فاعلية التضاد الفطري للمستخلصات الكحولية *Fraxinus syriaca* و *Gleditsia triacanthos* ضد فطريات التخزين في المخبر

Antifungal Activity of *Fraxinus Syriaca* and *Gleditsia Triacanthos* Ethanolic Extracts Against Storage Fungi in Vitro

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الملخص

جرت هذه الدراسة في عام 2019-2020 في مخابر كلية الزراعة - جامعة دمشق. في هذا البحث دُرس تأثير مستخلصات الأوراق والبذور واللحاء لكل من الدردار السوري (*Fraxinus syriaca*) والغلاديشيا ثلاثية الأشواك (*Gleditsia triacanthos*) في نمو مشيجة الفطرين *Botrytis cinerea* و *Penicillium digitatum* الممرضة للبندورة في ظروف المختبر. استخدمت طريقة تسميم الوسط المغذي لتقويم فاعلية التضاد الفطري. أظهرت النتائج أنّ المستخلصات الإيتانولية للنباتات المختبرة أظهرت تبايناً في فاعلية التثبيط تجاه الفطرين *B. cinerea* و *Penicillium digitatum*. أظهر المستخلص الإيتانولي لأوراق الدردار السوري أعلى فاعلية، حيث ثُبط نمو الفطرين *B. cinerea* والدردار السوري تماماً (100%) عند التركيز 1250ppm. تلاه مستخلص اللحاء. كانت فاعلية المستخلصات الإيتانولية لأوراق ولحاء وبذور الدردار السوري أعلى من المستخلصات الإيتانولية لأوراق ولحاء وبذور الغلاديشيا ثلاثية الأشواك ضد الفطرين المختبرين. من جهة أخرى كان الفطر *Botrytis cinerea* أكثر حساسية من الفطر *Penicillium digitatum* تجاه المستخلصات المختبرة جميعها. يمكن اعتبار مستخلصات الدردار السوري والغلاديشيا ثلاثية الأشواك كمصادر طبيعية لمركبات لها تضاد فطري لمعالجة أمراض النبات.

الكلمات المفتاحية: مستخلصات نباتية، فطريات *Gleditsia triacanthos* ، *Fraxinus syriaca* .

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Abstract

This study carried out in 2019-2020 in the faculty of Agriculture laboratory, Damascus Unv. The present investigation with deals effect of leaves, seed and bark extracts of *Fraxinus syriaca* and *Gleditsia triacanthos* in growth mycilum of two fungal *Botrytis cinerea* and *Penicillium digitatum*. For the antifungal evaluation, we used the Food Poison Technique. The results showed that the ethanolic extracts of the tested plants exhibited varying degrees of inhibition activity against the *Botrytis cinerea* and *Penicillium digitatum* fungi. The ethanolic extract of leaves from *Fraxinus syriaca* showed maximum activity. Where the inhibition of mycelium growth of *B. cinerea* and *Penicillium digitatum* were completely inhibited (100%) at 1250 ppm. Followed by the bark extract. Leaves, bark and seed of *Fraxinus syriaca* were more effective than the leaves, seed and bark extracts of *Gleditsia triacanthos* against the tested fungi. On the other hand, the fungi *Botrytis cinerea* was more sensitive than *Penicillium digitatum* for all the tested extracts. The extracts of *Fraxinus syriaca* and *Gleditsia triacanthos* could be considered as potential sources of antifungal compounds for treating diseases in plants.

Key words: Fungi, plant extracts, *Fraxinus syriaca* and *Gleditsia triacanthos*.

Introduction

Penicillium digitatum the cause of citrus green mold respect is important postharvest pathogens and cause serious losses annually (Palou, *et al*., 2001). *Botrytis cinerea* (the grey mold fungus) and other *Botrytis* species are important pathogens of nursery plants, vegetables, ornamental, field and orchard crops and stored and transported agricultural products. Considerable effort is invested in protecting the agricultural produce against *Botrytis* before and after harvest (Agrios, 2005).The control of these pathogens remains a challenge and is still based upon multiple applications of fungicides. Chemical control is effective and efficient but, at the same time, can leads to the development of pathogen resistance, chemical residues in fruit, phytotoxicity to other organisms or environmental and public health problems (Soylu, *et al*., 2005 and Al-Najar, 2007.).

The alternative choice may be the use of botanical fungicides that are easily biodegradable and safe, with minimal environmental impact and danger to consumers (Fawecett & Spencer, 1970). Many plants have antimicrobial activities that are related to their antimicrobial constituents, including alkaloids, terpenes, polysaccharide, esters, ketones, and quinones (Farzaei *et al*., 2015). Effective components extracted from plants have promising potential for this purpose because of their high efficacy, low toxicity, and selective characteristics (Sanei-Dehkordi *et al*., 2016). Hence, use of some safe bioactive compounds like essential oils has been proved beneficial in bringing down the physiological activities of fruits during storage and minimizing the overall qualitative and quantitative losses (Porat *et al*. 2002).

The anti-fungal activity of higher plants has long been thought to be an important factor for disease resistance and control against a wide range of fungi that infect crops (Nwachukwu & Umechurupa, 2001; Ramezani et al., 2002). Several studies have shown that natural products are capable of fungitoxic activity against a good number of micro-organisms (Dubey & Dwivedi, 1991; Oluma & Elaigwu, 2006). Extracts of plants have also exhibited marked effects on spore germination (Singh andDwivedi, 1990). Oluma and Garba (2004) found that the crude extract of *Eucalyptus globulus* Labill. and Ocimum *gratissimum* L. inhibited spore germination and reduced radial growth of *Pythium aphanidermatum* Edson by 44.5%-100%, with *E. globulus* being potent. Hadi and Sorkhi (2013) Reported *that the ethanol extracts of Urtica dioica, Matricaria chamomile, Cinnamomum zeylanicum* and *Mentha piperita* at 50, 25, 500 and 100 ppm respectively gave the maximum inhibition on the growth rate

of *Penicillium digitatum*in PDA media. Zaker (2013) investigated antifungal activity of peppermint, lavandula, eucalyptus, datura and nettle extract against *Alternaria sesame*. Results indicated that methanolic extracts of peppermint (15 and 10%), lavandula (15%) and eucalyptus (15%) were more effective than methanol: water extracts and completely inhibited the growth of the pathogen. Among tested extracts, methanolic extracts of peppermint (15%) and eucalyptus (15%) exhibited remarkable inhibition of the spore germination.

The genus Fraxinus (Oleaceae) is distributed mostly in the temperate regions and the subtropics of the Northern hemisphere (Wallander *et al*,2000). *Fraxinus syriaca* Boiss Description trees or rarely shrubs, deciduous or rarely evergreen. Leaves are odd-pinnate, opposite or rarely whorled at branch apices; petiole and petiolule are often basally thickened. Inflorescences are terminal or axillary toward end of branches, or lateral on branches of previous year, paniculate; bracts linear to lanceolate, caduceus or absent. Flowers are small, unisexual, bisexual, or polygamous. The fruit is a samara with elongated wing. The seed is usually one, ovate-oblong; endosperm fleshy; radicle erect (Hameed, 2011). The *Fraxinus* species have economical, commercial and medicinal importance (Stoyanov,1973, Wallander *et al*,2000). Many species attract considerable attention for their medicinal properties and find application in the folk medicine, as well as in the contemporary medicine (Shen, *et al*, 1993, Kruedener *et al*., 1995). Some species grow as garden plants; others are cultivated as ornamentals (Hosny,1998).

Fabaceae family is the third largest family of flowering plants after Orchidaceae and Asteraceae. This family is distributed throughout temperate and tropical regions of the world (Rundel, 1989). Among the Fabaceae is the genus Gleditsia, the Locust tree which comprises about 14 species of deciduous trees (Huxely et al., 1992), and is native to North America and Asia. Gleditsia species have been widely used in folk medicine. They are used for treatment of carbuncle, scabies, skin diseases and for treating apoplexy, headache, productive cough and asthma, also they are used as diuretic and expectorant (Miyase *et al*., 2010).

Honey locust (*Gleditsia triacanthos* L.) is a leguminous tree originating in the middle and eastern part of North America, which was in Southern Slovakia and Hungary widely planted in parks as ornamental species, round vineyards, gardens and fruit groves as thorn-hedge, along roads and fields as wind barrier, and as a component of floodplain forests (György, 2007).

The aim of the present study was to determine ethanol extracts of different parts (leave, seed and bark) of *Fraxinus syriaca* and *Gladitsia triacanthos* against two economically important plant disease, *Botrytis cinerea* and *Penicillium digitatum*. These diseases cause significant product losses storage fruits.

Materials and Methods

This study carried out in 2019-2020 in the faculty of Agriculture laboratory, Damascus University.

Plant material:

A sample of Leaves, seed and bark of *F. syriaca* and ***G. triacanthos*** were collected from the garden of Damascus D.C. Syria, Species were identified by a axonomist in the Dep. of Renewable Natural Resources and Ecology, Fac. of Agric., Damascus Univ. After washing with tap water, the plant material was surface sterilized with 1% sodium hypochlorite solution followed by thorough washing with sterilized water. These plant materials were dried at 40 ± °C in an electric oven and ground thoroughly to form powder. The powder was sealed in polyethylene bags until extraction.

Preparation of ethanolic plant extracts:

For investigations, ethanolic plant extracts were prepared by soaking 50 g of dry powder plant materials from each part in200 ethanol with stirring for 24 h and then filtered through double layers of muslin, and finally filtered again through Whatman filter paper No. (1) to remove plants debris and obtain a clear filtrate. The filtrates were evaporated and dried under reduced pressure and temperature below 45°C. The yield of the dry residues in relation to the starting plant material was calculated.

Fungal cultures :

Fungal cultures of *Penicillium digitatum* and *Botrytis cinerea* were isolated from tomato crop by hyphae point and monsporic technique and identified in the Faculty of Agriculture, Plant diseases Department, Damascus University (Barnett and Hunter. 1987.). The isolated fungi preserved in slants containing potato dextrose agar (PDA) till used. The tomato phytopathogenic fungi were provided from the culture collection of Plant diseases Department, Damascus University. The isolated fungus was maintained on potato dextrose agar (PDA) medium.

In vitro antifungal assays.

Inhibition of mycelial growth using the Food Poison Technique in PDA (Dhingra and Sinclair, 1995). Potato Dextrose Agar (PDA) was autoclaved and then maintained in a water bath at 40 °C. The plant extract residues were re-dissolved in 5 ml of methanol, and mixed with sterile potato dextrose agar medium to obtain the final concentration of obtain final concentrations of 150, 300, 500, 750, 1000 and 1250 ppm of each part extract and then poured in sterile Petri dishes (90 mm diameter) **.** For control, 2 ml of ethanol was added to the PDA medium. After the solidification of media, discs of 5 mm diameter of phytopathogenic fungi were cut from the periphery of 5 days old cultures and inoculated aseptically to the center of poured Petri dishes of treatment and control sets .There were three replicates per experiment. Plates were incubated for 7 days at 25 ±2 °C. The percentage inhibition of mycelia growth, in terms of fungitoxicity of the extracts, was calculated using the following formula Vincent:(1947).

$$\% \text{ inhibition} = [(Mc - Mt)] \times 100$$

Where, Mc is the average increase in mycelia growth in control an Mt is the average increase in mycelia growth in treatment (Singh and Tripathi, 1999).

Statistical Analysis:

The experiment was conducted, using completely randomized design (CRD), There were three replicates per experiment. Observed data was analyzed by SPSS20, using tow-way ANOVA. L.S.D (least significant difference) have been run to compare means statistically at significance level of (0.01)

Results

- Assay of *F. syriaca* against the tested fungi:

The results of the antifungal assay of the *F. syriaca* (ethanol extracts of Leaves, seed and bark) indicated that these plant exhibited antifungal activity against the both tested fungi at concentrations of 300,500, 1000 and 1250 ppm. The potential sensitivity of the extract was obtained against the two fungi tested and the percentage inhibition of mycelium growth were recorded and presented below in the table 1. Leaves extract of *F. syriaca* showed significantly superior antifungal activity against the tested fungi at all tested concentrations compare with the other treatment and control, where the percentage inhibition of mycelium growth of *Botrytis cinerea* were ,81.33, 95.14,100 and 100% , and *Penicillium digitatum*,were 74.23,87.38, 92.14 and 100% at 500, 750, 1000 and 1250 ppm. Followed by ethanol extract of bark extract. On the other hand, seeds extract of *F. syriaca* showed moderate antifungal activity against *Penicillium digitatum* and *Botrytis cinerea* at the tested concentrations where the percentage inhibition of mycelium growth of *Botrytis cinerea* and *Penicillium digitatum* were 89.36 and 63.12 at the maximum concentrate (1250 ppm), respectively. data analysis showed the differences between extracts, between concentrations, as well as between their interaction are significant (p<0.01).

Table 1: effects of ethanolic extracts of Leaves, seed and bark of *F. syriaca* on inhibition growth of *P. digitatum* and Botrytis cinerea on PDA.

Concen. (ppm)	*P. digitatum*			*B. cinerea*		
	Leaves	Bark	Seed	Leaves	Bark	Seed
	% inhibition					
Control	0	0	0	0	0	0
150	28.76	18.36	15.23	36.96	25.64	21.32
300	42.15	17.23	14.32	53.26	31.89	22.97
500	74.23	39.86	25.32	81.33	52.46	42.87
750	87.38	55.37	35.26	95.14	65.55	58.26
1000	92.14	63.98	43.16	100	79.84	71.21
1250	100	82.10	63.12	100	96.23	89.36

L.S.D (0.01) between concentrate= 5.23, between treatment= 7.98 , between interaction=3.65

- Assay of *Gladitsia triacanthos* against the tested fungi:

In the present work, the effect of different concentrations of extracts from three parts (Leaves, seed and bark) of *G. triacanthos* were studied on mycelia growth of *P. digitatum* and *B. cinerea* .The results of analysis of variance revealed that three parts extracts of *G. triacanthos* caused significant inhibition of mycelia growth of both fungi (Table 2). Furthermore, data analysis showed the differences between extracts, between doses, as well as between their interaction are significant ($p<0.01$). The comparison of means showed maximum inhibition of *P. digitatum* and *B. cinerea* growth was found at highest doses, 1000 and 1250 ppm (Table 2). It was followed by the concentration 500, 300 and 150 ppm of the plant extracts as compared to control which showed least inhibition on mycelia growth. The results in table 2 show that at all treatment concentrations of *G. triacanthos* extracts significantly reduced mycelia growth of *B. cinerea* in compared with *P. digitatum*. However, analysis of variance showed the differences between extracts, concentrations, as well as their interaction are significant ($p<0.01$). The comparison of means showed the extent of mycelia growth usually decrease by increasing the concentration of extracts and the maximum inhibition was usually observed at highest concentrations of extracts, 1000 and 1250 ppm .However, seed extract of *G. triacanthos* showed significantly superior antifungal activity against the tested fungi at all tested concentrations compare with the other treatment and control. Flowed by the leaves extract. On the contrast, the bark extract gave the lowest the percentage inhibition of mycelium growth of *P. digitatum* and *Botrytis cinerea*.

Table 2: effect of ethanolic extracts of Leaves, seed and bark of Gladitsia triacanthos on inhibition growth of *P. digitatum* and Botrytis cinerea on PDA.

Concen. (ppm)	*P. digitatum*			*B. cinerea*		
	Leaves	Bark	Seed	Leaves	Bark	Seed
	% inhibition					
Control	0	0	0	0	0	0
150	9.56	5.23	2.32	15.63	10.32	6.23
300	12.36	9.23	6.54	23.21	17.78	15.23
500	36.25	17.23	11.21	45.32	28.23	21.65
750	52.36	31.58	22.54	69.23	40.87	31.25
1000	67.58	56.35	43.56	75.87	60.65	54.23
1250	86.25	70.23	58.97	92.35	87.45	75.23

L.S.D (0.01) between concentrate= 3.87, between treatment= 3.21 , between interaction=2.87

Discussion

In this study, the ethanol extracts of *F. syriaca* and ***G. triacanthos*** (leaves, seed and bark) were tested. Experiment of plant extracts were conducted by the Food Poison Technique and identifying antifungal activities. The ethanol extracts of all plant parts displayed varied antifungal activity on *P. digitatum* and *B.cinerea*. The developments of mycelium of all pathogens have been significantly suppressed by compare with control. All plant extracts were showed antifungal effect depending on concentration increasing. In addition, the fungus *B.cinerea* has been observed the most sensitive diseases against plant extracts. According to the result of mycelium that percent of inhibition rates were found. These rates were displayed different level to compare the type of plants and parts used. As the concentration increases, the percent has been shown an increase in inhibition rates. the ethanol extracts of *F. syriaca* gave the superior antifungal activity against the tested fungi at all tested concentrations compare with the ethanol extracts of *Gladitsia triacanthos*.

May by du to exist coumarins, secoiridoids, phenylethanoids, flavonoids, and lignans in the composition of *Fraxinus* species. Kostova and Iossifova (2007)A wide range of chemical components including coumarins, secoiridoids, phenylethanoids, flavonoids, and lignans has been isolated from *Fraxinus* species. Extracts and metabolites have been found to possess antiinflammatory, immunomodulatory, antimicrobial, antioxidative, skin regenerating, photodynamic damage prevention, liverprotecting, diuretic and antiallergic activities. Some species find application in contemporary medicine.

Also these results in agreement with other investigators The ethyl ether fraction of the alcoholic extract of *F. excelsior* bark was inhibitory to *Bacillus subtilis* (Naimie and Bot, 1964). Extracts of the leaves of *F. excelsior* suppressed the growth of the fungi *Gloeosporum limetticola* and *Alternaria tennis* (Martin.*et al*.1966) .

Iossifova, *et al*., 1994 reported that grujic-Vacic and co-workers tested the antimicrobial activity of aqueous extracts of the leaves and the barks of *F. ornus* and *F. excelsior* against 11 microorganisms and found that the leaves of both species showed strong inhibition on the growth of Candida albicans with zones of inhibition of 25 and 22 mm, while the extracts of their barks expressed The antimicrobial activity of different groups of bark constituents of *F. ornus* was investigated.

In contrast, The main component responsible in *Gladitsia triacanthos*. is shown The seeds contain carbohydrates, fibers, starch, proteins, lipids, vitamins (A, B, K), considerably low content of glyceride oil (0.8 - 4.3%). The main components in triacylglicerols were found to be palmitic (34.4%), oleic (38.4%), stearic (16.5%) and linoleic acid (9.0%) (Mariod, 2008). However, the activity of *G. triacanthos* against tested fungi may by du to exist alkaloid compounds (Belikov et al., 1959) reported the isolation of the alkaloid triacanthine from the young leaves of *Gleditsia triacanthos*.

These results agreement with many articles were published about antifungal properties of medical plants against pathogenic fungi (Bajpai, *et al.*,2012 , Kalkışım,2012 and Al –Naser and Ezz Al-dden, 2015). Also, Ibrahim, 2016 demonstrated that the methanol extracts of the leaves from *Brachychiton populneus* showed the maximum antifungal activity against *Botrytis cinerea* and *Fusarium oxysporum* f. sp. *lycopersici* fungi.

Conclusion

The results of this study have shown that the ethanolic extracts of leaves, seed and bark of *F. syriaca* and *G. triacanthos* significantly reduce the mycelial growth of *P. digitatum* and *B.cinerea* on PDA the causal mold on storage fruits. Therefore, ethanolic extracts of *F. syriaca* and *G. triacanthos* determined activities showed that can be used as biopesticides. In addition, first time displayed antifungal properties of *F. syriaca* and *G. triacanthos* by this study. But it is important to state that further work needs to be carried out on the effects of this extracts on fungi under field conditions.

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