Detection of Mn – Dependent Chitinase for Wheat Root Rot Disease Control by Real time PCR

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Abstract

The use of some mineral salts with concentrations of 10, 30 and 50 mm Muller led to the inhibition of the activity of the enzyme chitinase, but to varying degrees. Manganese chloride MnCl2 showed more effect in stimulating the activity of the enzyme, as the effectiveness reached 74.87% in addition to calcium chloride CaCl2, which had a stimulating effect for the enzyme as well. The salts EDTA and NH4Cl had an inhibitory effect on the enzyme activity. The field experiment was conducted for the purpose of inducing systemic resistance of wheat plants with the fungus T. longibrachiatum under conditions of infection with the pathogen Fusarium oxysporum and evaluating the efficacy of the chitinase enzyme in improving plant growth for three varieties of wheat, which are Iba 99, Sham 6 and July 2 for 45 days, after which the vegetative characteristics were taken. For cultivated plants and the efficacy of the chitinase enzyme for the shoot and root system, the induction treatment (pathogenic fungus + T. longibrachiatum + MnCl2) proved its role in the highest reduction of the severity of infection with the pathogen fungus of wheat, reaching 18.05% and the high vegetative and root growth indicators under conditions of pathogen infection and for all the studied varieties. The results of induction were due to the increased expression of the chitinase enzyme gene. RT - PCR technique was used. It showed that all the studied cultivars with 99, Sham 6 and July 2 had higher expression than the control treatment.

Keyword *: Wheat , chitinase , $MnCl_2$, Trichoderma , RT-qPCR*

Introduction

Wheat Triticum L. belongs to the Poaceae family, and it is one of the oldest agricultural crops known to man, as it is grown on a large scale and produced in large quantities. Many of the economic losses in agricultural crops are due to its infection with fungi, and the fungi of roots rot the largest share, and at the forefront of these crops is wheat and barley. The symptoms of field infection are the absence of plants from some areas or the presence of scattered areas in the field of yellowish or wilted plants due to infection with the fungi of root rot, and the infection of different wheat plants with different types of fusarium, which affects the wheat yield. The severity of the disease was inversely related to the plant height. It also significantly contributed to reducing the number of heads and their infestation with mold[1]. There are many problems facing the process of growing the wheat plant, which cause a significant reduction in its production and quality, including the pathogens that afflict it in all stages of its growth and attack its various parts, causing the deterioration of its production in terms of quantity and quality and the occurrence of economic losses, especially when the appropriate conditions are available, including disease of seed rot, roots and death. Wheat seedlings caused by the fungus Pythium spp. , Rhizoctonia solani, Fusarium spp. and other fungal species [2].The fungus Fusarium sp. Fusarium head blight (FHB) for wheat is the most important problem in cereal cultivation, which leads to large quantitative losses and deterioration of the quality of grain fields and their products. Crop losses due to FHB can be excessive and in maximum cases up to 70% [3]. Fusarium head blight (FHB) mainly caused by F. graminearum is one

of the most destructive fungal pathogens of wheatgrass and very important economically[4][5].

Many studies have emphasized the importance of enzymes in biological control, including chitin degradation enzymes, as they received great attention in this regard and were developed as biopesticides or chemical defense proteins in genetically modified plants or microbial biological control agents. The biological control of the fungal diseases that carry the soil is interrelated with the production of chitinase, as the fungi and bacteria producing the enzyme chitinase are more virulent in inhibiting the growth and killing of pathogenic fungi [6]. The chitinase enzyme is one of the hydrolysis enzymes secreted by the plant when stimulating its resistance, and its secretion to this enzyme gives it protection from pathogens and increases its resistance to pathogens at different levels as it works to release N-acetyl glucosamine upon infection [7][8] .

Some enzymes show an absolute need for a specific inorganic ion for their catalytic activity, while other enzymes show increased activity when certain cations are added to the reaction medium. Some divalent cations can replace each other, but sometimes one of them competes with the other, while inhibitors reduce the activity Catalytic enzymes[9]. Found Lee[10] that the purified chitinase from Penicillium sp. LYG0704 was inhibited by (79 and 81)% in the presence of iron ions (Fe + 2) and mercury (Hg + 2)). Respectively, while the magnesium ions (Mg + 2) and molybdenum (Mo + 2) had an inductive effect on the activity of the enzyme by (26 and 22)%, respectively, at a concentration of 1 mmol at a temperature of 37 C for an hour.Told Yi [11]that zinc ions (Zn + 2), calcium (Ca + 2) and iron (Fe + 2) had an effect on the activity of the enzyme beta 1 - 3 clokinase purified from the fungus Trichoderma viride TP09. Due to the spread of wheat root rot disease and its increase in recent years And the importance of the chitinase enzyme in biological control and the role of T. RT-PCR cannula in detecting enzymatic activity. The study aimed:

1 -Use of RT - PCR technique to determine and quantify the expressivity of the chitinase gene.

2 -The effect of mineral salts in activating and inactivating the enzyme chitinase induced by wheatgrass, class Iba 99.

3 -The effect of the mineral inhibitor MnCl2 and the chitinase enzyme on pathogenicity of Fusarium oxysporum on the wheat plant.

Materials and Methods of Work

The Effect of Mineral Salts on the Effectiveness of chitinase

This experiment was conducted on pure chitinase induced from wheatgrass type Iba 99 bioavailable using the fungus Trichoderma longibrachiatum [12]. The salts have prepared Na_2MoO_4, MgCl2, K2SO4, ZnSO4, FeCl3, LiCl, $(NH_4)SO_4$, $MgSO_4$, CoCl2, MnCl, FeSO4, Na2SO4, $MnSO_4$, NH4Cl2, CaCl2, KCl, LiF, $CuSO_4$10, CaCl2, KCl3, LiF, $CuSO_4$, 3-D, 3, 3, 3, 3, 3, 3, 3, 3, 3, CaCl2, KCl, LiF, Cu 50) Milli-Muller and add 1 ml of each concentration to 1 ml of enzyme chitinase. Then incubate in a water bath at a temperature of 35 C for an hour, then take 1 ml of it and add 1 ml of pure chitin solution to it, then put it in a water bath at a temperature of 35 For an hour, then 1 ml of DNS was added and then placed in a boiling water bath at a temperature of 100 ° C for 5 minutes. After that, the tubes were cooled with tap water. The readings were taken on a spectrophotometer with a wavelength of 540 nm and then the enzymatic activity of chitinase was estimated.

Estimate the Efficacy of the Enzyme Chitinase

The method has been folloed by Tweddell [13] for the determination of the chitinase enzyme, as the reaction mixture consisted of adding 0.5 ml of a solution of chitin (1%) and 0.5 of the enzyme extract and incubated in a water bath at a temperature of 37 C for two hours. After which 1 ml of DNS was added and the mixture was placed in a water bath at a temperature of 100 M for 5 min, the tubes were cooled and the absorbance was measured in a Spectrophotometer at a wavelength of 540 nm. The standard curve for n-style glucose amine was adopted in estimating the enzymatic activity. The

enzymatic activity was defined as the amount of enzyme required to release 1 micromol of n-stylet glucose amine per minute and according to the reaction conditions.

Effect of mineral inhibitor $MnCl_2$ and chitinase enzyme on pathogenicity of Fusarium oxysporum on wheat plant.

The potting experiment was carried out in the 2019-2020 season. Mixed soil was cleaned and sifted well, then sterilized with 5% formalin, then covered with nylon for 10 days. Then the nylon was lifted to perform a 3-day aeration of the soil. Soil was distributed in the pots at a rate of 3 kg per pot and the experiment included 5 treatments of three varieties and three replications to produce 45 experimental units as follows:

Field experiment parameters

The experiment included three varieties of wheat (Sham 6, Tamuz 2, Iba 99). The experiment also included the following treatments:

- 1Treatment of healthy plants only.

-2Treatment of the pathogen F. oxysporum only.

-3Treatment of the pathogen F. oxysporum + Trichoderma longibrachiatum (T1).

-4Treatment of the pathogen F. oxysporum + MnCl2.

-5Treatment of the pathogen F. oxysporum + Trichoderma longibrachiatum (T1) + MnCl2.

A suspension of F. oxysporum spores was added at 1 x 1010 colony forming units (CFU) / ml at 100 ml per pot. After 3 days, a suspension of Trichoderm longibrachiatum (T1) was added at 1 x 1010 CFU / ml at 100 ml. For each pot, 15 seeds of each variety of wheat were planted separately for each pot. As for the treatment of manganese chloride MnCl2, 100 ml of it was added at a concentration of 10 mmol per pot after planting the seeds. The plant service was carried out from bush hoe (if any) and the irrigation was carried out according to the plant's need.

Studied traits

The studied traits were taken after 45 days of germination, except for the efficacy of chitinase, which were tested after two weeks of germination

Germination Rate

It was calculated by dividing the number of plants growing in pots for each variety of wheat by 3 replications for each treatment by the total number of seeds planted.

Chlorophyll Estimate

The percentage of chlorophyll in wheat plant leaves was estimated in the morning using a Chlorophyll meter, as three plants were taken from each repeat randomly for all varieties and treatments.

Weights Estimate for Dry and Wet Root and Vegetable Masses

Samples were taken randomly from the pots from all the duplicates, then the plants were cleaned and washed with water to get rid of soil residues on the roots. Then the weights were measured for the vegetative and root system (wet), after that, the plants were dried on sunlight until the weight stabilized and according to the dry weight (in grams) using the sensitive balance.

Estimate injury severity

According to Mckinney's equation[14], the percentage of injury severity was calculated in all treatments depending on the appearance of the injury and according to the pathological evidence adopted by it [15].

The degree appearance of the injury

0 The plant is healthy, the root system is large, and the roots are white

1 slight brown discoloration on the roots and yellowing of a specified number of leaves

2 Full coloration of the roots, with a complete

yellowing of leaves

3 The coloration extends from the roots to the bases of the stems

4 general death

The severity of the injury was estimated according to the following equation:

$$\text{Rity of the injury\%} \frac{\text{Number of plants in grade 0} \times 0 + \text{number of plants in degree 1} \times 1 + \text{number of plants in degree 4} \times 4}{\textit{Total examined plants} \times \textit{The hightest category}} \times 100\%$$

The Effectiveness of Chitinase in the Root System

The efficacy of the chitinase enzyme was estimated by the root group of the three varieties after two weeks of germination, as the plant was uprooted and washed well with sterile distilled water. After which the root group was separated from the vegetative and weighed 1 g per portion and placed in a ceramic mortar each part separately and 10 ml of acetate buffer solution was placed on top pH 5.6 The parts were crushed with the solution inside an ice bath until the plant part was well crushed and the plant cells were ruptured. After that, the solution was filtered and the solution was centrifuged at 5000 revolutions / minute for 10 minutes for the solution to get rid of the plant parts, the scent which represented the raw chitinase enzyme was collected and kept in the refrigerator at a temperature of 4 C until use, then the chitinase activity was estimated.

Extraction of RNA

The RNA was isolated from fungi and wheat plants of the three varieties using the GENEzol TM TriRNA Pure Kit provided by the Taiwanese company Geneaid, to extract the ribonucleic acid according to the instructions of the supplier.

Measurement of RNA Concentration and Purity

The concentration and purity of the extracted RNA was measured using a nucleic acid concentration and purity meter (NANO DROP). RNA purity was measured by dividing the RNA absorbance at length 230 by the absorbance product at 280, while the purity was calculated using the following equation: Concentration (µg / ml) = OD 260 x dilution factor x 40 µg / ml.

Converting RNA into cDNA

The conversion process was performed using the AccuPower RocketScript TM RT PreMix kit prepared from the Korean company BIONEER. The cDNA reaction mix includes 18 µl of template RNA (100pg), 2 µl of Oligo dt 20 (50 pmoles), after that, the reaction mixture was transferred to transparent white tubes of 0.2 ml, and the mixture was mixed with the Vortex device for 3 minutes at a speed of 3000 rpm. The reaction conditions as follow: Primer annealing (Oligo dT 20) at37 ℃ for 10 min, cDNA synthesis at 42 ℃ for 60 min and heat inactivation at 95 ℃ for 3 min.

Real Time PCR reaction

Prepare the qPCR mixture

The reaction mixture was prepared using the AccuPower GreenStar TM qPCR PreMix prepared from the Korean company BIONEER and according to the company's instructions, 5 ml of cDNA and 3 ml of the initiator (F + R) were added at a concentration of 10 pmole and 12 l DEPC - distilled water and then the mixture was applied In opaque white tubes of 0.2 ml for the kit and the Real Time PCR machine, then all the tubes were transferred to the Votrex machine to mix the mixture.

Prefixes used in the Reaction

The primers for the chitinase gene for wheatgrass were designed by Dr. Ahmed Abdul-Jabbar Sulaiman, prepared from Macrogen; the first primmer was Wheat

Primer "F: 5'-CTACACGTACGACGCCTTCA- 3' " and " R: 5'-TGGCCTTGCTTATCTCTTCC- 3' ",whose annealing temperature was 58 and it's produced band was 194 bp. The second primer was ADP "F: 5'-CCTCATGGTCGGTCTCGATG- 3' " and "R: 5'-GGATGGTGGTGACGATCTCT- 3' ". the annealing temperature of ADP was 59 and it's produced band was 80 bp.

The prefixes were dissolved with Nuclease Free Water to obtain a final concentration of 100 pmole as a storage solution and kept at a temperature of -20℃.Upon reaction, 10 pmole of the storage solution was prepared by taking 10 ml of the storage initiator and adding to it 90 ml of Nuclease Free Water to obtain a final volume of 100ml that was involved in the qPCR.

The program used to amplify the prefixes in a real-time polymerase chain reaction (qPCR) is shown below:

Step	Temperature	Time	Cycle
Predenaturation	95℃	3 min	1
Denaturation	95℃	20 Sec	40
Annealing	55℃	40 Sec	
Detection (Scan)		1min	
Melting	55 - 95℃	1 min	1
Incubated	25℃	1 min	

Statistical Analysis

The laboratory experiments and the CRD were carried out and the data were analyzed using the SPSS program, and the averages were compared according to the Least Significant Deferences LDS at a probability level of 0.05 [16].

Results and Discussion

Inhibition and Activation of Minerals on the Effectiveness of Chitinase

The results indicated that if Manganese Chloride recorded the highest activity of the enzyme, the percentage increase in activity was (74.82, 71.11, 66.33) %, followed by CaCl2 (43.76, 57.63, 73.98%). The lowest activity of the enzyme was recorded when using K2SO4, as the percentage increase in activity was (12.23, 7.03, 4.22% for the concentrations used respectively.

The results also indicated that there is a inhibitory effect for all EDTA concentrations, as the percentage of chitinase reductase activity was (22.75, 34.12, 56.43), followed by the salt NH4Cl, as the percentage of enzyme reductase was (18.87, 33.51, 42.32)%. These results were in agreement with Haider [17], who mentioned there is an induced effect of manganese chloride on the activity of the chitinase enzyme, but for calcium chloride the result was opposite to what appeared to us as it had a inhibitory effect on the enzyme activity. The results are also in agreement with those found by Adrangi [18] showed that manganese ions had an effect on the two forms of the intracellular enzyme (chi-56 and chi-64) purified from Massilia timonae. As for the effect of EDTA on decreasing the enzyme's effectiveness, it was identical to what Al- Fakihi [19] found in his study on the effectiveness of purified alpha amylase from malt type Iba 99, as it was also found a decrease in the

activity of the enzyme by increasing the concentration of EDTA, but its results were opposite to what we found with regard to manganese chloride. The activity of the enzyme decreased when incubated with this salt.

The higher activity in the presence of Mn ions may be attributed to the enzyme's need for this ion in the form of co-factor. The evidence for the enzyme's requirement for this ion is the lower activity when using the EDTA chelating agent. The decrease in activity when incubating the enzyme with the mineral salts under study may be attributed to the effect of these salts on the enzyme synthesis or at the enzyme's active sites on the one hand or in the base material on the other hand through the formation of complexes that impede the enzyme's attachment to the base material. The mechanism by which the positive and negative ions activating the enzyme works is also different, as the ion may change the interstitial direction of the protein in order to allow the correct association between the enzyme and its base material[20].

Effect of mineral inhibitor $MnCl_2$ and chitinase enzyme on pathogenicity of Fusarium oxysporum on wheat plant.

The Effect of Treatment with the Fungus T. longibrachiatum T1 and Manganese Chloride on Shoots Weight

The results listed in Table (1) show that the highest shoots weight at the level of treatments was when treating the fungus Tricho. + Mncl2 in the presence of the pathogen F. oxysporum, which reached 0.82 g, followed by the Trichoderma treatment in the presence of the pathogen, which reached 0.67 g compared to the weight of the shoot in the treatment of healthy plants (control), which amounted to 0.66 g. Whereas, the lowest weight of the shoot total was recorded in the MnCl2 treatment with the presence of The pathogenic fungus was 0.25 g, which did not differ significantly compared to the treatment of pathogenic fungi only (0.21 g). As for the average of the varieties, cultivar Iba 99 showed the highest weight of the shoot total, which reached 0.56 g, while the lowest weight of the shoots was 0.49 g in the Sham 6 cultivar. As for the interaction, the cultivar showed a positive 99 with the highest shoot weight of 0.86 g in the treatment of the pathogen + Tricho. + MnCl2 without significant differences with the control treatment compared to the minimum shoot weight of 0.17 g in the cultivar Cham 6 in the treatment of pathogenic fungi only.

Table (1) Effect of Treatment with the fungus T. longibrachiatum T1 and manganese chloride on shoots weight (gm) of three varieties of Iraqi wheat under conditions of infection with the pathogen F. oxysporum.

Average of transactions	Ibaa Class 99	Sham Class 6	July Class 2	Transactions
0.66	0.69	0.62	0.67	Control (healthy plant)
0.21	0.25	0.17	0.21	Pathogen fungi only
0.67	0.70	0.64	0.68	Pathogen fungi + Tricho.
0.25	0.28	0.21	0.25	Pathogen fungi + MnCl2
0.82	0.86	0.79	0.82	Pathogen fungi + Tricho. + MnCl2
	0.56	0.49	0.53	Average varieties
For Items = 0.071 Transactions = 0.084 Items x Transactions = 0.13				LSD 0.05

The Effect of Treatment with the Fungus T.longibrachiatum T1 and Manganese Chloride in Root Mass Weight

The results listed in table (2) show that the highest weight of the root group at the level of treatments was when treating the fungus Tricho. + Mncl2 in the presence of the pathogen F. oxysporum, which reached 0.53 g, followed by the treatment of Trichoderma in the presence of the pathogen, which reached 0.43 g compared to the weight of the root total in the treatment of healthy plants (control), which amounted to 0.4 g, while the lowest weight of the root group was recorded in the treatment of MnCl2 with the presence of The pathogenic mushrooms, which amounted to 0.15 gm, did not differ significantly compared to the treatment of pathogenic fungi only (0.13g).

As for average cultivars, cultivar Iba 99 showed the highest root total weight of 0.372 g, while the lowest root total weight was 0.29 g in Sham 6. With regard to the interaction, the cultivar showed a test of 99 with the highest root total weight of 0.59 g in the treatment of the pathogen + Tricho. + Mncl2, compared to the minimum root weight, was 0.11 g for the cultivar Cham 6, in the treatment of pathogenic fungi only.

Table (2) the effect of treatment with the fungus T. longibrachiatum and manganese chloride on the weight of the root system (gm) of three varieties of Iraqi wheat under conditions of infection with the pathogen F. oxysporum.

Average Transaction	Ibaa Class 99	Sham Class 6	July Class 2	Transactions
0.4	0.45	0.35	0.40	Control (healthy plant)
0.13	0.16	0.11	0.12	Pathogen fungi only
0.43	0.48	0.38	0.42	Pathogen fungi + Tricho
0.15	0.18	0.13	0.13	MnCl2+Pathogen fungi
0.53	0.59	0.48	0.51	Pathogen fungi + Tricho. + MnCl2
	0.37	0.29	0.316	Average varieties
For Items = 0.051 Transactions = 0.063 Items x Transactions = 0.086				LSD 0.05

The Effect of Treatment with the fungus T. longibrachiatum T1 and manganese chloride on the efficacy of the enzyme ketinase radical total.

The results listed in Table (3) show that the highest efficacy of the enzyme chitinase is in the area. Radical on the level of transactions was the treatment of Tricho mushrooms. + MnCl2 in the presence of the pathogen F. oxysporum, which reached 1.92 units / ml, followed by the treatment of Trichoderma in the presence of the pathogen, reaching 1.75 units / ml, compared to the effectiveness of the enzyme in the treatment of healthy plants (control), which amounted to 0.07 units / ml, while the lowest activity of the enzyme was recorded. Chitinase, MG. Radical treatment of MnCl2 in the presence of the pathogen, reaching 1.26 units / ml, which was not significantly different compared to the treatment of pathogenic fungi only (1.08 units / ml).

As for the average of the varieties, class Iba 99 showed the highest efficacy of the enzyme chitinase Mg. Radicals of 1.36 units / ml, while the lowest activity of

the enzyme chitinase was mg. Radical was 1.03 units / mL in the Sham 6 cultivar. As for the interaction, the cultivar showed 99 highest efficacy of the enzyme chitinase Mg. Radicals amounted to 2.07 units / ml in the treatment of pathogenic fungi + Tricho. + Mncl2 compared to the lowest efficacy of the Mg chitinase enzyme. Radicals amounted to 0.07 units / mL in the Sham 6 cultivar in the control treatment.

Table (3) the effect of treatment with the fungus T. longibrachiatum T1 and manganese chloride on the efficacy of the enzyme chitinase. Root (unit / ml) for three varieties of Iraqi wheat under conditions of infection with the pathogen F. oxysporum

Average of transactions	Class Ibaa 99	Class Sham 6	July class 2	Transactions
0.07	0.07	0.07	0.07	Control (healthy plant)
1.08	1.31	0.82	1.12	Pathogen fungi only
1.75	1.87	1.55	1.82	Pathogen fungi + Tricho.
1.26	1.50	0.96	1.31	Pathogen fungi + MnCl2
1.92	2.07	1.76	1.93	Pathogen fungi + Tricho. + MnCl2
	1.36	1.03	1.25	Average of Transactions
For Items = 0.061 Transactions = 0.066 Items x Transactions = 0.096				LSD 0.05

The Effect of Treatment with the Fungus T. longibrachiatum T1 and Manganese Chloride on Pathogen Severity

The results listed in Table (4) show that the highest severity of pathogen infection at the level of treatments was when treating the pathogen only. As it reached 75.44%, followed by MnCl2 treatment in the presence of the pathogen, reaching 73.94%, compared to the severity of pathogen infection in the treatment of healthy plants (control), which amounted to 0 %. While the lowest pathogen severity was recorded in Tricho treatment. + MnCl2 in the presence of the pathogen, reaching 18.05%, which does not differ significantly compared to the treatment of the fungus Trichoderma with the presence of the pathogen (23.71%). This is confirmed by the study of Hasan & Aldoury [21]. that there is a significant superiority in the severity of infection within the treatment of pathogenic fungi only compared to the rest of the treatments.

As for the average of the varieties, the Sham 6 variety showed the highest pathogen severity, which was 40.0%, while the lowest pathogen severity was 36.22%, in the IBA class 99. As for the interaction, the cultivar Sham 6 showed the highest pathogen intensity, which was 78.40% in the treatment of pathogenic fungi only, compared to the lowest pathogen severity, which was 17.05%, in the cultivar, Ibb 99, in the treatment of the pathogen + Tricho. + MnCl2.

Table (4) Effect of treatment with the fungus T. longibrachiatum T1 and manganese chloride on the severity of pathogen infection (%) for three varieties of Iraqi wheat under conditions of infection with the pathogen F. oxysporum

Average of transactions	Class IBA 99	Class Sham 6	July class 2	Transactions
0	0.0	0.0	0.0	Control (healthy plant(
75.44	71.37	78.40	76.55	Pathogen fungi only
23.71	22.66	25.63	22.85	Mushrooms. pathogen + Tricho
73.94	70.04	76.77	75	Pathogen fungi + MnCl2
18.05	17.05	19.23	17.87	Pathogen fungi + Tricho. + MnCl2
	36.22	40.00	38.45	Average varieties
For Items = 1.21 Transactions = 1.43 Items x Transactions = 3.05				LSD 0.05

Table (5) shows the results of gene expression and its values for the chitinase gene in the wheat plant

Transactions	Class Ebaa 99				Class Sham 6				2July class 2			
	Ct	ΔCT	ΔΔCT	Fold	Ct	ΔCT	ΔΔCT	Fold	Ct	ΔCT	ΔΔCT	Fold
Pathogen fungi only	29.21	4.75	-1.86	3.630077	15.97	-6.17	-3.38	10.41073	19.69	-1.43	0.06	0.959264
Pathogen fungi + Tricho.	24.38	3.32	-3.29	9.781122	20.02	2.68	-6.53	92.41147	27.55	-4.31	-2.82	7.061624
Mushroom pathogen + MnCl2	28.50	13.29	6.68	0.009753	19.42	5.14	-2.07	4.198867	19.21	-1.96	-0.47	1.385109
Pathogen fungi + Tricho. + MnCl2	17.73	-3.6	-10.21	1184.449	17.51	-4.74	-11.95	3956.475	17.55	-7.74	-4.25	19.02731

It was noticed from the results of the experiment that the treatment with the pathogenic fungus only gave the lowest values for the rate of germination, chlorophyll, plant height and dry weight for both vegetable and root parts; as well as, the experiment gave the highest rates for the dead cells, highest harshness and infection rates; this indicates that the virulence of the pathogenic fungus and the occurrence of damage in the plant, Pathogenic fungus enzymes such as cellulose, pectinase and protease cause degradation of the root and components of plant cells; as well as, the production of pathogenic fungus for fungal toxins that directly effect on plant growth, inversely the low vegetative growth traits and high indicators of injury[22]. The treatment

with MnCl2 alone did not positively affect on the characteristics of vegetative growth nor did it affect in the reduction of plant diseases and this was laboratory proven by Ahmed[23]as it may provide Mn element to the pathogen, while the treatment with *Trichoderma* improved the proportions of seed germination and all the characteristics of vegetative growth and reduced the incidence and severity of the infection due to the mechanisms' action of *Trichoderma* and their significant role in vital resistance such as antibiosis, competition, Mycoparasitism, Enzymes, resistance induction, production of plant hormones, increased readiness of elements and tolerance of external stress. In case of treating *Trichoderma*with MnCl2 treatment, it is observed that there is a significant superiority statistically to all the characteristics of vegetative plant growth and a decrease in infection indicators, which due to the role of *Trichoderma* and its direct and indirect mechanisms against the pathogen. It should be mentioned that the important factor is activation of "Chitinase", which is well known as Mn dependent enzyme.

Real Time PCR Gene Expression Results

RT-qPCR was used to detect and estimate the level of gene expression for the gene (Primer Chitinase Wheat) responsible for producing the enzyme chitinase in wheat plant. The ADP gene was used as a reference gene, and the relative quantitative expression method was used where data are presented for the reference gene where the expression is expressed. For reference genes in all cells of the organism under normal and pathological conditions, and although some reference genes are expressed at constant levels in most cases. There are genes whose expression may change depending on the situation, the interval (Ct) where Ct is the number of cycles required for the fluorescence to be emitted from the dye to reach the threshold level to detect the reaction.

The results listed in Table (5) show the gene expression values represented by the value of Cycle threshold (Ct), which indicates the degree of gene expression inversely (as the lower the value of Ct, the more the gene expression process increases), as well as the Fold Fold, where the results showed the high gene expression of the chitinase gene. In wheat plants, class Ibaa 99 for treatment No. 4 (pathogenic mushrooms + Tricho. + MnCl2), which amounted to 1184.449 compared to the control treatment, in which the value of the volt was 1, followed by treatment No. 2 for the same variety (pathogenic mushrooms + Tricho), reaching 9.78, i.e., it increased by approximately 9 times for the Control treatment, which equals 1.

As for the variety Sham 6, the results showed that the highest gene expression for treatment No. 4 (pathogen + Tricho) was 3956.475 compared to the Fold value for Control (1), followed by treatment No. 2 (pathogenic fungus + Tricho), reaching 92,411 compared to A control transaction.

As for the July 2 variety, the results of its gene expression showed that the highest value was recorded for treatment No. 4 (pathogenic fungus + Tricho), reaching 19.027, followed by treatment No. 2 (pathogenic fungus + Tricho), which reached 7, which increased by 7 times over the control treatment (1).

It is evident from the results shown above that the gene expression increased for the three cultivars by the last treatment No. 4 (pathogenic fungus + Tricho), but the highest gene expression was for type Sham 6. The reason for the high gene expression of the chitinase gene in Treatment No. (4) and for the three varieties is due to the treatment of plants with the fungus Trichodrema, which stimulates biological resistance and thus stimulates induced systemic resistance (ISR) in addition to the presence of salt (MnCl2), which acts synergistically with the fungus and leads to increased stimulation. The production of the enzyme chitinase and thus leads to the stimulation of acquired systemic resistance (SAR) in the plant, which lies in the stimulation of genes encoding some pathogen-related proteins such as the enzyme chitinase, which has a high activity in analyzing the cell wall of pathogenic fungi and improving plant growth[24][25]. This has been demonstrated in our previous experiments in induction of chitinase enzyme biosynthesis in 12 varieties of wheat plants, as the mushroom-induced

varieties showed the highest efficacy of the chitinase enzyme compared to the enzyme activity of the varieties without the inducement factor. As well as what was demonstrated in the experiment of steroids and enzyme inhibitors of a group A wide range of salts and three concentrations, as the MnCl2 salt recorded the highest activity of the enzyme and of the three concentrations. In another experiment on the effect of Mineralogenerator MnCl2 and the enzyme chitinase extracted from wheat induced by Trichoderma fungus on some pathogenic fungi. The results proved that the presence of the salt and enzyme mixture in the middle of the development of pathogenic fungi inhibits the growth of these fungi compared to the control plate. Thus, the salt and enzyme mixture inhibited the growth of pathogenic fungi due to the maximum effectiveness of the enzyme Manganese ions. The results of the gene expression were consistent with the findings of Al-Jassani [26]in his study of three varieties of date palms treated with salicylic acid and hydrogen peroxide. Which led to an increase in the gene expression of the gene responsible for the enzyme Superoxide SOD dismutase (which is considered one of the defense enzymes to reduce the damage caused by exposure to The plant under saline stress conditions). As the variety Burhi gave the highest gene expression when treated with salicylic acid 500 mg, when the value of Folding (3.36) was reached. As for the crescent variety, the highest value of gene expression in the treatment of salicylic acid was 250 mg, as the value of Folding (5.09) and the unknown variety gave the highest an expression value in the treatment of hydrogen peroxide at a concentration of 6%, as it reached (2.82).

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