Volume 13, Issue 3, Page 127-133, 2024 https://doi.org/10.46810/tdfd.1493162 Research Article

BİNGÖL UNIVERSITY

Türk Doğa ve Fen Dergisi
Turkish Journal of Nature and Science
www.dergipark.gov.tr/tdfd

# Evaluation of the Efficacy of Propolis Extracts Based on Different Solvents Against Some Plant Pathogenic Fungi

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(Received: 31.05.2024, Accepted: 27.08.2024, Online Publication: 26.09.2024)

**Keywords**
Propolis, Antifungal effect, *Fusarium oxysporum*, *Alternaria alternata*, *Verticillium dahliae*

**Abstract:** Propolis is a bee product produced as a natural defense mechanism by bees to protect their hives. It consists of plant resins, secretions from bees, and other substances collected from plants that, contain biologically active compounds with antimicrobial, antioxidant, and anti-inflammatory properties. Because of these characteristics, propolis has applications in various fields. In this study, pure propolis extracts obtained by Dimethyl Sulfoxide (DMSO), ethanol, methanol, glycerol, acetone and supercritical CO2 extraction of propolis were tested for their antifungal activity against three phytopathogenic fungi, *Fusarium oxysporum*, *Alternaria alternata* and *Verticillium* spp. Antifungal activity tests were conducted in vitro using zone inhibition measurements on a PDA medium. Our findings revealed that the antifungal efficacy of propolis and its effect on mycelial growth parameters varied depending on the type of propolis extract used, demonstrating a dose-dependent relationship. In the present study, the ethanol + propolis preparation was found to be more successful in inhibiting the growth of fungal hyphae at a dose of 200 µL for all fungi than other solvents. The other solvents showed different levels of inhibition depending on the fungal species. In general, acetone, DMSO, and glycerol preparations of propolis were less effective in inhibiting fungal growth. The results obtained indicate that ethanol-based propolis extracts have the potential for control agriculturally important phytopathogenic fungi.



# Farklı Çözücülere Dayalı Propolis Ekstraktlarının Bazı Bitki Patojenik Funguslara Karşı Etkinliğinin Değerlendirilmesi

**Anahtar Kelimeler**
Propolis, Antifungal etki, *Fusarium oxysporum*, *Alternaria alternata*, *Verticillium dahliae*

**Öz:** Propolis, arıların doğal savunma mekanizması olarak kovanlarını korumak için ürettikleri bir arı ürünüdür. Bitki reçinelerinden, arıların kendi salgılarından ve diğer bitkilerden topladıkları maddelerden oluşan propolis, antimikrobiyal, antioksidan ve anti-enflamatuar özelliklere sahip biyolojik olarak aktif bileşikler içerir. Bu özellikler nedeniyle, propolis çeşitli alanlarda kullanılmaktadır. Çalışmada, propolisin Dimetil Sülfoksit (DMSO), etanol, metanol, gliserol, aseton ve süperkritik CO2 ekstraksiyon yöntemiyle elde edilen saf propolis ekstraktları üç fitopatojenik fungus türü olan *Fusarium oxysporum*, *Alternaria alternata* ve *Verticillium dahliae*'a karşı antifungal aktiviteleri test edilmiştir. Antifungal aktivite testleri, PDA ortamında yapılan zona inhibisyonu ölçümleri kullanılarak in vitro olarak gerçekleştirilmiştir. Bulgularımız, propolisin antifungal etkinliğinin ve miselyal büyüme parametrelerinin, kullanılan propolis ekstrakt türüne bağlı olarak değiştiğini ve doza bağlı bir ilişki gösterdiğini ortaya koymuştur. Çalışmada, Etanol+Propolis çözücüsünün, diğer çözücülere kıyasla tüm funguslar için 200 µl'lik dozunun fungal hif gelişimini inhibe etmede daha başarılı olduğu tespit edilmiştir. Diğer çözüler fungus türüne bağlı olarak farklı inhibisyon sergilemiştir. Genel olarak, fungal inhibisyon için, propolisin aseton, DMSO ve gliserol preperasyonları daha etkisiz olmuştur. Sonuç olarak, etanol bazlı propolis ekstraktlarının tarımsal açıdan önemli fitopatojenik fungusları kontrol etme potansiyeline sahip olduğunu göstermektedir.

## 1. INTRODUCTION

Propolis is a resinous substance with strong adhesive properties that bees produce from different plant secretions and use to close holes in the hive and protect the hive entrance from invaders. Propolis, a product with a long history of traditional use dating back to 300 BC, is well-known for its various biological and pharmacological properties. These properties include antibacterial, antifungal, antiviral, antiprotozoal, local anesthetic, anti-inflammatory, and immunostimulant effects [1, 2, 3]. It has a color ranging from yellow-green to dark brown and an aromatic odor, depending on the source and age of collection [4]. Propolis contains approximately 300 bioactive compounds, the contents of which varies according to the source of collection and season. To date, more than 180 compounds, mainly polyphenols, have been identified as the components of propolis. Propolis also contains other compounds such as essential oils, aromatic acids, waxes, pollen, vitamins, resins, balsams and various trace elements [5, 6]. Under in vitro conditions, propolis has demonstrated effectively inhibit both gram-positive and gram-negative bacterial strains [7, 8]. This antibacterial activity is attributed to the presence of flavonoids (such as galangin, pinocembrin, and pinostrobin), aromatic acids, and esters in propolis solutions [9]. Propolis has also shown inhibitory activity against a broad spectrum of viruses and fungal agents. Studies have indicated its effectiveness against various viruses of human and animal origin, including adonovirus, coronavirus, and rotavirus [10]. Additionally, propolis has been found to have antifungal effects against microfungi such as different Candida spp., Trichosporon spp., and Pichia ohmeri [11, 12, 13].

In recent years, propolis extracts have gained attention for their potential antiphytopathogenic effects against agricultural pathogens. Several in vivo and in vitro studies have investigated the antifungal activity of propolis against phytopathogenic fungi [14, 15]. Various propolis supernatants, including those extracted using ethanol, methanol, olive oil, and water, have been found to exhibit fungicidal activity against numerous plant-pathogenic fungi [16, 17, 18, 19]. In conclusion, propolis demonstrated remarkable antimicrobial properties, making it a promising candidate for further research and potential therapeutic applications.

Phytopathological fungi such as *Fusarium*, *Alternaria*, and *Verticillium* are common plant pathogens that can cause serious damage to agricultural crops, leading to yield and economic losses [20]. The long-term use of conventional chemical fungicides can cause environmental pollution, resistance development and risks to human health [21]. Therefore, the search for natural and environmentally friendly antifungal agents is of great importance. The mycelial growth activity of propolis extracts is commonly evaluated using dilution and diffusion methods [22, 23]. Mycelial growth inhibition is typically determined by comparing the radial growth diameter of the mycelium in the negative control (without propolis) with that in the tested solution [24]. In line with this approach, our study aimed to investigate the mycelial inhibitory effects of different propolis extracts on F. oxysporum, A. alternata, and Verticillium spp. Specifically, we evaluated the dose-dependent antifungal activities of propolis extracts prepared using various solvents (ethanol, methanol, acetone, pure, glycerol) using agar diffusion methods under laboratory conditions. The results obtained from our study will not only help to determine the most effective solvent and dose of propolis but also contribute to demonstrating the usefulness of propolis as a natural protective agent against agricultural pathogens.

## 2. MATERIAL AND METHOD

### 2.1. Propolis Collection

Propolis was collected from plastic traps placed in beehives in Solhan district of Bingöl province (Turkey) and used in further studies.

### 2.2. Biological Material and PDA Medium

The test microorganisms, V. dahliae, A. solani, and F. oxysporum, were used from the available collection characterized in the Mycology Laboratory of Bingöl University, Faculty of Agriculture, Turkey. For inoculum preparation, all fungal species were grown for 7 days at 25 °C on Potato Dextrose Agar (PDA) (Merck, Darmstadt, Germany) prepared according to the company's instructions.



### 2.3. Preparing of Propolis Supernatants

In this study, six types of propolis supernatants were used: crude propolis purified by supercritical fluid extraction, acetone (ASP), ethanol (ESP), methanol (MSP), glycerol (GSP) and DMSO. The solvent concentration was 70% and the propolis/solvent ratio was used as 1/4 in the inhibition tests. For all supernatants, raw frozen propolis was pulverized using a grinder. The mixture of solvent and propolis in these proportions was incubated at 36 °C for 10 days on a magnetic stirrer, centrifuged at 1000 g for 5 minutes and then filtered. For the supercritical fluid extraction, 150 g of crude propolis was used, resulting in approximately 5 g of pure propolis supernatant. As in the previous method, it was homogenized with sterile distilled water. All the propolis supernatants were stored at +4 °C in the dark.

### 2.4. Antifungal Efficacy Assays

Antifungal assays were evaluated considering the inhibition of radial mycelium growth in the PDA culture medium [25]. Antifungal activity against phytopathogenic agents was tested in increasing doses (50, 100, and 200 µL) of each propolis supernatants added to the PDA medium. The negative control group consisted of PDA medium without supernatant and PDA medium with solvent added only at the concentrations indicated. All treatment and control groups were incubated at room temperature for one week. All tests

were performed in triplicate using a randomized design and radial fungal diameter was measured using a ruler and recorded. The percentage of inhibition was determined by assessing fungal growth in the control groups, following the equation provided by Deans and Sobada [26].

$$\text{Inhibition (\%)} = (gc - gt)/gc \times 100$$

Where gc refers to the mycelial growth diameter in the control plates; and gt is the mycelial growth diameter in the propolis suspension.

### 2.5. Statictical Analysis

Data were collected in triplicate using a factorial experimental design with randomized complete blocks. The statistical package program "JUMP 5.0" was used for the analysis. The data were analyzed using analysis of variance, and the treatment means were compared using Tukey's multiple comparison test.

## 3. RESULTS

Propolis possesses broad-spectrum anti-pathogenic properties against both plant and animal-derived agents. However, the direct use of propolis is not feasible. Therefore, the scientific community has been working to identify the most effective solvents for propolis extraction. Numerous studies have reported that ethanol is the most effective solvent for this purpose.Other commonly used solvents for propolis extraction include water, oil, propylene glycol, and glycerol [27]. Although propolis solutions have been tested worldwide against various fungal pathogens, the antifungal activity of this natural product varies across different studies [19]. However, there is a lack of research on the efficacy of different solvent extracts of propolis, particularly against plant pathogenic fungi. Literature screening reveals that most studies on the antiphytopathogenic effects of propolis have focused on ethanol preparations in relation to plant pathogens. Ethanol extracts from propolis of different origins have been shown to negatively affect mycelial growth in several plant pathogens [16, 17, 15, 28, 29, 30].

In this study, six propolis preparations at three different doses were tested against three phytopathogenic fungi (F. oxysporum, A. alternata, and V. dahliae). The results of this study showed that propolis, especially its ethanol solutions, exhibited fungicidal activity. Different rates of inhibition of fungal mycelial growth were obtained depending on the microorganism tested, dose and solvent.

### 3.1. Inhibition in Mycelial Growth Based on Fungus Species

In all tests, the smallest mean fungal diameter was observed in *V. dahliae* (19.60 mm), followed by A. solani (32.87 mm) and *F. oxysporum* (48.20 mm) (Fig. 1). The differences between mean fungal diameters were statistically significant (Table 1).



**Table 1.** Descriptive values indicating the fungus species-specific inhibition zone

| Fungal pathogens | Mean | Std. Deviation | Std. Error of Mean |
|---|---|---|---|
| ***A. solani*** | 32,8[b] | 9,1 | 0,9 |
| ***V. dahliae*** | 19,6[c] | 5,7 | 0,5 |
| ***F. oxysporum*** | 48,2[a] | 13,0 | 1,3 |
| **Average** | 33,5 | 15,2 | 0,8 |

a,b,c: the difference between different letters in the same column is statistically significant ($p \leq 0.01$)

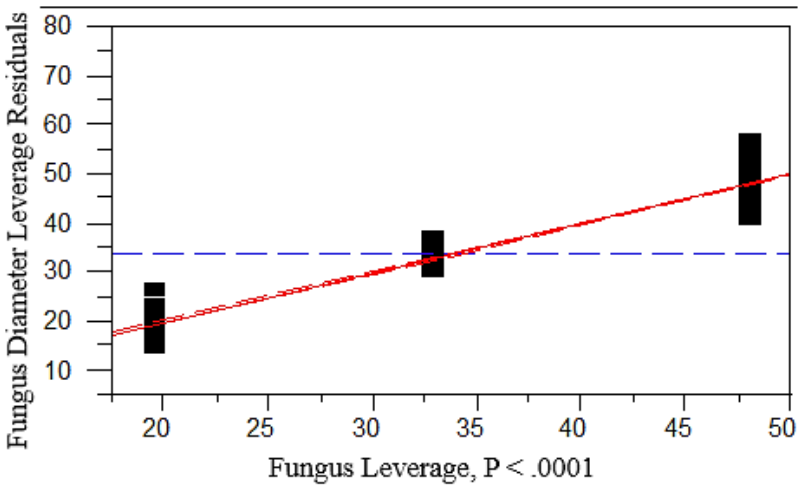


**Figure 1.** Diameter variation according to fungus species

### 3.2. Effect of Solvent and Propolis Extract Dose on Fungus Diameter

In this study, different solvents caused different rates of response inhibition in fungal species. The changes in fungal diameter caused by propolis solvents are summarized in Table 2. Statistically significant differences were found between the mean diameters. The lowest fungal diameter values were obtained in the ethanol+propolis (22.0), pure propolis (25.1) and

methanol+propolis (25.7) preparations, respectively (Fig. 2). This indicated that ethanol was the best solvent in all treatments. The highest fungal diameter value was observed in the solvents without propolis, indicating that solvent treatment alone has no activity in fungal inhibition. In addition, the mycelial growth diameter of the fungus decreased in all treated groups as the dose increased (data not shown).

**Table 2.** Fungal diameter values of solvent based propolis extracts

| Solvent | Mean | Std. Deviation | Std. Error of Mean |
|---|---|---|---|
| **Glycerol** | 43,7[b] | 18,1 | 3,5 |
| **Glycerol+propolis** | 35,7[d] | 16,2 | 3,1 |
| **Ethanol** | 46,6[a] | 18,6 | 3,5 |
| **Ethanol+propolis** | 22,0[h] | 11,4 | 2,2 |
| **DMSO** | 41,0[c] | 11,7 | 2,2 |
| **DMSO+propolis** | 29,6[f] | 9,5 | 1,8 |
| **Acetone** | 32,4[e] | 12,2 | 2,3 |
| **Acetone +propolis** | 30,3[f] | 12,9 | 2,4 |
| **Methanol** | 36,6[d] | 13,8 | 2,6 |
| **Methanol+propolis** | 25,7[g] | 10,2 | 1,9 |
| **Pure propolis** | 25,1[g] | 7,9 | 1,5 |
| **Total** | **33,5** | **15,2** | **0,8** |

a,b,c,d,e,f,g,h: the difference between different letters in the same column is statistically significant (p≤0.01)

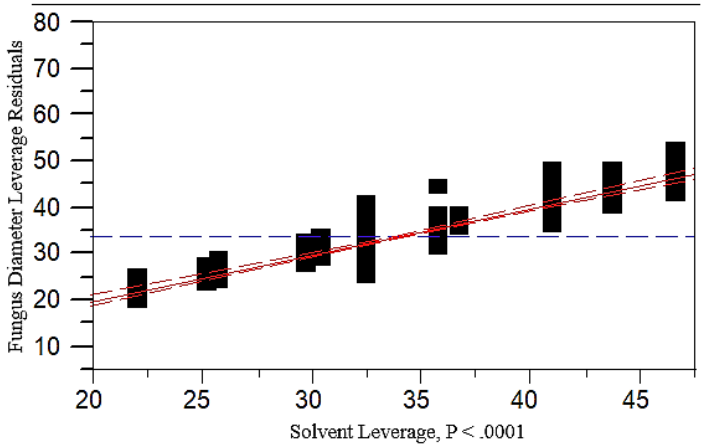


**Figure 2.** Change in fungus diameter with solvent treatment



### 3.3. Mean Values of Fungus-Solvent-Dose Interactions

The results showed that the effects of different solvent extracts of propolis varied according to the target fungal species. According to the analysis of variance, the model and effect tests were statistically significant (Table 3, Table 4), and r2 and adjusted r2 values were calculated as 0.98 and 0.97, respectively. The model indicated that approximately 97% of the variation in the diameter of the fungal hyphae was due to differences in the fungal species, dose and solvent used (Table 5).

**Table 3.** Analysis of Variance

| Source | DF | Sum of Squares | Mean Square | F Ratio |
|---|---|---|---|---|
| **Model** | 98 | 67807,333 | 691,912 | 147,9465 |
| **Error** | 198 | 926,000 | 4,677 | Prob > F |
| **C. Total** | 296 | 68733,333 | | <.0001 |

**Table 4.** Effect Tests

| Source | Nparm | DF | Sum of Squares | F Ratio | Prob > F |
|---|---|---|---|---|---|
| **Fungus** | 2 | 2 | 40576,242 | 4338,065 | <.0001 |
| **Solvent** | 10 | 10 | 17249,037 | 368,8239 | <.0001 |
| **Dose** | 2 | 2 | 2475,717 | 264,6825 | <.0001 |
| **Fungus *Solvent** | 20 | 20 | 5240,276 | 56,0246 | <.0001 |
| **Fungus *Dose** | 4 | 4 | 196,525 | 10,5054 | <.0001 |
| **Solvent*Dose** | 20 | 20 | 1287,246 | 13,7621 | <.0001 |
| **Fungus *Solvent*Dose** | 40 | 40 | 782,290 | 4,1818 | <.0001 |

**Table 5.** Summary of Model

| | |
|---|---|
| **RSquare** | 0,986528 |
| **RSquare Adj** | 0,97986 |
| **Root Mean Square Error** | 2,162584 |
| **Mean of Response** | 33,55556 |
| **Observations (or Sum Wgts)** | 297 |

In this study, the mean mycelial diameter varied depending on three variable factors. Depending on the solvent and dose, the mean mycelial diameter of *A. solani*, *V. dahliae*, and *F. oxysporum* was 32.8, 19.6 and 48.2, respectively. For all treatment groups, the highest dose (200 µL) of ethanol solution of propolis had the best inhibitory activity, according to the statistical analysis of the three-way interaction (Table 3). This is probably because ethanol allows efficient dissolution of biologically active components in propolis [31].

**Table 3.** Distribution of Means for Fungus-Solvent-Dose Interactions

| Treatment | *A. solani* | | | Average | *V. dahliae* | | | Average | *F. oxysporum* | | | Average |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 50µl | 100µl | 200µl | | 50µl | 100µl | 200µl | | 50µl | 100µl | 200µl | |
| **Glycerol** | 44,3±1,1 | 45,0±0,0 | 46,0±3,6 | 45,1±2,0 | 24,3±1,1 | 20,3±0,5 | 19,6±0,5 | 21,4±2,2 | 66,0±3,6 | 62,0±2,0 | 66,0±4,0 | 64,6±3,5 |
| **Glycerol + propolis** | 42,0±1,0 | 40,6±1,1 | 36,3±2,0 | 39,6±2,8 | 18,6±1,1 | 16,6±0,5 | 10,3±0,5 | 15,2±3,8 | 55,0±7,0 | 55,6±2,0 | 46,3±1,1 | 52,3±5,8 |
| **Ethanol** | 49,3±1,1 | 48,3±1,5 | 48,3±1,5 | 48,6±1,3 | 26,0±1,7 | 24,3±0,5 | 20,6±1,1 | 23,6±2,5 | 72,6±4,6 | 66,3±4,1 | 64,0±3,6 | 67,6±5,2 |
| **Ethanol + propolis** | 33,6±1,1 | 24,0±2,0 | 20,6±2,0 | 26,1±6,0 | 10,6±0,5 | 9,6±0,5 | 8,0±0,0 | 9,4±1,2 | 42,6±2,5 | 30,3±2,5 | 18,3±0,5 | 30,4±10,6 |
| **DMSO** | 43,0±2,6 | 39,3±0,5 | 35,6±1,1 | 39,3±3,5 | 31,3±6,1 | 25,3±0,5 | 28,6±1,1 | 28,4±4,0 | 56,0±1,7 | 53,6±1,1 | 56,3±3,2 | 55,3±2,2 |
| **DMSO + propolis** | 30,6±1,1 | 26,3±0,5 | 25,0±1,0 | 27,3±2,6 | 24,6±0,5 | 20,3±0,5 | 17,3±2,3 | 20,7±3,4 | 46,3±1,1 | 43,3±1,5 | 32,6±2,5 | 40,7±6,4 |
| **Acetone** | 31,6±1,5 | 26,3±1,1 | 26,6±1,5 | 28,2±2,8 | 24,0±1,0 | 20,0±3,0 | 19,0±1,7 | 21,0±2,9 | 51,6±2,0 | 44,6±7,2 | 47,6±6,8 | 48,0±5,9 |
| **Acetone + propolis** | 34,0±1,0 | 30,3±0,5 | 21,0±1,0 | 28,4±5,8 | 23,0±1,0 | 21,6±1,5 | 11,6±2,0 | 18,7±5,5 | 52,0±2,6 | 50,0±2,0 | 29,6±1,5 | 43,8±10,8 |
| **Methanol** | 33,6±0,5 | 31,6±1,1 | 31,3±1,1 | 32,2±1,3 | 23,3±0,5 | 22,6±0,5 | 22,3±0,5 | 22,7±0,6 | 55,6±1,5 | 56,0±1,0 | 53,3±1,5 | 55,0±1,7 |
| **Methanol + propolis** | 23,3±1,5 | 22,3±2,5 | 21,6±1,5 | 22,4±1,8 | 21,6±0,5 | 17,3±0,5 | 10,6±1,1 | 16,5±4,8 | 45,3±0,5 | 36,3±0,5 | 33,0±1,7 | 38,2±5,6 |
| **Pure propolis** | 27,3±0,5 | 24,0±1,0 | 20,6±1,1 | 24,0±3,0 | 20,3±0,5 | 17,6±1,1 | 14,3±0,5 | 17,4±2,6 | 41,0±1,7 | 32,0±1,7 | 28,6±1,1 | 33,8±5,6 |
| **Average** | 35,7±7,7 | 32,5±8,9 | 30,3±9,9 | **32,8±9,1$^{B}$** | 22,5±5,2 | 19,6±4,2 | 16,6±6,1 | **19,6±5,7$^{C}$** | 53,1±9,7 | 48,2±11,8 | 43,2±15,4 | **48,2±13,0$^{A}$** |

±: Standard deviation; ABC: The difference between the means shown with different letters in the same row is statistically significant (P≤0.01).

However, other best solvents of propolis showed different inhibition effects depending on the fungus species. For *A. solani*, the second best inhibitor of mycelial growth was pure propolis obtained by the CO2 extraction method, followed by methanol extract. DMSO and glycerol extracts were the least effective solvents. Compared to *A. solani*, glycerol and methanol were the second and third most inhibitory solvents of propolis extracts for *V. dahliae*. DMSO was the least effective solvent. For *F. oxysporum*, the second and third highest inhibition values were measured in pure propolis and acetone extracts. However, the most ineffective solvents for the growth of this fungal pathogen were acetone and glycerol. In parallel with the statistical analyses, the percentage inhibition values of different extracts of propolis on fungal pathogens were also calculated (Table 4).



**Table 4.** Fungus and dose-based percentage inhibition rates of propolis extracts

| Solvent/Fungus/Dose | *A. solani* (%) | | | *V. dahliae* (%) | | | *F. oxysporum* (%) | | |
|---|---|---|---|---|---|---|---|---|---|
| | 50µl | 100µl | 200µl | 50µl | 100µl | 200µl | 50µl | 100µl | 200µl |
| **Glycerol** | 47,88 | 50 | 48,88 | 73 | 77,44 | 78,22 | 26,66 | 31,11 | 26,66 |
| **Glycerol +propolis** | 53,33 | 54,88 | 59,66 | 79,33 | 81,55 | 88,55 | 38,88 | 38,22 | 48,55 |
| **Ethanol** | 45,22 | 46,33 | 46,33 | 71,11 | 73 | 77,11 | 19,33 | 26,33 | 28,88 |
| **Ethanol+Propolis** | 62,66 | 73,33 | 77,11 | 88,22 | 89,33 | 91,11 | 52,66 | 66,33 | 79,66 |
| **DMSO** | 52,22 | 56,33 | 60,44 | 65,22 | 71,88 | 68,22 | 37,77 | 40,44 | 37,44 |
| **DMSO+Propolis** | 66 | 70,77 | 72,22 | 72,66 | 77,44 | 80,77 | 48,55 | 51,88 | 63,77 |
| **Acetone** | 64,88 | 70,77 | 70,44 | 73,33 | 77,77 | 78,88 | 42,66 | 50,44 | 47,11 |
| **Acetone+Propolis** | 62,22 | 66,33 | 76,66 | 74,44 | 76 | 87,11 | 42,22 | 44,44 | 67,11 |
| **Methanol** | 62,66 | 64,88 | 65,22 | 74,11 | 74,88 | 75,22 | 38,22 | 37,77 | 40,77 |
| **Methanol+Propolis** | 74,11 | 75,22 | 76 | 76 | 80,77 | 88,22 | 49,66 | 59,66 | 63,33 |
| **Pure Propolis** | 69,66 | 73,33 | 77,11 | 77,44 | 80,44 | 84,11 | 54,44 | 64,44 | 68,22 |

Apart from the antifungal activity of ethanol extracts of propolis against plant pathogens, some studies have focused on other solvents. Özcan et al. [32] tested methanol extracts of propolis from five different regions of Turkey against *Alternaria alternata* and *Fusarium oxysporium f. sp. melonis*. All the propolis extracts showed complete inhibition at a concentration of 5 %. Yang et al.[14] compared the inhibitory effects of different solvent solutions of Chinese propolis (ethanol, water, petroleum ether, n-butanol, ethyl acetate) on *Penicillium italicum* mycelial growth. The results showed that ethyl acetate, ethanol, petroleum ether, n-butanol and water fractions were, in order, the most inhibitory at the same concentration (200 mg mL-1). Meneses et al. [16] tested different fractions of Colombian propolis (n-hexane/methanol fraction (EPEM), dichloromethane, ethyl acetate and methanol) against *Colletotrichum gloeosporioides* and Botryodiplodia theobromae. The results showed that two strains of *C. gloeosporioides* and *B. theobromae* were better inhibited by the dichloromethane and EPEM fractions, respectively. On the other hand, there

are very few studies on the DMSO extracts of propolis. Ertürk et al. [33] compared different solvents of propolis, including acetone, ethyl acetate, chloroform, ethanol, methanol, DMSO, and water, of animal-derived yeast *C. albicans* and other microorganisms. The DMSO solution of propolis showed weak inhibition against all tested microorganisms. Similarly, Ghasemi et al. [34] noted that PEE (propolis ethanol extract) exhibited broad-spectrum antibacterial activity against Gram-positive and Gram-negative bacteria compared to DMSO solutions. Solvent-based solutions showed different effects in a dose-dependent manner when comparing the antibacterial and antifungal activities of acetone and DMSO-based extracts against animal-derived pathogens. Overall, DMSO-based solutions have been reported to be more active than acetone-based solutions [35].

In this study, we found that ethanol was the most effective solvent for the extraction of propolis compared to other solvents. Our results indicate that ethanol extracts of propolis have the highest antiphytopathogenic activity. The superior performance of ethanol-propolis solutions in inhibiting fungal growth underscores the potential of this combination as a potent antifungal agent. Therefore, ethanol should be considered as the solvent of choice to maximise the bioactive properties of propolis in the control of plant pathogens. These results highlight the importance of the choice of the appropriate solvent in order to increase the efficacy of propolis in agricultural applications.

## 4. CONCLUSION

The study carried out shows that the effect values of the application models and variables were statistically significant. Considering the solvent and dose applications of propolis, the soil-borne pathogen V. dahliae showed a greater inhibition of hyphal colony diameter compared to the others, indicating that this pathogen is more sensitive to propolis preparations. In addition, it was found that the ethanol+propolis solvent was more effective in inhibiting fungal diameter growth at the high dose of 200 µL for all fungi compared to other solvents.

These findings suggest that propolis, particularly in combination with ethanol, has significant potential as a natural antifungal agent. Its effectiveness in inhibiting the growth of soil-borne pathogens like V. dahliae highlights the possibility of developing propolis-based treatments for managing plant diseases. This study contributes to the growing body of research exploring natural alternatives for disease control, and it paves the way for further investigations into the commercialization and practical application of propolis in sustainable agriculture.

## Conflicts of Interest

The article authors declare that there is no conflict of interest between them.

## Author's Contributions

The authors declare that they have made equal contributions to this article.

## REFERENCES


[1] Banskota AH, Nagaoka T, Sumioka LY, Tezuka Y, Awale S, Midorikawa K, et al. Antiproliferative activity of the Netherlands propolis and its active principles in cancer cell lines. In Journal of Ethnopharmacology. 2002;80(1):67–73.

[2] Abd-El-Rhman AM. Antagonism of Aeromonas hydrophila by propolis and its effect on the performance of Nile tilapia, Oreochromis niloticus. In Fish & Shellfish Immunology. 2009;27(3):454–459.

[3] Bankova V, Galabo, AS, Antonova D, Vilhelmova N, Di Perri B. Chemical composition of Propolis Extract ACF® and activity against herpes simplex virus. Phytomedicine. 2014; 21(11):1432-1438.

[4] Ghisalberti EL. Propolis:a review.Bee World. 1979;60:59-84.

[5] Castaldo S, Capasso F. Propolis, an old remedy used in modern medicine. Fitoterapia. 2002;73(1):1-6.

[6] Toreti VC, Sato HH, Pastore GM, Park YK. Recent progress of propolis for its biological and chemical compositions and its botanical origin. Evidence Based Complementary Alternative Medicine. 2013;697390. DOI: 10.1155/2013/697390.

[7] Hegazi AG. Propolis an overview. Congreso Internacional de Propóleos ,Buenos Aires 1-2nd, September 2000.

[8] Basim E, Basim H, Özcan M. Antibacterial activities of Turkish pollen and propolis extracts against plant bacterial pathogens. Journal of Food Engineering. 2006;77(4):992-996. DOI: 10.1016/j. jfoodeng.2005.08.027

[9] Marcucci MC. Propolis: Chemical Composition, Biological Properties And Therapeutic Activity. In Apidologie. 1995;26:83-99.

[10] Güney F, Yılmaz M. Propolisin kimyasal içeriği ile antibakteriyel, antiviral, antitümör, antifungal ve antioksidan aktivitesi. Arıcılık Araştırma Dergisi. 2013;10: 25-28.

[11] Lisa M, Leifertova I, Baloun J. Fungistatic effect of propolis. Folia Pharm Univ Carol. 1989;13:29-44.

[12] Matsuno T, Chen C, Basnet P. A tumoricidal and antioxidant compound isolated from an aqueous extract of propolis. Medical Science Research. 1997;25:583–584.

[13] Bogdanov S. Propolis: Composition, Health, Medicine: A Review. Bee Product Science, 2012. www.bee-hexagon.net
[14] Yang SZ, Peng LT, Su XJ, Chen F, Cheng YJ, Fan G, et al. Bioassay-guided isolation and identification of antifungal components from propolis against Penicillium italicum. Food Chemistry. 2011;127(1):210-215. http://dx.doi.org/10.1016/j.foodchem.2010.12.011
[15] Mattiuz BH, Ducamp-Collin MN, Mattiuz CFM, Vigneault C, Marques KM, Sagoua W, et al. Effect of propolis on postharvest control of anthracnose and quality parameters of 'Kent' mango. Scientia Horticulturae. 2015;184:60-168. DOI: 10.1016/j.scienta.2014.12.035
[16] Meneses EA, Durango DL, García CM. Antifungal activity against postharvest fungi by extracts from Colombian propolis. Química nova. 2009;32:2011-2017.
[17] Curifuta M, Vidal J, Salazar LA, Sánchez-Venegas J, Alvear M, Contreras A. The in vitro antifungal evaluation of a commercial extract of Chilean propolis against six fungi of agricultural importance. Ciencia e Investigación Agraria. 2012;39(2):347-359.
[18] Ali A, Wei YZ, Mustafa MA. Exploiting Propolis as an Antimicrobial Edible Coating to Control Post-harvest Anthracnose of Bell Pepper. Packaging Technology and Science. 2014. DOI: 10.1002/pts.2088
[19] Dudoit A, Mertz C, Chillet M, Cardinault N, Brat P. Antifungal activity of Brazilian red propolis extract and isolation of bioactive fractions by thin-layer chromatography-bioautography. Food Chemistry. 2020;327:1-8. DOI: 10.1016/j.foodchem.2020.127060
[20] Agrios GN. Plant pathology. 5th Edition, Elsevier Academic Press, Amsterdam, 2005;26-27:398-401.
[21] Lucas JA, Hawkins NJ, Fraaije BA. The evolution of fungicide resistance. Advances in applied microbiology. 2015;90:29-92.
[22] Quiroga EN, Sampietro DA, Soberon JR, Sgariglia MA, Vattuone MA. Propolis from the northwest of Argentina as a source of antifungal principles. Journal of Applied Microbiology. 2006;101(1):103-110. DOI: 10.1111/j.1365-2672.2006.02904.x
[23] Agüero MB, Svetaz L, Baroni V, Lima B, Luna L, Zacchino S, Tapia A. Urban propolis from San Juan province (Argentina): Ethnopharmacological uses and antifungal activity against Candida and dermatophytes. Industrial Crops and Products. 2014;57:166-173. DOI: 10.1016/j.indcrop.2014.03.009
[24] Bosio K, Avanzini C, D'Avolio A, Ozino O, Savoia D. In vitro activity of propolis against Streptococcus pyogenes. Letters on Applied Microbiology. 2000;31:174-177. DOI: 10.1046/j.1365- 2672.2000.00785.x
[25] Ronald MA. Microbiologia, Compania Editorial Continental S. A. de C. V., Mexico, 1 D. 1990. F. pp. 505.
[26] Deans SG, Sobada KP. Antimicrobial Properties of Marjoram (Origanum marjorana L.) Volatile Oil. Flavour Fragr. J. 1990;5:187-190.
[27] Arslan S, Perçin D, Silici S. The in vitro effects of propolis extracts prepared with different solvents on mutans streptococci. J Health Sci. 2010;19:68-73.
[28] Davari M, Ezazi R. Mycelial inhibitory effects of antagonistic fungi, plant essential oils and propolis against five phytopathogenic Fusariumspecies. Archives of Microbiology. 2022; 204:480.
[29] Er Y. In vitro and in vivo antimicrobial activity of propolis extracts against various plant pathogens. Journal of Plant Diseases and Protection. 2021;128(3):693-701.
[30] Costantin CR, de Oliveira HG, Roese LSM, Pancera MR, Silvestre WP, Siqueira GR, et al. Evaluation of the antifungal activity of propolis extracts from stingless bees on phytopathogenic fungi. Research, Society and Development. 2022;11(16):e445111638445-e445111638445.
[31] Moura SALD, Negri G, Salatino A, Lima LDDC, Dourado LPA, Mendes JB, et al. Aqueous extract of Brazilian green propolis: primary components, evaluation of inflammation and wound healing by using subcutaneous implanted sponges. Evidence-Based Complementary and Alternative Medicine. 2011.
[32] Özcan M, Ünver A, Ceylan DA,Yetisir R. Inhibitory effect of pollen and propolis extracts. Food/Nahrung. 2004;48(3):188-194.
[33] Ertürk Ö, Yavuz C, Sıralı C. The Antimicrobial Activity of Propolis from Ordu Province of Turkey. HARUM. 2011;28:11-16.
[34] Ghasemi FS, Eshraghi SS, Andalibi F, Hooshyar H, Kalantar- Neyestanaki D, Samadi A, et al. Anti-Bacterial Effect of Propolis Extract in Oil Against Different Bacteria. Zahedan J. Res. Med. Sci. 2017;19(3):7225.
[35] Ugur A, Arslan T. An in vitro study on antimicrobial activity of propolis from Mugla province of Turkey. Journal of Medicinal Food. 2004;7(1):90-94.