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# Short Paper

## Antifungal properties of propolis

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**RESUMEN**

**Propiedades antifúngicas de propóleos.**

Se prepararon extractos acuosos de propóleos a concentraciones de 0.5, 1, 2, 3 y 4%, estudiándose en medios de cultivo la inhibición por estos extractos de *Alternaria alternata, Aspergillus niger, Aspergillus parasiticus, Botrytis cinerea, Fusarium oxysporum* f.sp. *melonis* y *Penicillium digitatum.* La concentración más inhibidora de las 5 estudiadas fue la del 4%. Los microorganismos más afectados entre los hongos analizados, para todas las concentraciones, fueron *Alternaria alternata* y *Penicillium digitatum.* La concentración del 4% de extracto de propóleos mostró más del 50% de inhibición frente a todos los microorganismos estudiados.

*PALABRAS-CLAVE: Hongo - Inhibición - Propóleos.*

**SUMMARY**

**Antifungal properties of propolis.**

Water extracts at the concentration of 0.5, 1, 2, 3 and 4%, of propolis were prepared and investigated for inhibition of *Alternaria alternata, Aspergillus niger, Aspergillus parasiticus, Botrytis cinerea, Fusarium oxysporum* f.sp. *melonis* and *Penicillium* digitatum in culture media. The concentration of 4% was the most inhibitory of 5 concentrations studied. Microorganisms the most effected from all the concentrations among tested fungi were *Alternaria alternata* and *Penicillium digitatum.* The concentration of 4% of propolis extract showed more than 50% inhibition against all tested microorganisms.

*KEY-WORDS: Fungi - Inhibition - Propolis.*

## 1. INTRODUCTION

Propolis is a resinous hive product collected from plant buds by bees. Bee products such as honey, pollen and propolis are used for the treatment of several diseases. It is said that propolis was used as a perfect antibiotic agent (Grange and Davey 1990, Cherbuliez 1996, Feraboli 1996, Schmidt and Schmidt 1996). Propolis is a resin and its color changes from green to dark browning according plant source. Investigations have indicated that propolis contains wax, flavonoids, amino acids, essential oils, pollen, minerals and organic matters (Walker and Crane 1987, Crane 1990, Scheller *et al.*, 1990).

Recently, the propolis is commonly exported and/or imported. The countries which exported mainly are China, Argentina, Uruguay, Brazil, Canada and a part of east Europe countries (Crane 1990). It was pointed out that propolis had a strong antimicrobial, antiviral, antifungal and antioxidant properties (Ghisalberti 1979, Yamauchi *et al.*, 1992). Recently, investigations have indicated that interest for natural preservatives had increased. The use of propolis that is non-toxic as alternate preservative agent is considered by consumers, as safe (Ghisalberti, 1979). In the last thirty years, there has been considerable emphasis on the studies involving propolis (Ghisalberti 1979, Crane 1990, Yamauchi *et al.*,1992). It was established that flavonoids, benzoic acid, and derivatives found in propolis showed antibiotic, antiviral and antimycotic effect. Also, water extracts of propolis have been used for the treatment of several diseases observed in mammals (Linderfelser 1967, Ghisalberti 1979, König 1986, Walker and Crane 1987, Orgen 1988, Rosential 1989, Scheller *et al.*, 1989, Crane 1990, Grange and Davey 1990, Tatefuji *et al.*, 1993, Itah *et al.*, 1994, Diğrak *et al.*, l995, Marcucci 1995). The part that melted at the 70% alcohol of propolis had showed sinergist together with antibiotic activite. The same solution inhibited germination of vegetables such as potato, garlic, and cured the scalds from second degree, and showed anesthetic, antiseptic and antioxidant effect (König 1986, Orgen 1988, Scheller *et al.*, 1989, Yamauchi *et* al.1992, Hemeida and Abd. Alfattah 1993).

It is pointed out that its use for medicinal purposes has been carried out since antiquity. Also, propolis is used in cosmetic area day by day (Toth 1985, Toth 1988).It was reported that propolis has got antibacterial effect against *Bacillus subtilis, Bacillus alvei* and *Proteus vulgaris,* and galangin and flavor isolated from propolis had responsible for antibacterial effect. In other study, it was established that propolis contained essential oil (Ghisalberti 1979, Crane 1990 ). Powers (1964) established that propolis have a considerable antibiotic effect on *Salmonella, Staphylococcus aureus, Proteus vulgaris* and *Escherichia coli.* Rosential (1989) showed alcohol extract at the 30% concentration of propolis

to be active in inhibiting *Bacillus aureus* and *Escherichia coli*. Linderfelser (1967) analysed the propolis collected from different sources, and researched its antibacterial activity on 25-39 bacteria species *in vitro*, and reported that propolis was active against *Bacillus larvae* in culture media .

The purpose of this study was to determine effects of propolis at the different concentrations on the mycelial growth of *A. parasiticus*, *A. niger*, *A. alternata, B. cinerea, F. oxysporum* f.sp. *melonis* and *P. digitatum* (spoiled foods, especially stored foods) in microbiological media.

## 2. MATERIALS AND METHODS

**Propolis:** Propolis was collected from bee hives in Konya (Taşkent) in 1988 May.

**Mould:** The organisms used in this work were strain of *A.parasiticus* NRRL 2998, obtained from «USDA, Agricultural Res. Service, National Center for Agricultural Utilization Res. Service, Illinois, USA»; *A. alternata, A. niger* and *B. cinerea* were obtained from Department of Food Engineering, Faculty of Agriculture, Selçuk University; *F. oxysporum* f.sp. *melonis* and *P. digitatum* were obtained from Department of Plant Protection, Faculty of Agriculture, Selçuk University.

**Medium:** Czapek - Dox agar was used as main medium throughout the study. Propolis that used was extracted with hot water. Water extracts (0.5,1,2,3 and 4%) of propolis were prepared. Medium was separately prepared in each water extract. After, each medium was dispensed in 120 ml quantities into 250 ml Erlenmeyer flasks and sterilized by autoclaving at 121 °C for 15 min.

**Assessment of inhibition of fungal growth:** The effect of propolis at different concentrations (0.5,1,2,3 and 4%) was determined against *A. niger, A. parasiticus, A. alternata, B. cinerea, F. oxysporum* f.sp. *melonis* and *P. digitatum* growth using Czapek-Dox agar. Medium was dispensed into each petri plates as 20 ml. Five mm discs of the test fungi, cut from periphery of 7 days old cultures, were inoculated upside down separately onto each assay plate and incubated at 28 °C. The colony diameter was measured and percent mycelial inhibition calculated following (Deans and Svoboda 1990). Three replicates of each treatment were similarly maintained and averages calculated. Control sets were simultaneously run without using propolis.

$$I = (C - T/C) \times 100$$

I: Inhibition (%),

C: Colony diameter of mycelium from control petri plate (mm),

T: Colony diameter of mycelium from test petri plate (mm),

## 3. RESULTS AND DISCUSSION

The inhibitory effect of different concentration of propolis extracts were tested. The results and the most active concentration are given in Table I and Figure 1.

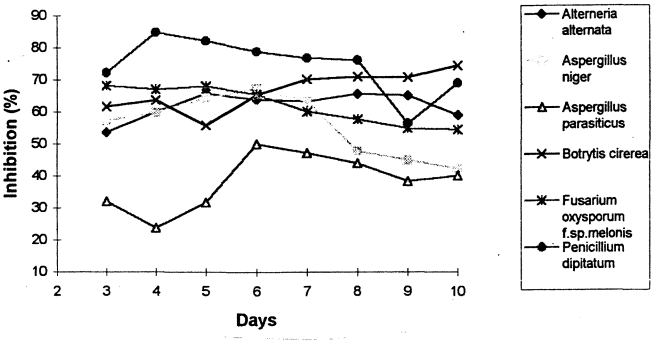


Figure 1
Antifungal effect of propolis at the 4% concentration on microorganisms used this study

Propolis extract at concentrations of 0.5, 1, 2 and 3% less effected on mycelial growth of test microorganisms but 4%. While all the concentrations showed inhibitory effect about 45-60% against *A. alternata* until six days of incubation, inhibitory activity was partly decreased on ten days of incubation. This decreasing varied in accordance with dose, effective material amount, effective level and microorganism species. Also, all of concentrations (except for 4%) had less effect than 50% rates against *A. niger, A. parasiticus, B. cinerea* (except for 2%) and *F. oxysporum* but more effected than about 50% (except for 1% on 3 days and 0.5% o 6, 7, 8, 9 and 10 days). Microorganisms the most effected from all the concentrations among tested fungi were *A. alternata* and *P. digitatum.* However, the concentration of 4% in accordance with other concentrations of propolis showed higher inhibitory effect. The same concentration (4%) against *A. parasiticus* showed more inhibitory effect as from 6 days of incubati on according to those of other concentrations.

As a result, plant species used by bees as propolis source are showed difference to region from region (König 1985). Also, while propolis collected by bees grown different region show similar properties due to some components, they show highly different properties because of some components (Marletto 1984). Only pinobanksin-3-acetate, 3-acetylpinobanksin, pinocembrin, p-coumaric acid and caffeic acid from 26 or more components isolated propolis extract showed considerably antimycotinic effect (Ghisalberti 1979). However, the same researcher reported that caffeic acid from propolis components showed fungistatic effect against *Helminthosporium carbonum.* Linderfelser (1967) reported that propolis inhibited 20 fungi growth in sampled 39 fungi. It was established that caffeic acid, benzyl cumarete, pinobanksin and pinocembrin found in propolis showed antimycotic properties.

Table I
**The inhibitory effects of different concentration of propolis extracts (inhibition %)**

| Days | Concentrations % | *Alternaria alternata* | *Aspergillus niger* | *Apergillus parasiticus* | *Botrytis cinerea* | *Fusarium oxysporum* | *Penicillium digitatum* |
|---|---|---|---|---|---|---|---|
| 3 | 0.5 | 45.6 | 28.6 | 18.9 | 40.4 | 3.4 | 58.2 |
| | 1 | 46.4 | 38.1 | 5.7 | 38.3 | 18.2 | 40.5 |
| | 2 | 50.7 | 41.7 | 9.4 | 40.4 | 29.5 | 65.8 |
| | 3 | 36.2 | 16.7 | 30.2 | 17.0 | 31.8 | 68.4 |
| | 4 | 43.6 | 57.1 | 32.1 | 61.7 | 68.2 | 72.2 |
| 4 | 0.5 | 55.6 | 16.8 | 22.2 | 38.2 | 12.8 | 73.1 |
| | 1 | 48.9 | 41.1 | 6.3 | 38.2 | 33.9 | 67.6 |
| | 2 | 60.0 | 30.5 | 9.5 | 40.0 | 24.8 | 76.6 |
| | 3 | 50.0 | 36.8 | 32.7 | 27.3 | 33.9 | 75.2 |
| | 4 | 60.0 | 60.0 | 23.8 | 63.6 | 67.0 | 84.8 |
| 5 | 0.5 | 51.9 | —* | 31.7 | 26.5 | — | 70.7 |
| | 1 | 46.3 | 43.0 | 9.8 | 35.3 | 33.8 | 66.9 |
| | 2 | 57.4 | 42.9 | 31.7 | 32.4 | 29.6 | 78.3 |
| | 3 | 53.7 | 37.1 | 32.0 | 25.9 | 23.2 | 76.4 |
| | 4 | 65.7 | 64.3 | 31.7 | 55.9 | 68.0 | 82.2 |
| 6 | 0.5 | 45.9 | — | 26.8 | — | — | 47.1 |
| | 1 | 42.6 | — | 36.6 | — | — | 52.9 |
| | 2 | 51.2 | 18.6 | 39.5 | 38.8 | — | 64.7 |
| | 3 | 51.2 | 30.2 | 38.9 | 28.6 | 26.2 | 70.6 |
| | 4 | 63.9 | 67.4 | 50.0 | 65.3 | 65.5 | 78.8 |
| 7 | 0.5 | 45.6 | — | — | — | — | 45.3 |
| | 1 | 45.6 | — | 40.0 | — | — | 53.5 |
| | 2 | 52.9 | 16.1 | 38.5 | 53.8 | — | 62.8 |
| | 3 | 54.4 | 21.8 | 39.4 | 41.5 | — | 65.1 |
| | 4 | 63.2 | 63.2 | 47.1 | 70.1 | 60.1 | 76.7 |
| 8 | 0.5 | 46.1 | — | — | — | — | 44.3 |
| | 1 | 45.5 | — | 38.0 | — | — | 52.3 |
| | 2 | 54.5 | — | 32.6 | 55.0 | — | 60.2 |
| | 3 | 57.1 | — | 34.0 | 43.0 | — | 56.3 |
| | 4 | 65.6 | 47.7 | 43.9 | 70.9 | 57.5 | 76.1 |
| 9 | 0.5 | 45.8 | — | — | — | — | 41.9 |
| | 1 | 48.2 | — | 37.1 | — | — | 50.7 |
| | 2 | 54.2 | — | 32.0 | 52.0 | — | 68.3 |
| | 3 | 56.6 | — | 30.0 | 48.5 | — | 56.4 |
| | 4 | 65.1 | 45 | 38.3 | 70.8 | 54.8 | 75.4 |
| 10 | 0.5 | 44.4 | — | — | — | — | 42.2 |
| | 1 | 44.4 | — | 37.8 | — | — | 60.0 |
| | 2 | 50.0 | — | 35.0 | 61.1 | — | 66.6 |
| | 3 | 51.1 | — | 38.0 | 45.6 | — | 54.5 |
| | 4 | 58.9 | 42.2 | 40.0 | 74.4 | 54.4 | 68.9 |

No inhibition.

## 4. CONCLUSIONS

As a result, it was established that propolis known as balsam matter which collected by bees from several plants showed antifungal effect. The higher over 4% of water soluble extracts of propolis will be probably higher inhibitory effect against fungi that spoiled foods.

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