Inhibitory effect of pollen and propolis extracts

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Bee pollen and propolis were collected from *Apis mellifera* colonies in five regions of Turkey. The antifungal properties of methanol extracts of pollen and propolis (2% and 5% concentrations) were determined on *Alternaria alternata* and *Fusarium oxysporium f.* sp. *melonis*. The least active concentration towards the tested fungi was 2% concentration of both extracts. The inhibitory effect of all propolis extracts on growth of *F. oxysporium* and *A. alternata* were generally higher when compared with pollen extracts. The growth of *A. alternata* and *F. oxysporium* were not affected at both concentrations of pollens. However, *F. oxysporium* against propolis extracts was more sensitive than *A. alternata* ($P < 0.01$). None of the pollen extracts tested completely inhibited mycelial growth of fungi used in our experiment. Percent inhibition of both pollen concentrations against *A. alternata* and *F. oxysporium* was lower than 50%. However, both concentrations of Alanya and Beyşehir propolis extracts were 100% effective on mycelial growth of *F. oxysporium* until the 7th day of incubation ($P < 0.01$). 2% Alanya and Beyşehir pollen extracts completely stimulated mycelial growth of *F. oxysporium* on the 7th day of incubation. Both concentrations of propolis extract showed more than 50% inhibition against *F. oxysporium*. It is suggested that high concentrations of propolis extract could be used as an antifungal agent against tested fungi.

1 Introduction

"Bee pollen" is actually pollen from flowers that is collected from bees as they enter the hive or is harvested by other means. Pollen granules stick to the bees' legs and other body parts as they help themselves to nectar (the precursor of honey) inside the flowers [1]. Pollens are the male reproductive cells of flowers. Flower pollens, bees' primary food source, contain concentrations of phytochemicals and nutrients and are rich in carotenoids, flavonoids and phytosterols [2]. Pollen or pollen products have been shown to have several beneficial applications for human use. Pollen has been successfully used for treatment of some cases of benign prostatitis 18–22 and for oral desensitisation of children who have pollen allergy [3, 4]. Pollen is essentially concentrated pollens from flowers and, as a result, its nutrient content may vary. As natural food, bee pollen contains most of the known nutrients. In addition to most vitamins and minerals, bee pollen also provides amino acids, enzymes and coenzymes, fatty acids, carbohydrates and 25% protein by weight. Bee pollen has antimicrobial effects but more common claim is that it increases energy levels [5, 6].

Propolis is a resinous substance collected by worker honey bees from the growing parts of trees and shrubs. The bees pack the propolis on their hind legs, and carry it back to their colony, where it is combined with beeswax and used by worker "hive" bees as a sealant and sterilant in the colony nest [7, 8]. Propolis has been used by man since early times, for various purposes, and especially as a medicine because of its antimicrobial properties [8]. Records from 12th century Europe describe medical preparations using propolis for the treatment of mouth and throut infections, and dental caries [9]. The most important pharmacologically active constituents in propolis are the flavones, flavonols and flavonones, and various phenolics and aromatics. Flavonoids play a major role in plant pigmentation. Flavonoids are thought to account for much of the biological activity in propolis [10, 11]. Active components of propolis showing an antibacterial effect include pinocembrin, galangin, caffeic acid and ferulic acid. Antifungal components include pinocembrin, pinobanksin, caffeic acid, benzyl ester, sakuraretin and pterostilbene. Anti-viral components include caffeic acid, lutseolin and quersetin [12–14]. Bee propolis protects the hive from harmful bacteria, viruses and fungi. It is said that propolis was used as a perfect antibiotic agenst [10, 14–18]. Recently, investigations have indicated that interest for natural preservatives had increased [7, 8, 19, 20]. The antimicrobial properties of propolis have been known for many years. Several published reports describe the effect of propolis on a variety of microorganisms as reviewed by Diğrak *et al.* [21], Grange [10] and Özcan [20]. Considerable variations in resistance of different microorganisms against propolis were observed [10, 20–23]. The use of propolis that is nontoxic as alternate preservative agent is considered by consumers as safe [7, 8].

The aim of the present study was to examine the inhibitory effect of pollen and propolis extracts at the different concentrations on mycelial growth of *Alternaria alternata* and *Fusarium oxysporiumf.* sp. *melonis* in microbial media.

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Keywords: Antifungal effect / Pollen / Propolis

2 Materials and methods

2.1 Materials

Bee pollen and propolis samples were collected from hives at different regions (Alanya, Antakya, Beyşehir, Hadim and Taşkent) of Turkey. The organisms (*Alternaria alternata, Fusarium oxysporium f.* sp. *melonis*) used in this experiment were obtained from the Department of Plant Protection, Faculty of Agriculture, Selçuk University. Czapek-Dox agar was used as main medium throughout the study. A 150 g sample of pollen and propolis was extracted for 8 h in a Soxhlet apparatus with 125 mL methanol at 70 °C. The crude extracts were pooled and concentrated in a rotary evaporator, then kept in small (20 mL) sterile color bottles under refrigerated conditions until usage. Pollen and propolis extracts were added to Czapek-dox agar with proper amounts in order to prepare 250 mL of 2% and 5% concentrations of extract-containing medium. Each medium was dispensed in 250 mL quantities into 500 mL Erlenmeyer flasks and sterilized by autoclaving at 121 °C for 15 min.

2.2 Assessment of inhibition of fungal growth

The effects of pollen and propolis extracts at two concentrations (2% and 5%) were determined on mycelial growth of *Alternaria alternata* and *Fusarium oxysporium f.* sp. *melonis* using Czapek-dox agar. Medium was dispensed into each petri plates as 20 mL. Five mm discs of the test fungi grown in Czapek-dox agar were cut from periphery of 7 days old cultures, then inoculated upside down separately onto each assay plate and incubated at 28 °C. Three replicates of each treatment were similarly maintained and averages calculated. Control sets were simultaneously run without using pollen and propolis extracts. The colony diameter (mm) was measured and percent mycelial inhibition calculated as following [24]: $I = (C–T/C) \times 100$ where is I is inhibition (%), C is the colony diameter of mycelium from a control petri plate (mm), and T is the colony diameter of mycelium from a test petri plate (mm).

2.3 Statistical analyses

Minitab program was used for statistical analysis [25]. The Duncan Multiple Range Test (DMRT) was applied at the comparison of the averages. Furthermore, in order to make single comparisons, LSD test was applied [26].

3 Results and discussion

The *in vitro* antifungal activity of pollen and propolis extracts at different concentrations was established against *Alternaria alternata* and *Fusarium oxysporium f.* sp. *melonis* (Tables 1–4). The inhibitory effect of all regions' pollen extracts on *A. alternata* were statistically significant ($P < 0.01$) which were stated as mean of regions per week in Table 1. The comparison of pollen extracts of Taşkent region to other regions according to incubation time (days), 3rd and 4th days were not effective but after these days the inhibition effect slightly increased. Beyşehir pollen extracts were slightly effective on the 3rd day of incubation but then showed the lowest effects, in comparison with the other regions after the 3rd day. Extracts from Alanya have the maximum effect at the 3rd day, then the effect decreased. Pollen extracts of Antakya had the highest effect at the 4th day but other days were lower than

Table 1. Antifungal effect of pollen on *Alternaria alternata* (mean values)

Days	Concentrations (%)	Regions						*P*	LSD (0.01)
		Taşkent	Beyşehir	Alanya	Antakya	Hadim	Mean of concentrations		
3	Control (0%)	33.0 a	33.0 a	33.0 a	33.0 a	33.0 a	33.0 a	<0.01	2.674
	2	30.7 a	26.0 bc	24.3bcd	30.0 a	31.0 a	28.4 b	<0.01	1.196
	5	22.3 d	23.3 cd	18.0 e	18.0 e	26.3 b	21.6 c		
Mean of regions		28.7 ab	27.4 bc	25.1 d	27.0 c	30.1 a	–	<0.01	1.544
4	Control (0%)	45.0 a	45.0 a	45.0 a	45.0 a	45.0 a	45.0 a	<0.01	2.674
	2	38.0 b	37.7 b	33.0 cd	34.7 c	38.3 b	36.3 b	<0.01	1.196
	5	33.0 cd	40.0 b	31.3 d	28.0 e	28.0 e	32.1 c		
Mean of regions		38.7 a	40.1 a	36.4 b	35.9 d	37.1 b	–	<0.01	1.544
5	Control (0%)	48.0 b	48.0 b	48.0 b	48.0 b	48.0 b	48.0 a	<0.01	2.674
	2	46.3 b	46.3 b	47.0 b	45.3 b	40.0 c	45.0 b	<0.01	1.196
	5	41.7 c	53.0 a	40.0 c	32.0 d	30.0 d	39.3 c		
Mean of regions		45.3 b	49.1 a	45.0 b	41.8 c	39.3 d	–	<0.01	1.544
6	Control (0%)	55.3 a	55.3 a	55.3 a	55.3 a	55.3 a	55.3 a	<0.01	2.674
	2	50.7 b	55.7 a	50.7 b	54.3 a	56.0 a	53.5 b	<0.01	1.196
	5	49.3 bc	54.7 a	47.3 c	37.0 d	31.7 e	44.0 c		
Mean of regions		51.8 b	55.2 a	51.1 b	48.9 c	47.7 c	–	<0.01	1.544
7	Control (0%)	58.0 ab	58.0 ab	58.0 ab	58.0 ab	58.0 ab	58.0 a	<0.01	2.674
	2	55.0 b	60.0 a	60.0 a	60.0 a	55.0 b	58.0 a	<0.01	1.196
	5	48.0 c	58.0 ab	40.0 d	40.0 d	33.0 e	43.8 b		
Mean of regions		53.7 b	58.7 a	52.7 b	52.7 b	48.7 c	–	<0.01	1.544
8	Control (0%)	58.0 de	58.0 de	58.0 de	58.0 de	58.0 de	58.0 b	<0.01	2.674
	2	70.0 b	73.0 a	65.0 c	73.0 a	58.0 de	67.8 a	<0.01	1.196
	5	55.0 e	60.0 d	58.0 de	47.0 f	35.7 g	51.1 c		
Mean of regions		61.0 b	63.7 a	60.3 bc	59.3 c	50.6 d	–	<0.01	1.544

Table 2. Antifungal effect of pollen on *Fusarium oxysporium* (mean values)

Days	Concentrations (%)	Regions						*P*	LSD (0.01)
		Taşkent	Beyşehir	Alanya	Antakya	Hadim	Mean of concentrations		
3	Control (0%)	40.0 a	40.0 a	40.0 a	40.0 a	40.0 a	40.0 a	<0.01	2.316
	2	39.0 ab	38.3 ab	34.7 cd	36.7 bc	36.7 bc	37.1 b	<0.01	1.036
	5	33.0 d	32.7 d	23.3 f	26.3 e	37.3 b	30.5 c		
Mean of regions		37.3 a	37.0 a	32.7 c	34.3 b	38.0 a	–	<0.01	1.337
4	Control (0%)	55.0 a	55.0 a	55.0 a	55.0 a	55.0 a	55.0 a	<0.01	2.316
	2	44.7 d	49.7 b	47.7 bc	50.0 b	45.0 d	47.4 b	<0.01	1.036
	5	47.0 cd	45.0 d	34.7 e	32.0 f	45.0 d	40.7 c		
Mean of regions		48.9 ab	49.9 a	45.8 c	45.7 c	48.3 b	–	<0.01	1.337
5	Control (0%)	67.0 a	67.0 a	67.0 a	67.0 a	67.0 a	67.0 a	<0.01	2.316
	2	51.7 e	58.3 cd	60.3 bc	60.0 bc	61.3 b	58.3 b	<0.01	1.036
	5	57.0 d	59.0 bcd	46.7 f	52.7 e	58.3 cd	54.7 c		
Mean of regions		58.6 bc	61.4 a	58.0 c	59.9 b	62.2 a	–	<0.01	1.337
6	Control (0%)	78.3 c	78.3 c	78.3 c	78.3 c	78.3 c	78.3 a	<0.01	2.316
	2	74.0 d	78.0 c	71.3 e	70.0 ef	71.3 e	72.9 b	<0.01	1.036
	5	85.7 a	67.7 f	58.3 g	55.0 h	81.3 b	69.6 c		
Mean of regions		79.3 a	74.7 c	69.3 d	67.8 e	77.0 b	–	<0.01	1.337
7	Control (0%)	85.0 b	85.0 b	85.0 b	85.0 b	85.0 b	85.0 a	<0.01	2.316
	2	88.0 a	88.0 a	88.0 a	82.0 c	82.0 c	85.6 a	<0.01	1.036
	5	88.0 a	80.0 c	58.0 d	58.0 d	88.0 a	74.4 b		
Mean of regions		87.0 a	84.3 b	77.0 c	75.0 d	85.0 b	–	<0.01	1.337
8	Control (0%)	90.0 a	90.0 a	90.0 a	90.0 a	90.0 a	90.0 a	<0.01	2.316
	2	90.0 a	90.0 a	89.0 a	88.0 ab	88.0 ab	89.0 a	<0.01	1.036
	5	90.0 a	86.0 b	65.0 c	60.0 d	90.0 a	78.2 b		
Mean of regions		90.0 a	88.7 a	81.3 b	73.3 c	89.3 a	–	<0.01	1.337

that. The effect of Hadim pollen extracts increased until the 5th day then it showed the maximum effect of incubation for the other days in comparison with the other regions extracts (Table 1). The inhibitory effect of pollen concentration change on *A. alternata* was significant ($P < 0.01$), stated as mean of concentrations per week in Table 1. It is apparent that the increase of concentration increased the inhibition effect until the 6th day but on the 7th day the 2% pollen extract lost its activity and on the 8th day 2% concentration was seen to be a stimulating agent (Table 1). Pollens' inhibition effects of interaction of concentration, day and region on *A. alternata* were statistically significant ($P < 0.01$). It was found that the inhibition effect of pollen extract of Hadim with 5% concentration was seen to be the maximum effective pollen extract after the 4th day in this study. Antakya region extract was as effective as Hadim region extract until the 4th day then it slightly decreased but its effectiveness was generally near to Hadim extracts (Table 1).

The effects of all regions' pollen extracts on inhibition of *F. oxysporium* were statistically significant ($P < 0.01$), stated as mean of regions per week in Table 2. Taşkent regions' pollen extract was generally not effective except for the 5th day. Inhibition effect of Beyşehir pollen extracts was detected on the 6th and 7th day. Pollen extracts of Antakya had the highest effects after the 5th day; 3rd and 4th day was also effective after Alanya extracts. Hadim extracts were generally not effective according to incubation time (Table 2). The effect of pollen concentration on inhibition of *F. oxysporium* was significant ($P < 0.01$), stated as mean of concentrations per week in Table 2. It is apparent that the increase of concentration increased the inhibition effect until the 6th day but after then the 2% concentration of pollen extract had lost its activity (Table 2). The inhibiton effects of interaction of concentration, day and region on *F. oxysporium* were statistically significant for pollen extracts ($P < 0.01$). It was found that the inhibition effect of pollen extract of Alanya and Antakya with 5% concentration was seen to be the maximum effective extracts. The effect of inhibition was seen to be very low at the 8th day for all factors (Table 2).

The effects of all regions' propolis extracts on inhibition of *A. alternata* were statistically significant ($P < 0.01$), stated as mean of regions per week in Table 3. Taşkent region' propolis extracts were not effective in comparison with other regions' for the incubation time period. Beyşehir propolis extracts were

Table 3. Antifungal effect of propolis on *Alternaria alternata* (mean values)

Days	Concentrations (%)	Regions						*P*	LSD (0.01)
		Taşkent	Beyşehir	Alanya	Antakya	Hadim	Mean of concentrations		
3	Control (0%)	33.0 a	33.0 a	33.0 a	33.0 a	33.0 a	33.0 a	<0.01	2.674
	2	25.3 b	9.3 d	9.67 d	15.0 c	15.3 c	14.9 b	<0.01	1.196
	5	17.3 c	10.3 d	9.67 d	0.0 e	0.0 e	10.5 c		
Mean of regions		25.2 a	17.5 b	17.4 b	16.1 b	16.1 b	–	<0.01	1.544
4	Control (0%)	45.0 a	45.0 a	45.0 a	45.0 a	45.0 b	45.0 a	<0.01	2.674
	2	28.3 b	17.0 e	17.0 e	20.0 d	23.3 a	21.1 b	<0.01	1.196
	5	19.3 de	16.7 e	14.0 f	6.0 g	20.7 c	15.3 c		
Mean of regions		30.9 a	26.2 b	25.3 b	23.7 c	29.7 cd	–	<0.01	1.544
5	Control (0%)	48.0 a	48.0 a	48.0 a	48.0 a	48.0 a	48.0 a	<0.01	2.674
	2	38.0 b	20.0 d	18.0 de	27.0 c	27.7 c	26.1 b	<0.01	1.196
	5	25.0 c	16.3 ef	14.7 f	12.0 g	25.0 c	18.6 c		
Mean of regions		37.0 a	28.1 cd	26.9 d	29.0 c	33.6 b	–	<0.01	1.544
6	Control (0%)	55.3 a	55.3 a	55.3 a	55.3 a	55.3 a	55.3 a	<0.01	2.674
	2	43.0 b	25.7 e	23.0 f	30.0 d	30.0 d	30.3 b	<0.01	1.196
	5	30.0 d	21.0 f	16.3 g	15.0 g	34.0 c	23.3 c		
Mean of regions		42.8 a	34.0 c	31.6 d	33.4 c	39.8 b	–	<0.01	1.544
7	Control (0%)	58.0 a	58.0 a	58.0 a	58.0 a	58.0 a	58.0 a	<0.01	2.674
	2	47.0 b	27.0 e	27.0 e	34.0 d	34.0 d	33.8 b	<0.01	1.196
	5	40.0 c	24.0 f	19.0 g	16.0 h	38.0 c	27.4 c		
Mean of regions		48.3 a	36.3 c	34.7 c	36.0 c	43.3 b	–	<0.01	1.544
8	Control (0%)	58.0 a	58.0 a	58.0 a	58.0 a	58.0 a	58.0 a	<0.01	2.674
	2	55.0 b	35.0 e	32.0 f	42.7 cd	45.0 c	41.9 b	<0.01	1.196
	5	44.7 c	30.0 fg	24.0 h	28.7 g	41.0 d	33.7 c		
Mean of regions		52.6 a	41.0 d	38.0 e	43.1 c	48.0 b	–	<0.01	1.544

slightly effective until the 4th day afterwards the effect increased slightly. Extracts from Alanya had the same effect as Beyşehir extracts until the 4th day but then Alanya propolis extracts were the most effective of the extracts. Propolis extracts of Antakya had a medium effect for all days of incubation. The effect of Hadim extracts were maximum on the 4th day the rest was not very effective (Table 3). The inhibitory effect of propolis concentration on *A. alternata* was significant ($P < 0.01$), stated as mean of concentrations per week in Table 3 for the whole incubation period. It is obvious that the increase of concentration increased the inhibition effect until the end of incubation time (Table 3). Propolis' inhibition effects of interaction of concentration, day and region on *A. alternata* were statistically significant ($P < 0.01$) (in italics). It was found that the propolis extracts of Antakya and Hadim with 5% concentration were the maximum effective extracts on the 3th day, with no fungal growth. After the 3rd day, Hadim regions' effect decreased but the effect of propolis extracts from the Alanya region slightly increased and reached its maximum on the 8th day (Table 3).

The effect of all regions' propolis extracts on inhibition of *F. oxysporium* were statistically significant ($P < 0.01$), stated as mean of regions per week in Table 4. Taşkent regions' propolis extracts were not effective in comparison with other regions for the incubation time period. Beyşehir and Alanya propolis extracts had the highest inhibitory effect until the 7th day but on the 8th day the extract from Alanya was the best, followed by Beyşehirs'. Propolis extracts of Antakya and Hadim had lower effects than that of others (Table 4). The effect of propolis concentration on inhibition of *F. oxysporium* was significant ($P < 0.01$), stated as mean of concentrations per week in Table 4. It is apparent that the increase of concentration increased the inhibition effect until the end of incubation time period (Table 4). The propolis' inhibition effects of interaction of concentration, day and region on *F. oxysporium* were statistically significant ($P < 0.01$) (in italics). It was apparent that the propolis extracts of Beyşehir and Alanya with 2% and 5% concentrations were the maximum effective extracts (Table 4).

None of the pollen extracts tested showed complete inhibition. The highest inhibition rate was 45% at the Alanya pol-

Table 4. Antifungal effect of propolis on *Fusarium oxysporium* (mean values)

Days	Concentrations (%)	Regions						*P*	LSD (0.01)
		Taşkent	Beyşehir	Alanya	Antakya	Hadim	Mean of concentrations		
3	Control (0%)	40.0 a	40.0 a	40.0 a	40.0 a	40.0 a	40.0 a	<0.01	2.316
	2	15.0 b	0.0 e	0.0 e	16.0 b	15.0 b	9.2 b	<0.01	1.036
	5	10.0 c	0.0 e	0.0 e	9.7 c	6.3 d	5.2 c		
Mean of regions		21.7 a	13.3 b	13.3 b	21.9 a	20.4 a	–	<0.01	1.337
4	Control (0%)	55.0 a	55.0 a	55.0 a	55.0 a	55.0 a	55.0 a	<0.01	2.316
	2	20.0 b	0.0 e	0.0 e	18.3 b	20.0 b	11.7 b	<0.01	1.036
	5	13.7 c	0.0 e	0.0 e	14.0 c	7.3 d	7.0 c		
Mean of regions		29.6 a	18.3 c	18.3 c	29.1 a	27.4 b	–	<0.01	1.337
5	Control (0%)	67.0 a	67.0 a	67.0 a	67.0 a	67.0 a	67.0 a	<0.01	2.316
	2	28.3 bc	0.0 f	0.0 f	26.7 c	30.0 b	17.0 b	<0.01	1.036
	5%	16.3 d	0.0 f	0.0 f	15.7 d	11.7 e	8.7 c		
Mean of regions		37.2 a	22.3 b	22.3 b	36.4 a	36.2 a	–	<0.01	1.337
6	Control (0%)	78.3 a	78.3 a	78.3 a	78.3 a	78.3 a	78.3 a	<0.01	2.316
	2	31.3 c	0.0 h	0.0 h	28.7 d	34.7 b	18.9 b	<0.01	1.036
	5	26.3 e	0.0 h	0.0 h	20.0 f	16.7 g	12.6 c		
Mean of regions		45.3 a	26.1 c	26.1 c	42.3 b	43.2 b	–	<0.01	1.337
7	Control (0%)	85.8 a	85.0 a	85.0 a	85.0 a	85.0 a	85.0 a	<0.01	2.316
	2	38.0 b	0.0 g	0.0 g	31.3 c	39.0 b	21.7 b	<0.01	1.036
	5	28.3 d	0.0 g	0.0 g	22.0 e	17.0 f	13.5 c		
Mean of regions		50.4 a	28.3 c	28.3 c	46.1 b	47.0 b	–	<0.01	1.337
8	Control (0%)	90.0 a	90.0 a	90.0 a	90.0 a	90.0 a	90.0 a	<0.01	2.316
	2	43.0 b	16.0 g	10.0 h	33.3 d	45.0 b	29.5 b	<0.01	1.036
	5	40.0 c	10.0 h	0.0 i	30.0 e	24.0 f	20.8 c		
Mean of regions		57.7 a	38.7 d	33.3 e	51.1 c	53.0 b	–	<0.01	1.337

len samples for both fungi. Also, all concentrations had lower effects than 50% rates against *A. alternata* and *F. oxysporium* (Table 5). However, both concentrations of Beyşehir and Alanya propolis extracts on mycelial growth of *F. oxysporium* were 100% effective at the 7th day of incubation (Table 6). But only the 5% levels of Antakya and Hadim propolis on *A. alternaria* growth were 100% effective for 3 days of incubation. In addition, 5% levels of all propolis samples (except for 5% level for the 6th and 7th day of Hadim region) on *A. alternata* growth showed higher inhibition rates than the 2% concentration during incubation (Table 6). Among the fungi tested, the most sensitive against propolis extract was *F. oxysporium*.

The present studies reveal that methanol extract of propolis is a natural antimicrobial agent and this explains the previously reported beneficial effects. Lindenfelser [27] reported that propolis inhibited the growth of 20 fungi out of sampled 39 fungi. It was established that caffeic acid, benzyl cumarete, pinobanksin and pinocembrin found in propolis showed antimycotic properties. The activity of a 10% ethanolic extract of propolis against 17 fungal pathogens was compared with that of Mylyt, an E. German propolis-containing preparation. The propolis extract inhibited *Candida* and all tested dermatophytes [18].

4 Concluding remarks

As a result, the inhibitory effect of propolis was found to be higher than that of pollen. The preservation action of pollen and especially propolis has recently received attention in the literature, where studies have reported that some foodborne bacteria and fungi may be inhibited by propolis extracts [10, 20, 23, 27–29]. Pollens and propolis from different regions showed highly varying properties because of chemical differences of some components. The flora and the plant species of the regions differ, causing diversity in constituents of pollen and propolis and as a result the antifungal and other properties of these products [20, 30]. The variation of inhibitory effects of tested extracts may be accounted for their constituents and the probable nonvolatile compounds of extracts [32, 33].

Table 5. Percent inhibition of pollen on *Alternaria alternata* and *Fusarium oxysporium*

Days	Concentrations (%)	Regions *(Alternaria alternata)*				
		Taşkent	Beyşehir	Alanya	Antakya	Hadim
3	2	7	21	26	9	6
	5	32	29	45	45	20
4	2	16	16	27	23	15
	5	27	11	30	38	38
5	2	4	4	2	6	17
	5	13	–10	17	33	38
6	2	8	–1	8	2	–1
	5	11	1	14	33	43
7	2	5	–3	–3	–3	5
	5	17	0	31	31	43
8	2	–21	–26	–12	–26	0
	5	5	–3	0	19	38
	Concentrations (%)	Regions *(Fusarium oxysporium)*				
		Taşkent	Beyşehir	Alanya	Antakya	Hadim
3	2	3	4	13	8	8
	5	18	18	42	34	7
4	2	19	10	13	9	18
	5	15	18	37	42	18
5	2	23	13	10	10	9
	5	15	0.4	30	21	13
6	2	5	14	9	11	9
	5	–9	26	26	30	–4
7	2	–4	–4	–4	4	4
	5	–4	9	32	32	–4
8	2	0	0	1	2	2
	5	0	4	28	33	0

Table 6. Percent inhibition of propolis on *Alternaria alternata* and *Fusarium oxysporium*

Days	Concentrations (%)	Regions *(Alternaria alternata)*				
		Taşkent	Beyşehir	Alanya	Antakya	Hadim
3	2	23	72	71	55	54
	5	48	69	71	100	100
4	2	37	62	62	56	48
	5	57	63	69	87	54
5	2	21	58	63	44	42
	5	48	66	69	75	48
6	2	22	54	58	46	46
	5	46	62	71	73	39
7	2	19	53	53	41	41
	5	31	59	67	72	34
8	2	5	40	45	26	22
	5	23	48	59	51	29
	Concentrations (%)	Regions *(Fusarium oxysporium)*				
		Taşkent	Beyşehir	Alanya	Antakya	Hadim
3	2	63	100	100	60	63
	5	75	100	100	76	84
4	2	64	100	100	67	64
	5	75	100	100	75	87
5	2	58	100	100	60	55
	5	76	100	100	77	83
6	2	60	100	100	63	56
	5	66	100	100	74	79
7	2	55	100	100	63	54
	5	67	100	100	74	80
8	2	52	82	89	63	50
	5	56	89	100	67	73

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Received March 24, 2003
Accepted January 8, 2004

