Evaluation of the efficiency of bio agents *Trichoderma harzianum* and *T*. *longibrachiatum* and some fungicides and a chemical compound against the fungus *Rhizoctonia sp.* that causes eggplant root rot disease in vitro

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Abstract

The study aimed to evaluate the efficiency of the bioagents Trichoderma harzianum and T. longibrachiatum, some fungicides and CuSO4.5H2O against the fungus Rhizoctonia sp. The causative agent of eggplant root rot in vitro. The results showed that the pathogenic fungus *Rhizoctonia sp.*, which was isolated from the roots of eggplant plants infected with root rot, appeared in a higher percentage than the rest of the isolates (36.11%) in all areas studied, so it was selected. The results of the pathogenicity test in dishes showed that the fungus Rhizoctonia sp. reduced the germination rate of eggplant seeds, reaching 20%, compared to the control treatment, which had a 93.3% germination rate. When the disease capacity was selected using plastic pots, the pathogenic fungus Rhizoctonia sp. led to an increase in the rate and severity of infecting eggplant seedlings, reaching 60 and 39.0%, respectively, compared to the control treatment in which the rate and severity of infection reached 0%. The pathogenic fungus Rhizoctonia sp. also reduced the germination rate, which amounted to 56.7%, compared to the control treatment of 90%. The results showed that the application of the fungicides Topsin-M and Beltanol at the recommended concentration of 1 g/L and 1 ml/L, respectively, led to the inhibition of the growth of the fungus Rhizoctonia sp. By 100% on the PDA culture medium compared to the fungicides Rizolex, Tachigazole and Medazim, the fungicide inhibition rate was 78.4, 68.23 and 48.10%, respectively. The two bioagents T.harzianum and T. longibrachiatum achieved a high antagonistic ability against the pathogenic fungus Rhizoctonia sp. on PDA medium, with the degree of antagonism reaching 1 and 2, respectively. The study also showed that the use of copper sulfate at the concentration of PPM 200 led to the inhibition of the growth of the pathogenic fungus Rhizoctonia sp. 100%.

Key words: Bio control, Copper sulphate, Fungicide ,Trichoderma harzianum , Trichoderma longibrachiatum

Introduction

The eggplant, Solanum melongena L., which belongs to Solanaceae family, is one of the most important vegetable crops of economic importance, especially in the tropics and subtropics of the world.In addition to crops belonging to the same family, such as tomatoes, potatoes and peppers (27). It is cultivated in Iraq during the winter in greenhouses and in the summer in the field (35). The eggplant crop is affected by many pests and diseases that cause severe damage, including fungal diseases caused by many fungi, including the fungus Rhizoctonia, which is among the most important and most dangerous pathogens for many agricultural crops.The danger of the fungus is due to its wide family range, which reaches more than 2500 plant species belonging to 125 genera belonging to different plant families, including the Solanaceae family. This fungus causes various diseases, including seed rot, seedling death, root and crown rot (49, 34).fungus form stone bodies of varying size that enable them to survive for several years under harsh environmental conditions (31). The fungus attacks the plant in its various stages of growth when the appropriate environmental conditions are available for its growth, and the infection increases with the presence of crop and weeds residues in the field with the availability of some stressful factors for the plant such as thirst, insects and nematodes

(33). Abdullah and Obeis (2) indicated the effect of isolates of the fungus R. solani on the severity of eggplant infection with root rot disease, which ranged between 46.6-86.60% compared to the control treatment without pathogenic fungus, which was zero%. It was also noted that the appearance of eggplant root rot disease caused by the fungus R. solani ranged between 33.3 - 83% (42). Several means have been used to control root rot disease caused by the fungus R. solani, including the fungicides Topsin-M, Beltanol Rizolex, Tachigazole, Medazim and other pesticides that were used to control and reduce the severity of infection with the pathogenic fungus (R.solani 17, 32, 11, 15).Baloch et al. (21) found that Topsin-M inhibited the pathogenic R.solani fungus that causes eggplant root rot disease by 100%. It was found that the presence of the pathogenic fungus R. solani percentage in the roots of tomato grown in soil treated with the pathogenic fungus and the bio controlling T.harzianum and copper sulfate factor amounted to 33.34% compared to the control treatment that contained only the pathogenic fungus R. solani, which amounted to 90%, which was reflected on the Indicators of tomato plant growth (8). The use of integration between chemical and bio control enhances the opportunity to reach effective resistance to plant diseases, and among the most widely used organisms is the fungus

Trichoderma spp. As an efficient biological resistance agent, due to its ease of isolation and propagation, and the availability of its growth requirements. as well as the multiplicity of its mechanisms of action. Many types of Trichoderma have been widely used in the field of bio control to soil fungi, including the fungus R. solani (29, 51, 22, 54).Some studies in Basra dealt with studying the effect of some bio agent and pesticides on the fungi of the eggplant shoot, including studying the effect of the bio agent T. harzianum and some pesticides on the fungus Botrytis cinera that causes gray rot on eggplant (52) and studying the effect of some fungicides on the fungi that cause eggplant leaf spot Such as Alternaria alternate, Cladosporium oxysporum and Curvularia lunata

(53) However, there have been few studies on the effect of these factors on the root system fungi of the eggplant crop in the region.Therefore, this study came for the purpose of knowing the effect of some bio agent and some chemical compounds on the fungus Rhizoctonia sp. The cause of eggplant root rot.

Materials and methods

Sample collection areas

Samples were collected from eggplant plants that showed symptoms of infection for the

agricultural season 2020-2021, from several areas in Basrah provaince, namely Al-Qurnah, Al-Madina, Al-Haritha, Shatt Al-Arab, Al-Zubair, Al-Lhais, Abu Al-Khasib, Al-Karma (field of the College of Agriculture, University of Basrah) and Safwan, The plants showing symptoms of infection were taken from each farm and brought to the laboratory after being placed in polyethylene bags for the purpose of isolating the pathogenic fungi from their roots.

Isolation and phenotypic and molecular diagnosis

Isolation was conducted from each sample of the infected eggplant plants on the day following the collection process. The roots of the affected plants were washed with running water to remove the soil attached to them. The infected plant parts were cut to a length of 1 then sterilized with 10% cm. sodium hypochlorite (NaOCl) solution from the commercial preparation for 2-3 minutes, then washed after sterilization with sterile distilled water to remove the traces of sterilization. The samples were dried on filter paper and four pieces were planted in each petri dish containing a nutrient medium (PDA) to which the antibiotic Chloramphenicol (250 mg / liter) was added. The dishes were incubated at 25°C for seven days, then the isolates of the fungi were purified, glass slides were prepared from them and stained with lactophenol dye, and they were diagnosed based on the phenotypic

and microscopic traits mentioned in (47, 57, 56, 38). The morphological diagnosis was confirmed based on the molecular technology of the isolate of the fungus under study. The hypoxic DNA was extracted from fungi using DNA extraction solutions, according to the manufacturer's Mini Kit Fungus Protocol (Geneaid Company).The percentage of appearance of fungi was calculated according to the following equation: -

Percentage of emergence = (number of Fungus appearances / total number of samples) x 100

Pathogenicity test of the pathogenic fungus Rhizoctonia sp. in dishes

The pathogenicity of Rhizoctonia sp. was tested. After his diagnosis, he prepared a sterile W.A (Water Ager) medium with an Autoclave at a temperature of 121°C and a pressure of 15 pounds/in2 and added the antibiotic Cholramphenacol (250 mg/L). The dishes were inoculated by taking a 0.5 cm diameter disc by a sterile cork piercer from near the edges of the Fungus colony at the age of seven days. The disc was placed in the center of the dish. Three dishes were used for the pathogenic fungus. As for the control treatment, three dishes were left containing WA medium only.The dishes were placed in

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the incubator at a temperature of 25 °C for three days, and after making sure of the occurrence of growth, the seeds of the Barcelona cultivar (non-moistened) were placed superficially sterilized with a solution of sodium hypochlorite (NaOCl) 10% of the commercial preparation in a circular motion at a distance of 1 cm from the edge and 10 seeds / Dish were used.

Then the dishes were placed in the incubator at a temperature of 25 °C, and the percentage of germination was calculated after 7 days of sowing the seeds according to the following equations:

Germination percentage = (number of germinated seeds / total number of seeds) x 100

Testing the pathogenicity of the pathogenic fungus Rhizoctonia sp. in pots

A soil mixture consisting of a mixture of agricultural soil and peat moss in a ratio of 1:3 soil: peat moss was used, the mixture was sterilized with an autoclave device at a temperature of 121 °C and a pressure of 15 pounds / inch² for an hour, then the sterilization was repeated after 24 hours. The fungus pathogenic vaccine was added. Rhizoctonia sp grown on millet seeds in 1% (w/w) sterilized soil. The soil and the inoculum were placed inside a cellophane bag and agitated well in order to distribute the pollen homogeneously in the soil. The soil contaminated with the fungus pollen was distributed to three pots at an average of 1000 gm of soil for each pot. Either the control treatment was added to it only sterilized millet seeds by 1% (weight / weight). The pots were irrigated with water, and after a few days, eggplant seeds were planted at a rate of 10 seeds for each root, at an average of three replications, leaving three replications as a ontrol treatment containing only sterile millet seeds.After the onset of infection symptoms

after six weeks, the percentage of infection severity was calculated according to the following pathological evidence:

0 = healthy roots 1 = secondary root rot 2 = secondary root rot and part of the main root

3 = main root rot without stem base rot 4 = main root rot and stem base rot

5 =plant death

Then, equation (43) was applied in (19) as follows:

The farms were filtered using the Whatman

No.1 filter paper, then the filter was passed

The severity of infection = $\frac{(Number \ of \ plants \ in \ grade \ 0 \ x \ 0) + - - + (Number \ of \ plants \ in \ grade \ 5 \ x \ 5)}{Number \ of \ examined \ plants \ \times \ the \ highest \ degree \ of \ infection} \times 100$

Effect of filtrate of the pathogenic fungus Rhizoctonia sp and the bio agent s T.harzianum and T.longibrachiatum on seed germination.

B.P.D (Broth Potato Dextrose) liquid media was prepared, then distributed in 250ml conical flasks at a rate of 100ml/ beaker. Sterilize the food medium with an osmosis device at a temperature of 121°C and a pressure of 15 pounds/in2 for 20 minutes. The flasks were cooled and each inoculated with three 0.5 cm diameter discs taken from the edge of the growing colony on the raw 7-dayold PDA culture media for each of the pathogenic fungi Rhizoctonia sp. And the bio agent s T.harzianum and T.longibrachiatum, each separately. The flasks were incubated at a temperature of 25 °C for 14 days with shaking every 48 hours during the incubation period. again through a fine filter whose holes diameter 0.22 μm (Millipore filter) to obtain a sterile filter. 10 Eggplant seeds of Barcelona cultivar were

sown superficially sterilized with sodium hypochlorite (NaOCl) 10% solution of the commercial preparation in the dishes containing sterile and moistened filter paper 6 ml of raw filtrate of the pathogenic fungus and the bio Rhizoctonia sp. agent s T.harzianum and T.longibrachiatum and both separately and 6 ml of sterile distilled water only as a control treatment, Then all the dishes were incubated in the incubator at a temperature of 25 ° C for 10 days, with the addition of fungus filtrate during the incubation period as needed. The percentage

of germinated seeds was then calculated according to the following equations:

germinated seeds % = number of germinated seeds / total number of seeds x 100

Antagonism efficiency test for the bio agent T.harzianum and T.longibrachiatum against the pathogenic fungus Rhizoctonia sp.

The double culture method was used to test the ability of the bio-control agent T.harzianum and T.longibrachiatum to antagonize the pathogenic fungus Rhizoctonia sp.The petri dish containing the sterile PDA nutrient medium was divided into two equal parts, then the center of the first section was inoculated with a disc diameter of 0.5 cm from the edge of the colony of each of the bio agent s T. 0.5 cm from the edge of the colony of the pathogenic fungus Rhizoctonia sp. 7 days old, leaving a comparison treatment inoculated with the pathogenic fungus only.Then the dishes were incubated under a temperature of 25°C, and after the growth of the pathogenic fungus in the control treatment was completed and it reached the edge of the dish, the degree of antagonism was calculated according to a scale (23) consisting of five degrees and as follows:

Degree	The description
1	Bioagent covers the whole dish.
2	Bioagent covers two-thirds of the dish.
3	The bioagent and the pathogenic fungus each cover half of the dish
4	Pathogenic fungus cover two thirds of the dish.
5	Pathogenic fungus cover the entire dish.

A bioagent is effective when the degree of antagonism is 1 or 2.

The effect of some fungicides on the percentage of inhibition of the pathogenic fungus Rhizoctonia sp. and the bio agent T.harzianum and T.longibrachiatum in vitro

Five fungicides were used in this test: Tachigazole, Medazim, Beltanol, Topsin-M and Rizolex. The PDA food medium was prepared and distributed in five glass beakers of 150 ml volume at a rate of 100 ml for each beaker and sterilized in an autoclave at a temperature of 121 °C and a pressure of 15 pounds / ing2. After sterilization, the five pesticides were added at the recommended concentration, namely, Topsin-M, Rhizolex (1 g/L), Beltanol, Medaz and Tachigazole (1 ml/L) separately.The flasks were shaken well for the purpose of homogenizing the pesticides with the medium and then poured into sterile Petri dishes with a diameter of 9 cm. Seven days each separately and with three replications for each treatment, leaving a control treatment free of pesticides for all fungi.The inoculated dishes were incubated in

the incubator at a temperature of 25 °C. After the growth in the control treatment reached the edge of the plate, the diagonal growth of the fungal colonies was measured by taking the average of two perpendicular diameters passing through the center of the plate from the back. The percentage of inhibition was calculated according to the Abbott equation in (55) as follows:

 The
 percentage
 of
 inhibition

 Average fungi colony diameter in control treatment – Average fungi colony diameter in the treatment
 × 100

 Average fungi colony diameter in control treatment
 × 100

Testing the efficiency of copper sulfate in the growth of the pathogenic fungus Rhizoctonia sp. and the bio agent T.harzianum and T. longibrachiatum.

it use the element copper in the form of aqueous copper sulfate, CuSO4.5H2O. Prepare a standard solution of aqueous copper sulfate at a concentration of 1000 ppm / liter

Quantities of the standard solution were transferred to 100 ml of PDA culture medium to obtain the final concentrations of 50, 100, 150 and 200 ppm.The flasks were shaken well to ensure the homogeneity of the mixture, then poured into a sterile 9 cm petri dish with three replicates for each concentration. harzianum and T. longibrachiatum separately. All dishes were incubated at 25°C, the growth of pathogenic fungi was calculated by measuring the average of two perpendicular diameters and the percentage of inhibition was calculated as in the previous paragraph. =

statistical analysis

Laboratory results were analyzed using Completely Randomized Design (C.R.D) and all means were compared using least significant difference test at 1% probability level (16).

Results and discussion

Isolation and phenotypic and molecular diagnosis

The results of isolation and diagnosis, which are shown in Table (1), showed that many types of fungi were associated with the roots of eggplant plants, and the most visible species in all samples was the fungus Rhizoctonia sp. So it was nominated to complete the rest of the experiments, where the percentage of its appearance reached 36.11%, followed by the fungi Fusarium solani. Fusarium oxysporum and Macrophomina phaseolina, with emergence rates of 30.55%, 25.92%, and 18.51%, respectively, This may be due to the frequent cultivation in these areas, which led to an accumulation of pollen and an increase in its density, or to the ability of many fungi to live in Saprobiontic of plant residues and organic materials, or their ability to secrete toxic substances in their growth environments,

which makes the dominance of fungal species on others (28, 24, 2). These results are in agreement with the results of several studies that indicated the emergence and spread of pathogenic soil fungi on eggplant (14, 13, 39, 42).The results of the molecular diagnosis also showed that the isolate of the fungus under study, Rhizoctonia sp., did not match the global isolates recorded according to the available information in the National Center for Biotechnology Information (NCBI) and the Gen Bank (Fig. 1). The isolates are in the genebank under bank code MZ506756.

isolated fungi	Appearance %
Rhizoctonia sp	36.11
fusarium solani	30.55
Fusarium oxysporum	25.92
macrophomina phaseolina	18.51

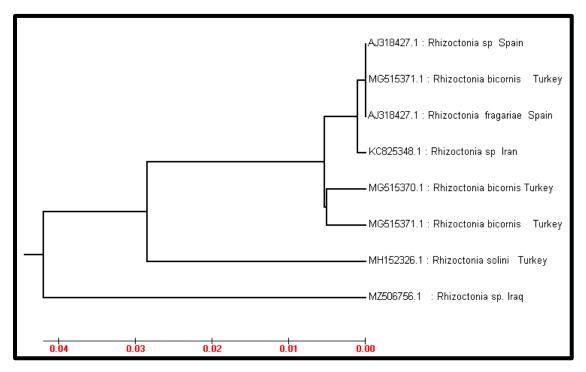


Figure (1) The germplasm tree of the pathogenic fungus Rhizoctonia sp. isolated from the roots of eggplant affected by root rot

Pathogenicity test of the pathogenic fungus Rhizoctonia sp. using eggplant seeds

The pathogenic fungus Rhizoctonia sp. achieved a significant reduction in the percentage of germination of eggplant seeds, where the germination rate reached 20% compared to the control treatment in which the germination rate reached 93.3% (Table 2). The result agreed with Khalil and Al-Khafaji (36), who indicated that the fungus R. solani that disease reduced causes root rot the germination rate of eggplant seeds to 21%

compared to the control treatment in which the germination rate was 98.66%. The ability of the fungus Rhizoctonia sp. to kill eggplant seeds may be due to the invasion of fungal hyphae of the seed coats, growth between starch grains, secretion of amylases enzymes, thus killing the cells and turning the seeds brown (40, 46), or to the secretion of some phytotoxic compounds such as Phenyl Acetic Acid (PAA) and its hydroxyl derivatives such as Beta-hydroxy and Para-hyderoxy, which cause the killing of seed embryos (41).

Treatments	germination*%
.Rhizoctonia sp	20
T. harzianum	96.7
T. longibrachiatum	86.7
Control	93.3
L.S.D. 0.01	27.4

 Table (2) The effect of fungi on the percentage of germination of eggplant seeds by seedling rot

 in dish

*Each number represents an average of three replicates.

Testing the pathogenicity of the pathogenic fungus Rhizoctonia sp. in pots

The results showed that the pathogenic fungus Rhizoctonia sp. led to an increase in the rate and severity of infection of eggplant seedlings, reaching 60 and 39.0%. respectively, compared to the control treatment free of pathogenic fungi. The pathogenic fungus reduced the germination rate, reaching 56.7%, compared to the 90% control treatment (Table 3). These results agree

with what a number of researchers found that the pathogenic fungus R. solani that causes root rot had a negative effect on the rate and severity of infection, as it ranged from slight to severe infection, which leads to complete destruction of the crop and causes 100% losses conditions when are appropriate (21,45). There are three metabolic activities of microorganisms of importance in the events of pathogenicity are the secretion of degrading enzymes, toxins and growth regulators (58, 43, 49).

 Table (3) Effect of the pathogenic fungus Rhizoctonia sp. on the percentage of eggplant seed

 germination and the percentage and severity of infection in the pots

Treatments	germination*%	infection severity*%	infection % *
. <i>Rhizoctonia</i> sp	56.7	39.0	60
Control	90	0	0
L.S.D. 0.01	30.6	10.6	26.5

*Each number represents an average of three replicates.

Antagonism efficiency test between the bio agent T.harzianum and T.longibrachiatum and the pathogenic fungus Rhizoctonia sp.

The results of the double culture experiment (Picture 1) showed a high antagonistic ability of T. harzianum against the pathogenic fungus Rhizoctonia sp. The degree of contrast is 1. This result was in agreement with several studies that confirmed the high antagonistic ability of T. harzianum against Rhizoctonia sp. on PDA culture medium (12, 9, 37, 13, 54). While the degree of antagonism reached 2 between the biological fungus T. longibrachiatum and the pathogenic fungus Rhizoctonia sp., and this result agreed with

Al-Abad (4) about the antagonism efficiency of T. longibrachaitum against the pathogenic fungus R. solani. The reason that Trichoderma spp. has such a high antagonistic property against pathogens is due to the direct parasitism of the pathogenic fungus on the mycelium of the pathogen and its wrapping around it and the breakdown of cell walls by the enzymes Chitinase, b-1-3-gluconase cellulase and Protease (3, 18).



Picture (1) Antagonism test for the bio agent T.harzianum and T.longibrachiatum against the pathogenic fungus Rhizoctonia sp. on PDA culture medium.

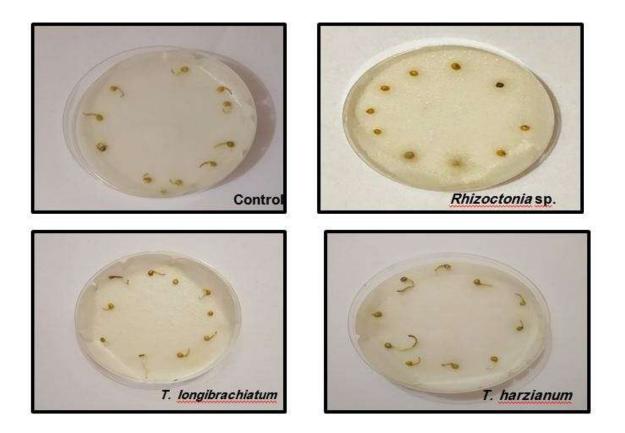
Effect of filtrate of pathogenic fungus Rhizoctonia sp and bio agent T.harzianum and T.longibrachiatum on seed germination.

Table (4) and Picture (2) showed that the filtrate of the pathogenic fungus Rhizoctonia sp. inhibited the growth of eggplant seeds by 100%. While the percentage of germination in the control treatment was 93.3%. While the percentage of germination in the two bio agent T.harzianum and T.longibrachiatum differed from the previous treatments, reaching 100 and 96.7%, respectively. This result agreed

with Al-Atabi (15). In which it was shown that the filter of the fungus R. solani reduced the percentage of germination to 67.33%, while the filter of the bio agent T.harzianum increased the percentage of germination to 85.65%.The filtrates of bio agent have an effect on bio agent of pathogens and increase the percentage of germination in addition to its penetration into seeds and induction of systemic resistance (30),While the pathogenic fungi filtrate has led to the rotting of the seeds due to its secretion of many toxins and enzymes that affect the vitality of the seeds and the failure of germination (50, 26, 44).

Table (4) Effect of filtrate of pathogenic fungus Rhizoctonia sp. and bio agent T.harzianumand T.longibrachiatum on germination rates of eggplant seeds in the dishes

Treatments	germination*%
.Rhizoctonia sp	0
T. harzianum	100
T. longibrachiatum	96.7
Control	93.3
L.S.D. 0.01	11.2



Picture (2) The effect of filtrate of the pathogenic fungus Rhizoctonia sp. and the bio agent T. harzianum and T. longibrachiatum on the germination rates of eggplant seeds in a dishes

Effect of some fungicides on the percentage of pathogenic fungus growth inhibition. Rhizoctonia sp and the bio agent T. harzianum and T. longibrachiatum in vitro

The results showed (Table 5) that there were significant differences between the pesticides Beltanol, Topsin-M. Rizolex. Tachigazole and Medazim in their effect on the growth of pathogenic fungi on PDA using the recommended culture media concentration for each pesticide, It was found that the two pesticides, Beltanol and Topsin-M, are among the most inhibiting pesticides, as the percentage of inhibition reached 100% for each of them, compared to the control treatment in which the growth rate was 9 cm. R. solani on PDA culture medium (11, 54). The reason for the lack of growth in the media containing culture the pesticide Beltanol is attributed to the action of the pesticide, which was used as a systemic fungicide, as it showed effectiveness in reducing the infection of fungi (25). The mechanism of the main effect of Topsin-M is focused on affecting the manufacture of DNA, cell division and the process of chromosome separation, and sometimes it leads to the breakage of chromosomes in the fungal cell (6).

It was also shown from the same table that the pesticides Beltanol and Tachigazole inhibited the growth of the bio agent T.harzianum and T.longibrachiatum by 100% when using the recommended concentration, while the pesticides Topsin-M, Rizolex and Medazim did not completely inhibit their growth. The least inhibition of them, where the percentage of inhibition reached 16.63 and 26.80%, respectively.The fungi growth excelled in low doses due to the fact that the active substance of the pesticide reaches the sensitive part in small quantities and interacts with the sensitive part and does not affect the fungus (5).

Table (5) The effect of some fungicides on inhibiting the growth of the pathogenic fungus Rhizoctonia sp. and the bio agent T. harzianum and T. longibrachiatum on PDA culture media

Pesticides	% inhibition *			
	T. longibrachiatum	T. harzianum	.Rhizoctonia sp	
Beltanol	100	100	100	
Topsin-M	26.8	16.6	100	
Rizolex	47.2	38.8	78.4	
Tachigazole	100	100	48.10	
Medazim	70.4	66.6	68.23	
Control	0	0	0	
L.S.D. 0.01	16.3	17.8	21.7	

Efficiency test of copper sulfate in the growth of the pathogenic fungus Rhizoctonia sp.

The results of this experiment (Table 6) showed that the element copper had a clear effect on reducing the national growth rate of the pathogenic fungus Rhizoctonia sp. compared to the control treatment and in all the concentrations used and with high significant differences. The results also showed that the effect of copper increased with the increase in the concentration used, with a significant difference, as the concentration of 200 mg / liter showed the highest inhibition

with the results of several studies in which it was indicated the effectiveness of copper sulfate in inhibiting the growth of plant pathogenic fungi such as R. solani (48, 8) and Fusarium oxysporum (10). The effectiveness of copper sulfate is due to the single and binary copper ions that have the ability to bind to many chemical groups present in the fungal cell, such as the amine group, the carboxyl group, and the thiol group to form complex compounds with it. , 5).or the effect may be due to the dissolved copper ion, which is attributed to the toxic effect of copper

rate of 100%. These results are in agreement

compounds, which are formed by atmospheric carbon dioxide or ammonia salts in water or by plant secretions or fungi secretions containing some acids such as malic acid, where dissolved copper interaction . with vital processes in The fungal cell (7).

Table (6) Effect of copper sulfate on inhibiting the growth of the pathogenic Rhizoctonia sp. onPDA medium

Copper sulfate concentration (mg/L)	% inhibition *	Pathogenic Fungus Growth Rate (cm)*
0	0	9
50	48.1	4.33
100	68.5	2.83
150	84.20	1.41
200	100	0.00
L.S.D. 0.01	14.73	1.44

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