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EFFICIENCY OF *TRICHODERMA HARZIANUM* AND BIO-FERTILIZER BOKASHI AND SALICYLIC ACID TO CONTROL OF FUNGI CAUSING EGGPLANT DAMPING OFF DISEASE

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Abstract

The aims of the study was to control the pathogens of Damping off disease on Eggplant in Babylon province, Iraq using some biological control agents and salicylic acid. The results showed the potential of *Tichoderma harzianum* and Effective Microorganisms (EM1) when tested with pathogenic fungi (*Rhizoctonia solani* (RS-1), *Fusarium solani* (FS-2) and *Macrophomina phaseolina* (MP-1)) under laboratory conditions. The results of the field experiment showed that all the treatment used in the experiment, which included *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. The treatment of *T. harzianum*, Bokashi and salicylic acid in the percentage of germination increased between 87.50-95.83%. And reduced the disease incidence and severity. The results showed that all the factors achieved a significant increase in the growth indicators of eggplant seedlings, namely plant length, wet and dry weight and leaf area compared to the treatment of fungus alone. The highest yield was recorded in the treatment of *T. harzianum*, bio-fertilizer and Salicylic acid, with a significant increase in plant height ranging from 28.33-30.00 cm^2 and wet and dry weight to 8.07-8.85 and 2.55-2.84 g Respectively and also contributed significantly to the increase and leaf area, which amounted to 23.80-24.67 cm^2. And increase leaf content of Chlorophyll.

Key words : Eggplant, damping off disease, biological control, Bokashi, salicylic acid.

Introduction

Eggplant yield in protected and exposed agriculture is affected by many pathogens of fungal, bacterial, viral and nematode diseases that cause severe damage (Bletsos *et al*., 1999; Janas *et al*., 2002; Sadeghi *et al*., 2008; Raigon *et al*., 2010). Eggplant Damping off disease and root rot is one of the most common diseases in nurseries and protected houses. It is widespread all over the world. It causes many soil- borne fungi. Because these organisms live in the soil, they often destroy the root mass without feeling their presence (Garret, 1977). *Fusarium solani*, *F. oxysporum*, *Rhizoctonia solani* and *Macrophomina phaseolina* are among the most important and prevalent soil diseases (Agrios, 2005; Dar *et al*., 2018). Several methods have been used to control of damping off and root rot diseases, including the use of chemical and agricultural control, resistant varieties and plant extracts (Ahmed *et al*., 2009; Salim *et al*., 2017; Shah *et al*., 2018). The intensive use of pesticides has been accompanied by negative impacts on the environment, human health, and non-target organisms (Brent, 1995 and Lorenz, 2009). As a result, consideration was given to alternatives, most notably the use of microorganisms in biological control programs to reduce inoculums of pathogens and increase quantity and quality of yield. The most important of these factors is *Trichoderma harzianum* and Plant Growth promoting Rhizobacteria (PGPR) are the already inhibitory or competitive of pathogens and stimulate the growth and defense of the host plant and other mechanisms that prompted many researchers interested in the study and development. (Benuzzi *et al*., 2004; Demir and Akkopru, 2007 and Kaewchai *et al*., 2009). Due to the importance of eggplant Damping off and the need for more information needed to control the disease and reduce its

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economic damage in safety methods, the study was aimed to isolate and diagnosis of pathogens causing damping off disease and control its by use some biological and microbiological EM-1 and chemically induced Salicylic acid under the laboratory and nursery conditions.

Materials and Methods

Isolation of some fungi associated with the roots of the infected eggplant seedlings and their diagnosis and testing of their pathogenicity

A number of field visits were carried out to nurseries and fields cultivated with Eggplant in Babylon governorate during the agricultural season 2016-2017. The province included the forward, Al- Nile district, Jebelah, Al-Mahawel, Al-Bada and Mashrooa Al-Mussaib (table 1). The samples of eggplant seedlings which were infected by rot of roots and stems close to the surface of the soil and carried out the isolation of fungus of plants used the Potato Dextrose Agar (PDA) medium added to the antibiotic Tetracycline at a concentration of 200 mg/L (Pathak, 1974). The fungi was identified for the level of Genus and species after the emergence of fungal mycelium based on the characteristics of the fungal colony and the nature of the fungal hypha and spores using the classification keys (Parmeter, Whitney, 1970; Ellis 1971, Booth, 1971, 1977; Sneh *et al*., 1996). The pathogenicity of all isolate *R. solani*, *F. solani* and *M. phaseolina* obtained through isolation from infected eggplant was tested. The method previously described by Bolkan and Bulter (1974) was tested. Some pathogenic fungi in the germination of eggplant seeds under the conditions of the nursery to obtain the most pathogenic fungi.

Table 1 : Districts collection of eggplant seedlings in some fields and nurseries of Babylon province for the agricultural season 2016-2017.

No. sample	Field site\ Babylon	Date\2017
1	Al- Nile	1\12
2	Jebelah	1\14
3	Al- Sabaghia	1\21
4	Al-Mahawel	1\23
5	Al-Bada	3\15
6	Mashrooa Al-Mussaib	3\22

Trichoderma harzianum against *Rhizoctonia solani*, *Fusarium solani* and *Macrophomina phaseolina* on PDA

T. harzianum fungus were obtained from the Plant Pathology Laboratory, Technical College of Al-Mussaib. Its antagonism ability was tested against the selected isolation of *R. solani*, *F. solani* and *M. phaseolina*. In the double culture method, the experiment was performed with 3 replicates. The dishes were placed in the incubator at 25±1°C for one week. The degree of antagonistic ability was estimated depend on keys which mentioned in Bell *et al*. (1982) have five to five degrees.

Activation of EM1 formula

The primary EM1 solution is inert and needs to be activated before adding it.EM-1 was activated in combination with molasses and warm water free of chlorine by 5: 5: 95, respectively. Solve the molasses well with sterile warm water, then add the EM solution and mix well the solution position in a sealed plastic barrel. Place a plastic barrel to prevent air contact with the solution and place the drum in a warm place away from the sun for 10 days in the summer (temperature 35-40^{0}C) and for one month in winter (15-20^{0}C). Open the barrel twice - 3 times to leak the formed gas and form a layer of deposit at the bottom of the barrel, and the pH of the solution should be between 3.5-4 using a pH meter (APNAN, 1995). It is used within a month and may not be activated again.

Test the anti-microbiological potential of EM1 against pathogenic fungi on the PDA medium

Antimicrobial ability of EM1 formula (which prepared in previous paragraph) was tested against pathogenic fungi *R. solani* (RS-1), *F. solani* (FS-2) and *M. phaseolina* (MP-1) on the PDA medium by adding the EM-1 formula using 3 concentrations 5, 10 and 15%. Repeat each treatment 3 times and leave 3 dishes without adding the EM-1 as a control. The dishes were incubated at 25±1°C for 7 days (Fatima *et al*., 2009). The growth rate of pathogenic fungi and the percentage of inhibition were calculated according to the following equation:

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

Where, r is the radius of the fungal colony against the bioagents and R is the radius of the fungal colony without the bioagents.

Preparation of bio fertilizer Bokashi

Bokashi is a Japanese word that means organic fertilizer fermented by the EM solution. The Bokashi was prepared from the following materials: 1 - wheat bran. (1:1) (size: size: size) Materials 1, 2 and 3 mixed the same proportions as the homogeneous mixture on a piece of plastic or thick nylon. The EM solution was added with continuous stirring until the moisture ratio reached about 30-40%. Then in black nylon bags and pressed well until the bag was filled, the bags were sealed and placed again in other bags and closed tightly to ensure that the air did not enter the mixture. The bags were left for a closed month in a warm, dark place away from direct sunlight.

The bags were opened and white growth was observed on the surface of the mixture with a distinctive smell and the disappearance of the odor of foul animal waste, which meant that manure was ready for use (APNAN, 1995).

Effectiveness of the *Trichoderma harzianum* and the biofertilizer and salicylic acid in the control of Eggplant damping off disease and its effect on some growth parameters under nursery conditions

This experiment was carried out under the conditions of the plastic house in the Imam district of Babylon governorate for the agricultural season 2017-2018 and using the seedling trays made of Styropor, the tray contains 11 × 19 holes. And 12 pits per treatment were used and the peat moss were used as a seed medium. The pathogenic fungi inoculum was prepared according to the Dewan method (1989). The local millet seeds were used for the preparation of fungal inoculums then the fungal inoculums were added to the treatments by 1% (weight: weight). The treatments of this experiment included the following : 1-*R.solani* alone (RS-1). 2- RS-1 + *T. harzianum* (Th). 3-RS-1 + Salicylic acid (SA). 4- RS-1 + Bokashi. 5- RS-1 + Th+SA. 6- RS-1 + Th+ Bokashi. 7- RS-1 + Th+SA + Bokashi. 8- RS-1 + Beltanol. The above treatments were repeated with pathogenic fungi *F. solani* (FS-2) and *M. phaseolina* (MP-1) each separately. 25- Control treatment (without adding pathogenic fungus). 26- Th(alone). 27- Sa (alone). 28- The Bokashi (alone). 29- Th + Sa .30- Th + Bokashi. 31- Th+SA +Bokashi. The medium of the planting (peat moss and Loam soil 1: 1) was well formed and quantities were placed on nylon pieces independently to be added treatments above. The *T. harzianum* inoculum. On local millet seeds was added. (1% weight / weight) before 5 days of planting by mixing with the medium of planting before being distributed to the holes and moistened with water to allow the fungus to grow (Sallam *et al*., 2008). While the fungicide Beltanol was added at a concentration of 1 ml/l after one day of addition of the pathogenic fungus. Where, bio-fertilizer Bokashi was added at a concentration 8% to the medium during planting (Al-Jarah, 2011). Salicylic acid was added at a concentration of 100 mg/l by soil watering and 2-3 mm/hole after planting seeds directly. The seeds were planted with local eggplant seeds (not treated with fungicides) of the Barcelona variety, with 3 seeds / hole. The plates were irrigated and fertilizer with hummus (0.5/100 L) and iron phosphate (1g/L). Seedling were separated after 4 weeks of planting. They were transferred to plastic bags of seedlings 10 × 10 cm. The same materials were added. Each according to the treatment to continue experiment until the export of seedlings and reach the age of delivery to the farmers. The complete random design was used with three replicates. The germination ratio was calculated after the germination of the seeds of the control treatment was completed (after 8 days) and according to the following equation:

% Germination = (Number of seeds grown / Total number of seeds) ×100.

The other results after 6 weeks of planting (the age of delivery to the farmer) were calculated, the disease incidence was estimated by using the following equation: % Disease incidence = (Number of infected seedlings\ Total number of total seedlings) × 100.

The severity of infection was calculated using the 6-degrees pathological index, as follows: 0 = no symptoms. 1 = 1-25% of the infected roots show rot. 2 = more than 25-50% of the roots have symptoms of rot. 3 = more than 50-75% of the roots show symptoms of rot. 4 = rot more than 75-100% of the roots and crown dark color. 5 = plant death. The percentage of severity according to Mckinney (1923) was calculated as follows:

Severity (%) = ((Plants in 1 degree ×1+… Plants in 5 degree ×5)/ all plants ×5) ×100%.

The length of seedling, wet and dry weight (average weight of 4 plants/replicate) and leaf area were calculated using the Planimeter system and the chlorophyll ratio using SPAD.

Results and Discussion

Isolation and diagnosis of fungi causing the damping off disease of eggplant seedlings

A microscopic test of the fungal growths of the infected plant parts on the PDA medium showed that there were species of fungi associated with the roots and bases of the stems of eggplant seedlings infected with damping off disease (table 2). *F. solani* was the most common fungus in most samples with varying rates of recurrence ranging from 20-67.5%, followed by *R. solani* and *M. phaseolina* at a rate of 44.16 and 29.16% respectively (fig. 1). This may be attributed to the repeated cultivation of Eggplant in these areas, which led to the accumulation of the inoculum and the increase in the annual density of the availability of the host and the appropriate environmental conditions. The results are consistent with the results of many studies of the emergence and spread of pathogenic soil fungi on Eggplant and caused of damping off disease on eggplant and other crops. The studies found that the isolated fungi were mainly *M. phaseolina*, *R. solani* and *Fusarium* sp. (Al-Isawi, 2010; Al Mamoori, 2014; Mwaniki *et al*., 2015; Mishra, 2017).

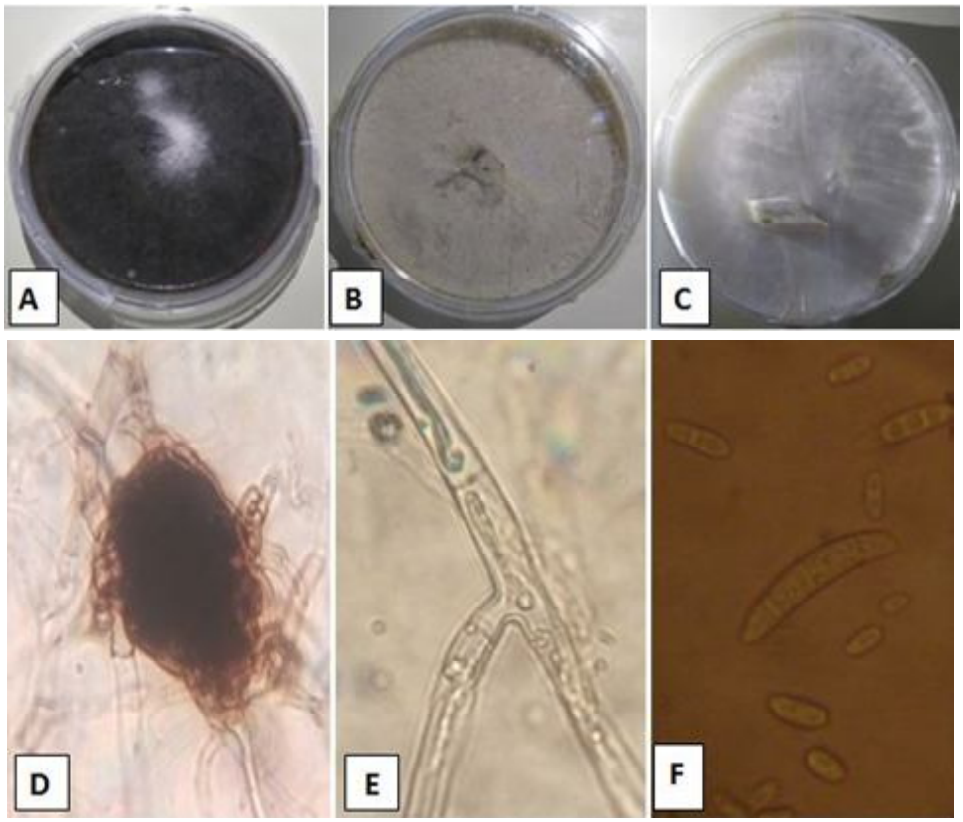

Fig. 1 : Cultural and microscopic properties of some fungi that cause damping off disease on Eggplant.. A. *Macrophomina phaseolina* colony of 7 days growing on the PDA. B. colony of *Rhizoctonia solani* fungus. C. colony *Fusarium solani*. D. The sclerotia of the *M. phaseolina* (100X). E. The diagnostic characteristics of *R. solani* describe the acute branching angle of modern developing fungal hyphae, which is a fixed diagnostic feature. And constricted near branching area and the septum close to the branching..F. Macro and Microconidia of *F. solani*.

The results of the isolation showed the presence of many fungi associated with the roots and stem bases of Eggplant appeared less frequency such as *Aspergillus niger* and *Botrytis* sp., *Penicilliuum* spp. and others. The isolates were given numbers to distinguished from other isolates.

Detection of pathogenic isolates using eggplant seeds

All fungi tested caused significantly reduced in eggplant seed germination. Table 3 shows that all isolates of the fungus *R. solani*, *F. solani* and *M. phaseolina* significantly reduced the percentage of eggplant seed germination under laboratory conditions. The RS-1, RS-4, RS-6 and FS-2 isolates were superior in reducing the percentage of germination that was between 0-6.67% compared with the control treatment, which had a germination rate of 100.00%. While the other isolates achieved a significant decrease in the percentage of eggplant seeds germination, which ranged between 10-50%. In addition, *M. phaseolina* isolated the area of the Nile area significantly reduced eggplant seed yield amounted to 23.33%.

The cause of the effect of the isolates is due to the level of secretions of fungi from toxic secondary metabolic compounds that kill the embryo and to the ability to produce the enzymes that are responsible for the rot in the seeds and then prevent them from germination. Some researchers also said that proteinase has a big role in determining the pathogenic ability For fungi *R. solani* (Weinhold, Sinclair, 1996; Mehrotra *et al*., 1997; Schwartz, 1999; Sett *et al*., 2000; Ramezani, 2008).

Results in table 4 indicated that all tested fungi *R. solani* (RS-1), *F. solani* (FS-2) and *M. phaseolina* (MP-1) were caused a significant reduction in eggplant seed germination under nursery conditions. *R. solani* (RS-1) prevented seed germination completely Compared to the control treatment without the addition of pathogenic fungi, which was germination rate of 100%. *F-solani* (FS-2) and *M. phaseolina* (MP-1) caused a significant reduction in germination rate of 23.33 and 26.67%, respectively.

R. solani attacks the host's seeds and causes them to be rotted and prevented from germinating. Seedlings are attacked before they emergence, resulting in a significant reduction in germination rates by killing the

Table 2 : Fungi associated with the roots of the infected Eggplant seedlings and their location and frequency in the samples.

Fungi	No. sample*	Replicate of fungus in samples (%)**	
		The highest ratio	Average
Aspergillus niger Van Tieghem	1,5	20	13.75
Botrytis sp.	5	12.5	12.5
Fusarium solani (Mart.) Sacc.	1-3, 5-6	67.5	35.0
Macrophomina phaseolina (Tassi) Goid.	1-3, 5	37.5	29.16
Penicilliuum spp.	4	7.5	7.5
Rhizoctonia solani Kuhn	1,4,6	87.5	44.16

*No. sample, numbers represent sample collection areas (Table 1).
%** repeat the fungus in the sample = (The number of fungus appeared in dishes\Total number of pieces used in the sample)x 100.

Table 3 : Detection of pathogenic isolates associated with the roots and bases of the stem of eggplant seedlings infected by damping off disease using eggplant seeds.

Isolate	% germination	Isolate	% germination
FS-1	50.000	**RS-6**	6.667
FS-2	3.333	**MP-1**	23.333
FS-3	16.667	**MP-2**	40.000
FS-5	10.000	**MP-3**	50.000
FS-6	13.333	**MP-5**	43.333
RS-1	0.000	**Control**	100.000
RS-4	3.333	**L.S.D.(P.0.05)**	**9.689**

RS = *Rhizoctonia solani*, FS = *Fusarium solani*, MP = *Macrophomina phaseolina*, the number next to the symbol represents the isolation number (isolation district).

Table 4 : Effect of *Rhizoctonia solani*, *Fusarium solani*, MP = *Macrophomina phaseolina* in germination of eggplant seeds under nursery conditions.

Isolates	% Germination
FS-2	23.333
RS-1	0.000
MP-1	26.667
Control	100.000
LSD (0.05)	**7.6867**

RS = *Rhizoctonia solani*, FS = *Fusarium solani*, MP = *Macrophomina phaseolina*, the number next to the symbol represents the isolation number.

seed or weakening the seed and delaying its emergence. Root rot and the bases of the seedling stems close to the surface of the soil, causing seedling to fall after they emerge on the soil surface and die (Agrios *et al*., 2005). This was confirmed by earlier studies that the fungi *F. solani, R. solani* and *M. phaseolina* are the leading fungi of eggplant damping off disease (Al-Isawi, 2010; Al Mamoori, 2014; Mwaniki *et al.*, 2015; Mishra, 2017).

Trichoderma harzianum against some pathogenic fungi in the laboratory

The results of this study (table 5) showed the potential of *T. harzianum* when tested with pathogenic fungi (*R. solani* (RS-1), *F. solani* (FS-2) and *M. phaseolina* (MP-1)). The results showed a difference in the effect of *T. harzianum* on fungi. *T. harzianum* achieved a high antagonistic ability against *F. solani* with an average of 1.33 degrees and1.67 degrees with *R. solani* while achieving a high degree of resistance to fungus *M. phaseolina* has reached one degree controlling the entire area of the dish then prevent pathogenic fungi from growing. Compared to the growth of pathogenic fungi in private as it was normal growth and filled the entire area of the dish. These results were consistent with Koicham and Sinha (2018) demonstrating the efficacy of *Trichoderma* isolates against pathogenic fungus *R. solani*, resulting in a high inhibitory rate of 78.82-94.11% when tested in double culture.

The antimicrobial potential of *T. harzianum* is due to a variety of mechanisms by which the fungus is affected by direct mycoparasitism, by twisting the fungus *T. harzianum* around the pathogen, penetrating it and emptying its cellular contents. Or it may be due to its ability to secrete antibiotics and some enzymes for the cell walls of fungus such as B-1,3- glucanase, Protease and Chitinase, or by meeting these mechanisms, such as parasitism, antimicrobial production, competition for food and place (Harman, 2000(.

The efficacy of EM-1 microorganism against pathogenic fungi on the PDA medium

The results of the test of antagonistic ability of Effective Microorganisms EM1 against pathogenic fungi.

Table 5 : Test of the antagonistic ability of *Trichoderma harzianum* against some fungi isolates that cause eggplant damping off disease.

Treatment	Degree of antagonistic ability
TH+FS-2	1.33
TH+RS-1	1.67
TH+MP-1	1.00
LSD (0.05)	**0.942**

* Each number represents an average of 3 replicates. RS = *Rhizoctonia solani*, FS = *Fusarium solani*, MP = *Macrophomina phaseolina*, TH = *Trichoderma harzianum*.

Table 6 : Efficiency of EM1 against pathogenic fungi *Rhizoctonia solani*, *Fusarium solani* and *Macrophomina phaseolina* in the PDA.

Treatment*	Concentration(%)	Inhibition (%)
FS-2+EM-1	15	94.44
RS-1+EM-1		90.73
MP-1+EM-1		98.15
FS-2+EM-1	10	88.88
RS-1+EM-1		81.48
MP-1+EM-1		92.59
FS-2+EM-1	5	54.22
RS-1+EM-1		61.22
MP-1+EM-1		72.28
FS-2	0	0.00
RS-1		0.00
MP-1		0.00
LSD	-	**4.814**

* Each number represents the rate of 3 replicates. RS = *Rhizoctonia solani*, FS = *Fusarium solani*, MP = *Macrophomina phaseolina*, EM = Effective Microorganisms.

solani (RS-1), *F. solani* (FS-2) and *M. phaseolina* (MP-1) causing the seedling disease on the PDA medium (table 6) were shown that EM1 formula have high antagonistic ability against pathogenic fungi. The highest degree in the inhibition of pathogenic fungi was in concentration 15%, ranging from 94.44 to 98.15%, followed by a concentration of 10% with an inhibition rate of 81.48-92.59%. In comparison to the control treatment, where the percentage of inhibition was 0%. The results agreement with a previous study that indicated that EM1 is highly efficient in inhibiting the growth of fungal and bacterial pathogens. It was found that the rate of inhibition of *R. solani* was 50% when adding 5% of the preparation (Castro *et al*., 1995) and agree with Al- Jarah (2011) when used for different concentrations of EM1 10-6-3% in laboratory where the percentage of inhibiting *R. solani* 88-78-59% for the three concentrations, has been indicated and the mechanism of inhibition of the preparation EM1, Of microorganisms against the pathogen. They also produce antifungal agents such as antibiotics that inhibit or reduce the growth of many pathogens (Higha, 2006).

Effect of *Trichoderma harzianum*, Bio-fertilizer Bokashi and Salicylic acid in seed germination and its efficiency in controlling Eggplant Damping off disease and its effect on some growth parameters under nursery conditions

The results of the field experiment (table 7) showed that all the treatments used in the experiment, which included *T. harzianum*, Bokashi and salicylic acid were individual or integrated, leading to a significant increase in the percentage of germination of eggplant seeds despite the presence of pathogenic fungi *R. solani* (RS-1), *F. solani* (FS-2) and *M. phaseolina* (MP-1), which cause Damping off disease, which reflects its inhibitory efficiency and its ability to control of pathogenic fungi and provide good protection for eggplant seeds and plants. The treatment of *T. harzianum*, Bokashi and salicylic acid in the percentage of germination increased between 87.50-95.83%. Followed by the interaction between the biological agent *T. harzianum* and the bio-fertilizer Bokashi, which had a germination rate of 83.33-91.67% and was close to the efficacy of Beltanol, which had a germination rate of 91.67-100.00% compared with the treatment of pathogenic fungus resulted in a significant reduction in germination Eggplant seeds with pathogenic fungi *R. solani* (RS-1), *F. solani* (FS-2) and *M. phaseolina* (MP-1) were 0.00, 25.00 and 20.83%, respectively. The results in table 7 showed that all the treatments used in the experiment to control the causes of eggplant damping off under nursery conditions resulted in a significant reduction in the disease incidence and severity of infection compared to the treatment of pathogenic fungi alone. The appearance of the symptoms of seed decay and pre-post emergence damping off and the roots and bases of stems rot from attacking the fungal hyphae of the pathogen by direct penetration of the tissue and the extension of fungal hyphae between the cells of the cortex or inside it sometimes causing the discoloration of brown and may occur before the arrival of fungal threads to it, Produce toxic substances and enzymes that help analyze the roots of plants, and thus reduce the absorption of nutrients and lack of growth of plants (Hall, 1991; Sett *et al*., 2000; Laemmlen, 2001 and Roman-Aviles, 2003).

The results (table 8) showed that all the control treatment showed a significant increase in Eggplant growth parameters, which include plant height, wet and dry weight, and leaf area compared to the treatment of

Table 7 : Effectiveness of some biocontrol agents and salicylic acid in reducing the disease incidence and severity of eggplant seed decay and damping off disease under nursery conditions.

Treatment*	Germination (%)	Disease incidence (%)	Severity (%)
FS-2	20.83	100.00	80.00
FS-2+ TH	79.17	41.67	36.67
FS-2+SA	58.33	50.00	40.00
FS-2+ Bokashi	75.00	41.67	36.67
FS-2+ TH+SA	83.33	33.33	30.00
FS-2+TH+Bokashi	87.50	33.33	26.67
FS-2+TH+SA+Bokashi	91.67	25.00	20.00
FS-2+ Beltanol	95.83	8.33	5.00
MP-1	25.00	100.00	73.33
MP-1+ TH	83.33	33.33	33.33
MP-1+SA	66.67	50.00	36.67
MP-1+ Bokashi	79.17	41.67	36.67
MP-1+ TH+SA	87.50	25.00	30.00
MP-1+TH+Bokashi	91.67	25.00	20.67
MP-1+TH+SA+Bokashi	95.83	25.00	16.67
MP-1+ Beltanol	100.00	8.33	6.67
RS-1	0.00	100.00	100.00
RS-1+ TH	75.00	41.67	33.33
RS-1+SA	54.17	58.33	36.67
RS-1+ Bokashi	70.83	50.00	36.67
RS-1+ TH+SA	79.17	41.67	30.00
RS-1+TH+Bokashi	83.33	33.33	26.67
RS-1+TH+SA+Bokashi	87.50	25.00	16.67
RS-1+ Beltanol	91.67	16.67	6.67
Control	100.00	8.33	3.33
TH	100.00	0.00	0.00
SA	100.00	0.00	0.00
Bokashi	100.00	0.00	0.00
TH+SA	100.00	0.00	0.00
TH+Bokashi	100.00	0.00	0.00
TH+SA+Bokashi	100.00	0.00	0.00
LSD (*P.*: 0.05)	**4.195**	**9.657**	7.616

* RS = *Rhizoctonia solani*, FS = *Fusarium solani*, MP = *Macrophomina phaseolina*, the number next to the symbol represents the isolation number. TH. = *Trichoderma harzianum*, SA = Salicylic acid.

pathogenic fungi alone. The highest yield was recorded in the treatment of *T. harzianum*, bio-fertilizer and Salicylic acid, with a significant increase in plant height ranging from 28.33-30.00 cm, wet and dry weight to 8.07-8.85 and 2.55-2.84g, respectively and also contributed significantly to the increase leaf area, which amounted to 23.80-24.67 cm^2. The Effective microorganisms EM1contained in this preparation belong to several species of aerobic and anaerobic bacteria such as photosynthesis *Rodopseudomonas* spp. Actinomycetes, which secrete various substances such as amino acids and carbohydrates that promote plant growth and antibiotic release, inhibit the growth of some pathogenic microbes (Lavania *et al*., 2006; Ojha *et al*., 2008; Edmisten *et al*., 2010).

The results in table 8 showed that all the treatments used to control the pathogenic causes of eggplant egg death and when added separately or in combination with each other and without the addition of pathogenic fungi achieved a significant increase in growth parameters of eggplant seedlings, namely plant length, wet and dry weight and leaf area compared to control treatment (without adding pathogenic fungus). And a significant improvement in the treatment of interference between *T. harzianum* and bio-fertilizer Bokashi and Salicylic acid with the highest result in the length of the plant at 32.33 cm and the increase in the wet and dry weight to reach 9.22 g and 3.17g respectively, and contributed significantly to increase the leaf area and was 29.67cm^2, while the treatment of the interaction between *T. harzianum* and Bokashi significantly increased the length of the seedlings and wet and dry weight and the leaf area to reach 30.23cm, 8.92, 2.97 g and 28.12 cm^2, respectively. The results showed that the treatment of the interaction between chemical resistance inducer and *T.harzianum* and also contributed to a significant increase in plant growth under the nursery conditions represented by the length of the plant and wet and dry weight and leaf area.

The results in table 9 showed the efficiency of the biocontrol agents used in the study by increasing the leaf content of chlorophyll. The treatment of the interaction between *T. harzianum* and bokashi increased significantly in the chlorophyll ratio to 22.667. Followed by *T. harzianum* and the treatment of Bokashi, which was 21.300 and 21.333 respectively, compared with control and *F. solani* treatments, which were19.800 and 17.100, respectively.

The positive effect of certain *T. harzianum* isolates is to stimulate plant growth to produce plant growth regulators that work in concert with other mechanisms, such as increasing nutrients readiness and facilitating plant uptake (Reyes *et al*., 2006; Sankar *et al*., 2011). Harman (2000) found that the plants inoculated with *Trichoderma* spp. has a significantly larger total roots compared to non-inoculated treatment, and found that the *T. harzianum* works to form a dense and deep root mass and thus achieves the physiological benefits of Maize

Table 8 : Effectiveness of some biocontrol agents and Salicylic acid in improves the growth parameters of eggplant seedlings under nursery conditions.

Treatment*	Plant weight (g)		High of plant (cm)	Leaf area (cm^2)
	Wet	Dry		
FS-2	2.13	1.27	16.33	18.67
FS-2+ TH	6.33	2.20	25.67	20.67
FS-2+SA	5.33	1.50	23.00	20.12
FS-2+ Bokashi	7.07	2.40	26.00	21.33
FS-2+ Bokashi	7.07	2.40	26.00	21.33
FS-2+ TH+SA	7.63	2.77	27.33	22.00
FS-2+TH+Bokashi	8/20	2.43	28.33	23.33
FS-2+ TH+SA +Bokashi	8.74	2.80	29.67	24.00
FS-2+ Beltanol	6.37	2.10	24.67	20.80
MP-1	2.20	1.29	16.67	18.83
MP-1+ TH	6.37	2.27	26.17	20.80
MP-1+SA	5.10	1.33	23.33	21.33
MP-1+ Bokashi	7.17	2.07	26.50	21.67
MP-1+ TH+SA	7.90	2.80	27.67	22.33
MP-1+TH+Bokashi	8.23	2.56	28.67	24.33
MP-1+TH+SA +Bokashi	8.80	2.84	30.00	24.67
MP-1+ Beltanol	6.03	2.16	25.00	21.00
RS-1	0.00	0.00	0.00	0.00
RS-1+ TH	5.23	1.77	15.67	20.33
RS-1+SA	5.00	1.20	22.67	21.00
RS-1+ Bokashi	6.27	2.10	25.33	21.00
RS-1+ TH+SA	7.17	2.37	27.00	22.00
RS-1+TH+Bokashi	6.90	1.63	28.00	22.33
RS-1+ TH+SA +Bokashi	8.07	2.55	28.33	23.80
RS-1+ Beltanol	5.53	2.05	23.67	20.00
Control	6.50	2.40	25.67	23.33
TH	7.47	2.84	28.00	27.33
SA	6.47	1.93	26.33	24.67
Bokashi	7.67	3.13	28.67	27.67
TH+SA	7.57	2.87	28.33	27.67
TH+Bokashi	8.92	2.97	30.23	28.12
TH+SA+Bokashi	9.22	3.17	32.33	29.67
LSD (*P.*: 0.05)	**0.359**	**0.315**	**0.8462**	0.5046

* RS = *Rhizoctonia solani*, FS = *Fusarium solani*, MP = *Macrophomina phaseolina*, the number next to the symbol represents the isolation number. TH. = *Trichoderma harzianum*, SA = Salicylic acid.

plant and other plants, especially in the dry growth season. This fungus can dissolve phosphatic rocks through the production of organic acids. Kapri and Lakshmi (2010) found that the use of *T. harzianum* resulted increase in plant growth, nutrient uptake, gradual alteration of pH, increasing phosphorus release from the triple superphosphate. Krupa *et al*. (2014) found that the use of *T. viride* contributed to the increased growth parameters of tomato plants and recommended its use as an alternative to chemical pesticides in the against *Fusarium* sp. Salicylic acid leads to the synthesis of hydrogen peroxide H, O_2 and Enzyme Peroxidase, which has a significant effect in the decomposition of fungal cell walls and works to increase the thickness of the walls of host plant cells by increasing the material of lignin. It also stimulates genes responsible for plant resistance against many pathogens, as well as increasing plant tolerance for various environmental stresses (Stuver and Custers, 2001; Ton *et al*., 2002; Uquillas *et al*., 2004).

Table 9 : Effectiveness of biocontrol agents increased leaf content of chlorophyll.

Treatments *	Chlorophyll ratio
FS-2	17.100
TH	21.300
Bokashi	21.333
SA	20.033
TH+Bokashi	22.667
Control	19.800
LSD (*P.*: 0.05)	**0.4845**

*FS= *Fusarium solani*. TH=*T.harzianum* ¡ SA= Salicylic acid.

Conclusion

We conclude from the present study that the main causes of eggplant damping off disease in this study are fungi *Rhizoctonia solani*, *Fusarium solani* and *Macrophomina phaseolina* where it was the most isolated fungi repeated. And the efficiency of the use of *T.harzianum* and bio-fertilizers Bokashi and salicylic acid single or integrated with a significant increase in the percentage of germination and reduce the disease incidence and severity of the disease and achieve a significant increase in plant height and wet and dry weight and leaf area and better treatment of triple interference.

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