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Wild honey inhibits growth of some phytopathogenic fungi *in vitro*

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Summary. Wild honey was diluted to 1000 ppm with sterile distilled water and tested *in vitro* for inhibition of the plant pathogenic fungi *Fusarium oxysporum*, *Rhizoctonia solani*, *Alternaria solani*, *Stemphylium solani*, *Colletotrichum* sp., and *Phytophthora infestans*. Wild honey was effective against all these fungi, particularly *A. solani* and *P. infestans*, the causal agents of early and late blight diseases respectively; also against *R. solani* and *F. oxysporum*, and to a less extent against *S. solani* and *Colletotrichum* sp. This is the first report on the inhibiting effect of wild honey against plant pathogenic fungi.

Key words: antifungal activity, *in vitro* inhibition.

Introduction

It is well established that honey inhibits a broad spectrum of bacterial species and there are many reports of its bactericidal as well as its bacteriostatic activity. There are also reports of honey being an antifungal agent. These reports were comprehensively reviewed by Molan (1992a,b).

In almost all reports on honey as an antibacterial agent, the selection or floral source of the honey is not considered. Aristotle, around 350 BC, and Dioscorides, about 50 AD (Gunther, 1959), recommended that honey collected in specific regions and seasons (and therefore presumably from different floral sources) be used for the treatment of particular aliments, but modern clinical practitioners have not heeded these views, nor indeed the laboratory findings that large differences exist in the antibacterial potency of different honeys. These differences were recognized more than 40 years ago, and a method was then devised to determine the "inhibine number" of a type of honey as a measure of its antibacterial activity (White *et al.*, 1963). The "inhibine number" is the degree of dilution to which a honey will retain its antibacterial activity, representing a sequential dilution of honey in steps of 5%, from 25 to 5%. A study of 345 honey samples from New Zealand (Allen *et al.*, 1991a) found many honeys with low activity and 36% of samples with activity near or below detectable levels. The main variations in overall antibacterial activity were due to variations in the level of hydrogen peroxide in the honey. Hydrogen peroxide, produced from the oxidation of glucose by the enzyme glucose oxidase, which is formed in the glands of bees (White, 1978), is the principal antimicrobial factor in honey.

In this study, the antifungal effects of diluted

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wild honey on six species of fungi collected from diseased plants in Jordan were investigated. This is the first report on wild honey as an agent inhibiting the growth of plant pathogenic fungi.

Materials and methods

Wild honey was extracted from the hives of wild bees in the Jordan Valley. Honey was kept at 4°C away from direct light before use. A voucher specimen was deposited in the author's research laboratory at the Department of Biotechnology, Faculty of Agricultural Technology, Al-Balqa' Applied University, Al-Salt, Jordan.

Six species of fungi were isolated from samples of different crops collected from five locations in Jordan: *Alternaria solani* and *Phytophtora infestans* from potato leaves; *Rhizoctonia solani* from the root of cucumber plants; *Fusarium oxysporum* from potato roots; *Stemphylium solani* from tomato leaves; and *Colletotrichum* sp. from the stems of Dieffenbachia. All fungal isolates were identified microscopically, and samples of each fungus were deposited in the fungal collection bank at the Department of Biotechnology, Al-Balqa' Applied University, Al-Salt, Jordan.

Fungal isolates were maintained on potato dextrose agar (PDA) (Difco, Detroit, MI, USA), and the cultures were stored at room temperature and subcultured once a month (Deans and Svoboda, 1990).

A 1000-ppm dilution of wild honey was prepared using sterile distilled water (SDW). The solution was shaken vigorously in a volumetric flask to dissolve the honey (Carter, 1968). Two-ml aliquots of solution were evenly distributed on PDA in each Petri plate. Control plates received 2 ml SDW each. Plates were left overnight to allow the solutions to be absorbed through the media.

With a 10-cm-long spring-loaded plunger of 5 mm diameter, a plug of inoculum from the actively growing margin of a 7–10-day-old Petri plate culture of each fungal isolate was removed and placed in the center of each plate with the mycelium face down. Each isolate was inoculated onto eight plates and incubated for 7 days at room temperature (~22°C). Eight control plates receiving only SDW were run along with each fungal isolate and crude extract.

Starting two days after inoculation, radial growth was recorded daily for 7 days or until the plates were overgrown. Percent fungal inhibition due to wild honey was calculated using the following formula: $\% \text{ Inhibition} = [(C-T)/C] \times 100$, where C is the mean diameter of the control colonies, and T the mean diameter of the colonies grown on inhibitory media with honey (Daouk *et al*., 1995). The mean values of three trials and standard deviations were calculated.

Results and discussion

Diluted wild honey had an antimicrobial effect *in vitro* on phytopathogenic fungi. It was highly effective against the early blight fungus *Alternaria solani*, of which it inhibited colony growth by 42±4.3%, and against the potato pathogen *Phytophthora infestans*, with a mean inhibition of 38.2±3.3%. Wild honey further inhibited *R. solani* and *F. oxysporum*, and, to a less extent, *S. solani* and *Colletotrichum* sp. (Table 1).

Antimicrobial activity in honey is mainly due to hydrogen peroxide produced enzymatically in the honey (Adcock, 1962; White *et al*., 1963). The glucose oxidase enzyme is secreted from the hypopharyngeal gland of the bee into the nectar to assist in the formation of honey by the nectar (Haydak *et al*., 1975). The hydrogen peroxide and the acidity produced serve to preserve the honey, but the hydrogen peroxide acts in this way only during the honey ripening process. Diluted wild honey was used in this study because full-strength honey has only negligible levels of hydrogen peroxide, which is short-lived in the presence of transition metal ions and ascorbic acid in the honey, being catalyzed by them into oxygen and water (Stinson, 1960). Glucose oxidase is practically inactive in full-strength honey, and gives rise to hydrogen peroxide only when the honey is diluted. This is because the acidity produced by the enzyme lowers the pH to a point which is too low for the enzyme to work. When honey is diluted, enzyme activity increases by a factor of 2,500–50,000, giving a "slow-release" antiseptic at a level which is antimicrobial but not tissue-damaging (White *et al*., 1962).

The evidence that honey contains other antimicrobial factors is mainly because the peroxide-generating system does not account for all of the antimicrobial activity observed (Radwan *et al*., 1984; Morse, 1986). Furthermore, heating honey inactivates the glucose oxidase, and while this entails

Table 1. *In vitro* inhibition of fungal growth by wild honey solution.

Trial[a]	Inhibition (%)[b]					
	Alternaria solani	*Phytophthora infestans*	*Rhizoctonia solani*	*Fusarium oxysporum*	*Stemphylium solani*	*Colletotrichum* sp.
1	41.9	41.6	19.7	11.7	0.9	0.9
2	43.1	39.8	21.6	8.3	0.7	0.8
3	40.8	33.3	28.5	6.8	0.9	0.8
Mean	41.9	38.2	23.3	8.9	0.8	0.8
S.D.	4.3	3.3	2.8	0.7	0.01	0.01

[a] Three separate trials were conducted, and the mean value and the standard deviation (SD) were calculated.

[b] Percent inhibition in each trial was the mean of eight replications for each species of fungus. Inhibition = $[(C-T)/C] \times 100$, where C is the mean diameter of mycelium of the controls, and T the mean diameter of mycelium from each fungus. Treatments were based on 1000 ppm wild honey solution.

the loss of activity against some species, it is retained against others (Dold *et al*., 1937). In fact, several chemicals with antibacterial activity have been identified in honey by various researchers: pinocembrin, terpenes, benzyl alcohol, 3,5-dimethoxy-4-hydroxybenzoic acid (syringic acid), methyl 3,5-dimethoxy-4-hydroxybenzoate (methyl syringate), 3,4,5-trimethoxybenzoic acid, 2-hydroxy-3-phenylpropionic acid, 2-hydroxybenzoic acid and 1,4-dihydroxybenzene. However, the quantities of these chemicals were far too small to account for any significant antibacterial activity.

Honeys from different sources have different antimicrobial activities. Honeydew honey from the conifer forests of the mountainous regions of central Europe has particularly strong antibacterial activity (Buchner, 1966; Chambonnaud, 1966; Sedova and Usmanov, 1973). Honey from manuka (*Leptospermum scoparium*) in New Zealand also has strong antibacterial activity, about half of which consists of its exceptionally strong non-peroxide activity (Allen *et al*., 1991b). Thus it is important that honey intended as an antimicrobial agent should be of a type assayed in the laboratory and known to be antimicrobially active. Such honey must also be stored at a low temperature and not be exposed to light, so that its glucose oxidase activity is not lost. Although all honey will stop the growth of bacteria because of its high sugar content, when the sugars are diluted, as by body fluids, this antibacterial action is lost. The other antimicrobial components then become important.

In a recent study at Michigan State University, Kirk (2002) compared chlorine dioxide and hydrogen peroxide as potential sanitary control agent of dry rot (*Fusarium* sp.), soft rot (*Erwinia carotovora*) and late blight (*Phytophthora infestans*), and examined the secondary infections they caused in potatoes. These sanitary agents reduced tuber breakdown in storage without causing significant damage to the tubers. Kirk (2002) found that hydrogen peroxide, which is one of the components of diluted honey, has the potential to control plant pathogenic fungi, and his findings therefore validate the results of the present study.

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