*Egypt. J. Phytopathol.,* Vol. **42**, No. 2, pp. 171-186 (2014)

# Effect of Pomegranate (*Punica granatum L.*) Fruits Peel on some Phytopathogenic Fungi and Control of Tomato Damping-off

**Tahany G.M. Mohamad* and Amal A. Khalil****

* *Central Agricultural Pesticides Laboratory, Agric. Res. Centre, Giza, Egypt.*

** *Plant Pathol. Res. Inst., Agric. Res. Centre, Giza, Egypt.*

**Pomegranate** peel powder and its extract were used to study their efficiency against some phytopathogenic fungi and tomato damping-off caused by *Fusarium oxysporum*. *In vitro*, methanolic extract of pomegranate peel caused inhibitory effect to the linear growth of different phytopathogenic fungi, *i.e. Botrytis cinerea, Colletotrichum dematium, Fusarium oxysporum, F. solani, Phoma* sp. and *Rhizoctonia solani,* which isolated from different hosts. Also, pomegranate peel extract (PPME) effectively decreased the linear growth and spore germination of *F. oxysporum,* when the greatest decrease was recorded at 4000 ppm. Under greenhouse conditions, application of pomegranate peel powder as seed treatment or soil drench decreased pre- and post-emergence damping-off of tomato seedlings caused by *F. oxysporum* compared with the untreated check. Treating tomato seedlings or soil with peel extract before sowing provided a good protection against damping-off. In this concern, soil drench was more effective than seedling treatment. Analysis of methanolic extract of pomegranate peel revealed that it contained different compounds of antifungal compounds including three flavonoids, three tannins, four phenolic compounds, one glycosides, along with four sterols.

**Keywords:** Flavonoids, *Fusarium oxysporum,* gas chromatography, glycosides, phenolic compounds, sterols and tannins.

Tomato (*Lycopersicon esculentum* Mill) is one of the most economic vegetable crops cultivated at different localities in Egypt and all over the world for either local consumption or exportation purposes (Tampoare *et al.*, 2013). It is subjected to attack by many soilborne fungal diseases specially damping-off and wilt diseases cause considerable losses either in the nurseries or in the field (Abdel-Kader *et al.*, 2012). Farmers usually apply synthetic fungicides as preventive and therapeutic measures to check 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 Hollomon, 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 *in vitro* and *in vivo* antimicrobial activity against many pathogenic fungi and bacteria (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.

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 *Fusarium oxysporum*, to check tomato damping-off. Also, phytochemical analysis of pomegranate extract using gas chromatography mass spectrometric (GC-MS) technique was carried out.

## Materials and Methods

*Phytopathogenic fungi:*

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

*Preparation of pomegranate peel methanolic extract (PPME):*

Fresh fruits of pomegranate (*Punica granatum* L.) were manually peeled. Collected peels were then rinsed with distilled water and dried in an oven by hot air at 50°C for 48h and then 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 Whattman No.1 filter paper. The extract was concentrated at 40°C using rotary evaporator (Heidolph, LABOROTA A 4000-Germany) and then dried in an oven at 50°C for 48h. Finally, using the dried extract, different concentrations of PPME, *i.e.* 31.3, 62.5,125, 250, 500, 1000, 2000 and 4000 ppm, were prepared (Tayel *et al.*, 2009).

*Effect of (PPME) on the linear growth of some pathogenic fungi:*

The anti-fungal activity of PPME was *in vitro* studied using the poisoned food technique (Singh *et al.*, 2008). PDA medium was autoclaved and amended with the abovementioned PPME concentrations after reasonable cooling to 35-45°C then vigorously shake along with one ml of streptomycin before pouring into Petri dishes. Dishes contained PDA medium only were used as check. Discs (4-mm-diam.), taken from 1-week-old of each of the six tested fungal species cultures, were aseptically transferred into the centre of the prepared dishes and then sealed with parafilm before incubation at 25±2°C until the fungal growth in the check treatment reached the edges of the dishes (Agarwal *et al*., 2001). Three dishes were used as replicates for each treatment.

*Effect of (PPME) on the linear growth of F. oxysporum:*

One ml of each PPME concentration, *i.e.* 250, 500, 1000, 2000 and 4000 ppm, was added to PDA medium and then poured in Petri dishes as mentioned before. Plates containing only PDA medium were used as check treatment. Each plate was

inoculated with a disc of 8-day-old culture of *F. oxysporum* and then the plates were incubated at 25±2°C until the fungal growth covered the whole surface in the check plates, when the linear growth in different treatments was determined. Three replicates were used for each concentration. Fungal growth inhibition (%) was calculated using the following formula:

$$\text{Fungal growth inhibition (\%)} = \frac{C - T}{C} \times 100$$

Whereas: C= Fungal growth in the check and T= Fungal growth in the treatment.

*Effect of PPME on F. oxysporum spore germination:*

Different concentrations of PPME (250, 500, 1000, 2000 and 4000 ppm) were tested. One drop from the spore suspension of *F. oxysporum* was added to each of the aforementioned concentration and dropped by sterilized pipette on glass slides. Other glass slides, with spore suspension drop, were prepared using distilled water as check treatment. All slides were incubated at 25±2°C for 18 h. Three replicates were used for each concentration. Spore germination (%) was recorded using microscopic examination and inhibition (%) of germination was calculated.

*Assessing of $EC_{50}$:*

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

*Greenhouse experiments:*

These experiments were carried out using *F. oxysporum* only.

*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-diam.) of 8-day-old culture of *F. oxysporum* and incubated at 25±2°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 drench with pomegranate peel powder on the incidence of pre- and post-emergence damping-off caused by F. oxysporum:*

*a) Seed treatment:*

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

*b) Soil drench:*

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 treatment at the rate of 6 seedlings/pot. Four replicates were transplanted for each treatment. The growing seedlings in all treatments were examined periodically. Pre- and post-emergence damping-off were recorded 15 and 30 days after transplanting.

*Effect of tomato seedlings treatment and soil drench with pomegranate peel extract on damping-off incidence:*

*a) Seedling treatment:*

Four-week-old tomato seedlings were dipped in PPME (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 check 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.

*b) Soil drench:*

Pots (30-cm-diam.) containing soil infested with 4% inoculum level of *F. oxysporum* were treated with pomegranate peel extract (10 ml/ pot), transplanting was made with uninoculated 4-week-old tomato seedlings. Six transplants were transplanted in each pot and four replicates were used. The plants in all treatments were examined periodically and the root rot incidence 60 days after sowing was recorded.

*Phytochemical analysis of PPME:*

The preliminary qualitative phytochemical analysis for the presence of various phytochemical compounds was performed using the methanolic extract. Presence of carbohydrates was determined by Molish's test (Sadasivam and Balasubramanian, 1985). Presence of reducing sugars 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 was confirmed by ferric chloride and gelatine tests, respectively, (Mace, 1963). The presence of flavonoids was investigated by lead acetate test (Kosalec *et al*., 2005).

*GC-MS Analysis of PPME:*

The qualitative and quantitative compositions of the 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 spectrophotometric 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 Song and Keane (2006).

## Results

*Antifungal potential of PPME:*

The preliminary determination of the *in vitro* antifungal activity by using the poisoned food technique of the methanolic extract of pomegranate peel was studied against the six phytopathogenic fungi, *i.e. Botrytis cinerea, Fusarium oxysporum, F. solani*, *Phoma* spp., *Colletotrichum dematium* and *Rhizoctonia solani*.

Results presented in Table (1) reveal variation in the antifungal activity of the methanolic extract, that extract displayed high inhibitory effect on the growth of *B. cinerea, C. dematium, F. oxysporum, F. solani, Phoma* sp. and *R. Solani* at concentrations ranging from 1000 to 4000 ppm.

**Table 1. Screening inhibitory potential of PPME at different concentrations on linear growth of six pathogenic fungi**

| Concentration (ppm ) | Fungal growth inhibition (%) | | | | | |
|---|---|---|---|---|---|---|
| | *B. cinerea* | *C. dematium* | *F. oxysporum* | *F. solani* | *Phoma* spp. | *R. solani* |
| Check | 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 |

The *in vitro* antifungal activity of PPME expressed as a minimum effective concentration ($EC_{50}$) of 50% of mycelial growth with the corresponding 95%. Confidence limits are shown in Table (2). It is clear that the antifungal activity was increased with increasing the concentrations of the extract. *F. solani* and *C. dematium* showed greater sensitivity to PPME than *Phoma* sp., *R. solani*, *B. cinerea* and *F. oxysporum*. The $EC_{50}$ of the tested phytopathogenic fungi was 266.4, 272.5, 305.6, 307.9, 318.4 and 787.4 ppm, respectively.

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

| Tested fungus | $EC_{50}$ | 95% Confidence limit | |
|---|---|---|---|
| | | Lower | Upper |
| *B. cinerea* | 318.4 | 240.4 | 419.4 |
| *C. dematium* | 272.5 | 207.6 | 353.3 |
| *F. oxysporum* | 787.4 | 340.7 | 2998.4 |
| *F. solani* | 266.4 | 194.2 | 361.3 |
| *Phoma* sp. | 305.6 | 186.1 | 491.8 |
| *R. solani* | 307.9 | 281.3 | 336.7 |

*Effect of different concentrations of PPME on the linear growth and spore germination of F. oxysporum:*

Different concentrations of PPME were evaluated for their effects on the linear growth and spore germination of *F. oxysporum*. Data in Table (3) show that PPME significantly decreased the linear growth and spore germination of *F. oxysporum* compared to untreated check. Increasing the concentration was associated with an additional decrease with maximum records for linear growth and spore germination inhibition(s) at 4000 ppm, being 76.7 and 82.7%, respectively.

**Table 3. Effect of different concentrations of PPME on the linear growth and spore germination of *F. oxysporum***

| 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 treatment and soil drench with peel powder on pre- and post-emergence damping-off caused by F. oxysporum:*

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 highest efficiency, being 4.2 and 92.3%, respectively. Soil drench with pomegranate peel powder caused more reduction than seed treatment. The percentages of damping-off reached 76.9 and 46.1%, respectively.

*Effect of seedling treatment and soil drench with pomegranate peel extract before sowing on incidence of tomato root rot:*

Data presented in Table (5) indicate that the used treatments significantly decreased disease incidence compared to the check. Fungicide treatment recorded the lowest (4.2%) value, followed by soil drench (8.3%), whereas, seedling treatment recorded the lowest effect (20.8%). However, the variations among the three treatments were in significant.

**Table 4. Effect of tomato seeds treatment and soil drench with peel powder on pre- and post-emergence damping-off caused by *F. oxysporum***

| Treatment | Damping-off (%) | | Total (%) | Efficiency (%) |
|---|---|---|---|---|
| | Pre-emergence | Post-emergence | | |
| Seed treatment | 12.5a * | 16.7ab | 29.2 | 46.1 |
| Soil drench | 8.3a | 4.2b | 12.5 | 76.9 |
| Fungicide (Flowsan) | 0.0a | 4.2b | 4.2 | 92.3 |
| Check | 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).

**Table 5. Effect of seedling treatment and soil drench with pomegranate peel extract before sowing on incidence of tomato root rot**

| Treatment | Disease incidence (%) | Efficiency (%) |
|---|---|---|
| Seedling treatment | 20.8b* | 58.3 |
| Soil drench | 8.3b | 83.3 |
| Fungicide (Rizolex T) | 4.2b | 91.6 |
| Check | 50.0a | - |

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

*Preliminary phytochemical determination of PPME:*

The preliminary qualitative phytochemical analysis of PPME carried out in (Table 6A). 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.

**Table 6A. Determination of some phytochemical groups in PPME 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 | + |
| Gelatine test | Tannins | + |
| Lead acetate test | Flavonoids | + |

(+) Present ; (-) Absent

*GC-MS analysis of PPME:*

Qualitative analysis of PPME using GC-MS analysis which led to identify number of compounds from the GC of methanol extract of pomegranate peel (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 (6B). 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, being 11.77, 9.38, 6.66, 5.97 and 5.58 %, respectively. Figs. 2 to7 show the mass spectrum of the most abundant components of methanol extract of pomegranate peel.

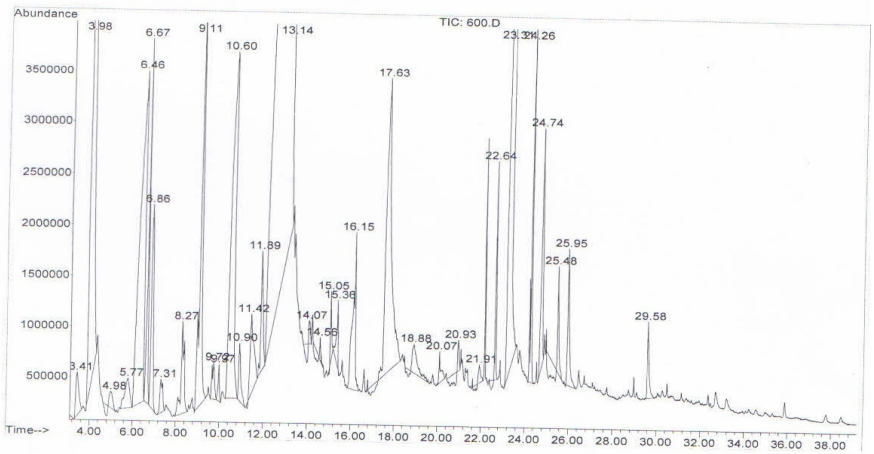


**Fig. 1. The chromatogram of all compounds produced from GC-MS analysis of methanolic extract of pomegranate peel.**

Data presented in Tables (6 A , B) and Figs. (1-7) reveal that the methanolic extract of pomegranate peel contained many different compounds which have antifungal activities. These compounds include 5,5-oxy-dimethylene-bis (2-furaldehyde), 9-Octadecanoic acid, and 2H-Pyran,3,4-dihydro which belong to the flavonids group, with average rate (13.87%). 2-Furancarboylic acid, methyl ester, 2-Thiophenacroxylic acid, 5-methyl, and 2-6-Pyridinediamine which belong to the 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 belong to the phenolic group, with average rate (4.52%). 1,3-cyclohexanedione, 2-methyl which belong to the 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 which belong to the Sterols group, with average rate of 19.85%.

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

| Peak | Chemical group | Rate | Compound name | Molecular formula |
|---|---|---|---|---|
| 25 | Flavonids | 11.77 | 5,5-oxydimethylene-bis (2-furaldehyde) | $C_{12}H_{10}O_5$ |
| 27 | Flavonids | 1.5 | 9-Octadecanoic acid | $C_{10}H_2O_2$ |
| 4 | Flavonids | 0.6 | 2 H- Pyran,3,4-dihydro | $C_4H_4O_2$ |
| 10 | Tannins | 3.59 | 2-Furancarboylic acid, methyl | $C_6H_6O_3$ |
| 14 | Tannins | `1.09 | 2-Thiophenacroxylic acid, 5-methyl | $C_6H_6O_2$ |
| 29 | Tannins | 0.96 | 2-6-Pyridinediamine | $C_7H_6O_4$ |
| 7 | Phenolic compound | 1.65 | 1H-Pyran-4-one,2,3-Dihydro3-5-dihydroxy-6-methyl | $C_6H_8O_4$ |
| 19 | Phenolic compound | 1.69 | 1H-Indol-3-acetic acid, 2-methyl | $C_{11}H_{11}NO_2$ |
| 24 | Phenolic compound | 0.65 | n-Hexadecanonic acid | $C_{16}H_{32}O_2$ |
| 1 | Phenolic compound | 0.53 | 3-Amino-S-triazole | $C_2H_4N_4$ |
| 9 | Glycosides | 1.1 | 1,3-cyclohexanedione,2-methyl | $C_7H_{10}O_2$ |
| 5 | Sterols | 6.66 | 2,5-Furandione,3-methyl | $C_6H_4O_3$ |
| 20 | Sterols | 5.97 | Propanedioic acid ethyl-,diethyl ester | $C_6H_{16}O_4$ |
| 13 | Sterols | 5.58 | 4H-pyran-4-1,2,3-dihydroxy-6-methyl | $C_6H_8O_4$ |
| 26 | Sterols | 1.64 | 9-octadecanoic acid, methyl ester | $C_{19}H_{36}O_2$ |

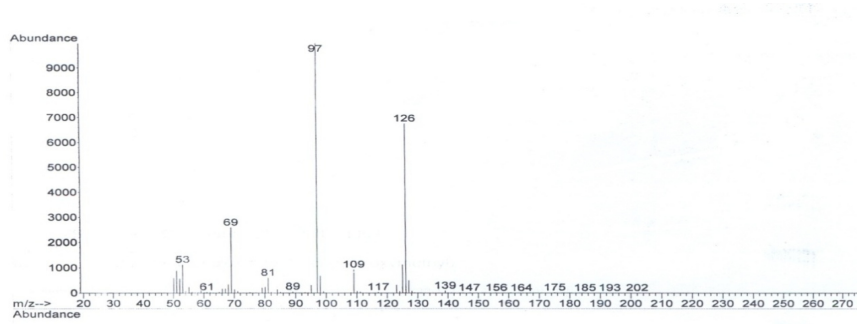


**Fig. 2. Mass spectrum of 2-furancarboxaldehyde, 5-(hydroxymethyl).**

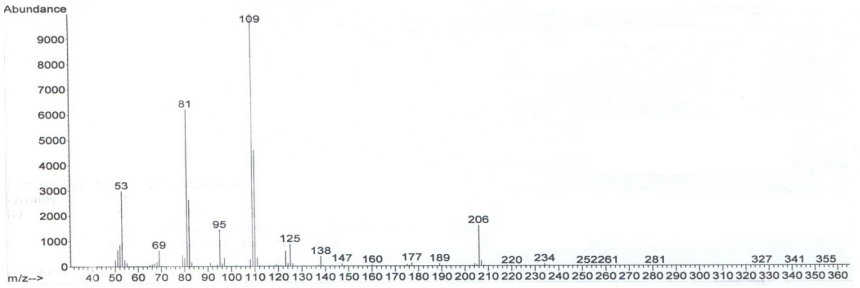


**Fig. 3. Mass spectrum of 5,5-oxy-dimethylene-bis (2-furldehyde).**

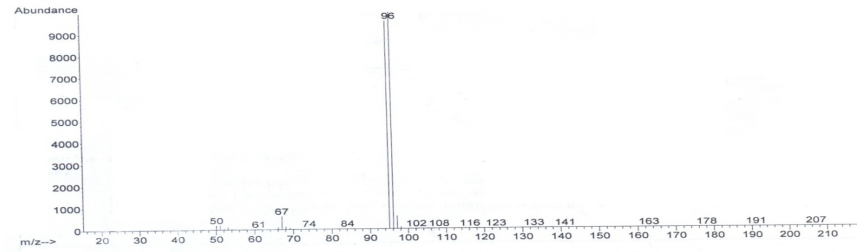


**Fig. 4. Mass spectrum of 2-furancarboxaldehyde.**

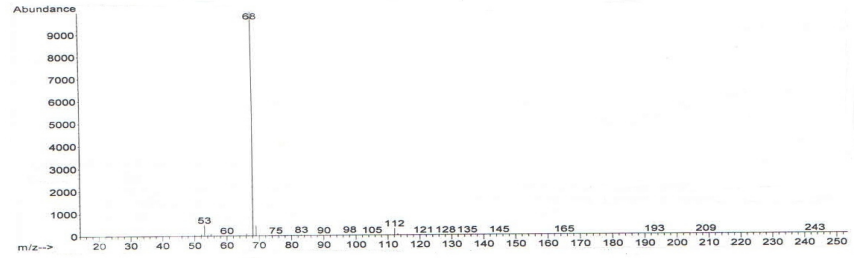


**Fig. 5. Mass spectrum of 2, 5-furandione,3-methyl.**

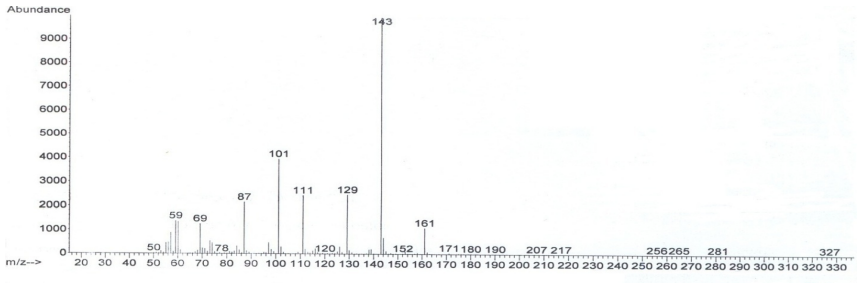


**Fig. 6. Mass spectrum of propanedioic acid ethyl-, diethyl ester.**

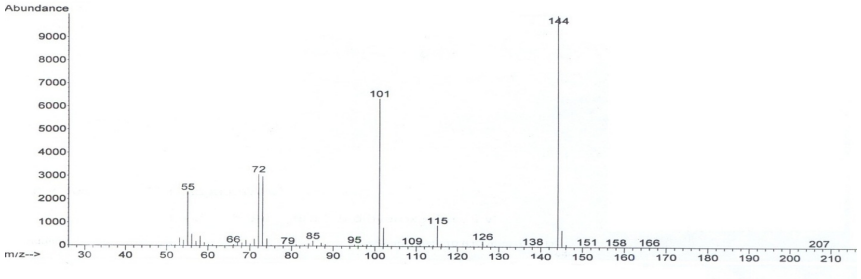


**Fig. 7. Mass spectrum of 4H-pyran-4-one, 3-hydroxy-2-methyl.**

## Discussion

In recent years, the use of synthetic pesticides in plant diseases protection programs around the world has resulted in disturbances of the environment, pest resurgence and plant resistance to pesticides and lethal effect to non-target organisms in the agro-ecosystems in addition to direct toxicity to the users (Prakash *et al*., 2008; Asogwa *et al*., 2010; Bajpai *et al*., 2010). Therefore, considering the deleterious effects of synthetic pesticides on life supporting systems, there is an urgent need to search for alternative approaches for the management of plant pathogenic microorganisms. The uses of some natural compounds such as essential oils and plant extracts for biological check of pathogens have been noticed. Several studies on the fungi-toxic activities of plant secondary metabolites have been reported (Ojala *et al*., 2000; Kordali *et al*., 2003; Field *et al*., 2006 and Lee, 2007).

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 extracts effectively decreased the linear growth and spore germination of *Fusarium oxysporum* (the causal of tomato root rot disease) and the maximum reduction was recorded at 4000 ppm. The presence of some phyto-compounds 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 has 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 *Penicillium citrinum* and *Rhizopus oryzae*, respectively.

Results of the present investigation showed that, pomegranate extract reduced the linear growth of *Alternaria alternata*, *F. oxysporum*, *Phoma destructive*, *R. solani* and *Sclerotium rolfsii* with different degrees of activity against the tested fungi. Similar results were recorded by Al-Askar (2012) and Mangang and Chhetry (2012).

Under greenhouse conditions, application of pomegranate peel powder as seed treatment or soil drench before sowing tomato seeds and transplanting tomato transplants in soil infested with *F. oxysporum*, effectively decreased the incidence of pre- and post-emergence damping-off compared to untreated infected check. At the same time, seedling treatment or soil drench with pomegranate peel extract before sowing in soil infested with *F. oxysporum* provided good protection against root rot incidence. Soil drench 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, *i.e. Aspergillus* spp., due to application of pomegranate peel extract. Also, pronounced decrease was found in citrus green mould disease (*Penicillium digitatum*) as a result of application with pomegranate peel extract (Tayel *et al*., 2009).

Phytochemical analysis of PPME revealed that the extract contained different compounds including three flavonoids, four phenolic compounds, three tannins and one glycosides. Similar findings were also reported by many researchers

(Hegde *et al*., 2012; Chebaibi and Filali 2013 and Uma *et al*., 2013). Moreover, these compounds were reported to have antimicrobial activities (Kannaiyan *et al*., 2013).

Several phenolic compounds have *in vitro* antifungal activities. For example, phenolic compounds extracted from olive plants showed *in vitro* antifungal activity against *Phytophthora* spp. Also, 4-O-glucoside and 7-O-glucoside completely inhibited conidial germination of *Neurospora 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 invasion in plant (Mandavia *et al.,* 1999).

Flavonoids are a group of naturally occurring agents and have been shown to possess good antimicrobial activity. Maussaoui *et al*. (2010) indicated that flavonoid compounds isolated from the aerial parts of *Launaea resedifolia* have antimicrobial activity against *Streptococcus* sp., *Enterobacter* sp. and *Candida albicans*. Whereas, Jayshree *et al*. (2012) tested three different flavonoid compounds (dimethoxy flavones 3,6- dimethoxy flavone, 6,2-dimethoxy flavone and 6,3- dimethoxy flavone) against some fungi. They found that all the three compounds showed *in vitro* excellent antifungal activity against *Aspergillus flavus, A. fumigatus, A. niger, Rhizopus* sp. and *Candida albicans.*

The *in vitro* toxicity of tannins was documented against several fungi including *Botrytis cinerea, Aspergillus niger, Colletotrichum graminicola* and *Penicillium* sp (Lattanzio *et al.,* 2006). Furthermore, tannic acid exhibited antimicrobial activity against *Aspergillus niger, A. fumigatus, A. flavus, Penicillium granulatum* and *P. granulosum* at concentration 3%. This indicating that our methanolic extract of pomegranate peel is good source of secondary metabolites having an important role.

The present work refers to the possible use of agro-industrial plant wastes as antimicrobial agents against phytopathogenic fungi to overcome pesticide pollution and maintaining the environmental safety.

## Acknowledgement

The researchers express their thankful to Dr. Islam. N. Nasr (Central Agricultural Pesticides Laboratory) for helping in (GC-MS) analysis.

## References

Abdel-Kader, M.M.; El-Mougy, Nehal S.; Aly, M.D.E. and Lashin, S.M. 2012. Integration of biological and fungicidal alternatives for checking foliar diseases of vegetables under greenhouse conditions. *Int. J. Agric. forestry,* **2**(2): 38-48.

Agarwal, M.; Walia, S.; Dhingra, S. and Khambay, B.P.S. 2001. Insect growth inhibition, antifeedant and antifungal activity of compounds isolated/derived from *Zingiber officinale* Roscoe (ginger) rhizomes. *Pest Manage. Sci*., **57:** 289-300.

Al-Askar, A.A. 2012. *In vitro* antifungal activity of three Saudi plant extracts against some phytopathogenic fungi. *J. Plant Prot. Res.*, **52**(4): 458-462.

Asogwa, E.U.; Ndubuaku, T.C.N.; Ugwu, J.A. and Awe, O.O. 2010. Prospects of botanical pesticides from neem, *Azadirachta indica* for routine protection of cocoa farms against the brown cocoa mirid Sahlbergella singularis in Nigeria. *J. Med. Plant Res*., **4**: 1-6.

Bajpai, V.K.; Cho, M.J. and Kang, S.C. 2010. Check of plant pathogenic bacteria of Xan-thomonas spp. by the essential oil and extracts of *Metasequoia glyptostroboides* Miki ex Hu *in vitro* and *in vivo*. *J. Phytopathol*., **158:** 479-486.

Barnard, M.; Padgitt, M. and Uri, N.D. 1997. Pesticide use and its measurement. *Int. Pest Check*, **39**: 161-164.

Brent, K.J. and Hollomon, D.W. 1998. Fungicide Resistance: The assessment of risk. Monograph No. 2. FRAC, Global Crop Protection Federation, Brussels, Belgium, pp. 1-48.

Chebaibi, Amina and Filali, F.R. 2013. Bacterial activity and *Punica granatum* leave extracts. *J. Medicinal Plants Res.*, **7**(14): 887-891.

Dahham, S.S.; Ali, M.N.; Tabassum, H. and Khan, M. 2010. Studies on antibacterial and antifungal activity of Pomegranate (*Punica granatum* L.) *American-Eurasian J. Agric. & Environ. Sci*., **9**(3): 273-281.

Evans, W.C. 1997. An index of medicinal plants. *A Textbook of Pharmacognosy*. Vol. 7(5), 14th Ed., pp 12-14.

Field, B.; Jorden, F. and Osbourn, A. 2006. First encounters-deployment of defence related natural products by plants. *New Phytol*., **172:** 193-207.

Gomez, K.A. and Gomez, A.A. 1984. *Statistical Procedures for Agricultural Research*. John Wiley and Sons, NY, USA. 680pp.

Hegde, C.R.; Madhuri, M.; Swaroop, T.; Nishitha, Das; Arijit1 Bhattacharya Sourav and Rohit, K.C. 2012. Evaluation of antimicrobial properties, phytochemical contents and antioxidant capacities of leaf extracts of *Punica granatum* L. ISCA. *J. Biol. Sci*., **1**(2): 32-37.

Ishii, H. 2006. Impact of fungicide resistance in plant pathogens on crop disease check and agricultural environment. *Japan Agric. Res. Q*. 40: 205-211.

Jayshree, N.; Narayanan, N. and Sriram, L. 2012. Antibacterial, Antifungal and Antimycobacterial studies on some synthetic dimethoxy flavones. *Asian J. Pharm. Clin. Res.*, **5**(1): 101:103.

Kannaiyan, M.; Thambidurai, P.; Raja, V.; Belli T.S.; Ponnusamy, V. and Nooruddin, T. 2013. Antimicrobial activity and qualitative phytochemical analysis of *Punica granatum* Linn. *J. Medicinal Plants Res*., **7**(9): 474-479.

Kokate, C.K. 1999. *Practical Pharmacognosy*. 4th Ed., Vallabh Prakashan Publication, New Delhi, India.

Kordali, S.; Cakir, A.; Zengin, H. and Duru, M.E. 2003. Antifungal activities of the leaves of three Pistacia species grown in Turkey. *Fitoterapia*, **74:** 164-167.

Kosalec, I.; Pepeljnjak, S.; Bakmaz, M. and knezevic S.V. 2005. Flavonoid analysis and antimicrobial activity of commercially available propolis products. *Acta Pharma*, **55**: 423-430.

Lattanzio, V.; Veronica, M.; Lattenzio, T. and Cardinali, A. 2006. Role of phenolic in the resistance mechanisms of plant against fungal pathogens and insects. Phytochemistry. *Advances in Res*., **2006**: 23-67.

Lee, H.S. 2007. Fungicidal property of active component derived from *Acorus gramineus* rhizome against phytopathogenic fungi. *Bioresour. Technol*., **98:** 1324-1328.

Mace, M.E. 1963. Histochemical localization of phenols in healthy and diseased tomato roots, *Phytochem*., **16**: 915-925.

Mandavia, M. K.; Gajera, H.P; Andharia, J.H; Khanda, R.R and Parameswaran, M. 1999. Cell wall degrading enzymes in host pathogen interaction of fusarium wilt of chickpea: Inhibitory effect of phenolic compound. *Indian Phytopathol.*, **52**(3): 285-288.

Mangang, H.C and Chhetry, G.K.N. 2012. Antifungal properties of certain plant extracts against *Rhizoctonia solani* causing root rot of French bean in organic soil of Manipur. *Int. J. Sci. Res. Publ.*, **2** (5): 1-4.

Maussaoui, F.; Zellagui, A.; Segueni, N and Touil, A. 2010. Flavonoid constituents from Algerian *Launaea resedifolia* (O.K.) and their antimicrobial activity. *Rec. Nat. Prod*., **4**(1): 91-95.

Ojala, T.; Remes, S.; Haansuu, P.; Vuorela, H.; Hiltunen, R.; Haahtela, K. and Vuorela, P. 2000. Antimicrobial activity of some coumarin containing herbal plants growing in Finland. *J. Ethnopharmacol*., **73:** 299-305.

Prakash, A.; Rao, J. and Nandagopal, V. 2008. Future of botanical pesticides in rice, wheat, pulses and vegetables pest management. *J. Biopest.*, **1**: 154-169.

Ramkrishnan S. and Rajan R. 1994. *Text Book of Medical Biochemistry.* Orient Longman, New Delhi, India, 715 pp.

Sadasivam, S. and Balasubramanian, T. 1985. Practical Manual (Undergraduate), Tamil Nadu Agricultural University, Coimbatore, 2 pp.

Sadik, M.S and Asker, M.S. 2014. Antioxidant and antitumor activities of pomegranate (*Punica granatum*) peel extracts. *World J. Pharmaceutical Sci.*, **2**(11): 1441-1445.

Satish, S.; Mohanan, D.C.; Raghavendra, M.P. and Ravessha, K.A. 2007. Antifungal activity of some plant extracts against important seed borne pathogens of *Aspergillus* sp. *J. Agric. Technol.*, **3**(1): 109-119.

Singh, P.; Srivastava, B.; Kumar, A.; Kumar, R.; Dubey, N.K. and Gupta, R. 2008. Assessment of *Pelargonium graveolens* oil as plant-based antimicrobial and aflatoxin suppressor in food preservation. *J. Sci. Food & Agric.*, **88**: 2421-2425.

Song, W. and Keane, A.J. 2006. Parameter screening using impact factors and surrogate- based ANOVA techniques, AIAA-2006-7088. 11th AIAA/ISSMO Multidisciplinary Analysis and Optimization Conference, Renaissance Portsmouth, Portsmouth, Virginia, USA.

Steffens, J.J.; Pell, E.J. and Tien, M. 1996. Mechanisms of fungicide resistance in phytopathogenic fungi. *Curr. Opin. Biotechnol*., **7**: 348-355.

Tampoare, G.B.; Adazabra, A.N. and Miller, G.K. 2013. Analysing the economic benefit of fresh tomato production at the Tono irrigation scheme in upper east region of Ghana. *Elixir Agric.*, **58**: 14613-14617.

Tayel, A.A.; Al-Baz A.F.; Salem, M.F. and El-Hadary, M.H. 2009. Potential application of pomegranate peel extracts for the check of citrus green mould. *J. Plant Dis. Prot.*, **116**(6): 252-256.

Uma, M.R.; Manasa, N.; Chinna, E.M. and Sindhura, S. 2013. Comparative evaluation of antimicrobial activity of *Punica Granatum* leaves extracts. *Internat. J. Pharmacy Practice & Drug Res.*, **2**(2): 98-104.

*(Received 12/10/2014;*
*in revised form 25/11/2014)*

# تأثير متبقيات قشور ثمار الرمان على بعض الفطريات الممرضة للنبات ومقاومة سقوط البادرات فى الطماطم

تهانى جابر محمدين* ، أمل عبد الوهاب خليل**
* المعمل المركزى للمبيدات - قسم بحوث مستحضرات المبيدات - مركز البحوث الزراعية - الجيزة.
** معهد بحوث أمراض النباتات - مركز البحوث الزراعية - الجيزة.

فى هذا البحث أستخدم قشر الرمان فى صورة مسحوق أو مستخلص كحولى لدراسة مدى كفاءته ضد مرض سقوط بادرات الطماطم المتسبب عن الفطر فيوزاريوم اوكسسبورم ، تحت ظروف المعمل 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 وكذلك تثبيط انبات جراثيم الفطر. وجد أن أعلى تثبيط 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 بالمستخلص 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 وفلافونيد وتانينات وجليكوسيدات وستيرولات.