Available online at http://bajas.edu.iq
https://doi.org/10.37077/25200860.2019.280
College of Agriculture, University of Basrah

Basrah Journal of Agricultural Sciences

ISSN 1814 – 5868 Basrah J. Agric. Sci., 32(Spec. Issue 2): 320-336, 2019 E-ISSN: 2520-0860

A Study of the Effect of Bioagent *Trichoderma harzianum* Rifai, the Fungicide Topsin-M and their Interaction on Root Rot Disease of Okra *Abelmoschus esculentus* in the Field

Yehya A. Salih* & Noor M. Mansoor

Department of Plant Protection, College of Agriculture, University of Basrah, Iraq

***Corresponding author e-mail: yehyasalih@gmail.com**

Received 19 October 2019; Accepted 17 December 2019; Available online 28 December 2019

Abstract: This study aimed to investigate the effect of interaction between *Trichoderma harzianum* and the fungicide Topsin-M on root rot disease that infected okra in the field. Three fungi were isolated from the root of okra that infected with root rot disease: *Fusarium solani*, *Rhizoctonia solani* and *Macrophomina phaseolina*. The pathogenicity of these fungi was tested and found to be they cause root rot disease on okra, the disease severity was 41.7, 6.7 and 31.7% respectively. The laboratorial experiments showed that *T. harzianum* had a high antagonism ability with degrees of 1 and 2 against the pathogenic fungi *M. phaseolina*, *F. solani* and *R. solani* respectively. Also, it was found that the fungicide Topsin –M inhibited the growth of all pathogenic fungi with a percent of 100%, while it inhibited the bioagent fungus growth with a percent of 50.4 %, therefore it be recommended for the interaction experiments. The field results showed that using of bioagent *T. harzianum* and fungicide topsin-M significantly reduced the infection percentage and severity disease of the pathogenic fungi *F. solani*, *R. solani and M. phaseolina* to 65.3, 21.20, 13.20, 46.20, 25.70 and 18.20% respectively, compared to each pathogenic fungus alone which were 71.00, 60.20, 60.20, 66.80, 80.20 and 60.20% respectively. The interaction between the bioagent *T. harzianum* and topsin-M led to increase the plant height, fresh and dry weight of shoot and root systems and the fruit productivity of the examined okra plants .

Keywords: Okra, Fungal disease, Fungicide, Biological control, Basrah.

Introduction

The okra *Abelmoschus esculentus* (L.) Moench belongs to the Malvaceae family. It is believed that African continent is the origin of okra, particularly Ethiopia and Sudan. And then distributed cross Eastern Mediterranean, Arabian Peninsula, India, Europe and America during the 13th and 17th centuries (Matloob *et al*., 1989). Iraq ranks fifth globally in okra production with rate of 0.102 million tons (FAO, 2014). Okra is the third most cultivated crop in Iraq for summer time, after cucumber and watermelon with an area of 28401 donum and the sixth in terms of production at an average rate of 15833 tons (SCO, 2016). The planted area of okra in Basrah governorate for the year 2016 was estimated at 21948 donum with a productivity of 22.99 tons.donum^{-1}, for the year 2017 estimated at 21948 donum and productivity of 2402 tons.donum^{-1} and for the year 2018 was

estimated at 21948 donum and productivity of 2180 tons.donum^{-1} (Planning & Follow-up, 2018).

Okra is infected with many pests, including fungal diseases that have been more virulent and spreader under greenhouse conditions. One of the most important fungal diseases is damping–off, and root rot, which is a common disease on okra crop in open fields and greenhouses. This disease caused by many fungi, including *Fusarium* spp. *Rhizoctonia solani* and *Macrophomina phaseolina* (Rahim & Dawar, 2015). As a result of the increasing damage of plant diseases and harmful effect of chemical pesticides, biocontrol has been used to eliminate several pathogens (Farah & Sahera, 2016). In recent decades, biocontrol of pathogens has obtained a broad interest using some bioagents such as fungi and bacteria that have high efficiency in inhibiting growth of several plant pathogens. These bioagents include species of *Trichoderma,* such as *T. harzianum* which achieved success in the field and greenhouse experiments (Zeilinger & Omann, 2007). Alwan (2014) found that *T. harzianum* has reduced the infection of cucumber root rot disease that caused by *M. phaseolina*. Salih & Al-Maarich (2016) indicated that the use of *T. harzianum* gave highest percentage of seed germination and lowest percentage of seed rot in wheat that caused by *R. solani* in Northern Basrah. Matrood (2018) reported that *T. harzianum* has reduced the rate of Septoria leaf spot in eggplant that caused by *Cladosporium cladosporioides.* Chemical control is one of the most common methods known against plant diseases in the fields and greenhouses. These methods include chemical compounds, that are toxic and inhibit the germination, growth and reproduction of pathogens (Al-Adel, 2006). El-Habbaa *et al*. (2016) reported that topsin-M reduced the severity of die back disease of grape plants and their effect on defence-related enzymes. Al-Fadhl & Al-Janabi (2017) showed the role of topsin-M to reduce disease severity of *R. solani*, that causes root rot in Betonia plant in Najaf province and led to a significant increase in fresh and dry weight. Shailbala & Kumar (2017) reported that topsin-M reduced the severity of *Colletotrichum falcatum*, which causes red rot on sugar cane and improved growth indicators as well as. Al-Kaabi *et al*. (2009) studied the interaction between benlate and *T. harzianum* that gave the best results in germination rate with lower infection severity and higher dry weight, decreasing the severity of infection and increasing the dry weights of both shoot and root systems, which differed significantly from the comparative treatment of pathogenic fungi.

Mohammad (2012) reported that using coefficients of *Trichoderma* spp. and benomyl have increased the efficiency of biocontrol. Salih & Al-Maarich (2016) studied the effect of interaction between bioagent *T. harzianum* and moncut on wheat bare patch disease that caused by *Rhizoctonia solani* in Basrah. Al-Fadhl & Al-Janabi (2017) reported the effect of some biological agents and topsin-M on root rot disease that caused by *R. solani*. Recently, root rot infections have been noticed on okra in different regions of Basrah province with serious damages, therefore this research was carried out to study the following aims: (i) Isolation and identification of the fungi causing okra root rot disease, (ii) the pathogenicity of the isolated fungi, (iii) the effect of *T. harzianum* on okra root rot disease, (vi) the effect of the interaction between fungicide topsin -M and bioagent *T. harzianum* on the growth of pathogenic fungi, and (v) the percentage of infection and

disease severity and the plant growth parameters.

Materials & Methods

Isolation and identification of the pathogenic fungi

Okra plants were collected from Medinah, Faihaa, Garmat-Ali, Hartha, Nashwa and the fields of College of Agriculture at Basrah province. The roots of infected plants were washed with tap water to remove the stuck mud, then cut into small pieces 1-1.5 cm. After that, the small pieces of roots were sterilized superficially for 2-3 minutes by 10% concentration of sodium hypochlorite solution (NaOCl). The pieces were removed from the solution and washed with distilled water to remove the sterilization effects. Next, The pieces were dried using sterile filter paper Whatman No.4, and then put in a petri dish (9 cm diameter) containing sterile PDA with an antibiotic chloramphenicol (250 $mg.l^{-1}$). Five pieces per a dish and three replicates were applied, after that the dishes incubated at a temperature of 25 ± 2 ºC for four days. Fungal growth were examined, and then isolated fungi were purified on the same medium. The isolated fungi were identified by phenotypic characters based on Parmeter & Whitny (1970), Sinclair (1982) and Lesslei & Summurel (2006).

Pathogenicity test

Pathogenicity of the fungi isolated from the roots: *F. solani*, *R. solani* and *M. phaseolina* was tested according to Bolkan & Butler (1974). The plates containing water agar medium were inoculated with a 0.5 cm disk from seven days colony of growing fungus using PDA medium. Then, these plates were incubated at a temperature of 25 ± 2 º C for three days. After that, the seeds of okra were surface sterilized superficially using a solution of 10% sodium hypochlorite (NaOCl) and circularly cultured near the edge of the plate. Ten seeds per plate and three replicates per treatment were applied, while the control treatment contained only okra seeds. Then the plates were incubated at a temperature of 25 ± 2 º C and the results were recorded after seven days of planting by calculating the percentage of germination as the following equation :

$$\text{Percentage of germination} = \frac{\textbf{\textit{Number of germinated seed}}}{\textbf{\textit{Total seeds}}} \text{ X } 100$$

Evaluation of disease severity was calculated according to the examined disease index as follow:

Disease index of the fungus of *F. solani* according to Al-Hasnawy (2017)

0 = The plant is healthy, and the root system is white colour.

1 = Discolouration 1-25 from the root light brown colour.

2 = Discolouration of more than 25-50 dark brown colour.

3 = Discolouration of more than 50-75 of the root in a dark brown colour with yellowing of leaves.

4 = Discolouration of more than 75-100 of the root in a dark colour with the death of infected plant.

Disease index of the fungus of *R. solani* according to Al-Hasnawy (2017)

0 = The plant is healthy, and the root system is white colour.

1 = Discolouration of the root in a yellowish-brown colour and ulceration with a diameter less than 10 mm around the stem.

2 = Dark brown discolouration of the root and canker with diameter of 11-20 mm around the stem.

3 = Reddish-brown canker fully surrounding the stem.

4 = Plant death.

Disease index of the fungus of *M. phaseolina* according to Fayyadh (1997)

0 = Healthy plant.

1 = 1/4 of the root system is coloured blackish-brown and yellowing of the lower leaves and discolouration of the stem base.

2 = 1/2 of root system is coloured blackish-brown and yellow 1/4 shoots system with discolouration 2-3 cm from the stem base and observation the sclerotia easily.

3 = Discolouration 3/4 of the root system with blackish brown colour and yellowing the whole plant with discolouration 4-6 cm from the stem base and observation the sclerotia easily.

4 = Discolouration of the root system with blackish brown colour and the death of the whole plant and observation of sclerotia sufficiently in the area of infection.

The Mickenny (1923) equation cited from Al- Waily (2004) was used to calculate the disease severity as the following :

$$\%\text{Disease severity} = \frac{Sum\ (Infected\ plants\ number\ in\ each\ degree \times degree\ number}{Total\ infected\ plants \times Highest\ degree} \times 100$$

The percentage of infection also, calculated according to the following equation:

$$\%\ Infection = \frac{\text{Number of infected plants}}{\text{Number of total plants}}\ X\ 100$$

Testing of the antagonism ability of *T. harzianum* against the pathogenic fungi on PDA.

Aghighi *et al.* (2004) method was followed to test the ability of bioagent *T. harzianum* against the pathogenic fungi *F. solani, R. solani* and *M. phaseolina* on PDA. A Petri dish containing sterile PDA was divided into two equal parts, the first part was inoculated with a disk (0.5 cm diameter) taken from the seven days old colony margin of *T. harzianum*, while the second part was inoculated with a disk (0.5 cm diameter) taken from the seven days old colony margin of each pathogenic fungus, whereas, the control treatment was inoculated with a pathogenic fungus only. Three replicates for each treatment were applied. All Petri dishes were incubated at 25±2 ºC in the incubator. When the fungal growth in the control treatment reached to the plate margin, the antagonism was calculated according to Bell *et al*. (1982) scale which consists of five degrees as following:

1 The antagonism fungus covers all the plate
2 The antagonism fungus covers two thirds of the plate.
3 The antagonism fungus covers half of the plate.

4 The pathogenic fungus covers two thirds of the plate.

5 The pathogenic fungus covers all the plate

Effect of some fungicides on the growth of pathogenic fungi and *T. harzianum* on PDA.

Four fungicides: betanol, topsin-M, trimax-plus and rizolex with recommended concentrations were applied in this test. The medium Potato Dextrose Agar (PDA) was prepared, poured in 150 ml conical flasks (100 ml for each flask) and sterilized in an autoclave at 121^{o}C and 15 pounds.inch^{-2} for 30 minutes. The above four fungicides in concentrations of 1ml.l^{-1} for the first fungicide and 1gm.l^{-1} for each of three other fungicides, were added to each flask, well homogenized and poured in the plates. Then, the plates were inoculated with a disk (0.5 cm diameter) taken from the seven days old colony margin of each fungus with three replicates for each treatment. The control treatment was prepared by adding distilled water in place of fungicides. All plates were incubated at 25±2 ^{o}C in the incubator. When the fungal growth in the control treatment reached to the plate margin, the radial growth was measured by taking an average of two vertical diameters passed throughout the reverse of plate centre. The inhibition percentage was calculated according to Abbott equation (1925) which cited from Shaaban & Al-Mallah (1993) as the following:

$$\% \, Inhibition = \frac{\text{Control radial growth} - Treatment\ radial\ growth}{\text{Control radial growth}} \ X\ 100$$

Field experiment

The field experiment was carried out in the fields of the College of Agriculture, University of Basrah in a greenhouse. The greenhouse (12×5m) was prepared and divided into four rows (1m between rows) and 50 cm between plant and another one. *T. harzianum* obtained from Department of Plant Protection laboratories was added in an average of 1% w/w of grown fungus on millet seeds and left for three days with irrigation. After that the pathogenic fungi *F. solani*, *R. solani*, and *M. phaseolina* grown on millet seeds at 1% w/w individually (Dewan, 1989), were mixed with the soil thoroughly and continuously irrigated for three days, a drip irrigation system was used. Then five seeds of okra (local variety) per each pit were planted.

All treatments were performed with three replicates, The following treatments were applied :

Control, *F. solani* (F.s), *F. solani* + *T. harzianum* (F.s+T.h), *F. solani*+topsin –M (F.s+TM), *F. solani* + *T. harzianum* + topsin-M (F.s+T.h+TM), topsin-M (TM), *T. harzianum* (T.h), *T. harzianum* + topsin –M (T.h+TM), *R. solani* (R.s), *R. solani* +*T. harzianum* (R.s+T.h), *R. solani* + topsin-M (R.s+TM), *R. solani* + *T. harzianum* + topsin-M (R.s+T.h+TM), *M. phaseolina* (M.p), *M. phaseolina* +*T. harzianum* (M.p+T.h), *M. phaseolina* + *T. harzianum* (M.p+TM), *M. phaseolina* + *T. harzianum* + topsin-M (M.p+T.h+TM).

The percentage of disease severity and percentage of infection were calculated after four months as prescribed in the previous paragraph.

Parameters & Features

The parameters : Plant height (cm), fresh and dry weights of shoot and root systems (g), fruit number, fruit weight (g) and fruit productivity per plant (g.plant^{-1}) were measured for each experimental unit at the end of the experiment (after four months).

Statistical analysis

All experiments were carried out according to Complete Random Design (CRD) and the mean differences between the averages were compared with Least Significant Difference (LSD), all treatment were repeated for three times (Al-Rawi & Khalaf-Allah, 1980). All statistical analyses were conducted by using Genstat discovery edition program.

Results &discussion

Isolation and identification of pathogenic fungi

Three fungi *Fusarium solani*, *Rhizoctonia solani*, and *Macrophomina phaseolina* were isolated from infected okra plant roots at Basrah province from different regions named Medinah, Faihaa, Garmat-Ali, Hartha, Nashwa and the fields of College of Agriculture (Fig. 1 & Table 1). Their morphological and microscopical characters agreed with Watanabe & Shiyome (1975), Sinclair (1982), and Lesslei & Summurel (2006).

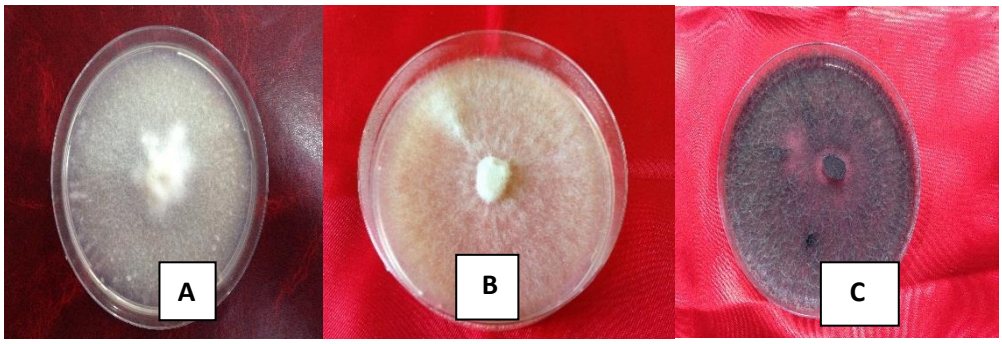


Fig. (1): Colonies of fungi isolated from the roots of infected okra plants on PDA.

A*: F. solani*, B*: R. solani* and C*: M. phaseolina*.

Table (1): Fungi isolated from the roots of infected okra plants at different regions of Basrah province.

Region	Fungi
1-Medinah	*Fusarium solani* *Macrophomina phaseolina*
2-Faihaa	*Fusarium solani*
3-Garmat-Ali	*Fusarium solani* *Macrophomina phaseolina*
4-Hartha	*Fusarium solani* *Macrophomina phaseolina*
5-Nashwa	*Fusarium solani*
6-Fields of College of Agriculture	*Fusarium solani* *Macrophomina phaseolina* *Rhizoctonia solani*

As shown in the same table above, *F. solani* has appeared in all regions, *R. solani* appeared only in the fields of College of Agriculture, while *M. phaseolina* appeared in four regions: Medinah, Garmat-Ali, Hartha and the fields of College of Agriculture. Also, the three fungi were found in the fields of College of Agriculture only .

Pathogenicity test

The results of the pathogenicity test (Table 2) showed that all the isolated fungi were significantly pathogenic compared to control treatment, which had 0.00% disease severity. The disease severity on okra plants treated with *F. solani* was 41.70%, *R. solani* was 6.70% and *M. phaseolina* was 31.33%. The percentage of infection on okra plants treated with *F. solani* was 67.33%, *R. solani* was 53.70%, and *M. phaseolina* was 61.66% compared to the control treatment (without pathogenic fungus) which was 0.00%. These results agreed with Salih & Al-Maarich (2016), Radhi *et al*. (2016), Musawy *et al*. (2017) and Al-Juboory (2018), who confirmed that the pathogenic fungi *F. solani*, *R. solani* and *M. phaseolina* increased the disease severity compared with the control treatment. The pathogenic fungi differed in their pathogenicity, so this difference may be due to their genomes, biochemical effects or mode of action, however there were three metabolic activities considered as essential factors for pathogenicity, including enzymes, mycotoxins and growth regulators which effect on the host plant either combined or individually (Neergaard, 1997).

Table (2): Disease severity and infection percentage (Mean±SD)by *Fusarium solani, Rhizoctonia solani* and *Macrophomina phaseolina*.

Pathogenic fungi	% Infection	% Disease severity
F. solani	67.33±10.91	41.70±10.71
R. solani	53.70±3.75	6.70±6.79
M. phaseolina	61.66±8.08	31.33±5.87
Control	0.00±22.75	0.00±9.79
L.S.D	15.60	31.93

Testing the antagonism ability of *Trichoderma harzianum* against the pathogenic fungi *F. solani, R. solani* and *M. phaseolina*

The results showed that *T. harzianum* gave a high antagonism with degrees of 1 and 2 against *M. phaseolina*, *F. solani* and *R. solani* respectively according to Bell *et al*. (1982) (Fig. 2). This result was in agreement with Jasim & Al-Qurani (2012), Alwan (2014), Matrood (2015) and Salih & Al-Maarich (2016) who reported the high antagonism ability of *T. harzianum* against the pathogenic fungi *F. solani*, *R. solani* and *M. phaseolina*. The antagonism ability of *T. harzianum* was due to many different mechanisms such as direct parasitism, antibiotic exudates, degrading enzymes and competition (Sivan & Chet, 1989; Limon *et al*., 1999 and Saad, 2001).

Effect of some fungicides on the growth of pathogenic fungi and *T. harzianum*

The results explained (Fig. 3) that the fungicide beltanol inhibited all pathogenic

fungi growth in a percent of 100%, while trimax–plus has inhibited the growth of *R. solani* and *M. phaseolina* in a percent of 100% for each one, but the inhibition percentage of *F. solani* was 85%, whereas topsin–M has inhibited the growth of *R. solani* and *M. phaseolina* with a percent of 100% for each one, while it inhibited *F. solani* growth with a percent of 72.8 %. Also, the results showed that rizolex inhibited the growth of *R. solani*, *M. phaseolina* and *F. solani* with percent of 100, 93.5 and 0.2 % respectively. The results agreed with Wannas (2011), Alwan (2014) and Al-Maarich (2015) who found that the fungicide beltanol

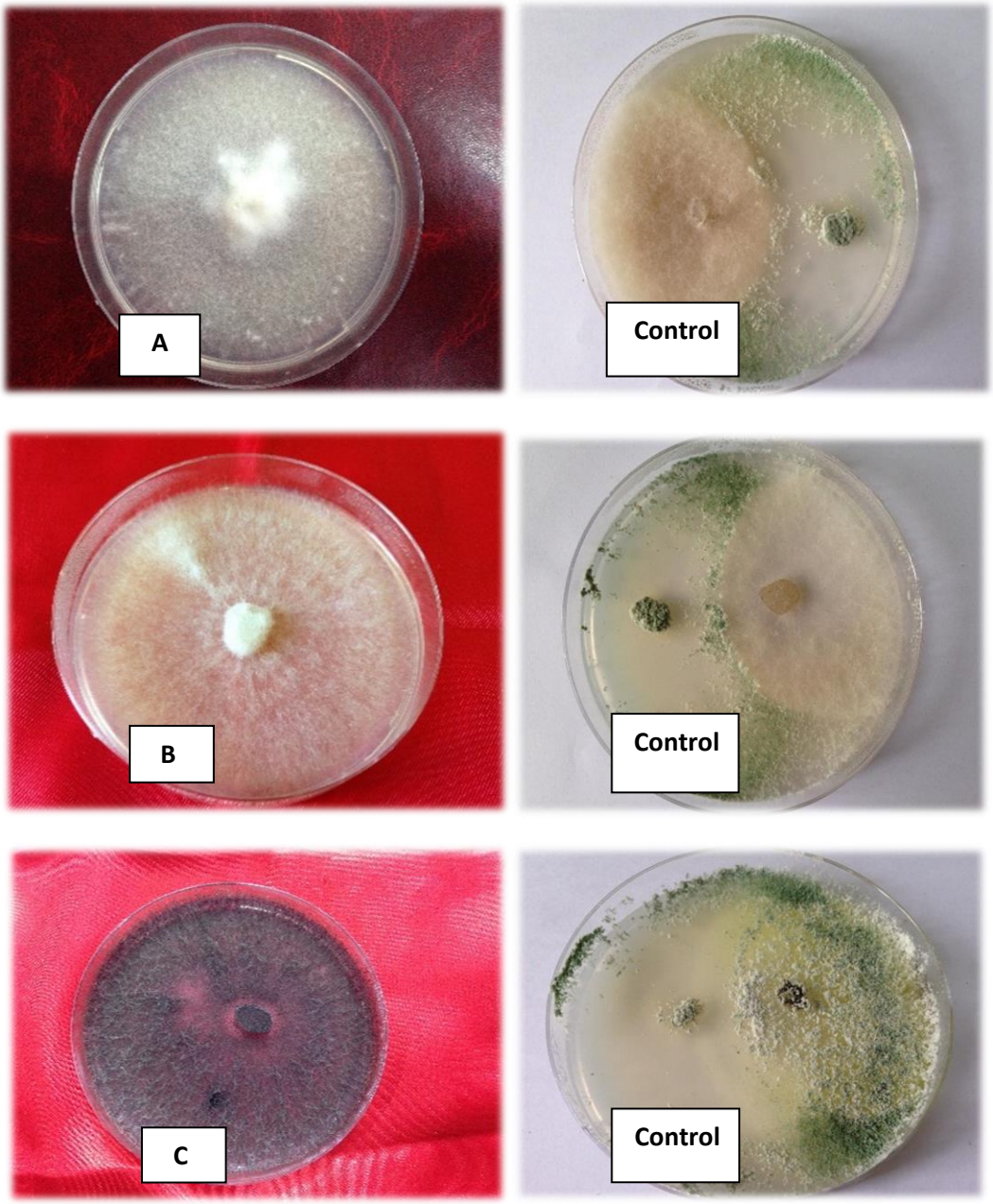
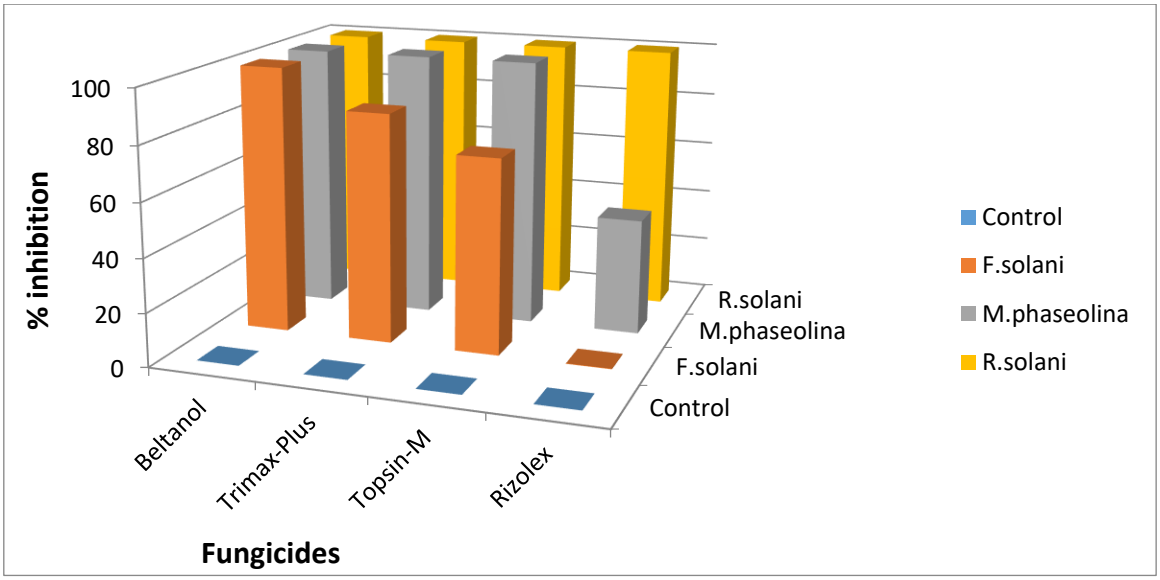


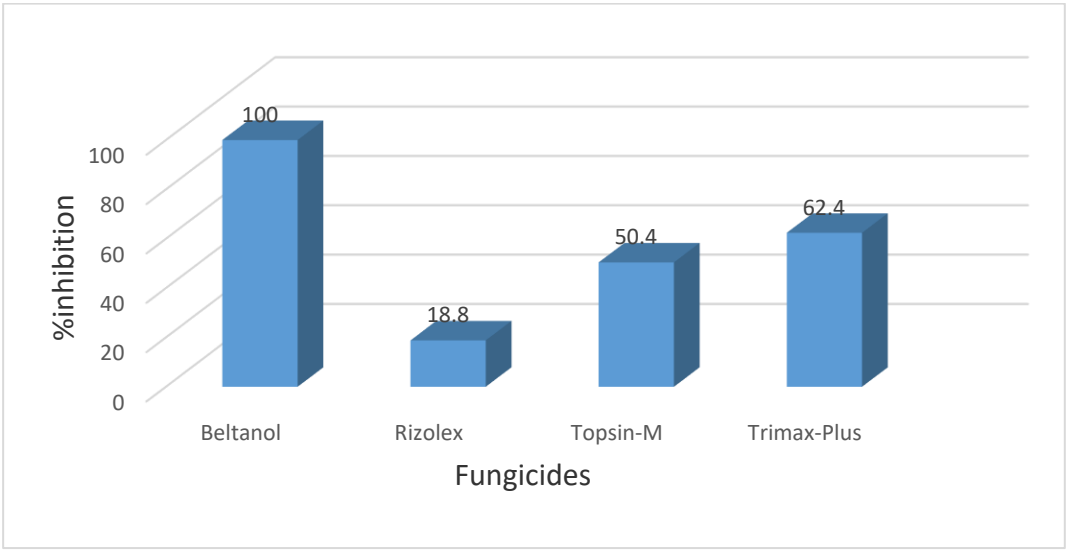
Fig. (2): Testing the antagonism ability of *T. harzianum* against the pathogenic fungi *F. solani*, *R. solani* and *M. phaseolina* on PDA.A: *T. harzianum*+*F. solani*, B: *T. harzianum* +*R. solani* and C: *T. harzianum* + *M. phaseolina*

inhibited the growth of *R. solani*, *F. solani* and *M. phaseolina*. These results were also, in agreement with Abd-El-Kader *et al*. (2010) and Al-Fadhl & Al-Janabi (2017) who confirmed that the fungicide topsin-M inhibited the growth of these pathogenic fungi. The study also agreed with Smiley *et. al*. (1990) and Jasim (2007) who showed that the fungicide rizolex inhibited the growth of *R. solani* with a percent of 100% .On the other hand, Fig. (4) showed that the fungicides beltanol, trimax–plus, topsin–M and rizolex inhibited the growth of bioagent *T. harzianum* with percent of 100, 62.4, 50.4 and 18.8 % respectively.



L.S.D$_{0.01}$= 0.05

Fig. (3): Effect of some fungicides on the growth of pathogenic fungi *F. solani, R. solani* and *M. phaseolina.*



L.S.D$_{0.01}$ = 0.05

Fig. (4): Effect of some fungicides on the growth of bioagent *T. harzianum.*

This result was in full agreement with Al-Maarich (2015) who found that beltanol inhibited the fungus growth with a percent of 100 %, While it had disagreement with the

same author who noticed that topsin-M inhibited the fungus growth with a percent of 100%.

Effect of bioagent *T. harzianum* and fungicide topsin-M and their interaction on the percentage of infection and disease severity by okra root rot disease.

The results of table (3) showed that the pathogenic fungi *F. solani*, *R. solani* and *M. phaseolina* increased the disease severity and infection percentage of root rot disease with 71.00, 60.20, 60.20, 66.80, 80.20 and 60.20% respectively compared to the control treatment, which was 0.00%. The results in the same table also, showed that the interaction between treatments of *T. harzianum* and topsin-M with the pathogenic fungi *F. solani*, *R. solani* and *M. phaseolina* (F.s + T.h + TM, R.s + T.h + TM and M.p+ T.h + TM) have reduced the infection percentage of root rot to 65.30, 21.20 and 13.20%, respectively, compared to 71.00, 60.20 and 60.20% of pathogenic fungi alone.

Table (3): Effect of bioagent *T. harzianum* and fungicide topsin-M and their interaction on the percentage of infection and disease severity (Mean±SD)of root rot disease on okra plant.

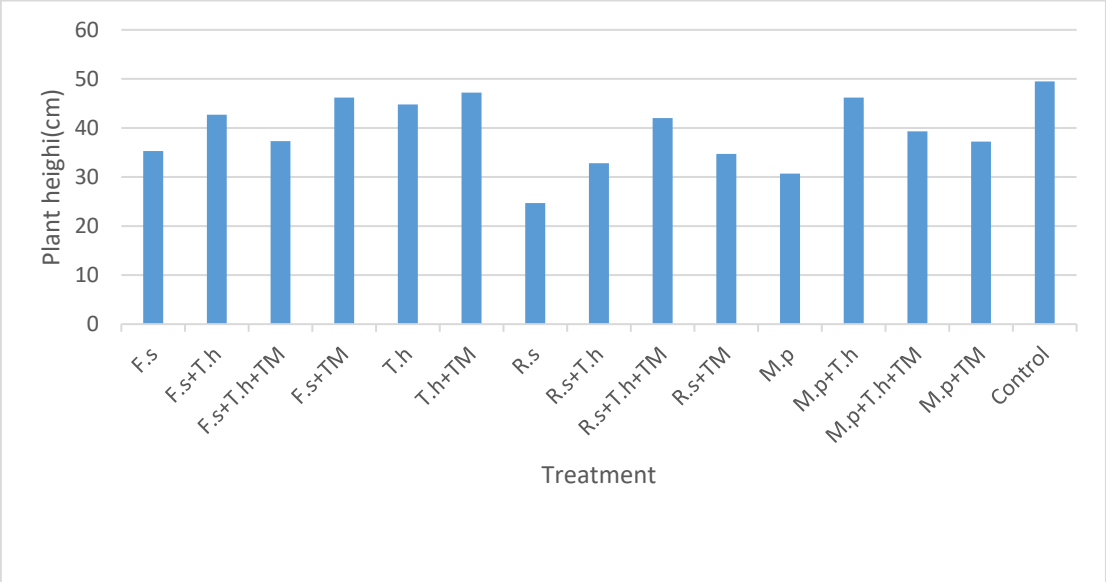
Treatments	% Disease Severity	% Infection
F.s	66.80±16.28	71.00±18.67
F.s + T.h	40.66±3.61	60.31±13.01
F.s+ T.h+ TM	46.20±6.28	65.30±15.68
F.s+ TM	20.66±6.38	26.00±4.15
T.h	0.00±16.72	0.00±17.15
T.h + TM	0.00±16.72	12.33±10.99
R.s	80.20±23.61	60.20±13.01
R.s + T.h	68.00±17.28	20.66±6.82
R.s + T.h + TM	25.70±4.05	21.20±6.32
R.s + TM	10.00±11.72	24.00±5.15
M.p	60.20±13.61	60.20±13.01
M.p + T.h	43.40±4.78	53.33±9.51
M.p + T.h + TM	18.20±7.38	13.20±10.65
M.p + TM	22.00±5.72	25.33±4.49
Control	0.00±16.72	0.00±17.15
$L.S.D_{0.05}$	0.19	0.48

F.s: *F. solani*, R.s: *R. solani*, M.p: *M. phaseolina*, T.h: *T. harzianum*, TM: topsin–M.

Also these treatments have reduced the disease severity to 46.20, 25.70, and 18.20 % respectively compared to 66.80, 80.20 and 60.20 % for the pathogenic fungi respectively. These results are agreed with Ghisalberti *et al*. (1990), who reported that *T. harzianum* could produce antibiotics pyrones that inhibits pathogenic fungi and thus protect the seeds from the pathogenic fungus effect. Al-Murad & Al-Taei (2006) found that the using of *T. harzianum* have reduced the rate of damping-off caused by fungi *F. solani* and *R. solani* in bean plant. *Trichoderma* has the potential to stimulate plant growth by increasing root absorption of nitrogen (N) and dissolving nutrients and making it more available for plants such as zinc, copper, iron and manganese (Altomare *et al*., 1999). In a study conducted by Mukhtar (2008), the results showed that the treatment of okra seeds with *T. viride* have increased the yield. Gajera *et al*. (2012) explained that when seven species of *Trichoderma* were used against *M. phaseolina*, they inhibited the growth of pathogenic fungus and observed under the microscope the *Trichoderma* fungus wrap around the pathogen and penetrate the wall by the apressoria and then break it down by producing enzymes chitinase, protease, cellulose and B-1,3 glucanase.

Effect of bioagent *T. harzianum* and fungicide topsin-M and their interaction on the height of okra plant, the fresh and dry weight of the shoot and root system in the presence of fungi causing root rot disease.

Fig. (5) showed that the interaction between bioagent *T. harzianum* and fungicide topsin-M in the presence of pathogenic fungi led to increase the height of okra plants significantly in the treatments: F.s+ T.h + TM, R.s + T.h + TM and M.p+ T.h + TM up to 37.3, 42.00 and 39.8 cm respectively compared with the pathogenic fungi *F. solani*, *R. solani* and *M. phaseolina* alone which were 35.3, 24.7 and 30.7 cm respectively. As shown in table (4),



L.S.D $_{0.05}$ =16.9

Fig. (5): Effect of bioagent *T. harzianum* and fungicide topsin-M and their interaction on the height of okra plant in the presence of fungi causing root rot disease.

F.s: *F. solani*, R.s: *R. solani*, M.p: *M. phaseolina*, T.h: *T. harzianum*, and TM: topsin –M.

Table (4): Effect of bioagent *T. harzianum* and fungicide topsin M on dry and fresh weight of shoot (Mean±SD)and root system of okra in the presence of fungi causing root rot disease.

Treatments	Dry weight (g)		Fresh weight (g)	
	Root system	Shoot system	Root system	Shoot system
F.s	0.71±0.42	3.86±0.90	1.79±1.46	10.50±0.12
F.s+T.h	0.92±0.28	5.58±0.07	0.93±1.08	5.10±2.67
F.s+T.h+TM	1.84±0.16	6.26±0.40	4.61±0.63	11.80±0.43
F.s+TM	0.67±0.40	4.36±0.50	1.60±0.74	13.10±1.31
T.h	0.90±0.28	5.50±0.06	2.33±0.38	12.36±0.88
T.h+TM	1.26±0.10	4.33±0.56	1.90±0.59	15.10±2.31
R.s	0.15±0.65	0.71±2.33	1.73±0.71	8.10±1.17
R.s+T.h	0.62±0.43	5.06±0.15	2.51±0.29	9.00±0.70
R.s+T.h+TM	3.41±0.89	10.89±2.55	7.42±2.10	26.40±7.86
R.s+TM	1.33±0.07	5.23±0.06	2.56±0.26	11.40±0.39
M.p	0.82±0.33	3.96±0.78	1.58±0.73	8.60±1.04
M.p+T.h	3.53±1.03	4.92±0.28	6.63±1.76	5.66±2.47
M.p+T.h+TM	1.86±0.05	7.29±0.96	4.24±0.67	15.70±2.40
M.p+TM	0.43±0.50	1.83±1.76	0.83±1.13	5.70±2.45
Control	4.20±1.36	12.23±3.43	7.36±2.21	19.90±4.95
$L.S.D_{0.05}$	2.13	7.47	4.67	16.06

F.s: *F. solani*, R.s: R. solani, M.p: *M. phaseolina*, T.h: *T.* harzianum, and TM: topsin –M.

the interaction between *T. harzianum* and topsin-M in the presence of pathogenic fungi led to increase fresh and dry weight of shoot and root system up to 11.80, 4.61, 6.26, 1.84, 26.40, 7.42, 10.89, 3.41, 15.70, 4.24, 7.29 and 1.86 g respectively, compared to the control treatment of pathogenic fungi *F. solani*, *R. solani* and *M. phaseolina*, which were 10.5, 1.79, 3.86, 0.71, 8.10, 1.73, 0.71, 0.15, 8.60, 1.58, 3.96 and 0.82 g respectively. The positive effect of *T. harzianum* on increasing plant height rate and the rate of the dry weight of root and shoot system may be due to its role in increasing nutrient availability in the rhizosphere zone (Altomare *et al.*, 1999). These results were agreed with Al-Juboory *et al.*, (2018) who refer that the decreasing in the percentage of infection and disease severity were accompanied by a significant increase in the rate of shoot and root system length, fresh

and dry weight when *T. harzianum* was applied.

Effect of bioagent *T. harzianum* and fungicide topsin-M and their interaction on the production of okra in the presence of fungi causing root rot disease

The results in table (5) showed that the use of bioagent *T. harzianum* and the fungicide topsin-M together significantly reduced the negative effect of pathogenic fungi. It was treatments of pathogenic fungi *F. solani*, *R* found that the interaction between the. *solani* and *M. phaseolina* and the bioagent *T. harzianum* significantly increased the number of fruits to 2.40, 2.73 and 5.00 fruit respectively, compared with the treatments of pathogenic fungi alone, which got a number of fruits reached 2.00, 0.80 and 0.20 fruit respectively, and led to increase fruit weight to 3.70, 3.30 and 3.30 g respectively,

Table (5): Effect of bioagent *T. harzianum* and fungicide topsin M and their interaction on the productivity parameters (Mean±SD) of okra in the presence of fungi causing root rot disease.

Treatment	Fruit production per plant (kg)	Weight of particular fruit (g)	Number of fruits. Plant^{-1}
F.s	1.20±2.78	1.40±0.68	2.00±0.04
F.s+T.h	7.20±0.26	3.70±0.33	2.40±0.20
F.s+T.h+TM	1.66±2.62	1.20±0.74	1.40±0.34
F.s+TM	4.20±1.23	3.60±0.33	1.30±0.42
T.h	5.50±0.67	2.10±0.36	1.76±0.19
T.h+TM	10.43±1.79	3.36±0.27	3.36±0.61
R.s	2.50±2.10	3.40±0.30	0.80±0.64
R.s+T.h	12.26±2.71	3.30±0.48	2.73±0.24
R.s+T.h+TM	15.30±4.23	4.50±0.83	2.30±0.15
R.s+TM	7.46±0.31	3.30±1.13	2.13±0.01
M.p	0.50±3.15	3.40±0.28	0.20±0.04
M.p+T.h	12.70±2.83	3.30±0.27	5.00±1.47
M.p+T.h+TM	3.50±1.60	3.20±0.26	1.20±0.46
M.p+TM	0.40±3.22	3.40±1.21	0.70±0.72
Control	17.33±5.24	4.30±0.73	4.33±1.09
L.S.D$_{0.05}$	0.17	0.16	0.61

F.s*: F. solani*, R.s: *R. solani*, M.p: *M. phaseolina*, T.h: *T.* harzianum, and TM: topsin –M.

compared with the treatments of pathogenic fungi alone, which were 1.40, 3.40 and 3.40 g respectively. Also the fruit yield per plant significantly increased to 7.20, 12.26 and 12.7 kg respectively, compared with the treatment of pathogenic fungi alone, which amounted to 1.20, 2.50 and 0.50 kg, respectively. They were followed by the interaction of F.s + T.h + TM, R.s + T.h+ TM and M.p + T.h + TM, which where the number of fruits reached 1.40, 2.30 and 1.20 fruit respectively, and the average weight of the fruit reached 1.20, 4.50 and 3.20 g and the fruit yield per plant also increased to 1.66, 15.30 and 3.50 kg respectively, compared with the treatment of pathogenic fungi alone, which were previously mentioned in the above paragraph. The results obtained from the experiment showed a great compatibility between the fungicide and bioagent *T. harzianum* in reducing the negative effects of the pathogens and improving plant growth, these results are consistent with the results of many researches involving the use of these factors against many different pathogens (Juber, 1996; Mukhtar, 2008).

Conclusions

It was found that *T. harzianum* has an antifungal and inhibitory activity against pathogenic fungi causing root rot disease of okra *R. solani*, *F. solani*, and *M. phaseolina*. Also, it was found that there was a possibility of using bioagent *T. harzianum* in the programs of biological control for root rot diseases in okra. Finally, there was a positive effect of interaction between *T. harzianum* and fungicide topsin–M for reducing okra root rot disease and increasing the plant growth parameters.

Acknowledgements

We are so grateful for the Department of Plant Protection, College of Agriculture for its financial support. Also we record our thanks for Dr. Abdulnabi A. Matrood for providing the isolate of *T. harzianum*. Finally, we thank Prof. Asaad Y. Ayed, Department of Animal Production, College of Agriculture for helping in the statistical analyses.

References

Abbott, W.S. (1925). A method of computing the effectiveness of insecticides. J. Econ. Entomol., 18: 265-267. (C.F. Shaaban & Al-Mallah,1993).

Abd-El-Kader, M.M.; El-Mougy, N.S.; Aly, M.D.E. & Lashin, S.M. (2010). First report of ashy stem blight caused by *Macrophomina phaseolina* on *Aeonium canariense* in Egypt. J. Plant Pathol. Microbiol., 1: 1-11.

Aghighi, S.; Shahidi–Bongjar, G. H.; Rawashdeh, R.; Batayneh, S. & Saadoun, I. (2004). First report of antifungal spectra of activity of Iranian Actinomycetes strains against *Alternaria* strains, *Alternaria alternata, Fusarium solani*, *Phytophthora megasperma*, *Verticillium dahlia* and *Saccharomyces cerevisiae*. Asian J. Plant Sci., 3(4) : 463-471.

Al-Adel, K.M. (2006). The pesticides, essential concepts and their role in the agriculture and health. Coll. Agric. Univ. Baghdad. 442pp. (In Arabic).

Al-Fadhl, F. & Al-Janabi, M.M. (2017). Assessment the effect of some bio agents and Topsin-M in controlling root-rot disease on Petunia plants. Kufa J. Agric. Sci., 9(1): 393-404. (In Arabic).

Al-Hasnawy, E.M. (2017). Evaluation of using some biological control factors of

control seeds rot and damping-off disease on okra plant caused by *Fusarium solani* and *Rhizoctonia solani*. Karbala J. Agric. Sci., 5(4): 250-260. (In Arabic).

Al-Juboory, H.H.; Hassan, A.K. & El-Humairy, Y.N. (2018). Effect of some bio inducers in controlling the pathogen *M. phaseolina* causing root and stem charcoal rot of strawberry. Arab J. Plant Prot., 36(2): 154-163. (In Arabic).

Al-Kaabi, A.N.K.; Al-Shagiri, A. & Salih, A.M. (2009). Effect of some fungicides and bioagent fungi in controlling the seed rot disease and damping- off of tomato caused by pathogenic fungi *Rhizoctonia solani* Kühn, *Fusarium solani* Marti. Qadisiyah J. Pure Sci., 14(3): 46-56. (In Arabic).

Al-Maarich, E.R.M. (2015). A study of bare patch disease caused by *Rhizoctonia solani* Kuhn on wheat in fields at North of Basrah and its biological and chemical control. M. Sc. Thesis, Coll. Agric., Univ. Basrah. 115pp. (In Arabic).

Al-Murad, N.Y. & Al-Taei, A.K.M. (2006). Biological control of root rot of beans. The 9th Arab Conf. Plant Prot.: 214. (Abst.). (In Arabic).

Al-Musawy, M.A.; Lahouf, A.A. & Jafar, O.H. (2017). Isolation and identification of the pathogens causing seed decay and damping-off disease on wheat and control by using some biological and chemical factors. J. Karbala Univ. Sci., 4(1): 112-132. (In Arabic).

Al-Rawi, K.M. & Khalaf Allah, A.M. (1980). Design and analysis of Agricultural experiments. College of Agriculture and Forestry Publishing. Univ. Mosul: 486pp. (In Arabic).

Altomare, C.; Novell, W.A.; Bjorkman, T. & Harman, G.E. (1999). Solubilization of phosphates and micro nutrients by the plant growth promoting and biocontrol fungus *Trichoderma harzianum* Rifai 1295-22. Appl. Environ. Microbiol., 65(7): 2926-2933.

Al-Waily, D.S.A. (2004). Studies on tomato damping-off and its integrated control in Zubair and Safwan farms. Ph.D. Thesis, Coll. Sci., Univ. Basrah. 110pp. (In Arabic).

Alwan, K.F. (2014). Activity of some bioagents against *Fusarium solani* and *Macrophomina phaseolina* causing cucumber root rot disease in Babylon Province. Al-Mouthana J. Agric. Sci., 2: 20-27. (In Arabic).

Bell, D.K.; Wells, H.D. & Markham, C.R. (1982). *In vitro* antagonism of *Trichoderma* species against six fungal plant pathogens. Phytopathology, 72: 379-382.

Bolkan, H.H. & Butler, E.E. (1974). Studies on heterokaryosis virulence of *Rhizoctoni solani* . Phytopathology, 64: 513-522.

Dewan, M.M. (1989) . 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. Western Australia. 210pp.

El-Habbaa, G.M.; Abdou, M.M.; Mahdy, F.; Mohamed, G. & El-Shaery, S.A. (2016). Biological and chemical control of grapevine die-back disease and their effect on defence related enzymes. Int. J. Sci. Eng. Res., 7(3): 201-212.

FAO (Food and Agriculture Organization of the United Nations). (2014): FAOSTAT, http://www.fao.org/faostat/en/#data/QC.

Farah, S. & Sahera, N. (2016) . Antifungal activity of *Pseudomonas aeuroginosa* and *Bacillus subtilis* against pathogens of cucurbitaceous fruits. Int. J. Innov. Res. Sci. Eng. Tech., 5: 1-5.

Fayyadh, M.A. (1997). Response of different genotypes of sunflower *Helianthus annus* L. for infection by *Macrophomina phaseolina* (Tassi) Goid and the role of some biological methods for control. Ph.D. Thesis, Coll. Agric., Univ. Baghdad. 106pp. (In Arabic).

Gajera, H.P.; Bambharolia, R.P.; Patel, S.V.; Khatrani, T.J. & Goalkiya, B.A. (2012). Antagonism of *Trichoderma* spp. against *Macrophomina phaseolina*: Evaluation of coiling and cell wall degrading enzymatic activities. J. Plant Pathol. Microb., 3: 7. DOI: 10.4172/2157-7471.1000149

Ghisalberti, E.L.; Narbey, M.J.; Dewan, M.M. & Sivasithamparam, K. (1990). Variability among strains of *Trichoderma harzianum* in their ability to reduce take-all and to produce pyrones. Plant Soil, 121: 287-291.

Juber, K.S. (1996). Pathogenic complex between nematode *Melodogyne javanica* and fungi *Fusarium solani* and their biological control. Ph.D. Thesis, Coll. Agric. Univ. Baghdad. 79 pp. (In Arabic).

Jasim, N.S. (2007). A study of root and stem bases rot disease of the beans caused by *Rhizoctonia solani* in Basrah province and its biological and chemical control . Ph.D Thesis, Coll. Agric. Univ. Basrah. 123pp. (In Arabic).

Jasim, N.S. & Al-Qurani, J.T.A. (2012). Effect of Salicylic acid (SA) against the fungus *Macrophomina phaseolina* (Tassi) Goid and development of charcoal rot disease on sunflower *Helainthus annus* L. Basrah J. Agric. Sci., 25(2): 58-71. (In Arabic).

Leslie, J.F. & Summerell, B.A. (2006). The *Fusarium* laboratory manual photographs. Suzanne Bullock: 388pp.

Limon, M.C.; Pintor-Toro, J.A. & Benitez, T. (1999). Increased antifungal activity of *Trichoderma harzianum* transformants that overexpress a 33-kDa chitinase. Phytopathology, 89: 254-261.

Matloob, A.N.; Ezdeen, S.M. & Kareem, S.A. (1989). Vegetable Production. Mosul Univ. Publ., Iraq. 337pp. (In Arabic).

Matrood, A.A. (2015). Induction of systemic resistance in sunflower against fungus *Macrophomina phaseolina* the causal agent of charcoal rot disease using *Glomus* sp. and some species of *Trichoderma.* Ph.D. Thesis, Coll. Agric., Univ. Kufa. 127 pp. (In Arabic).

Matrood, A.A. (2018). Biological control of eggplant leaf spot disease caused by *Cladosporium cladosporioides*. Arab J. Plant Protec., 36(3): 192-198. (In Arabic).

Mickenny, H.H. (1923). Influence of soil temperature and moisture on infection of wheat seedling by *Helminthosporium sativum*. Agric. Res., 26: 195-217. (C.F. Al-Waily, D.S.A., 2004).

Mohammad, S.Y. (2012). Chemical and biological control of damping–off and root rot disease of bean caused by *Rhizoctoni solani*. J. Rafidain Agric., 40(4): 206-216. (In Arabic).

Mukhtar, I. (2008). Influence of *Trichoderma* species on seed germination in okra. Mycopathology, 6: 47-50.

Neergaard, P. (1997). Seed Pathology. Vol. I and II. McMillan Press. London. 1187pp.

Parmeter, J.R. & Whitney, H.S. (1970). Taxonomy and nomencleature of the imperfect stage. In: Parmeter, J.R. (Ed.), Univ. California Barkley. Los Angeles. 255pp.

Planning & follow-up. (2018). Basrah Agriculture Directorate. Basrah province-Iraq. (In Arabic).

Radhi, K.H.; Kadhem, Z.K. & Rashed, Y.D. (2016). Biological control of root rot disease of eggplant caused by *Fusarium solani*. Karbala J. Agric. Sci., 3(1): 99-115. (In Arabic).

Rahim, S. & Dawar, S. (2015). Seed borne mycoflora associated with okra *Abelmoschus esculentus* (L.) Moench. Pakistan J. Bot., 47: 747-751.

Saad, N.A. (2001). Interference between root knot nematodes *Meloidogyny javanica* and *Rhizoctonia solani* in eggplant and biological control. M.Sc. Thesis, Coll. Agric., Univ. Baghdad. 87pp. (In Arabic).

Salih, Y.A. & Al-Maarich, E. R. M. (2016). Effect of interaction between bioagent *Trichoderma harzianum* and fungicide Moncut on wheat bare patch disease caused by *Rhizoctonia solani* in Basrah Northern. Babylon J. Pure Sci., 4(24): 968-984. (In Arabic).

SCO (Central Statistical Organization Iraq). (2016). Production Report of Vegetables and Secondary Crops by Provinces for Year 2015, Baghdad: 56pp.

Shaaban, A. & Al-Mallah, N.M. (1993). Pesticides. Mosul Univ. Publ., Mosul, Iraq. 520pp. (In Arabic).

Shailbala, A. & Kumar, A. (2017). Effect of thiophanate methyl on red rot disease (*Colletotrichum falcatum*) and yield attributes of sugarcane. J. Pharmacogn. Phytochem.,1: 163-167.

Sinclair, J.B. (1982). Compendium of Soybean Disease. 2nd ed. American Phytopathol. Soc. St. Paul. MN. 104pp.

Sivan, A. & Chet, I. (1989). The possible role competition between *Trichoderma harzianum* and *Rhizoctonia solani* on rhizosphere colonization. Phytopathology, 79(2): 198-203.

Smiley, R. W.; Wilkins, D. E. & Klepper, E. L. (1990). Impact of fungicide seed treatments on *Rhizoctonia* root rot, take-all, eyespot and growth of winter wheat. Plant Disease, 74: 782-787.

Wannas, R.A. (2001). Isolation and identification of the pathogenic fungi associated with root rot of cowpea and integration in resistance. M.Sc. Thesis, Coll. Agric. Univ. Kufa. 106pp. (In Arabic).

Watanabe, T. & Shiyomi, M. (1975). Hyphal morphology of *Rhizoctonia solani* Kuhn and related fungi isolated from sugarcane in Taiwan. Trans. Mycol. Soc. Japan, 16: 253-263.

Zeilinger, S. & Omann, M. (2007). *Trichoderma* biocontrol signal transduction pathways involved in host sensing and mycoparasitism. Vienna Univ. Technol., 227-234.