Effectiveness evaluation of Trichozone for \textit{Trichoderma harzianum} and Fulzyme for \textit{Bacillus subtilis} and \textit{Pseudomonas fluorescens} in curbing causes of charcoal rot disease on the watermelon plant

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Abstract
The results of the study showed the inhibitory effect of the preparation of Trichozone and Fulzyme included the biocontrol agents \textit{Trichoderma harzianum} and Fulzyme bacterial preparation in \textit{Bacillus subtilis} and \textit{Pseudomonas fluorescens} in the laboratory in the growth of colonies of the pathogenic fungus \textit{Macrophomina Phaseolina} on the PDA agricultural medium, as the two preparations at three concentrations 2.5, 5 and 7.5\% achieved in a case of singularity or their combination together a significant inhibition of the pathogenic fungi colony diameter compared to the control treatment, as the interaction between the two factors of Trichozone and Fulzyme achieved the lowest diameter of the growth of that colony as it reached 2.3 cm and a rate of inhibition of 74.44\% at the third concentration 7.5\%. The results of the study showed the effectiveness of Trichozone and Fulzyme in field to inhibit the charcoal disease on the watermelon plant caused by the pathogenic fungus \textit{M. phaseolina}, where significant superiority was achieved in treating the combined of the trichozone and Fulzyme and by treating the seeds and watering the seedlings in reducing the severity of infection and reducing the loss in the studied growth characteristics of wet biological weight, dry weight of the root system, plant length and chlorophyll ratio, with an effect average reached 0.17, 1212.1 gm, 3.43 gm, 241.3 cm and 62.8 spad, respectively. Control of pathogen contaminated fungus and untreated achieved averages of 0.63, 731.6 gm, 1.81 gm, 145.2 cm and 50.7 spad, respectively. Moreover, the results of the study showed a significant difference in the effect of the same treatment with the difference in the method of application that treatment, the method of using the seed treatment and watering the seedlings superiority on the seed treatment only and for all studied treatments.

Keywords: Bacillus subtilis, Trichoderma harzianum, Macrophomina phaseolina

1. Introduction
\textit{Citrullus lanatus} (watermelon) belongs to the Cucurbitaceae family, and comes in third place for its importance among vegetable crops, after cucumber and tomato crops, in terms of cultivated areas. This crop is characterized by its economic and nutritional value, as its fruits contain carbohydrates represented by sugars, fibers and minerals such as iron and calcium, addition the carotene and lycopene dyes [20]. Watermelon plant, like all plants of the cucurbit family, has many pathogens, including root pathogens, and among these pathogens is the fungus \textit{Macrophomina phaseolina}, which determines the cultivation of a watermelon in the world including Iraq, the charcoal rot disease has caused economic losses in its production areas it has high temperatures in Pakistan, South Africa, Brazil, Australia and India [6,24]. \textit{M. phaseolina} fungus it is one of the plant pathogens endemic to the soil, as it affects more than 500 plant Host, and has the ability to survive for long periods of 3-5 years in the absence of its Hosts in the form of spherical or irregular stone bodies according to the abundance of dry conditions. Also, the infection occurs early, but the symptoms appear only in the stressful conditions of the plant during the flowering period and the close maturity of the crop that the pathogen exploits [9,23]. Chemical pesticides were used to treat the soil and seeds to reduce the impact of pathogens such as Tachigren, Rival and Beltanol, as some of them gave high effectiveness against those causes on different crops, and despite the effectiveness of these pesticides, they do not consistent with modern strategies in the world, that work to reduce the use of pesticides, because of its negative effects on non-target organisms, human health and the environment, as well as the emergence of strains resistant to the action of pesticides, therefore modern trends are to find safe alternatives to the environment for chemical
pesticides, and these methods include using microorganisms and activating their biological activity to increase their efficiency in curbing pathogens, including fungi and bacteria located in the rhizosphere [11]. Biological control gained wide interest in the use of organisms in pest control. Among these organisms are the species that belong to the fungus Trichoderma, including the fungus T. harzianum, which has achieved wide success in the field in open and protected agriculture, because of its many influential and controlling mechanisms in its environmental medium, due to its enzymatic ability and its ability to compete with other organisms in addition to facilitating plant feeding requirements and reducing its stress factors [29], in addition to this, some bacterial genera, including Pseudomonas fluorescens and Bacillus subtilis, have proven to be highly efficient against fungi that cause root rot, such as Fusarium spp. and Rhizoctonia solani [17,26].

Due to the importance of the charcoal rot disease on the watermelon crop and the lack of extensive studies on it in Iraq, therefore we decided to study some methods in controlling the disease, unlike the traditional methods used, including activating the biological control elements and encouraging their efficiency in curbing the pathogenic fungus.

2. Materials and methods

2.1 Isolation and diagnosis of pathogenic fungi M. phaseolina

Samples of an infected watermelon were brought from the vegetable fields in Al-Shirqat district/ Salah Al-Din province during the agricultural season 2018, which showed symptoms of yellowing and wilt of the plant and a blackish tint for the crown area. The plants were pulled out and placed in polyethylene bags and transferred to the laboratories of the College of Agriculture at Tikrit University, then cut These parts of the roots and crown area after cleaning them into small pieces about 4-5 mm, and sterilized superficially with sodium hypochlorite solution (NaOCl) at a concentration of (1%) for three minutes, then washed with distilled water and dried those parts between the folds of sterile filter paper and then these parts were distributed on Petri dishes Contains a nutritional medium sterilizer (Water Agar), and added antibiotic Amoxicillin at a concentration of 250 mg/ L to prevent bacterial growth, and incubated dishes at a temperature of 27 ± 2 ° C for five days, and after the growth of fungal colonies in the form of light white fluffy growth, a portion of the edge of the colony was transferred to Petri dishes which contains a sterile culture medium (Potato Dextrose Agar) and antibiotic added to encourage the growth of the fungal colony and the formation of stone bodies. After four days of incubation and the appearance of a blackish-brown color on the nutritional medium, a portion of the colony's edge was taken and loaded onto a glass slide for microscopic examination and observation of the stone bodies in irregular shapes. The classification key referred to by [28] was used and that isolation was adopted from the colony fungi and propagation for use in the study hubs.

2.2 Propagation of pathogenic pollen on millet seeds

Local millet seeds Panicum miliaceum were used and washed well with water to get rid of impurities and the dust sticking to it, then dried them at laboratory temperature and then distributed on 500 ml glass flasks weighing 150 grams of millet per flask and then moistened with distilled water, then closed the orifice of the flask by means of a tight cotton seal and covering it with an aluminum slide, then put the flask in the Autoclave at a temperature of 121 °C and a pressure of 1.5 kg/ cm² for 20 minutes and after the expiration of the sterilization period and the temperature of the flasks decreased slightly, was pollinated the flasks from the edge a newly grown colony at the age of 72 hours using tablets with a diameter of 0.5 cm using a cork borer and at the rate of 5 tablets/ flask. The flask orifice was closed using medical cotton and then placed in the incubator at a temperature of 27 ± 2 °C for a period of two weeks, with interest the flasks shaking every two days to homogenize the distribution of the fungus vaccine and until the growth is complete covering the seeds with black-brown fungi growth and covering all millet seeds with stone bodies. The pathogenic vaccine was kept in the refrigerator until it was used in field experiments.

2.3 Test of suspensions of biological control agents in inhibition of the pathogenic fungi colony M. phaseolina in Laboratory

The use of Trichoderma harzianum and B. subtilis + P. fluorescens to inhibit the pathogenic growth colony of M. phaseolina. Trichoderma was obtained from Trichozone bio-product of Al-Joud Agricultural Company in the amount of 2 gm/ L, where it was transplanted into the culture medium PDA in Petri dishes, then took part of the colony's edge of the fungus and implanted on a liquid culture medium (PSB), then incubated for a period of 7 days at a temperature of 27 ± 2 °C, taking into account the shaking of the flask from time to time, after which the filtrate was separated from the biomasses in the isolation room and in sterile atmospheres using a centrifuge at 3000 rpm for 3 minutes, then the leachate is pure with a special filter measuring 22 µm, inside a medical injection piston to obtain a pure leachate free of fungus and bacterial cell structures, it was placed inside test tubes and marked and stored in the laboratory refrigerator until use.

The bacteria (B. subtilis and P. fluorescens) were obtained from the Fulzyme product from JH Biotech, Inc. American, and in the same way was planted on the PDA culture medium and incubated colonies at 37 °C for 48 hours and then purified and

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taken part of them separately on a liquid culture medium (PSB) and in the same previous method was filtered to obtain the filtrate of bacteria and was marked and saved in the refrigerator until use. A filtrate of these biological agents was used at three concentrations (2.5, 5 and 7.5) ml/100 ml filtrate Trichoderma and bacteria (P. fluorescens + B. subtilis) to poison the PDA culture medium separately and their interaction with the shaking of the medium well until mixed, and poured mixture in petri dishes, after their hardening, they were marked by the type and concentration of the filtrate added to them, and then pollinated the middle of all dishes with a disk of the newly growing pathogenic fungus colony with a diameter of 0.5 cm.

The experiment was carried out in a laboratory according to Completely Randomized Design (CRD) and with three replicates, so that each treatment includes three dishes, in addition to the treatment of the contaminated pathogen without poisoning the culture medium with the above filtrates.

After that all dishes were incubated for the treatments at a temperature of 27 ± 2 °C for a period of 7 days, and after the completion of the growth of the fungus colony for the comparison treatment, the readings of the fungus colony diameter were taken at the rate of two orthogonal diameters and calculating the rate of inhibition according to the following equation:

\[
\text{Inhibition}\% = \frac{\text{Comparison colony diameter} - \text{Treatment colony diameter}}{\text{Treatment colony diameter}} \times 100
\]

### 2.4 Field experiment

Preparation of bio-pesticide solutions: Add 2 gm of vital products Trichozone to *T. harzianum* and Fulzyme to bacteria (*B. subtilis* + *P. fluorescens*) separately to 1 liter of water and shake well and leave for 20 minutes, then the solutions are filtered with a piece of agricultural gauze to get rid of the carrier and obtain effective biological formulations used 30 ml in each bore, as well as fumbling the seeds that were used for planting with these treatments with biological preparations for field experiment treatments.

The experiment was carried out on the field at 10/7/2018 on the wasteland of mixture soil with an area of 900 m² with dimensions of 15 × 60 m, where it was plowed and smoothed and then were divided according to the drip irrigation system, then fertilizing it with compound fertilizer NPK (15.15.15), The cultivation lines were sterilized by injecting the 5% formalin solution into the drip network and covering it by polyethylene. After three days, the irrigation process was restored to maintain soil moisture and homogeneity of the distribution of formalin vapors, and leave the polyethylene cover for a month to increase the efficiency of the sterilization process with the thermal effect of solar radiation to the summer temperature in June. The land was divided equally into two parts and each section includes the treatment method of the biological product (Seed treatment and seed treatment + watering the seedlings). Each section includes three sectors and between each sector and another 4 m. The polyethylene covers were removed from the cultivation lines during 10 days before planting to ventilate it and get rid of residual formalin vapors, and then the planting bores were determined at a distance of 40 cm between one bore and another. After that, these bores were contaminated with the pathogenic vaccine *M. phaseolina*, which is carried on the seeds of local millet, at 5 gm per bore, excluding some inequities from pollution, which represents the control treatment of a healthy.

After three days, those bores were planted by watermelon seeds of the hybrid variety Topylid-F1 produced by Sacata Company after they were washed with lukewarm water to remove the fumigation residues of those seeds with the fungicide that was treated by the producing company, and then the seeds were treated according to their treatments with the biological product except for the seeds of the healthy and polluted control treatments.

The experiment was carried out with the Randomized Complete Block Design (RCBD) system, and it includes one sector on 6 treatments and one treatment includes 16 plants taking into consideration leaving a distance of 1 m between one treatment and the other so that the one sector includes the following treatments:

- **A-** Trichozone treatment of the fungicide *T. harzianum* + *M. phaseolina*.
- **B-** Fulzyme treatment of bacteria (*B. subtilis* + *P. fluorescens*) + *M. phaseolina*.
- **C-** Treatment of Trichozone + Fulzyme + *M. phaseolina*.
- **D-** Treatment contaminated only with pathogenic fungus *M. Phaseolina* (control of 1 contaminated witness).
- **E-** Treatment not contaminated with the pathogenic fungus vaccine (control 2 healthy witness).
- **F-** Treatment of the fungicide Tacharezol and the active substance Hymexazol 40%. Use the fungicide at a rate of 1.5 ml/L according to the recommendations of the producing company.

After 10 days of germination and until the formation of three real leaves, the process of watering the plants of the included treatments (treatment of seeds + watering the seedlings) was carried out by solutions of biological control agents and fungicide at a rate of 30 ml/ plant with the exception of control treatments, adding water only and when the treatment plants reached the flowering stage and the beginning of the fruit contract, and the emergence of pathological symptoms in the treatment of a witness contaminated with the fungus only. Data were recorded on the number of infected plants, and then the distribution of those infected plants according to the degree of their infection depending on the pathological evidence consisting of 6 categories (Table 1), which he mentioned [8,14].
Table 1. Index disease.

| Class | Appearance of injury |
|-------|---------------------|
| 0     | No satisfactory pathological display (healthy plant) |
| 1     | Yellowing of 20% of the vegetative system, at the rate of (3-5) basic leaves of the plant |
| 2     | Yellowing 21-40 of the vegetative system, at a rate of (6-10) leaves |
| 3     | Yellowing 41-60 of the vegetative system with partial wilting of one branch of the plant |
| 4     | Yellowing 61-80 of the vegetative system, with wilting more than one branch and ulceration brown in the crown |
| 5     | Withering of all branches of the plant (the death of the whole plant) |

The severity of the injury was calculated according to the formula [21]:

\[
\text{Infection severity} = \frac{\text{The total number of infested plants in each category} \times \text{its category}}{\text{Total number of plants tested} \times \text{highest category in index disease}}
\]

After that, three plants were randomly selected in each treatment, and they were adopted to calculate some growth characteristics, including wet biological weight, dry weight of the root system, plant length, and chlorophyll ratio. The data was analyzed using the SAS program, according to the Duncan test, under a 5% probability level.

3. Results and discussion

The results of Table (2) indicate the inhibitory effect of biocontrol agents’ filtration on the growth of colonies of the pathogenic fungus *M. phaseolina* on the culture medium PDA, from which it appears that the filtrate of these biological preparations and for three concentrations of 2.5, 5 and 7.5% all of them achieved in the case of unilateral or interaction together a significant inhibition of the diameter of the pathogenic fungus colony compared to the control treatment as the interaction between the two factors of the two Trichozone and Fulzyme achieved the lowest diameter of the growth of that colony at the third concentration 7.5% the lowest diameter of the pathogenic fungus colony, as it reached 2.3 cm. and for a rate of inhibition of 74.44%, in addition to the superior treatment at the two concentrations (2.5 and 5)% as it reached (2.3, 2.43) cm, respectively. It is also noticeable that the treatments differed in their individual effects, as the treatment of Trichozone for *T. herzainum* and all concentrations in inhibiting the pathogenic fungi were significantly superior.

Table 2. Influence of the filtrate of biological preparations on inhibition of the pathogenic fungus colony

| Treatments | Concentration (%) | Mean of Treatment |
|------------|------------------|-------------------|
|            | Diam. (cm)       | Inhib. (%)        | Diam. (cm) | Inhib. (%) | Diam. (cm) | Inhib. (%) |
|            | 2.5              | 5                 | 7.5        |            |            |            |
| Trichozone (T)+ M.Ph 3.96 c*      56       3.33 e 63           3.00 f 66.66 3.43 c 61.88 |
| Fulzyme(F)+ M.Ph 4.66 b 48.22 3.76 cd 58.22           3.60 de 60 4.006 b 55.48 |
| T+F+ M.Ph 2.63 g 70.77 2.43 gh 73           2.30 h 74.44 2.45 d 72.73 |
| Control M.Ph 9 a 0 9 a 0 9 a 0 0 |
| Mean of conc. 5.067a 43.74 4.633b 48.55 4.475c 50.27 |

* - The values followed by the same letter do not differ significantly according to the Duncan test at P≤ 0.05.

Table (1) shows the presence of a significant inhibitory effect of the filtrate of the biological preparations on inhibiting the pathogenic fungus colony when tested individually or Mutual, the effectiveness of Fulzyme is due to the activity of *B. subtilis* in addition to the enzymatic inducers contained in the preparation that work on the production of five antibiotics such as Bacilomycin, Bacitracin, Bacinolin and subtiline, and these antigens inhibit the growth of the fungus, as they secrete Chitinase and β-1,3 glucanase enzymes, which analyze the cell walls of pathogenic fungi [27]. In addition to containing bacteria, *P. florescens*, which secrete a group of metabolically inhibiting compounds inside the fungus cells, such as pseudobactin and pyoverdin, which inhibit the growth of the fungus colony, which leads to analysis of cell walls and secretion of some of the volatile compounds [22]. The main factor of biological control agents inhibiting plant pathogenic fungi in general is the metabolic activity of their antibiotics produced by *P. fluorescens* strains and these antibiotics have a chemical synthesis in the form of various rings containing nitrogen (N-Containing heterocyclic) as Pyrrole-type, Phenazine, Indolderivatives and Pyoluteorin compounds, as well Other non-nitrogen antagonists such as DAPG, this compound is produced by some colonies of *P. fluorescens* which are an antibiotic with a broad spectrum of phenolic molecules [12].
The efficacy of the bacteria *P. fluorescens* and *B. subtilis* in its antagonistic ability may be due to the production of different types of compounds that have the ability to inhibit the pathogens of the plant. These compounds include peptide antibiotics and analytic enzymes such as Protease, Chitinase and Glucanase [7]. The results of that study agree with Fayadh [15] that bacteria *P. fluorescens* have a high ability to inhibit the growth of *M. phaseolina* in the food medium (PDA). Omran et al. [22] showed that *P. fluorescens* have a higher inhibition ability than *B. subtilis* on the fungus *M. phaseolina* in the PSA culture medium. Whereas, 10-1 dilution of *B. subtilis* inhibited the fungus by 47.21%, While *P. fluorescens* were inhibited and the same dilution at 100% compared to the control treatment. These results are consistent with what was indicated by [25] study that stated that *T. harzianum* fungus gave the highest degree of inhibition to *M. phaseolina* that causes eggplant root rot compared to other biomycetes *T. viride*, *T. polysporum* and *T. hamatum*. The antagonistic efficacy of the Trichozzone product, including *T. harzianum*, can be attributed to the effectiveness of this fungus against many pathogenic fungi according to the phenomenon of fungal parasitism, since the fungi spinning of *T. harzianum* wraps around the pathogenic fungi spinning into spirals or pressurized structures that penetrate the spinning cells and intrusive on it [18]. In addition to its ability to secrete some enzymes that break down the fungi spinning of the pathogen such as Protease, Glucanase and Chitinase, these enzymes break down the multiple sugars of the pathogen as well as break down the cell walls of the pathogen [13], or may be due to its ability to secrete some toxic compounds. Barakat et al. [10] stated that the inhibitory effect of *T. harzianum* filtrate colony is due to its ability to produce toxic compounds such as Trichothecin, Gliotoxin, Viridin, Trichodermin, and Pyrones, or it may be due to the presence of several analyzing enzymes such as Protease, Esterase, B-glucosidase, and Phosphoamidase, they pointed out that strong isolates produce large quantities of these compounds, while weak isolates did not notice their production of such compounds. The current results are consistent with several studies that indicated the efficacy of the filtrate of biological fungi, including *T. harzianum*, in inhibiting the growth of many pathogens of the plant, including *M. phaseolina* [2,5].

The results in Table (3) indicate the effect of treatments of the biological product in reducing the severity of the incidence of charcoal rot disease on the watermelon plant, both treatments Trichozone and Fulzyme contributed to a decrease in the severity of the infection with a significant difference from the treatment of the contaminated infection with the pathogen and without treatment through the rate of influence of each of them individually or mutual together, as well as the superiority of the interference of the two preparations T + F in achieving the highest reduction in the severity of infection and significantly superiority the treatment of the fungicide Charlesol, as it reached 0.17 and 0.19, respectively, compared to the control treatment (2) contaminated with pathogenic fungi only, in addition to that the treatment of interaction between the two treatments of both preparations and their impact rate contributed to reducing loss and a difference significantly from the control treatment (2) healthy without treatment. The effect of the treatment of fungicide Tacharezol was 0.36 and 0.38, respectively.

### Table 3. Effect of Trichozzone, Fulzyme and Tacharezol fungicide on the severity of infection.

| Treatments          | Treatment of seeds | Treatment of seeds + seedling watering | Mean of the treatment effect |
|---------------------|--------------------|---------------------------------------|-----------------------------|
| control (1) healthy without treatment | 0.0 i              | 0.0 i                                 | 0.0 f                       |
| control (2) polluted without treatment | *0.63 a*           | 0.63 a                                | 0.63 a                      |
| Trichozone + M.Ph   | 0.41 d             | 0.40 e                                | 0.405 c                     |
| Fulzyme + M.Ph      | 0.49 b             | 0.46 c                                | 0.475 b                     |
| T+F + M.Ph          | 0.18 g             | 0.16 h                                | 0.17 e                      |
| Fungicide Tacharezol+ M.Ph | 0.20 f           | 0.18 g                                | 0.19 d                      |
| Mean of the method effect | 0.38 a            | 0.36 b                                |                             |

* - The values followed by the same letter do not differ significantly according to the Duncan test at P≤ 0.05.

As for the effect of Trichozzone and Fulzyme treatments on some studied growth characteristics, plant length, biological weight, dry weight of the root system, and the percentage of chlorophyll as in Table (4, 5, 6 and 7), it is clear that all treatments of both preparations and their impact rate contributed to reducing loss and a difference significantly from the control treatment (2) contaminated with pathogenic fungi only, in addition to that the treatment of interaction between the two vital preparations (T + F) excelled in achieving less significant loss, although it did not differ significantly in some traits with the effect of the treatment of fungicide Tacharezol for the characteristics of vegetative growth in Plant length, biological weight, and dry weight of the root system and the percentage of chlorophyll as the average effect of that treatment was 241.3 cm, 1212.1 gm, 3.43 gm and 62.8 spad respectively, compared to the rate of fungicide Tacharezol which reached 234.8 cm, 1202.4 gm, 3.33 gm and 61.8 spad, respectively.
Table 4. Effect of Trichozone, Fulzyme and Tacharezol Fungicide on Plant Length (cm).

| Treatments                              | Treatment of seeds | Treatment of seeds + seedling watering | Mean of the treatment effect | Amount of loss/cm |
|-----------------------------------------|--------------------|----------------------------------------|------------------------------|-------------------|
| control (1) healthy without treatment   | 285.9a             | 285.9a                                 | 285.9 a                      | 0                 |
| control (2) polluted without treatment  | 145.2 g            | 145.2 g                                | 145.2 e                      | 140.7             |
| Trichozone + M.Ph                       | 183.4 d            | 190.3 d                                | 186.8 c                      | 99.1              |
| Fulzyme + M.Ph                          | 161.1 f            | 170.5 fc                               | 165.8 d                      | 120.1             |
| T+F + M.Ph                              | 237.7 bc           | 244.8 b                                | 241.3 b                      | 44.6              |
| Fungicide Tacharezol+ M.Ph              | 231.4 c            | 238.06 bc                              | 234.8 b                      | 51.1              |
| Mean of the method effect               | 207.43 b           | 212.46 a                               |                             |                   |

* - The values followed by the same letter do not differ significantly according to the Duncan test at P≤ 0.05.

Table 5. Effect of Trichozone, Fulzyme and Tacharezol Fungicide on Biological Weight of Plant (gm).

| Treatments                              | Treatment of seeds | Treatment of seeds + seedling watering | Mean of the treatment effect | Amount of loss/gm |
|-----------------------------------------|--------------------|----------------------------------------|------------------------------|------------------|
| control (1) healthy without treatment   | *1455.6 a          | 1455.6 a                               | 1455.6 a                     | 0                |
| control (2) polluted without treatment  | 731.6 g            | 731.6 g                                | 731.6 e                      | 724              |
| Trichozone + M.Ph                       | 1035.5 e           | 1157.3 e                               | 1096.4 c                     | 359.2            |
| Fulzyme + M.Ph                          | 942.7 f            | 1115.5 d                               | 1029.1 d                     | 426.5            |
| T+F + M.Ph                              | 1132.1 c           | 1292.1 b                               | 1212.1 b                     | 243.5            |
| Fungicide Tacharezol+ M.Ph              | 1124.9 cd          | 1279.9 b                               | 1202.4 b                     | 253.2            |
| Mean of the method effect               | 1070.4 b           | 1172 a                                 |                             |                   |

* - The values followed by the same letter do not differ significantly according to the Duncan test at P≤ 0.05.

It is also noticed that the method of using the biological preparation treatment has differed its effect significantly, as the method of using the biological preparation (treatment of seeds + watering of seedlings) superiority on (treatment of seeds only) and for all studied characteristics as well as a significant difference in the case of the interaction of both preparations together with the treatment of use of each product Individually and for each method of use, the treatment of the biological product Trichozone and the method of treating seeds + seedling watering was superior to the treatment of the biological product Fulzyme in reducing the loss of some characteristics studied compared to the control treatment, as it reached in the treatment of Trichozone 359.2 gm and 1.62 g and in the control treatment (2) it reached 724 gm and 2.35 gm for the weight biological and dry weight of the root system, respectively.

Table 6. Effect of Trichozone, Fulzyme and Tacharezol Fungicide on Dry Weight of Root System (gm).

| Treatments                              | Treatment of seeds | Treatment of seeds + seedling watering | Mean of the treatment effect | Amount of loss/gm |
|-----------------------------------------|--------------------|----------------------------------------|------------------------------|------------------|
| control (1) healthy without treatment   | *4.16 a            | 4.16 a                                 | 4.16 a                       | 0                |
| control (2) polluted without treatment  | 1.81 f             | 1.81 f                                 | 1.81 g                       | 2.35             |
| Trichozone + M.Ph                       | 2.41 e             | 2.67 d                                 | 2.54 d                       | 1.62             |
| Fulzyme + M.Ph                          | 2.31 de            | 2.43 de                                | 2.37 de                      | 1.79             |
| T+F + M.Ph                              | 3.28 c             | 3.58 b                                 | 3.43 bc                      | 0.73             |
| Fungicide Tacharezol+ M.Ph              | 3.24 c             | 3.43 bc                                | 3.33 c                       | 0.83             |
| Mean of the method effect               | 2.868 b            | 3.013 a                                |                             |                   |

* - The values followed by the same letter do not differ significantly according to the Duncan test at P≤ 0.05.
The results of the treatments of the above mentioned for both the fungal and bacterial biologic preparations show a decrease in the severity of the infection in comparison to the control treatment (2). This effect was reflected directly in the contribution of improving the studied characteristics of the watermelon plant with charocal rot diseases such as the biological weight of the plant, dry weight of the root system, length of plant, and the percentage of chlorophyll. The effect of the bio-preparation Trichozone is attributed to the activity of *T. harzianum* as one of the biological control agents that have been applied widely from use in plant disease control programs, especially pathogens that settle in the soil due to the enzymatic activity affecting the antigen and inhibition of pathogens present in the rhizosphere as well as analysis of organic matter in the soil, increased readiness and absorption of nutrients due to the penetration of the fungus thread into the skin cells of the epidermis and the cortex of the roots treated with *T. harzianum* [4]. It is clear from the results shown by the mutual treatment of the biological and bacterial preparations to the synergistic action between biological control agents in fighting the pathogen, and this is consistent with what was indicated by [19] in combating seed rot and seedlings death disease and caused by the fungus *solani Rhizoctonia* on the cotton plant using *P. fluorescens* and *T. harzianum*, as the interaction treatment between the two biological agents achieved a high germination rate and a reduction in the severity of infection, in addition to reducing the loss in the growth characteristics such as plant height and dry weight of the root and vegetable system compared to the control treatment (fungus pathogenic). Abed *et al.* [3] indicated the importance of the role of bacteria in providing essential nutrients for the plant and thus increasing the efficiency of biological processes, which was positively reflected in the increase in the percentage of total chlorophyll and the root and vegetative systems of the plant. It is also evident from the results of the treatments with fungal and bacterial preparations, the induction of the plant’s internal resistance. Hassan and Al-Samarai [16] indicated the Appearance of new protein bundles compared to the healthy plants for the interaction between bacteria *Azotobacter chroococcum* and *T. harzianum* when analyzing the protein of potato leaves treated with *A. chroococcum* and *T. harzianum* with presence of pathogen of black crust disease in potato fungus *R. solani*.

Also in this direction, Abed *et al.* [1] found that when treating the soil with *T. harzianum* to improve growth indicators for hot pepper, the effect was significant, as the treatment with biological fungus achieved the highest average mean for the height of the plant, dry weight of the roots, number of branches, plant yield, number of fruits and dry weight of the vegetative system and the percentage of chlorophyll compared to the control treatment (without pollination), that scored the lowest mean for all traits. **Table 7.**

| Treatments                    | Treatment of seeds | Treatment of seeds + seading watering | Mean of the treatment effect | Amount of loss/spad |
|-------------------------------|--------------------|---------------------------------------|-----------------------------|----------------------|
| control (1) healthy without treatment | 70 a               | 70 a                                  | 70.0 a                      | 0                    |
| control (2) polluted without treatment | 50.7 e             | 50.7 e                                | 50.7e                       | 19.3                 |
| Trichozone + *M. Ph*          | 56.1 bc            | 57.6 cd                               | 56.8 c                      | 13.2                 |
| Fulzyme + *M. Ph*             | 54.7 de            | 55.9 d                                | 55.31 cd                    | 14.69                |
| T+F + *M. Ph*                 | 61.9 c             | 63.7 b                                | 62.8 b                      | 7.2                  |
| Fungicide Tacharezol+*M. Ph*  | 60.8 c             | 63.1 b                                | 61.9 bc                     | 8.1                  |
| Mean of the method effect     | 59.03 b            | 60.16 a                               |                             |                      |

* - The values followed by the same letter do not differ significantly according to the Duncan test at P≤ 0.05.

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