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Research Article

Role of Hydrogen Peroxide (H_2O_2) and *Trichoderma koningii* in Reducing Root Rot Disease of Tomato Caused by *Fusarium solani*

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Abstract: Two isolates of the pathogenic fungus *Fusarium solani* the causative of tomato root rot disease named Fs1 and Fs2 were isolated from tomato plants infected with root rot disease. Results showed that both examined isolates had significant effects on the percentage of seed germination of tomato and damping-off disease. Thus, isolate Fs1 was more effective than isolate Fs2, as it recorded a percentage of germination and damping-off 56.6 and 71.6% respectively, compared to the control treatment, which recorded 100 and 0% respectively. It was found that the use of hydrogen peroxide (H_2O_2) at concentrations of 50, 100 and 200 ppm had a significant effect on the fungal radial growth, dry weight (DW) and sporulation of *F. solani*. Antifungal activity of H_2O_2 appeared even at the lowest concentration (50 ppm), which inhibited radial growth to 49.33%, and decreased the dry weight to 498 mg, and the sporulation to 3.75×10^6 spores. Additionally, the results indicated significant inhibitory effects of hydrogen peroxide (H_2O_2) and the bioagent *Trichoderma koningii* on *F. solani* growth. It was noticed that H_2O_2 has compatible effects with *T. koningii*, where the antagonistic ability of *T. koningii* against *F. solani* increased when the concentration of H_2O_2 had increased. The results revealed that the treatment *F. solani* + H_2O_2 (200 ppm) + *T. koningii* significantly reduced the percentage of damping-off and plant survival to 9.40 and 5.39% respectively, in comparison with the control treatment and *F. solani* alone treatment, which reached 20.22, 14.48, 34.32 and 67.41% respectively.

Keywords: Biological Control, Damping-off, *Fusarium solani*, Hydrogen Peroxide, Stimulation of Resistance, *Solanum lycopersicum L.*

1. INTRODUCTION

Tomato (*Solanum lycopersicum* L.) is the most important economic vegetable produced in the world in terms of cultivated land area and consumption, with high nutrients including vitamins such as A, B and C, in addition to fructose, fats, protein, sucrose and some minerals including calcium, magnesium and phosphorus [1]. Tomato also contains antioxidant compounds including phenolic compounds, carotenoids and ascorbic acid [2].The total production of fresh tomato in the world reached to 180 million tons in 2019 with an area of 5 million hectares of agricultural land [3].The production of tomato crops in Iraq was estimated at 400,542 tons in an area of 79,269 dunums for the year 2020, and the production of Misan Governorate was estimated at 159,306 kg in an area of 53 dunums [4]. Tomato is a vulnerable plant that usaually infects with several pathogens, most importantly the pathogenic fungus *F. solani*, which causes wilting, root rot and damping-off diseases and causes great economic losses. Soil-borne diseases caused by many fungi such as *F. solani* were considered a problem for crop production, especially for vegetables. These pathogens often live for long periods on the host plant residues or organic matter in the soil. As these soil-borne fungi have become resistant to chemical fungicides, so, biological control is considered a safe and effective new alternative to fungicides in controlling plant diseases [5, 6].

Using the bioagent microorganisms for controlling soil borne fungi is considered as one of the most acceptable procedures, because of their safety for humans, animals, non-target organisms

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and environment, while chemical fungicides may lead to contaminate the soil and have negative effects on human and animal health [7]. Several fungi have been applied for controlling plant diseases biologically. The important one is *Trichoderma* spp., this importance could be attributed to their inhibition mechanisms against pathogens, including parasitism, competition, producing antibiotics, etc. [8]. Many studies showed that *Trichoderma* promotes plant growth and development and has the ability to induce plant defense responses against different pathogens [9]. Zaghloul *et al.* [10] indicate that *Trichoderma* reduced root rot disease of tomato and stimulated a high plant production.

There are many previous studies were carried out in southern of Iraq on the ability of several species of the bioagent *Trichoderma* including *T. harzianum*, *T. longibrachiatum* for controlling damping off and root rot disease of tomato, eggplant and cucumber crops causing by *Fusarium solani* and *F. oxysporum*, [11-13]. Additionally, it was noticed that *T. harzianum, T. longibrachiatum*, *T. koningii* and *T. viride* significantly decreased the fusarium wilt disease of tomato which is caused by *F. oxysporum* f.sp. *lycopersici* [14, 15]. The use of inducing factors like hydrogen peroxide (H_2O_2), is one of the most important and modern methods for controlling plant diseases by activating different protective mechanisms designed to prevent the reproduction and spread of pathogens, such as defense mechanisms, rapid production of reactive oxygen types, modifications in cell wall composition and accumulation of secondary antimicrobial metabolites such as phytoalexins, they activate or synthesize defense peptides and proteins [16, 17]. The current study aimed to evaluate efficiency of the bioagent *T. koningii* and the inducing factor hydrogen peroxide (H_2O_2), individually or in combination for inhibiting the growth of the pathogenic fungus *F. solani*, as well as their ability to control root rot disease in tomato.

2. MATERIALS AND METHODS

The laboratorial experiments were performed in the laboratories of Plant Protection Department / College of Agriculture/University of Misan. The pot experiments were performed in the Agriculture College wooden canopy/University of Misan, in the year 2022.

2.1. Isolation and Identification of the Pathogenic Fungus *Fusarium solani*

Symptomatic plant materials were collected randomly from different fields. Infected roots were separated and washed carefully with running tap water and dried by using filter papers. Pieces with 1 cm length for each one was prepared and sterilized with 2% sodium hypochlorite (NaOCl) solution for 2-3 minutes, washed with sterile distilled water and dried again by using filter papers. Five sterilized pieces were put in each Petri dish containing sterilized PDA medium (200 gm potato, 20 gm dextrose and 20 gm Agar) with Chloramphenicol at a concentration of 250 mg/l. The plates were put in the incubator at a temperature of 25 ± 2 °C for a period of 3-5 days. The fungus was identified depending on its macro and micro scopic features according to Leslie and Summerell [18].

2.2. Pathogenicity Test

The pathogenicity of two isolates of *F. solani* was tested. Soil sterilized with formalin, left for about ten days and put in 1 kg plastic pots with equal quantities. The inoculum of *F. solani* which is grew on the seeds of local millet (*Panicum miliaceum* L.) was mixed well with the soil and added to each pot at a rate of 1% w/w. Millet seeds free from pathogenic fungus were added as control treatment at the same rate [19]. The soil was irrigated with water and the pots were covered with polyethylene bags for three days. Ten superficially sterilized seeds of tomato were put in each pot. Three replicates of each treatment including the control were applied. After one week, the germinated seed percentage was estimated as follows:

$$\%\ Germination = \frac{No.\ of\ geminated\ seeds}{Total\ No.\ of\ seeds} \times 100$$

The percentage of damping-off was calculated after one month as follows:

$$\%\ Damping - off = \frac{No.of\ dead\ seedlings}{Total\ No.of\ seedlings} \times 100$$

2.3. Effect of Hydrogen Peroxide (H_2O_2) on the Growth of *Fusarium solani*

This test was carried out using the most virulence isolates of the fungus *F. solani*. The technique of poisoned culture medium was used, where PDA medium was treated with concentrations of 50, 100 and 200 ppm of H_2O_2 and poured into Petri

dishes (9 cm diameter). A disc (0.5 cm diameter) was taken from an edge of 7-day-old pure colony of *F. solani* and put in the center of each plate, the control treatment was left without adding H_2O_2. Each treatment was replicated for three times. All plates were kept in incubator at a temperature of 25 ± 2 °C. The fungal radial growth was measured when the fungal growth in the control treatment reached the edge of the plate, the percentage of fungal growth was calculated by using the equation suggested by Ahmed [20] as follows:

$$Reduction\ in\ linear\ growth = \frac{G1 - G2}{G1} \times 100$$

Where, G1 = Linear growth of the fungus in the control. G2 = Linear growth of the fungus in the treatment.

2.4. Effect of Hydrogen Peroxide (H_2O_2) on Dry Weight of *Fusarium solani* Biomass

The toxicity of hydrogen peroxide (H_2O_2) at concentrations of 50, 100 and 200 ppm were examined against the growth of *F. solani* in potato dextrose broth (PDB) medium (three flasks for each concentration). Equal discs with a diameter of 0.5 cm for each one were taken from an edge of 7-day-old colony of *F. solani* and added to 250 ml flasks containing 50 ml sterile PDB with concentrations of 50, 100 and 200 ppm H_2O_2. The control treatment had no H_2O_2. All flasks were transferred to the incubator and incubated for ten days at a temperature of 25 ± 2 °C [21]. The mass dry weight (mg MDW per 100 ml liquid medium) of different treatments was measured after separating the fungal mass by filtration of the PDB with filter paper. The samples were dried at 60 °C for two days by using the oven. The percentage of fungal growth inhibition was calculated according to Sutton *et al*. [22] as follows:

$$\%Inhibition = \frac{MDW of\ control - MDW of\ treatment}{MDW of\ control} \times 100$$

2.5. Effect of Hydrogen Peroxide (H_2O_2) on Sporulation Rate of *Fusarium solani*

After calculating the growth rate of the different treatments in the media, *F. solani* isolate was grown in 9 cm diameter Petri dishes supplied with PDA medium treated with three concentrations of hydrogen peroxide, 50, 100 and 200 PPM, with three replicates for each concentration, and incubated for 10 days at a temperature of 25 ± 2 °C. One disc with a diameter of 0.5 cm was taken by a sterile cork borer and put in each test tube containing 10 ml of sterile distilled water. Each treatment was replicated three times. All tubes were shaken well for about five minutes with a vibrator to release spores from the conidiophores. The rate of sporulation of *F. solani* in 1 ml was calculated by using a hemocytometer, and the rate of the number of spores in a 0.5 cm disc was calculated by multiplying the rate of the number of spores in 1 ml × 10.

2.6. *In vitro* Effect of Combination of H_2O_2 and *Trichoderma koningii* on the Growth of *Fusarium solani*

Hydrogen peroxide (H_2O_2) at the concentrations of 50, 100 and 200 PPM were added to sterile PDA medium and poured into Petri dishes (20 ml /dish) and left to solidify. Each plate was divided into two equal parts. A disc with a diameter of 0.5 cm was taken from the edge of 7-day old colony of the bioagent *T. koningii* and placed in the center of one part of the plate, while the other part has been inoculated with a 0.5 cm disc of the pathogenic fungus *F. solani*. A control treatment was applied by inoculating the center of the Petri dish with a 0.5 cm disc of *F. solani* only. Each treatment was triplicated. All plates were transferred to the incubator with a degree of 25 ± 2 °C. Both pathogenic and bioagent fungal growth was measured regularly. When the growth of *F. solani* in control treatment reached the plate edge, the percentage of fungal growth inhibition (MGI%) for all treatments was estimated according to EL-Ashmony *et al*. [21] according to the following equation:

$$MGI\% = \frac{(dc - dt)}{dc} \times 100$$

Where, dc = fungal colony diameter in the control, dt= fungal colony diameter in the treatment.

2.7. Preparation of the Fungal Inoculum of the Pathogenic and the Bioagent Fungi

Inoculum of *F. solani* and the bioagent *T. koningii* were prepared according to [19]. as follows: Local millet seeds (*Panicum miliaceum* L.) were washed with tap water carefully. 150 gm of the seeds were put in each 250 ml flask and autoclaved for 1h at a temperature of 121 °C and a pressure of

15 pound/inch^2. Each flask was inoculated with 5 discs taken from the edge of a 7-day-old colony of each of *F. solani* and *T. koningii* separately. Finally, the flasks were transferred to the incubator and incubated for 14 days at a temperature of 25 ± 2 °C with shaking them well every two days to distribute the fungal inoculum on the seed surface.

2.8. The Efficacy of Hydrogen Peroxide (H_2O_2) and Bioagent *T. koningii* Treatments in Reducing *F. solani* the Causal Agent of Tomato Root Rot Disease in Pots

Plastic pots (5 kg capacity) containing sterilized soil with formalin were prepared. The bioagent *T. koningii* grown on millet seeds was added to the soil with an average of 1% w/w, mixed well and left for three days with irrigation [19]. The pathogenic fungus *F. solani* was added to the soil with same rate after three days with continuous irrigation. After another three days, ten seeds of local variety of tomato treated with two concentrations of hydrogen peroxide (H_2O_2) 50 and 200 ppm each separately were planted in each pot. Control treatment was left without adding anything. Each treatment was triplicated. All pots had been distributed in the plastic house randomly. The pots were irrigated as the field capacity. The treatments were conducted as follows:

Control, pathogenic fungus *F. solani*, bioagent *T. koningii*, H_2O_2 (50 ppm), H_2O_2 (200 ppm), *F. solani* + *T. koningii, F. solani* + H_2O_2 (50 ppm), *F. solani* + H_2O_2 (200 ppm), *F. solani* + *T. koningii* + H_2O_2 (50 ppm), *F. solani* + *T. koningii* + H_2O_2 (200 ppm).

After 30 days, the percentage of damping-off was calculated, while the percentage of disease severity was calculated after 90 days based on five-grade disease index suggested by Dorrance *et al*. [23], where 0 = no root rot appearance, 1 = appearance ulceration or visible discoloration on 1-33% of the roots, 2 = root rot in approximately 34-50%, 3 = root rot in approximately 51-80%, 4 = root rot more than 81% or plant death.

The disease severity index (DSI) was estimated according to Liu *et al*. [24] as the following equation:

$$DSI = \frac{\Sigma d}{d\,max \times n} \times 100$$

Where, d represents the disease rating possible, d max refers to the maximum disease rating and n represents the total number of plants examined in each replicate.

Statistical analysis

A completely randomized design (CRD) was applied for laboratory and pot experiments. To compare the means, the least significant difference (LSD) test was applied with a probability 0.01 for laboratory experiments and 0.05 for pot experiments. Statistical program (SPSS) version 23 was used for analyzing data.

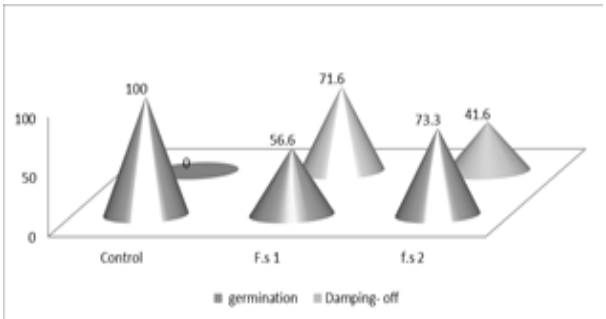
3. RESULTS AND DISCUSSION

3.1. Isolation and Identification of *Fusarium solani*

Two isolates of *F. solani* were isolated from the infected roots of tomato. The colony of *F. solani* showed a white to cream mycelium on PDA, reverse pale, septate hyphae, macroconidia straight with 3-4 septa, microconidia fusiform or kidney shaped with no or one septum, Chlamydospores spherical or ovoid shaped, produced singly or in pairs. These characteristics have been agreed with [25, 26].

3.2. Pathogenicity Test of the Two Isolates of *F. solani* in Pots

The results (Figure 1) elucidated that the two isolates of *F. solani* (Fs1 and Fs2) had a significant effect on tomato seeds germination percentage and damping-off percentage. It was found that the percent of germination and damping-off in the treatment Fs1 were 56.6 and 71.6% respectively, and significantly differed from the isolate Fs2 which recorded 73.3 and 41.6% respectively. Both isolates were significantly differed from the control treatment, which amounted to 100 and 0% respectively. This



L.S.D $_{0.01}$ (Germination) = 6.8 L.S.D $_{0.01}$ (Damping-off) = 16.1

Fig. 1. Pathogenicity test of *F. solani* isolates in the pots.

result agreed with [12, 13], who confirmed that *F. solani* caused root rot disease on cucumber and eggplant in the pots. The differences between the effect of both isolates could be attributed to the genetic differences between them and differences in their ability to produce fusaric acid, the potent phytotoxin [27], as well as its production of pectin and cellulose-degrading enzymes such as phosphatase, pectinase, cellulase, methylesterase and pectinmethylhydrase and production of toxins, phenolic and glycosidic compounds [28].

3.3. *In vitro* Effect of Hydrogen Peroxide (H_2O_2) on the Growth of *F. solani*

Table (1) showed the effect of H_2O_2 on fungal radial growth, fungal dry weight (DW) and sporulation of *F. solani* which varied according to the examined concentrations, the concentration 50 ppm had the lowest effect where the fungal radial growth, fungal dry weight and sporulation amounted to 49.33 mm, 498 mg and 3.75×10^6 spores/ml respectively, while the concentration 200 ppm gave a highest effect and significantly reduced the fungal radial growth, fungal dry weight and sporulation, as they reached 22.04 mm, 124 mg and 0.93×10^6 spores/ml respectively, and significantly differed from other concentration and control treatments that recorded 85.30 mm, 738.00 mg, and 6.98×10^6 spores/ml, respectively. It was also noticed that the inhibition percentage of *F. solani* had significantly increased with the increasing of H_2O_2 concentrations, thus, our results are in accordance with the results of El-Ashmony *et al*. [21] who found that H_2O_2 had affected on the pathogen growth significantly. The effect of H_2O_2 may be explained by their reactivity, which had been toxic to cells at high concentrations [29]. Finnegan *et al*. [30] explained that H_2O_2 also may contains multiple models of structural oxidation and stages different oxidative stresses of proteins, amino acids and differences in its effect towards the different enzymes produced by microorganisms.

3.4. Effect of H_2O_2 and *Trichoderma koningii* Treatment on *F. solani* Growth *In vitro*

Table (2) showed that the treatment of *T. koningii* increased the inhibition percentage of *F. solani* when it was interacted with H_2O_2. It was found that increasing of H_2O_2 concentration led to increase the inhibitory effect of the bioagent *T. koningii* against *F. solani*, where the concentration 200 ppm gave the highest percentage which reached 58.48% compared to the control treatment, which recorded 0.00. The high antagonistic ability of *T. koningii*

Table 1. *In vitro* effect of H_2O_2 on fungal growth, fungal dry weight and sporulation of *F. solani.*

Treatments	Fungal radial growth (mm)	% Inhibition	Fungal dry weight (mg/50ml) liquid medium	Sporulation (X10^6 spores/ml)
Control	85.30	0.00	738	6.98
H_2O_2 (50 ppm)	49.33	45.03	498	3.75
H_2O_2 (100 ppm)	37.24	61.47	278	2.37
H_2O_2 (200 ppm)	22.04	78.20	124	0.93
$L.S.D_{(0.01)}$	2.074	2.040	139.6	1.228

Table 2. Effect of interaction of H_2O_2 and *Trichoderma koningii* on the growth of *F. solani in vitro.*

Treatments	% Inhibition of linear growth of *F. solani*
Control	0.00
H_2O_2 (50 ppm) + *T. koningii*	42.22
H_2O_2 (100 ppm) + *T. koningii*	52.32
H_2O_2 (200 ppm) + *T. koningii*	58.48
$L.S.D_{(0.01)}$	1.279

may be attributed to its production of several enzymes that can degrade cell wall and release a number of mycotoxins that can inhibit the growth of the pathogen [31]. Also, it was found from previous studies that the different species of *Trichoderma* can produce many extracellular enzymes including protease, cellulase, poly galacturonase, B-1,3-gluconase and chitinase, and produce many antibiotics like trichodermin, viridin, besides, they can produce toxic compounds like gliotoxin which can reduce pathogenic fungi growth [32,33].

3.5. Effect of Hydrogen Peroxide (H_2O_2) and Bioagent *T. koningii* Treatment in Reducing Tomato Damping-off Caused by *F. solani* and Survival Plants in Pots

It was noticed from Table (3) that there was a significant decrease in damping-off and survival plants depending on the response of *F. solani* to different concentrations of H_2O_2 alone or in combination with the bioagent *T. koningii*, as the highest concentration of H_2O_2 (200 ppm) recorded a significant decrease in damping-off and survival plant which reached17.78 and 10.55%, respectively. *T. koningii* also reduced damping-off and survival plants significantly to 17.48 and 10.70% in comparison with control and pathogen treatments which amounted to 20.22, 14.48, 34.32 and 67.41% respectively. Additionally, the combination treatment of *F. solani* + H_2O_2 (200 ppm) + *T. koningii* was found to be the best treatment which led to decrease the percentage of damping-off and plant survival significantly to 9.40 and 5.39% respectively. These results agreed with Al-Abbas and Salih [12], Al-Mansoury and Salih [13], and Hassan and Salih [14, 15], who confirmed that *Trichoderma* species such as *T. harzianum*, *T. longibrachiatum*, *T. koningii*, and *T. viride* led to decrease root rot, damping-off and fusarium wilt diseases in okra, cucumber, eggplant and tomato caused by *F. oxysporum* and *F. solani* significantly compared to the pathogen treatments. The results also agreed with Abdel-Monaim *et al*. [17] who mentioned that the high rates of tomato damping-off disease before and after emergence caused by *Pythium* sp., *F. oxysporum* and *F. solani* were reduced significantly as a response to inducing factor H_2O_2. Copes [34] indicated that H_2O_2 activated the plant defense mechanisms by increasing lignin and suberin contents in plant and increasing the plant defending against pathogens, H_2O_2 also, plays an essential role in strengthening cell walls at the site of the attack by pathogens. The reason for decreasing disease severity and root rot may be attributed to the presence of *T. koningii* which can protect the plant from the disease infection, promote its growth and supply some essential minerals [35]. It was found from some previous studies that *Trichoderma* spp. stimulate the plants

Table 3. Effect of interaction of H_2O_2 and bioagent *T. koningii* in reducing damping-off disease of tomato causing by *F. solani* and survival plants in pots under greenhouse conditions.

Treatments	% Damping-off	Survival plants
Control	20.22	14.48
F. solani	34.32	67.41
T. koningii	17.48	10.70
H_2O_2 (50 ppm)	19.22	12.80
H_2O_2 (200 ppm)	17.78	10.55
F. solani + *T. koningii*	25.33	46.22
F. solani+ H_2O_2 (50 ppm)	30.75	54.75
F. solani+ H_2O_2 (200 ppm)	15.15	14.44
F. solani + H_2O_2 (50 ppm) + *T. koningii*	12.36	7.75
F. solani + H_2O_2 (200 ppm) + *T. koningii*	9.40	5.39
$L.S.D._{(0.05)}$	1.49	1.59

to make changes in their metabolism and lead to changes in the plant response to pathogens and environmental stress and help the plants to tolerate the hard-environmental conditions, also they produce some compounds that have toxic action against the pathogens like viridin, koninginin, azaphilones, pyrones and steroids [36-38].

4. CONCLUSIONS

The main objective of this study was to find the best procedure to reduce the pathogen *F. solani* the causative of damping of and root rot on tomato plants by using the inducing agent hydrogen peroxide (H_2O_2) and the bioagent *T. koningii*. Results revealed that the disease severity had been decreased with the increasing of the hydrogen peroxide concentration, as well as using the bioagent *T. koningii*, which gave positive results in controlling and reducing this disease when it used alone or in combination with the inducing factor (H_2O_2) at different concentrations. Damping-off disease decreased significantly when the bioagent *T. koningii* and H_2O_2 were used together.

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6. CONFLICT OF INTEREST

The authors declare no conflict of interest.

7. REFERENCES

1. M.A.R. Alvarenga (Ed.). Tomate produção em campo, casa de vegetação e hidroponia. 2nd edn. *Universidade Federal de Lavras, Lavras* (2013).
2. B. George, C. Kaur, D.S. Khurdiya, and H.C. Kapoor. Antioxidants in tomato (*Lycopersium esculentum*) as a function of genotype. *Food Chemistry* 84: 45-51 (2004).
3. FAOSTAT. Crops and Livestock Products. *Food and Agriculture Organization of the United Nations* (2021). http://www.fao.org/faostat/en/#data/TP.
4. Central Statistical Organization. Production of secondary crops and vegetables by governorates for the year 2020. *Agricultural Census*. Chapter Three (2021) (In Arabic).
5. Y.S. Gonzalez, Y. Dijkxhoorn, A. Elings, J. Glover-Tay, I. Koomen, R.C. van der Maden, G. Nkansah, and P. Obeng. Vegetables business opportunities in Ghana. *Wageningen UR: Wageningen, The Netherlands* (2014).
6. M. Shenashen, A. Derbalah, Hamza, A. Mohamed, S. Tuzun, S. El Safty. Recent trend in controlling root rot disease of tomato caused by *Fusarium solani* using alumina silica nanoparticles. *International Journal of Advanced Research in Biological Sciences* 4(6): 105-119 (2017).
7. A.H. Gaigole, G.N. Wagh, and A.C. Khadse. Antifungal activity of Trichoderma species against soil borne pathogens. *Asiatic Journal Biotech Resour*ces 2(4): 461 (2011).
8. G.E. Harman, C.R. Howell, A. Viterbo, I. Chet, and M. Lorito. *Trichoderma* species–opportunistic, avirulent plant symbionts. *Natural Reviews Microbiology* 2: 43-56 (2004).
9. R.R. Waghunde, R.M. Shelake,and A,N. Sabalpara. *Trichoderma*: A significant fungus for agriculture and environment. *African Journal of Agricultural Research* 1(22): 1952-1965 (2016).
10. R.A. Zaghloul, A. Ehsan, N. Hanafy, A. Neweigy, and A.K. Neamat. Application of biofertilization and biological control for tomato production. *12th Conference of Microbiology, Cario, Egypt, March 18-22, 2007* pp. 198-212 (2007).
11. Y.A. Salih, and B.A. Al-Mansoury. Evaluation of the efficiency of some bioagents and their interaction with the fungicide topsin-m in the controlling eggplant root rot disease caused by *Fusarium oxysporum*. *International Journal of Agricultural and Statistical Sciences* 17(Supplement 1): 2153-2162 (2021).
12. H.H. Al-Abbas, and Y.A. Salih. Evaluation of the efficiency of the bioagent *Trichoderma longibrachiatum* against root rot disease of cucumber plant caused by the fungus *Fusarium solani*. *International Journal of Agricultural and Statistical Sciences* 18(Supplement 1): 1395-1401 (2022).
13. B.A. Al-Mansoury, and Y.A. Salih. The integrated control of eggplant root rot disease caused by *Fusarium solani* in the greenhouse. *Annals of Forest Research* 66(1): 1441-1455 (2023).
14. N.A. Hasan, and Y.A. Salih. Effect of the bioagents *Trichoderma harzianum and T. longibrachiatum* on tomato fusarium wilt disease caused by *Fusarium oxysporum* f.sp. lycopersici and some plant growth parameters under greenhouse conditions. *NeuroQuantology* 20(10): 9816-9826 (2022).

15. N.A. Hasan, and Y.A. Salih. Evaluation of efficiency of the bioagents *Trichoderma koningii* and *T. viride* in controlling the pathogenic fungus *Fusarium oxysporum* f.sp. *lycopersici* in the pots. *Misan Journal Academic Studies* 22(45): 264-279. (2023). (In Arabic).
16. U. Malolepsza, and S. Rozalaska. Nitric oxide and hydrogen peroxide in tomato resistance: nitric oxide modulates hydrogen peroxide level in o-hydroxyethylorutin- induced resistance to *Botrytis cinerea* in tomato. *Plant Physioogy. Biochemistry*. 43: 623-635. (2005).
17. M.F. Abdel-Monaim, M.A. Abdel-Gaid, and H.A. Armanious. Effect of chemical inducers on root rot and wilt diseases, yield and quality of tomato. *International Journal of Agricultural Sciences* 7: 211–220 (2012).
18. J.F. Leslie, and B.A. Summerell (Eds.). The *Fusarium* Laboratory Manual. *Blackwell Publishing, Hoboken* (2006).
19. M.M. Dewan. Identity and frequency of occurrence of fungi in root of wheat and rye grass and their effect on take-all and host growth. Ph.D. Thesis. *The University of Western Australia* (1989).
20. M.F. Ahmed. Effect of adding some biocontrol agents on non-target microorganisms in root diseases infecting soybean and broad bean plants. M.Sc. Thesis. *Faculty of Agriculture, Benha University, Moshtohor, Egypt* (2005).
21. R.M.S. EL-Ashmony, M.R. Abdel-Latif, EL.S. Abdou and A.A. Gala. Influence of Hydrogen peroxide (H_2O_2) on *Trichoderma harzianum* potentiality to control sunflower root/collar rot pathogen *Sclerotium rolfsii*. *Egyptian Journal of Phytopathol*ogy 45(2): 103-113 (2017).
22. L.M. Sutton, and M.J. Starzyk. Procedure and analysis of a useful method in determining mycelial dry weights from agar plates. *Applied Microbiology* 24(6): 1011-1012 (1972).
23. A.E. Dorrance, S.A. McClure and S.K. Martin. Effect of partial resistance on Phytophthora stem rot incidence and yield of soybean in Ohio. *Plant Disease* 87: 308–312 (2003).
24. L. Liu, J.W. Kloepper, and S. Tuzun. Induction of systemic resistance in cucumber against fusarium wilt by plant growth-promoting rhizobacteria. *Phytopathology* 85: 695-698(1995).
25. A.M. Lopez-Lopez, R. Allende-Molar, K.C. Correia, J.M. Tovar-Pedraza, I. Marquez-Zequera, S. Tuzun, R.S. Garcia-Estrada. First report of stem canker of tomato caused by *Fusarium striatum* in Mexico. *Plant Disease* 105: 497–499 (2021).
26. M.H. Abass, Q.H. Madhi, and A.A. Matrood. Identity and prevalence of wheat damping-off fungal pathogens in different fields of Basrah and Maysan provinces. *Bulletin of the National Research Centre* 45: 51(2021).
27. S.H. Wen, T. Yanglan, W. Shuangxia, M.G. Donald, D.S. Sarah, L. Yucai, W. Cheng, F. Yingying, W. Zhouping, and W. Aibo. Mycotoxigenic potentials of Fusarium species in various culture matrices revealed by mycotoxin profiling. *Toxins* 9(1): 6 (2017).
28. A.S. Kashyap, N. Manzar, F. Ahamad, J. Tilgam, P.K. Sharma, and A.K. Saxena. First report of root rot disease in green gram (*Vigna radiata*) caused by *Ectophoma multirostrata* in India. *Plant Disease* 106: PDIS11212400PDN (2022).
29. A.A. Ali. Role of hydrogen peroxide in management of root rot and wilt disease of thyme *plan. Journal of Phytopathology and Pest Management* 5(3): 1-13 (2018).
30. M. Finnegan, E. Linley, S.P. Denyer, G. McDonnell, C. Simons, and J.Y. Maillard. Mode of action of hydrogen peroxide and other oxidizing agents: differences between liquid and gas forms. *Journal of Antimicrobial Chemotherapy.* 65: 2108–2115. (2010).
31. A. Puyam. Advent of Trichoderma as a bio-control agent- A review. *Journal of Appied Natural Sci*ence 8(2): 1100-1109 (2016).
32. E.E. Demirici, E.Dane and C. Eken. In vitro antagonistic activity of fungi isolated from sclerotia on potato tubers against *Rhizoctonia solani*. *Turkish Journal of Biology* 35: 4 (2011).
33. H.P. Gajera, R.P. Bambharolia, S.V. Patel, T.J. Khatrani, and B.A. Goalkiya. Antagonism of *Trichoderma* spp. against Macrophomina phaseolina: Evaluation of coiling and cell wall degrading enzymatic activities. *Plant Pathology and Microb*iology 3: 7 (2012).
34. W.E. Copes. Concentration and intervals of hydrogen dioxide applications to control *Puccinia hemerocallidis* on daylily. *Crop Pro*tection 28: 24–29 (2009).
35. E. Humberto, D. Daniel, and C. Luis. Patents on endophytic fungi for agriculture and bio and phytoremediation applications. *Microorganisms* 8: 1237(2020).
36. R.A. Azevedo, P.L. Gratão, C.C. Monteiro, and R.F. Carvalho. What is new in the research on cadmium induced stress in plants? *Food Energy Secur*ity 1: 133–140 (2012).
37. F. Vinale, K. Sivasithamparam,E. Ghisalberti,

S. Woo, M. Nigro, R. Marra, N. Lombardi, and A. Pascale. Trichoderma secondary metabolites active on plants and fungal pathogens. *The Open Mycology Journal* 8(Suppl-1, M5): 127-139 (2014).

38. R.A.A. Khan, S. Najeeb, S. Hussain, B. Xie, and Y. Li. Bioactive secondary metabolites from *Trichoderma* spp. against phytopathogenic fungi. *Microorganisms* 8(6): 817 (2020).