The inhibitory effect of four different plant extracts to control some fungi infecting tomato plants

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التأثير التثبيطي لأربعة مستخلصات نباتية لمقاومة الفطريات التي تصيب نبات الطماطم

المستخلص:

يختلف النشاط التضادي لكل مستخلص نباتي باختلاف كلا من الفطريات وتركيز المستخلص. حيث تزداد النسبة المئوية لتثبيط النمو الفطري بزيادة تركيز المستخلص– لوحظ أن حساسية أو مقاومة الفطريات ضد فعل كل مستخلص نباتي تعتمد على اختلاف الفطريات اتجاه المركبات الكيمائية لكل مستخلص. وجد أن مستخلص الثوم والزعتر كانا ذو قدرة فائقة وتأثير تضادي فعال ضد نمو الفطريات الممرضة للنبات (60.14، 56.31% على التوالي) في المتوسط. وأظهرت مستخلصات كلا من الحلبة والزنجبيل أنهما ذات قدرة تضاد قليلة من حيث تأثيرهما على نمو الفطريات (41.41، 356.60% على التوالي) في المتوسط. ولوحظ ان استعمال 10% من مستخلص الثوم تحت ظروف الصوبة أدى إلى مقاومة الفطر *Fusarium.solani* (أحد مسببات موت البادرات) حيث زادت النسبة المئوية للنباتات القائمة الحية إلى 60% عند مقارنتها بالكنترول (6.66%) وأن استعمال الثوم بمعدل 5% لمقاومة الفطر *Fusarium.oxysrprum* (المسبب الثاني للمرض) أدى إلى زيادة في النسبة المئوية للنباتات القائمة (46.67%) بالمقارنة مع الكنترول (13.33%). لوحظ أن استعمال الزعتر بمعدل 20% لمقاومة الفطر، *Rizoctonia.solani*، *Sclerotinia sclerotiorum* أدى إلى زيادة النسبة المئوية للنباتات السليمة القائمة إلى 33.33، 33.34% بالمقارنة بالكنترول لكلا منها (0.0%).

الكلمات المفتاحية: *الفطريات، الأمراض، المستخلص النباتي، نبات الطماطم.*

Abstract:

Fungal diseases of cultivated crops remain the principal limitation to increase agricultural production every year. In the present research, it was found that the antifungal activity of each tested plant extract was differed with its used concentration. The in vitro studies revealed that inhibition percentage of fungal growth was increased with increasing of applied concentration. The sensitivity or resistance of fungi was depending on their variability.Garlic extract, followed by thyme exhibited a good antifungal effect since they caused a severe reduction of the fungal growth, being 60.14 and 56.31%, respectively.

On the contrary, the extract of ginger and fenugreek had least activity in this concern, being 35.60 and41.41%, respectively Under the greenhouse conditions, it was noticed that use of 10% of garlic extract led to control of *Fusarium solani* which causes On the contrary, the extract of ginger and fenugreek had least activity in this concern, being 35.60 and41.41%, respectively Under the greenhouse conditions, it was noticed that use

of 10% of garlic extract led to control of *Fusarium solani* which causes seedlings damping-off disease of tomato since it increases the percentage of survival plants to 60.00 % if compared to the control (6.66%).

Meantime, usage of 5% of the same extract, i.e. garlic extract was the second treatment in its effect to control *Fusarium oxysporum* compared to the control, the analogous values of survival plants were 46.67 and 13.33%, respectively.

On the other hand, it was found that use of thyme extract at the rate of 20% to control either *Rhizoctonia solani* or *Scleotinia sclerotiorum* led to 33.33% of survival tomato plants compared with the control. being 0%.

Keywords: *Fungal, diseases, plant extract inhibition.*

Introduction:

The protection of plant from various pathogens remains a primary concern of agriculture scientists (Guleria and Kumar, 2006). In the greenhouse and fields, the use of fungicides is considered the most mean of controlling fungal diseases. Although this method has been effective, some major problems are involved besides their negative effect on human and animal health and also on the agro – ecosystem (Pinto *et al.*, 1998).

The plants have ability to synthesize aromatic secondary metabolites and have antimicrobial effect (Das *et al.*, 2010).

One of the major pharmacological properties of medicinal plants is their antimicrobial activity (Bansod and Rai, 2008). Plant extracts have been reported to be effective antimicrobials

against food and grain storage fungi, foliar pathogens and soil – borne fungi (Bowers and Locke, 2000).

Therefore, the application of plant extracts for control diseases is non-hazardous and friendliness to the environment.

Seedling damping-off and root rot disease is worldwide spread in crop growing area and causing significant economic losses

The present research aims to study the *in vitro* antifungal activity of four different aqueous extracts namely; garlic, thyme, ginger and fenugreek on the growth of four pathogenic fungi that caused damping- off and root rot disease of tomato caused by *Rhizoctonia solani, Fusarium solani, Fusarium oxysporam* and *Sclerotinia sclerotiorum*. In addition to the effect of some extracts that were more active in vitro studies to control the disease under the greenhouse conditions was evaluated.

Materials and methods:

1- Source of the plant materials:

Four different plants namely: fenugreek (*Trigoneila foenumgraecum*L.), garlic (*Allium sativum* L.), ginger (*Zingiber officinale* L.) and thyme (*Thymus vulgaris*L.) were collected from the local markets at Tarhona- Libya.

2-Used fungi:

Four different fungi(*Fusarum solani, Fusarium oxsporum, Rhizoctonia solani* and *Sclerotinia sclerotiorum*) were isolated from infected tomato with seedlings damping – off and root rot disease and used in this research. All the above fungi were maintained on Potato Dextrose + Agar medium (PDA) in the refrigerator until use.

3- **Preparation of aqueous plant extracts:**

Plants were washed with tap water to remove any dust, and then left to dry at room temperature. The dried samples were soaked with distilled water for 3 days and dried at 30C for 3 days in a hot air oven, then crushed to make powder. One hundred gms. of each plant were extracted in a blender by adding 100ml of distilled water (1:1W/v).

The obtained extracts were individually filtered through What man N0.1 filter Erlenmeyer flasks, plugged with separately cotton and heated at 50 C° for 15 minis. to avoid contamination and kept in the refrigerator for a maximum time of one week.

4- Antifungal activity of plant extracts in vitro:

The antifungal activity of aforementioned four plant extracts (fenugreek, garlic, ginger and thyme)was evaluated by poisoned food technique as described by (Bansal and Gupta, 2012) for their efficacy against of the growth of previous our phytopathogenicfungi.

Different concentrations (1, 5, 10, 15 and 20%) of each plant extract were prepared with sterilized distilled water and incorporated to sterilized PDA medium, just before solidification and thoroughly mixed with the medium.

Each concentration was poured in sterilized Petri dishes.

The medium without any extract was used as control.

The mycelia discs of the phytopathogenic fungi were prepared using a sterilized cork borer (5mm in diameter) from10-days old cultures margin and placed in the center of each Petri dish.

Each treatment was replicated three times. All the plates were incubated at 25±2 C°. The fungal growth was measured (mm) when the growth in the control treatment reached to the edge of plate. The growth inhibition percentage was calculated by using the following formula (Nwachukwu and Umechuruba, 2001).

$$\% \text{ MGI} = \frac{x1 - x2}{x1} \times 100$$

Where:

%MGI refers to% of mycelia growth in inhibition.

X1 refers to the diameter (mm) of the growth in the control.

X2 refers to the diameter (mm) of the growth in any treatment.

5- Antifungal activity of plant extracts under the greenhouse conditions:

In this part, the plant species extract that proved to be more active on the fungal growth inhibition *in vitro*, were used to evaluate their role to control tomato seedlings ping damping- off and root rot disease according to the method of (Watterson, 1986).

5.1. Inoculums preparation:

Barley medium consisting of 75gm barley grains and 100ml of distilled water was prepared in a 500ml glass bottles. The medium was sterilized and left to cool, then inoculated with (5mm) disc of 15 days- old agar culture of each used fungus. The inoculated bottles were incubated at 25±2 C° for 15 days.

5.2. Pots and soil infestation

Plastic pots (No.15) were rinsed in 5% formalin solution for 10 mines. and left to air

dry for 10 days. The soil was sterilized using the autoclave.

5.3. Inoculation process

The aforementioned prepared pots were filled with the sterilized soil. The inoculum of each desired fungus was thoroughly mixed with the soil at the rate of 3% of soil weight (Elian, 1978). The inoculated soil was irrigated periodically for 14 days before seed planting to ensure each of the inoculum spreading.

The tomato seeds cultivar Rey Kharandy were surface sterilized as usually with sodium hypochlorite solution (2%), then soaked in each tested concentration of the active extracts for 24 hrs. that resulted from the in vitro studies (5 and 10% of garlic extract and 20% of thyme extract). Five seeds of tomato were grown in each pot. Other post that inoculated with each fungus and the tomato seeds were soaked for the same period in sterilized distilled water instead of the extract was used as control. Three post were employed for each treatment. After 2 and 4 weeks of sawing, the percentage of pre- and post-emergence damping-off was determined, respectively. Also, the percentage of survival tomato plants was calculated after 6 weeks from sowing.

Statistical analysis

The analysis of variance according to the procedure of (Snedecor and Cochran, 1980) Significant used to verify was. the Differences between treatments.

Results and discussion:

Antifungal activity of aqueous plant extracts in vitro the aqueous extract of four different medicinal plants were screened against the growth of four pathogenic fungi to investigate their antifungal activities by poisoned food technique.

1- Effect of aqueous extract of thyme on the growth inhibiton of four phytopathogenicfungis:

Data obtained are presented in Table (1).

It was found that there was significant increase in the inhibitory effect of the mycelia growth with the increase of concentration from1 to 20 %. At 20 % of thyme extract, the growth of all tested fungi was completely inhibited (100%) and this might be attributed to that highly concentration of inhibitory effect. Meantime, the action of thyme extract was significantly differed from one fungus to another. *Fusarium oxsporaum* was more sensitive, followed by *Rhizoctonia solanion* the contrary: *Fusarium solani* was most resistant to the action of plant extract.

The corresponding values of growth inhibition were 69.23, 61.56 and 41.30 %, respectively.

Table (1): The inhibitory effect of aqueous extract of thyme (*Thymus vulgaris* L.) at different concentrations on the growth of four phytopathogenic fungi.

Phytopathogenic fungi	Growth inhibition (%) Extract concentration (%)					Average
	1	5	10	15	20	
Fusarium oxysporum	**27.81**	**38.89**	**87.96**	**91.48**	**100.0**	**69.23**
Fusarium solani	**21.30**	**25.00**	**27.78**	**32.41**	**100.0**	**41.30**
Rhizoctonia solani	**23.15**	**32.41**	**63.89**	**88.33**	**100.0**	**61.56**
Sclerotinia sclerotiorum	**17.62**	31.48	**34.36**	**82.41**	**100.0**	**53.15**
Average	**22.47**	31.95	**53.50**	**73.66**	**100.0**	**56.31**
Control	**0.0**	**0.0**	**0.0**	**0.0**	**0.0**	

L.S.D. (0.05) for fungi =1.957, 0.649, 0.205, and 0.444 respectively.

The obtained results are in agreement with those recorded by (ALRahmah *et al.*, 2013) who studied the fungicidal activity of methanolic plant extracts of thyme for its antifungal efficiency
On tomato phytopathogenic fungi (*Fusarium oxsporaum*, *Pythium aphanidermum* and *Rhizoctonia solani*)

2- Effect of fenugreek aqueous extract on the growth inhibition of four phytpathogenic fungi:

Data in Table (2) show that as the concentration of extract increased, the percentage of pathogen growth inhibition also significantly increased.
No fungal growth inhibition even using 20% of fenugreek extract concentration and this indicates that this extract has least potential to inhibit the growth. On the other hand, the pathogenic fungi were differed in their sensitivity to the action of fenugreek extract. *Fusarium solani* was significantly more sensitive, whereas *Sclerotinia sclerotiorum* and *Rhizoctonia solani* were significantly more resistant to the extract. The analogous values were 55.62, 20.37 and 40.74 %, respectively. This variation among the pathogenic fungi might be due to difference in their genetic construction and / or difference in the chemical and structural composition of the fungal cell walls.

Table (2): The inhibitory effect of aqueous extract of fenugreek (Trigonella foenum-graecum L.) at different concentrations on the growth of four phytopathogicfungi:

Phytopathogenic fungi	Growth inhibition (%) Extract concentration (%)					Average
	1	5	10	15	20	
Fusarium oxysporum	**35.19**	**43.52**	**45.37**	**47.22**	**48.15**	**43.89**
Fusarium solani	**47.22**	**48.15**	**50.82**	**64.11**	**67.78**	**55.62**
Rhizoctoniasolani	**19.44**	**33.33**	**35.18**	**52.78**	**62.96**	40.74
Sclerotinia sclerotiorum	**11.11**	**11.11**	**13.89**	**18.52**	**47.22**	**20.37**
Average	**18.24**	**33.78**	**36.32**	**45.66**	**56.53**	**41.41**
Control	**0.0**	**0.0**	**0.0**	**0.0**	**0.0**	

L.S.D. (0.05) for fungi: 0.052, 0.213, 4.564 and 0.218, respective

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The obtained data are in disagreement with those recorded by (Makai and Balatincz, 1998) who stated that the use of plant extracts that contain a lot of active compounds are considered to be successful alternative to the chemicals in improving the shelf life of fruits and vegetables. The extract of fenugreek seeds is rich inprotein, fat, carbohydrates, fluid materiels, nutrients and vitamins, beside contains many active substances such as alkaloids and glycosides.

Also (Othman et al., 2020) determined the antifungal activities of nine kinds of powdered plant extracts and five essential oils in vitro against four of the most common fungal species Aspergillus spp. *Penicillium sp.*

3- Effect of ginger aqueous extract on the growth inhibition of four phytopathogenicfungi:

It was found a significant increase of the inhibitory effect on the mycelia growth accompanied with concentration increased (Table 3) only the growth of *Rhizoctonia solani* was completely inhibited at the highest concentration, i.e. 20%.

Fusarium solani was more sensitive to the action of ginger extract. On the other hand, *Fusarium oxsporum*, followed by *Rhizoctonia solani* were more significantly resistant to the extract. The corresponding values were 42.93, 21.86.and 38.54%, respectively.

Table (3): The inhibitory effect of aqueous extract of gingre (*Zingiberofficinole* L.) at different concentrations on the growth of four phytopathogicfungi:

Phytopathogenic fungi	Growth inhibition (%) Extract concentration (%)					Average
	1	**5**	**10**	**15**	**20**	
Fusarium oxysporum	**11.11**	**11.11**	**19.44**	**23.18**	**44.44**	21.86
Fusarium solani	**24.26**	**34.07**	**45.74**	**51.67**	**58.89**	42.93
Rhizoctoniasolani	**13.92**	**14.88**	**21.29**	**42.59**	**100.00**	38.54
Sclerotinia sclerotiorum	**14.88**	**17.59**	**44.44**	51.85	**66.46**	39. 54
Average	**16.04**	**19.41**	**32.73**	**42.32**	**67.45**	39. 05
Control	**0.0**	**0.0**	**0.0**	**0.0**	**0.0**	

L.S.D. (0.05) for fungi =0.103, 0.251, 0.239 and 0.122, respectively

The obtained data are in the line of those recorded by (Shovan *et al.*, 2008) who reported that ginger extract gave 39.83% reduction in colony diameter at the highest concentration due to its fungi static and fungicidal activity. The low effect on the other hand indicates that it has least potent to inhibit the growth among all the studied plant extracts.

On the contrary, the results are somewhat in agreement with those recorded by (Ramanathan *et al.*, 2004) who found that thyme extract showed complete suppression on colony growth of *Fusarium oxsporum* and Rhizoctoniasolani, followed by extract of ginger. The difference between these results might be due to varied of the fungal species.

4- Effect of garlic aqueous extract on the growth inhibition of four phytopathogenicfungi:

Data in Table (4) reveal that the percentage of growth inhibition of the pathogen was significantly increased as concentration of garlic extract was increased. On the average, at 1 % concentration, the growth inhibition was 19.13%, whereas at 20%, the growth inhibition reached to 88.20% at 20% concentration, the growth of each of *Rhizoctoniasolani*, *Fusarium oxsporum* and *Fusarium solani* was completely inhibited being 100%. In addition, the growth of the *Fusariumo xsporum* was also completely inhibited at 5% of garlic extracts while *Fusarium solani* was completely inhibited at 10%. Only *Sclerotinia sclerotiorum* still grows at all used concentrations and this might be due to that, this fungus formed sclerotia in the cultures that resistant to some compounds of the extract. Therefore, *Sclerotinia sclerotiorum* proved to be more resistant to the action of extract, followed by *Rhizoctonia solani*. The corresponding values were 35.37 and 52.97 %, respectively.

Table (4): The inhibitory effect of aqueous extract of garlic (*Allium sativum* L.) at different concentrations on the growth of four phytopathogic fungi:

Phytopathogenic fungi	Growth inhibition (%) Extract concentration (%)					Average
	1	5	10	15	20	
Fusarium oxsporaum	20.37	**100.0**	**100.0**	**100.0**	**100.0**	84.07
Fusarium solani	**13.89**	**26.85**	**100.0**	**100.0**	**100.0**	68.15
Rhizoctoniasolani	**15.77**	**22.22**	**44.45**	**82.41**	**100.0**	**58.97**
Sclerotinia sclerotiorum	**26.39**	**27.78**	**34.73**	**35.18**	**52.78**	**35.37**
Average	**19.13**	**44.13**	**69.80**	**79.40**	**88.20**	60.14
Control	**0.0**	**0.0**	**0.0**	**0.0**	**0.0**	

L.S.D. (0.05) for fungi =0.104, 0.290, 0.175 and 0.165, respectively.

The obtained results are in the line with those reported by (Shukla and Dwivedi, 2012) who used garlic to control two Fusarium species that causing wilt Also disease, (Tagoe *et al*., 2011) indicated that garlic had potential antifungal properties (contains essential oils with sulfur compounds, vitamins, hormones and antiseptic substances as allicin).

From all the above-obtained results, it could be concluded that *Allium stivum* was the best-effected extract, followed by *Thymus vulgaris*

5- Effect of some plant extracts at specific concentrations on the control of seedlings damping-off and root rot disease of tomato under the greenhouse conditions:

Under the greenhouse conditions, the effect of two plant extracts i.e. thyme at 20% and garlic at 5 and 10% were evaluated for their role to control seedlings damping-off and root rot disease of tomato using cultivar Rey Khrandy.

Results in the (5) dealing with the effect of different treatments on the percentage of pre-emergence damping –off reveal that use 5 and 10% of garlic extract significantly reduced the infection with the disease caused by *Fusarium oxsporum* and *Fusarium solanito* 20.00 % and 0.0, respectively compared to the control (46.67 and26.67 %) respectively. On the other hand, thyme extract at 20% also significantly reduced the infection caused by *Rhizoctonia solani* to 46.67 % in comparison with the control, being 66.67. On the contrary, thyme at 20% significantly increased the infection caused by *Sclerotinia sclerotiorum* to 53.33% compared to the control (40.00%).This might be due to produce developing sclerotia and led the fungus to be resistant against the preliminary of its present in the soil.

Table (5): Effect of soil infestation, individually, with some fungi that previously isolated from infected from tomato plants on the percentage of pre-, post- emergence damping – off and percentage of survival tomato plants:

Soil infestation treatments	Pre-emergence	Post-emergence	% survival tomato plant
Fusarium oxsporum+immersed tomato seeds in 5%garlic extract	**20.00**	**33.33**	**46.67**
Control	**46.67**	**40.00**	**13.33**
Fusarium solani +immersed tomato seeds in 10%garlic extract	**0.0**	**40.00**	**60.00**
Control	**26.67**	**66.67**	**6.66**
Rhizoctonia solani +immersed tomato seeds in 20% thyme extract	**46.67**	**20.00**	**33.33**
Control	**66.67**	**33.33**	**0.0**
Sclerotinia sclerotiorum+immersed tomato seeds in 20% thyme extract	**53.33**	**13.33**	**33.34**
Control	**40.00**	**60.00**	**0.0**

L.S.D. (0.05) =17.23

The obtained results are in agreement with those reported by (Shukla and Dwivedi, 2012) who used garlic extract to control two fusarial species that causing with disease (AL-Rahmah *et al.,* 2013) Concerning with the effect of two used plant extracts on the percentage of post- emergence damping off, results in Table (5) indicate that thyme extract at 20% significantly reduced the infection with post- emergence damping-off caused by *Sclerotinia sclerotiorum* to 13.33% compared with the control((60.00%), followed by that caused by *Rhizoctonia solani*(20.0 and 33.33%, respectively).

Meantime, usage of 5% and 10% garlic extract also decreased the infection caused by *Fusarium oxsporum* and *Fusarium solani* to 33.33 and 40.0% compared to the control, being 40.0 and 66.67%, respectively. Dealing with the effect of the disease on the percentage of survival tomato plants, results in Table (5) show that usage of 10% of garlic extract in the presence of *Fusarium solani* in the soil led to a significant increase of the percentage of survival plants to 60.00% if compared with the control (6.66%). Meantime, the soil infested with *Fusarium oxsporum* and use of 5% of garlic extract was the second in this respect, being 46.67 % and 13.33 % respectively. The least percentage of survival tomato plants was obtained when the soil was individually infested with *Sclerotinia sclerotiorum* and *Rhizoctonia solani.*The analogous values were 33.34 and 33.33%, respectively compared with the control of each one (0.0%).

The variation in the fungi toxicity of plant extracts against the phytopathogenic fungi might be due to considerable variations in their constituent's fungal species (Manorantitham, *et al,* 2001). Itcould be concluded that the action of plant extract in vitro was in par ell of its activity under greenhouse conditions.

Recommendations

1-Plant extracts have a great potential as antifungal compounds against most microorganism. Thus, the can be used in the treatment of plant diseases caused by resistant fungi to other methods of control.

2- Further studies are needed to find new plants that have more effect on the in vitro growth of most pathogenic fungi and hence decrease of plant diseases.

3- More studies should be carried out to determine the major active components of the excellent plant extracts that have a high antifungal activity.

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