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EFFECT OF POMEGRANATE (*Punica granatum*L.) FRUITS PEEL ON SOME PHYTOPATHOGENIC FUNGI AND CONTROL OF TOMATO DAMPING-OFF

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ABSTRACT

Pomegranate peel powder and its extract were used to study their efficiency against damping-off disease caused by *Fusarium oxysporum* f. sp. *lycopersici*. *In vitro*, methanolic extract of pomegranate peel caused inhibitory effect to the linear growth of different phytopathogenic fungi isolated from different hosts including:

Botrytis cinerea, Colletotrichum dematium, F. oxysporum, F. solani, Phoma sp. and *Rhizoctonia solani.* Also, Pomegranate peel extract (PPE) effectively decreased linear growth and spore germination of *F. oxysporum* and greatest decrease was recorded at 4000 ppm under greenhouse conditions. Application of pomegranate peel powder as seed treatment or soil treatment deceased pre and post-emergence damping-off caused by *F. oxysporum* f. sp. *lycopersici* compared with untreated infected control. Treating tomato seedlings or soil with peel extract before sowing provided a good protection against damping off and soil treatment was more effective than seedling treatment. Methanolic extract of pomegranate peel revealed that it contained different compounds of antifungal activity including three flavonoids, three tannins, four phenolic compounds, one glycosides along with four sterols.

Keywords: *Fusarium oxysporum* f. sp. *lycopersici*, Linear growth, spore germination, Gas chromatography-mass spectrometry (GC-MS)analysis, antifungal activity,flavonoids, tannins, phenolic compounds, glycosides, sterols.

INTRODUCTION

Tomato (*Lycopersicon esculentum* Mill) is one of the most economic vegetable crops cultivated at different locations in Egypt and all over the world for either local consumption or export purposes (Tampoare *et al.,* 2013). It is subjected to attack by many soil borne fungal diseases specially the damping-off and wilt diseases causing considerable losses either in nurseries or in the field (Abdel-Kader *et al.,* 2012). Farmers usually apply synthetic fungicides as preventive and therapeutic measures to control plant diseases, though the hazardous effect for the environmental pollution (Steffens *et al.,* 1996 and Ishii, 2006) and the risk of pathogenic microorganisms (Barnard *et al*., 1997 and Brent and Holomon, 1998).

Recently, certain natural products of plant origin have been evaluated as a source of antimicrobial agents against a variety of phytopathogenic fungi (Dahham *et al.,* 2010, Al-Askar 2012 and Mangang and Chhetry 2012).

The extract of peel, seed and whole fruit of pomegranate (*Punica granatum* L.) have antimicrobial activity against many pathogenic fungi and bacteria *in vitro* and *in vivo* (Dahham *et al*., 2010 and Al-Askar, 2012. In this

respect, Dahham *et al.,* (2010) and Sadik and Asker (2014) identified different compounds which have antimicrobial activity from various parts of pomegranate fruit, including phenols, flavonoids, condensed tannins, hydrolyable tannins and polyphenols. In searching for compounds from plants that are active against plant pathogenic fungi, we found that the methanol extract of pomegranate peel is highly active against most of plant pathogens tested. So, the aim of the present study is to evaluate the anti-fungal activity of methanolic extracts of pomegranate peel against the phytopathogenic fungi So, the present study aimed to evaluate the antifungal activity of pomegranate peel extract against some phytopathogenic fungi as well as its effect on the linear growth, spore germination of *F. oxysporum* f. sp. *lycopersici*, to control tomato damping-off. Also, phytochemical analysis to pomegranate extract using GC-Ms technique was carried out.

MATERIALS AND METHODS.

Preparation of methanolic extract of pomegranate peel:

Fresh fruits of Pomegranate (*Punica granatum* L.) were manually peeled. The collected peels were then rinsed with distilled water and dried in an oven at (50°C) for 48h, and were powdered to get 60-mesh size using a mixing grinder (Dahham *et al*., 2010). The powdered peels (2g) of pomegranate were macerated with 20 ml of 80% methanol at room temperature. The macerated material was strained through Whatman No1 filter paper. The extract was concentrated at 40°C by using Rotary Evaporator (Heidolph, LABOROTA A 4000-Germany) then dried in the oven at 50°C for 48h. Finally, by using the dried extract, different concentrations of methanolic extract i,e 31.3, 62.5,125, 250, 500, 1000, 2000 and 4000 ppm were prepared (Tayel *et al.,* 2009).

Phytopathogenic fungi used

Six authentic phytopathogenic fungi isolated from different hosts, were used in this study. *Botrytis cinerea* (pea)*, Colletotrichum dematium* from (soybean)*, F. oxysporum, F. solani, Phoma* sp. and *Rhizoctonia solani* from (tomato). Kindly were provided by Seed Pathol. Res. Dept., Plant Pathol. Res. Instit., ARC. The tested fungi were regularly subcultured and maintained on potato dextrose agar (PDA) medium in a refrigerator at 5 ±1˚C**,** throughout the course of the study.

Effect of (PPE) on the linear growth of some fungi.

The anti-fungal activity of methanolic extract of pomegranate peel was studied *in vitro* by a poisoned food technique (Singh *et al*., 2008). PDA medium was autoclaved and the methanolic extract was added after reasonable cooling to 30-35 ˚C and vigorous shaking along with one ml of streptomycin before pouring into Petri-dishes, The prepared concentrations of methanolic extracts were i,e 0.0 , 31.3, 62.5, 125, 250, 500, 1000, 2000 and 4000 ppm were added to PDA medium and then poured into three Petri-dishes. Petri-dishes contained PDA medium only were used as control. A disc (4 mm diameter) of any of the six fungal species, i,e *B. cinerea, C. dematium, F. oxysporum, F. solani, Phoma* sp. and *R. solani* was cut from 1-week-old culture and inoculated to the

center of the poured plates. Plates were sealed with Parafilm then incubated. Three replicates were used for each treatment at 25 ± 2˚C until the fungal growth in the control dishes was almost completed (Agarwal *et al*., 2001).

Effect of (PPE) on the linear growth of *F. oxysporum* f. sp. *lycopersici:*

One ml from each concentration of PPE (250, 500, 1000, 2000 and 4000 ppm) was mixed with reasonably cooled PDA medium and then poured in Petri-dishes. Plates containing only PDA medium were used as control treatment. All plates were inoculated with discs of 8 days old culture of *F. oxysporum F. oxysporum* and then incubated at 25˚C. When the mycelial growth covered the whole surface in the control plates, the linear growth in different treatments was determined. Three replicates were used for each concentration. The decreased percentage in mycelial growth of *F. oxysporum F. oxysporum* was calculated using the following formula:

$$\text{Percentage of inhibition or fungal growth} = \left(\frac{C - T}{C}\right) x100$$

Where:

C= Fungal growth of the control.

T = Fungal growth of the treatment.

Effect of PPE on spore germination of *F. oxysporum* f. sp. *lycopersici*:

Different concentrations of PPE (250, 500, 1000, 2000 and 4000 ppm) were tested. One drop from spore suspension of *F. oxysporum F. oxysporum* was added to the mentioned concentrations were pipetted by sterilized pipette on glass slides. Other glass slides, with drop from spore suspension, were prepared using distilled water as control treatment. All slides were incubated at 25˚C for 18 h. Three replicates were used for each concentration. Percentage of spore germination was recorded after microscopic examination and percentage of inhibition in germination was calculated as mentioned before.

Assessing of EC_{50}:

EC_{50} values were determined by the linear regression (LPd line Computer Program) of the probit of the tested fungus percentage inhibition vs. logs the concentrations (ppm) of the tested extract. The EC_{50} notation used to indicate the effective concentration (ppm) that causes 50% growth inhibition.

Greenhouse experiments:

Preparation of fungal inoculum:

Corn meal-sand medium (3:1w/w) in 500 ml glass bottles was autoclaved at 121˚C for 30 minutes. The sterilized bottles were then inoculated with discs (5 mm in diam.) of 8 days old culture of *F. oxysporum* and incubated at 25˚C for 15 days. Fungal inoculum of *F. oxysporum* was mixed thoroughly with the potted sterilized soil at the rate of 4 % inoculum level (w/w). The infested soil was adequately watered for one week to enhance growth and distribution of the fungal inoculum.

Effect of tomato seed treatment and soil application with pomegranate peel powder on the incidence of pre and post- emergence damping-off caused by *F. oxysporum* f. sp. *lycopersici*.

a- Seed treatment

Tomato seeds (Beto ,cv) were treated with Arabic gum (1%) as a sticker and then coated with pomegranate peel powder at the rate of 10g/ kg seed. Another group of seeds was treated with the fungicide Flowsan 42.7 % FS at the rate of 3g/ kg seed. Untreated seeds were used as a control treatment. Then seeds were sown in pots (30-cm-diam.) containing soil infested with 4% inoculum level of *F. oxysporum* f. sp. *lycopersici*. Six seeds were sown in each pot and four replicates were used for each treatment.

b- Soil treatment:

Pomegranate peel powder was added to pots (30-cm-diam) containing soil infested with 4% inoculum level of *F. oxysporum* at the rate of 100g/ pot, and then the pots were transplanted with untreated tomato seedlings at the rate of 6 seedlings per pot. Four replicates were transplanted in each pot. The growing seedlings in all treatments were examined periodically. Pre and post emergence damping-off was recorded 15 and 30 days after transplanting.

Effect of tomato seedling treatment and soil treatment with pomegranate peel extract on disease incidence.

Seedling treatment:

Four-week old tomato seedlings were dipped in PPE (4000 ppm) for 1h. Other seedlings were dipped in the fungicide Rizolex T 50% at the rate of 3g/L. Untreated seedlings were used as control treatment. The seedlings were transplanted in pots containing soil infested with 4% inoculum level of *F. oxysporum*. Six seedlings were transplanted in each pot and four replicates were used for each treatment.

Soil application:

Pots (30 cm-diameter) containing soil infested with 4% inoculum level of *F. oxysporum* f. sp. *lycopersici* were treated with pomegranate peel extract (10 ml/ pot), Transplanting was made with uninoculated 4-week-old tomato seedlings. Six Trans plants were sown in each pot and four replicates were used, plants in all treatments were examined periodically and the disease incidence 60 days after transplanting was recorded.

Phytochemical analysis of PPE:

The preliminary Qualitative phytochemical for the presence of various phytochemical compounds was performed using the methanolic extract. Presence of carbohydrates was determined by Molish's test. Presence of reducing sugar was detected by Benedict's test. (Ramkrishnan and Rajan 1994). Alkaloids in the extract were evaluated by Mayer's test. The sterols and Glycosides were determined by Salkowski's test and Borntrager’s test, respectively. (Evans 1997). The saponins were analyzed by Froth's test (Kokate, 1999). The occurrence of phenolic compounds and tannins were confirmed by ferric chloride and gelatin tests, respectively (Mace, 1963). The presence of flavonoids was investigated by lead acetate test (Kosalec, *et al.,* 2005).

GC-MS Analysis of PPE:

The qualitative and quantitative compositions of the methanolic extract of pomegranate peel extract were studied by GC-MS analysis Agilent 6890 gas chromatography (equipped with an Agilent mass spectrometric detector, with a direct capillary interface and fused silica capillary column PAS-5 ms, 30 mm x 0.25 µm film thickness). Samples were injected under the following conditions: Helium was used as carrier gas at approximately 1ml/min., pulsed split less mode. The solvent delay was 3 minutes and the injection size was 1.0 µl. The mass spectophotometric detector was operated in electron impact ionization mode an ioning energy of 70 e.v. scanning from m/z 50 to 500. The ion source temperature was 230°C and the quadruple temperature was 150°C. The electron multiplier voltage (EM voltage) was maintained 1250 v above auto tune. The instrument was manually tuned using perflurotributyl amine (PFTBA). The GC temperature program was started at 60°C then elevated to 280°C at rate of 8°C /min, and 10 min hold at 280°C. The Detector and injector temperature were set at 280°C and 250°C, respectively. Wiley and Nist 05 mass spectral database was used in the identification of the separated peaks.

Statistical analysis:

Completely randomized design in factorial arrangement with three replicates. according to Gomez and Gomez, 1984 was used. The least significant difference (L.S.D) between means was checked according to (Waller and Duncan 1969).

RESULTS

Antifungal potential of PPE:

The preliminary determination of the antifungal activity *in vitro* by using the poisoned food technique of the methanolic extract of pomegranate peel was studied against the six phytopathogenic fungal species: *B. cinerea, C. dematium, F. oxysporum, F. solani, Phoma* sp. and *R. solani.* The results (Table, 1) reveal variations in the antifungal activity of the methanolic extracts, that extract displayed high inhibitory effect on the growth of *B. cinerea, F. oxysporum, F. solani*, *Phoma* spp, *C. dematium* and *R. solani*, at concentrations from ranging between 1000 to 4000 ppm.

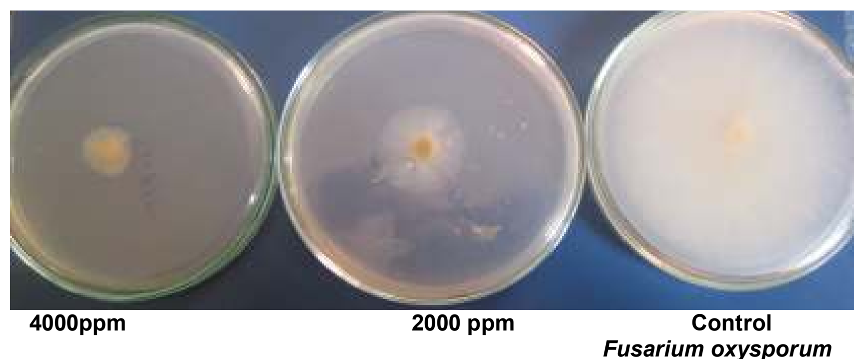
The *in vitro* antifungal activity of PPE expressed as a minimum effective concentration (EC_{50}) of 50 % of mycelial growth with the corresponding 95 % Confidence limits is shown in Table (2). It is clear that the antifungal activity increased with increasing the concentrations of the extracts. *F. solani* and *C. dematium* showed greater sensitivity to MPE than *Phoma* spp, *R. solani*, *B. cinerea* and *F. oxysporum.* The EC_{50} of the tested phytopathogenic fungi was 266.40, 272.47, 305.59, 307.88, 318.36 and 787.41 ppm, respectively.

Table (1) Screening inhibitory potential of PPE at different concentrations on linear growth of six fungi.

Concentration (ppm)	Tested fungi Percentage of Inhibition%					
	B. cineara	***C. dematium***	***F. oxysporum* f. sp. *lycopersici***	***F. solani***	***Phoma* spp**	***R. solani***
Control	0.0	0.0	0.0	0.0	0.0	0.0
31.3	16.0	20.9	7.7	7.70	13.7	21.8
62.5	24.1	29.1	13.2	16.7	22.3	29.3
125	44.1	38.5	20.9	30.7	33.4	37.9
250	49.6	48.7	30.7	48.3	46.2	47.2
500	55.2	58.9	42.1	66.3	59.3	56.6
1000	66.3	68.6	54.2	81.1	71.5	65.6
2000	72.6	77.1	65.9	90.0	81.6	73.8
4000	83.0	84.2	76.3	90.0	89.1	80.9
LSD ≥ 0.05	0.9	1.5	1.3	1.4	1.3	0.9

Table (2) Effective concentration (EC_{50}) of 50 % of the mycelial growth of six pathogenic fungi.

Tested Fungi	EC_{50}	95 % Confidence limit	
		Lower	Upper
B. cinerea	318.36	240.4	419.4
C. dematium	272.47	207.6	353.3
F. oxysporum	787.41	340.7	2998.4
F. solani	266.40	194.2	361.3
Phoma sp	305.59	186.1	491.8
R.solani	307.88	281.3	336.7



4000ppm **2000 ppm** **Control**

Fusarium oxysporum

Fig. (1): Effect of different concentrations of PPE on the liner growth and spore germination of *F. oxysporum* f. sp. *lycopersici.*

Different concentrations of PPE were used to study their effect on the linear growth and spore germination of *F. oxysporum* f. sp. *lycopersici*. Data in Table (3) and Fig. (1) showed that PPE significantly decreased the linear growth and spore germination of *F. oxysporum* f. sp. *lycopersici* compared to untreated control. Increasing the concentration was associated with an additional decrease with maximum records for linear growth and spore germination inhibition (s) at 4000 ppm, being 76.6 and 82.6 % respectively.

Table (3):- Effect of different concentrations of PPE on the linear growth and spore germination of *F. oxysporum* f. sp. *lycopersici.*

Concentration (ppm)	Linear growth (cm)	Inhibition (%)	Spore germination (%)	Inhibition (%)
0.0	9.0	-	91.4	-
250	6.3	30.0	69.4	24.1
500	5.2	42.2	60.8	33.4
1000	4.1	54.4	56.7	37.9
2000	3.1	65.5	32.2	64.7
4000	2.1	76.7	15.8	82.7
LSD≥ 0.05	1.9	-	14.6	-

Effect of tomato seed application and soil treatment with peel powder on pre and post- emergence damping off caused by *F. oxysporum* f. sp. *lycopersici*.

Data presented in Table (4) show that application of pomegranate peel powder and the fungicide Flowsan significantly decreased damping-off and recorded the highest survivals and the lowest damping-off and the highest efficiency, being 4.2 and 92.3 % respectively. Soil treatment with pomegranate peel powder was more higher than seed treatment in reducion. The efficiency of damping-off being 76.9 % and 46.1 %, respectively.

Table (4) Effect of tomato seed application and soil treatment with peel powder on pre and post- emergence damping off caused by *F. oxysporum* f. sp. *lycopersici*.

Treatments	Damping off (%)		Total (%)	Efficiency (%)
	Pre-emergance	Post-emergence		
Seed treatment	12.5a *	16.7ab	29.2	46.1
Soil treatment	8.3a	4.2b	12.5	76.9
Fungicide (Flowsan)	0.0a	4.2b	4.2	92.3
Control	20.8a	33.3a	54.2	-

***Means within the same column followed by the same letter are not significantly different according to Duncan's multiple range test (P≥0.05).**

Effect of seedling treatment and soil treatment with pomegranate peel extract before transplanting on disease incidence of tomato.

Data presented in Table (5) indicate that the used treatments significantly decreased disease incidence compared to untreated control. The fungicide treatment recorded maximum decrease (4.2 %) followed by soil treatment with PPE (8.3%). Whereas, seedling treatment with the same extract gave the lowest effect, being 20.8%.

Table (5):- Effect of seedling treatment and soil treatment with pomegranate peel extract before transplanting on disease incidence of tomato.

Treatments	Disease incidence (%)	Efficiency (%)
Seedling treatment	20.8b*	58.3
Soil treatment	8.3b	83.3
Fungicide (Rizolex T)	4.2b	91.6
Control	50.0a	-

***Means within the same column followed by the letters are significantly different according to Duncan's multiple range test (P≥0.05).**

Preliminary phytochemical determination of PPE.

Plants produce phytoalexins are considered as a defensive tool in response to microbial invasion. (Glazebrook and Ausubel, 1994).The Preliminary qualitative phytochemical analysis of methanolic extract of pomegranate peel extract was carried out for detection of secondary metabolites is presented in Table (6-A). The results indicate that the alkaloids and saponin were totally absent showing the negative test. Carbohydrates, reducing sugar, sterols, glycosides, phenolic compounds, tannins and flavonoids were found to be present by the qualitative test. The similar findings were also reported by (Prashanth *et al.,* 2001; Hegde *et al*., 2012; Satheeh, 2012; Amina and Filali, 2013; Kannaiyan *et al.,* 2013 and Uma *et al.,* 2012). This indicating the methanolic extract of pomegranate peel is good source of secondary metabolites having an important role in metabolism.

Table (6-A). Determination of some phytochemical groups in PPE by different tests.

Test	Phytochemical compound	Reaction
Molish's test	Carbohydrates	+
Benedict's test	Reducing sugar	+
Mayer's test	Alkaloids	-
Salkowski's test	Sterols	+
Borntrager's test	Glycosides	+
Froth's test	Saponins	-
Ferric chloride test	Phenolic compounds	+
Gelatin test	Tannins	+
Lead acetate test	Flavonoids	+

+ Present
- Absent

GC-MS analysis of PPE:

Qualitativ analysis of the methanol extract of pomegranate peel was done using GC-MS analysis led to identification number of compounds (Fig.1). The active principals with their retention time (RT), molecular formula, molecular weight and concentration in the methanol extract of pomegranate peel are presented in Table (6-B). Thirty components were detected in the methanol extract. 2-furancarboxaldehyde, 5-(hydroxymethyl)- was found to be the major component of the methanol extract with 39.71 % of the total peak area followed by 5,5-oxy-dimethylene-bis (2-furldehyde) , 2-furancarboxaldehyde, 2, 5-furandione,3-methyl-, propanedioic acid ethyl-, diethyl ester and 4H-pyran-4-one,3-hydroxy-2-methyl 11.77, 9.38, 6.66, 5.97 and 5.58 %, respectively. Figs. 2-7 showed the mass spectrum of the most abundant components of methanol extract of pomegranate peel.

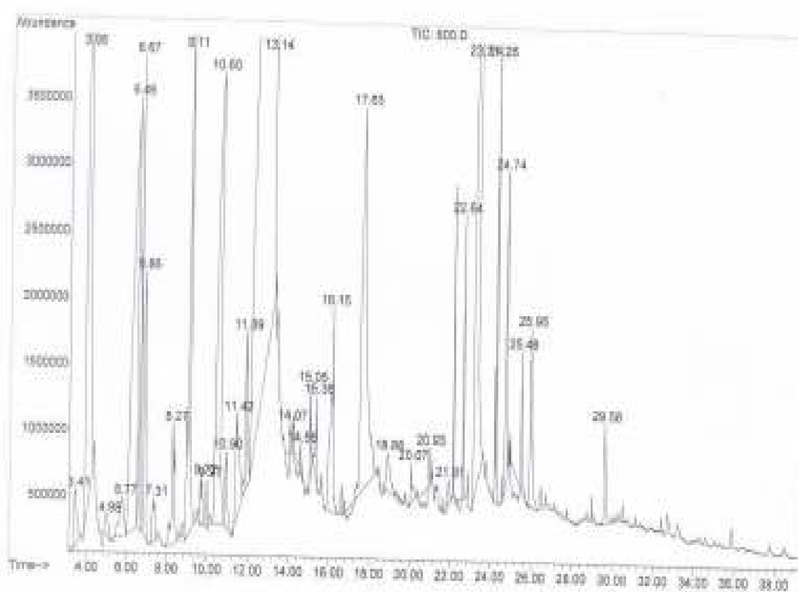


Figure 1. The chromatogram of all compounds produced from GC-MS analysis of methanolic extract of pomegranate peel.

Data presented in Tables (6-B) and Figs. (1-7) revealed that chemical analysis of methanolic extract of pomegranate peel contain many different compounds which have antifungal activities. These compounds including 5,5-oxy-dimethylene-bis (2-furaldehyde), 9-Octadecanoic acid, and 2H-Pyran,3,4-dihydro which belonged to Flavonids group, with average rate (13.87%). 2-Furancarboylic acid, methyl ester, 2-Thiophenacroxylic acid, 5-methyl, and 2-6-Pyridinediamine which belonged to Tannins group, with average rate (5.64%).1H-Pyran-4-one,2,3-Dihydro 3-5-dihydroxy-6-methyl, 1H-Indol-3-

acetic acid,2-methyl, n-Hexadecanonic acid, and 3-Amino-S-triazole which belonged to phenolic group, with average rate (4.52%). 1,3-cyclohexanedione,2-methyl belonged to glycosides group, with rate of (1.10%). 2,5-Furandione,3-methyl, propanedioic acid ethyl-,diethyl ester, 4H-pyran-4-one,2,3-dihydroxy-6-methyl, and 9-octadecanoic acid, methyl ester belonged to Sterols group, with average rate (19.85%).

Table (6-B): The main components identified by GC-MS in the methanolic extract of Egyptian pomegranate peel.

Peak	RT	Area%	Name	Molecular Formula	Molecular Wight
1	3.41	0.53	3-Amino-S-triazole	$C_2H_4N_4$	84.04
2	3.98	9.38	2-Furancarboxaldehyde	$C_5H_4O_2$	96.02
3	4.98	0.14	4-cyclopentene-1,3-dione 1 H-Imidazole,1-ethyl-	$C_5H_4O_2$	96.02
4	5.77	0.6	2H-Pyran,3,4-dihydro-	$C_4H_4O_2$	84.02
5	6.46	6.66	2,5-Furandione,3-methyl-	$C_5H_4O_3$	112.02
6	6.67	1.48	2-Furancarboxaldehyde,5-methyl	$C_6H_6O_2$	110.04
7	6.86	1.65	4H-pyran-4-one,2,3-Dihydro-3,5-dihydroxy-6- methyl-	$C_6H_8O_4$	144.04
8	7.31	0.37	(s) -(-)-4-hydroxy-2-methyl-2-cyclopentenone	$C_6H_8O_2$	112.05
9	8.27	1.10	1,3-cyclohexanedione,2-methyl-	$C_7H_{10}O_2$	126.07
10	9.11	3.59	2-Furancarboylic acid, methyl ester	$C_6H_6O_3$	126.03
11	9.72	0.36	4H-pyran-4-one,3-hydroxy-2-methyl	$C_6H_8O_2$	112.05
12	9.97	0.2	Butanoic acid,3-methyl-,2-methyl propyl ester	$C_9H_{18}O_2$	158.13
13	10.6	5.58	4H-pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6- methyl	$C_6H_8O_4$	144.04
14	11.42	1.09	2-Thiophenacroxylic acid,5-methyl	$C_6H_6O_2S$	142.01
15	11.89	0.93	5-formyl -2-furfuryl methanoate	$C_7H_6O_4$	154.03
16	13.14	39.71	2-Furancarboxaldehyde, 5-(hydroxymethyl)-	$C_6H_6O_3$	126.03
17	15.05	0.27	Cyclopentanone,2-octyl-	$C_{13}H_{24}O$	196.18
18	15.36	0.37	2,6-dimethyl cyclohexanone-5-Acetoxymethyl-2-furaldehyde.	$C_8H_{14}O$	126.10
19	16.15	1.69	1H-Indol-3-acetic acid,2-methyl	$C_{11}H_{11}NO_2$	189.08
20	17.63	5.97	Propanedioic acid ethyl-, diethyl ester	$C_9H_{16}O_4$	188.10
21	18.87	0.51	Cyclohexane,1,2 ,4,5-tetraethyl cyclohexane	$C_{14}H_{28}$	196.22
22	20.07	0.23	Tetradecanoic acid	$C_{14}H_{28}O_2$	228.37
23	21.91	0.10	2-chloro-5-methoxybiphenyl	$C_{13}H_{11}clo$	218.05
24	22.64	0.65	n-Hexadecanoic acid	$C_{16}H_{32}O_2$	256.424
25	23.31	11.77	5,5-oxy-dimethylene-bis (2-furaldehyde)	$C_{12}H_{10}O_5$	234.05
26	24.26	1.64	9-Octadecanoic acid, methyl ester	$C_{19}H_{36}O_2$	296.27
27	24.74	1.50	9-Octadecanoic acid	$C_{10}H_{20}O_2$	172.2464
28	25.48	0.06	3-Hydroxy-3-methoxycarbonyl-pentan EDIOIC acid dimethyl ester	$C_9H_{14}O_7$	234.o7
29	25.94	0.96	2,6-Pyridinediamine	$C_5H_7N_3$	109.06
30	29.58	0.51	2,2,9,9-Tetramthyl dec-5-ene-3,7-diyne	$C_{14}H_2O$	188.16

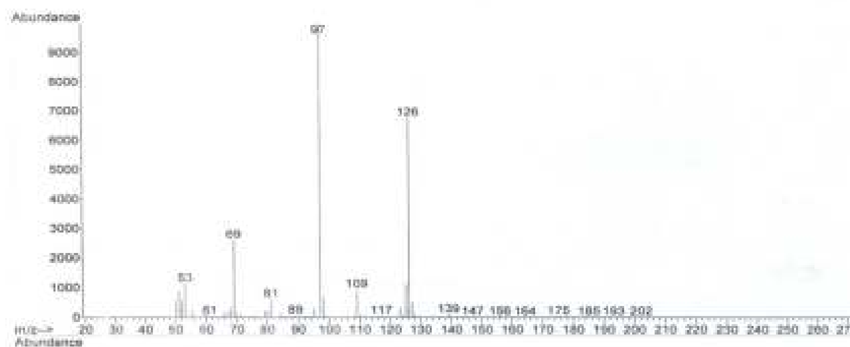


Fig. 2. Mass spectrum of 2-furancarboxaldehyde, 5-(hydroxymethyl).

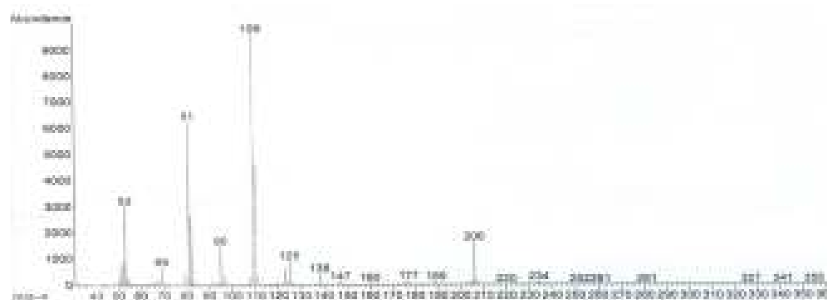


Fig. 3. Mass spectrum of 5,5-oxy-dimethylene-bis (2-furldehyde).

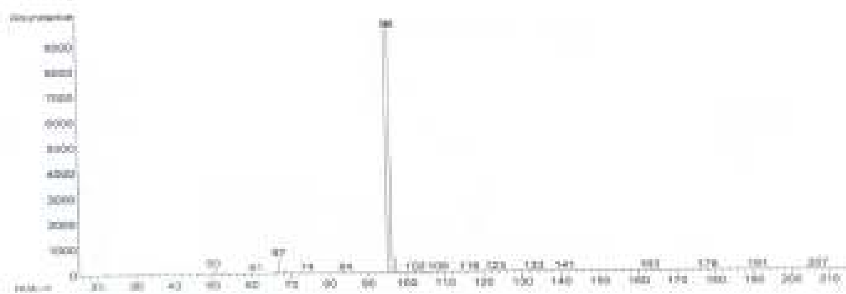


Fig. 4. Mass spectrum of 2-furancarboxaldehyde.

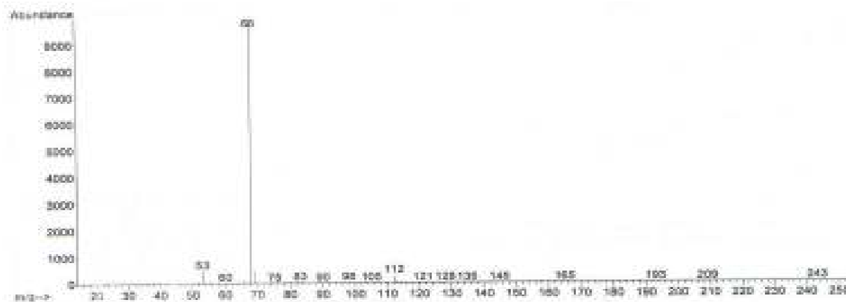


Fig. 5. Mass spectrum of 2, 5-furandione,3-methyl.

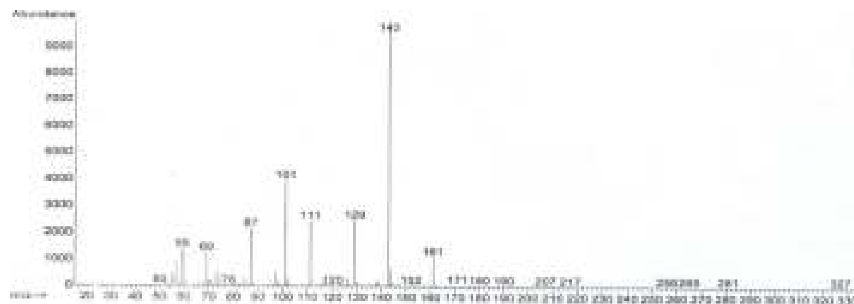


Fig. 6.Mass spectrum of propanedioic acid ethyl-, diethyl ester.

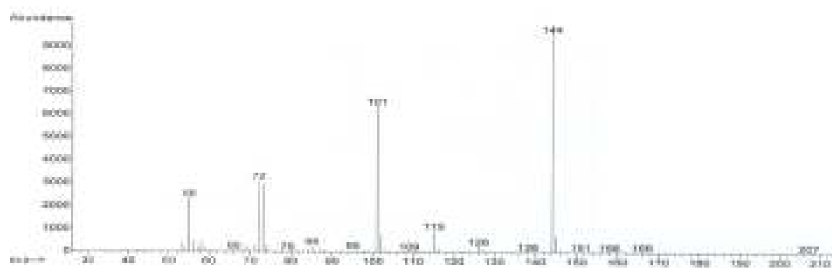


Fig. 7. Mass spectrum of 4H-pyran-4-one,3-hydroxy-2-methyl

DISCUSSION

In vitro obtained results indicated that pomegranate peel extract exhibited inhibitory effect on the linear growth of six phytopathogenic fungi isolated from different hosts, with minor variation among them. Furthermore, the tested extract effectively decrease the linear growth and spore germination of *F. oxysporum* (The causal of tomato damping off disease) and the maximum reduction was recorded at 4000 ppm. The presence of some

phytocompounds in the pomegranate peel extract may be responsible for the inhibitory effect (Dahham *et al*., 2010). The antifungal activity of pomegranate extract against some phytopathogenic fungi have been previously reported by Dahham *et al*., 2010) who tested different pomegranate extracts on linear growth of different fungi. They found that the highest antifungal activity was recorded on *Aspergillus niger* followed by *Penicillum citrinum* and *Rhizopus oryzae* respectively.

Similarly, pomegranate extract reduced linear growth of *Alternaria alternata*, *F. oxysporum*, *Phoma destructive*, *R. solani* and *Sclerotium rolfsii* with different degrees of activity against the tested fungi (Al-Asker, 2012 and Mangang and Chhetry, 2012).

Under greenhouse conditions, application of pomegranate peel powder as seed treatment or soil application before sowing and transplanting tomato transplants in soil infested with *F. oxysporum. F. oxysporum* effectively decreased pre and post emergence damping-off compared to untreated infected control. At the same time, seedling treatment or soil treatment with pomegranate peel extract before sowing in soil infested with *F. oxysporum* provided good protection against disease incidence. Soil treatment was more effective than seedling treatment. Obtained results are in agreement with those reported by Satish *et al.,* (2007), who found antifungal activity against seed-borne pathogens of *Aspergillus* spp as the result of application pomegranate peel extract. Also, pronounced decrease was found in citrus green mould disease (*Penicillium digitatum*) as a result of application of pomegranate peel extract Tayel *et al*., 2009. In another study, soil treatment with pomegranate leaf extract before sowing, effectively reduced root rot disease of French bean caused by *Rhizoctonia solani*, under greenhouse and field conditions.

Phytochemical analysis of PPE revealed that the extract containing different compounds including three flavonoids, four phenolic compounds, three tannins and one glycosides. These compounds were reported to have antimicrobial activities.

Several phenolic compounds are directly antifungal *in vitro.* For example, phenolic compounds extracted from olive plants showed antifungal activity against *Phytophthora* spp. *in vitro*. Also 4-O-glucoside and 7-O-glucoside completely inhibited conidial germination of *Nurospora crassa* (Lattanzio *et al.,* 2006). Furthermore, phenolic compounds might have a major role to play in disease resistance through the inactivation of fungal cell wall degrading enzymes (cellulose and pectin methyl esterase), thereby restricting the degradation of the cell wall and the process of fungal invation in plant (Mandavia *et al.,* 1999).

As for tannins, *In vitro* toxicity of tannins is well documented for several fungi including *B. cinerea, A. niger, C. graminicola* and *Penicillum* sp (Lattanzio *et al.,* 2006). Furthermore, tannic acid exhibited antimicrobial activity against *A. niger, A. fumigatus, A. flavus, P. granulatum* and *P. granulosum* at concentration 3%. This indicating the methanolic extract of pomegranate peel is good source of secondary metabolites having an important role.

The present work refers to the possible use of agriculture waste as antimicrobial agents against phytopathogeny to overcome pesticide pollution and maintaining environment safety.

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تأثير قشر الرمان على بعض الفطريات الممرضة للنبات و مقاومة مرض سقوط بادرات الطماطم

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فى هذا البحث تم أستخدام قشر الرمان فى صورة مسحوق أو مستخلص كحولى لدراسة مدى الكفاءة ضد مرض سقوط بادرات الطماطم المتسبب عن الفطر فيوزاريوم اوكسسبورم فورما سبيشيالس ليكوبرسيسى, تحت ظروف المعمل و قد أدى أستخدام مستخلص قشر الرمان الى تثبيط النمو الطولى لبعض الفطريات الممرضة للنبات والمعزولة من عوائل مختلفة وتشمل :

Botrytis cinerea, Colletotrichum dematium, Fusarium oxysporum, Fusarium solani, Phoma spp and *Rhizoctonia solani.*

كما أدى استخدام مستخلص قشر الرمان أيضا الى تثبيط نمو الفطر فيوزاريوم اوكسسبورم فورما سبيشيالس ليكوبرسيسى وكذلك تثبيط انبات جراثيم الفطر. ووجد أن أعلى تثبيط النمو الفطرى كان عند تركيز ٤٠٠٠ جزء فى المليون.

تحت ظروف الصوبة وجد أن معاملة بذور الطماطم او البادرات او التربة قبل الزراعة بمسحوق قشر الرمان ادى الى انخفاض نسبة موت البادرات مقارنة بالكنترول الغير معامل. و كانت معاملة التربة بمستخلص قشر الرمان أفضل من معاملة البذرة.

و لقد أثبت التحليل الكيماوى (باستخدام الغاز اللونى الطيفى الكتلى(GC-MS) لمستخلص قشر الرمان الى احتواء المستخلص على العديد من المركبات ذات التأثير المثبط لنمو المسببات المرضية و تشمل مركبات فينولية و فلافونيد و تانينات وجليكوسيدات و ستيرولات.