peat moss was used in a ratio of 2:1, which was sterilized using commercial formalin at a rate of 20 ml / liter of water (commercial formalin concentration 40).Where formalin was used at a rate of 3 liters of water / m3 of soil, and sprinkled on the soil after collecting it on plastic cover and stirring it, and it was covered well with transparent plastic cover for a period of 7 days under the sun. Diameter 24 cm from the top, 22 cm from the bottom, and a depth of 18 cm [10]. It was planted with seedlings of eggplant of three varieties (Barcelona, Samara, Zumurud) at the age of 30 days. Three replicates were made for each cultivar, with three plants for each replicate. The pots were placed inside the plastic house, and the necessary agricultural operations such as irrigation and fertilization were carried out. The experimental transactions are as follows:

1-BR (Barcelona cultivar) 2- ZM (Zumurud cultivar) 3- SM (Samara cultivar) 4- BR+ T.h 5- ZM+ T.h 6- SM+ T.h 7- BR + B.s 8- ZM + B.s 9- SM + B.s 10- Beltanol+ BR + V.n 11- Beltanol+ ZM + V.n 12- Beltanol+ SM + V.n 13- BR +T.h + B.s 14- ZM + T.h + B.s 15- SM + T.h + B.s 16- BR + V.n 17- ZM + V.n 18- SM + V.n 19- BR + T.h + V.n 20- ZM + T.h + V.n 21- SM + T.h + V.n 22- BR + B.s+ V.n 23- ZM + B.s+ V.n 24- SM + B.s+ V.n 25- BR +T.h+ B.s+ V.n 26- ZM +T.h+ B.s+ V.n 27- SM +T.h+ B.s+ V.n .

The bacterial suspension grown on the culture medium Nutrient Broth was added to the soil at a rate of 25 ml / pot at a concentration of 22.8×10^{10} colony forming units / ml (CFU / ml), while the fungus T. harzinum loaded on millet seeds was added to all treatments that required its addition at an average of2 g / kg .
The Beltanol pesticide treatment was conducted at a rate of 1 g / liter of water for two-time sprays of 10 days between one spray and the other. The first spray was seven days before adding the pathogenic fungus to the treatments that require its addition, and after 7 days had passed from adding the vital treatments and the systemic pesticide .The treatment of pathogenic and growing fungus was added to millet seeds, and the addition of the pathogenic fungus was enhanced after 10 days by adding it in a suspended form of the growing pathogenic fungus pollen to the culture medium (P.D.A) after placing it in a mixer in the amount of 1 plate / 500 ml of water for each plant.
Follow-up and readings were taken after the inoculation process was conducted, and two months after adding the treatments until symptoms appeared on the plant (comparison). The severity of the infection was calculated according to the following gradation:
= 0no infection. 1 = Weak infestation, with symptoms of wilting covering 1-25% of the leaves of the plant. 2 = Medium infestation, symptoms of wilting, including 26-50% of the leaves of the plant. 3 = Severe infection, symptoms of wilting, including 51-75% of the leaves of the plant. 4 = very severe infection, death of the plant, symptoms of wilting, including 76-100% of the leaves of the plant, or the death of the entire plant.
The severity of the infection was calculated according to [36] .equation, as follows:

$$\textbf{Infection severity \%} = \frac{(Number\ of\ plants\ degree\ 0 \times 0 + \ldots\ldots + Number\ of\ plants\ degree\ 4 \times 4)}{Total\ number\ of\ plants\ tested\ x\ 4} \times 100$$

Averages of height, fresh and dry weight of vegetative and root system were calculated for the three eggplant cultivars (Barcelona, Samara, Zumurud) for all treatments.

8- Evaluation of the efficiency of bio-control factor represented by *B.subtillus* bacteria, *T.harzinum,* selected plant varieties (Barcelona, Samara, and Zumurud), and Beltanol in the severity of infection with the pathogenic fungus *V. nubilum* and some growth parameters of eggplant in the plastic house and under field conditions.

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This experiment was carried out in the Babylon province in the plastic house of the Technical College of Musayyib / Al-Furat Al-Awsat Technical University for the agricultural season 2022-2023, where the soil was plowed, leveled and smoothed, and DAP fertilizer was added to it. The terraces were fertilized with fermented and decomposed animal manure in moderate quantities.A drip irrigation system was connected, and The Randomized Complete Block Design (RCBD)was used for this experiment, with three replicates for each treatment, and for each replicate, three plants, where previously prepared seedlings were planted, which include the three varieties (Barcelona, Samara, and Zumurud) at the age of 30 days, and the distance between one plant and another was 50 cm, and after 45 days had passed from Cultivation of seedlings in the plastic house, the parameters were added as in greenhouse experiment. The bio control factor represented by *B.subtillus* bacteria growing on its media was added to the soil at an amount of 50 ml/plant [31]. At a concentration of 22.8 × 10^{10} CFU 1 ml, and after a week, the bio-control factor vaccine represented by the fungus T. harzianum, loaded on millet seeds, was added at a rate of 20 g / kg to the treatments that required its addition [10] , and the Beltanol pesticide treatment was carried out at a rate of 1 ml / liter Water for two time sprays of 10 days between one spray and another, and the first spray was seven days before adding the pathogenic fungus to the treatments that require its addition. As for the treatment of the pathogenic fungus *V. nubilum*, it was added after seven days of inoculation with its treatments, at a rate of 20 gm \ and the infection was reinforced by a suspended action of the pathogenic fungus inoculum grown on the food medium P.D.A, in the amount of 1 dish \ 500 ml water \ plant after mixing it with a hand mixer and adding it to the treatments that require addition .After 7 days of inoculation with the experimental treatments, irrigation, fertilization and control of insect pests, whiteflies, aphids and spider mites were carried out, according to the instructions of the Iraqi Ministry of Agriculture for eggplant cultivation in Iraq.The results were taken after 4 months of cultivation by calculating the percentage of the severity of infection with wilt disease according to the gradient that was used in the previous point. The length, fresh and dry weight of the vegetative total and the root total of the three eggplant cultivars (Barcelona, Samara, Zumurud) were measured.

9-statistical analysis

A complete randomized design (C.R.D) was used for laboratory experiments and the Green House experiment. The Statistical Package For Socil Science (SPSS) program was used in data analysis to study the effect of different treatments on the studied traits, and the results were compared using the value of the Least Significant Difference (L.S.D. Least Significant Difference).

Results and discussion :-

1- Isolation and phenotypic identification of the pathogenic fungus *Verticillium nubilum*:

The results of isolation and diagnosis (Table 1) showed the presence of wilt disease of the eggplant plant in all regions of the Babylon province that were visited, with a percentage of infection ranging between 13-52% and severity of infection ranging between 26-72%. The field of the aljadwal algarbiu area, which amounted to 44%.The reason for the high rate of infection in these areas is due to the fact that they are areas dedicated to the cultivation of eggplant, in which the crop is grown annually and repeatedly, or because of the cultivation of crops belonging to the Solanaceae family in these fields, which led to the accumulation of pathogenic fungal inoculum, especially Sclerotia, which reside in the soil for a long time and to suit their environmental conditions, especially Temperatures, the duration of its stay in the soil may reach five years [19,51]. As the results showed, the lowest infection rate was

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in Al-Dulaimi samples / the Al-Musauib project, and the reason for this is due to the difference in the locations of the fields from which the disease was isolated and the difference in environmental factors, or because these areas were planted with eggplant crops for the first time, in addition to interest in soil and crop service operations.It is known that environmental factors, such as humidity and temperature, have a significant impact on increasing the fungus pollen, as well as increasing the pathogenicity of fungi, as all these factors affect the plant, making it more susceptible to infection with the pathogen. The symptoms were characterized by yellowing of the edges of the lower leaves of the plant, which is in the shape of the letter V, then they dry and fall, and at a later time the upper leaves are affected and then the leaves of the plant fall completely, accompanied by a change in the color of the wood texture due to stimulation of the plant to secrete tyloses and gums and the presence of fungal parts in the vessels that carry the plant, which The brown discoloration of the walls of these vessels along the plant from the bottom to the top,Fungi were diagnosed at the genus level after the appearance of fungal growths, based on the nature of the mycelium, the characteristics of the fungal colony and the structures they form, using the approved taxonomic keys [52].

Table (1) Isolation and identification *of verticillium wilt* disease of eggplant plants for some fields in Babylon province for the season 2022-2023

sample number	Location	Cultivars	Field area/dunum	Infection rate (%)	Severity of infection(%)
1	**Babylon / Dulaimi**	**Abd Aswad local**	**1**	**13**	**26**
2	**Babylon / Srideep**	**Abd Aswad local**	**8**	**20**	**49**
3	**Babylon / Aljadwal Algharbiu**	**Local Syrian**	**10**	**44**	**70**
4	**Babylon / Al-Azzawiya**	**Abd Aswad local**	**2**	**52**	**72**

Isolation and diagnosis of the pathogen:

Molecular Diagnosis Using Polymerase Chain Reaction (PCR) Technology

The result of electrophoresis of DNA extracted from mushrooms under study on an agarose gel showed the presence of two bands with molecular weight (500 bp - 650 bp) Figure (1) using clonal spacer primers ITS1 and ITS4 and this result confirmed the ability of these primers to amplify the rDNA of fungi, where they were used in many studies [37].

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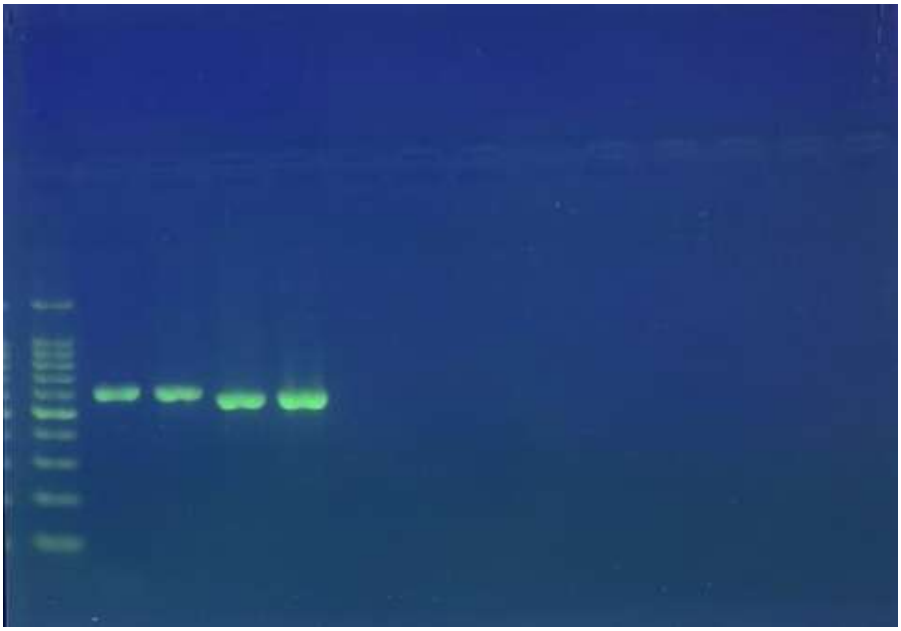

Figure (1) Electrophoresis of the genetic material of *Verticillium* spp. isolates. Nucleotide sequencing study.

The results of the nucleotide sequence of the fungus showed that the first isolate, V1, of type cephalosporum.V, showed a 92% match with the global isolates in the NCBI global gene bank.While the V2-V3-V4 isolates matched 95% with the type of *V. nubilum*, the nucleotide sequences of the fungus were registered in the International Genebank Organization and accession number MH864644.1 and LT560383.1 were obtained and became a reference for Iraq, the Middle East and the world **.**

2- Pathogenicity test:

Evaluation of eggplant seedlings for the presence of the virulent strain of *Verticillium* spp:

The results showed (Table 3) that isolates of *Verticillium* spp. The experiment caused a significant decrease in the percentage of germination of eggplant seeds It was noted that there was a large variation in the pathogenicity of the fungus isolates, where the Babylon/Azzawiya (V4) isolate was excelled in its pathogenicity to the isolates, which had a clear effect in reducing the percentage of germination, reaching 4% (Picture 1),Compared to the isolates of the large project / Dulaimi, Sreideb, and aljadwal algharbiu belonging to the province of Babylon (V2, V1, V3), which gave a germination rate of 73% -72% - 44%, respectively.The reason for the variation of the isolates in their effect on the percentage of daughters of eggplant seeds may be due to the genetic difference between the isolates collected from different regions.The reduction of germination of eggplant seeds was also due to the treatment of *Verticillium* spp. For its ability to produce lytic enzymes that have the ability to break down the structural barriers of plant cells, which have an important role in pathogenic processes, in addition to the toxic effects that this fungus produces on plant cells[55].

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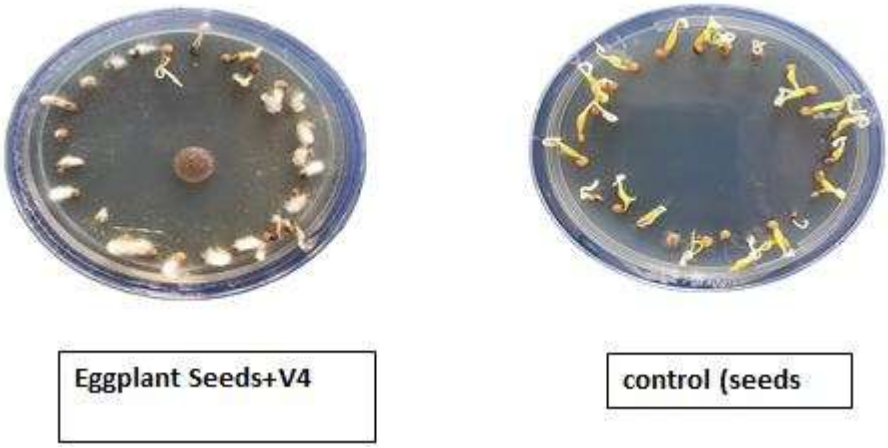

Picture (1) shows the pathogenicity of the fourth isolate in its effect on eggplant seeds.

Table (3) shows the pathogenicity test of the four isolates of the pathogenic fungus *Verticillium* spp and its effect on eggplant seeds on PDA culture medium.

treatments	number of germinated seed	Germination%
V1	18.25	73
V2	18	72
V3	11	44
V4	1	4
control	25	100
L.S.D	1.67	6.54

V= *Verticillium* spp.

3- Testing the antagonistic ability of *B.subtillus* bacteria, *Trichoderma harzianum* and Beltanol against the pathogenic fungus *Verticillium nubilum* on PDA:

The results (Table 2) of this test showed the presence of a high antagonistic ability of the biocontrol elements represented by *B.subtillus* and *T.harzianum* against the pathogenic fungus in the laboratory.The results showed that the tested bacteria *B.subtillus* had a significant effect in obtaining the highest inhibition rate for the growth of the pathogenic fungus, where the inhibition rate reached 97.15% for the pathogenic fungus.Compared to the control treatment (only fungi) without adding the bacterial suspension, which amounted to 0.0%, this is due to the mechanism of its effect, which is through the production of antibiotics that are secreted outside the bacteria cells and volatile hydrocarbons, which are responsible for inhibiting the growth of pathogens [27,1,50]. *B. subtilis* produces more than 66 types of antibiotics, most of which are peptides, including subsporin, neocidin, eumycin, bacillomycin, subtilin, mycosubtilin, bacilysin, and others.

The results of the investigation of the bio fungus *T. harzianum* also showed a high antagonistic ability against the pathogenic fungus, of which the percentage of inhibition was 91.07% compared to the control treatment, which amounted to 0.00. The antagonistic ability of *T. harzianum* is due to

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the various mechanisms by which it affects the pathogenic fungus through direct parasitism, by wrapping the fungal biofilm around the fungal hyphae of the pathogenic fungus, penetrating it and absorbing cellular contents, or through competition for food and place, secretion of antibiotics and some of the degrading enzymes. to the cell walls of pathogenic fungi such as Protase, glucanase, 1,3-B, and others [23]. These results are consistent with what was shown by [30]. The efficiency of the *T. harzianum* isolate against many pathogenic fungi, as shown in Table (2), obtained a 100% inhibition rate for the pathogenic fungus using the fungicide Beltanol

Compared to the control treatment, which amounted to 0.0%, as shown in (Fig. 8), this result was confirmed by many studies that proved that the use of Beltanol pesticide on the PDA medium led to the complete inhibition of the growth of pathogens [6,26,4]. The effective effect of the chemical pesticide Beltanol is due to the fact that it is a systemic pesticide with high efficiency against many pathogenic fungi, and the result of this effectiveness is the formation of chelating compounds with copper in the tissues of the host, which facilitates the process of passing into the cells of pathogenic fungi and killing them [35].

Table (2) represents the test of the antagonistic ability of *B.subtilis*, *T. harzianum* and the fungicide Beltanol against the pathogenic fungus *V. nubilum* in laboratory conditions

No.	treatments	Colony Diameter (cm)	inhibition%
1	B.S + V.n	0.3	97.15
2	T.H + V.n	0.8	91.07
3	V.n + Bentanol	0.0	100
4	fungus alone	9.0	0.0
5	L.S.D	0.23	2.58

***T. harzianum* = T.h , *B.subtillus* = B.s , *V. nubilum* = V.n**

Figure (1) The antagonistic ability of *B.subtillus* bacteria, *Trichoderma harzianum*, and Beltanol against the pathogenic fungus *Verticillium nubilum*, with control treatment, is placed on PDA culture medium.

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4- Evaluation of the efficiency of biological factors represented by the fungus *T.harzianum*, bacteria *B.subtillus*, selected plant varieties (Barcelona, Samara, and Zumurud), and the pesticide Beltanol in the severity of infection with the pathogenic fungus *V. nubilum* and some growth parameters of eggplant in greenhouse and under the conditions of greenhouse

Table (3) showed that the biocontrol factors represented by the fungus *T. harzianum* and bacteria *B.subtillus* and the treatment of the fungicide Beltanol gave a significant reduction in the severity of infection with *verticillium wilt* of eggplant plants by the pathogenic fungus *V. nubilum* compared to the control treatment (a single plant).The interaction treatment between the biofactors *T. harzianum* and *B.subtillus* with the presence of the pathogenic fungus *V. nubilum* excelled on the rest of the treatments, as it achieved a reduction in the severity of the infection, which with the Barcelona cultivar reached 4.60.%This was followed by Samara and Zumurudah cultivars, which amounted to 5.84, 6.93,% respectively, compared to the percentage of infection intensity with the pathogenic fungus treatment alone, which reached 83.33, 91.66, %and 94.44 %respectively with Barcelona, Samara and Zumurudah cultivars.

The reason for the excellent interactions between bacteria and fungi in reducing the severity of the infection and increasing plant growth indicators is that the interaction between microorganisms gives the best results as a result of using the many mechanisms that these organisms possess against plant pathogens. Also, the reason may be due to the synergistic effect between the biocontrol factor used in the experiment in stimulating plant systemic control against pathogens [18,32]. This was followed by the treatment of the biological fungus *T. harzianum* with the pathogenic fungus *V. nubilum*, and the intensity of infection with Barcelona and Samara Zumurud cultivars was 16.66, 22.22, %and 25.00, % respectively.Compared to the control treatment of the pathogenic fungus alone, the reason is that the fungus *T. harzianum* is a biological control agent that has high effectiveness against pathogenic fungi as it strengthens the roots of the host plant and helps it facilitate the uptake of minerals, including iron, in the root zone [33,59]. *T. harzianum* produces other antigens such as harzianolide, 6-pentyl-pyrone (6-PP), harzianic acid and aspinolide. The root also shows antifungal and phytopathogenic activity, stimulates plant defenses and provides them with protection against pathogens [54,6]. The treatment of *B.subtillus* with the pathogenic fungus *V. nubilum* gave a reduction in the severity of infection with the pathogenic fungus, which reached 19.44, 25.00, % and 27.77 % with the Barcelona, Samara and Zumurud cultivars, respectively.Compared to the control treatment of pathogenic fungi alone, this is due to the ability of *B. subtillus* bacteria to decompose fungal hyphae through its secretion of many decomposing enzymes such as Protease, Lipase, Amylase and Chitinase, which have a role in the decomposition of chitin, which is the main component of the cell walls of most fungi [39,7]. Also, the treatment with the fungicide Beltanol led to a significant reduction in the severity of the infection, which amounted to 8.33, 11.11, % and 13.88, % respectively, compared to the treatment of the pathogenic fungus alone. The effect of the chemical pesticide Beltanol on fungi is due to the fact that the active substance in the pesticide sulfate hydroxyquinoline-8 works in association with metals and heavy elements such as iron, sulfur, and copper, which leads to killing the pathogen, because these elements are necessary for the survival of the pathogen [6]. Table (3) showed that all the interaction treatments for biological factors achieved a significant increase and significant differences in the growth parameters of eggplant plants such as height, fresh and dry weight of the vegetative total and the root total in the presence of the pathogenic fungus, compared with the treatment of the pathogenic fungus

alone. The interaction treatment between the bio factors *T. harzianum* and *B.subtillus* in the presence of the pathogenic fungus *V. nubilum* for the three eggplant cultivars (Barcelona, Samara, Zumurud), gave the highest values of growth parameters for eggplant plants, such as plant height, root length, fresh and dry weight of the vegetative set and the root set, where they reached with Barcelona cultivar 66.00, 29.97 cm and 462.93, 94.43, 57.91 and 13.77 g, respectively and with Samara cultivar 55.87 , 25.34 cm , 354.81 , 77.88 , 47.06 , 11.77 gm , respectively , and Zumurud cultivar 51.05 , 22.84 cm , 320.53 , 66.14 , 29.58 , 9.97 gm , respectively, compared with the treatment of pathogenic fungus B. single plant length, root length and rate The fresh and dry weight of the vegetative growth and root with Barcelona cultivar were 38.49, 20.10 cm and 142.97, 27.16, 23.99 and 5.83 g, respectively and with the Samara cultivar 32.30, 18.14 cm and 121.34, 22.06, 20.29 and 4.27 gm, respectively, and with the Zumurud cultivar 27.17, 11.72 cm and 11.72, 18.37, 17.87 and 3.43 gm, respectively. The reason for the interaction treatment between the biofactors among them in reducing the percentage of the severity of the infection and the increase in the growth standards is that the interaction between the biocontrol factors leads to achieving better results, because each biocontrol has different mechanisms in resisting the pathogen and by combining these mechanisms we get It has a greater suppression of the effect of the pathogen and achieves better results than if the bio-control was used alone. Among these mechanisms is the stimulation of the production of growth regulators, which play an important role in the growth process of the plant [56,57].

And that the interaction between biocontrol factors has a greater effect in reducing the severity of infection with plant pathogens compared to if a biofactor was used alone and its effect in raising plant growth standards significantly on different plant families [9,25,47]. The treatment of *B.subtillus* in the presence of the pathogenic fungus gave a significant increase in growth parameters such as plant height, root length, fresh and dry weight of the vegetative growth and the root , as it reached 57.25, 24.89 cm with the Barcelona cultivar and 337.48, 54.84, 41.35 and 8.23 g, respectively, and with the Samara cultivar. 43.66 , 22.13 cm and 211.24 , 43.48 , 41.27 , 7.37 gm, respectively, and with Zumurud cultivar 41.36 , 20.15 cm and 185.91 , 38.79 , 30.58 , 5.53 gm respectively,Comparison with the treatment of pathogenic fungus alone. And that the bacteria *B.subtillus* has an effective role in the rhizosphere, as it works to stabilize pathogens and compete for nutrients, as well as inducing systemic resistance to the plant, as well as decomposing fungal filaments through its secretion of a number of analyzing enzymes such as Protease, lipase, and Amylase. The hydrolyzer of starch and the enzyme Chitinase, the hydrolyzate of Chitin, which is the main component of the cell walls of most higher fungi, including the imperfect ones [2,44]. Also, the treatment with the fungicide Beltanol led to a significant reduction in the severity of the infection, which amounted to 8.33, 11.11, and 13.88, respectively, compared to the treatment of the pathogenic fungus alone. The effect of the chemical pesticide Beltanol on fungi is due to the fact that the active substance in the pesticide sulfate hydroxyquinoline-8 works in association with metals and heavy elements such as iron, sulfur, and copper, which leads to killing the pathogen, because these elements are necessary for the survival of the pathogen [6].

Table (3) showed that all the interaction treatments for biological factors achieved a significant increase and significant differences in the growth parameters of eggplant plants such as height, fresh and dry weight of the vegetative total and the root total in the presence of the pathogenic fungus, compared with the treatment of the pathogenic fungus alone. The interaction treatment between the bio factors *T. harzianum* and *B.subtillus* in the presence of the pathogenic fungus *V. nubilum* for the three eggplant cultivars (Barcelona,

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Samara, Zumurud), gave the highest values of growth parameters for eggplant plants, such as plant height, root length, fresh and dry weight of the vegetative set and the root set, where they reached with Barcelona cultivar 66.00, 29.97 cm and 462.93, 94.43, 57.91 and 13.77 g, respectively and with Samara cultivar 55.87 , 25.34 cm , 354.81 , 77.88 , 47.06 , 11.77 gm , respectively , and Zumurud cultivar 51.05 , 22.84 cm , 320.53 , 66.14 , 29.58 , 9.97 gm , respectively, compared with the treatment of pathogenic fungus B. single plant length, root length and rate The fresh and dry weight of the vegetative growth and root with Barcelona cultivar were 38.49, 20.10 cm and 142.97, 27.16, 23.99 and 5.83 g, respectively. and with the Samara cultivar 32.30, 18.14 cm and 121.34, 22.06, 20.29 and 4.27 gm, respectively, and with the Zumurud cultivar 27.17, 11.72 cm and 11.72, 18.37, 17.87 and 3.43 gm, respectively. The reason for the interaction treatment between the biofactors among them in reducing the percentage of the severity of the infection and the increase in the growth standards is that the interaction between the biocontrol factors leads to achieving better results, because each biocontrol has different mechanisms in resisting the pathogen and by combining these mechanisms we get It has a greater suppression of the effect of the pathogen and achieves better results than if the bio-control was used alone. Among these mechanisms is the stimulation of the production of growth regulators, which play an important role in the growth process of the plant [56,57]. And that the interaction between biocontrol factors has a greater effect in reducing the severity of infection with plant pathogens compared to if a biofactor was used alone and its effect in raising plant growth standards significantly on different plant families [9,25,47]. The treatment of *B.subtillus* in the presence of the pathogenic fungus gave a significant increase in growth parameters such as plant height, root length, fresh and dry weight of the vegetative growth and the root , as it reached 57.25, 24.89 cm with the Barcelona cultivar and 337.48, 54.84, 41.35 and 8.23 g, respectively, and with the Samara cultivar. 43.66 , 22.13 cm and 211.24 , 43.48 , 41.27 , 7.37 gm, respectively, and with Zumurud cultivar 41.36 , 20.15 cm and 185.91 , 38.79 , 30.58 , 5.53 gm respectively,Comparison with the treatment of pathogenic fungus alone. And that the bacteria *B.subtillus* has an effective role in the rhizosphere, as it works to stabilize pathogens and compete for nutrients, as well as inducing systemic resistance to the plant, as well as decomposing fungal filaments through its secretion of a number of analyzing enzymes such as Protease, lipase, and Amylase. The hydrolyzer of starch and the enzyme Chitinase, the hydrolyzate of Chitin , which is the main component of the cell walls of most higher fungi, including the imperfect ones [2,44]. The treatment of the bio fungus *T. harzianum* in the presence of the pathogenic fungus achieved a significant increase in growth parameters such as plant height, root length, fresh and dry weight of the vegetative growth, and root system, as it reached 68.17, 32.21 cm with the Barcelona cultivar and 490.58, 98.30, 59.49 and 14.80 g, respectively, and with the Samara cultivar. 57.84, 27.22 cm, 364.93, 80.92, 50.59, and 13.03 cm, respectively, and with Zumurud cultivar 52.12, 25.02 cm, and 297.50, 69.64, 43.19, and 10.43 g, respectively.Compared with the treatment of pathogenic fungi alone, the bio factor is characterized by a high ability to protect eggplant plants from infection with pathogenic fungi, and the reason for this is due to the possession of many chemical compounds, which are known as antibiotics [23]. and that the biofactor *T. harzianum* acts as a competitor to fungal pathogens, especially when nutrients are few [34,12]. It has a high ability to stimulate acquired resistance (SAR) [21]. and the amazing capabilities that enable it to secrete enzymes that dissolve the cell walls of pathogenic fungi and parasitize on them, and its enzymes, chitinases and proteases, which work to colonize nutrients in the soil [53].

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Table (3) Evaluation of the efficiency of the biofactors represented by the fungus *T.harzianum*, bacteria *B.subtillus*, selected plant cultivar (Barcelona, Samara and Zumurud) and the pesticide Beltanol in the severity of infection with the pathogenic fungus *V. nubilum* and some growth parameters of eggplant plant in the plastic house and under the conditions of greenhouse

Root system weight (g)		root system length)cm(	Vegetative weight (g)		Vegetative growth length)cm(	severity of infection%	treatments	No.
dry	fresh		dry	fresh				
7.50	29.87	24.58	51.86	241.54	58.23	0.00	BR	1
4.27	22.80	17.73	30.06	151.11	43.42	0.00	ZM	2
6.63	26.83	20.89	39.65	194.62	47.24	0.00	SM	3
9.70	43.11	29.38	59.66	309.27	65.67	0.00	BR+ T.h	4
6.87	36.99	22.34	42.37	214.33	49.74	0.00	ZM+ T.h	5
8.70	30.04	25.70	52.24	276.14	53.61	0.00	SM+ T.h	6
8.83	42.82	25.31	58.04	290.08	61.74	0.00	BR + B.s	7
5.83	29.99	22.04	39.55	194.67	43.41	0.00	ZM + B.s	8
7.87	39.98	23.56	47.30	231.55	46.65	0.00	SM + B.s	9
6.97	29.56	24.26	49.40	221.52	62.41	8.33	Beltanol +BR + V.n	10
3.87	23.21	18.65	26.17	134.54	42.50	13.88	Beltanol +ZM + V.n	11
5.93	27.83	21.43	35.98	175.75	47.13	11.11	Beltanol +SM + V.n	12
14.80	59.49	32.21	98.30	490.58	68.17	0.00	BR +T.h + B.s	13
10.43	43.19	25.02	69.64	297.50	52.12	0.00	ZM + T.h + B.s	14
13.03	50.59	27.22	80.92	364.93	57.84	0.00	SM + T.h + B.s	15
5.83	23.99	20.10	27.16	142.97	38.49	83.33	BR + V.n	16
3.43	17.87	11.72	18.37	103.93	27.17	94.44	ZM + V.n	17
4.27	20.29	18.14	22.06	121.34	32.30	91.66	SM + V.n	18
8.83	41.88	27.92	55.95	248.58	60.60	16.66	BR + T.h + V.n	19
5.63	35.81	21.01	39.64	160.33	44.58	25.00	ZM + T.h + V.n	20
7.60	40.50	23.87	49.26	201.91	51.09	22.22	SM + T.h + V.n	21
8.23	41.35	24.89	54.84	337.48	57.25	19.44	BR + B.s+ V.n	22
5.53	30.58	20.15	38.79	185.91	41.36	27.77	ZM + B.s+ V.n	23
7.37	41.27	22.13	43.48	211.24	43.66	25.00	SM + B.s+ V.n	24
13.77	57.91	29.97	94.43	462.93	66.00	5.55	BR +T.h+ B.s+ V.n	25
9.97	29.58	22.84	66.14	320.53	51.05	11.11	ZM +T.h+ B.s+ V.n	26
11.77	47.06	25.34	77.88	354.81	55.87	8.33	SM +T.h+ B.s+ V.n	27
0.5170	9.354	1.501	1.722	41.02	2.148	5.268	LSD 0.05	28

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* Each number represents the average of three replicaties, *T. harzianum* = T.h, *B.subtillus* = B.s, *V. nubilum* = V.n, Barcelona cultivar = BR, Zumurud cultivar = ZM, Samara cultivar = SM.

5- Evaluation of the efficiency of bio-control agents represented by *B.subtillus* bacteria, *T.harzinum*, selected plant varieties (Barcelona, Samara, and Zumurud) and Beltanol in the severity of infection with the pathogenic fungus *V. nubilum* and some growth parameters of eggplant in the plastic house and under field conditions.

The results of Table (4) showed that all the treatments used in it, which included the biocontrol factors represented by the fungus *T.harzianum* and the bacteria *B.subtillus*, led to a significant reduction in the severity of the infection in varying proportions compared with the treatment of the pathogenic fungus alone on the plant, and that the treatment of the interaction between the bio fungus *T. harzianum* and the bacteria *B.subtillus* in the presence of the pathogenic fungus *V. nubilum* It excelled on the rest of the treatments, as it achieved a reduction in the severity of the infection, which reached 2.77, 5.55, % and 8.33 % with the Barcelona, Samara, and Zumurud cultivars, respectively, compared to the severity of the infection with the pathogenic fungus treatment alone, which amounted to 77.77, 180.55, % and 83.33, % respectively.

The reason for the interactions between bacteria and fungi excelling in reducing the severity of infection and increasing plant growth indicators is that the interaction between microorganisms gives the best results as a result of using many of the mechanisms that these organisms possess against plant pathogens. The reason may also be due to the cooperative effect. Among the bio control factors used in the experiment in inducing systemic resistance of selected eggplant cultivars against the pathogenic fungus [18,32]. and these results were consistent with many studies that showed that the interaction between bio control factors has a high effect in reducing the severity of infection with plant pathogens, compared to if a biological agent was used alone [25]. This was followed by the treatment of the biological fungus *T. harzianum* against the pathogenic fungus *V. nubilum*, whose infection intensity with Barcelona, Samara and Zumurud cultivars was 13.88, 19.44, 22.22,% respectively, compared with the treatment of the fungus alone with the three cultivars. Also, the treatment of *B. subtillus* with the pathogenic fungus *V. nubilum* reduced the severity of infection with the pathogenic fungus, which reached 16.66, 22.22, % and 25.00 % with the Barcelona, Samara and Zumurud cultivars, respectively.

And that *B. subtilis* bacteria secrete a group of antifungal metabolites (lipopeptides) such as Surfactin and iturin A that have an effect on the pathogenic fungus penetrating host cells and thus lead to inhibition of its growth [42]. In addition to its ability to produce many antibiotics such as Subtenolin, Bacillin, Bacillomycin and Bactracin, which degrade the cytoplasm of mycelium and deform the apices of mycelium [13,38]. Beltanol treatment also achieved a significant reduction in the severity of infection, where the intensity of infection for the three cultivars Barcelona, Samara, and Zumurud reached 5.55, 8.33,% and 11.11%, respectively. The infection caused by it, in addition to the formation of chelating compounds with copper in the tissues of the host, and this facilitates its passage into the cells of the pathogenic fungus, and then it is liberated and leads to killing the pathogen [35]. The results of Table (4) show the positive effect of bio factor treatments on the significant increase in the average length, fresh and dry weight of vegetative and roots, compared with the pathogenic fungus treatment alone and that all the interactions between the bio factors and the presence of the pathogenic fungus achieved a significant increase in the length, fresh and dry weight of the vegetative and root total of the selected eggplant cultivars. The interaction treatment between the bio fungus

T. harzianum and bacteria *B.subtillus* with the presence of the pathogenic fungus *V. nubilum* excelled on the rest of the treatments, which achieved the highest values of growth parameters for eggplant plants such as height, fresh and dry weight of the vegetative growth and the root , which reached with the Barcelona cultivar 96.12, 40.59 cm and 708.88 cm. 139.60, 96.96, and 23.04 gm, respectively, and with Samara 84.38, 37.39 cm, and 604.94, 109.90, 85.55, and 18.98 gm, respectively and with Zumurud cultivar 74.37, 30.30 cm and 502.99, 96.44, 65.91 and 13.46 gm, respectively, compared with the single fungus treatment, in which the plant length, root length, and average fresh and dry weight of vegetative and roots of Barcelona cultivar were 66.92, 21.42 cm, and 214.75, 4. 3.28 , 42.32 , 7.90 gm, respectively, and with Samara 59.86, 18.14 cm and 202.57, 38.38, 30.28, and 6.41 gm, respectively and with the Zumurud cultivar, 52.98, 15.36 cm and 150.54, 28.37, 15.43 and 3.33 g, respectively. It turned out that the reduction that occurs to the root system as a result of the influence of fungal pathogens negatively affects the vegetative system, which in turn leads to a decrease in the amount of yield [41,58,40]. Many studies have shown that the use of interaction treatments between bio-control factors results in an increase in yield compared to if a bio-control factor was used alone.

The reason for the significant increase in the growth parameters of the selected eggplant cultivars in the overlap treatments is due to the cooperative effect between the bio control mechanisms possessed by these bacteria and the two biofungi used in the experiment.This was followed by the treatment of the interaction between the biological fungus *T. harzianum* against the pathogenic fungus *V. nubilum*, where the mean length, fresh and dry weight of the shoot and root system of Barcelona cultivar were 87.65, 34.35 cm and 359.97, 59.51, 97.41, 18.57 g, respectively, and with Samara cultivar 81.72 and 29.78. cm and 275.73 , 51.25 , 84.00 , and 14.67 gm , respectively , and with the Zumurud cultivar 69.80 , 36.12 cm , and 227.78 , 39.62 , 61.58 , 12.40 gm , respectively ,

compared with the pathogenic fungus treatment alone, The reason for this is that *Trichoderma* spp. It has the ability to produce indol asitic acid (IAA), which enhances the root growth of the plant and thus changes the root structure, which leads to an increase in root mass and an increase in the root space for microbial colonization beneficial to the plant and the enhancement of nutrient absorption [6]. The bacteria *B.subtilis* against the pathogenic fungus *V. nubilum* achieved a significant increase in the growth parameters, where the length, fresh and dry weight of the vegetative and root system of Barcelona cultivar reached 82.57, 31.13 cm and 384.04, 79.23, 87.99 and 19.46 g, respectively and for the Samara cultivar it was 78.00, 25.72 cm and 285.81, 58.57, 82.24 and 15.00 gm, respectively, and for the Zumurud cultivar it was 67.49, 21.60 cm and 233.85, 43.83, 51.67 and 11.90 gm, respectively, compared with the treatment of pathogenic fungi alone,The reason for this is that the bacteria *B.subtilis* has an effective role in the rhizosphere, as it works to compete with pathogens for food and inhibit them, as well as analyze fungal hyphae by secreting a number of analyzing enzymes such as Protease that analyzes protein, Lipase that analyzes fats, Amylase that analyzes starch, and the enzyme Chitinase that analyzes matter Chitin, which is considered the main component of the cell walls of most higher fungi, including the imperfect ones [2,44]. Also, the treatment with the chemical pesticide Beltanol reduced the intensity of infection with the pathogenic fungus of the selected eggplant cultivars treated with it, compared with the treatment of the pathogenic fungus alone. This was reflected positively on the growth parameters of the eggplant plant, and this is consistent with several studies that demonstrated the inhibitory effectiveness of Beltanol on pathogenic fungi [28,7]. The results of the field experiment matched the results of the laboratory experiments and the

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results of the pots experiment. As for the interaction treatments between the fungus *T. harzianum* and the bacterium *B.subtilis* alone without the addition of the pathogenic fungus, no infection with the pathogenic fungus *V. nubilum* was shown.

factors only, whether they were interaction or individually, all of which achieved a

The table also shows that there was a significant increase in the growth parameters of the three eggplant cultivars (Barcelona, Samara, and Zumurud) in the treatments that included bio control

significant increase in length, fresh and dry weight of the vegetative and root.

Table (4) Evaluation of the efficiency of biological resistance elements represented by *B.subtillus* bacteria, *T.harzinum* fungus, selected plant cultivars (Barcelona, Samara, and Zumurud) and Beltanol in the severity of infection with the pathogenic fungus *V. nubilum* and some growth parameters of eggplant in the greenhouse and under field conditions.

Root system weight (g)		root system length)cm(	Vegetative weight (g)		Vegetative growth length)cm(	severity of infection%	treatments	No.
dry	fresh		dry	fresh				
11.43	65.66	28.32	53.46	345.88	81.18	0.00	BR	1
4.63	28.56	19.39	28.80	181.25	63.18	0.00	ZM	2
8.90	42.13	23.27	41.44	243.84	72.34	0.00	SM	3
20.04	100.33	37.60	141.48	631.57	92.79	0.00	BR+ T.h	4
13.48	65.22	28.57	83.05	423.90	71.95	0.00	ZM+ T.h	5
16.80	86.45	31.29	113.67	512.33	82.39	0.00	SM+ T.h	6
19.06	96.50	32.65	129.16	570.48	87.52	0.00	BR + B.s	7
11.77	54.41	25.39	59.52	316.56	70.05	0.00	ZM + B.s	8
15.00	85.49	28.63	107.80	304.79	79.21	0.00	SM + B.s	9
12.79	65.31	27.91	64.91	345.31	80.98	5.55	Beltanol +BR + V.n	10
6.56	30.78	21.02	35.79	180.94	63.43	11.11	Beltanol +ZM + V.n	11
8.94	41.18	21.52	48.77	242.75	71.66	8.33	Beltanol +SM + V.n	12
24.76	113.85	41.21	197.79	945.62	110.24	0.00	BR +T.h + B.s	13
14.72	67.24	31.57	86.61	434.84	83.62	0.00	ZM + T.h + B.s	14
20.70	88.17	39.35	152.90	686.00	94.77	0.00	SM + T.h + B.s	15
7.90	42.32	21.42	43.28	214.75	66.92	77.77	BR + V.n	16
3.33	15.43	15.36	28.37	150.54	52.98	83.33	ZM + V.n	17
6.41	30.28	18.14	38.38	202.57	59.86	80.55	SM + V.n	18
18.57	97.41	34.35	59.51	359.97	87.65	13.88	BR + T.h + V.n	19
12.40	61.58	36.12	39.62	227.78	69.80	22.22	ZM + T.h + V.n	20
14.67	84.00	29.78	51.25	275.73	81.72	19.44	SM + T.h + V.n	21
19.46	87.99	31.13	79.23	384.04	82.57	16.66	BR + B.s+ V.n	22
11.90	51.67	21.60	43.83	233.85	67.49	25.00	ZM + B.s+ V.n	23
15.00	82.24	25.72	58.57	285.81	78.00	22.22	SM + B.s+ V.n	24
23.04	96.96	40.59	139.60	708.88	96.12	2.77	BR +T.h+ B.s+ V.n	25
13.46	65.91	30.30	96.44	502.99	74.37	8.33	ZM +T.h+ B.s+ V.n	26
18.98	85.55	37.39	109.90	604.94	84.38	5.55	SM +T.h+ B.s+ V.n	27
1.1691	2.099	5.810	3.749	83.27	2.655	4.404	LSD 0.05	28

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* Each number represents the average of three replications, *T. harzianum* = T.h, *B.subtillus* = B.s, *V. nubilum* = V.n, Barcelona cultivar = BR, Zumurud cultivar = ZM, Samara cultivar = SM.

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