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In vitro Evaluation of Insecticidal and Antifungal potencies of fruit peel extracts of pomegranate (*Punica granatum*)

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ABSTRACT

Different fruit peel extracts of Punica granatum were tested in vitro for their insecticidal and antifungal activities against two pests (Myzus persicae and Phthorimaea operculella) and four fungi (Botrytis cinerea, Fusarium sambucinum, Penicillium digitatum and Aspergillus niger). In fact, significant mortalities were recorded on adults treated with aqueous, ethanol and methanol extracts with mortalities of 29%, 53% and 55%, respectively. For potato tuber moth, similar effects were observed on the first larval penetration of Phthorimaea operculella into potato tubers and the number of eggs. Additionally, all extracts tested were found to be effective in checking the mycelial growth only for Penicillium digitatum and Fusarium sambucinum as compared to the control. But no significant difference between control and treated fungi was found in the case of Botrytis cinerea and Aspergillus niger. The treatment with these botanical extracts may be promising in protecting plants from pests and diseases infections.

Keywords: Punica granatum, extracts, biological activities.

INTRODUCTION

The overzealous and indiscriminate use of most of the synthetic fungicides has created different types of environmental and toxicological problems. Recently, in different parts of the world, attention has been paid towards exploitation of higher plant products as novel chemotherapeutants in plant protection. The popularity of bo-tanical pesticides is once again increasing and some plant products are being used globally as green pesticides [1, 2].

Yet, there is an urgent need to develop low cost safe control alternatives and environmental-friendly. Considerable efforts have been focused on plant derived materials, potentially useful as commercial bioinsecticides. *Punica granatum*, commonly known as pomegranate belongs to family Punicaceae. It is a native shrub of central Asia, especially parts of Iran in the Transcaucasia-Caspian region [3] from where it has spread to the rest of the world [4, 5].

Pomegranate is an important crop known by its taste and nutritional and medicinal properties [6-8]. Several studies have reported the antimicrobial [9] and insecticidal [10, 11] activities of extracts from different tree parts, such as bark, leaves, fruit, and fruit peel.

The aim of the present study is to evaluate the insecticidal and antifungal activities *in vitro* of the aqueous, ethanol and methanol fruit peel extracts from *P. granatum* against pests as Myzus persicae and *Phthorimaea operculella* and plant diseases as *Botrytis cinerea*, *Fusarium sambucinum*, *Penicillium digitatum* and *Aspergillus niger*.

MATERIALS AND METHODS

Preparation of pomegranate peel extracts.
Pomegranates, *P. granatum* cv. Kalaii were obtained from local market. The fruits were washed and the peels were manually removed, dried at room temperature (20 to 25°C) and powdered to get 0.5 mm size. About 100 g of the powder was extracted by stirring using a magnetic stirrer with 300 ml of ethanol, methanol and water for 24 h each at 25°C.

The extract was sieved through Whatman filter paper to remove peel particles. After filtration, the ethanol and methanol extracts were let to evaporate at room temperature during 48 h and the aqueous extract was evaporated under vacuum at 100°C.

Insects
Green peach aphids, *Myzus* persicae Sulzer, were collected from pepper crop leaves in the Regional Centre of Research on Horticulture and Organic Agriculture (CRRHAB) and directly used in the experiments.

Colonies of the potato tuber moth *Phthorimaea operculella* have been maintained for many years in the Entomology Laboratory of the Regional Research Centre on Horticulture and Organic Agriculture (CRRHAB). *P. operculella* colonies were kept in standard conditions at 27 °C and 65 % humidity. Larvae were fed on potato tubers.

Fungal strains
The four test fungal species, *Botrytis cinerea*, *Fusarium sambucinum*, *Penicillium digitatum* and *Aspergillus niger* were obtained from the laboratory of Phytopathology in the CRRHAB. These fungi are among the most important pathogenic fungi of economical significance to plants. They were cultured during 7 days at 25°C on potato dextrose Agar (PDA) medium amended with 300 mg/l of streptomycin sulphate before use.

Bioassays
Insecticidal activity assay against *Myzus persicae*
Twenty mg of each crude extract was dissolved in distilled water to obtain the final concentration of 2%. 5 µl of each solution (ethanol, methanol and aqueous) was sprayed directly on pepper leaves attacked by *Myzus persicae*. The control received 5 µl of distilled water. The leaves containing the insects were placed in petri dishes measuring 9cm*1.3 cm coated with filter paper. Plates were maintained in a climatic chamber at 25 ± 2ºC, relative humidity of 70 ± 10% and photoperiod of 12 h. The assessment of mortality rate was recorded after 24 hours.

Insecticidal activity assay against *Phthorimaea operculella*
To examine the percentage of larval penetration of *P. operculella*, the first larval instar was used because it searches and mines into the host [12]. At first, each potato tuber was dipped in 1 ml of 5% methanolic fruit peel extracts of *P. granatum*. When solvent was evaporated and tubers were dried, they (five potato tubers) per treatment were transferred into plastic boxes with ventilated lids kept at 25±2ºC, relative humidity of 70 ± 10% and photoperiod of 16: 8 (Light : Dark). Infested tubers were introduced on each box. Therefore, larval penetration was recorded with the number of individuals moving into potatoes. For the oviposition-preference activity, the number of eggs was determined under a binocular microscope.

Antifungal activity assay
Efficacy of aqueous, ethanol and methanol fruit peel extracts of *P. granatum* were studied *in vitro* against plant fungi by poisoned food technique. 20 mg of each extract was dissolved in 1 ml of distilled water and versed on Potato Dextrose Agar as basal culture medium. For the control, distilled water was used. The test fungi were incubated at 25±2°C in the dark. On the fourth day, the mycelial growth of fungi was recorded.

Statistical analyses
Five replications were performed for each test. For statistical comparison among several means, all the data were subjected to a one-way analysis of variance (ANOVA) followed by mean comparisons (at $P = 0.05$) and Student-Newman-Keuls (SPSS 11.0).

RESULTS AND DISCUSSION

Insecticidal activities
All extracts of fruit peel of pomegranate that were examined in the present study had a significantly high toxicity effect on *Myzus persicae* as compared to untreated control. This effect was shown in Figure 1.

Similar findings were obtained by Mohammad [13] and Ben Hamouda *et al.* [11] where the ethanol extracts also induced mortality of *Tribolium confusum* and *Tribolium castaneum* larvae and adults. Gandhi *et al.* [10] indicated that spraying of this powder led to 40% to 85% of *Tribolium castaneum* adult mortality.

Moreover, results given in figure 2 demonstrate that ethanolic, methanolic and aqueous peel fruit extracts of pomegranate had a high preventive effect on potato tuber moth larval penetration with 77% less than the untreated control. Mohammad [8] reported a strong repellent effect (86.7%) caused by ethanol extract of pomegranate fruit peel after two hours of exposure at a concentration of 2.5% for *Tribolium confusum*. However, Ben Hamouda *et al.* [11] showed that only ethanol extract showed a low repellent activity against *Tribolium castaneum*.

According to Figure 3, applying all extracts of peel fruit of pomegranate on potato tubers reduced egg laying of the *P. operculella* whereas the pest preferred to lay eggs on un-treated tubers. The average number of laid eggs on tubers treated with ethanol, methanol and aqueous extracts were 4.8, 6.6 and 8.4 eggs/tuber, respectively. Therefore, the pest preferred to oviposit on non-treated tubers with a mean of 16 eggs/tuber. These results demonstrated that pomegranate extracts had inhibitory effects against the pest.

Koide *et al.* [14] reported that toxicity caused by *P. granatum* is due to the astringent properties of tannins contained in the peel fruit which stop insect's infestation. Moilanen and Salminen [15] showed that peel is rich in ellagitannins that are considered as toxic against insects.

Antifungal activities

The antifungal properties of ethanol , methanol and aqueous extracts of fruit peel pomegranate were evaluated *in vitro* against *Botrytis cinerea*, *Fusarium sambucinum*, *Penicillium digitatum* and *Aspergillus niger* using PDA as basal medium as par Poisoned Food Technique [16]. Results revealed that all extracts tested were found to be effective in checking the mycelial growth only for *Penicillium digitatum* and *Fusarium sambucinum* as compared to the control (table 1). Percent inhibition of the test pathogen with all plant extracts ranged from 8.9 to 26.79 % in the case of *F. sambucinum* with the highest effect of ethanolic extract; and from 27.58 to 40.24% for *P. digitatum* with the highest effect of methanolic extract. Dahham *et al.* [17] also confirm the inhibitory effect of the methanol extract against *P. digitatum* which was attributed to phenols. Moreover, Azzouz and Bullerman [18] and Tehranifar *et al.* [19] reported that peel fruit extracts of *P. granatum* are able to inhibit the growth of certain potato rot diseases.
However, no significant difference between control and treated fungi was found in the case of *Botrytis cinerea* and *Aspergillus niger*. Al-Zoreky [9] showed that aqueous extract had no inhibitory activity on *A. niger*. Similarly, Aguilar *et al.* [20] reported that some *Aspergillus* species tolerated the tannins contained in ellagitannins and used them as carbon source.

These preliminary data suggest that the ethanol and methanol extracts of the pomegranate fruit peel should be further investigated in order to determine its chemical composition and to elucidate more its insecticidal and antifungal potentialities.

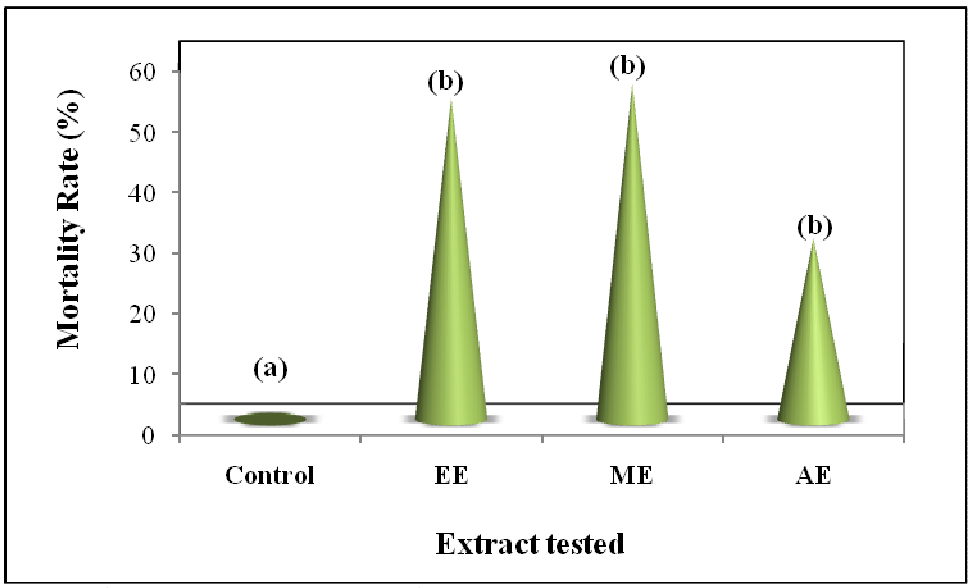

Figure 1: Mortality rate of *Myzus persicae* adults treated by foliar application with ethanol (EE), methanol (ME) and aqueous (AE) fruit peel extracts of pomegranate as compared to the untreated control. Bars attributed by the same letter are not significantly different according to the Student-Newman-Keuls test ($P \leq 0.05$).

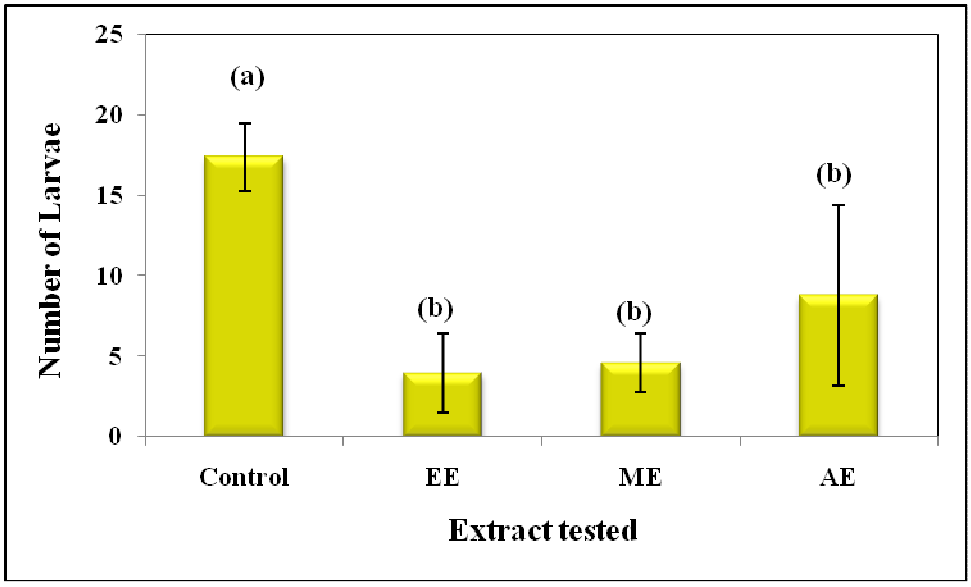

Figure 2: Effect of ethanolic (EE), methanolic (ME) and Aqueous (AE) extracts on the first larval penetration of *Phthorimaea operculella* into potato tubers as compared to the untreated control. Bars attributed by the same letter are not significantly different according to the Student-Newman-Keuls test ($P \leq 0.05$).

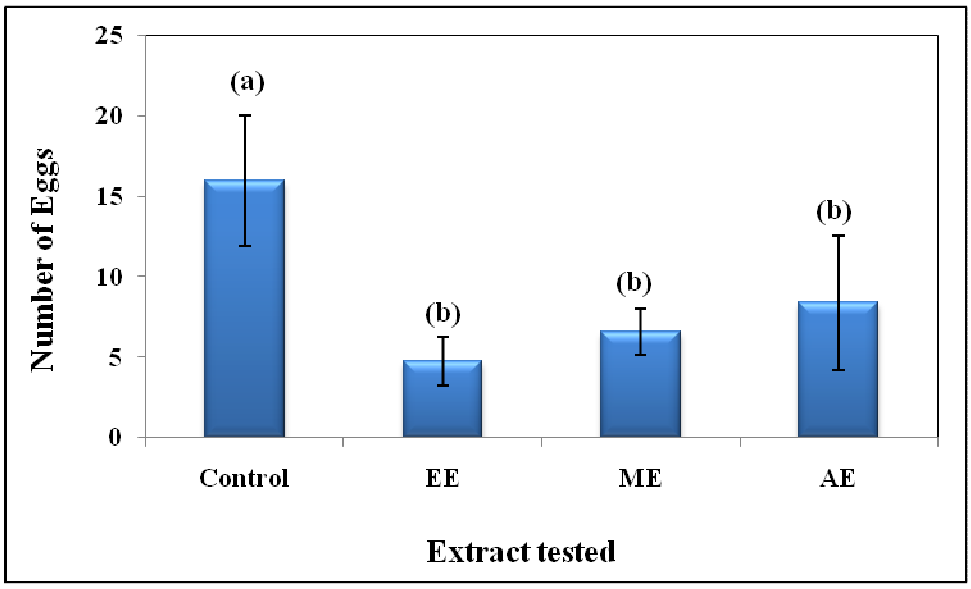

Figure 3: Effect of ethanol (EE), methanol (ME) and aqueous (AE) fruit peel extracts of pomegranate on the eggs number of *Phthorimaea operculella* as compared to the untreated control. Bars attributed by the same letter are not significantly different according to the Student-Newman-Keuls test ($P \leq 0.05$).

Table 1.Evaluation of fruit peel extracts on mycelia growth of *Penicillium digitatum* and *Fusarium sambucinum*.

	Ethanolic extract	Aqueous extract	Methanolic extract	Control
Penicillium digitatum	21±1	21±1	17,33±2,08	29±0,73
Fusarium sambucinum	27,33±2,51	34±3,6	35,66±5,13	37,33 ±2,51

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