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Biological and chemical control of *Fusarium graminearum* contaminating barley seeds under green house conditions

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A B S T R A C T

This study evaluated the antagonistic activity of fungal biocontrol agent *Trichoderma viride UPM 29* in preventing the growth of pathogenic fungus *Fusarium graminearum* isolated from barley seeds stored in Nineveh seed production companies. The greenhouse results showed that samples of barley seeds of the Mosul Aswad 1 were planted in treated soil with the biological resistance *Trichoderma viride UPM 29* alone, showed high germination rates, reaching 96.66%, followed by the treatment with the chemical pesticide Raxil 2%, which reached 90%. It also showed a significant increase in the rate of plant height, the fresh and dry weight of the shoot and root systems, the length of the root system, the number of tillers, and productivity characters such as the individual and biological weight of plant and the weight of a thousand seeds.While treatment with the pathogenic fungus *Fusarium graminearum* caused a significant decrease in the percentage of seed germination, plant height rate, and characteristics of the vegetative and root systems, such as fresh and dry weight and the number of tillers per plant, thus negatively affecting productivity. However, adding the chemical pesticide Raxil at a rate of 2% and the bio resistance agent led to a decrease in the negative effect for treatment with pathogenic fungi and for all mentioned vegetative, root and production characters.

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Introduction

Barley, scientifically known as Hordeum vulgare, plays a vital role as a significant grain. It serves as a crucial feed source for cattle, a staple food for humans, and the primary agricultural commodity for brewing beer. In 2016, the global production of barley amounted to 141 million metric tons, positioning it as the fourth most abundant grain commodity worldwide, following corn, wheat, and rice. [1] . Barley ranks as the world's fourth most widely grown cereal, following corn (Zea mays ssp. mays), common wheat (Triticum aestivum), and rice (Oryza sativa). [2]. Seeds serve as the foundation for agriculture and the production of food. The majority of essential crops, such as cereals, are cultivated from seeds, which play a vital role in the diets of both humans and animals. It is important to acknowledge that seeds can harbor various microorganisms, including fungi, which can potentially cause issues for the seeds. This can have detrimental effects on human health, agricultural output, and the well-being of animals. Exposure to fungi carried by seeds can result in the transmission and spread of infections, leading to plant diseases and impacting the quality and lifespan of seeds, ultimately resulting in decreased crop productivity. Moreover, if contaminated seeds contain mycotoxins, it can pose significant health risks to both humans and animals if consumed. [3]. In the past, barley was mainly utilized as animal feed because it couldn't be made into dough and lacked flavor and color. But as living standards improved, the focus shifted towards the nutritional benefits of food. [4] suggest that substituting barley flour for wheat flour can boost nutritional value. Improper harvesting methods and poor conditions for drying, handling, packaging, storage, and transportation can lead to fungal growth[5]. Barley storage is significantly impacted by fungal contamination, presenting a significant obstacle. Fungi thrive in suitable storage conditions, leading to the degradation of vital nutrients in barley. Consequently, the quality of barley declines, potentially jeopardizing consumer interest.[6]. The microbial community found on or within barley seeds is composed of various species belonging to five distinct groups: viruses, bacteria, fungi, micromycetes, and protozoa. Among these groups, bacteria and fungi have the most significant impact on the properties of barley grains. This is mainly because they are often present in larger quantities and many of them have the ability to utilize grains as a source of nutrients. The composition of fungal species present in or on barley seeds and grains undergoes changes throughout the production process, starting from cob and grain growth, continuing through grain harvest, and concluding with barley burning .[7]. Biocontrol agents provide not only effective disease control but also present safe and environmentally friendly alternatives. The concept of biocontrol revolves around the introduction of antagonists into agricultural systems. By harnessing the power of a living and reproducing biocontrol agent, it becomes feasible to continuously and naturally suppress pathogens without the need for chemical intervention. In contrast, chemical methods can disturb the microbiological community, leading to an unfavorable environment for the growth and prosperity of beneficial organisms. [8]. Therefore, employing antagonists directly would offer a more reliable method to introduce microorganisms into the soil, with the objective of biologically managing soil-borne plant pathogens. Extensive research has demonstrated that biocontrol agents produce a wide range of antibiotic compounds and effectively prey on other harmful fungi. [9]. T. viride has been widely acknowledged as an exceptionally effective biocontrol agent in combating soil-borne diseases in field crops, specifically Fusarium wilt. [10]. The objective of this study is to

1. Study of the pathogenicity of Fusarium graminearum under greenhouse conditions to evaluate its effect on seed germination and some characteristics of the shoot and root system and productivity of barley plants.
2. Evaluation of the antagonistic ability of the biocontrol agent Trichoderma viride against pathogenic fungus Fusarium graminearum under greenhouse.
3. Study the effect of the biocontrol agent Trichoderma viride and on shoot and root characteristics of barley variety Mosul Aswad 1

Material and Methods:

The effect of the pathogenic fungus *Fusarium graminearum* and the biological control agent *Trichoderma viride* UPM 29 on the germination of barley seesds and their plants under greenhouse conditions. This experiment was conducted in a completely randomized design, with three replicates , Sandy clay soil was autoclaved and transferred in to sterilized pots each containing 5 kg soil, pots were inoculated with *Fusarium graminearum* and the bioresistant *Trichoderma viride UPM* 29 growing on PDA for 7 days at the rate of petri dish / pot which mixed with the surface of the soil at depth 1-3 cm according to .[11]. Then the pots kept in greenhouse for 3 days before sowing the seeds , Pots containing non-inoculated soil were used as control . Three replicates were used per treatments. Pathogen freeseeds were surface sterilized and planted (10 seeds / pot) in both inoculated and non-inoculated soils. The duration of the experiment lasted four months (from 12/15/2023 to 4/15/2024), and for the purpose of confirming the pathogenic fungus, the seeds that did not germinate were detected and the pathogenic fungus re-isolated from them. The percentage of germination was calculated, in addition to calculating the average plant height, the wet and dry weight of the root and shoot groups, the number of shoots, the length of the tiller, the individual weight, the biological weight of the plant, and the weight of a thousand seeds. The results were analyzed statistically and compared according to Duncan's multinomial test. The experiment consisted of the following treatments:

1. Soil contaminated with Trichoderma viride UPM 29
2. Soil contaminated with the pathogenic fungus (Fusarium graminearum, isolated from barley seed Mosul Aswad 1 variety).
3. Soil contaminated with pathogenic fungi Fusarium graminearum, addition of Trichoderma viride UPM 29.
4. Soil contaminated with the chemical pesticide Raxil at concentration 2%
5. Soil contaminated with pathogenic fungi Fusarium graminearum addition of chemical pesticide Raxil at concentration 2%
6. Autoclave soil (as control)

Result and Discussion:

Percentage of seed germination of barley seeds

The results in Tab . (1) showed no significant differences between the various treatments, while the treatment with the biocontrol agent achieved the highest germination percentage, reaching 96.66%, and did not differ significantly from the rest of the treatments except for treatment with the pathogenic fungus alone, which achieved the lowest germination rate, reaching 70%.

Table 1. The effect of different treatments on the percentage of seed germination of barley

Treatments	Percentage of seed germination%
Autoclave soil (control)	90 A
Soil treated with *T.viride UPM 29*	96.66 A
Soil treated with pathogenic fungus *F.graminearum* alone	70 B
Soil treated with pathogenic fungi *F.graminearum,* addition of *T.viride* UPM 29.	86.66 A
Soil treated with the chemical pesticide Raxil + pathogenic fungus	90 A
Soil treated with the chemical pesticide Raxil only	90 A

*Numbers that have the same alphabet vertically have no significant difference according to Duncan's multinomial test at a significance level of 0.05.

The effect of different treatments on the Characteristics of the vegetative and root system of barley plants

1.Plant height rate (cm)

The results of Table (2) show that the treatment with the biological resist agent Trichoderma viride had the highest rate of plant height, which amounted to 58,330 cm, and did not differ significantly from the control , which was 58,110 cm. The lowest significant plant height was in the treatment with the pathogenic fungus alone, which amounted to 52,077 cm, and did not differ significantly from the treatment with the chemical pesticide Raxil added to pathogenic fungi amounted to 53.663 cm.

Table 2. The effect of different treatments on plant height rate (cm) of barley

Treatments	Plant height rate(cm)
Autoclave soil (control)	85.110 A
Soil treated with *T.viride UPM 29*	85.330A
Soil treated with pathogenic fungus *F.graminearum* alone	53.663A
Soil treated with pathogenic fungi *F.graminearum,* addition of *T.viride* UPM 29.	75.773 A
Soil treated with the chemical pesticide Raxil + pathogenic fungus	52.077A
Soil treated with the chemical pesticide Raxil only	56.687 A

*Numbers that have the same alphabet vertically have no significant difference according to Duncan's multinomial test at a significance level of 0.05.

2.The length of the root system

The results in Table (3) showed that the highest significant value for the average length of the root system was achieved in the treatment with the chemical pesticide alone, and it reached 25.5 cm and did not differ significantly from the treatments with the bio resistance agent , the control treatment, the treatment with the bio resistance agent to which the pathogenic fungus was added, and the treatment with the chemical pesticide Raxil to which the pathogenic fungus was added, it was 24.333, 22.49, 21.99, and 20.25 cm. As for the least significant value for the length of the root system, it was in the treatment with the pathogenic fungus only and amounted to 19.10 cm.

Table 3. The effect of different treatments on the length of the root system (cm) of barley plant

Treatments	The length of the Root
Autoclave soil (control)	22.493 ABC
Soil treated with *T.viride UPM 29*	42.333AB
Soil treated with pathogenic fungus *F.graminearum* alone	19.100C
Soil treated with pathogenic fungi *F.graminearum,* addition of *T.viride* UPM 29.	21.993ABC

Soil treated with the chemical pesticide Raxil + pathogenic fungus	20.250C
Soil treated with the chemical pesticide Raxil only	25.500A

*Numbers that have the same alphabet vertically have no significant difference according to Duncan's multinomial test at a significance level of 0.05.

1.Wet weight of shoots (g) of barley plants

The results in Table (4) showed that there are significant differences in the relative average wet weight of barley plants. The highest average wet weight was in the treatment with the chemical pesticide Raxil plus the pathogenic fungus, as it differed significantly from the rest of the treatments and amounted to 10.71 gm , while there was a significant decrease in the wet weight in the treatment with pathogenic fungi only it amounted to 4.703 gm. Treatment with the bio resist agent plus the pathogenic fungus also had a positive effect on the wet weight of the barley plant, reaching 8.5733 gm when compared to the treatment of the pathogenic fungus alone.

2.Dry weight of shoots (g) of barley plants

The results in Table No. (4) show that there are significant differences in the shoot dry weight of barley plants, where the highest average dry weight in the treatment with the biological resistance agent to which the pathogenic fungus was added reached 4.2733 gm, and this value did not differ significantly from the dry weight value in the soil treatment with the chemical pesticide Raxil only it reached 4.7133 gm, while the least significant value for dry weight was in the treatment with the pathogenic fungus alone and amounted to 1.953 gm.

Table 4. The effect of different treatments on wet and dry weight of barley plant shoot

Treatments	Wet weight(gm)	Dry weight(gm)
Autoclave soil (control)	8.9833C	3.4000B
Soil treated with *T.viride UPM 29*	8.6433C	3.2600B
Soil treated with pathogenic fungus *F.graminearum* alone	4.7033D	1.9533C
Soil treated with pathogenic fungi *F.graminearum,* addition of *T.viride* UPM 29.	8.5733C	4.2733A
Soil treated with chemical pesticide Raxil + pathogenic fungus	10.7100B	3.233B
Soil treated with the chemical pesticide Raxil only	13.0633A	4.7133A

*Numbers that have the same alphabet vertically have no significant difference according to Duncan's multinomial test at a significance level of 0.05.

3.Wet weight of the root mass (g) of barley plants

Table (5) shows the effect of different treatments on the wet weight of the root system of barley plants, where the treatment with the chemical pesticide Raxil only achieved the highest value for the dry weight of the root system, which amounted to 1.7333 gm and was significantly different from the rest of the treatments, followed by the treatment with the chemical pesticide Raxil plus the pathogenic fungus, which amounted to 1.3300 gm and differed significantly form the rest of the treatments, then the treatments for the bio resistance agent to which the pathogenic fungus was added, the control treatment, the treatment with the bio resistance agent only, and the treatment with the pathogenic fungus alone, and amounted to (1.0233, 0.8333, 0.7733, and 0.6300) gm , respectively.

4.Dry weight of root mass (g) of barley plants

The results in Table (5) indicate that the lowest significant value for the dry weight of the root system was in the treatment with the pathogenic fungus, which caused a significant decrease of 0.393 gm compared to the control treatment, which had a value of 0.380 gm. The highest value for the dry weight of the root system was in the treatment with the chemical pesticide Raxil only and amounted to 0.41333 gm, which did not differ significantly from the control treatments, the treatment with the bio resistance agent alone, the treatment with the bio resistance to which the pathogenic fungus was added, and the treatment with the chemical pesticide Raxil to which the pathogenic fungus was added, and amounted to (0.380, 0.3033, 0.3933, and 0.2933) gm, respectively.

Table 5. The effect of different treatments on wet and dry weight of barley plant root

Treatments	Wet weight(gm)	Dry weight(gm)
Autoclave soil (control)	0.38000A	0.8333CD
Soil treated with *T.viride UPM 29*	0.38000	0.7733D
Soil treated with pathogenic fungus *F.graminearum* alone	0.27000A	0.6300D
Soil treated with pathogenic fungi *F.graminearum,* addition of *T.viride* UPM 29.	1.0233C	0.39333A
Soil treated with chemical pesticide Raxil + pathogenic fungus	1.3300B	0.29333A
Soil treated with the chemical pesticide Raxil only	1.7333A	0.41333A

*Numbers that have the same alphabet vertically have no significant difference according to Duncan's multinomial test at a significance level of 0.05

5.The number of tillers of barley plants

The results in Tab:(6)showed that the number of tillers for the barley plant recorded a significant decrease in the pathogenic fungus treatments and amounted to 2.3, while its value in the treatment with the chemical pesticide Raxil only reached 4.6.

Table 6. The effect of different treatments on the number of tillers of barley plants

Treatments	Number of points
Autoclave soil (control)	4.3333 A
Soil treated with *T.viride UPM 29*	4.0000A
Soil treated with pathogenic fungus *F.graminearum* alone	2.3333B
Soil treated with pathogenic fungi *F.graminearum,* addition of *T.viride* UPM 29.	4.3333A
Soil treated with the chemical pesticide Raxil + pathogenic fungus	4.000A
Soil treated with the chemical pesticide Raxil only	4.667A

*Numbers that have the same alphabet vertically have no significant difference according to Duncan's multinomial test at a significance level of 0.05

6. The spike length of barley plants

The results in Table (7) show that there are no significant differences between the spike length values in all treatments. The highest spike length value was achieved in the treatment with the chemical pesticide Raxil only and amounted to 6.4133, while the lowest value was achieved in the treatment with the pathogenic fungus and amounted to only 5.710.

Table 7. The effect of different treatments on the spike length of barley plants

Treatments	The length of the barley spike (cm)
Autoclave soil (control)	5.7100 A
Soil treated with *T.viride UPM 29*	6.3167A
Soil treated with pathogenic fungus *F.graminearum* alone	5.9000
Soil treated with pathogenic fungi *F.graminearum,* addition of *T.viride* UPM 29.	6.3267A
Soil treated with the chemical pesticide Raxil + pathogenic fungus	6.2267A
Soil treated with the chemical pesticide Raxil only	6.4133 A

*Numbers that have the same alphabet vertically have no significant difference according to Duncan's multinomial test at a significance level of 0.05

The effect of different treatments on the productivity of barley plants

1.Individual plant weight (g) of barley plants

The results of Table (8) show that the highest significant value for individual plant weight was in the treatment with the chemical pesticide Raxil to which the pathogenic fungus was added, which differed significantly from the rest of the treatments and amounted to 0.56667 grams, then followed by the treatment with the chemical pesticide Raxil alone and amounted to 0.48667 grams, then the treatment with the biological resistance to which the pathogenic fungus was added. Then the treatment of the bio resistance agent alone, then the control treatment, amounted to 0.493333, and 0.35000 g, respectively, while the treatment of the pathogenic fungus alone recorded the least significant value for plant weight, 0.28333 gm.

Table 8. The effect of different treatments on Individual plant weight (g) of barley plants

Treatments	The Individual plant Weight (g)
Autoclave soil (control)	0.35000D
Soil treated with *T.viride UPM 29*	0.40667C
Soil treated with pathogenic fungus *F.graminearum* alone	0.28333E
Soil treated with pathogenic fungi *F.graminearum,* addition of *T.viride* UPM 29.	0.493333B
Soil treated with the chemical pesticide Raxil + pathogenic fungus	0.56667A
Soil treated with the chemical pesticide Raxil only	0.48667B

*Numbers that have the same alphabet vertically have no significant difference according to Duncan's multinomial test at a significance level of 0.05

2.The biological plant weight (g) of barley plants

The results of Table (9) show that the highest significant value for the biological plant weight was in the treatment with the chemical pesticide Raxil to which the pathogenic fungus was added, and it amounted to 25.487 grams, then it was followed by the treatment with the chemical pesticide Raxil alone and it reached 24.607 grams, then the treatment with the bio resistant agent alone, then the treatment with the bio resistant agent to which the pathogen fungus was added then the control treatment reached 22.847, 22.720 and 18.950, while the pathogenic fungus treatment alone recorded the lowest significant value for plant weight and was 18.167 g.

Table 9. The effect of different treatments on the biological plant weight (g) of barley plants

Treatments	Biological plant Weight (g)
Autoclave soil (control)	18.167 B
Soil treated with *T.viride UPM 29*	22.847A
Soil treated with pathogenic fungus *F.graminearum* alone	18.950B
Soil treated with pathogenic fungi *F.graminearum,* addition of *T.viride* UPM 29.	22.720A
Soil treated with the chemical pesticide Raxil + pathogenic fungus	25.487A
Soil treated with the chemical pesticide Raxil only	24.607A

*Numbers that have the same alphabet vertically have no significant difference according to Duncan's multinomial test at a significance level of 0.05.

3.The weight of 1000 seeds (g) of barley plants

The results in Table (10) indicate that the highest significant value for the weight of a thousand seeds was in the treatment with the bio resistance agent to which the pathogenic fungus was added and it amounted to 33.533 gm then it was followed by the treatment with the chemical pesticide Raxil, to which the pathogenic fungus was added, and it amounted to 31.237, then the treatment with the chemical pesticide Raxil alone , the bio resistance agent alone ,the control treatment reached 26.387, 25.230, and 23.110, while the treatment of the pathogenic fungus alone recorded the lowest significant value for the weight of a thousand seeds, which was 16 grams.

Table 10 . The effect of different treatments on the weight of 1000 seeds (g) of barley plants

Treatments	weight of 1000 seeds (g)
Autoclave soil (control)	23.110BC
Soil treated with *T.viride UPM 29*	25.230AB
Soil treated with pathogenic fungus *F.graminearum* alone	16.000C
Soil treated with pathogenic fungi *F.graminearum,* addition of *T.viride* UPM 29.	33.533A
Soil treated with the chemical pesticide Raxil + pathogenic fungus	31.237AB
Soil treated with the chemical pesticide Raxil only	26.387AB

*Numbers that have the same alphabet vertically have no significant difference according to Duncan's multinomial test at a significance level of 0.05

Several research studies have shown that Fusarium spp. growth can impact germination capacity. additionally, Fusarium spp. may generate unknown proteinases during infection, leading to the synthesis or activation of barley proteinases involved in the germination process[12].Numerous research studies have provided evidence of a decline in the quality of various cereals due to the existence of Fusarium species.[13].Pathogenic fungi have the ability to secrete cellulase and pectinase enzymes, which cause root ulceration, seed rot, and seedling death, in addition to secreting some compounds with a toxic effect on plants, such as Phenyl Acetic Acid and its alpha- and beta-hydroxy derivatives, which lead to the killing of seed embryos .[14]. Fusarium infection, caused by a fungus, has the potential to cause two crop diseases: Fusarium Head Blight (FHB) and Fusarium damaged kernels (FDK). These diseases can significantly impact the yield, kernel quality, and seed germination percentage, leading to reduced agricultural productivity.[15] Furthermore, the occurrence of Fusarium spp. in barley grains is associated with gushing, which refers to the excessive foaming and eruption of beer when it is opened .[16,17] .Fusarium spp. infection can result in the formation of diverse hydrolytic enzymes, including cutinases, proteinases, xylanases, and cellulases. These enzymes that degrade cell walls are essential for the pathogenicity of the fungus and are likely to be involved in the colonization of barley grains. Among these enzymes, proteinases are the most crucial, and protein degradation can significantly influence the malting or brewing quality of the diseased grain. .[18].Rotting roots cause loss of shoot numbers, seed atrophy and shrinkage, and loss of nutritional value, which leads to significant crop losses. [19] .The pathogenic fungus *F. oxysporum* produces metabolic substances that cause comprehensive seed rot and abnormal growth of seedlings. Symptoms of wilting appear on the infected seedlings, as they begin to bend at the top, and the leaves turn yellow and begin to dry. When the stems of the wilted seedlings fall, a black scar appears in the area of separation from the interior tissue. [20,21]. The Trichoderma fungus produces compounds and secondary metabolites that increase root growth, which may be the reason behind the increase in the wet and dry weight of the root system [22].These results are consistent with results reported by [23] that use of the T.viride with the pathogenic fungi Rhizctonia spp and F.solani resulted in an improvement in the growth characteristics of the black cumin seed plant the dry weight of the root and shoot values also increased significantly due to the ability of the bio resistance agent to provide nutrients and minerals for the plant.

Conclusion:

The results of the sensitivity of the barley variety Mosul Black 1 to the pathogenic fungus F. graminearum that added to the soil before planting showed that it caused a significant decrease in the percentage of seed germination, the rate of plant height, the wet and dry weight of the shoot and root systems, the number of tillers , the length of the root system, and in productivity characters that included plant weight. Individual, biological, and weight of one thousand seeds. Also showed the efficiency of isolation of the bio resistant agent Trichoderma viride UPM 29 as biological control agent against the pathogenic fungus F. graminearum. That decreased the effect of the pathogenic fungus on the characteristics of germination, vegetative and root systems, and productivity.

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