arastirma.tarimorman.gov.tr/bursagida
ISSN 1303-3107
e-ISSN 2822-2873

Gıda ve Yem Bilimi - Teknolojisi Dergisi / Journal of Food and Feed Science - Technology 35:78-87 (2025/2) 10.56833/gidaveyem.1733947

## Original Article/Özgün Araştırma

# Antifungal Activity of Bee Pollen Extracts Against Selected Filamentous Fungi

# Arı Poleni Ekstraktlarının Filamentöz Mantar Türlerine Karşı Antifungal Etkisi

**Neslihan Ulubayram[1,2*], Aycan Yigit Cinar[3]**

[1] *Department of Food Processing, Vocational School of Altıntas, Kutahya Dumlupınar University, KUTAHYA, TÜRKİYE*
[2] *Department of Food Engineering, Graduate School of Natural and Applied Sciences, Bursa Uludag University, BURSA, TÜRKİYE*
[3] *Department of Food Engineering, Faculty of Engineering and Natural Sciences, Bursa Technical University, BURSA, TÜRKİYE*
*(By author order/Yazar sıralamasına göre)*
***ORCID ID:*** *0000-0001-5880-4261, Öğr. Gör.*
***ORCID ID:*** *0000-0003-2038-725X, Doç. Dr.*
**Corresponding author/Sorumlu yazar: neslihan.ulubayram@dpu.edu.tr*

**Geliş Tarihi : 12.02.2025**
**Kabul Tarihi : 11.06.2025**

## Abstract

**Objective:** Pollen is a natural bee product known for its rich bioactive content and positive effects on health. Although previous research has highlighted the antibacterial and antifungal properties of bee pollen, there is still a lack of comprehensive studies investigating its inhibitory effects on fungi, particularly molds. Our research seeks to contribute to filling this gap by assessing the antifungal potential of bee pollen against selected fungal species.

**Materials and methods:** In this study, the antifungal properties of bee pollen obtained by combining two multifloral samples from the Bursa region were evaluated. Ethanolic and methanolic extracts of the bee pollen at varying concentrations (1%, 5%, 7.5%, and 10%) were tested for their inhibitory effects against *Alternaria alternata*, *Penicillium chrysogenum*, *Fusarium culmorum*, and *Aspergillus flavus*.

**Results and conclusion:** Both pollen extracts exhibited dose-dependent antifungal activity against all tested fungal strains, with the methanol/water extract demonstrating significantly greater efficacy than the ethanol/water extract. The highest antifungal activity among all tested fungi was consistently observed at the 10% extract concentration. Among the tested strains, *F. culmorum* demonstrated the highest sensitivity, with a 7.5% concentration effectively inhibiting its growth. Given the promising antifungal potential, further investigation is needed to identify the primary bioactive compounds and elucidate their mechanisms of action.

**Keywords:** Bee pollen, antifungal activity, filamentous fungi, mold

## Öz

**Amaç:** Polen, zengin biyoaktif içeriği ve sağlık üzerine olumlu etkileri ile bilinen doğal bir arı ürünüdür. Polen üzerinde yürütülen bilimsel çalışmalar, onun antibakteriyel ve antifungal özelliklerini ortaya koymaktadır. Ancak, özellikle küf mantarları üzerinde arı poleninin inhibisyon etkisine dair yeterli çalışma bulunmamaktadır. Araştırmamız, arı poleninin seçilmiş mantar türlerine karşı antifungal potansiyelini değerlendirerek literatüre katkıda bulunmayı amaçlamaktadır.

**Materyal ve yöntem:** Bu çalışmada, Bursa bölgesinden temin edilen iki multifloral polen örneğinin birleştirilmesiyle elde edilen arı poleninin antifungal özellikleri değerlendirilmiştir. Polenin farklı konsantrasyonlarda hazırlanan etanolik ve metanolik ekstreleri (%1, %5, %7,5, %10), *Alternaria alternata, Penicillium chrysogenum, Fusarium culmorum* ve *Aspergillus flavus* olmak üzere dört filamentöz mantar türüne karşı test edilmiştir.

**Tartışma ve sonuç:** Her iki polen ekstresi de test edilen tüm mantar türlerine karşı doza bağlı antifungal aktivite göstermiş olup, metanol/su ekstresinin etanol/su ekstresine kıyasla belirgin şekilde daha yüksek etkinlik sergilediği gözlemlenmiştir. En yüksek antifungal aktivite, tüm mantar türlerinde %10'luk ekstre konsantrasyonunda kaydedilmiştir. Test edilen türler arasında *F. culmorum*, %7,5'lik konsantrasyonda dahi

etkili bir inhibisyon göstererek en yüksek duyarlılığı sergilemiştir. Elde edilen umut verici sonuçlar doğrultusunda, polenin antifungal potansiyelini sağlayan biyoaktif bileşenlerin tanımlanması ve etki mekanizmalarının aydınlatılması için ileri çalışmalara ihtiyaç duyulmaktadır.

**Anahtar kelimeler:** Arı poleni, antifungal aktivite, filamentli mantarlar, küf

## 1. Introduction

Pollen is typically yellow or orange in color and a powdery material, produced by blooming plants and collected by bees to serve as a nutrient source for the development and maintenance of the colony. Besides being a nutrient food for bees, bee pollen has been used in human nutrition and apitherapy for many years. Composition of pollen mainly depends on botanical and geographic origin, therefore some pollen types are classified as highly nutritious, while others are characterized by their therapeutic properties (Kieliszek et al., 2018). In general, bee pollen contains essential nutrients like carbohydrates, amino acids, proteins, lipids, vitamins, minerals and bioactive substances such as phytosterols, phenolic compounds, phytochemicals and flavonoids. Due to its nutrients, it is considered as a potential source of energy and proteins for human consumption (Kroyer and Hegedus, 2001; Campos et al., 2010). With contribution to nutrition, bee pollen exhibits antioxidants, anti-inflammatory, hepatoprotective, anti-carcinogenic, antibacterial, anti-fungicidal, and anti-atherosclerotic activities (Campos et al., 2010; Komosinska-Vassev et al., 2015; Alvarez-Suarez, 2017). Owing to its bioactive components and nutritional benefits, pollen is generally consumed as a functional food and therapeutic agent. While bee pollen contains compounds with potential bioactive and therapeutic properties, further extensive studies are necessary to fully understand its applications in therapy (Denisow and Denisow-Pietrzyk, 2016).

Recent studies have underlined the antibacterial and antifungal features of bee pollen, attributing these effects primarily to its rich bioactive compounds such as flavonoids, phenolic compounds, and phenol amides (Rodríguez-Pólit et. al., 2023). Studies investigating the correlation between phenolic content and antimicrobial activity in bee pollen have identified kaempferol, quercetin, and caffeic acid as key bioactive compounds (Ilie et. Al.,2024). These components act through multiple mechanisms, including bacterial membrane disruption, ion channel interference, and ATP synthesis inhibition against diverse bacterial pathogens (Bakour et al., 2019). Also, the precise mechanisms underlying the differential effects of these phenolics on microbial growth remain to be fully understood. Further research is necessary to know the relationships between these two and specific antifungal efficacy of individual phenolic constituents.

Research has shown that bee-collected pollen exhibits antimicrobial effect against diverse pathogens, including bacteria and fungi. The most sensitive microorganisms are Gram positive bacteria, particularly *Staphylococcus aureus*, as shown by Carpes et al. (2007), Fatrcová-Šramková et al. (2013) and Pascoal et al. (2014). Some studies have also shown an inhibitory activity opposed to Gram negative bacteria, such as *Pseudomonas aeruginosa* and *Escherichia coli*, along with yeasts and fungi (Carpes et al., 2007). Studies about antibacterial effects of pollen are considerably more comprehensive and more than those addressing its antifungal properties. Notably, investigations into fungal activity have predominantly focused on *Candida* spp., common yeast species frequently associated with such research (Didaras et al., 2020). The most extensive study conducted on the antifungal activity of bee pollen evaluated its efficacy against a wide range of fungal species, including *Aspergillus niger*, *Aspergillus flavus*, and *Aspergillus fumigatus*, as well as seven different yeast strains: *Candida krusei, Candida albicans, Rhodotorula mucilaginosa, Candida glabrata, Geotrichum candidum, Candida parapsilosis* and, *Candida tropicalis* (Kacániova et al., 2012). However, there is not sufficient research on the inhibitory effects of bee pollen against fungi, especially molds. Also, studies exhibit considerable variation depending on the solvent utilized. This variability underscores the importance of solvent selection in determining the efficacy of bioactive compounds, highlighting the need for careful optimization in antimicrobial studies. In this study, the antifungal activity of bee pollen, especially on filamentous molds, was investigated and the comparative results obtained using different solvents were evaluated. The inhibitory potential and efficacy of the bee pollen on causers of food spoilage and potentially toxigenic fungi was investigated.

## 2. Materials and Methods

Pollen was prepared by mixing two multifloral bee pollen samples which were obtained from different beekeepers in Kestel and İnegöl regions of Bursa, Turkey. While the flora of these regions has been investigated in previous studies, palynological analysis could provide valuable insights for further research into regional variations in pollen composition.

The microscopic filamentous fungi (*Alternaria alternata, Penicillium chrysogenum, Aspergillus flavus and Fusarium culmorum*) were provided by the culture collection of Department of Food Engineering, Faculty of Engineering and Natural

Sciences, Bursa Technical University. Sabouraud dextrose agar (SDA) used as the main medium in the study.

For the preparation of sample extracts, the method reported by Morais et al. (2011) was used, with minor modifications. Prior studies have shown that bee pollen compounds are soluble in alcohol-based solvents and, to some extent, in water. Considering these findings, a solvent mixture combining ethanol/methanol and water was formulated to enhance the extraction of such bioactive compounds. Methanol/water (3:1, v/v) and ethanol/water (3:1, v/v) were used as solvents. Pollen (50 g) was extracted 150 ml of solvent by maceration with stirring at room temperature for 24 h and the solution was filtered by Whatman filter paper No. 4 and the solid residue was re-extracted. Then, they were stored in small (20 mL) colorful bottles under refrigeration until use.

The fungi were cultivated on Sabouraud dextrose agar (SDA) petries for seven days at 30 °C and the spores were harvested with 10 mL of 0.1% Tween 80 (Merck, Darmstadt, Germany) solution sterilized by membrane (0.45 μm) filtration. The spore suspensions were adjusted with the same solution to give a final concentration of $10^6$ spore/mL and used the same day. Pollen extracts were added to Sabouraud dextrose agar with proper amounts to prepare of 1%, 5%, 7.5 % and 10% (v/v) concentrations of extract-containing medium. Agar solutions, adjusted to the desired concentrations, were poured into sterile Petri dishes and point-inoculated with 2 mL of spore suspension in the center. Plates were incubated at 30 °C for 7 days. Culture medium without pollen extracts was used as a control and methanol/ethanol solvents were substituted to determine inhibition effect of solvents (Yigit and Korukluoglu, 2007). The fungus was incubated at 30°C for seven days. The Petri plates, in which no growth was observed were incubated for 14 days. After seven days of incubation, the colony diameter was measured, and percent mycelial inhibition was calculated as follows.

$$I = (C - T/C)\, x\, 100$$

Where I is the inhibition (%), C is the colony diameter of mycelium from the control Petri plate (in mm), and T is the colony diameter of mycelium from the test Petri plate (in mm) (Özcan, 2004). Three replications were made for each concentration and the microorganism, and averages and standard deviations were calculated.

The statistical analyses were performed using Minitab, version 22 (Minitab LLC, PA, USA). The obtained data was expressed as mean ± standard deviation (SD). Differences between means were determined by one-way analysis of variance (ANOVA) at a significance level of $p<0,05$, followed by Tukey's test as a post-hoc analysis.

## 3. Results and Discussion

Existing research often focuses on a narrow range of fungal species. Results generally varied significantly due to factors such as botanical origin, extraction methods, and the microorganisms tested. The choice of solvent also critically influences outcomes, complicating data interpretation. These limitations are obstacles to fully understanding the antifungal potential of bee pollen. Our study aimed to contribute to this field by increasing the available data and it was observed that both pollen extracts demonstrated dose-related antifungal effect on all test fungi. The antifungal activities of methanol/water and ethanol/water extracts of pollen against pathogenic fungus are shown in Table 1.

Among the applied extract concentrations, the 10% dose showed the strongest antifungal activity against all tested fungal species. *Alternaria alternata* showed statistically significant inhibition ($p<0.05$) across tested concentrations (5%, 7.5% and 10%) of both methanol and ethanol extracts. The inhibition zones decreased from 64.3 ± 0.6 mm to 29.5 ± 1.7 mm at the highest concentration (10%) of the methanol/water extract, while the ethanol/water extract showed a more dramatic reduction from 85 mm to 3.42 ± 2.8 mm. At 7.5% concentration, the inhibition of *A. alternata* was 46% for the methanol/water extract and 44% for the ethanol/water extract. The highest inhibition was observed at 10% concentration, with the methanol/water extract achieving 54% inhibiting and the ethanol/water extract reaching 60% (Figure 1). According to Özcan et al. (2004), the percent inhibition of pollen at concentrations of 2% and 5% against *A. alternata* was found to be lower than 50%. This may be attributed to the relatively low concentration used, as evidenced by the findings in our study, where at a 5% concentration, the methanol extract exhibited 37% inhibition, while the ethanol extract demonstrated 25% inhibition against *A. alternata*. As reported by Cabrera and Montenegro (2013), aqueous extract, did not exhibit a complete inhibitory effect on the fungi; however, it resulted in delayed growth when compared to the control group. The study result reveals the importance of the extraction method.

**Table 1.** Inhibition zone diameters of methanol/water and ethanol/water extracts against fungi at different concentrations (in mm ± S.D.)

| Fungi | Extract Type | Control | 1% | 5% | 7.5% | 10% |
|---|---|---|---|---|---|---|
| *Alternaria alternata* | M | 64.3 ± 0.6$^{a}$ | 59.5 ± 0.0$^{b}$ | 40.7 ± 0.3$^{c}$ | 34.8 ± 0.6$^{d}$ | 29.5 ± 1.7$^{e}$ |
| | E | 85.0 ± 0.0$^{a}$ | 85.0 ± 0.0$^{a}$ | 63.8 ± 1.6$^{b}$ | 4.73 ± 0.6$^{c}$ | 3.42 ± 2.8$^{d}$ |
| *Aspergillus flavus* | M | 66.3 ± 0.6$^{a}$ | 64.5 ± 0.0$^{a}$ | 36.3 ± 1.0$^{b}$ | 31.2 ± 0.6$^{c}$ | 27.2 ± 0.6$^{d}$ |
| | E | 85.0 ± 0.0$^{a}$ | 85.0 ± 0.0$^{a}$ | 78.7 ± 1.2$^{a}$ | 4.82 ± 1.6$^{b}$ | 3.67 ± 1.5$^{b}$ |
| *Penicillium chrysogenum* | M | 14.3 ± 0.6$^{a}$ | 19.2 ± 0.6$^{a}$ | 13.0 ± 0.7$^{ab}$ | 9.80 ± 0.6$^{bc}$ | 5.00 ± 0.7$^{c}$ |
| | E | 27.3 ± 0.6$^{a}$ | 25.3 ± 0.4$^{a}$ | 16.7 ± 0.6$^{a}$ | 15.0 ± 1.4$^{b}$ | 12.3 ± 0.6$^{b}$ |
| *Fusarium culmorum* | M | 61.0 ± 1.0$^{a}$ | 14.2 ± 0.6$^{b}$ | 12.5 ± 0.0$^{b}$ | 9.50 ± 0.0$^{b}$ | - |
| | E | 41.0 ± 1.0$^{a}$ | 17.7 ± 0.6$^{b}$ | 15.8 ± 0.1$^{c}$ | - | - |

(−) No inhibition zone, M: Methanol extract, E: Ethanol extract Within a column, means with distinct letters differ significantly ($p < 0.05$).

Similarly, the methanol/water extract reduced *Aspergillus flavus* growth from 66.3 ± 0.6 mm to 27.2 ± 0.6 mm, while the ethanol/water extract showed a more pronounced reduction, from 85 mm to 3.67 ± 1.53 mm. At 10% concentration, the methanol/water extract achieved the highest inhibition of Aspergillus flavus at 59%, while the ethanol/water extract reached 57% inhibition, demonstrating the strong dose-dependent antifungal potential of both extracts at higher concentrations (Figure 1). Research about the antifungal activity of pollen on *Aspergillus species* (*Aspergillus flavus, Aspergillus fumigatus, Aspergillus niger, Aspergillus ochraceus and Aspergillus versicolor)* provided bee pollen has an antifungal activity but not completely inhibited mycelial growth (Özcan, 2004; Fatrcová-Šramková et al., 2016). A study used three different strains of filamentous fungi, *A. niger, A. flavus, A. fumigatus* has confirmed that pollen had antifungal effect on selected fungus. In this study, methanol and ethanol were used for extraction, with concentrations of 99.9% and 70% methanol (aqueous, v/v) and 96% and 70% ethanol (aqueous, v/v), respectively. The antifungal activity of the 70% aqueous methanol extract of pollen was found greater than the ethanol extract with the same concentration on *A. flavus* (Kacániová et al., 2012) similarly to our findings. The methanol/water extract demonstrated greater effectiveness overall compared to the ethanol/water extract, especially at low concentrations. The study supported earlier research and contributed to understanding pollen's antifungal effect.

*Penicillium chrysogenum* exhibited notable inhibition across all concentrations of both methanol/water and ethanol/water extracts. The growth zones of mold decreased progressively with increasing extract concentrations, reaching their lowest values at the highest concentration (10%), where significant growth suppression was observed. Academic literature has a limited number of studies investigating the antimicrobial activity of bee pollen against *Penicillium spp*. For instance, in a study by Fernandes et al. (2024), which focused on selected fungal species, it was reported that bee bread exhibited no inhibitory effect on *Penicillium* sp. In this study, the broth microdilution method was utilized, and measurements were performed employing a spectrophotometric technique. Despite these findings, a separate study examining hydroxycinnamic acid amides from bee pollen revealed that *Penicillium verrucosum* was the most susceptible mold to these antifungal compounds. The compounds not only inhibited the radial growth of *P. verrucosum* but also affected its ability to sporulate (Kyselka et al., 2018). It is important to consider that the current findings may be influenced by the methodologies employed. Results show a need for further research to determine the efficacy of antifungal components and determine the relationships between these components and their antimicrobial activities.

In our study, *Fusarium culmorum* showed absolute growth inhibition at the highest concentration (10%) of both extracts. Furthermore, the 7.5% concentration of ethanol/water extract inhibited the growth of *F. culmorum*. This result is significant, as *F. culmorum* is a known plant pathogen responsible for crop diseases. Research has demonstrated that *F.culmorum* is more sensitive than other species of molds. In these studies which tested the fungistatic activity of aqueous extracts of plants and essential oils against *A. Alternata, A. candidus, A. niger*, F. culmorum and *Penicillium* sp., observed *F.culmorum* was the most sensitive one (Magro et al., 2006; Sitara et al., 2008).

Generally, *A.alternata* and *A.flavus* showed resistance to all extracts, *F.culmorum* was the most sensitive strain as both extracts has low inhibiton percentage. Considering all the results, antifungal activity was observed against all test microorganisms. While the findings align with

some studies in literature, there are also studies that present contrasting results. Erkmen and Özcan, (2008) carried out a study on Turkish bee pollen with methanol extracts of (0.02% to 2.5% and mentioned that pollen showed no activity on selected spoilage and pathogenic microorganisms which includes *Candida albicans, Saccharomyces cerevisiae, A. alternata* and *F. oxysporium*. Özcan et al., 2004 used methanol extract with the concentrations of 2,5% and 5% on same fungus. None of the tested pollen extracts were able to fully suppress the mycelial growth of the fungi. Additionally, the inhibition percentage for both concentrations of pollen against *A. alternata* and *F. oxysporum* was less than 50%. Due to these results, the increase in inhibition percentages depends on doses and the usage of lower doses may have provided unsatisfactory results about inhibition efficacy of bee pollen.

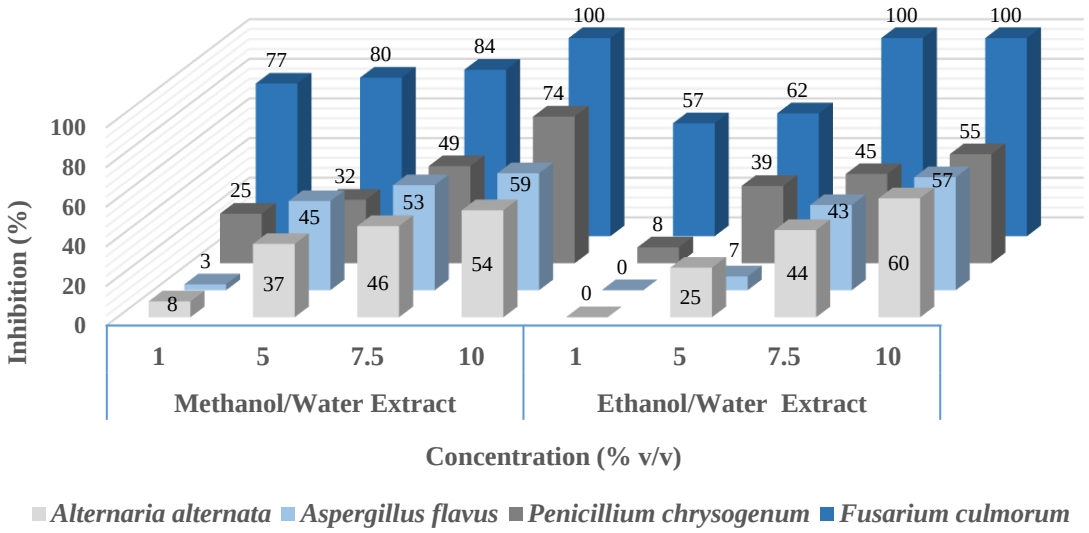


**Figure1.** Inhibition percentage of fungi with different amounts of bee pollen extracts

The methanol/water extract generally exhibited higher antifungal activity at lower concentrations in comparison to the ethanol/water extract. However, the ethanol/water extract exhibited a more precise effect at higher concentrations, particularly against *Fusarium culmorum*, where complete inhibition was observed. The choice of solvent and concentration plays an essential role in the efficacy of antifungal activity. The differences in the antifungal effect with tested extracts may be assigned to their ability to dissolve polar compounds like flavonoids and phenolics which show good antimicrobial activity (Parekh et al., 2006; Wang and Weller, 2006).

Methanol and ethanol are the most accepted extraction agents for antimicrobial analysis, followed by water. The choice of solvent may influence the extraction efficiency because of its specific chemical affinity for certain active components. For instance, water has been shown to effectively extract flavonoids, including quercetin and kaempferol-linked glycosides (Didaras et al., 2020). Akhir et al. (2017) extracted bee bread using ethanol and hexane, and showed ethanol exhibited higher antioxidant and antimicrobial activity compared to hexane-extracted samples. Since antimicrobial activity is primarily attributed to phenolic compounds, the ethanolic aqueous solution appears to facilitate greater extraction of these bioactive constituents. In another study, among the tested solvents (95% ethanol, 70% ethanol, dichloromethane, and hexane), 70% ethanol extraction yielded the highest extract amount (41.1% w/w) and demonstrated the strongest antioxidant activity. These results suggest that the aqueous-ethanolic solution (70%) is more efficient in extracting bioactive compounds as we used in our study (Rashid et al., 2023). Methanol was also used alongside ethanol as both are widely used in similar studies. Methanol is preferred due to its potential to extract bioactive antifungal compounds despite its known toxicity (Suurbaar et al., 2017; Kabra et al., 2019).

Moreover, existing studies have demonstrated that the effect of solvent on antifungal activity is specific to microorganisms. For instance, *A.*

*fumigatus* exhibited the highest susceptibility among *Aspergillus spp.* when tested against the 70% ethanol extract, whereas it displayed the highest resistance against the 70% methanol extract (Kacániová et al., 2012). These results highlight the importance of solvent selection and concentration in influencing antifungal efficacy, underlining the need for Detailed analysis of these parameters in future studies.

The antifungal activity of bee pollen cannot be properly evaluated without considering the key bioactive compounds. Flavonoids such as quercetin and kaempferol glycosides interfere with fungal cell wall synthesis and increase membrane permeability, break cellular integrity. Phenolic acids, including caffeic acid and ferulic acid, show antifungal effect by inducing oxidative stress, which damages proteins and DNA. Additionally, the enzyme glucose oxidase generates hydrogen peroxide, a potent antimicrobial agent that suppresses fungal proliferation (Bhattacharya et al., 2023).

Non-polar solvent extracts, rich in lipophilic fractions, have demonstrated efficacy against common foodborne fungi like *Penicillium* and *Aspergillus* by disrupting their lipid membranes. Organic acids also make the environment too acidic for fungi to survive (Kaškonienė et al.,2020). Importantly, the interaction of these diverse compounds likely produces synergistic effects, enhancing the overall antifungal activity.

Besides phenolic compounds, macronutrient composition of bee pollen also significantly influences its antimicrobial activity. Phospholipids of bee pollen contribute to its antifungal activity by affecting the fluidity and permeability of the fungal cell membrane. The proteins and amino acids in bee pollen may further enhance its antifungal properties by acting as antimicrobial peptides. Additionally, compounds such as alkaloids and terpenes, which are less studied but gaining attention, could play a role in the antimicrobial mechanisms of bee pollen (Didaras et al., 2020).

## 4. Conclusion

Fungal pathogens threaten food security by damaging vegetables, fruits and grains, and contaminating them with toxins. In this study, the fungal pathogens were specifically selected based on their documented potential for toxicity and their capacity for plant diseases. The results proved that bee pollen has an antifungal effect of these fungal pathogens. In the future, besides therapeutic usage of it, pollen and its bioactive components may be used as bio preservative agents or to increase the functionality of food. Additional research is necessary to identify the phenolic compounds responsible for antifungal activity, elucidating their structure-activity relationships, and correlating these findings with the product's origin are crucial for understanding its bioactive potential and ensuring reproducible efficacy.

## 5. Acknowledgments

A preliminary version of this study was presented as an oral presentation at [IX International Scientific Agriculture Symposium, in October 2018, Bosnia and Herzegovina]. The current manuscript has been expanded and revised for publication.

## 6. References

Akhir, R. A. M., Bakar, M. F. A., & Sanusi, S. B. (2017, October). *Antioxidant and antimicrobial activity of stingless bee bread and propolis extracts.* In AIP conference proceedings (Vol. 1891, No. 1). AIP Publishing.

Alvarez-Suarez, J. M. (Ed.). (2017). *Bee Products-Chemical and Biological Properties.* Springer.

Cabrera, C., and Montenegro, G. (2013). Pathogen control using a natural Chilean bee pollen extract of known botanical origin. *Ciencia e investigación agraria: revista latinoamericana de ciencias de la agricultura*, 40(1), 223-230.

Campos, M. G. R., Frigerio, C., Lopes, J., and Bogdanov, S. (2010). What is the future of Bee-Pollen. *Journal of ApiProduct and ApiMedical Science*, 2(4), 131-144.

Carpes, S. T., Begnini, R., Alencar, S. M. D., and Masson, M. L. (2007). Study of preparations of bee pollen extracts, antioxidants and antibacterial activity. *Ciência e Agrotecnologia*, 31(6), 1818-1825.

Denisow, B., and Denisow-Pietrzyk, M. (2016). Biological and therapeutic properties of bee pollen: a review. *Journal of the Science of Food and Agriculture*, 96(13), 4303-4309.

Didaras, N. A., Karatasou, K., Dimitriou, T. G., Amoutzias, G. D., and Mossialos, D. (2020). Antimicrobial activity of bee-collected pollen and beebread: State of the art and future perspectives. *Antibiotics*, 9(11), 811.

Erkmen, O., and Özcan, M. M. (2008). Antimicrobial effects of Turkish propolis, pollen,

and laurel on spoilage and pathogenic food-related microorganisms. *Journal of medicinal food*, 11(3), 587-592.

Fatrcová-Šramková, K., Nôžková, J., Kačániová, M., Mariassyova, M., Rovna, K., and Stričík, M. (2013). Antioxidant and antimicrobial properties of monofloral bee pollen. *Journal of Environmental Science and Health, Part B*, 48(2), 133-138

Fatrcová-Šramková, K., Nôžková, J., Máriássyová, M., and Kačániová, M. (2016). Biologically active antimicrobial and antioxidant substances in the Helianthus annuus L. bee pollen. *Journal of Environmental Science and Health, Part B*, 51(3), 176-181.

Fernandes, K. E., Frost, E. A., Kratz, M., and Carter, D. A. (2024). Pollen products collected from honey bee hives experiencing minor stress have altered fungal communities and reduced antimicrobial properties. *FEMS Microbiology Ecology*, 100(7), fiae091.

Ilie, C. I., Spoiala, A., Geana, E. I., Chircov, C., Ficai, A., Ditu, L. M., & Oprea, E. (2024). Bee bread: a promising source of bioactive compounds with antioxidant properties—first report on some antimicrobial features. *Antioxidants*, 13(3), 353.

Kabra, A., Sharma, R., Hano, C., Kabra, R., Martins, N., & Baghel, U. S. (2019). Phytochemical composition, antioxidant, and antimicrobial attributes of different solvent extracts from myrica esculenta buch.-ham. Ex. D. Don leaves. *Biomolecules*, 9(8), 357.

Kacániová, M., Vuković, N., Chlebo, R., Haščík, P., Rovna, K., Cubon, J., ... and Pasternakiewicz, A. (2012). The antimicrobial activity of honey, bee pollen loads and beeswax from Slovakia. *Archives of Biological Sciences*, 64(3), 927-934.

Kaškonienė, V., Adaškevičiūtė, V., Kaškonas, P., Mickienė, R., & Maruška, A. (2020). Antimicrobial and antioxidant activities of natural and fermented bee pollen. Food Bioscience, 34, 100532.

Kieliszek, M., Piwowarek, K., Kot, A. M., Błażejak, S., Chlebowska-Śmigiel, A., and Wolska, I. (2018). Pollen and bee bread as new health-oriented products: A review. *Trends in Food Science & Technology*, 71, 170-180.

Komosinska-Vassev, K., Olczyk, P., Kaźmierczak, J., Mencner, L., and Olczyk, K. (2015). Bee pollen: chemical composition and therapeutic application. Evidence-Based *Complementary and Alternative Medicine*, 2015(1), 297425.

Kroyer, G., and Hegedus, N. (2001). Evaluation of bioactive properties of pollen extracts as functional dietary food supplement. *Innovative Food Science and Emerging Technologies*, 2(3), 171-174.

Kyselka, J., Bleha, R., Dragoun, M., Bialasová, K., Horáčková, S., Schätz, M., ... and Synytsya, A. (2018). Antifungal polyamides of hydroxycinnamic acids from sunflower bee pollen. *Journal of Agricultural and Food Chemistry*, 66(42), 11018-11026.

Magro, A., Carolino, M., Bastos, M., and Mexia, A. (2006). Efficacy of plant extracts against stored products fungi. *Revista Iberoamericana de Micología*, 23(3), 176-178.

Morais, M., Moreira, L., Feás, X., and Estevinho, L. M. (2011). Honeybee-collected pollen from five Portuguese Natural Parks: Palynological origin, phenolic content, antioxidant properties and antimicrobial activity. *Food and Chemical Toxicology*, 49(5), 1096-1101.

Özcan, M. (2004). Inhibition of Aspergillus parasiticus NRRL 2999 by pollen and propolis extracts. *Journal of medicinal food*, 7(1), 114-116.

Özcan, M., Ünver, A., Ceylan, D. A., and Yetisir, R. (2004). Inhibitory effect of pollen and propolis extracts. *Food/Nahrung*, 48(3), 188-194.

Parekh, J., Jadeja, D., and Chanda, S. (2006). Efficacy of aqueous and methanol extracts of some medicinal plants for potential antibacterial activity. *Turkish Journal of Biology*, 29(4), 203-210.

Pascoal, A., Rodrigues, S., Teixeira, A., Feás, X., and Estevinho, L. M. (2014). Biological activities of commercial bee pollens: Antimicrobial, antimutagenic, antioxidant and anti-inflammatory. *Food and Chemical Toxicology*, 63, 233-239.

Rashid, Z. M., Abd Rashid, K. A., & Anwar, N. Z. R. (2023). Heterotrigona Itama Bee Bread Extracts: Effect of Solvent Polarity on Extraction Yield, Chemical Characteristics and Antioxidant Activity. *Journal Of Agrobiotechnology*, 14(1), 44-53.

Rodríguez-Pólit, C., Gonzalez-Pastor, R., Heredia-Moya, J., Carrera-Pacheco, S. E., Castillo-Solis, F., Vallejo-Imbaquingo, R., ... and Guamán, L. P. (2023). Chemical properties and biological activity of bee pollen. *Molecules*, 28(23), 7768.

Sitara, U., Niaz, I., Naseem, J., and Sultana, N. (2008). Antifungal effect of essential oils on in vitro growth of pathogenic fungi. *Pakistan Journal of Botany*, 40(1), 409.

Suurbaar, J., Mosobil, R., & Donkor, A. M. (2017). Antibacterial and antifungal activities and phytochemical profile of leaf extract from different extractants of Ricinus communis against selected pathogens. *BMC research notes*, 10, 1-6.

Wang, L., and Weller, C. L. (2006). Recent advances in extraction of nutraceuticals from plants. *Trends in Food Science and Technology*, 17(6), 300-312.

Yigit, A., and Korukluoglu, M. (2007). The effect of potassium sorbate, NaCl and pH on the growth of food spoilage fungi. *Annals of Microbiology*, 57(2), 209-215.