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Antifungal Activity of *Pomegranate* and Oak Galls Extracts Against *Penicillium* spp. and *Aspergillus niger*

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ABSTRACT

Different factorial experiments were conducted during 2008-2009 in the laboratories of Biology Department, College of Science, University of Salahaddin-Erbil, to determine the effects of aqueous and ethanol extracts of pomegranate (*Punica granatum* L.) and oak gall (*Quercus infectoria* L.) at 0, 5, 10, 15 and 20% of raw extracts to control *Penicillium* spp. and *Aspergillus niger* after 48, 96 and 168 hours of incubation, using completely randomized design (CRD) with four replications. The results revealed that all the plant extract concentrations used were effective against the two fungi. Ethanol extracts were more efficient against the growth of mycelia of the two fungi than aqueous extracts. *Penicillium* spp. was more sensitive to the plant extracts of pomegranate and oak gall more than *Aspergillus niger.* There were significant interactions between time of incubation and plant extract concentrations in their effects on the growth of mycelia. The two fungi renewed part of their activity after 168 hours of application of plant extracts.

Keywords: Antifungal activity, Pomegranate, Oak gall, *Penicillium* spp. , *Aspergillus niger.*

تأثير المستخلصين قشور الرمان و البلوط العفصى على الفطرين *Penicillium* spp., *Aspergillus niger*

الملخص

أجريت عدة تجارب معملية بالتصميم العشوائي الكامل وأربع مكررات لكل معاملة في مختبرات قسم علوم الحياة في كلية العلوم بجامعة صلاح الدين-أربيل خلال 2008-2009 لمعرفة تأثير المستخلصات المائية والكحولية لقشرة ثمار الرمان وثمار البلوط العفصي بتراكيز 0، 5، 10، 15 و20% في مكافحة فطري

Penicillium spp. و *Aspergillus niger* . بعد 48، 96 و168 ساعة من المعاملة والحضن دلت النتائج على أن التراكيز المختلفة كانت مؤثرة في نمو الفطرين وكان تأثير المستخلصات الكحولية أكثر من تأثير المستخلصات المائية في التأثير في نمو المايسيليوم لكلا الفطرين. فطر *Penicillium* spp. كان أكثر حساسية تجاه مستخلصات النباتين من *Aspergillus niger* ووجد تداخل بين تراكيز المستخلصات وفترات الحضن في التأثير في نمو المايسيليوم للفطرين. كلا الفطرين استعادا نموهما جزئياً بمرور الوقت.

الكلمات الدالة: مضاد فطري، رمان، وثمار البلوط، فطر بنسيليوم، فطر اسبرجلس.

INTRODUCTION

The plant fungal diseases have traditionally been controlled by chemical fungicides. The development of resistant strains of pathogens against various chemical fungicides (Lin, 1981 ; Witte, 1998) and their harmful effects on soil biosphere and causing health hazards for humans and animals which found to pose carcinogenic risk due to their residual toxicity (Anonymous, 1998 ; Sarmamy, 2001) make the use of these chemicals limited. Because of these problems associated with the use of chemicals, researchers are trying to use environmentally safe alternative methods of fungal controls to reduce synthetic fungicides side effects. Plant extracts as natural products are widely used to control pests, since few last decades (Taiga *et al*., 2008; Joseph *et al*, 2008; Sarmamy *et al*., 2010 ; Basno, 2009). Plant extracts show antibacterial effects (Sarmamy and Al-Juboory, 2005) and antifungal activity against wide range of fungi (Aba Alkhail, 2005; Basm and Khalil, 2007; Sarmamy and Saleem, 2009). Pomegranate (*Punica granatum*) Punicaceae and oak galls (*Quercus infectoria*) Fagaceae are two well known medicinal plants grown in the region (Dohuk, Erbil and Suleymania). Galls are formed as a result of a pathologic swelling that infects the branches of normal oak by a female insect *Cynipisgalle*. The gall is formed when the insect drills the bud and puts its eggs in the wood (Hamawendi, 2006). The gall contains 50-70% tannic acid, starch, resin, qurcetin, calcium salts and acids. The gall is used in folk medicine in the case of pyorrhea, gum bleeding, diarrhea, gastritis enteritis, clean skin burns, wounds and eczema (Al-Rawi and Chachravarty, 1988). It is also used in tannery, dying, and ink industry as well as being an important source for tannic acid.

The aim of the present study was to evaluate the antifungal activity of the tow plant extracts that widely distributed in the region such as oak gall and epicarp of pomegranate extracted with cold distilled water and ethanol against tow *Penicillium* spp. and *Aspergillus niger.*

MATERIALS AND METHODS

Preparation of extracts and culture

Epicarp of pomegranate fruits and oak galls were obtained from the local market. Plant segments (parts) were washed with tap water then by distilled water, dried by spreading them

on a plastic sheet in the laboratory at the room temperature with occasional mixing to prevent fungal growth. Each plant parts were ground separately by electrical grinder and extracted by macerating 100gm of the powder of each plant material in 200ml of distilled water or 90% ethanol, and were put in an electric shaker for 24 hrs. Plant extracts were filtered by passing them through four folded layers of gauze and filtered by Buchnner apparatus and filter paper (Whatman No.4), then sterilized by passing them through bacterial filter (Seitz). Ethanol extract was concentrated by rotary vacuum evaporator at 45˚C. (Gull *et al*., 1988 ; Harborn, 1973).The extracts were kept in the refrigerator until use (Harborn, 1984). Concentrations of 0, 5, 10, 15 and 20% of the raw extracts were prepared and added to the sterilized (PDA) medium, mixed well then 20 ml of the mixture (PDA medium + plant extract) were poured in each 9cm sterilized Petri dishes. The medium without extract was served as control 0%. Mycelial discs of fungi were prepared using a cork borer (5 mm diameter) from the margin of 5 days old culture of the two tested fungi and placed at the centre of Petri dishes after solidification of PDA medium. Each treatment was replicated four times. Plates were incubated in an incubator at 25º C. Fungal growth after 48, 96, and 168 hrs was measured by taking the mean of the two diameters taken at right angles for each colony.

Preparation of fungi

Aspergillus niger was isolated from infected onion bulbs while *Penicillium* spp. was isolated from infected citrus fruits and pure cultures of the two isolated fungi were identified on the bases of morphological and microscopically characteristics according to the key of Barnett and Hunter (1972) and Bessy (1968).

RESULTS AND DISCUSSION

Effects of pomegranate extract on controlling studied fungi

Effects of aqueous extract of pomegranate:

The results show (Table 1) that the aqueous extract of pomegranate affected significantly the growth of mycelium of *Penicillium* spp. and *A. niger* at all extract concentrations used (5, 10, 15 and 20%) by reducing the mycelial growth of *Penicillium* spp. and *A. niger* to 7 and 20.58-28.08 mm compared with control (53 and 60.67 mm) for the tow fungi respectively. Time of incubation affected significantly the growth of mycelia. Figure 1 shows that the effect of aqueous extract on the growth of mycelia of *Penicillium* spp. The growth decreased with time and no differences between the extract concentrations in their effects were observed. The effect of aqueous extract on the growth of *A. niger* was more effective with time than *Penicillium* spp. (Figure 2). The mycelial growth was 7-12mm after 68 hours of incubation and increased to 33.5 mm at 20% of the extract after 168 hours of incubation. It seems that *A. niger* recovered part of its activity after 168 hours of application of the plant extracts. The interaction between extract percentages and time is clear and the highest effects were 7mm compared with 81 mm for control after 168 hours.

Effects of ethanol extracts of pomegranate

The different ethanol extract concentrations affected significantly the mycelial growth of the studied fungi (Table 1). In the case of *Penicillium* spp. all extract concentrations affected significantly and reduced mycelial growth (16.58-20.83 mm) compared with control (53 mm),

and there is no significant difference between the different concentrations in their effects. In the case of *A. niger* the ethanol extracts affected significantly and reduced the mycelial growth to 29.33 mm at concentration of 20% compared with 60.67 mm for control.

Time of incubation affected on the growth of mycelia significantly. Figure 3 shows that all concentrations of ethanol extracts of pomegranate at the three times affected mycelial growth of *Penicillium sp* but the effects reduced after 168 hours. Figure 4 shows slight effects of all extract concentrations on the growth of *A. niger* except 20% and the fungus was recovered its activity after 96 and 168 hours of treatment. Data in table 1 show that *Penicillium* spp. was sensitive more than *A. niger* to pomegranate extracts.

Effects of oak galls extract on controlling the studied fungi

Effects of aqueous extract:

Table 2 shows that the aqueous extract of oak galls at all concentrations affected significantly the growth of mycelia of *Penicillium* spp. and *A. niger* and reduced the mycelial growth of the two fungi to 15.33-23.83 mm and 31.08-48.25 mm respectively in comparison with 53 and 60.67 mm for the control of the two fungi respectively. Time of incubation also affected the growth of mycelia significantly and the effects of extracts reduced as the time increased. It seems that *A. niger* is resistant to plant aqueous extracts more than *Penicillium* spp. The mycelial growth was 34.5 mm after 48 hours of application and increased to 81.75 mm after 168 hours of application. Figure 5 shows significant interactions between time of incubation and extract concentrations, and the highest effect was at concentration of 20% and 48 hours of incubation (60.5 mm) compared with control and 168 hours of incubation (70 mm) for *Penicillium* spp. and 23 mm at concentration of 20% after 48 hours of incubation compared with 81.75 mm in control after 168 hours of incubation in case of *A. niger* (Figure 6).

Effects of ethanol extract on controlling of studied fungi

Table 2 shows that ethanol extract of oak galls at all concentrations affected significantly the growth of mycelia of *Penicillium* spp. and *A. niger* and reduced the mycelia growth of the two fungi to 14.0-26.25 mm and 31.5-48.25 mm in comparison with 60.5 and 65.75 mm for control of the two fungi respectively. Time of incubation after application of extracts affected significantly the growth of mycelia of the two fungi. After 48 hours of incubation the mycelial growth of *Penicillium* spp. was 14.8 mm increased to 40.8 mm after 168 hours and in the case of *A. niger* increased from 26.5 mm to 51 mm (Figures 7 and 8).

There were interactions between time of incubation and plant extract concentrations in their effects on the growth of mycelia for the two fungi. In the case of *Penicillium* spp. the highest effect was in concentration 20% after 48 hours of incubations (7 mm) compared with 70 mm for control and 168 hours of incubation. In the case of *A. niger* the highest effect was 23.5 mm compared with 81.75 mm in control after 168 hours of incubation.

Ethanol extract of *Pomegranate* and *oak galls* were more effective on controlling *A. niger* and *Penicillium* spp. than aqueous extract and caused significant reduction in mycelial growth for the two fungi (Tables 1 and 2). This may be due to the differences between the chemical components of the two plant extracts and most of the biological active chemicals are soluble in ethanol more than water such as volatile oils, tannin and glycosides and these components are

physiologically active against fungi. oak galls containing tannins (Paaverurve and Raal, 2010), which have inhibitory effects on fungi (Vonshak *et al*., 2003), while pomegranate antifungal activity may be due to polyphenols and punicalagins (Seeram *et al*., 2006; Mertens *et al*., 2006; Plumb *et al*., 2002).

A. niger was more resistant against extracts of pomegranate and oak galls than *Penicillium* spp. This may be due to differences between the two fungi in their genetic constructions or may be due to the differences in the chemical and structural composition of the cell walls of the two fungi.

Table 1: Effects of aqueous and ethanol extracts of Pomegranate on the growth of *Penicillium* spp. and *Aspergillus niger*

Treatments		**Pomegranate Extract (%)**			
		Aqueous		**Ethanol**	
Time (hrs)	Conc. (%)	*Penicillium* spp. Mycelial Growth (mm)	*Aspergillus niger* Mycelial Growth (mm)	*Penicillium* spp. Mycelial Growth (mm)	*Aspergillus niger* Mycelial Growth (mm)
		Mean ± S. E.	Mean ± S.E	Mean ± S. E.	mean± S. E.
48	0	28.5 ± 1.56 c	34.5±0.65	28.50±1.56	34.5 ±0.65
	5	7± 0.00d	12±0.41	13.75±1.38	33.0 ±0.71
	10	7± 0.00d	10.5±0.29	11.25±0.75	31.75 ±1.18
	15	7± 0.00d	7±0.00	9.50±0.50	27.50±1.04
	20	7± 0. 00d	7±0.00	9.00±1.00	17.50±1.04
	X⁻	11.3± 1.99 a	14.20±2.38 a	14.40± 1.72a	28.85±1.89 a
96	0	60.5± 0.50b	65.75±3.33	60.5±0.50	65.75±3.33
	5	7± 0.00d	29.75±1.93	18.75±0.75	67.50±1.44
	10	7± 0.00d	28.5±0.96	18.00±0.71	63.00±1.22
	15	7± 0.00d	23±1.73	16.25±0.75	63.00±1.08
	20	7± 0.00d	21.25±0.48	15.5±1.33	28.75±1.25
	X⁻	17.7± 4.88a	33.65± 3.81b	25.80± 4.00a	57.60± 3.41b
168	0	70± 2.04a	81.75±1.18	70.0±2.04	81.75±1.18
	5	7± 0.00d	42.5±1.04	30.00±1.23	77.75±0.85
	10	7± 0.00d	38.5±0.65	29.75±0.25	74.00±1.69
	15	7± 0.00d	35.25±1.65	27.75±3.98	75.25±1.03
	20	7± 0.00d	33.5±0.96	25.25±1.03	41.75±0.86
	X⁻	19.6± c	46.30± 4.12b	36.55 ±3.89b	69.50± 3.30c
X⁻	0	53± 4.33a	60.67± 6.02a	53.00± 5.41a	60.67± 6.01a
	5	7±0.00 b	28.08± 3.84 b	20.83± 2.14b	59.42± 5.80a
	10	7± 0.00b	25.83± 3.51b	19.67± 2.33b	56.25± 5.45a
	15	7± 0.00b	21.75± 3.56b	17.83± 2.30b	54.25± 5.84a
	20	7± 0.00b	20.58± 3.29b	16.58± 2.10b	29.33± 3.04b
	X⁻	16.20±2.30	31.38±2.64	25.58±2.25	51.98±2.75

* Means with different letters in a same column show significant differences as determined by Duncan's multiple range tests (α = 0.01).

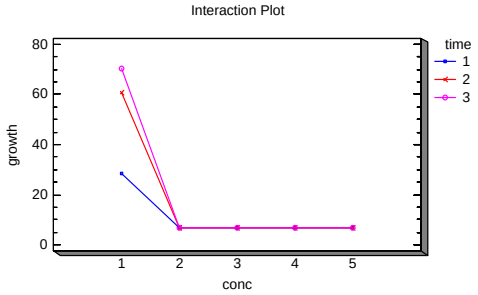

Fig. 1: Effect of interactions between Conc. of aqueous extracts of pomegranate and time of incubation on mycelial growth of *Penicillium* spp.

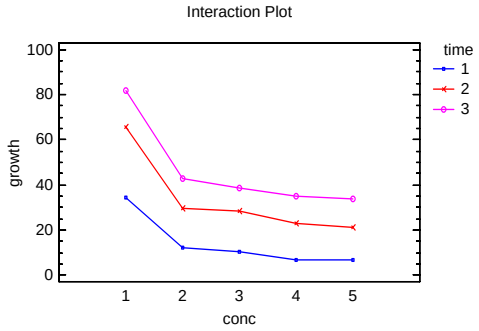

Fig. 2: Effect of interactions between conc. of aqueous extracts of pomegranate and time of incubation on mycelial growth of *Aspergillus niger*

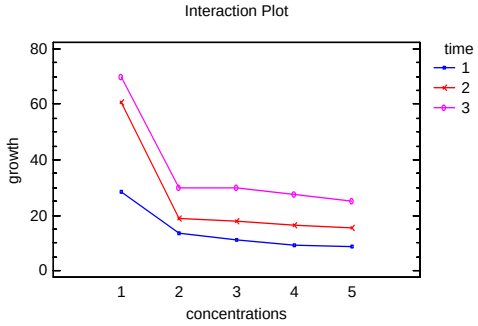

Fig. 3: Effect of interactions between conc. of ethanol extracts of pomegranate and time of incubation on mycelial growth of *Penicillium* spp.

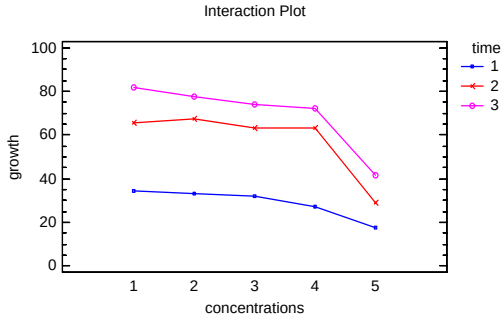

Fig. 4: Effect of interactions between conc. of ethanol extracts of pomegranate and time of incubation on mycelial growth of *Aspergillus niger*

Table 2: Effects of aqueous and ethanol extracts of Oak galls on the growth of Penicillium spp. and *Aspergillus niger*.

Treatments		Oak galls Extract (%)			
		Aqueous		Ethanol	
Time (hours)	Conc. (%)	*Penicillium* spp. Mycelial Growth (mm)	*Aspergillus niger* Mycelial Growth (mm)	*Penicillium* spp. Mycelial Growth (mm)	*Aspergillus niger* Mycelial Growth (mm)
		mean± S.E.	mean± S.E	mean± S.E	mean± S.E
48	0	28.5±1.56	34.5±0.65	28.50±1.68	34.5±0.65
	5	11.50±0.65	34.25±1.49	13.00±0.41	34.25±1.49
	10	11.00±0.58	26.75±1.38	11.75±0.63	26.75±1.38
	15	10.75±0.45	24.0±1.47	11.25±1.25	24.00±1.47
	20	9.5±0.50	23.5±1.45	7.00±0.00	23.50±1.49
	X^-	14.25±1.68 a	28.6±1.23a	14.30±1.96a	26.50 ± 1.33a
96	0	60.5±0.50	65.75±3.33	60.50±0.50	65.75±3.33
	5	25.25±0.75	48.25±1.44	26.25±0.48	48.25±1.44
	10	22.75±0.85	38.5±0.95	23.50±0.95	38.50±0.96
	15	19.50±0.50	34.75±1.25	19.50±2.53	34.75±1.25
	20	14.25±0.45	31.5±0.95	14.00±0.71	31.50±0.95
	X^-	28.45±3.78 b	43.75± 2.92b	28.75±3.80b	40.70± 3.31b
168	0	70.0±2.04	81.75±1.18	70.0±2.04	81.75±1.18
	5	34.75±0.85	62.25±1.03	47.50±2.50	62.25±1.03
	10	32.00±1.23	48.75±1.44	34.75±1.89	48.75±1.49
	15	28.50±1.45	43.0±1.08	28.50±1.55	43.00±1.58
	20	22.25±1.31	38.25±0.63	21.25±1.25	38.25±0.67
	X^-	37.50±3.89 b	54.8± 3.62c	40.80±3.94b	51.00± 4.12c
X^-	0	53.0± 4.33a	60.67± 6.02a	53.00±5.08c	60.67±6.01a
	5	23.83± 2.90b	48.25± 3.52ab	28.92±4.35b	44.33±3.52b
	10	21.92±2.64b	38.00± 2.8bc	23.33±2.91ab	35.17±2.17bc
	15	19.58±2.24b	33.92± 2.44bc	20.42±2.57ab	32.33±1.89cd
	20	15.33±1.65b	31.08± 1.9c	14.08±1.81a	24.50±1.47d
	X^-	26.73±2.24	42.38± 2.11	27.82±2.35	39.4±2.22

*Means with different letters in a same column show significant differences as determined by Duncan's multiple range tests ($\alpha = 0.01$).

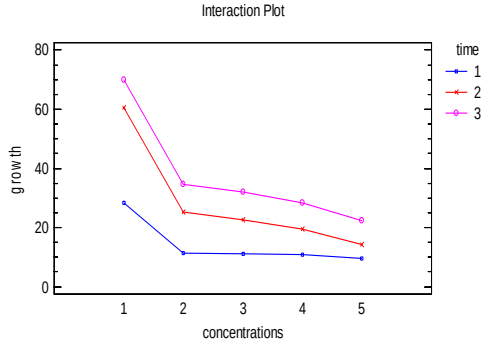

Fig. 5: Effect of interactions between conc. of aqueous extracts of oak galls and time of incubation on mycelial growth of *Penicillium* spp.

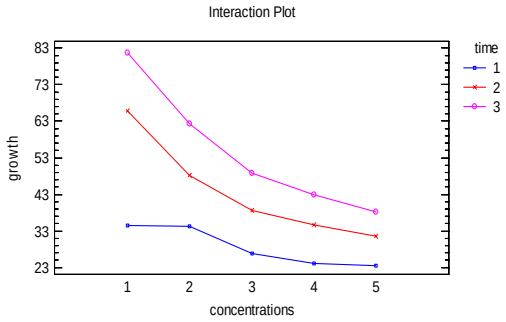

Fig. 6: Effect of interactions between conc. of ethanol extracts of oak galls and time of incubation on mycelial growth *Aspergillus niger.*

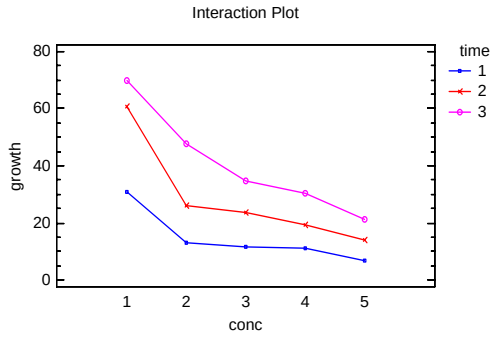

Fig. 7: Effects of interactions between conc. of ethanol extracts of oak galls and time of incubation on mycelium growth of *Penicillium* spp.

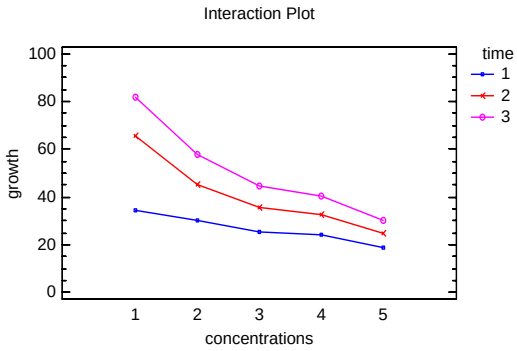

Fig.8: Effects of interactions between conc. of ethanol extracts of oak galls and time of incubation on mycelium growth of *Aspergillus niger.*

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