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Article

Inducing Resistance Against Seed Rot and Damping-off Disease Infecting Bell Pepper Using Some Antioxidants and its Reflection on Seedling Protection Under Greenhouse Conditions

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Abstract

A greenhouse experiment was performed to assess the efficacy of some bio-control agents and glutathione to induce resistance in pepper plants against damping off disease caused by the fungus *Rhizoctoniasolani*. The fungus *Trichodermaviride* was highly efficient in inhabiting *R.solani* in growth medium when it scored 1.33, the highest antagonistic ability. The bacterium *Azospirillum brasilense*could inhibit the pathogenic fungus on PDA growth medium by 100% at 10^{-5} concentration. Glutathione and Beltanol pesticide control scored 100% growth inhibition at 3000 and 2000 mg/Lm, respectively. Greenhouse experiment showed *T.viride*, *A. brasilense* and glutathione (G) combination treatment decreased infectivity and disease severity to 0.00 and 0. 00%, respectively, compared to 56.67 and 55.00% for *R.solani* only treatment. While (G + *R. solani*), (*T. viride* +*R.solani*), (*T. viride* + G +*R.solani*) and (*A. brasilense* + G +*R.solani*) scored 3.33, 33.33, 10.00 and 10.00% infectivity and 1.67, 1.67. 6.67 and 8.33 % disease severity, respectively. Besides, the combination (*T. viride*+ *A. brasilense* + G) scored the highest plant height, dry and wet weights and total chlorophyll content, which were 100.00 cm, 87.33 and 32.08 gm/plant and 60.00 SPAD unit, respectively.

Keywords: Seed Rot, Damping off Disease, Bell Pepper, Greenhouse Conditions

Introduction

Bell pepper Capsicum annuum L. is an essential solanaceous crop due to its nutritional value for humans and animals. It ranks 3rd in marketing values after tomato and potato[1,2,3,4,5]. It contains many vitamins (A, B, C, E and K), essential for human health and wellness. Each 100 g of pepper fruits comprises 1.2 g protein, 4.8 g carbohydrates and fluorine, required for strengthening bone and teeth[5,6]. Dell pepper is infected by several foliar and root dresses, including those caused by soil fungi. These pathogens can cause severe losses in fruit production as they infect plants at different growth stages, causing root rot and damping off diseases[7,8]. Rhizoctoniasolani is one of Iraq's most widespread and high incidents among other fungal species. It has been reported to be one of the primary

pathogens that cause seed rot, damping off, pre and post-emergence death, and root and crown rot diseases, mostly in recent years[9,10]. The intensive usage of chemical pesticides can harm human health and the environment. Hence, more attention has been paid to other controlling approaches, including induced resistance using biological and non-biological factors[13,14]. indicated[15]Trichoderma spp is an effective biological agent against root rot pathogens. Azospirillum brasilense could promote plant growth by producing Indole acetic and lactic acids in the rhizosphere. At the same time, it showed[17] that applying glutathione at 200 ml could enhance plant resistance against root rot diseases. This study was aimed at the efficacy assessment of some induced resistance factors to protect bell pepper seedlings against the infection of R. solani and their effectiveness on pepper plant growth.

Materials and Methods

Isolation, identification and pathogenicity test of R. solani

R. solani isolate was provided by Plant Pathology Lab./Plant Protection Dept,/Agriculture Research Directorate -Iraqi Ministry of Agriculture. The isolate was grown on Potato Dextrose Agar (PDA) in Petri plates and incubated in an inverted position at 25 C°±2. Inoculum of the pathogenic fungus was prepared following 18 using millet seeds Panicummiliaceum local verity. Pathogenicity test was confirmed following Koch's postulates using a sterilized mixture of sandy soil with peat moss at a (1:1) ratio. The inoculum of R. solani grown on millet seeds was added to the soil mixture at a 1% (W/W) rate and to 2 kg pots. Sterilized millet seeds were used in the control treatment. All pots were watered, covered with perforated polyethylene bags, and kept for 3 days. Pots were uncovered, sown with bell pepper seeds (local verity), and sterilized for 2 min with 2% sodium hypochlorite. Ten seeds per pot with 3 replicates were used. Ten days after sowing, infectivity percent was calculated for the next 5 days for complete seed germination of control treatment, based on the following formula:

$$\text{Infectivity \%} = \frac{No.of\ infected\ plants}{total\ plant\ number} \text{ X } 100$$

Efficacy assessment of T. viride to inhibit R.solani growth on PDA

T. viride was provided by Plant Pathology Lab./Agriculture Directorate -Iraqi Ministry of Science and Technology. The antagonistic activity of T. viride against R.solani was tested using the dual culture method. This method was performed in 9 cm Petri plates containing PDA medium. The plate was divided into two equal parts. The 1st part was inoculated with a 0.5 mm disc sliced from a 5-day growth stage culture of the pathogenic fungus. The 2nd part was inoculated with a 0.5 mm disc from a 7-day growth stage culture of T. viride. This test was performed in 3 replicates, and 1 ml of sterilized water was used in the T. viride-free control treatment. Plates were incubated for 5 days at 25 C°±2. The antagonistic activity was calculated based on a 1-5 scale as follows:

Class description

Antagonistic fungus growth covers the entire plate area

Antagonistic fungus growth covers 2/3 of the plate area, while the pathogenic fungus covers the remaining area

Antagonistic fungus growth covers half of the plate area, while the pathogenic fungus covers the other half

Antagonistic fungus growth covers 1/3 of the plate area, while the pathogenic fungus covers the remaining 2/3 area

Pathogenic fungus growth covers the entire plate area

The biological agent is active against the pathogenic fungus when it scores 2 or less antagonism value.

Efficacy assessment of Azospirillum brasilense to inhibit R. solani and T. viride under laboratory conditions

A. brasilensebacterial isolate, confirmed by CHB50 biochemistry tests, was provided by Central Lab./Dept. of Soil and Water Resources/College of Agricultural Engineering Sciences/University of Baghdad. The bacterial isolate was grown on nutrient broth (NB) for 48 h at 25 Cº±2, then A ten-fold serial dilution up to 10^{-9} was performed. One ml of each concentration was added to a Perti-plate containing 15-20 ml of unsolidified PDA medium with 3 replicates at each concentration. A bacterial-free control was included using 1 ml of sterilized NB medium. The plates were rotated to mix the medium up to solidify, and then 0.5 cm discs from 5 days growth stage R. solani were placed in the middle of the plates. The plates were incubated for 5 days at 25 Cº±2 then inhibition percentages were calculated as follows:

$$\text{Inhibition \%} = \frac{\text{radial growth rate of the control} - \text{radial growth rate of treatment}}{\text{adial growth rate of the control}} \text{ X 100}$$

One ml of the concentration that scored the best inhibition against the pathogenic fungus was added to a plate containing PDA, and 0.5 discs from T. viride were placed in the middle of the plate. Three replicates were used, and 1 ml of NB medium was added for bacterial-free control. Plates were incubated for 7 days at the same temperature, and inhibition percentages were calculated.

Efficacy assessment of glutathione to inhibit R. solani and the biological agents T. viride and anA.brasilense under laboratory conditions.

The poisoned food technique was used to test the inhibitory effect of glutathione against R.solani, T.viride, an A, and brasilense. Three concentrations of 1000, 2000 and 3000 mg/L were prepared in flasks containing PDA, well shaken before medium solidification and poured into 9 cm Petri plates. A plate of each concentration was inoculated with a 0.5 cm disc, taken from the edge of 5 and 7-day growth stage R. solani and T. viride cultures. A. brasilense treatment, the 3 concentrations were prepared in NA medium, poured into the plates, and 1 ml/plate of the bacterial suspension at 10^{-5} concentration was spread. Control treatments included glutathione-free PDA, and NB media inoculated with R. solani, T. viride and A. brasilense. All treatments were incubated at 25 Cº±2 for 5, 7 and 1 days for R. solani, T. viride and A. brasilense. Inhibition percent was calculated by measuring two perpendicular diameters of the fungal growth for R. solani and T. viride. At the same time, it was based on counting the number of colonies for A. brasilense. All treatments were performed in 3 replicates.

Fungicidal activity assessment of Beltanol against R. solani on PDA.

The fungicidal activity of the pesticide Beltanol (Chinosol 50%) from (Probelte, Spain) was tested against R.solani using the poisoned food technique. Based on the active ingredient, the concentrations 500, 1000, 1500 and 2000 mg/L were prepared in PDA medium and poured into 9 cm Petri plates. A plate of each concentration was inoculated with a 0.5 cm disc from the 5-day growth stage of R. solani in the middle. All treatments were prepared in 3 replicates, and Beltanol-free PDA control was included. All treatments were incubated at 25 Cº±2 for 5 days. Inhibition percent was calculated by measuring two perpendicular diameters of the fungal growth.

Efficacy assessment of some bio-combinations to induce resistance in pepper plants against R. solani infection under greenhouse conditions.

This experiment was performed in a greenhouse at the Plant Protection Dept./College of Agricultural Engineering Sciences/University of Baghdad during Spring 2021-2022. The soil was prepared and sterilized using Beltanol pesticide mixed with 2 ml/m2 of irrigation water. One month later, random soil samples were tested for pathogens' incidence using the trap plant method to confirm the absence of any pathogen in treated soil[20]. The soil was plowed and harrowed. RCBD was performed, and the experimental field was divided into 3 blocks with 1 m^2 ridges (3 treatments with 3 replicates of each). Pepper seeds from a local variety were sown (2 seeds per hole). About 100 ml of bacterial inoculum at 7X 10^6 Cfu/ml was used for each treatment. The pathogenic fungus inoculum, loaded on millet seeds, was added 15 days of sowing, at 10 g/hole rate, by digging a slot by the hole[18,21]. Sterilized millet seeds inoculum was used for control treatment at the same rate. About 100 ml/hole of glutathione at 3000 mg/L concentration was added. T. viride was added at a 10 g/hole rate during the seed sowing. Following the manufacturer's recommended dose, the pesticide was added for 2 days of pathogen addition at a rate of 100 ml/ hole. All necessary agricultural processes were performed during the experiment. Plants were fertilized with Urea and triple superphosphate at 6 and 10 g/m^2 rates, respectively. The following treatments were included

Sterilized soil only
Soil + R. solani
Soil + R. solani +Glutathione
Soil + R. solani + A. brasilense
Soil + R .solani +T. viride
Soil + R. solani +Glutathione +A. brasilense
Soil + R. solani + Glutathione + T. viride
Soil + R. solani +T. viride + A. brasilense
Soil + R. solani +T. viride + A. brasilense+ Glutathione
Soil + R. solani + Beltanol

After five days of pathogenic fungus inoculum addition, post-emergence infectivity percent was calculated up to 40 days. Ninety days later,4 plants were selected randomly to measure height and leaf chlorophyll content using SPAD meter, infectivity percent, and dry and wet weights.

Results

Pathogenicity test of R. solani

The pathogenic fungus could decrease the germination of pepper seeds up to 3.3% under greenhouse conditions compared to 100% for the control treatment (Table (1) . Fig(1)). R. solani pathogenicity may affect germination due to the production of enzymes that hydrolase pectin and cellulose at the first stage of plant growth.

Treatment	Germination%
Control	100
R.solani	3.3
L.S.D$_{0.05}$	9.25

Table 1. Pathogenicity test of the fungus *R. solani* in pots.

Each number represents the average of 3 replicates

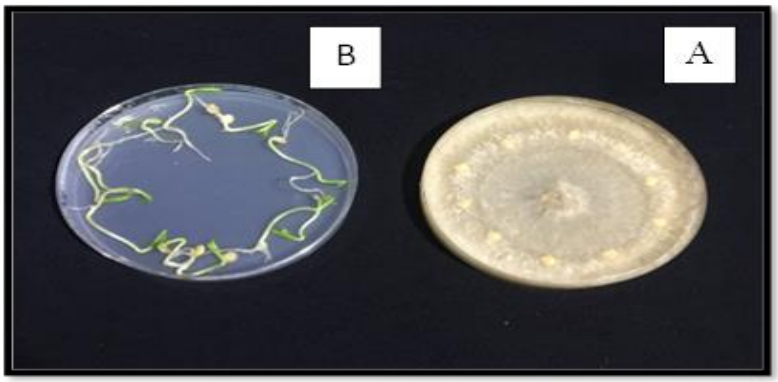

Figure 1. Pathogenicity test of the fungus R. solani in WA medium on pepper seeds.

A: Seeds with the pathogenic fungus.

B: Seeds without the pathogenic fungus.

Efficacy assessment of T. viride to inhibit R. solani growth on PDA.

T. viride showed high antagonistic activity against R. solani when it scored a 1.33 antagonism value, the highest scale, 5 days of inoculation on the PFA medium. The activity of T. viride continued to inhibit the pathogenic fungus R. solani. A direct contact was observed between the bio-agent and the pathogenic fungus colonies as R. solani growth was restricted to the plate edge due to the T. viride (Fig. 2). It was noted that T. viride hyphae covered R. solani growth, indicating a parasitic activity of the bio-agent against pathogenic fungi.

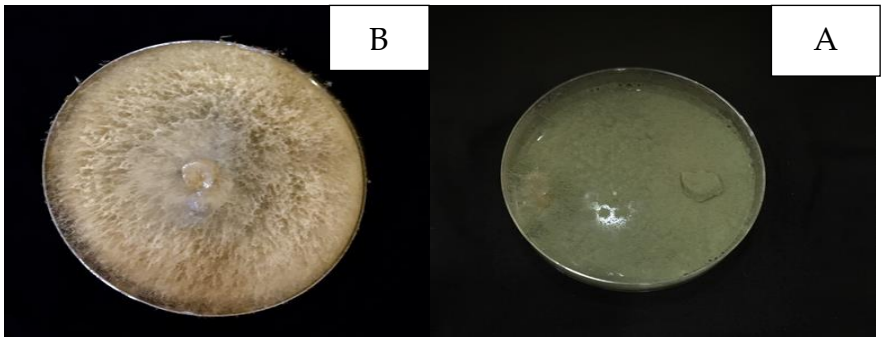

Figure 2. Antagonistic activity of T. viride against R. solani on PDA medium.

A: T. viride against R. solani. B: R. solani only.

Efficacy assessment of A. brasilense to inhibit R. solani and T. viride under laboratory conditions.

Application of the bacterium A. brasilense as a biological agent inhibited R. solani growth up to 78.62%, while the inhibition against T. viride was 9.99% Table (2). Fig(3).

treatments	Mean of colony diameter (cm)	%Inhibition
Control Tr.	9.00	**0.00**
Tr. + Az.	8.10	**9.99**
L.S.D$_{0.05}$	0.42**	**4.72****
Control *R.solani*	9.00	**0.00**
R.solani + Az.	1.92	**78.63**
L.S.D$_{0.05}$	0.43**	**4.74****

Table 2. Test the antagonistic activity of A. brasilense against R. solani and T. viride in laboratory conditions. Each number represents the average of 3 replicates.

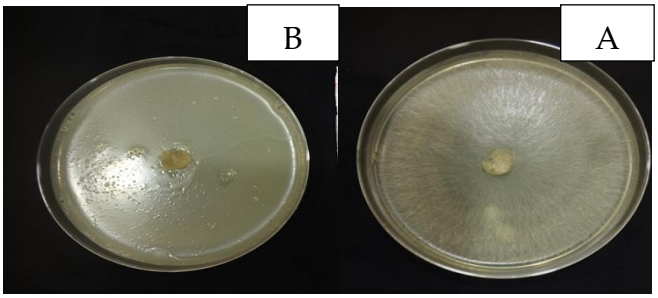

Figure 3. The antagonistic activity of A. brasilense against R. solani. A. R. solani.A.brasilense against R. solani.

Efficacy assessment of glutathione to inhibit R. solani and the biological agents T. viride and A. brasilense under laboratory conditions.

Glutathione added to the PDA medium could decrease. solani growth up to 6.46, 1.83 and 0.0 cm at concentrations 1000, 200 and 3000 mg/ml, respectively, scoring inhibition 28.18, 79.62 and 100.00 % compared to 9.0 cm and 0.00% in the control treatment, respectively (Table 3). In T. viride, the diameters of colonies treated with glutathione were 8.9, 8.43 and 7.7 for the same concentrations, scoring inhibition 1.11, 6.29 and 14.44, respectively. For A.brasilense treated with glutathione at the same concentrations on NA medium, the averages of colony numbers were 39,33, 40.00 and 40.00 colonies, scoring 1.66, 0.00 and 0.00% inhibition, respectively, compared to 40.00 colonies and 0.00% in the control treatment, respectively (Table 4).

Fungi	concentrations (mg/L)	Average of colony diameter (cm)	%Inhibition
R.solani	0	9.00	0.00
	1000	6.463	28.150
	2000	1.833	79.630
	3000	0.00	100.00
T.viride	0	9.00	0.00
	1000	8.900	1.110
	2000	8.433	6.3000
	3000	7.700	14.440
L.S.D$_{0.05}$	Treatment concen-	0.335	3.741
	trations of interac-	0.237	2.645
	tions	0.474	5.291

Table 3. Efficacy assessment of glutathione inhibits R. solani and the biological agents T. viride under laboratory conditions. Each number represents the average of 3 replicates.

concentrations (mg/L)	No. of bacterial colonies	Inhibition%
1000	39.333	**1.667**
2000	40.000	**0.000**
3000	40.000	**0.000**
Control	40.000	**0.000**
L.S.D$_{0.05}$	ns	**ns**

Table 4. Efficacy assessment of glutathione against *A. brasilense* on NA medium. Each number represents the average of 3 replicates.

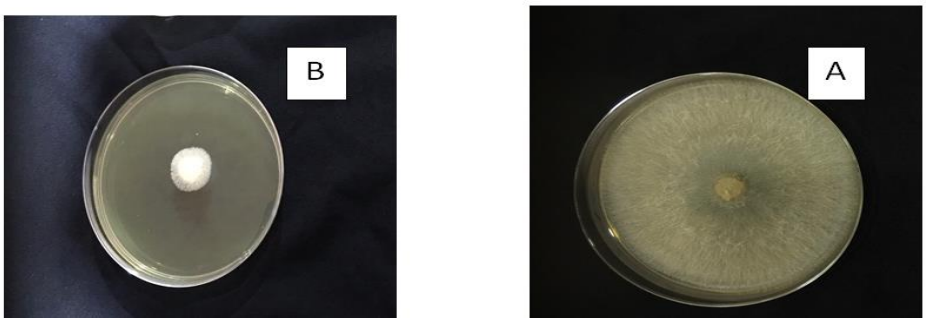

Fig (4): Glutathione efficacy to inhibit *R. solani*. R. solani only. B. R. solani treated with glutathione.

Fungicidal activity assessment of Beltanol against R. solani on PDA.

Beltanol pesticide inhabited R. solani growth up to 100% at 2000 mg/L compared to control treatment (Table (5) . Fig(5)). It scored 26.03, 64.07 and 93.70 inhibitory percent at concentrations 500, 1000 and 1500 mg/L, respectively.

concentrations (mg/L)	Average of colony diameter (cm)	Inhibition%
500	6.66	**26.03**
1000	3.23	**64.07**
1500	0.57	**93.70**
2000	0.00	**100.00**
Control	9.00	**0.00**
L.S.D$_{0.05}$	0.80**	**8.83****

Table 5. Fungicidal activity assessment of Beltanol against R. solani on PDA medium. Each number represents the average of 3 replicates.

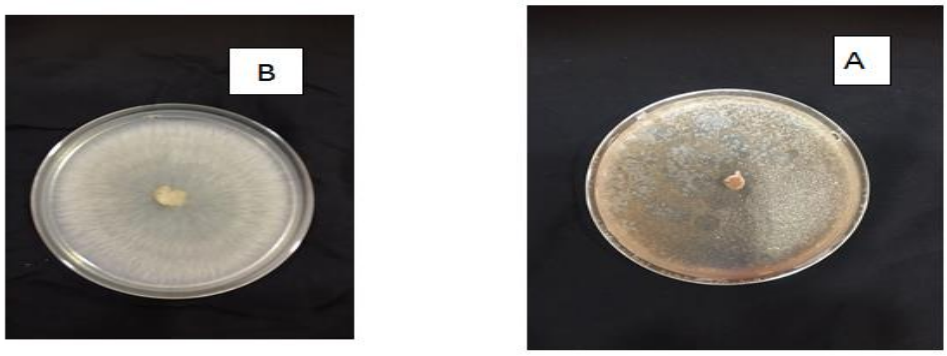

Fig (5): Fungicidal activity assessment of Beltanol against *R. solani. A.Beltanol against R. solani. BR solanine.*

Efficacy assessment of some bio-combinations to induce resistance in pepper plants against R. solani infection under greenhouse conditions.

All treatments could decrease infectivity and disease severity percentages of R. solani compared to pathogen control (Table 6). The combination T.viride +A. brasilense + glutathione scored the lowest infectivity, and disease severity percentages were 0.00 and 0.00%, compared to pathogen control with 56.67 and 55.00 %, respectively. Whereas Glutathione, T. viride + glutathione, T. viride, and A. brasilense + glutathione treatments scored 3.33, 3.33, 10.00 and 10.00% infectivity, and 1.67, 1.67, 6.67 and 8.33% disease severity, respectively followed by T. viride + A. brasilense scoring 13.33 and 8.33% infectivity and disease severity, respectively. Infectivity and disease severity of other treatments ranged from 16.67-23.33% and 13.33-20.00%, respectively.

Treatments	Infectivity%	%Disease severity
Control	0.00	**0.00**
G + *R. solani*	3.33	**1.67**
Tr + *R. solani*	10.00	**6.67**
Tr + G + *R. solani*	3.33	**1.67**
Az + G + *R. solani*	10.00	**8.33**
Az + Tr + *R. solani*	13.33	**8.33**
G +Az +Tr + *R. solani*	0.00	**0.00**
Beltanol + *R. solani*	23.33	**20.00**
Az + *R. solani*	16.67	**13.33**
R. solani	56.67	**55.00**
$L.S.D_{0.05}$	10.80**	**7.99****

Table 6. Efficacy assessment of some bio-combinations to induce resistance in pepper plants against R. solani infection under greenhouse conditions.

Each number represents the average of 3 replicates.

Tr : Trichoderma viride

Az :Azosprillium brasilense

G: Glutathione

The different treatments affected plant growth parameters, including plant height and dry and wet weights (Figs. 6-8). Pepper plants treated with G + T. viride + A. brasilense combination scored 100 cm, 87.33 and 32.08 g/plant maximum height, wet and dry weights, respectively, compared to pathogen and pathogen-free

treatments; that was 42.00 cm, 36.28 and 14.37 g/plant and 66.33 cm, 69.23and 24.30 g/plant, respectively. Whereas plants treated with the combinations, Az. + G + R. solani and G + Tr. + R. solan scored 77.00 cm, 80.43 and 30.14 g/plant and 75.67 cm, 78.79 and 28.13 g/plant of the same plant parameters, respectively. Other treatments ranged 64.33 -70.00 cm, 68.37-74.68 and 22.48-26.08 g/plant of the above plant parameters, respectively, under greenhouse conditions.

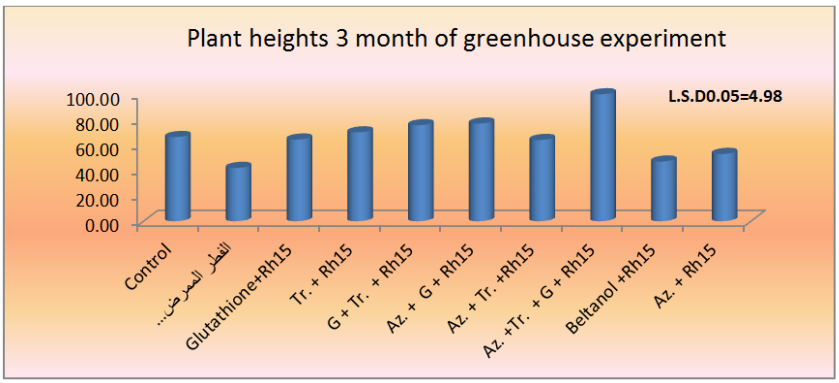

Figure 6. Effectiveness of some bio-factors on pepper plant heights under greenhouse conditions.

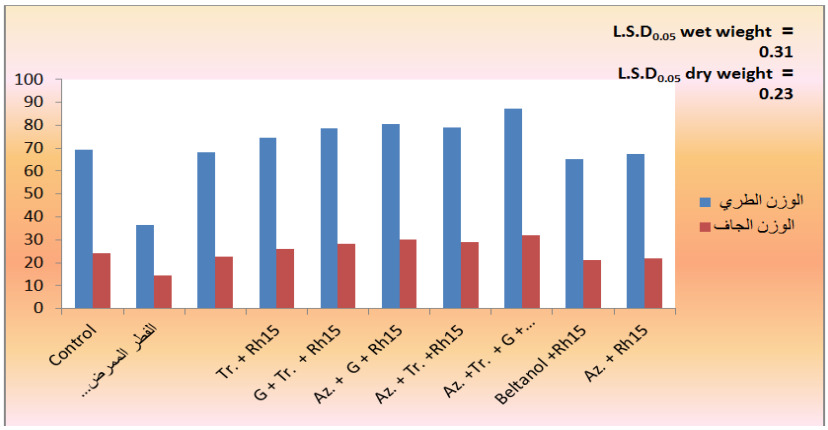

Figure 7. Effectiveness of some bio-factors on pepper plant dry and wet weights.

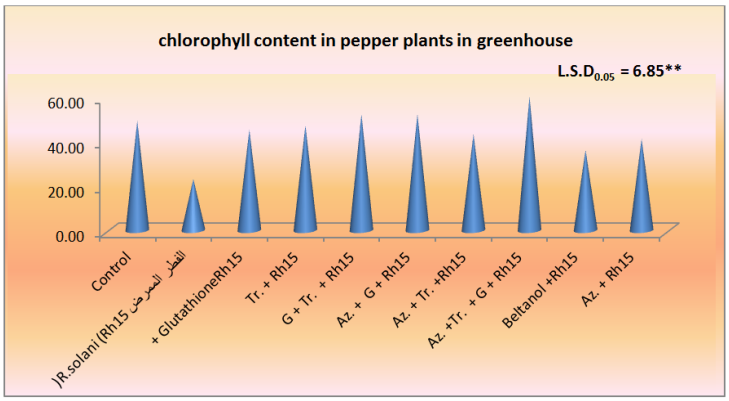

Figure 8. Effectiveness of different treatments on chlorophyll content under greenhouse conditions.

Discussion

The enzymes, including pectinase, methyl esterase and pectin-lyase, enable the pathogen to penetrate the host and cause the disease.

The feature of the bio-agent may be related to its hyphae being smaller in diameter, which enables twisting its hyphae around the pathogenic fungi. In addition, bio-agent hyphae are some enzymes produced by, including chitinase and cellulose, that break down the pathogenic fungal cell walls, resulting in penetration and parasitism. Besides the competition on nutrients, the production of some organic volatiles, Including ethyl hexadecanoate, azetidine and 2-phenyl ethanol, or enzymes, including chitinase and β-1,3-glucanase may inhibit the pathogenic fungi and limit their spread[23].

The inhibition activity of this bacterium may be due to the production of metabolites, organic compounds, indole lactic acid, some enzymes and antibiotics, gibberellins and cytokinins[16,24]. In addition, their roles in increasing nitrogen, phosphor and potassium make the plant resist pathogens[25]. Due to its inhibition activity. brasilense was used against several plant pathogens, including R. solani[26].

Glutathione inhibition efficacy can be attributed to its activities to produce metabolites, decrease the effect of reactive oxygen and role as an antioxidant. It damages the fungal mycelia and new growth, preventing the fungal spread. Glutathione is involved in plant stress resistance, preventing or decreasing roots' interaction with pathogens, as it comprises amino acids[27]—similarly, [28] glutathione to control Rhizoctonia infecting okra.

Beltanol's active ingredient,8-hydroxyquinoline neutral sulfate, can control R. solani by forming chelating agents with the copper element inside host tissues, which enables penetration of the active ingredient into the pathogen cells and then eliminates it or decreases the infection[29,30].

The activity of these agents to decrease infectivity and disease severity may trigger the plant's defense system to produce pathogenesis-related proteins (PRP) as the time required to induce systemic resistance in pepper plants ranges from 3 to 5 days[31]. Hence, this mechanism, alongside other biological agent activities, including antibiotic production, parasitism and space and water completion. Glutathione has many antifungal properties, including metabolite production and decreasing the effect of reactive oxygen. Besides its role as an antioxidant and in plant stress resistance, glutathione has a proteinaceous composition including many active amino acids that have a role in constructing an environment unsuitable for fungal growth and boosting root growth of pepper plants so that they can be much more active against pathogens[32,33]. Glutathione can increase root efficacy absorption of nutrients, in addition to the efficacy of photosynthesis [32,33,34]. The antagonistic activity of T. viride to suppress R. solani may be through the production of many antifungal metabolites, including, polyketides, steroids, isonitriles and peptaibols, that inhabit the pathogenic fungus growth[35].In addition, T. viride may inhibit the mycelium growth by producing enzymes that break down the cell walls of the pathogenic fungus[36].

On the other hand, T. viride may enhance peroxidase and chitinase production and activities in treated plants[37]. The completion of space and nutrients may be related to T. viride antagonistic activity, and this fungus may enhance plant growth through an increase in the availability of nutrients. Similarly, the antagonistic activity of the bacterium A. brasilense may be related to the high completion of nutrients and root metabolites, resulting in pathogen eviction or elimination from the rhizosphere[16]. Besides, this bacterial bio-agent releases a number of enzymes against several root fungal pathogens including R. solani[38,39]A. brasilense can induce systemic resistance by releasing some enzymes, including proteinases, chitinases, β-1,3-glucanases and Polyphenol oxidases. These metabolites trigger the accumulation of PRP in plants at infection sites, have high activity to hydrolyze the fungal cell walls and increase

the systemic activity of phytoalexins and enzymes, including peroxidases and phenylalanine ammonia-lyase (PAL)[24].

Similarly, the chlorophyll content increased in pepper plants treated with bio-agents and glutathione (Fig8). Pepper plants treated with the G + T. viride + A. brasilense combination scored the highest total chlorophyll content, 60.00 SPAD. Most studies indicated Trichoderma spp. and bio-fertilizers increased plant parameters, including chlorophyll content[40]. Thus. Besides offering the best protection to seedlings against pathogens, combining more than one bio-factor can significantly increase plant parameters, including plant height and dry and wet weights on different crops[41,42] Conceivably, T. viride improved plant growth parameters through enhancing physiological processes controlled by growth regulators. These naturally produced phytohormones, including auxins (i.e., indole acetic acid IAA) and gibberellins (GAS), significantly control and regulate plant growth[43].

Similarly, A. brasilense may improve plant growth through different mechanisms, including producing phytohormones, including IAA, GAs and other plant growth regulator-like molecules, that increase cell division and metabolic rates[44]. Glutathione might improve plant growth parameters by increasing nitrogen levels or chlorophyll in leaves by providing nutrients or acting as a growth regulator like molecule 45. Compared to the pathogen, the decrease in dry and wet weights of pepper plants treated with the pathogenic fungus R. solan was related to the infection. Respiration and carbohydrate consumption rates are increased in diseased plants, which may decrease the wet and dry weights, resulting in weak plants. Besides, root infection can minimize water and nutrient uptake in plants[46].

Conclusions

The diagnosed isolate of the pathogenic fungus R.solani was highly pathogenic on tested pepper seeds. Glutathione, T.viride and Azospirillumbrasilense have a high antagonistic ability against the pathogenic fungus R.solani in the PDA medium. Using the biostimulant Glutathione alone or combined with Azospirillumbrasilense protected the pepper plant from infection with pathogenic fungi and increased plant growth parameters under field conditions. The effectiveness of biological factors in inducing systemic resistance in plants increases its efficiency in absorbing element N and its readiness for the plant, increasing growth parameters regarding plant heights and total chlorophyll content.

References:

1. Thang, P.; Ripening, TN Behavior of capsicum (*capsicum annuum* L.) fruit Thesis for the degree of doctor of philosophy.Univ. ofAdelaide, SouthAustralia.pp. **2007**. 149.
2. Faostat, Available online: http://www.fao.org/(accessed on 12 March 2020).**2018**.
3. Mannai, S.; Jabnoun-Khiareddine, H.; Nasraoui, B.;Daami,R.M.Rhizoctonia root rot of Pepper (*Capsicum annuum*) : Comparative pathogenicity of causal agent and biocontrol attempt using fungal and bacterial Microbiology .**2018**; 9 (2) : 1000431 – 1000440 .
4. Tripodi, P.; Kumar, S. The Capsicum Crop: An Introduction. **2019**; 10(2) : 1-8.
5. Parisi, M., Alioto, D., Tripodi P. Overview of Biotic Stresses in Pepper (*Capsicum* spp.): Sources of Genetic Resistance, Molecular Breeding and Genomics. *International Journal of Molecular Sciences.* **2020***; 21 (7):* 2587.
6. Mishra , R.; Nanda, S.; Rout, E.; Chand, S.K.; Mohanty , J.N.; Joshi, R.K.Differential expression of defense – related genes in chilli pepper infected with anthracnose pathogen Colletotrichumtruncatum. Physiol.Mol.Plant Pathol. **2017**; 97: 1-10.

7. Yang, H.W.Ye.; Zeng, J.; Ma.D.;Rong, Z.; Zheng. M. Xu.Endophytic fungal communities associated with field-grown soybean roots and seeds in the Huang-Huai region of China. *Peer. J.*, **2018***; 30(6):* 4713- 4713.
8. Hyder , S.; Gondal, AS; Rizvi, Z.F.; &Inam – UL – Haq, M., Biological control of chili damping off disease , caused by *Pythium myriotylum*. *Frontiers in Microbiology*. **2021**. *12(1):*35-42.
9. Abbas, A., Khan, S.U.; Khan,W.U.; Saleh, T.A.; Khan, M.H.U.; Ullah, S.;Ikram, M. Antagonist effects of strains of *Bacillus* spp. against *Rhizoctoniasolani* for their protection against several plant diseases: Alternatives to chemical pesticides. ComptesRendusBiologies. **2019**; 342: 124-135.
10. Khrieba, M. Damping-off Caused by *Pythium* Species: Disease Profile and Management.2020; 31: 257-269.
11. Aboutorabi, M.A. Review on the Biological Control of Plant Diseases using Various Microorganisms. *Journal of Research in Medical and Dental Science,* **2018***; 6(4)* : 30-35.
12. Lahlali, R.; Ezrari, S.; Radouane, N.; Kenfaoui, J.; Esmaeel, Q.; El Hamss, H.;Belabess, Z.; Barka, EA Biological Control of Plant Pathogens: A Global Perspective. Microorganisms.**2022**; 10(3):596-599.
13. Llorens, E.; García-Agustín, P.; Lapeña, L. Advances in induced resistance by natural compounds: towards new options for woody crop protection. Scientia Agricola, **2017**; 74: 90-100.
14. Mhlongo, M.I; Piater, L.A; Madala, N.E; Labuschagne, N.; Dubery, I.A. The Chemistry of Plant-Microbe Interactions in the Rhizosphere and the Potential for Metabolomics to Reveal Signaling Related to Defense Priming and Induced Systemic Resistance. Front Plant Sci. Feb.**2018**; 9.9:112.
15. Ruangwong, O.U.; Wonglom, P.; Suwannarach, N.; Kumla J.; Thaochan, N.; Chomnunti, P.; Pitija, K.; Sunpapao A. Volatile Organic Compound from *Trichoderma asperelloides* TSU1: Impact on Plant Pathogenic Fungi. *J Fungi (Basel). Mar* . **2021***; 7(3)*:187-276.
16. Pedraza, R.; Filippone, M.; Fontana, C.; Salazar, S.; Ramírez, A.; Sierra-Cacho, D.; Baca, B.*Azospirillum*. **2020**; 6(5):73-105.
17. Huang, H.; Ullah, F.; Zhou, D.X.; Zhao, Y. Mechanisms of ROS Regulation of Plant Development and Stress Responses. Front. PlantSci. **2019**; (10):800.
18. Dewan, M.M. 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. Univ. West Australia. **1989**; 210 pp.
19. Bell, DK; Wells, H.D.; Markham, C.R. In vitro antagonism of *Trichoderma* species against six fungal plant pathogens. Phytopathology, **1982**; 72 (4) : 379-382.
20. Escobar, F.J.; Gallego, M. Benlloch, J.;Membrillo, J. Infant &P.Algaba . Treatments of oak decline using pressurized injection capsules of antifungal materials . European j. Forest Pathology . **1999**;29 : 29 – 30.
21. Fayyad, MA; Response of different genotypes of sunflower Helianthus Annus L. to infection with the fungus *Macrophomina phaseolina* (Tassi) Goid and the role of some biological methods in resistance. PhD thesis - College of Agriculture - University of Basra, **1997**; 91 p.
22. Zheng, N.L.P.; Zhang, F.Y.; Huang, GE.W.K.; Kong, L.A.; peng, D.L.; Liu . Conidia of one *Fusarium-solani*isolate from a soybean-production field enable to be virulent to soybean and make soybean seedlings wilted. *Journal of integrative agriculture,* **2018***; 17(9)*: 2042-2053.
23. Phoka, N.; Suwannarach N.; Lumyong S.; Ito, S.I.; Matsui K.; Arikit S.Sunpapao A. Role of Volatiles from the Endophytic Fungus *Trichodermaasperelloides* PSU-P1 in Biocontrol Potential and in Promoting the Plant Growth of *Arabidopsis thaliana*. J. Fungi (Basel). Dec. **2020**; 6.6(4):339-341.
24. Liu, H.; Carvalhais, L.C.; Crawford, M.; Singh, E.; Dennis, P.G.; Pieterse, C.M.; Schenk, P.M. Inner Plant Values: Diversity, Colonization and Benefits from Endophytic Bacteria. Front. Microbiol. **2017**; 8:2552.
25. Hassan, M.;Abd, S.; El-Abbasi, I.; Mikhail, M. Inducing Resistance against Faba Bean Chocolate Spot Disease. **2018**;1-7.
26. Santos, M.S.; Nogueira, M.A.;Hungria, M. Microbial Inoculants: Reviewing the Past, Discussing the Present and Previewing an Outstanding Future for the Use of Beneficial Bacteria in Agriculture. AMB Express . **2019**; Dec 21;9(1):205.
27. Balint-Kurti, The plant hypersensitive response: Concepts, control and consequences. Mol. Plant Pathol. **2019**; 20: 1163–1178.
28. Bittsánszky, A.;Ravishankar, V.;Oros, G. Response of glutathione conjugation system to soil borne *Rhizoctonia* infection of okra. ActaPhytopathologicaetEntomologicaHungarica. **2012**; 47: 191-202.

29. Al-Mayahi, S.H.A.; Isolation and Identification of Soybean Glycin max (L.) Merrill fungus causing damping-off disease and induction of its resistance. Master Thesis, Plant Protection Department, College of Agricultural Engineering Sciences, University of Baghdad. p: **2020**; 35-37.
30. Kassoub, F.T.Y.; Induction of systemic resistance of sesame plants against seed rot and seedling death. Master Thesis . Plant Protection Department - College of Agricultural Engineering Sciences / University of Baghdad. **2021**; 56 p.
31. Liorens, E.; García-Agustín, P.; Lapeña, L. Advances in induced resistance by natural compounds: towards new options for woody crop protection. Scientia Agricola, **2016**; 74: 90-100.
32. Gullner, G.; Zechmann, B.; Künstler, A.; Király, L.The Signaling Roles of Glutathione in Plant Disease Resistance. In Glutathione in Plant Growth, Development, and Stress Tolerance, 1st ed.; Hossain, M.A.; Mostofa, M.G.; Diaz-Vivancos, P.; Burritt, D.J.Eds. Springer International Publishing AG: Cham, Switzerland, pp .**2017**; 331–357.
33. Dumont, S.;Rivoal, J. Consequences of Oxidative Stress on Plant Glycolytic and Respiratory Metabolism. Frontiers in Plant Science Sci. **2019**; 18; 10: 166.
34. Hussein, M.M.; Okasha, E.M.; Mehanna, HM Response of cotton plant to glutathione rates under saline conditions . *middle east j.APPI. Sci* , **2014***; 4(1)*: 47 – 53.
35. Sallam, N.;Abd-Elrazik, A.A.; Hassan, M.; Koch,E. Powder formulations of Bacillus subtilis ,*Trichoderma*spp.andConiothyriumminitans for biocontrol of onion white rot.Phytopathology and Plant Protection. **2009**; 42(2):142-147.
36. Radjacommare, R.; Venkatesan, S.;Samiyappan, R. Biological control of phytopathogenic fungi of vanilla through lytic action of Trichoderma species and Pseudomonas fluorescens .Archives of Phyto. and Plant Prot. **2010**; 43(1):1-17.
37. Hassan, M.; Ahmed, H.M.; Kamel, S.M.; Abd El-Hamed, W. &Yousef, H. Biological control of damping-off and root rot disease caused by *Rhizoctoniasolani* on cucumber plants. *Fayoum Journal of Agricultural Research and Development.* **2021***; 525*.
38. Vejan, P.R.; Abdullah, T.; Khadiran, S.; Ismail, A.Nasrulhaq Boyce Role of Plant Growth Promoting Rhizobacteria in Agricultural Sustainability–A Review. Molecules. .**2016**; 21: 573. pmid:27136521.
39. Zaheer, M.S.; Raza, M.A.S.; Saleem, M.F.; Khan, I.H.; Ahmad, S.; Iqbal, R. Manevski, K. Investigating the Effect of *Azospirillumbrasilense* and Rhizobium pisi on Agronomic Traits of Wheat (*Triticumaestivum*L.). Arch. Agron. Soil Sci. **2019**; 65: 1554–1564.
40. Said, FH Integrated management of chemical, organic and biological fertilizers and their effect on the growth and productivity of some genotypes of cucumber plants. PhD thesis, Department of Horticulture and Landscape Engineering. College of Agriculture - University of Baghdad, **2015**; 128p.
41. Hussein, S.N. Molecular identification and integrated management of the Fusarium f. sp. cucumerinum the causal agent of Fusaruim wilt disease of *Cucum issativus* L. in Iraq. *Journal of Experimental Biology and Agricultural Sciences.* **2016***; 4(4)*: 389-397.
42. Al Juboory, H.H.; Juber, K.S.; Hussein, S.N. Identification, pathogenicity and controlling of the *Macrophominaphaseolina* (Tassi) Goid the causal agent of the charcoal rot disease on watermelon. *Journal of University of Duhok,* **2016***; 19(1)*: 558-564.
43. Harman, J.E. An overview of the mechanisms and uses of *Trichoderma* spp., Phytopathology, .**2006**; 96 (2): 190-194
44. Zhang, J.; Sajid, H.;Futao, Z.; Lianfeng, Z.; Xiaochuang, C.; Shengmiao Y.; Qianyu J. Effects of *Azospirillumbrasilense*and *Pseudomonas fluorescens* on nitrogen transformation . *Journal of Soils and Sediments.* . **2017***; 18(4):*34-64.
45. Al-Aboudi, M.Q.J. Effect of foliar spraying with ketathione for different stages on the growth and yield of maize. Master Thesis .Musayyib Technical College. Al-Furat Al-Awsat Technical University in resistance. PhD thesis - College of Agriculture - University of Baghdad, **2019**; 91 p.
46. Al-Juboory, H.; Hassan, A.; El-Humeiri, Y.N. Effect of some bioinducers in controlling the pathogen *Macrophomina phaseolina* that causes root and stem charcoal rot of strawberry. *Arab Journal of Plant Protection.* **2018***; 36*: 154-163.

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