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The Effects of organic fertilizer complement by addition Biological control agents on *Rhizoctonia solani* Kühn Causing of Eggplant root rot Disease

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Abstract

The research was aimed to study of some plant extracts, *Trichoderma harzianum* and Plant growth promoting rhizobacteria *Azotobacter chroococcum* and addition of organic fertilizer made of Date palm leaves in control Eggplant root rot disease caused by *Rhizoctonia solani* fungus. Results of the field survey showed the presence of Eggplant root rot disease in all the surveyed districts Baghdad, Babylon and Karbalaa province with disease incidence of 33.3-83.0% and severity of 16-60%. *T. harzianum* and *A. chroococcum* have highly antagonistic ability against of pathogenic isolates of *R. solani* under laboratory conditions. The plant extract that used in study (Cinnamon, ginger, Milk thistle, Crack willow) had against effect on the growth of pathogen on PDA. superior of Cinnamon extract which was prevent growth of pathogen. The results of the field experiment and lath house condition indicated that Integrated treatment with the *T. harzianum* and *A. chroococcum* caused significant reduction in disease severity to 33.33% and caused significantly enhanced eggplant growth parameters. The organic fertilizer of Date palm leaves increased the Efficiency of biological control agents. The integrated treatment between *T. harzianum* and *A. chroococcum* and organic fertilizer of Date palm leaves showed decreased in disease severity to 20.00% then increasing the plant growth parameters into 17.33 cm, 18.33 and 2.77g respectively. These results were appeared for the first time in Iraq that *T. harzianum*, *A. chroococcum* and organic fertilizer of Date palm leaves showed high antagonistic with *R. solani* and decreased severity of Eggplant root rot disease.

Keywords: Eggplant, Root rot disease, pathogenic fungus, Bio-agent and Plant extracts.

1. Introduction

Eggplant (*Solanum melongena* L.) in Iraq is one of the economically and nutritionally important crops, and the cultivated areas have increased, especially in greenhouses to meet the growing market need. The area under cultivation reached 25705 dunums, producing 104402 tons [1]. The eggplant crop is affected by many plant pathogens and pests that cause severe damage during the growth season [2]. *Rhizoctonia solani* is the most important soil borne fungi caused many diseases and is widespread on many economic agricultural crops, including eggplant. [3, 4]. This pathogen affects the seedlings of most vegetable crops, causing significant losses through death or rot of seeds and root rot and damping off of seedlings pre and post emergence, forcing farmers to replant [5, 6]. Several methods have been used to control the disease, the most important of which is the use of chemical pesticides, but the excessive and wrong use of these toxic substances led to environmental pollution and adverse effects on humans, animals, plants, soil, water, air and natural imbalance [7, 8]. The biological method and Integrated Pest Management (I.P.M) has recently appeared as environmentally friendly alternatives, in addition plant extracts have been used. Some plants contain substances that have the potential to inhibit the growth of many plant pathogens, especially fungi and bacteria, as well as less harmful, faster decomposition and difficulty resistance to them by pathogens [9]. Species belonging to the genus *Trichoderma* and bacteria that stimulate plant growth, including the genus *Azotobacter*, are among organism produce the antibiotics that have given encouraging results at the level of greenhouse and field experiments in combating some of the most important root rot causes and improving plant growth and production parameters [10, 11, 12, 13, 14]. Recently, there have been many calls to abandon or reduce the use of fertilizers and chemical pesticides, and to pay attention to the quality of the product and the return of the use of organic fertilizers [15, 16]. Due to the importance of eggplant root rot disease and to try to find ways of disease combating with modern methods, the study aimed



to: isolate and diagnose the cause of eggplant root rot disease, test the pathogenicity of its isolates, study the effectiveness of some biological agents, plant extracts and organic fertilizer of palm fronds against pathogenic fungus and its effects on the plant.

2. Materials and methods

2.1 Field survey and collection of samples to isolate the pathogenic fungus of eggplant seedlings

Eggplant seedlings were taken from some fields in Babylon, Baghdad and Karbala governorates randomly starting from 28/9 to 26/10/2015 (Table 2). The percentage and severity of the infection were calculated.

$$\% \text{Infection} = (\text{number of infected plants} / \text{number of tested plants}) \times 100$$

The severity of the infection was calculated according to the following pathological index: 0 = a healthy root. 1=1 - 25% of the root in a light brown color 2= Discoloration of more than 25 - 50% of the root in a dark brown. 3= Coloring more than 50 - 75% of the root in a dark brown color. 4= Coloring more than 75 - 100% of the root in a dark color without the death of the plant. 5= Death of plant. The severity was calculated according to the Mckinney equation [17]. As follows:

$$\text{Severity} = ((\text{number of plants in degree } 0 \times 0) + \dots + (\text{number of plants in degree } 5 \times 5) / \text{total number of plants examined} \times \text{highest degree of infection}) \times 100.$$

Isolation was carried out from each sample of infected eggplant and *Rhizoctonia solani* isolates was identified [18]. The fungal inoculum was prepared according to Dewan method [19] Used local millet seeds *Panicum miliaceum*. The same method was used to prepare the *Trichoderma harzianum* inoculum.

2.2 Effect of aquatic plant extracts of Cinnamon, Ginger, Milk thistle and Willow on the growth of *Rhizoctonia solani*.

Four plants were selected to study their effect against the *R. solani*. Table 1.

Table 1. Plants used in the study

Common name	Scientific Name	Used parts	Site of collection
Cinnamon	<i>Cinnamomum zeylanicum</i>	Bark	Local markets
milk thistle	<i>Silybum marianum</i>	Leaves	Al-Mussaib Technical institute
willow	<i>Salix alba</i>	Leaves, stem	Mahaweel -Azawea
ginger	<i>Zingiber officinale</i>	Rhizomes	Local markets

The method of Seema et al [20] was used in the preparation of aqueous extracts by mixing 150 g of plant powder for each plant sample separately with 1000 ml of distilled water, then leave the mixture for 24 hours under laboratory conditions, then filter the mixture and sterilized in an autoclave at 121 ° C for 5 minutes. Mix the aqueous extract of the selected plants with the PDA medium after sterilization. The dishes were inoculated at the center with a 0.5 cm disc from a fungal colony of the *R. solani*. The plates were incubated at 25 ° C [21]. The percentage of inhibition was calculated using the following equation:

$$\% \text{ Inhibition} = [(R - r) / R \times 100].$$

Where R is the growth of fungus in control treatment and r is the growth of fungus in control treatment.

2.3. Evaluation of aqueous extract of Cinnamon and *Azotobacter chroococcum*, *Trichoderma harzianum* and Bentanol in infection of *Rhizoctonia solani* and some growth parameters of eggplant plants under wood canopy conditions

This experiment was carried out in the wooden canopy of the Department of Plant Production Techniques on 11/3/2017 using plastic pots diameter of 12.5 cm and a capacity of 1 kg in which sterile soil which was planted by Syrian type of eggplant seedlings at the age of 21 days at the rate of five seedlings per pot. The experiment included the following treatment: 1- *R. solani* (Rh) alone. 2- Rh + *Azotobacter chroococcum* (Ac) 3- *R. solani*+ Cinnamon (Ci) 4- Rh + *Trichoderma harzianum* 5- Rh + Ac+ Ci 6- Rh + Th+ Ci 7- Ci 8 - Ac 9 - Th 10 - Ac+ Ci 11 - Th+ Ci 12 - Rh + Bentanol 13 - control without any addition. The bacterial *A. chroococcum* inoculum was added at a rate of 20 ml / pot (2 x 10⁶ colony forming units / ml).T.

harzianum was added by 1% w / w three days before the pathogen was added. aqueous extract of Cinnamon has been added at the rate of 25 ml after the addition of the pathogenic fungus inoculum during planting. The results were calculated after 40 days of the experiment by estimating the disease incidence and severity and calculated the plant height and the soft and dry weight.

2.4 The effect of organic palm frond fertilizer and biocontrol agents in controlling eggplant root rot disease and its effect on some growth parameters under field conditions.

The field experiment was conducted in the plastic house of the Department of Biological Control Techniques Department for the spring season 2018. the treatments were included the following: 1- *R.solani* (Rs) 2- Rs + *T. harzianum* (Th) 3- Rs + *A. chroococcum* (Ac) 4- Rs + Ac + Th 5- Rs + Palm Fertilizer 6- Rs + Ac + Palm Fertilizer 7- Rs + Th + Palm Fertilizer 8- Rs + Ac + Palm Fertilizer 9- Rs + Ac + Th + Palm Fertilizer 10- Rs + Beltanol 11- Control (No Addition) 12- Th 13- Ac 14- Ac + Th 15- Palm Fertilizer 16- Th + Palm Fertilizer 17- Ac + Palm Fertilizer 18- Th + Ac + Palm Fertilizer. The *R. solani* inoculum growth on millet seeds was added to all treatments that required to be added by making an incision along the center and continuously in equal quantity for all treatments by using a 250 cm³ flask containing the fungal inoculum for each treatment and then covered with moist soil from the center. The chemical pesticide Beltanol was added at a concentration of 1 ml / L water watering a day after the addition of pathogenic fungus. The *T. harzianum* was added a week before contamination of soil with pathogenic fungi at a rate of 6 gm². The *A.chroococcum* was added by 25 ml from a 3-day-old growing farm on liquid N.B medium a week before planting. The organic fertilizer for palm fronds (obtained from the Ministry of Agriculture / Organic Fertilizers Division - National Project for Manufacturing Organic Fertilizers in Babylon) was added to all treatments that require a week before planting at a rate of 20 g / experimental unit. Eggplant seedlings of class Barcelona age 30 days was cultivated. At the end of the experiment (after 30 days). The severity of root rot disease and plant length and wet, dry weight was estimated.

3. Results and discussion

3.1 Field survey for the damping off diseases on eggplant seedlings

The results showed the presence and prevalence of eggplant root rot and damping off disease in all the surveyed areas in Babylon, Baghdad and Karbala governorate. The highest incidence was in the samples of Babylon/Al-Yassia and Al-Siyahi districts, which reached 62.5 and 80.0% respectively and the severity rates were 55 and 60% respectively (Table 2). This districts were cultivated annually, resulting in the accumulation of the pathogenic fungus inoculum, particularly Sclerotia, which remains in the soil for as long as five years [22, 23].

Table 2. Temporal and spatial distribution of eggplant field locations surveyed.

No. sample	District	Data	Disease incidence%	Severity%	Isolate symbol
1	Karbala\ Al - Atishy	2017/9/28	80.0	24	-
2	Karbala\ Al-Hussainiyah	2017/9/28	83.0	23	Rs-1
3	Babylon \ Mussaib	2017/9/30	75.0	25	-
4	Baghdad\ Latifiya	2017/10/8	33.3	25	Rs-2
5	Baghdad \ Krayat	2017/10/8	80.0	16	Rs-3
6	Babylon \Badaa	2017/10/24	80.0	20	Rs-4
7	Babylon \ Al-Siyahi	2017/10/25	62.5	55	Rs-5
8	Babylon \ Al-Yassia	2017/10/25	80.0	60	Rs-6
9	Babylon \ Khanfara	2017/10/26	40.0	30	Rs-7
10	Babylon \ Imam	2017/10/26	80.0	20	Rs-8

3.2 Isolation of *Rhizoctonia solani* from infected plant parts

The results of the isolation of *R. solani* showed 8 pure isolates of *R. solani* from Karbala / Al-Hussainiyah, Babylon / Khanfara, Badaa, Imam, Al- Yassia, Baghdad, Al-Krayat and Latifiya fields. The results are consistent with several studies indicated that *R. solani* is the most frequent isolate from the roots of infected plants, including eggplant, and is a major pathogen of damping off and root rot in many fields [24, 25, 26, 27]. The results also showed that *R. solani* did not appear in the samples of Karbala / Al-Atishy and Babylon / Mussaib areas. The isolation results also showed the appearance of a number of fungi associated with infected roots such as *Aspergillus niger*, *A. flavus* and *Fusarium* sp. And *Penicillium* sp. *Macrophomina phaseolina* and *Rhizopus* sp. with lower appearance rates.

3.3 Effect of aqueous extract of Cinnamon, Ginger, Milk thistle and Willow on the growth of pathogenic fungus *R.solani* using food poisoning method with medium.

The results shown in Table 3. showed that the aqueous extracts of Cinnamon, Ginger, Milk thistle and Willow (5, 10 and 15%), respectively, achieved inhibitory activity against *R. solani* compared with non-extract control and significant superiority of the treatment of Cinnamon extract by decreasing the growth of pathogenic fungus to 0.00- 1.00cm. The increase in inhibition was directly proportional to the increase in concentration to reach the highest readings of water extracts of Cinnamon, Ginger, Milk thistle and Willow at 15% concentration of 37.00, 40.00, 26.00, 63.67, 100%, respectively. The results are consistent with what many researchers have found to be effective in using some plant extracts to inhibit the growth of pathogenic fungus *R.solani* [20, 28]. These plant extracts are inhibited because they contain active substances such as alkaloides, Glycosides, Phenols, Sapanins, Lectins, Tanins, Flavinooids, Volatile oil and many other substances [29].

Table 3. Effect of Cinnamon, Ginger, Milk thistle and Willow on the growth of pathogenic fungus *R.solani*.

Plant name	Concentration%	Colony diameter(cm)	Inhibition (%)
Cinnamon	5	1.0	88.90
	10	0.0	100
	15	0.0	100
Ginger	5	5.3	40.3
	10	4.2	53.3
	15	3.2	63.67
Milk thistle	5	7.5	16.70
	10	7.0	22.2
	15	6.7	26.00
Willow (leaf)	5	6.0	33.33
	10	5.5	38.4
	15	5.4	40.00
Willow (bark)	5	6.1	32.22
	10	5.9	34.44
	15	5.7	37.00
Control	0	9.0	0.00
L.S.D	-	0.12	1.512

3.4 Effect biological control agents and aqueous extract of Cinnamon and their efficiency in protecting eggplant seedlings from *Rhizoctonia solani* infection under wood canopy conditions.

The results Table 4. of the treatment of eggplant seedlings with bio-control agents and aquatic plant extract showed a reduction in the disease incidence and severity and an increase in plant growth parameters. Significantly superior to the interference treatment between *T. harzianum* and aqueous extract of Cinnamon in which the infection rate was reduced to 22.2% and the severity of the infection was 4.44% followed by the interaction of *A. chroococcum* with the aqueous extract of Cinnamon which had an infection rate and severity 33.33% and 6.66%, respectively. The length of the plant and the wet and dry weight were also increased by addition the control treatments, the interaction between *T. harzianum* and the Cinnamon extract improve the growth criteria, which reached 11.70 cm and 4.20 g and 1.17 g and the treatment of the interaction between *A. chroococcum* with aqueous extract, which reached 10.80 cm 4.00 g and 0.93 g respectively, approaching and without significant differences from the most efficient Pesticides used to combat damping off and root rot disease, Beltanol, which in turn achieved a significant reduction in the incidence of eggplant damping off and improved seedling growth in the treatment of 11.1% and the severity of infection 2.22% compared to the pathogen alone, which was the disease incidence and severity of infection at 100 and 92%, respectively. Thus reducing morale The results showed that the addition of biological agents and aqueous extract without the addition of pathogenic fungi achieved significant increase in plant growth parameters compared to the control treatment without any addition. The length of the plant and the weight of wet and dry at 12.33 cm and 4.57 g and 1.27 g. This result is consistent with what many researchers have said that *A. chroococcum* is highly competitive with pathogenic fungus, giving scope for use in integrated plant disease management programs. One of the qualities that has enabled these bacteria to control pathogenic fungi is their rapid growth In the medium in which they live, their high competitiveness enables them to settle in the Rhizosphere zone and exploit the available food sources and their ability to produce Sidrophores and secrete some antibiotics that analyze the cytoplasm of fungal threads in addition to producing and for Indol acetic acid and gibberellins plant hormones [30, 31]. Also, *T. harzianum* has the ability to secrete certain chemical compounds that have a pathogenic inhibitory effect. It is also characterized by its ability to fungal parasitism by wrapping the fungal hyphae of the biological control fungus around the hyphae of pathogenic fungi then analyzed by enzymes which are thought to play an important role in inhibiting the develops of the pathogen, which hinders the occurrence of infection [11]. The effect of the Cinnamon extract is due to the fact that it contains anti-pathogenic compounds and may stimulate several biochemical defenses in the plant such as stimulating plant enzymes against fungi, including Chitinase, Chitosan and β -1,3 – glucanase, as well as chemical and synthetic changes in the cell wall. It is due to the amount of phenolic compounds associated with the wall and callus deposition [32].

3.5 The field experiment

The results of this experiment (Table 5) showed that the agents used have significantly reduced the negative effects of the pathogenic fungus and provided good protection for eggplant plants from infection. *A. chroococcum* (Ac) and *T. harzianum* were highly efficient in controlling the pathogen and significantly reduced the disease incidence. The treatment of the *A. chroococcum* and *T. harzianum* was effective in controlling *R. solani* and reduced the severity of infection to 33.33% and contributed significantly increase to the studied plant growth parameters of plant height, wet and dry weight to 12.67 cm, 17.33 and 2.40 g. Respectively. As for the role of the chemical pesticide Beltanol in the control of the disease, the results showed its high effectiveness in influencing the pathogenic fungi and thus reduce the incidence and severity of the resulting infection. The results showed that the addition of organic fertilizer to palm fronds reduced the severity of the pathogenic fungus. The addition of organic fertilizer to palm fronds increased the efficiency of the biological agents in its antagonism with the pathogen. The integration of treatment between organic fertilizer for palm fronds and *T. harzianum* and *A. chroococcum* and the presence of the pathogenic fungus reduced the severity of infection to 20.00% and significantly increased plant height, wet and dry weight to 17.33 cm, 18.33 and 2.77 g respectively, and significant difference on the treatment of integration between *T. harzianum* and *A. chroococcum* without the addition of organic fertilizer.

Table 4. Effect of Biocontrol agents and their efficiency in controlling *Rhizoctonia solani* under wood canopy conditions.

Treatments*	Diseases incidence (%)	Severity (%)	Plant high (cm)	weight (g)	
				Wet	Dry
Rs-1	100.0	92.00	6.07	1.03	0.39
Ac+ Rs-1	44.4	17.74	8.54	3.30	0.97
Ci+ Rs-1	55.5	22.17	8.20	3.03	0.77
Th+Rs-1	33.3	13.33	9.10	3.47	1.10
B+Rs-1	11.1	2.22	12.00	4.37	1.23
Ci +Ac+Rs-1	33.3	6.66	10.80	4.00	0.93
Ci + Th + Rs-1	22.2	4.44	11.70	4.20	1.17
Control	0.0	0.00	12.33	4.57	1.27
Ac	0.0	0.00	13.23	4.80	1.60
Ci	0.0	0.00	12.83	4.67	1.43
Th	0.0	0.00	13.60	5.00	1.67
Ci +Ac	0.0	0.00	13.83	5.20	1.73
Ci + Th	0.0	0.00	14.23	5.60	1.90
L.S.D.(0.05)	17.91	5.65	0.33	0.17	0.14

*Each number represents an average of 3 plants / refined, and three replicates. RS-1 = *Rhizoctonia solani*, Ac = *Azotobacter chroococcum*, B = Beltanol, Ci=Cinnamon, Th = *T. harzianum*, the number next to the symbol represents the isolation number.

On the other hand, the addition of biocontrol agents alone and integrated with organic fertilizer resulted in a significant increase in plant height and wet and dry weight of eggplant plant and a significant superiority for the treatment of the integration between *Azotobacter* and *T. harzianum* in the presence of organic fertilizer for palm fronds which improved plant growth into 23.67 cm, 26.67 and 3.20 g respectively. The effect of adding organic fertilizer to palm fronds may be attributed to the fact that it is a locally produced and pasteurized fertilizer as it does not contain harmful pathogens due to high temperatures of more than 60°C during fermentation and preparation. On the other hand, the addition of organic fertilizer has altered the pathogen's environment and made it unsuitable for its growth. Improved the effectiveness of biological agents as it provided a nutritional base for its growth, which increased its effectiveness towards the pathogen as well as the efficiency of organic fertilizer in increasing the readiness of the plant of nutrients and thus increase its resistance to the disease and increase its growth and production. The results are consistent with the findings of Abd El-Gaid and Nassef [16] that the addition of organic palm frond fertilizer improved the germination and growth of many vegetable seedlings, including eggplant and superior to imported organic fertilizer, where it was found that the organic fertilizer of palm frond contains good amounts of nitrogen and high levels Phosphorus and potassium are higher than those in imported compost. These findings are consistent with Benuzzi et al. [9] that *Azotobacter* is an important inhibitor of pathogenic fungi as well as its important role in increasing plant growth and development. Almammory and Matloob [33] found the effectiveness of *T. harzianum*, Bio-fertilizer Bokashi and salicylic acid alone or interrelated, led to a significant increase in the percentage of germination of eggplant seeds and provide good protection for eggplant seeds and plants and contrast to the *R. solani* that caused the damping off disease of eggplant seedlings.

Table 5. Efficacy of *Trichoderma harzianum*, *Azotobacter chroococcum*, and Palm Fertilizer in Severity of Eggplant Root Rot Caused by *Rhizoctonia solani* under Field Conditions.

Treatments*	Severity (%)	Plant height (cm)	Weight (g)	
			Wet	Dry
Rs-1	93.33	5.33	3.67	0.67
Th+ Rs-1	46.67	10.67	11.33	1.77
Ac+Rs-1	40.00	11.00	11.67	2.03
Th+Ac+Rs-1	33.33	12.67	17.33	2.40
Po +Rs-1	73.33	7.33	4.50	0.83
Po+Th+Rs-1	40.00	13.33	14.33	1.97
Po+Ac+Rs-1	33.33	14.67	15.33	2.27
Po+Ac+Th+Rs-1	20.00	17.33	17.33	2.77
Bel +Rs-1	6.67	16.33	14.67	2.03
Control	4.44	17.13	16.50	2.23
Th	0.00	20.33	17.67	2.57
Ac	0.00	19.33	17.33	2.46
Ac+Th	0.00	21.33	19.33	2.80
Po	0.00	19.33	17.22	2.43
Po+Th	0.00	21.00	23.67	2.97
Po+Ac	0.00	20.67	23.00	2.90
Po+Ac+Th	0.00	23.67	26.67	3.20
(0.05) LSD	11.38	1.29	1.12	0.098

*Each number represents an average of 3 plants / refined, and three replicates. RS-1 = *Rhizoctonia solani*, Ac = *Azotobacter chroococcum*, B = Beltanol, Ci=Cinnamon, Th = *T. harzianum*, Po= organic fertilizer for palm fronds, the number next to the symbol represents the isolation number.

Conclusions

The presence of Eggplant root rot disease in all the surveyed districts Baghdad, Babylon and Karbalaa province. The integrated treatment between *T. harzianum* and *A. chroococcum* and organic fertilizer of Date palm leaves showed decreased in disease severity then increasing the plant growth parameters. These results were appeared for the first time in Iraq that *T. harzianum*, *A. chroococcum* and organic fertilizer of Date palm leaves showed high antagonistic with *R. solani* and decreased severity of Eggplant root rot disease.

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