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# Antifungal activity of olive cake extracts

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**Summary.** Powdered, dried olive (*Olea europaea*) cake was extracted with hexane, methanol and butanol. Six phenolic compounds, coumaric acid, ferulic acid, oleuropein, caffeic acid, protocatechuic acid and cinnamic acid, were isolated from these extracts after fractionation. The fractions were tested for their antifungal activity against *Verticillium* sp., *Fusarium oxysporum*, *Rhizopus* sp., *Penicillium italicum*, *Rhizoctonia solani*, *Stemphylium solani*, *Cladosporium* sp., *Mucor* sp., *Colletotrichum* sp. and *Pythium* sp. Strongest activity was reported against *Fusarium oxysporum* and *Verticillium* sp. No effect was observed against *Alternaria* sp.

**Key words:** phenolic compounds, *Olea europaea*, Jordan, antifungal activity, olive cake.

## Introduction

In Jordan, olive (*Olea europaea* L.) is officially considered the national crop. The amount of olives produced in 1998 was 177,000 tons, the greatest crop of all fruit trees in Jordan (Anonymous, 1999). Almost every house, backyard and sidewalk in Jordan has one or more olive trees.

Virgin residue, or residue, is the solid obtained from pressing olives, and consists of fragments of skin, pulp, stone and seed. It retains the natural fat and moisture of olives. Dried press olive cake is the residue from which the main particles of stone have been removed. The amount of olive cake from mechanical processing varies according to the type of olive and the method of pressing and ranges from 25 to 50 percent of the weight of olives pressed. After an exceptionally dry year, the figure may be as high as 70 percent, but the usual percentage is between 35 and 40 percent (Frezzotti *et al.*, 1956; Al-Bana and Hijazi, 1987; Kivitsakis, 1990; Qalalweh, 1995).

The commercial value of olive cake depends on its water and fat content. Moisture usually accounts for between 20 and 30 percent of the weight, while the fat content varies from a minimum of about 2.5 percent of the weight to 10–12 percent, and in some cases even more, depending on the extraction process used. In addition to its

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use as stock feed for animals, the residue can also be used as a fertilizer, after it has been properly steeped to avoid fermentation, which would damage the plants (Al-Momany and Al-Saket, 1989). The stone fragments obtained from the husks are also excellent as fuel, developing almost as many calories as coke. Excellent ash is obtained from burnt virgin residues and the exhausted residue of skins after the fat and the fragments of olive stones have been extracted. This ash is a good fertilizer owing to its phosphorus, potash and calcium contents (Frezzotti *et al*., 1956; Mohammad *et al*., 1993).

Studies have also shown the importance of natural chemicals as a possible source of non-phytotoxic, systemic and easily biodegradable alternative pesticides (Fawcett and Spencer, 1970; Bye, 1978). Surveys have shown that the extracts of many plant species have antifungal activities (Osborn, 1943; Spencer *et al*., 1957; Dixit and Tripathi, 1975; Lapis and Dumancas, 1978; Franje, 1984; Guesin and Reveillere, 1984; Deans and Svoboda, 1990).

Studies on the antifungal effects of olive cake are rare. The antimicrobial capacity of eight phenolic compounds isolated from olive cake and identified by Abo-Zaid *et al*. (1993) was tested by Aziz *et al*. (1998) against the pathogenic bacteria *Escherichia coli*, *Klebsiella pneumonia* and *Bacillus cereus,* and the mycotoxigenic fungi *Aspergillus flavus* and *A. parasiticus*. The antimicrobial activity found ranged from no effect to complete growth inhibition. Some researchers report that most phenolic derivatives have a fairly broad spectrum of action which is both bacteriostatic and fungistatic. That these derivatives inhibited *Saccharomyces cerevisiae*, *Aspergillus niger* and *Trychophyton rubum* was reported by Baranowski *et al*. (1980) and by Gourma *et al*. (1989). Several food products are susceptible to contamination by many species of fungi, including *Aspergillus*, *Penicillium*, *Claviceps*, *Alternaria* and *Phoma* (Aziz, 1987). The effect of antimicrobial agents on the growth and aflatoxin production of the *Aspergillus flavus* group was assessed by Uraih *et al*. (1977) and El-Far *et al*. (1992). No further research has been conducted on the effect of olive cake extracts on plant pathogenic fungi. Therefore, we investigated the possible antifungal effect of eight olive cake extracts on eleven of the most destructive plant pathogenic fungi frequently isolated from crops and soils in Jordan and worldwide (Table 1).

## Materials and methods

### Extraction and purification of active compounds

Olive cake (7 kg) was obtained in 1998 from a local olive oil mill (Zobia area, 70 km northwest of Amman, Jordan) and percolated with 96% ethanol (11 l). The solvent was evaporated to leave an extract (754 g) which was dissolved in water (1 l) and then extracted three times, with 500 ml hexane, chloroform and butanol respectively. The butanolic extract (E1=100 g) was chromatographed by silica gel column (350 g, 5x90 cm). Fractions were collected and eluted with a gradient $CHCl_3$-meth-

Table 1. Fungal isolates used to study the antifungal activity of olive cake extracts obtained from different locations in Jordan.

| Fungus | Host | Plant part | Location | Date of isolation |
|---|---|---|---|---|
| *Verticillium* sp. | Tomato | Roots | Mafraq | 12 July 1999 |
| *Rhizopus* sp. | Tomato | Leaves | Yadoda | 30 June 1999 |
| *Fusarium oxysporum* | Cucumber | Root | Jerash | 23 June 1999 |
| *Penicillium italicum* | Pepper | Stem & leaves | Jerash | 05 June 1999 |
| *Rhizoctonia solani* | Cucumber | Roots & stem | Jerash | 05 June 1999 |
| *Stemphylium solani* | Tomato | Stem & leaves | Jerash | 05 June 1999 |
| *Alternaria* sp. | Cucumber | Leaves | Jerash | 23 June 1999 |
| *Cladosporium* sp. | Petunia | Stem | Jerash | 23 June 1999 |
| *Mucor* sp. | Gerber | Roots | Amman | 21 July 1999 |
| *Colletotrichum* sp. | Dieffenbachia | Stem | Baqa' | 23 June 1999 |
| *Pythium* sp. | Thyme | Roots & stem | Yadoda | 14 June 1999 |

anol (100–30%). Fractions that were similar according to TLC analysis using petroleum ether:ethyl acetate (7:3) as a solvent system were combined. The phenolic compound detected in the *n*-hexane extract (E2=2.6 g) was purified by column chromatography (125 g, 2.5x90 cm) using petroleum ether:$CHCl_3$ (100:70 v/v). The $CHCl_3$ extract (E8) was analysed by TLC (silica gel, eluent as above).

The structure of the pure fractions was established by $^{1}$H-NMR, $^{13}$C-NMR, MS and by direct comparison with authentic samples.

**Antifungal activity**

The fungi used in this study were collected from various locations in Jordan (Table 1). All fungal isolates were identified at species or genus level and deposited in the fungal collection bank at the Department of Biotechnology of Al-Balqa' Applied University, Al-Salt, Jordan.

Fungal isolates were maintained on potato dextrose agar (PDA, Difco Laboratories, Detroit, MI, USA), and the cultures were stored at room temperature and subcultured once a month (Deans and Svoboda, 1990). The medium (15 ml per plate) was dispensed to sterile Petri plates and allowed to cool down before use. The isolates were allowed to grow for 7–10 d before they were used in the microbial studies.

Three olive cake crude extracts (E1, E2 and E8) and five butanolic fractions (E3–E7) (Table 2) were diluted with sterile distilled water (SDW) to give a final concentration of 1000 ppm each (Carter, 1968). Each extract solution was evenly distributed on PDA in the designated Petri plates at 2 ml of solution per plate. Control plates received 2 ml of SDW each. Plates were left overnight for the solutions to be absorbed through the media.

A plug of inoculum from the actively growing margin of a Petri plate culture of each fungal isolate (Table 1) was placed face down in the center of each Petri plate using a 10-cm-long spring-loaded plunger of 5-mm diameter. Each isolate from each extract was inoculated onto four plates and incubated for 9 d at room temperature (~22°C). Four control plates receiving SDW only were run along each fungal isolate and crude extract, following the same procedure as in the samples.

Starting two days after inoculation, radial growth was recorded daily for 7 d, or until the plates were overgrown. The percentage of fungal growth inhibition caused by each crude extract was calculated as follows: percentage inhibition = [(growth in control – growth in sample)/ (growth in control) x 100] where growth was measured in mm as colony diameter (Daouk *et al*., 1995). The values reported for percent inhibition were the means of four determinations each. Standard errors were calculated and are shown.

Table 2. Inhibition of fungal growth (%)[a] by the organic extracts from olive cake and some of their phenolic components.

| Fungus | Extract[b] | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | E1 | E2 | E3 | E4 | E5 | E6 | E7 | E8 |
| *Verticillium* sp. | 100.0 | 27.4±1.2 | 100.0 | 100.0 | 17.2±2.6 | 100.0 | 65.7±6.6 | 11.4±1.3 |
| *Fusarium oxysporum* | 100.0 | 100.0 | 100.0 | 69.4±4.9 | 0.0 | 100.0 | 38.1±4.8 | 100.0 |
| *Rhizopus* sp. | 85.0±0.8 | 54.2±4.8 | 10.6±3.6 | 75.0±3.2 | 78.4±1.7 | 16.9±5.4 | 17.7±4.4 | 66.3±5.5 |
| *Penicillium italicum* | 59.3±5.5 | 0.0 | 58.8±5.9 | 55.9±4.4 | 70.1±0.7 | 59.4±5.2 | 29.5±2.2 | 0.0 |
| *Rhizoctonia solani* | 44.2±3.1 | 27.3±0.4 | 70.5±5.9 | 29.9±3.0 | 0.0 | 88.2±1.2 | 41.4±4.6 | 31.5±0.03 |
| *Stemphylium solani* | 1.7±0.0 | 2.1±0.1 | 1.2±0.4 | 0.8±0.8 | 1.2±0.4 | 4.1±0.5 | 10.7±4.5 | 1.2±0.1 |
| *Alternaria* sp. | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |
| *Cladosporium* sp. | 2.6±0.3 | 7.2±0.7 | 1.9±0.2 | 1.6±0.2 | 5.7±0.9 | 1.9±0.6 | 24.1±1.8 | 22.7±2.0 |
| *Mucor* sp. | 49.2±4.1 | 10.6±0.8 | 53.3±0.5 | 13.8±0.8 | 35.1±3.6 | 54.5±6.0 | 20.7±3.1 | 7.5±0.9 |
| *Colletotrichum* sp. | 31.5±3.5 | 15.0±1.0 | 7.5±0.8 | 30.0±2.9 | 40.0±4.9 | 22.0±3.1 | 29.7±3.3 | 42.5±2.5 |
| *Pythium* sp. | 0.0 | 16.7±0.7 | 0.0 | 0.0 | 35.8±2.6 | 29.6±1.9 | 10.7±1.1 | 47.6±3.4 |

[a] Percentage of inhibition (growth in control – growth in sample/growth in control x100) is the mean ± SE of four determinations.
[b] E1, butanol crude extract; E2, caffeic acid; E3, coumaric acid; E4, protocatechuic acid and oleuropein; E5, ferulic acid; E6, cinnamic acid; E7, mixture of compounds; E8, unidentified flavonoids.

## Results and discussion

Five known compounds were isolated from the butanol extract, and their structures were established as protocatechuic acid (from fraction E4 to E6), coumaric acid (E3), ferulic acid (E5), cinnamic acid (E6) and oleuropein (E4). Fraction E7 revealed a mixture of compounds that have not been identified. The hexane extract (E2=2.6 g) mainly revealed caffeic acid. Inspection of the $CHCl_3$ extract (E8) with TLC revealed different flavonoids. None of these flavonoids were purified because of their scarcity.

Antifungal activity assays (Table 2) clearly indicated that these phenolic compounds had a good antifungal activity.

With the exception of *Alternaria* sp., all fungi tested showed various degrees of sensitivity to five or more of the extracts tested. There was a total growth inhibition of *Verticillium* sp. when the PDA plates were fortified with extracts E1 (butanol crude extract), E3 (coumaric acid), E4 (protocatechuic acid) or E6 (cinnamic acid) (Table 2). Percent inhibition with other extracts ranged from 11.4% to 65.7%. With *Fusarium oxysporum*, extracts E1, E2 (caffeic acid), E3, E6 and E8 (unidentified flavonoids) caused total growth inhibition. Only E5 (ferulic acid) had no effect on the growth of *F. oxysporum*. Percent inhibition of *F. oxysporum* with E4 and E7 (mixture of compounds) was 69.4±4.9 and 38.1±4.8 respectively.

For *Rhizopus* sp., 85% of fungal growth was inhibited with E1, followed by E5 (78.4%), E4 (75%), E8 (66.3%) and E2 (54.2%). E3, E6 and E7 had a weaker antifungal effect against *Rhizopus* sp. About 70% of *Penicillium italicum* growth was inhibited when the medium was fortified with E5. E1, E3, E4 and E6 inhibited growth by between 55.9 and 59.4%. E7 had a low inhibition activity, and E2 and E8 had no effect. Against *Rhizoctonia solani* E5 was not effective, E2, E4 and E8 showed slight inhibitory activity, E1 and E7 moderate activity, while E6 was the most effective (88.2%) followed by E3 (70.5%). Moderate antifungal activity was observed when the samples E1 and E7 were used, while low activity was observed with E2, E4 and E8. All extracts showed very low antifungal activity against both *Stemphylium solani* and *Cladosporium* sp. Moderate growth inhibition of *Mucor* sp. was observed when extracts E1, E3 and E6 were added to the growth medium; lower inhibition, ranging between 7.5 and 35%, was obtained with the other extracts. E5 and E8 were moderately effective against *Colletotrichum* sp., the other extracts less so, with growth inhibition ranging from 7.5 to 31.5%. Moderate inhibition activity (47.6%) was obtained against *Pythium* sp. when E8 was added to the medium, lower activity (10.7–35.8%) with E2, E5, E6 and E7, and no activity was obtained with E1, E3 and E4.

The importance of indigenous products for plant disease control has been investigated in other studies, and encouraging results are reported (Misra and Dixit, 1976; Chaudhuri, 1982; Mahmood, 1985; Akhtar *et al*., 1986; Asthana *et al*., 1986; Chaturvedi *et al*., 1987; Al-Abed *et al*., 1993). However, this is the first paper on the antifungal activity of olive cake extracts against phytopathogenic fungi.

Olive cakes used as fertilizers also enhance the effect of the mycorrhizal fungus *Glomus fasciculatum* inoculated on olive seedlings. When different amounts of olive cakes were added to olive seedlings inoculated with *G. fasciculatum* to study its effect on plant growth for 1.5 years under greenhouse conditions, it was found not only that the mycorrhizal olive seedlings grew better than the non-mycorrhizal plants (Al-Momany and Al-Saket, 1989), but that the addition of olive cakes to the inoculated plants at 10 or 20% of soil weight gave an additional improvement in growth.

The findings indicate that olive cakes used as fertilizer also control fungal growth (Table 2). This could have a very significant impact on olive cultivation by reducing the need for pesticides, and consequently lowering costs. From the environmental point view, this would reduce the adverse effect of chemical pesticides on the environment by transforming olive cake, which is currently a waste product left in large quantities in olive press stations, into an effective and inexpensive fertilizer and antifungal agent. The findings in this study are in agreement with the notes and results reported by Frezzotti *et al*. (1956) and Mohammad *et al*. (1993).

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