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Effect of Aqueous Extracts of *Punica* Granatum and *Quercus Infectoria* against *Penicillium* Spp. *and Cladosporium* Spp.Mycelial Growth

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Abstract

The experiments were conducted during 2011-2012 in the laboratories of Biology Department, Faculty of Science and Health, University of Koya , Kurdistan region Iraq by using completely randomized design (CRD) with three replications. The effects of aqueous extracts of pomegranate (*Punica granatum* L.) and oak gall (*Quercus infectoria* L.) at concentrations were determined against (0, 10, 20 and 25%) of raw extracts to control growth of *Penicillium* spp. and *Cladosporium* spp. after different times of incubation . The results revealed that *Penicillium* spp. was more sensitive to both extracts than *cladosporium* spp.. The aim of the present study was to evaluate the antifungal activity of the two plant extracts (oak gall and pomegranate) against *Penicillium* spp. and *Cladosporium* spp.

Keywords: Antifungal activity, *Penicillium* spp., *Cladosporium* spp., *Punica granatum*, *Quercus infectoria.*

1. Introduction

The plant fungal diseases have traditionally been controlled by chemical fungicides. The development of resistant strains of pathogens against various chemical fungicides (Lin, K.C. 1981 , Witte, W. 1998) and their harmful effects on soil biosphere, Sarmamy, A. O. I. (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 are one of several non-chemical control alternatives that inspiring great interest due to their availability, non-toxicity and friendliness to the environment Aqil, F., M. Zahin, etal,(2010). A number of

antifungal compounds of diverse skeletal patterns have been found in plants, these compounds belong mainly to six broad chemical groups such as phenol and phenolic acids, coumarins and pyrones, flavonoids, isoflavonoids, steroids, alkaloids, and miscellaneous compounds (Mitra, S.R., Choudhuri, A.and Adityachaudhury, N1984) . *Penicillium* spp. is an important post harvest pathogen that not only causes decay on fruits but also produces the carcinogenic mycotoxin patulin in spoiled fruit and processed fruit. *Cladosporium* spp. are pigmented moulds (dematicae) widely distributed in the air as well as decayed organic matter, and they are food contaminants. Some species are most widely distributed in the tropics and subtropics (Hoog GS,. *etal*, 2000 , Dixon DM, Polak-Wyss A, 1991). Plant extracts show antibacterial effects and antifungal activity against wide range of fungi (Sarmamy, A. O. I. ; Al-Juboory, B. A. 2005 Aba Alkhail, A. A. 2005 , Aba Alkhail, A. A. 2005 , Sarmamy A. O.I. ; Saleem, Z. K. 2009) .

2. Materials and Methods

2.1 Preparation of Fungi

Penicillium spp. and *Cladosporium* spp. were isolated from infected kiwi and onion respectively. 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).

2.2 Preparation of Extracts and Culture:

The epicarp of pomegranate fruits and oak galls were obtained from the local market at koya city. They were washed and dried at room temperature, each plant parts were ground separately by mortal pestle and weight 50gm of the powder of each plant material were added to 100ml of distilled water and put in an electric shaker for 24 hrs. Plant extracts were filtered by passing them through four folded layers of gauze. The extracts (50%w/v of each plant was used as stock solution) were kept in the refrigerator until use (Harborne, J. B. 1984) .

Table 1. Preparation of plant extracts concentrations

Plant extract Concentration	Stock solution 50% ml	Distilled water ml
Control 0%	0	20ml
10%	4 ml	10ml
20%	8ml	10ml
25%	10ml	0

After preparation of each concentration added to the sterilized potato dextrose agar medium (PDA) supplemented by the chloramphenicol was added to the media for preventing bacterial growth, 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. Each treatment

was replicated three times. Plates were incubated in an incubator at 25 ℃. Fungal growth after 5,7, and 9 days for *Penicillium* spp. and *Cladosporium sp* was measured by taking the mean of the two diameters taken at right angles for each colony.

3. Results and Discussion

3.1 Effects of Pomegranate Extract on Controlling Studied Fungi

Effects of aqueous extract of pomegranate:

The results show in (Table 2) that the aqueous extract of pomegranate affected significantly the growth of mycelium of *Penicillium* spp. and *Cladosporium* sp. at all extract concentrations used (10, 20 and 25%) by reducing the mycelial growth of *Penicillium* spp. and *Cladosporium* sp to 31.6 and 30-29 mm compared with control (42.6 mm) for the two fungi respectively. The growth of *Penicillium* sp and *Cladosporium* sp directly decrease according to concentration of plant extract (10,20and 25%) respectively that is shown by decrease of diameter of fungal colony both fungal isolate as compare to the control, the diameter of colony at 25% is smaller than other because contains highly concentrated of inhibitory affect. The diameter of *Cladosporium* sp is smaller than the *Penicillium* sp because *Penicillium* growth faster and also may be due to difference between the two fungi in their genetic construction or may be due to the difference in the chemical and structural composition of the cell walls. Time of incubation also affected the growth of mycelia significantly and the effects of extracts reduced as the time increased. The mycelial growth was 20 mm after 5 and 7 days of application. Figure 1 shows significant interactions between time of incubation and extract concentrations, and the highest effect was at concentration of 25% and 5 days of incubation (20 mm) compared with control and 7 days of incubation (30 mm) for *Penicillium* spp. and 13 mm at concentration of 25% after 5 days of incubation compared with 43 mm in control after 7 days of incubation in case of *Cladosporium* spp. (Figure 2).

The time of incubation also affected the growth of mycelia significantly and the effect of extract reduced as a time increase. Oak gall containing tannins Paaverurve, V. M. ; Raal, A. (2010) which have inhibitory effect on fungi Vonshak, A. etal,(2003), while pomegranate antifungal activity may be due to polyphenols and punicalagins (Seeram, N. P.; etal, 2006). The result of affect of epicarp of pomegranate on the *Penicillium* sp and *Cladosporium* spp. is shown in the table (2).

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Table 2. effect of aqueous extract of pomegranate on *Penicillium* sp and *Cladosporium* spp. on mycelia growth.

Treatment		Fungal species	
Time (days)	Concentration (%)	*Penicillium* spp. Mycelia growth (mm) Mean ±SD	*Cladosporium* spp. Mycelia growth (mm) Mean ±SD
5	0	42 a ±6.9	23a ±4.9
	10	31.6b ±4.7	18b ±2.8
	20	30b ±8.1	15.3b ±0.4
	25	29 c ±6.6l	14.6c ±12.7
7	0	55a ±8.1	37.3a ±6.1
	10	43.3b±6.2	33.3b±1.2
	20	36.3b ±5.3	25.3b±4.4
	25	35.6c ±7.5	25c ±12.5
9	0	64a ±10.1	47.6a ±6.2
	10	50 b ±8.1	38.6b ±6.2
	20	40b ±5.4	28.6b ±2.7
	25	39 c ±6.4	28c ±1.4

Means with different letters in a same column show significant differences as determined by Duncan's multiple range tests ($\alpha = 0.01$) it is (0.066) for *Penicillium* spp. And (0.114)for *Cladosporium* spp.

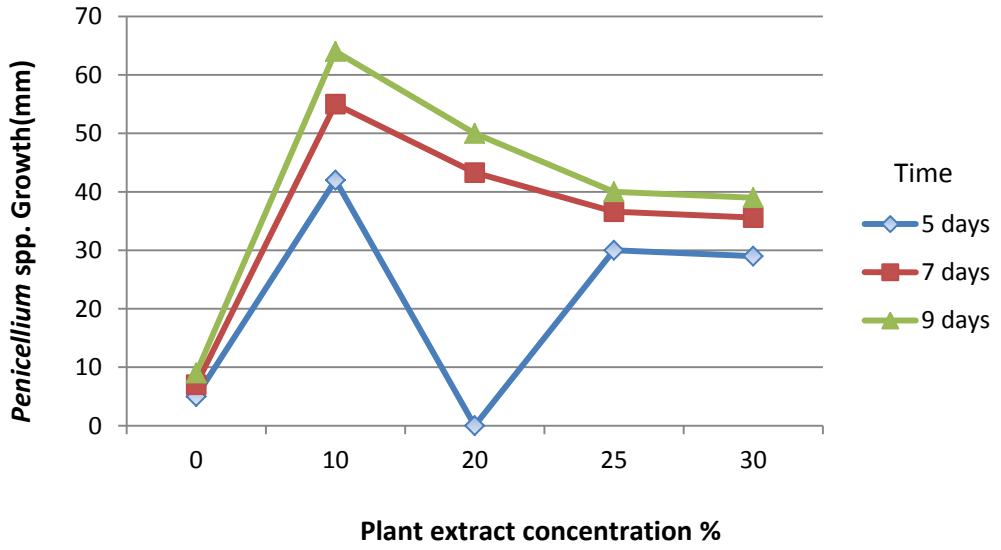

Fig. 1. Effect of interactions between Conc. of aqueous extracts of pomegranate and time of incubation on mycelia growth of *Penicillium* spp.

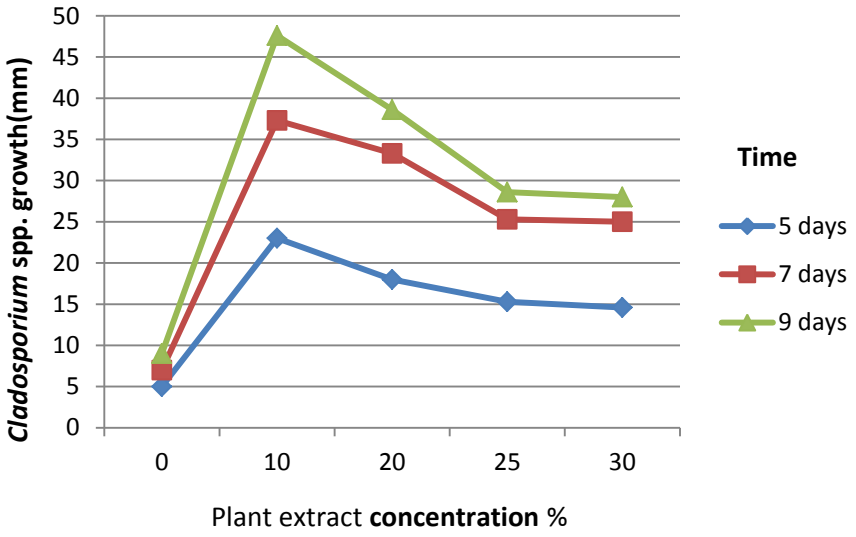

Fig. 2. Effect of interactions between conc. of aqueous extracts of pomegranate and time of incubation on mycelia growth of *Cladosporium* spp.

3.2 Effects of Oak Galls Extract on Controlling the Studied Fungi

Table 3 shows that the aqueous extract of oak galls at all concentrations affected significantly the growth of mycelia of *Penicillium* spp. and *Cladosporium* sp. Reduced the mycelia growth of the two fungi to (29-14.6 and 29.6-15.3 mm compare with 42.6 and 23 mm for the control respectively. Time of incubation also affected the growth of mycelia significantly and the effects of extracts reduced as the time increased. Figure 3 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. (Figure 4) show the mycelia growth was 35-25mm after 5 days of incubation and decreased to 24 mm at 20% of the extract after 5days of incubation. The interaction between extract percentages and time is clear and the highest effects were 45mm compared with 70 mm for control after 9 days.

Table 3. effect of aqueous extract of oak gall on *Penicillium* spp. and *Cladosporium* spp. Mycelia growth.

Treatment		Fungal species	
Time (days)	Concentration (%)	*Penicillium* spp. Mycelia growth (mm)Mean ± SD	*Cladosporium* spp. Mycelia growth (mm) Mean ± SD
5	0	42.6 a ±6.0	23 a ±4.9
	10	31.3 b ±5.7	16.3 b ±2.0
	20	29.6 b ±8.9	16 b ±2.1
	25	29 c ±3.9	14.3 c ±3.0
7	0	55 a ±7.0	37.3 a ±6.1
	10	39.6 b ±7.0	27.3 b ±1.2
	20	34 b ±7.4	24.6 b ±3.8
	25	33.3 c ±5.4	17.6 c ±3.8
9	0	64 a ±5.6	47.6 a ±3.8
	10	46.6 b ±8.8	31 b ±1.6
	20	40 b ±9.3	29.6 b ±5.2
	25	38.3 c ±5.4	20 c ±2.1

Means with different letters in a same column show significant differences as determined by Duncan's multiple range tests ($\alpha = 0.01$) it is (0.035) for *Penicillium* spp. And (0.33)for *Cladosporium* spp.

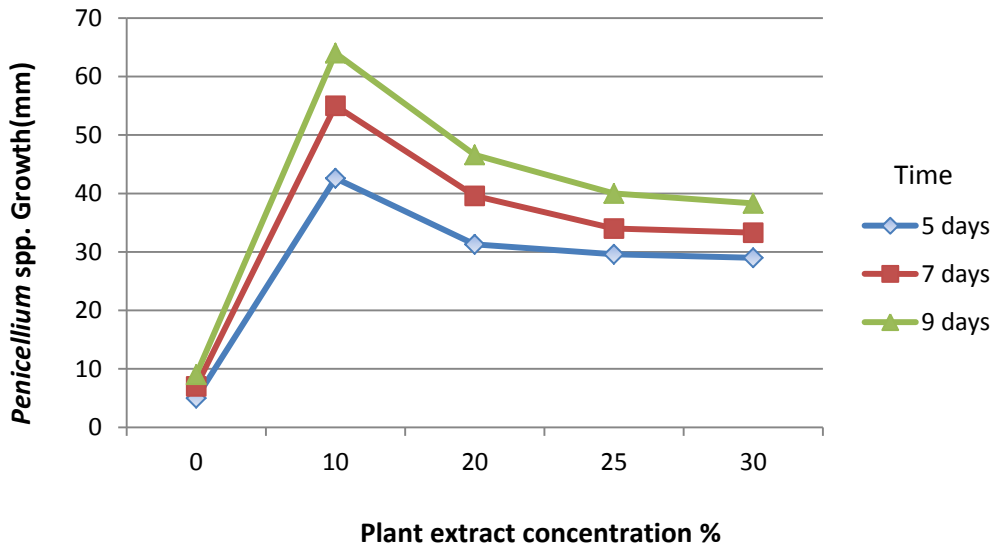

Fig. 3. Effect of interactions between conc. of aqueous extracts of oak and time of incubation on mycelial growth of *Penicillium* spp.

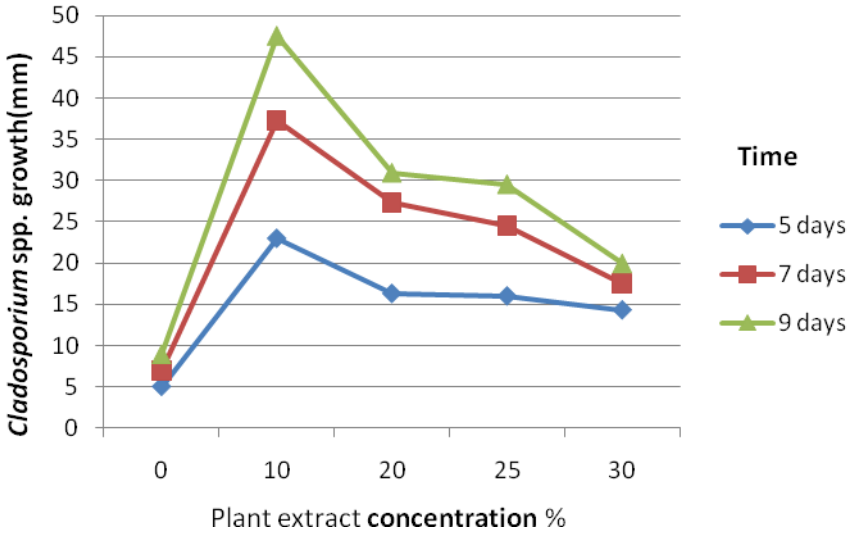

Fig. 4. Effect of interactions between conc. of aqueous extracts of oak and time of incubation on mycelia growth of *Cladosporium* spp.

4. Conclusion

The aqueous extract of pomegranate affected significantly the growth of mycelium of *Penicillium* spp. and *Cladosporium* sp by reducing the mycelial growth Time of incubation also affected the growth of mycelia significantly and the effects of extracts reduced as the time increased.

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