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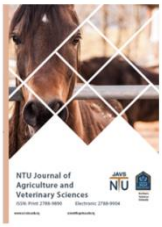

Effect of Bio Control Agents on Controlling Root Rot and Damping-off Disease of Cucumber Seedlings Caused by *Rhizoctonia solani*

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Cucumissativus, *Rhizoctoniasolani*, root rot, *Trichodermaharzianum*

Root rot and seedlings damping-off disease caused by *Rhizoctonia solani* is the most damaging disease of cucumber. This disease causes reducing yield in cucumber. This study conducted laboratory and greenhouse conditions to find out the efficacy of biological control agents, *Trichoderma harzianum, Bacillus subtilis,* the chemical compound Bion, and fungicide revanol. The results of pathogenicity test of R. solani on cucumber seeds showed that R. solanihas led to a significant reduction in the percentage of germination and seed rot compared to the control treatment, which amounted to 100%. The results showed the ability of all control agents used in this study to reduce the incidence of the disease. The Bion (benzothiadiazole) treatment showed significant effect and outperformed the other treatments in increasing the activity of the peroxidase enzyme, which was recorded at 14,440 (unit. minute^{-1}.g wet weight^{-1}). It also achieved the highest significant increase in total phenolics 1.52 (mg $^{-1}$. gram wet weight $^{-1}$). Biological control agent *T. harzianum* has outperformed on other treatments in causing an increase in the activity of the polyphenol oxidase enzyme, it has recorded 1811.7 (unit. minute $^{-1}$ .gram of wet weight $^{-1}$ leaf).

Introduction

Cucumber (*Cucumis sativus* L.) is one of the important crops that belong to the Cucurbit family. It is grown all over the world on a large scale in the tropical and subtropical regions of the world. Its original homeland is South Asia (Adams et al,1992). Cucumbers are infected with many pathogens, including root rot and seedling death caused by the fungus *R. solani*, which is the main cause of seedling death and root rot(Moataza, 2006).Root rot and seedling deadly disease affect cucumbers at the seedling and ripening stage in protected cultivation and causes significant yield losses (Aljawasim et al,2020 and Cao et al,2020). Many methods have been used to exterminate fungi, including chemical control, but the repeated use of this type of control has passive effects on the environment, including its impact on the effectiveness of non-targeted organisms, its impact on human health, and disruption of the natural balance in the soil, in addition to the high cost and the emergence of resistance types(Nicolopoulou-Stamati et al,2016 and Baćmaga et al,2019). Therefore, there must be an alternative to pesticides, which is the use of biological control against plant pathogens(Wang et al,2007). Species of Trichoderma fungi and Bacillus bacteria have been widely used to treat diseases caused by fungi (Awad and Fayyadh,2018 and Mahmoud,2015). The use of biological control agents, including Trichoderma and Bacillus species against the fungus *R. solani*, which causes root rot and seedling death of cucumber plants, has led to a significant reduction in the infection rate, and thus these treatments increased plant growth(Hassan et al,2021). The use of the chemical compound Bion has led to enhanced plant growth and its ability to induce systemic resistance, in addition to being safe and easy to use and does not cause environmental pollution(Roy et al,2017 and Ambhore et al,2018). Given the importance of this disease in cucumber fields in most regions, we decided to conduct this study.

Materials and Methods

Isolation of the pathogen

Samples of cucumber plants that have shown symptoms of seedling death and root rot have been collected during visits to infected cucumber fields in different areas of Nineveh Governorate. They have been kept in clean polyethylene bags and transported to the laboratory of the Plant Protection Department - College of Agriculture and Forestry - University of Mosul. The samples have been washed with water for 25 minutes to remove dust and plankton. Then, the root system and the crown area have been cut into small parts approximately 4-5 mm long. They have been sterilized on surface with sodium hypochlorite at a concentration of 1% for 2-3 minutes and washed with sterile distilled water.Then, they have been dried between folds of filter paper. The sterilizer has been transferred to Petri dishes containing the nutrient medium, Potato dextrose agar (PDA), which has been previously sterilized in a steam autoclave at a temperature of 121 °C and under a pressure of 1.5 kg/cm2 for 20 minutes. When the medium temperature reaches 50°Cthe antibioticAmoxicillin was added and the dishes have been incubated in the incubator at a temperature of ±25°C for 5 days, after which the isolates have been purified.

Propagation of pathogenic fungus on millet groats

have used local millet seeds, *Panicum miliaceum*, to propagate the pathogenic fungus after washing them with water. The seeds have been soaked in water for six hours Then, they have been filtered with a piece of gauze. After that, every 100 grams of millet seeds have been placed in 250 ml glass beakers. Later, I have closed the mouth of the beakers with a cotton tampon and sterilized. At the end of the sterilization period, the flasks have been removed and then left to cool. The flasks have been inoculated by placing 5 discs from the edge of the 7-day-old fungal colony with a diameter of 0.5 cm using a cork drill. It has been left in the incubator at a temperature of 25±2°C for 15 days, taking care to shake the flasks constantly every 2-3 days to distribute the fungal inoculum to all the seeds (Dewan and Sivasithamparam,1988).

Testing the pathogenicity of the fungus *R. solani* in the laboratory

An experiment has been made to study the pathogenicity of the fungus *R. solani*, which causes root rot and death of cucumber seedlings, according to the method(Mishra and Behr,1976). By calculating the germination capacity of four varieties of cucumber seeds (Dalal, Karm, Fox, and Beit Alfa), Petri dishes have been prepared containing the mycelium of the pathogenic fungus grown on Potato Dextrose Agar, the *R. solani* isolate has been incubated, and the cucumber seeds have been then washed with water transferred to 1% sodium hypochlorite for one minute Then, they have been washed several times in sterile distilled water. After that, cucumber seeds have been distributed on the surface of the nutrient medium (PDA) contaminated with the fungus in Petri dishes, with 10 seeds per plate, while the control seeds have been placed on the surface of the nutrient medium (PDA). without fungus (comparison). The plates have been incubated with

the seeds in the light at room temperature (20±2°C), and after 7 days the number of Germinated and non- Germinateds seeds has been calculated according to the following equation:

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

The effect of biological and chemical pesticide on pathogenic fungal growth

The experiment has been conducted in the greenhouse of the Department of Plant Protection - College of Agriculture and Forestry - University ofMosul under natural field conditions to test the efficiency of the biological agents represented by the fungus *T. harzianum*T22, the bacteria *B. subtilis*, the chemical pesticide Revanol, and the stimulating agent Bion. The treatments are as follows:

1- Positive control treatment (contaminated with pathogenic fungi)
2- Negative control treatment (without contamination with pathogenic fungi)
3- *R. solani* + *T. harizanum* T22
4- *R. solani* + *B. subtilis*
5-*R. solani* + the chemical pesticide Revanol
6- *R.solani* + bion compound at a concentration of 10 mg/l
7- *R. solani* + bion compound at a concentration of 20 mg/l
8- *R. solani* + bion compound at a concentration of 30 mg/l

Plastic pots have been used in this test, containing soil sterilized with formalin at a concentration of 5%, in addition to peatmoss in a ratio of 1:1. The effect of biological agents and chemical pesticide on the pathogenic fungus has been tested. The seeds have been superficially sterilized with a 1% sodium hypochlorite solution for 2-3 minutes and washed with water and planted in plastic pots containing soil contaminated with the pathogenic fungus grown on millet seeds at a rate of 10 g/pot, at a depth of 2 cm, where biological agents have been used, including *T. harzianum* T22 and *B. subtilis* and the chemical pesticide Revanol, in addition to three concentrations of the compound Bione. , planted in anvils containing previously sterilized soil contaminated with pathogenic fungi.

Estimation of the effectiveness of the peroxidase enzyme (unit.min^{-1}.gram fresh weight^{-1})

The activity of the peroxidase enzyme has been measured with the Guaiacol test according to the method (Müftügil,1985).

Estimate the activity of the polyphenol oxidase enzyme(unit.min^{-1}.gram fresh weight^{-1})

To estimate the effectiveness of the polyphenol oxidase enzyme the method of (Shi et al,2002) has been used.

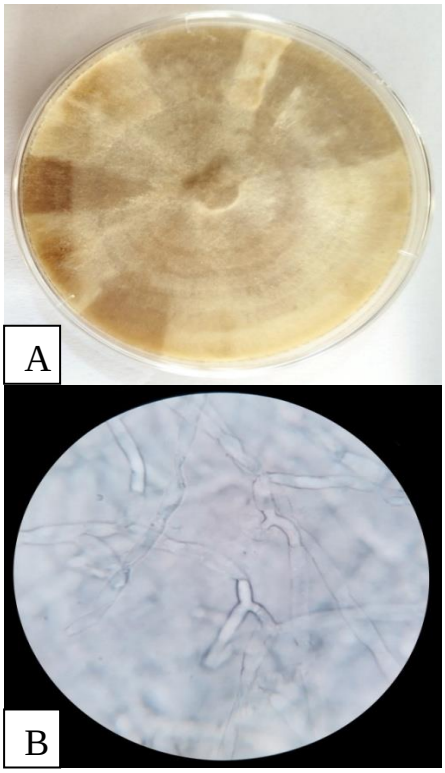
Determination of total phenols (mg.g fresh weight^{-1})

The extraction method has been used with methanol: water 80:20 according to the method described before(Aberoumand and Deokule,2008 and Chandra et al, 2014).

Results and discussion

Isolation and diagnosis

The results of isolation and diagnosis from the roots of cucumber plants that have shown symptoms of seedling death and root rot have shown the presence of the fungus *R. solani*, which is distinguished by its brown colour on the PDA nutrient medium. Microscopic examination has shown that the mycelium is divided into many branches and the presence of narrowing in the branching areas, in addition to the presence of transverse septa near it. From the point of emergence of the branch, these characteristics are consistent with what is mentioned by (Parmeter and Whitney,1970).



A-Rhizoctoniasolani fungi on PDA
B- Rhizoctonia solani fungi Mycellium with a strength of 40x

Fig (1) Cultural and microscopic characteristics of the fungus *Rhizoctonia solani*

Transactions	Items				Effect of Transactions
	Dalal	Karm	Fox	Beit Alfa	
Nagative comparison	12413g-l	12180 g-l	13647 g-e	14477 g-e	13179 c-b
Positive comparison	11643g-l	17860 a-b	11797 g-l	12927 g-e	13557 a-b
T. harzianum T22	12763g-h	13160 g-e	9613 j-l	11383 g-l	11730 c
B. subtilis	8847 m-l	10300 j-l	9363 m-l	18893 a	11851 c
Revanol	13867g-e	14053 g-e	8450 m	10017 j-l	11597 c
Bion Concentration 10	12047g-l	16750 c-b	12023 g-l	16940 c-b	14440 a
Bion Concentration 20	8753m-l	16557 c-b	14670 g-e	8777 m-l	12189 c-b
Bion Concentration 30	12883g-e	16457 c-e	12360 g-l	15240 c-e	14235 a
Items effect	11652 c	14665 a	11490 c	13582 b	

Testing the pathogenicity of the fungus *R. solani* in the laboratory

It is clear from Figure (2) that the fungus *R. solani* has caused a significant reduction in the germination rate.as compared with the control treatment, which has amounted to 100%, and that the reason for the failure of germination and the rotting of the seedlings is owed to the fungal high ability to secrete the enzymes Cellulase and Pectinase, which decompose and disintegrate the cells and cause their rot, as well as the ability of the fungus to Secretion of toxic secondary metabolites that cause seed rot (Ajayi-Oyetunde and Bradley,2018 and Tjimune et al,2022).

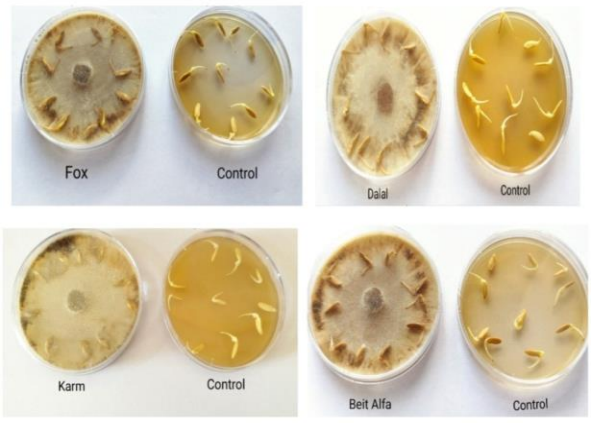


Fig (2) The pathogenicity of the fungus *R. solani* on the seeds of four varieties of cucumber in the laboratory.

The effect of the fungus *T. harzianum* T22, the bacteria *B. subtilis*, Bion, and the pesticide Revanol on the activity of the peroxidase enzyme (unit. min $^{-1}$.gram, wet weight $^{-1}$leaves)

The results in table (1) have shown that the bion treatment is significantly superior to all treatments, as it has increased the activity of the peroxidase enzyme, which are recorded at 14440 (units. Min^{-1}.gram wet weight^{-1}). It has not differed significantly from the bion at a concentration of 30, while it has differed from the rest of the treatments and has been recorded as a pesticide. Revanol has the lowest average peroxidase activity activity of 11597 (units per minute $^{-1}$gram wet weight^{-1}). According to the results of the effect of the varieties, it is clear from the table that the Karm variety has given the highest effectiveness of the peroxidase enzyme, reaching 14665 (units. Min^{-1}.gram wet weight^{-1}). According to the results of the interactions between the varieties and treatments, it has been found that the Beit Alfa variety treated with the bacterium *B. subtilis*has giventhe highest average value for the peroxidase enzyme, which has reached 18893(units. Min^{-1}.gram wet weight^{-1}), while the lowest value has been for the Fox variety treated with the pesticide Revanol, which has reached 8450 (units. Min^{-1}.gram wet weight^{-1}).

Table 1. Effect of the fungus *T. harzianum* T22, the bacterium *B. subtilis*, Bion, and the pesticide Revanol on the activity of theperoxidase enzyme (unit. Min^{-1}.gram. wet weight $^{-1}$ leaves).

Transactions	Items				Effect of Transactions
	Dalal	Karm	Fox	Beit Alfa	
Nagative comparison	2026.7 b-c	1213.3 f-h	1133.3 f-h	920.0 f-h	1323.3 c
Positive comparison	1573.3 d-f	1066.7 f-h	1826.7 c-d	3313.3 a	1945.0 a
T. harzianum T22	1653.3 d-f	2446.7 b	1246.7 f-h	1900.0 c-d	1811.7 a-b
B. subtilis	1473.3 f-h	1813.3 c-d	1666.7 d-f	1240.0 f-h	1548.3 b-c
Revanol	853.3 f-h	513.3 j-I	560.0 I-j	933.3 f-h	715.0 d
Bion Concentration10	846.7 f-h	313.3 j	506.7 I-j	500.0 I-j	541.7 d
Bion Concentration20	646.7 j-h	846.7 f-h	406.7 I-j	640.0 h-j	635.0 d
Bion Concentration30	833.3 f-h	786.7 j-h	513.3 I-j	693.3 h-j	706.7 d
Items effect	1238.3 a-b	1125.0 a-b	982.5 b	1267.5 a	

* Similar letters for each factor are not significantly different
according to Duncan's multinomial test at the 5% probability level.

The effect of the fungus *T. harzianum* T22, the bacteria *B. subtilis*, Bion, and the pesticide Revanol on the activity of the polyphenol oxidase enzyme (unit. min $^{-1}$.gram, wet weight $^{-1}$leaves)

Table 2.Effect of the fungus *T. harzianum* T22, the bacterium *B. subtilis*, Bion, and the pesticide Revanol on the activity of the polyphenol oxidase enzyme (unit. Min^{-1}.gram. wet weight $^{-1}$ leaves).

* Similar letters for each factor are not significantly different according to Duncan's multinomial test at the 5% probability level.

The results in table (2) have been shown that the*T. harzianum* T22 treament is significantly superior to all treatments, as it gave the highest average activity of the polyphenol oxidase enzyme, which are recorded at 1811.7 (units. Min^{-1}.gram wet $weight^{-1}$). And the bion concentration of 10 recorded the lowest average . For the 1effectivenessof the polyphenol oxidase enzyme, it reached 541.7 (units. Min^{-1}.gram wet $weight^{-1}$). Through the results of the effect of the varieties, it is clear from the table that the Beit Alfa variety gave the highest average for the polyphenol oxidase enzyme, reaching 1267.5 (units. Min^{-1}.gram wet $weight^{-1}$) and through the results of the interacions between the varieties and treatments, it was that the Karm variety treated with the fungus *T. harzianum* T22 gave the highest average of the polyphenoloxidase enzyme, reaching 2446.7(units. Min^{-1}.gram wet $weight^{-1}$). While bion at a concentration of 10 recorded the lowest average for polyphenoloxidase enzyme, reaching 313.3 (units. Min^{-1}.gram wet $weight^{-1}$).

Table 3. Effect of the fungus *T. harzianum* T22, the bacterium *B. subtilis*, Bion, and the pesticide Revanol on the content of phenols (mg $^{-1}$gram wet $weight^{-1}$) in cucumber plants under conditions of infection with the fungus *R. solani*

Transactions	Items				Effect of Transactions
	Dalal	Karm	Fox	Beit Alfa	
Nagative comparison	1.25 e-f	1.17 f-k	1.57 b-c	1.13 k-m	1.28 b-c
Positive comparison	1.33 e-f	1.63 a-b	1.16 k-m	1.48 e-f	1.40 a-b
T. harzianum T22	1.20 f-k	1.08 k-m	1.25 e-f	1.24 e-f	1.19 c-d
B. subtilis	1.18 f-k	1.23 e-f	1.80 a	1.08 k-m	1.32 b-c
Revanol	1.34 e-f	0.88 m	1.25 e-f	0.98 k-m	1.12 d
Bion Concentration10	1.40 e-f	1.36 e-f	0.91 l-m	1.31 e-f	1.24 b-d
Bion Concentration20	1.43 e-f	1.50 e-f	1.58 b-c	1.54 c-e	1.52 a
Bion Concentration 30	1.50 e-f	1.04 k-m	1.48 e-f	1.51 e-f	1.38 a-b
Items effect	1.33 a-b	1.24 b	1.38 a	1.28 a-b	

* Similar letters for each factor are not significantly different according to Duncan's multinomial test at the 5% probability level.

The effect of the fungus *T. harzianum* T22, the bacterium *B. subtilis*, Bion, andthe pesticide Revanol on the content of phenols (mg^{-1}.g wet $weight^{-1}$)

It is noted from the results of table (3) that bion has given the highest average total phenols content, which amounted to 1.52 (mg^{-1}.g wet $weight^{-1}$) at a concentration of 20, while the pesticide Revanol has recorded the lowest average total phenols content, reaching 1.12 (mg^{-1}.g wet $weight^{-1}$),which has not differed from the bion treatment at concentration 10 and the *T. harzianum* T22 treatment, but it has differed from the rest of the treatments. From the results of the effect of the varieties, it is clear from the table that the Fox variety recorded the highest average for the total phenolic content, reaching 1.38 (mg^{-1}.g wet $weight^{-1}$) while the Karm variety has given the lowest average for the total phenolic content, reaching 1.24 (mg^{-1}.g wet $weight^{-1}$).. According to the results of the interactions between the varieties and treatments, it has been found that the Fox variety treated with the bacteria *B. subtilis* recorded the highest average for the total phenols content, reaching 1.80(mg^{-1}.g wet $weight^{-1}$), while the Karm variety has recorded the lowest average for the total phenols content in the treatment with the pesticide Revanol which amounted to 0.88 (mg^{-1}.g wet $weight^{-1}$).

These results may be owed to the protection provided by the compound Bion, which rapidly signals defense-related genes (Buzi et al,2004). Bion activates the Systemic Acquired Resistance mechanism and produces a large number of antimicrobial toxins (Chen et al, 2010 and Govindappa et al,2010). These results agree with what is reported by (El-Fiki et al,2007 and Nafie and Mazen,2008). That treating plants with the compound Bion has led to an increase in the activity of the enzymes polyphenol oxidase, peroxidase, glucanase-3-1β, and chitinase, and this is attributed to its ability to be transported systemically in the plant and activate proteins associated with diseases and phenolic compounds. Increasing lignin strengthens the plant cell wall and hinders the attack of pathogens (Walters et al,2005).Has pointed out that the use of the bionic compound has caused the stimulation of plant genes by increasing the activity of peroxidase and polyphenol oxidase enzymes, as well as the catalase enzyme, which inhibit pathogenic fungi and cause an increase in the activity of these enzymes.(Gupta et al,2014 and El-Rayes et al,2022) pointed out that the biological control agent *T. harzianum* negatively affects the growth of the fungus *R. solani*, as it has inhibited the growth of mycelium. This is ascribed to the ability of the fungus *T. harzianum* to secrete some enzymes that degrade the cell walls of the pathogen, as well as its secretion of some chemicals that it inhibits growth by wrapping the hyphae of the biocontrol agent on the mycelium of the pathogen.

Conclusions

Through the research, we conclude that the use of biological control agents represented by the fungus *Trichoderma harzianum* and the bacteria *Bacillus subtilis* has caused a significant reduction in the incidence of the disease and an increase in the rate of germination of cucumber seeds, and that the use of the fungus *T. harzianum* T-22 and the bionic compound have caused the induction of systemic resistance in cucumber plants, as the activity of peroxidase and polyphenol oxidase enzymes and the total phenols content increase.

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