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THE ROLE OF POMEGRANATE (*PUNICA GRANATUM)* HUSKS AND CITRUS (*CITRUS AURANTIUM*) HUSKS EXTRACTS IN REDUCING THE GROWTH OF SOME PATHOGENIC FUNGI OF THE PLANT

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Abstract

The emergence of many problems faced by agricultural products, most notably the consumption of these plants in the fields and homes as a result of disease. A great attention to environmentally friendly methods has been putted to solve these problems. Therefore in this study plant hot aqueous (water) extract and alcoholic extract were used from the crusts of Pomegranate and Citrus individually and in combination to inhibited the growth of (*Fusarium oxysporum*), which causes vascular wilt in the tomato and (*Plasmopara viticola*), which causes downy mildew in grapes. The results were analyzed with LSD and Duncan test and there were significant differences among most results of extracts, best effects were found in the combine extracts, due to the synergistic activity for both mixed extracts that led to major inhibition effects on fungal growth compared with control growth treatment. The aim of the study is to use natural products as pesticide to control plant pathogens because most fungal chemical pesticides have become toxic to the environment, humans, animals and other soil organisms, as well as their lack of efficiency due to the emergence of resistant fungal strains.

***Key word*:** Alcoholic extract, Hot water extract, Vascular wilt, Downy mildew.

Introduction

The largest range of plant pathogens are Fungi which are heterogeneous eukaryotic organisms, they do not had photosynthetic pigments. They are responsible for many plant diseases. Plant cells are damaged or these plants suffer from stress when they infected. The origin sources of fungal infections are contaminated soil, air, water and the bad use from farmers and workers for some chemical compounds such as fertilizers and pesticides (Rahmani *et al*., 2017). Many fungi had the ability to be varied in their growth and development when grown on different media or system growth and this gave them a wide range for infections. (Anjisha and Vrinda, 2012). It was habitual that there are many kinds of fungi can do infections for some important products or crops such as some vegetables and fruits, for example some species of *Fusarium* and *Plasmopara* can be ahead for bad damages for plants during growth period and after harvest. All species of Fusarium spp. are varying from the nature of its susceptibility to the plant, some cause rot or spotting and others cause wilt or ulceration. *Fusarium oxysporum* is a soil borne pathogen with a high level of host specificity. This fungal cause disease on a wide range of agricultural crops and wilt on tomato, *F. oxysporum* has the ability to produce many metabolic compounds and toxins, these substances play a large role in the pathogenesis of the fungus. Although of plant pathogenicity, *Fusarium oxysporum* had a pathogenic site to humans and animals. The diseases associated with Fusarium include infection to the eyeball also the fungal infection of the nail that can cause fingernails and other infection is of the skin that can result in an extreme rash or internal bleeding.(Berlin and Eitrem, 2005). *Plasmopara viticola* is also one of fungal that made problems for crop production causing downy mildew disease in grapevines. It is clearly that this disease poses a big threat wherever viticulture is cultured and founded. *P. viticola* attacks all green parts of grapevines including the leaves, the clusters and young fruit.4 In warm, humid weather. (Yin *et al*., 2017 and Goker *et al*., 2007). On other side it is found that plants and their products are commonly used in the cure of a lot of diseases from long time. Recent study demonstrated that medicinal plants shows a great role in disease controlling, inhibition and killing according to their ability of attack and biological activities (Rahmani *et al*., 2015 and Rahmani, *et al*., 2014). All parts of pomegranate (Punica sp.) extracts shows antimicrobial and antioxidant activities for many kind of bacteria and fungi (mold and yeast),(Nozohour *et al*., 2018 and Foos *et al*., 2014) Results confirmed that the highest antifungal activity was noticed against many fungus pathogenic species, and antimicrobial capacity against yeast cells such as Candida genus (Dahham *et al*., 2010 and Anibal *et al*., 2013). Also there are some type of plants found to be very active against some microbes including bacteria and fungi, but many effects mostly were reported against Gram-positive and negative bacterial strains. Essential oils and specific composition of Citrus would be divided into two groups. First group is rich with a compounds known as monoterpene hydrocarbons and second point according subjected to oxygenate monoterpenes. (Al-Ani and Aziz, 2013 and Tiina *et al*., 2015).

Materials and Methods

Pathogenic Fungi Isolation

Fusarium oxysporum isolates was obtained from the plant parts (stems and roots) of the tomato crop which showed the symptoms of the infection. The frequency of fungus was 60% compared to other isolated fungi from these plants. *Plasmopara viticola* was collected from different infected leaves locations of grapevine. The frequency of fungus was 55% compared to other types of fungus species.

Preparation of husks powder

Each samples (pomegranate and Citrus husks were collected from the local market and washed several time with tab water and then with distilled water after that the cleaned husks were dried under controlled conditions at room temperature, to avoid any contamination. Then husks crushed using mortar and then placed in the electric mixer until it

became accurately powder so they can easily be used for extraction.

Preparing the Extracts

(a) The aqueous hot extraction

Hot extraction was done according to (Dahiru *et al*., 2005) for each sample

- 250 ml of Boiling Distal Water (DW) was add for 50 gm. of plant powder samples.
- Incubate it for 60 min in shaker incubator at 30 °C.
- The extract was filtered to get pure liquid.
- The pure liquid was centrifuged for 3000 r\20 min, tow separated layers will appear.
- The first layer (supernatant) was taken then concentrated and dried.
- the extracts resulted are H1(hot extract of pomegranate), H2 (hot extract of Citrus) and H3 1:1 (H1 + H2).

(b) The Methanolic Extraction:

Methanolic extraction was done for each sample as it referred in (Shafighi *et al*., 2012).

- A volume of dry weight 50 gm. from husk powder for each plant has been put on filter paper (Whatmann No.1).
- Extraction by using Soxhlate apparatus with 500 ml of solvent (mixture of Methanol and water 3:1) for four hours.
- The extractions were concentrated by rotary evaporator.
- vi- the extracts resulted are M1 (methanolic extract of pomegranate) M2 (methanolic extract of Citrus) and M3 1:1 (M1+M2).

Antifungal assay

The extracts were tested for antifungal activity by agar diffusion assay (Sharifi *et al*., 2014).

- Each pathogenic tested fungi was cultured on plats of Sabouraud dextrose agar (SDA). Then the growth will transported to Sabouraud dextrose broth.
- 6mm as wells were made in agar plates.
- Then they were filled with 50 µL of extracts that were prepared (treatments) and add for it 50 µL of fungi
- Six different treatments extract (H1, H2, H3, M1, M2 and M3) and the sterility control treatment was the medium without fungi and medium with fungi but without extract spouse to be growth control.
- All plates were incubated for 5 days at 25 ºC.

Statistical analysis

Statistical analysis of results were done according to statistical program SAS was used to analyze the results obtained. Two analytical tests were done, differences were compared with the least significant difference (LSD) probability, and Duncan test was done also with significant level of ($P < 0.05$). (SAS, 2012)

Results and Discussion

The identification and classification of pathogenic fungi

Identification was achieved by placing a drop of the stain on clean slide with the aid of a mounting needle, lactophenol was used. The species encountered were identified and classified accordance to(Cheesbrough *et al*., 2000).

Fungi Classification

***(Fusarium oxysporum*)**

- Eukaryote\ Fungi\ Ascomycota\ Sordariomycetes\ ypocreales\ Fusarium\

***(Plasmopara viticola*)**

- Heterokonta\ Oomycota \ Peronosporales\ Peronosporaceae\ Plasmopar.

Plant (samples) Classification

- Pomegranate (*Punica granatum*) classification (Kumari *et al*., 2012)
- Plantae\ Angiosperms\ Eudicots\ Rosids\ Myrtales\ Lythraceae\ Punica.
- citrus (*Citrus aurantium*) classification (Karthikeyan and Karthikeyan, 2014)
- Plantae\ Angiosperms\ Eudicota\ Sapindales\ Rutaceae\ Citrus.

Antifungal Assay

The plants extracts activity appeared after incubation for five days, a significant differences among some results at different treatments for both fungi were reported in this study. As it shown in table (1) that there were a significant differences among all results comparing with the control, it was clear that the results show graduated activity, treatment H3 is best followed by H2, H1 respectively for inhibition the growth of both fungi *F. oxysporum* and *P. viticola*, and there were no significant differences between the two fungi at each specific aqueous treatment.

Table 1: Effect of different hot aqueous extraction treatments of pomegranate and Citrus husks powder on the growth of *Fusarium oxysporum* and *Plasmopara viticola*

Pathogenic fungi	Growth diameter(mm) after use Treatment H1	Growth diameter(mm) after use Treatment H2	Growth diameter(mm) after use Treatment H3	Growth diameter (mm) of the fungi control	LSD
Fusarium	5.2 ± 0.12bc	6.2 ± 0.15b	3.5 ± 0.11c	9.0 ± 0.26a	2.61*
Plasmopara	4.4 ± 0.09bc	6.0 ± 0.13b	2.8 ± 0.06b	8.0 ± 0.17a	2.35*
LSD	0.962 NS	0.558 NS	0.831 NS	1.22 NS	---
Means having with the different small letters in same row referred to significantly differences. * (P<0.05).					

The inhibition growths measured by mm, all data are mean of three replications

The methanolic extractions shows a significant differences after five days of incubation among all data that tested for the three type of extracts M1, M2 and M3 graduated respectively compared with the control growth, treatment M3 found to be the most promised mix that gave a very good results of inhibition activity for both tested fungi *F. oxysporum* and *P. viticola* as it noticed and there were no significant differences between the two fungi at each prepared treatment unless in M2, the best results were found in M3, M2, M1. table (2).

Table 2 : Effect of different Methanolic extraction treatments of pomegranate and Citrus husks powder on the growth of *Fusarium oxysporum* and *Plasmopara viticola*.

Pathogenic fungi	Growth diameter (mm) after use Treatment M1	Growth diameter (mm) after using Treatment M2	Growth diameter (mm) after using Treatment M3	Growth control diameter (mm) of the fungi	LSD
Fusarium	3.5 ± 0.06b	3.0 ± 0.05b	1.5 ± 0.03b	b9.0 ± 0.26a	2.59*
Plasmopora	4.0 ± 0.10bc	5.2 ± 0.09b	2.5 ± 0.03c	8.0 ± 0.17a	2.41*
LSD	1.63 NS	1.75*	1.08 NS	1.,22 NS	---
Means having with the different small letters in same row reffered to significantly differences. * (P<0.05).					

The inhibition growth measured by mm, all data are mean of three replications

This study was in agreement with many studies such as with (Shnee *et al*., 2013 and Rongai *et al*., 2016 and Rongai *et al*., 2012), they all reported that the antifungal activity of pomegranate aqueous extract were rising with the increasing of concentration of the extract thus the inhibition increased significantly. (Fawole *et al*., 2012 and Zarei *et al*., 2010) Demonstrated that some pomegranate methanol extracts contain high ratio of total phenolic and presence by a combination of poly phenols and organic acids (Fawole *et al*., 2012 and Orak *et al*., 2011). The fresh Citrus sinensis essential oils (EO) was obtained by vapor distillation, the EO vapors in plant act as a cause for inhibitory the growth of many pathogenic fungi growth decreased when there were un increasing in EO concentration (Maria *etal*., 2013). There are many studies reviewed the possibility of antifungal effect of plants parts on the fungi *Plasmopara sp*. by using several types of extracts from these plants parts and their juice (Elina *et al*., 2017). The results of this study was conforming with results of many Scientific researches (Dingle and McGee, 2003) and all these researches including present study ensuring that different concentrations of wild, farms, gardens and greenhouse plants were very efficacious in limiting the growth of *Plasmopara sp.* and specially *P. viticola* sporulation, germination and as a result its growth (Musetii *et al*., 2003 and Musetii *et al*., 2006).

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