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المجلة العراقية لبحوث السوق وحماية المستهلك

INDUCING SYSTEMIC ACQUIRED RESISTANCE IN PEPPER PLANTS AGAINST *RHIZOCTONIA SOLANI*

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ABSTRACT

This study was initiated to assess the efficacy of some biological materials separately or mixed to control *Rhizoctonia* root rot disease caused by the fungus *Rhizoctonia solani*. In vitro efficacy assessment showed; glutathione could inhabit fungal growth up to 100% at concentration 3000 mg/L. Whereas, the bacterium *Azospirillum brasilense* scored 78.63% inhibitory at 10^{-5} concentration. The fungal bio-agent *Trichoderma viride* scored 1.33 highest antagonistic activity 5 days of inoculation on PDA medium. Under greenhouse conditions, (Tr + Az + G+ *R. solani*) and (Tr + G+ *R. solani*) combination treatments could decrease *R.solani* infectivity and disease severity up to 0.00% compared to 73.33 and 68.33% for control treatment, respectively. Similarly, these two treatments could induce systemic acquired resistance (SAR) when scored the highest polyphenol oxidase (PPO) activity 6 and 12d of pathogenic fungus inoculation compared to healthy control. They scored 82.14 and 67.07, 78.12 and 65.33 absorbance increase rate (AIR)/min/g fresh leaf weight, respectively, compared to 41.67, 40.08 for AIR/min/g fresh leaf weight, respectively, for healthy control. Amongst other treatments, (Az + *R.solani*) scored 11.553% highest protein content compared to 9.433% for healthy control.

Keywords: Pell pepper, *A. brasilense*, Glutathione, *R. solani*, *T. viride*

استحثاث المقاومة الجهازية المكتسبة في نبات الفلفل ضد الفطر *Rhizoctoni solani*

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الخلاصة

اجريت هذه الدراسة لتقيم كفاءة بعض المواد الاحيائية بصورة مفردة او بالخلط فيما بينها في الحد من مرض تعفن الجذور الرايزوكتوني في بادرات الفلفل المتسبب عن الفطر *Rhizoctonia solani* .

اظهرت نتائج دراسة التقييم المختبري لكفاءة المستحثات (Glutathione ،*Azospirillum brasilense* ،*Trichoderma viride*) في تثبيط النمو الشعاعي للفطر الممرض اذ حقق الـ Glutathione نسبة تثبيط بلغت 100% عند التركيز 3000 ملغم/ لتر، في حين ان البكتريا *A.brasilense* اعطت نسبة تثبيط بلغت 78.63% عند التخفيف 10^{-5}، اما الفطر الاحيائي *T. viride* اعطى نسبة تضاد بلغت 1.33 حسب السلم المعتمد وهي اعلى قيمة في السلم بعد خمسة ايام من التلقيح في الوسط الزرعي PDA .

اما في ظروف البيت البلاستيكي فقد حققت معاملة الخلط بين (Tr + Az + G+ *R.solani*)، ومعاملة الخلط بين (Tr + G + *R.solani*) في خفض نسبة الاصابة وشدة الاصابة بالفطر *R.solani* اذا بلغت نسبة الاصابة والشدة فيها 0.00 و 0.00 % لكليهما على التتابع تليها بقية المعاملات، قياسا الى نسبة وشدة الاصابة في معاملة المقارنة والتي بلغت 73.33 و 68.33 % على التتابع فضلا عن تسببها في زيادة الوزن الطري والجاف للنبات معنويا على باقي المعاملات الاخرى، كما اثبتت العوامل كفاءتها في استحثاث المقاومة الجهازية من خلال زيادة فعالية انزيم (PPO) بولي فينول اوكسديز بعد 6 و 12 يوماً من اضافة الفطر الممرض قياساً بمعاملة المقارنة (من دون فطر ممرض) فقد حققت معاملة الخلط بين (Az +Tr + G + *R.solani*)، ومعاملة الخلط بين (Tr + G + *R.solani*) اعلى نسبة بالمحتوى الانزيمي فقد بلغت 82.14 و 67.07 ، 78.12 و 65.33 معدل التغير بالامتصاص الضوئي/ دقيقة/ غم وزن طري لاوراق النبات على التتابع في حين بلغ معدل التغيير بالانزيم بمعاملة المقارنة (من دون فطر ممرض) والتي بلغت

41.67، 40.08 معدل التغير بالامتصاص الضوئي/ دقيقة/ غم وزن طري لاوراق النبات على التتابع تلتها بقية المعاملات الاخرى. اما المحتوى البروتيني فقد حققت معاملة (Az + *R.solani*) اعلى محتوى للبروتين بلغ 11.553%، في حين بلغ معدل المحتوى البروتيني بمعاملة المقارنة (من دون فطر ممرض) 9.433 % تليها بقية المعاملات الاخرى.

الكلمات المفتاحية: الفلفل، بكتريا الازوسبرلم، الكلوتاثيون، رايزوكتونيا سولاني، الفطر الاحيائي الترايكو فيرادي.

INTRODUCTION

Bell pepper *Capsicum annuum* L. is cultivated worldwide due to its nutritional values, as it has a high content of vitamins and antioxidants (**Parisi *et al*., 2020**). Based on Central Agency for Statistics and Information Technology recent statistics, bell pepper production area was 21189 dunam (1 dunam= 2,500 m^2) with a total 45498 tons production in Iraq. Damping off disease is one of the major threat to bell pepper production worldwide (**Hyder *et al*., 2020**). It can be caused by several pathogens including *R. solani* (**Abbas *et al*., 2019**). The use of some microorganisms to induce plant systemic resistance has recently been introduced (**Mhlongo *et al*., 2018**). The plant resistance can be activated through biological and non-biological factors. Accordingly, a group of natural and chemical plant defenses are formed which increase the plant resistance against pathogens (**Zechmann *et al*., 2020 ; Hassan, 2021)**. The bio-agent *Trichoderma* spp. can induce resistance through many mechanisms including the increase in the formation of some compounds, including terpenoids and phenols, highly toxic to pathogens (**Hassan, 2021**). Moreover, it increases the activity of defiance enzymes including chitinase and polyphenol oxidase in addition to defense hormones in plant (**Contreras-Cornejo *et al*., 2016**). The bacterium *Azospirillum* was applied to induced systemic resistance in plants through soil and root treatment, as this bacterium could increase cell wall thickness of plants, activate some genes involved in the expression of pathogenic related protein and increase the activity of the defense enzymes in plant (**Zechmann *et al*., 2020**). Due to the importance of induced systemic resistance (ISR) as an alternative approach to chemical fungicides, this study was aimed at investigating some ISR mechanisms in bell pepper plant using *Trichoderma viride* and *Azosprillum brasilens* against the pathogenic fungus *R.solani* under greenhouse condition.

MATERIALS AND METHODS

Pathogenicity test of *R.solani*

An isolate of *R.solani*, provided by Plant Pathology Laboratory/ Departments of Plant Protection /Agriculture Research Directorate, was grown on Potato Dextrose Agar (PDA) medium in Petri dishes kept inverted at 25±2 °C. The inoculum of pathogenic fungus was prepared following **(Dewan,1989)** by growing the fungus on local millet seeds *Panicum miliaceum.* Koch's postulates were performed to confirm the fungus pathogenicity by adding fungal inoculum grown on millet seeds to 2 kg pots filled with sterilized soil mixture (1 soil:1 peat-moss) at 1% (w/w). A sterilized millet seed treatment was included as a control. All pots were watered and sealed with polyethylene bags for three days then pots were unsealed and pepper seeds (surface sterilized with 2% sodium hypochlorite for 2 min) were sown at rate 10 seeds/treatment with 3 replicates. Ten days after sewing, infectivity percent was calculated, in every 5d interval for 3 weeks until the complete germination of seeds in control treatment, following equation:

$$\text{Infectivity (\%)} = \frac{No.of\ infected\ plants}{Total\ plant\ number} \times 100$$

Efficacy assessment of *T. viride* to inhabit *R.solani* on PDA medium

An isolate of *T. viride* was provided by Plant Pathology Laboratory/ Agriculture Research Directorate /Iraqi Ministry of Science and Technology. The antagonistic activity of *T. viride* against *R.solani* was tested using dual culture assay. The 9 cm diameter Petri plates, containing PDA was divided into two equal sectors. One sector was inoculated with a 0.5 disc sliced from 5d growth *R.solani* culture. Whereas, the opposite sector was inoculated with a 0.5 disc taken from 7 d growth *T. viride* culture. Three replicates were used and a non-biological agent control treatment was included. Plates were incubated for 5d at 25±2 °C and the antagonistic activity based on **Bell *et al*., (1982)** method using the following 1-5 scale:

Class	Description
1	Antagonistic fungus growth covers the entire plate area
2	Antagonistic fungus growth covers 2/3 of plate area while the pathogenic fungus covers the remaining area
3	Antagonistic fungus growth covers half of the plate area while the pathogenic fungus covers the another half
4	Antagonistic fungus growth covers 1/3 of plate area while the pathogenic fungus covers the remaining 2/3 area
5	Pathogenic fungus growth covers the entire plate area

The biological agent has an antagonistic activity against the pathogenic fungus when scores 2 or less.

In vitro efficacy assessment of *A. brasilense* to inhibit *R.solani* and *T. viride* bio-agent

A local isolate of the bacterium *A. brasilense* was kindly provided by Central laboratory Department of Soil and Water Resources, College of Agricultural Engineering Sciences/ University of Baghdad. The provided bacterium was isolated from soil and confirmed by CHB50 biochemistry tests. *A. brasilense* isolate was grown on Nutriant Broth (NB) medium for 48 h at 25 °C ±2, then ten-fold serial dilutions up to 10^{-9} were prepared. About 1 mL of each bacterial concentration was mixed with 15 -20 mL unsolidified PDA medium in a petri-plate through plate rotating. When solidified, 0.5 cm discs from the edge of 5d growth stage *R. solani* culture were placed in the middle of plates. Three replicates for each treatment were made. One Ml of sterilized NB medium was used as a bacterial free control treatment. All plates were incubated for 5d at 25 °C ±2 then inhibition percent was calculated as follows:

$$\text{Inhibition (\%)} = \frac{C-T}{C} \times 100$$

Where C = The average of colony diameter in control.
T = The average of colony diameter in treatments.

The inhibitory effect of the About 1 mL of the concentration scored the best inhibition against the pathogenic fungus was added to plate containing PDA medium, then 0.5 disc from *T. viride* was placed in the middle of plate. Three replicates were used and 1 mL of PDA medium was added as a bacterial free control. Plates were incubated for 7d at 25 °C ±2 and inhibition percent was calculated.

In vitro efficacy assessment of glutathione to inhibit the pathogenic fungus *R.solani* and the bio-agents *T.viride* and *A. brasilense*

To select the best glutathione concentration and identify its effect on the bio-agents, the poisoned food technique was performed to test the inhibitory effect of glutathione against *R. solani*, *T. viride* and *A, brasilense*. Three concentrations 1000, 2000 and 3000 mg/L were prepared in flasks containing PDA, well shaken before medium solidification and poured into 9 cm Petri-plates. Plate of each concentration was inoculated with 0.5 cm disc, taken from the edge of 5 and 7d growth stage *R. solani* and *T. viride* culturesm respectively. For *A. brasilense* treatment, the 3 concentrations were prepared in NA medium , poured into the plates and 1mL/plate of the bacterial suspension at 10^{-5} concentration was spread. Glutathione free PDA and NB media inoculated with *R. solani*, *T. viride* and *A. brasilense* , respectively were included as control treatments. All treatments were incubated for 5, 7 and 1d at 25 °C ±2 for *R. solani*, *T. viride* and *A. brasilense* , respectively. Inhibition percent was calculated based on measuring two perpendicular diameters of the fungal growth for *R. solani* and *T. viride*. For *A. brasilense* the inhibitory percent was calculated based on counting the number of colonies. All treatments were performed in 3 replicates.

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

Fungicidal activity of the pesticide Beltanol (Chinosol 50%), produced by (Probelte, Spain), against *R. solani* was tested, using poisoned food technique. The concentrations 500, 1000, 1500 and 2000 mg/L were tested based on the active ingredient. Thee 4 concentrations were prepared in PDA medium and poured in 9 cm Petri-plates. Plates of each treatment were inoculated with 0.5 cm disc taken from 5d growth stage of *R. solani* at the middle. Three replicates of each treatment were prepared and Beltanol free PDA control was included. All treatments were incubated for 5d at 25 °C ±2. Inhibition percent was calculated based on by measuring two perpendicular diameters of the fungal growth.

Inducing systemic resistance of bell pepper seedlings against *R.solani* using some bio-agents in pots under greenhouse conditions

This experiment was performed in a greenhouse at Department of Plant Protection/ College of Agricultural Engineering Sciences/ University of Baghdad for the spring agricultural season of 2021-2022 . Soil mixed with peat-moss and autoclaved for 20 min at 121 °C and a pressure of 1.5 kg/cm^3, left to cool and the sterilization repeated twice in every 24 h interval. Sterilized mixture was distributed in plastic pots at 4 kg/pot rate. The following treatments were applied

1. Soil only
2. Soil + *R. solani*
3. Soil + Glutathione (G)
4. Soil + *A. brasilense* (A)
5. Soil + *T. viride* (T)
6. Soil + *R. solani* + Glutathione
7. Soil + *R. solani* + *A. brasilense*
8. Soil + *R. solani* + *T. viride*
9. Soil + *R. solani* + Glutathione + *A. brasilense*
10. Soil + *R. solani* + Glutathione + *T. viride*
11. Soil + *R. solani* + *A. brasilense*+ *T. viride*
12. Soil + Glutathione + *A. brasilense*

13. Soil + Glutathione + *T. Viride*
14. Soil + *A. brasilense*+ *T. viride*
15. Soil + *R. solani* + Glutathione+ *A. brasilense* + *T. viride*
16. Soil + Glutathione+ *A. brasilense* + *T. Viride*
17. Soil + *R. solani* + Beltanol

Each pot was sewed with 5 bell pepper seeds from a local variety, surface sterilized with 2% sodium hypochlorite for 2 min, then washed with distilled water. About 100 mL of glutathione was added to soil in pots at 3000 mg/L concentration. Around 100 mL /pot of bacterial inoculum was added at 7×10^6 Cfu/mL concentration. *T.viride* was loaded on millet seeds and 40 g/pot was used. Beltanol pesticide was applied based on the by dose recommended by manufacturer which was 100/pot at 1 mL/L concentration. The pathogenic g was added 15d of sewing through contaminating the soil with 40 g/pot of fungal inoculum loaded on local millet seeds. The control treatment included sterilized soil and 40 g/pot of millet seeds but without fungal inoculum. Similar steps were followed with glutathione, *A.brasilense* and *T.viride* for control treatment but without fungal inoculum. About combination treatments, each factor was added at half concentration. A completely randomized design (CRD) for 17 treatments in 3 replicates was used. Leaf yellowing and seedling wilt symptoms were noticed 6d of adding fungal inoculum. Infectivity percent was calculated following the equation :

$$\text{Infectivity (\%)} = \frac{No.of\ infected\ seedlings}{Total\ seedling\ no.} \times 100$$

Plant samples were collected two months of cultivation, and disease severity was calculated based on 0-4 scales **(Alwan, 2014)** as follows:

0= Not infected plants
1=1-25% of root is rotten
2= More than 25-50% of root is rotten
3= More than 50-75% of root is rotten
4= More than 75-100% of root is rotten or plant death

Disease severity percent was calculated based on **Mckinney (1923)** as follows: highest

$$\text{Disease severity (\%)} = \frac{No.\ of\ plants\ in\ class\ (0\times0)+\cdots+No.of\ plants\ in\ class\ (4\times4)}{Total\ \ no\ of\ all\ examined\ plants\times highest\ class} \times 100$$

The infection of pathogenic fungus was confirmed by microscopic examination. Plant roots were collected, surface sterilized, cultured on PDA and examined under light microscope. Fresh and dry weights were estimated in plant samples following the estimation of disease severity percent. Plant samples were dried in electric oven for 2d at 50 °C to ensure the stability of the weight. Some biochemical parameters were tested to identify induced systemic resistance in seedlings against the pathogenic fungus. Leaf samples were collected from plants treated with previously mentioned bio-agents, inoculated with *R. solani* and thought to be systemically resistance induced against the disease 6 to12d of inoculation. Polyphenol oxidase (PPO) was estimated following the method priviously described **Ojha & Chatterjee (2012).** Protein content was estimated in leaf samples collected 30 d after inoculation using Microkeldahl apparatus to estimate total nitrogen content **(Black,1965)** through multiplying nitrogen percent by 6.25 factor to obtain the total leaf protein percent **(Scheffelen *et al*., 1961)**.

RESULTS AND DISCUSSION

Pathogenicity test of *R. solani*

Pathogenicity test showed *R. solani* significantly decreased seed germination of bell pepper up to 3.3% under greenhouse conditions, compared to 100% in control treatment (without pathogenic fungus) (Table 1) (Figure,1). The high pathogenicity may be related to the enzyme activity produced by the fungus that degrade pectin and cellulose at the first stages of plant growth. These enzymes including pectinase, pectin methyl esterase and pectinlyase, can be involved in host penetration and disease initiation **(Toghueo, 2019)**.

Table (1): Pathogenicity test of *R. solani* in pots.

* Treatments	Germination (%)
Control	100
R.solani	3.3
$L.S.D_{0.05}$	9.25

***Each number represents an average of 3 replicates.**

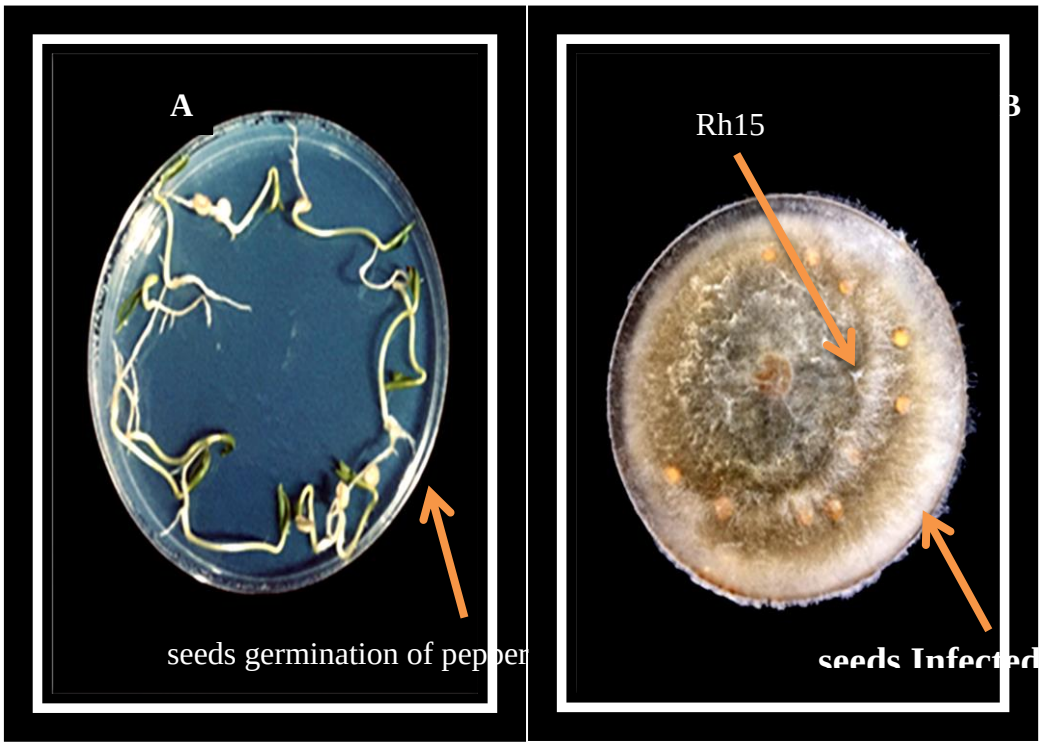

Figure (1): Pathogenicity of *R. solani* on pepper seeds.
A: Seeds without the pathogenic fungus (control).
B: Seeds with the pathogenic fungus.

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

In vitro tests confirmed the high antagonistic activity of the bio-agent fungus *T. viride* against *R. solani* when score 1.33 highest antagonistic percent based on the scale used, 5d of

inoculation o PDA. *T. viride* could inhibit the *R. solani* growth a direct contact between the bio-agent and pathogen colonies was noted. The growth of the pathogenic fungus was restricted to the plate edge due to *T. viride* (Figure. 2). It was noticed that the hyphae of *T. viride* were covering *R. solani*, indicating the bio-agent fungus activity against pathogenic fungi. This feature of is characteristic to the bio-agent as its tiny hyphae are smaller in diameter enables twisting around those in the pathogenic fungi. Besides, some enzymes produced by bio-agent hyphae, including chitinase and cellulose, enable breaking through the cell walls of the pathogenic fungus resulting in penetration and parasitism. Other features of antagonism are the competition on nutrients, production of some organic volatiles, including, ethyl hexadecanoate, azetidine and 2- phenyl ethanol, and/or enzymes, including chitinase and β-1,3-glucanase, that may be involved in inhibiting the pathogenic fungi and limit their spread **(Phoka *et al.*, 2020)**.

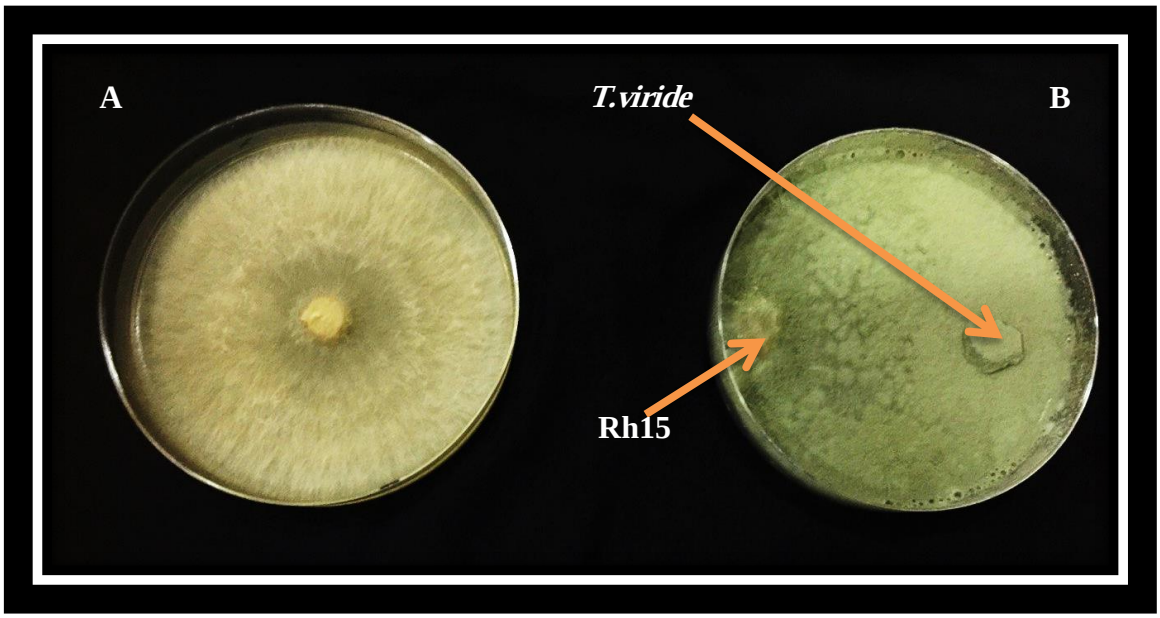

Figure (2): Antagonistic activity of *T. viride* against *R. solani* on PDA medium.
A: *R. solani* only.
B: *T. viride* against *R. solani*.

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

In vitro tests of the bacterium *A. brasilense* as a biological agent could inhibit *R. solani* growth up to 78.63%, while the inhibition against *T. viride* was 9.99% (Table 2 and Figure 3). 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 **(Pedraza *et al.*, 2020)**. In addition, *A. brasilense* roles involve in increasing nitrogen, phosphor and potassium enables the plant to resist pathogens **(Nia,2015)**. Due to its inhibition activity *A. brasilense* was used against several plant pathogens, including *R. solani* **(Santos *et al.*, 2019)**.

Table (2): Test the antagonistic activity of *A. brasilense* against *R. solani* and *T. viride* in laboratory conditions.

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

*Each number represents an average of 3 replicates
**There are significant differences at the $L.S.D_{0.05}$ level of the treatments

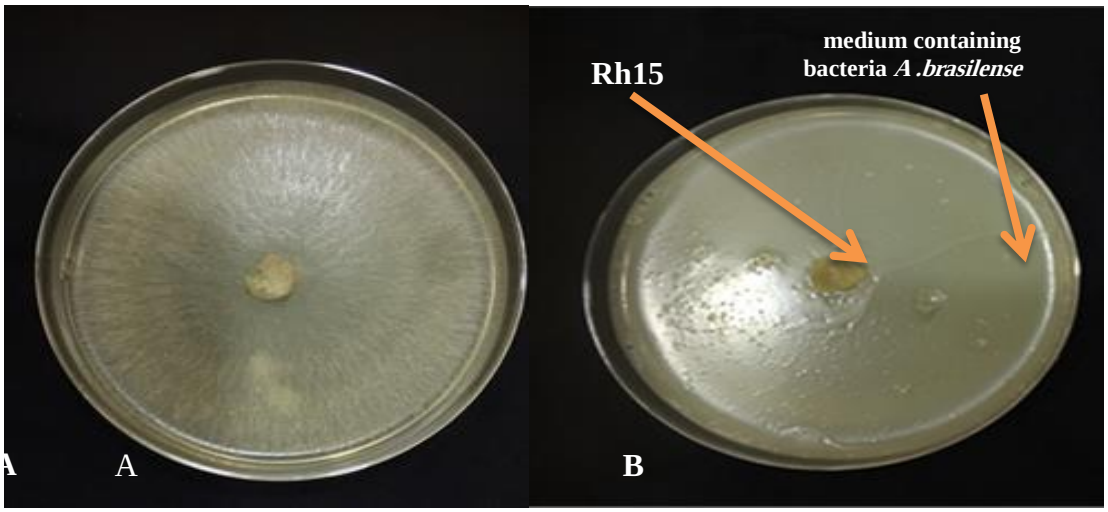

Figure (3): the antagonistic activity of *A. brasilense* against *R. solani*.
A: *R. solani*.
B : *A. brasilense* against *R. solani*.

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

In vitro tests confirmed glutathione added to PDA medium could decrease *R. solani* growth up to 6.46, 1.83 and 0.0 cm at 1000, 200 and 3000 mg/mL concentrations, respectively, scoring inhibition 28.15, 79.63 and 100.00 % compared to 9.0 cm and 0.00% in control treatment, respectively (Table 3). Whereas, in *T. viride*, the diameters of colonies treated with glutathione were 8.9, 8.43 and 7.7, at 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 control treatment, respectively (Table 4). 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 **(Balint-kurti *etal*., 2019)**. Similarly, **Bittsanszky *et al*., (2012)** used glutathione to control *Rhizoctonia* infecting okra.

Table (3): Efficacy assessment of glutathione to inhibit *R. solani* and the biological agents *T. viride* under laboratory conditions.

* 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
$L.S.D_{0.05}$	Concentrations	0.335	3.741
	Treatments	0.237	2.645
	Interactions	0.474	5.291

***Each number represents an average of 3 replicates**.

Table (4): Efficacy assessment of glutathione against *A. brasilense* on NA medium.

* 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

*Each number represents an average of 3 replicates.

*ns There are no significant differences at the $L.S.D_{0.05}$ level of the treatments.

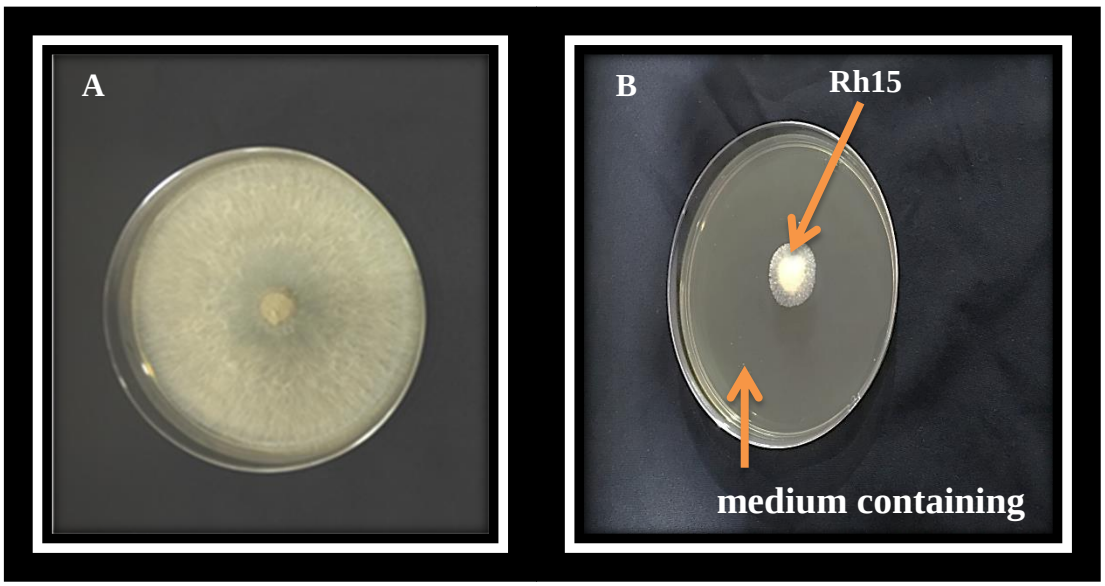

Figure (4): Glutathione efficacy to inhibit *R. solani*.
A : *R. solani* only .
B : *R. solani* treated with glutathione .

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 and Figure, 5). Whereas, it scored 26.03, 64.07 and 93.70 inhibitory

percent at concentrations 500, 1000 and 1500 mg/L, respectively. Beltanol active ingredient, 8-hydroxyquinoline neutral sulphate can control *R. solani* through forming chelating agents with copper element inside host tissues which enables penetration of the active ingredient into the pathogen cells then eliminate it or decrease the infection **Al-Mayahi (2020)** .

Table (5): Fungicidal activity assessment of Beltanol against *R. solani* on PDA medium.

* Concentrations (mg/L)	Mean 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

***Each number represents an average of 3 replicates.**

****There are significant differences at the $L.S.D_{0.05}$ level of the treatments.**

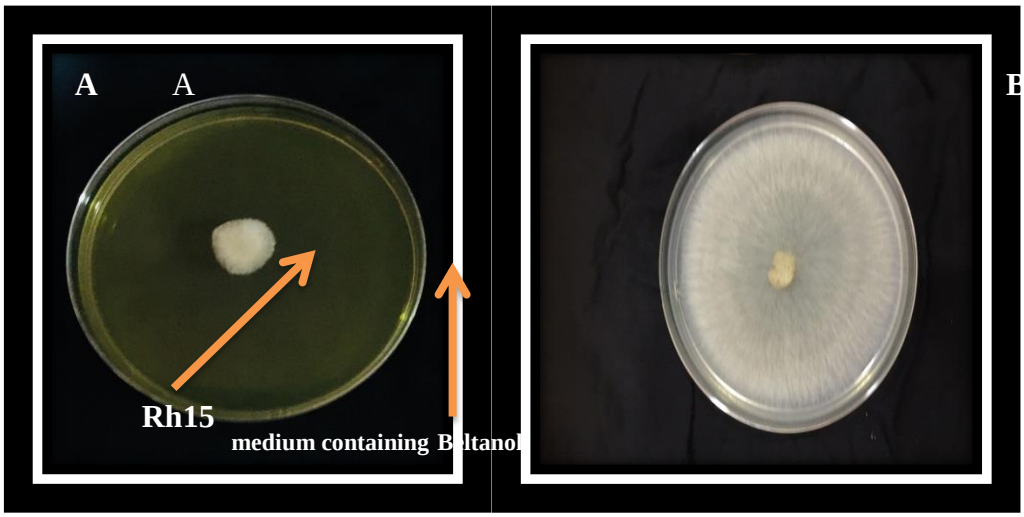

Figure (5): Fungicidal activity assessment of Beltanol against *R. solani*.
A: Beltanol concentration (2000 mg/L) against *R. solani*.
B: *R. solani* only.

Inducing systemic resistance of bell pepper seedlings against *R. solani* using some bio-agents in pots under greenhouse conditions

All treatment could decrease infectivity and disease severity percentages of *R. solani* compared to pathogen control (Table 6). The combination (*R.solani*+Az+T + G) scored the lowest infectivity and disease severity percentages that were 0.00 and 0.00% compared to pathogen control with 73.33 and 68.33 %, respectively. Whereas, both (*R. solani* + Tr + G) and (*R. solani* + Az + G) combination treatments scored 0.00 and 0.00% infectivity disease severity, respectively followed by other treatments. The absence of infectivity and disease severity in these combinations may be related glutathione efficiency to produce metabolites and reduce reactive oxygen, its role as an antioxidant and in plant stress resistance. Besides, glutathione has a proteinaceous composition including many active amino acids that have a role to construct an environment unsuitable for fungal growth and boost root growth of pepper plants, so they can be much active against pathogens **(Gong *et al.*, 2017)**.

Table (6): Inducing systemic resistance of bell pepper seedlings against *R. solani* using some bio-agents in pots.

* Treatments	Infectivity(%)	Disease severity(%)
Control	0.00	0.00
R.solani	73.33	68.33
Glutathione (G)	0.00	0.00
A.brasilens (Az)	0.00	0.00
T.viride (Tr)	0.00	0.00
G + *R.solani*	6.67	3.33
Az + *R.solani*	33.33	26.67
Tr + *R.solani*	26.67	21.67
Az + G + *R.solani*	0.00	0.00
Tr + G + *R.solani*	0.00	0.00
Tr + Az + *R.solani*	6.67	1.67
G + Az	0.00	0.00
G + Tr	0.00	0.00
Tr + Az	0.00	0.00
Tr + Az + G + *R.solani*	0.00	0.00
Tr + Az + G	0.00	0.00
Beltanol + *R.solani*	20.00	16.67
** $L.S.D_{0.05}$	15.88	12.75

***Each number represents an average of 3 replicates.**
***There are significant differences at the $L.S.D_{0.05}$ level of the treatments.**

The highest polyphenol oxidase (PPO) content was in the combination (*R.solani* + G + Az + Tr) after 6 and 12d when scored 82.14 and 67.07, the rate of change in light absorption/min/gm fresh weight of plant leaves respectively, followed by the (*R.solani* + G + Tr) which scored 78.12 and 65.33, respectively (Table 7). Whereas, (*R.solani* + Az) scored the highest protein content which was 11.553%, followed by other treatments (Figure. 6).

Table (7): The effectivity of inducing factors tested on the increase of PPO content after 6 and 12d of inoculation with *R. solani* in pots.

*Treatments	Average measurement ppo after 6d in pots	Average measurement ppo after 12d in pots
Control	41.67	40.08
Glutathione (G)	64.90	54.53
A.brasilense (Az)	55.26	47.20
T.viride (Tr)	53.18	45.12
Az + G	71.12	61.32
G + Tr	73.42	62.14
Tr + Az	74.14	62.66
Az + Tr + G	78.32	66.14
R. solani (Rh15)	60.28	44.63
G + Rh15	73.42	62.17
Tr + Rh15	66.12	54.18
Az + Rh15	68.12	57.33

Tr + G + Rh15	78.12	65.33
Az + G + Rh15	76.19	64.13
Az + Tr + Rh15	70.80	60.18
Az + Tr + G + Rh15	82.14	67.07
Beltanol + Rh15	0.00	0.00
**L.S.D$_{0.05}$	2.85	2.11

***Each number represents an average of 3 replicates.**

***There are significant differences at the L.S.D$_{0.05}$ level of the treatments.**

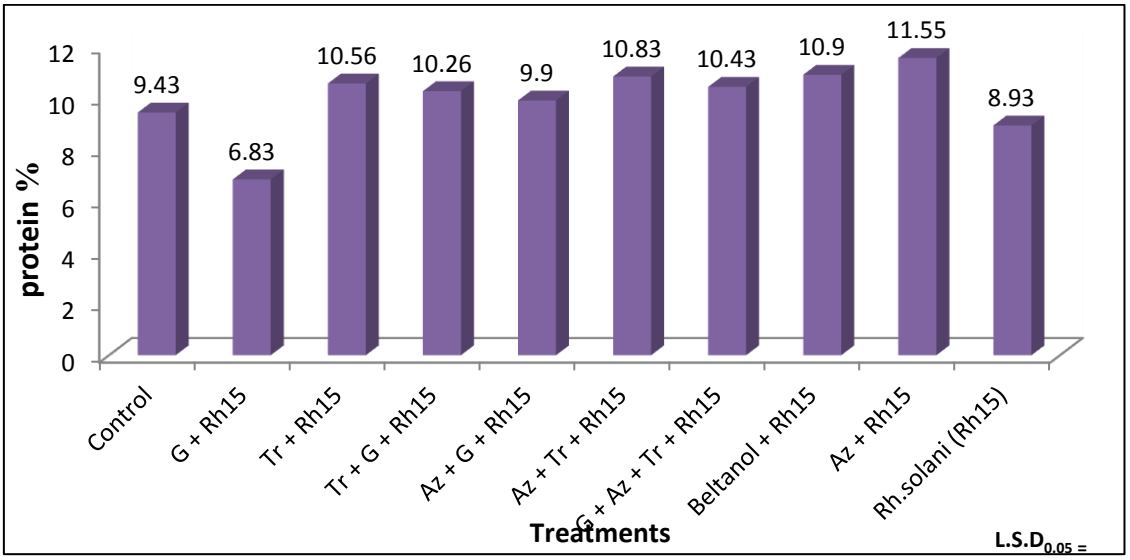

Figure (6): Effect of treatments of some biological factors on protein activity in potted pepper plants under greenhouse conditions.

** L.S.D$_{0.05}$ = 0.256 There are significant differences of the treatments.

The combination (Az.+ Tr. + G + *R.solani*) could improve plant growth when scored the highest fresh and dried weights which were 8.30 and 3.90 g/plant, respectively (Table 8) Whereas, plant fresh and dry weight scored 4,80 and 1.20 g/plant, respectively, in pathogen control treatment. The combinations treatment may improve growth parameters through increasing the nutrient availability in bell pepper leaves which may result in a positive improvement of growth indicators **(Al-Aboudi, 2019)**.

Table (8): The effectivity of bio-agents on plant fresh and dry weights in pots.

* Treatments	Fresh weight g/plant	Dry weight g/plant
Control	7.10	3.23
Glutathione (G)	8.50	3.87
A.brasilense (Az)	8.10	3.40
T.viride (Tr)	9.80	4.70
Az + G	9.50	4.30
G + Tr	9.27	4.10
Tr + Az	8.90	4.03
Az + Tr + G	10.00	4.90
R. solani	4.80	1.20
G + *R.solani*	7.00	2.77
Tr + *R.solani*	5.80	2.20
Az + *R.solani*	5.30	1.97

Tr + G + *R.solani*	7.50	3.37
Az + G + *R.solani*	8.10	3.80
Az + Tr + *R.solani*	8.10	3.97
Az + Tr + G + *R.solani*	8.30	3.90
Beltanol + *R.solani*	6.20	2.53
** $L.S.D_{0.05}$	0.48	0.28

***Each number represents an average of 3 replicates.**

****There are significant differences at the $L.S.D_{0.05}$ level of the treatments.**

CONCLUSION

Glutathione, *T.viride* and *A. brasilense* have high antagonistic ability against the pathogenic fungus *R.solani* on PDA medium. The use of the biostimulant Glutathione alone or in combination with *Azospirillum brasilense* provided protection for pepper plants from pathogenic fungi and increased plant growth parameters under greenhouse conditions.

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