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Comparative Effects of Crude Libyan Propolis and Commercial Propolis Extracts on Aspergillus Ochraceus, Aspergillus Niger, and Penicillium spp. in Vitro

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ABSTRACT

Propolis, a resinous substance collected by honeybees, exhibits antimicrobial properties attributed to its flavonoid and phenolic content, which vary significantly with geographical origin. This study evaluated and compared the antifungal efficacy of crude Libyan propolis (CLP) and commercial propolis extract (CPE) against Aspergillus niger, Aspergillus ochraceus, and Penicillium spp. using mycelial growth inhibition on agar (10, 25, and 50 mg mL^{-1}) and biomass dry-weight quantification in broth (1, 5, and 10 mg mL^{-1}), with all experiments performed in triplicate and data analysed by three-way ANOVA. Both propolis types significantly inhibited fungal growth compared with controls ($p < 0.001$). CLP showed superior activity against A. niger (64.7% inhibition at 25 mg mL^{-1}) compared with CPE, which achieved comparable inhibition (45.1%) only at 50 mg mL^{-1}, a twofold higher concentration. Against A. ochraceus, CLP exhibited maximal inhibition at 10 mg mL^{-1} (76.4%), while CPE provided stable inhibition (58.8–67.0%) across concentrations. In biomass assays, Penicillium spp. was most sensitive to CPE at 10 mg mL^{-1} (1.42 g), and a hormetic effect was observed at 1 mg mL^{-1} CPE, where biomass exceeded controls by up to 86%. These findings demonstrate that both CLP and CPE possess species-specific antifungal activity. CLP was particularly effective against A. niger, whereas CPE showed greater activity against Penicillium spp. The observed biphasic and hormetic responses underscore the importance of dose optimisation. Libyan propolis therefore represents a promising natural antifungal agent for food preservation.

التأثيرات المقارنة للبروبوليس الليبي الخام ومستخلصات البروبوليس التجارية على فطريات Aspergillus ochraceus وAspergillus Niger وPenicillium spp. في المختبر

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الكلمات المفتاحية:
البروبوليس الليبي الخام.
مستخلص البروبوليس التجاري.
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العفن الأسود.
الرشاشية الصفراء (الأوكراوية).

الملخص

يعتبر البروبوليس (العكبر) مادة راتنجية يجمعها نحل العسل، وتتميز بخصائص مضادة للميكروبات تُعزى إلى محتواها من الفلافونيدات والمركبات الفينولية. قيمت هذه الدراسة الفعالية المضادة للفطريات للبروبوليس الليبي الخام (RLP) ومستخلص البروبوليس التجاري (CPE) ضد Aspergillus niger، Aspergillus ochraceus، Penicillium spp. باستخدام تثبيط نمو الغزل الفطري على الآجار (10، 25، 50 ملجم/مل) والوزن الجاف للكتلة الحيوية في الوسط السائل (1، 5، 10 ملجم/مل). أُجريت التجارب بثلاثة مكررات، وحللت البيانات باستخدام تحليل التباين (ANOVA) عند $p < 0.05$. أظهر كلا النوعين من البروبوليس تثبيطاً معنوياً مقارنة بالضبط ($p < 0.001$).

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تفوق البروبوليس الليبي ضد Aspergillus niger (64.7% عند 25 ملجم/مل) مقارنة بـ CPE (41.1% عند 50 ملجم/مل). ضد Aspergillus ochraceus، حقق RLP أقصى تثبيط (76.4% عند 10 ملجم/مل). لوحظ تأثير تنشيطي عند 1 ملجم/مل من CPE، حيث تجاوزت الكتلة الحيوية الضبط بنسبة 86%. تثبت النتائج أن كلاً من RLP و CPE يمتلكان نشاطاً مضاداً للفطريات متخصصاً بالنوع، ويمثل البروبوليس الليبي عاملاً طبيعياً واعداً لحفظ الأغذية.

1. Introduction

Propolis, or "bee glue," is a resinous substance collected by honeybees (*Apis mellifera*) from plant exudates such as buds and sap flows [1]. It is used within the hive for structural reinforcement and antimicrobial protection and is widely recognised for its broad-spectrum antimicrobial, antifungal, anti-inflammatory, and antioxidant activities [2,3].

Its chemical composition varies significantly with geography, flora, and climate [4]. Typically, propolis contains resinous compounds, wax, essential oils, pollen, and minor organic constituents, with flavonoids and phenolic acids identified as the primary bioactive components [2,5]. These compounds include pinocembrin, galangin, chrysin, quercetin, caffeic acid, and ferulic acid derivatives.

Libyan propolis has gained attention due to Libya's strong ecological contrast between Mediterranean coastal zones and Saharan desert regions [6]. This diversity produces chemically distinct propolis profiles. A comprehensive analysis of Libyan samples identified five phytochemical clusters, confirming substantial regional variability [6]. Libyan propolis is rich in flavonoids and phenolics, alongside tannins, alkaloids, and saponins, with reported quantitative variation across samples [7,8]. Such compositional diversity is expected to influence biological activity.

Propolis exhibits antimicrobial effects through multiple mechanisms, including disruption of fungal cell membranes, inhibition of cell wall synthesis, enzymatic interference, and suppression of mycotoxin production [3,9]. Its antifungal potential against food spoilage fungi has been widely reported [10]. Libyan raw propolis (RLP) has demonstrated dose-dependent inhibition against *Aspergillus niger*, *Aspergillus ochraceus*, and *Penicillium* spp., with strong activity reported at higher concentrations [11]. Similar activity has been observed in regional Libyan samples, including Misurata propolis, against multiple fungal species [10].

These fungi are major agents of food spoilage and mycotoxin contamination. *A. niger* is associated with fruit and vegetable decay, *A. ochraceus* produces ochratoxin A, and *Penicillium* spp. contribute to spoilage of dairy and bakery products and may produce patulin [12,13]. Growing demand for natural preservatives has intensified interest in plant-derived antifungal agents [14,15].

Despite promising results for raw Libyan propolis, commercial extracts differ due to variations in origin, extraction methods, and standardisation [16,17]. Commercial propolis is typically sourced from temperate regions and exhibits more uniform chemical profiles [2,17]. Given Libya's unique botanical diversity, its propolis may display distinct or enhanced antifungal activity. However, direct comparative studies between Libyan and commercial propolis under identical experimental conditions remain limited.

Therefore, this study evaluates and compares the antifungal activity of Libyan propolis and commercial propolis extracts against *A. niger*, *A. ochraceus*, and *Penicillium* spp. using parallel solid and liquid assays. The work aims to determine species-specific efficacy patterns, dose–response behaviour, and the potential of Libyan propolis as a regionally derived natural antifungal agent for food preservation applications.

2. Materials and Methods

This study investigated the antifungal efficacy of raw Libyan propolis (RLP) and commercial propolis extract (CPE) against *A. niger*, *A. ochraceus*, and *Penicillium* spp. using two distinct methodological approaches: mycelial growth inhibition and a multi-method biomass quantification approach. PDA (solid medium) was used for the mycelial growth inhibition assay to measure radial colony growth, while PSB (liquid medium) was used for the biomass assay to ensure uniform dispersion of the extract and enable subsequent determination of mycelial dry weight. Both media shared the same potato dextrose base to maintain nutritional consistency across assays.

2.1 Propolis Samples

Two propolis samples were utilized in this study. Raw Libyan propolis (RLP) was sourced directly from local beekeepers in Libya. The propolis was collected from hives located in the northern agricultural belt of Libya (a region spanning multiple towns; exact town/region unknown because the material was pooled from several apiaries). This region is known for its distinct floral diversity [7, 11]. The second sample, a commercial propolis extract (CPE) used in this study, was a 5:1 extract powder (1500 mg equivalent, 300 mg per capsule) from NOW Foods (Bloomingdale, IL, USA; brand: NOW Foods). The raw propolis was collected from domestic and overseas beekeepers, and the product was made and quality tested in the USA with globally sourced ingredients. The CPE is standardized to 70% total propolis constituents and 7% flavones. The extraction method was water-based and alcohol-free. procured for comparative analysis.

2.2 Fungal Strains

The fungal strains investigated included *Aspergillus niger, Aspergillus ochraceus,* and *Penicillium* spp. These strains were previously isolated and identified from spoiled food products (specifically bread) in a prior study [11]. The strains were re-cultured on Potato Dextrose Agar (PDA) plates and incubated at 27°C to obtain actively growing cultures. For all experiments, 5 mm diameter disks were cut from the leading edge of 7-day old fungal cultures using a sterile cork borer.

2.3 Mycelial Growth Inhibition

The antifungal activity of RLP and CPE on fungal mycelial growth was assessed using the direct contact agar phase method on PDA plates [18]. Three concentrations of each propolis sample were prepared: 10 mg/ml, 25 mg/ml, and 50 mg/ml. These concentrations were achieved by dissolving 0.1 g, 0.25 g, and 0.5 g of propolis, respectively, in 10 ml of dimethyl sulfoxide (DMSO). The propolis solutions were then incorporated into cooled, molten PDA before solidification. Control plates included sterile distilled water and 2% DMSO to account for potential solvent effects. Each treatment, including controls, was performed in triplicate.

The plates were inoculated centrally with a 5 mm fungal disk and incubated at 27 °C. After 7 days, when the control fungi had reached full growth, the radial growth of the fungal colonies was measured in two perpendicular directions. The percentage of fungal growth inhibition was calculated using the formula described by [18]:

Growth inhibition (%) = [(growth in control – growth in sample) / growth in control] × 100.

The minimum inhibitory concentration (MIC) was defined as the lowest extract concentration that resulted in little to no fungal growth after 7 days.

2.4 Biomass Dry Weight Comparison

For biomass dry weight assessment, Potato Sucrose Broth (PSB) was prepared, and 100 ml aliquots were dispensed into 250 ml Erlenmeyer flasks. These flasks were then sterilized via autoclaving at 121°C for 15 minutes. Upon cooling, the pH of the broth in each flask was adjusted to 5.6 using two drops of lactic acid to create optimal conditions for fungal growth.

RLP and CPE were incorporated into the PSB at three final concentrations: 1 mg/ml, 5 mg/ml, and 10 mg/ml. Fungal disks (5 mm diameter) from 7-day old cultures of *A. niger, A. ochraceus,* and *Penicillium* spp. grown on PDA were introduced into the respective flasks; no further standardization of spore density or biomass was performed. Each fungus-propolis concentration combination was tested in triplicate. Control flasks contained only the nutrient medium and fungal disks without any propolis.

The flasks were incubated in a shaker incubator at 120 rpm for 10 days

at 25 ± 2°C to promote submerged fungal growth. Following incubation, the fungal biomass was harvested by vacuum filtration using pre-weighed Whatman No. 2 filter paper. The wet weight of each fungal biomass was recorded. Subsequently, the fungal biomasses were dried in an oven at 70°C for 48 hours to determine the constant dry weight. The mold biomass was determined by measuring the dry weight of the mycelium after drying at 70 °C to constant weight. following the method described by [19,20]. All reported values represent the average of three replicates.

3. Statistical Analysis

The inhibition of radial mycelial growth and biomass data were analyzed using Three-way ANOVA (Univariate Analysis of Variance) and Post-hoc comparisons using Tukey's HSD test (α = 0.05). All statistical analyses were performed using IBM SPSS Statistics version 26.0 (IBM Corp., Armonk, NY, USA).

4. Results and Discussion

This study evaluated the antifungal efficacy of raw Libyan propolis (RLP) and commercial propolis extract (CPE) against *A. niger, A. ochraceus*, and *Penicillium* spp. using mycelial growth inhibition on solid media and biomass dry weight quantification in liquid culture.

4.1 Mycelial Growth Inhibition

A three-way analysis of variance (ANOVA) was performed on mycelial growth (mm) with propolis type (Control, RLP, CPE), concentration (0, 10, 25, 50 mg/ml), and pathogen (*A. niger*, *A. ochraceus*) as fixed factors.

Mycelial Growth of *A. niger* and *A. ochraceus* Following Treatment with RLP and CPE is shown in **Table** 1. Both propolis types significantly inhibited mycelial growth compared with the control ($p < 0.001$), Table 2.

Table 1: Mean Mycelial Growth (± Standard Deviation) of *A. niger* and *A. ochraceus* Following Treatment with RLP and CPE

Pathogen	Propolis Type	10 mg/ml	25 mg/ml	50 mg/ml	2% DMSO Control	Sterile Distilled Water Control
A. niger	RLP	5.00 ± 0.00 ^{b}	3.00 ± 1.00 ^{a}	3.83 ± 0.76 ^{ab}	8.50 ± 0.00 ^{c}	8.50 ± 0.00 ^{c}
A. ochraceus	RLP	2.00 ± 0.50 ^{a}	3.50 ± 0.50 ^{ab}	3.17 ± 0.76 ^{ab}	8.50 ± 0.00 ^{c}	8.50 ± 0.00 ^{c}
A. niger	CPE	6.83 ± 0.29 ^{b}	6.17 ± 0.29 ^{b}	4.67 ± 0.76 ^{a}	8.50 ± 0.00 ^{c}	8.50 ± 0.00 ^{c}
A. ochraceus	CPE	2.83 ± 0.70 ^{a}	3.67 ± 0.58 ^{ab}	3.50 ± 0.87 ^{ab}	8.50 ± 0.00 ^{c}	8.50 ± 0.00 ^{c}

Note: Values in the same row (within each pathogen) with different superscript letters are significantly different (Tukey's HSD, $p < 0.05$). The 2% DMSO control did not significantly differ from the sterile distilled water control ($p > 0.05$, Tukey's HSD). Data for both controls are shown separately for transparency.

The three-way ANOVA revealed a significant main effect of propolis type ($p < 0.001$) and a significant main effect of pathogen ($p < 0.001$). Significant two-way interactions were observed for propolis type × pathogen ($p = 0.002$), indicating that the relative efficacy of RLP versus CPE differed between the two fungal species. Simple effects analysis (using Tukey HSD within each pathogen) revealed that RLP was significantly more effective than CPE against both *A. niger* (RLP 3.94 mm vs. CPE 5.89 mm, $p < 0.001$) and *A. ochraceus* (RLP 2.89 mm vs. CPE 3.33 mm, $p < 0.05$).

Table 2: Percentage Inhibition of Mycelial Growth by Raw Libyan Propolis (RLP) and Commercial Propolis Extract (CPE)

Pathogen	Propolis Type	10 mg/ml	25 mg/ml	50 mg/ml	2% DMSO Control	Sterile Distilled Water Control
A. niger	RLP	41.18% ± 0.00 ab	64.71% ± 11.76 b	54.94% ± 8.94ab	0	0
	CPE	19.65% ± 3.41 a	27.41% ± 3.41 a	45.06% ± 8.94 ab	0	0
A. ochraceus	RLP	76.47% ± 5.88 b	58.82% ± 5.88 ab	62.71% ± 8.94 ab	0	0
	CPE	66.71% ± 8.24 ab	56.82% ± 6.82 ab	58.82% ± 10.24 ab	0	0

Note: Values in the same row (within each pathogen) with different superscript letters are significantly different (Tukey's HSD, $p < 0.05$). The 2% DMSO control did not significantly differ from the sterile distilled water control ($p > 0.05$, Tukey's HSD). Data for both controls are shown separately for transparency

Furthermore, the concentration × pathogen interaction ($p < 0.001$) reflects that the reduction in growth with increasing concentration was more pronounced for *A. niger* than for *A. ochraceus* Table 1. Post-hoc comparisons using Tukey's HSD test (α = 0.05) showed that all propolis-treated groups (RLP and CPE at all concentrations) produced significantly lower mycelial growth than the untreated control ($p < 0.001$). Both propolis types (RLP and CPE) significantly reduced mycelial growth compared to the untreated control at all tested concentrations (Dunnett's test, $p < 0.001$; see Table 3 for full ANOVA results. The 2% DMSO solvent control produced mean colony diameters of 8.50 ± 0.00 mm for *A. niger* and for *A. ochraceus*, which were not significantly different from the sterile distilled water control (8.50 ± 0.00 mm for both species; $p > 0.05$), confirming that DMSO alone had no antifungal activity.

Table 3: Summary of SPSS Results

Source	Df	F	p	Partial η^2
Propolis Type	1	30.311	<.001	0.558
Concentration	2	1.098	.350	0.084
Pathogen	1	23.087	<.001	0.490
Propolis Type × Concentration	2	2.180	.135	0.154
Propolis Type × Pathogen	1	11.951	.002	0.332
Concentration × Pathogen	2	15.262	<.001	0.560
Propolis Type × Concentration × Pathogen	2	3.098	.064	0.205

Note: Significant effects ($p < 0.05$) are shown in bold. The model explained 91.1% of the variance ($R^2 = 0.911$, adjusted $R^2 = 0.862$).

Percentage inhibition ranged from 19.65% (CPE at 10 mg/ml against *A. niger*) to 76.47% (RLP at 10 mg/ml against *A. ochraceus*); however, due to low statistical power, differences among concentrations within each propolis type and pathogen were not significant (see Limitations).

RLP exhibited a non-monotonic, biphasic response against *A. niger,* Fig.1**,** with inhibition increasing up to 25 mg/mL (peak inhibition 64.7%) and then declining at 50 mg/mL (54.9%), suggesting a hormetic or paradoxical effect at higher concentrations. In contrast, RLP showed a more conventional monotonic dose-response against *A. ochraceus,* with inhibition decreasing gradually from 76.5% at 10 mg/mL to 58.8% at 25 mg/mL and 62.7% at 50 mg/mL."

This pattern may reflect a biological phenomenon analogous to the paradoxical effect (also known as the Eagle effect) described for Aspergillus species exposed to certain antifungals [21].

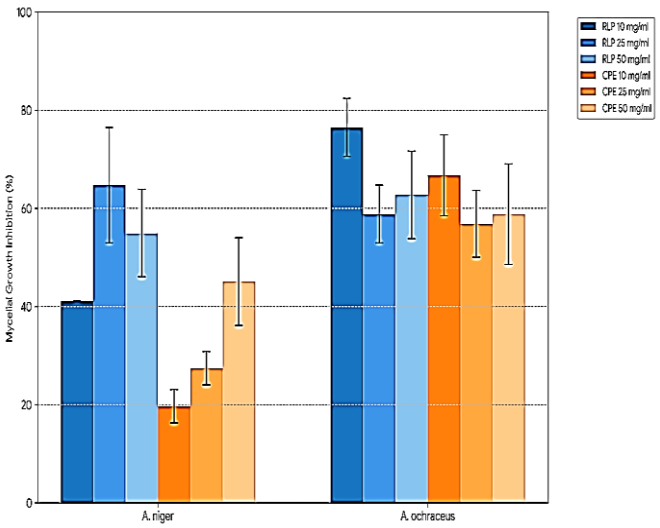

Fig. 1: Comparing the percentage inhibition of mycelial growth for Aspergillus niger and Aspergillus ochraceus treated with Raw Libyan Propolis (RLP) and Commercial Propolis Extract (CPE) at concentrations of 10, 25, and 50 mg/ml. Error bars represent standard deviation (n=3).

Aspergillus species, particularly non-fumigatus species like *A. niger* and *A. tubingensis*, are known to exhibit reduced susceptibility to azoles, and in some studies, this susceptibility profile includes a non-monotonic response [22]. This phenomenon, while frequently

documented with echinocandins (e.g., caspofungin) in Aspergillus, has also been observed in studies investigating the complex physiological responses of Aspergillus to azole antifungals such as voriconazole or isavuconazole [21]. The proposed mechanisms for this paradoxical effect include: (i) at low or sub-inhibitory concentrations, Aspergillus species may show increased growth as a compensatory stress response; (ii) at intermediate concentrations, growth is inhibited through the expected fungistatic action targeting ergosterol biosynthesis; and (iii) at very high concentrations, some isolates exhibit renewed growth mediated by upregulation of alternative pathways, such as ABC transporter efflux pumps or compensatory ergosterol synthesis pathways, allowing the fungus to survive at supra-MIC concentrations [21,22].

Species within the Aspergillus section *Nigri*, including *A. niger,* frequently exhibit high resistance to itraconazole and reduced susceptibility to voriconazole compared to *A. fumigatus* [23]. This non-monotonic response can complicate susceptibility testing, making the determination of the Minimum Inhibitory Concentration (MIC) challenging, as the fungus may appear resistant at low concentrations, susceptible at intermediate concentrations, and then resistant again at high concentrations [24]. Further studies are warranted to elucidate whether the observed biphasic response to RLP involves similar molecular mechanisms, including efflux pump activity or alterations in membrane composition.

CPE demonstrated stable inhibition of *A. ochraceus* (58–66% across all concentrations) and a gradual increase against *A. niger*, reaching 45.06% at 50 mg/ml. The non-linear response of RLP against *A. ochraceus* suggests a biphasic effect, likely due to optimal activity of specific bioactive compounds at lower concentrations or antagonistic interactions at higher doses [25].

Remarkably, RLP achieved 76.5% inhibition at only 10 mg/mL against *A. ochraceus*, whereas a study by [11] reported 88.8% inhibition but at a substantially higher concentration of 68 mg/mL. This indicates that the propolis extract used in the current study reaches near-maximal inhibitory activity at a much lower concentration, suggesting a rapid plateau in the dose-response curve [26,27], exemplifying how a nearly 7-fold increase in concentration leads to only modest gains in biological activity, a phenomenon noted in antifungal pharmacology.

The stable performance of CPE reflects its standardized composition (70% propolis, 7% flavones), which ensures consistent bioavailability of active principles [17, 28].

4.2 Biomass Dry Weight Comparison

In liquid culture, both propolis types reduced fungal biomass in a dose-dependent manner, with species specific patterns **Figures 2 and 3**. For RLP, biomass of *A. niger* and *Penicillium* spp. decreased substantially at 10 mg/ml, reaching 2.21 g and 2.46 g, respectively, while *A. ochraceus* remained relatively less affected (4.54 g). Fig.2.

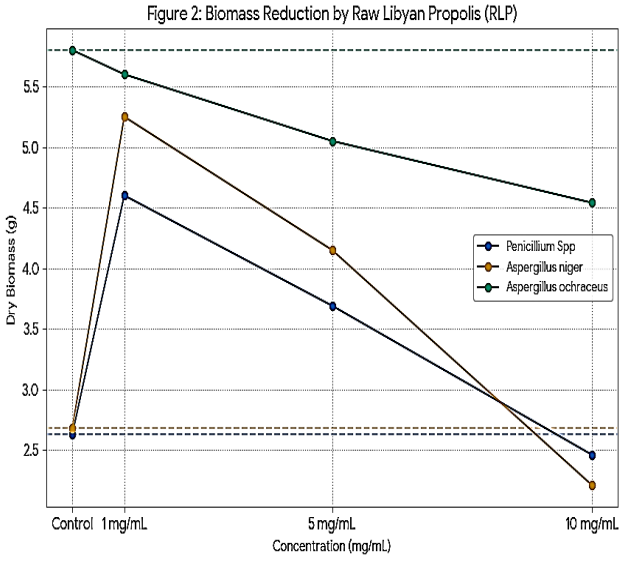

Fig. 2: Biomass Reduction of *Penicillium* spp., *A. niger,* and *A. ochraceus* by Raw Libyan Propolis (RLP)

For CPE, a hormetic effect was observed at 1 mg/ml: biomass of *Penicillium* spp. and *A. niger* exceeded control values by 86% and 76%, respectively. At 5 and 10 mg/ml, strong inhibition occurred, with *Penicillium* spp. biomass dropping to 1.42 g at 10 mg/ml, Fig.3.

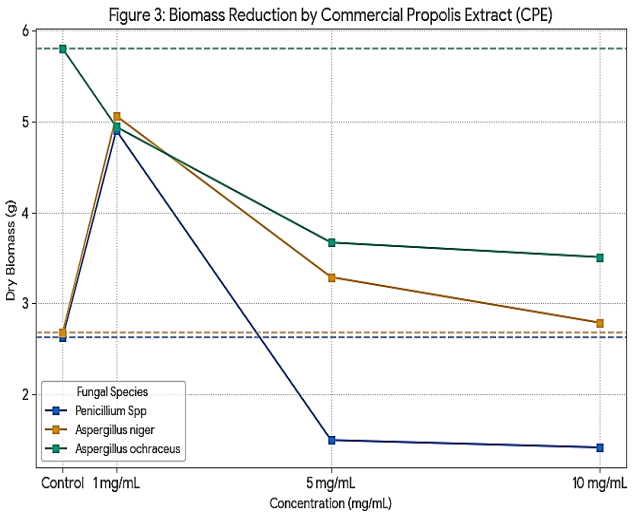

Fig. 3: Biomass Reduction of *Penicillium* spp., *A. niger*, and *A. ochraceus* by Commercial Propolis Extract (CPE)

The stimulatory effect at low CPE concentrations is consistent with hormesis, in which subinhibitory doses may provide nutrients or trigger stress responses that temporarily enhance fungal growth [14, 25, 29]. The low-dose stimulatory effect of CPE on fungal growth, while consistent with classical hormesis, may also involve several non-mutually exclusive mechanisms. First, propolis contains not only antimicrobial phenolics but also amino acids, vitamins, trace minerals, and carbohydrates; at sub-inhibitory concentrations, these constituents could act as supplementary nutrients, directly fueling fungal metabolism. This interpretation is supported by evidence that propolis enhances mycelial development in edible mushrooms when used as an enrichment additive [30]. Second, a mild stress-induced adaptive response (hormesis proper) remains plausible, whereby sub-lethal doses activate stress-response pathways that temporarily enhance growth a phenomenon well documented in fungi exposed to low doses of fungicides and other chemicals [31,32]. Third, the xenohormesis hypothesis proposes that heterotrophs have evolved to sense chemical cues from stressed autotrophs as early environmental warnings; because propolis is derived from plant resins collected by bees, its bioactive compounds may be perceived by fungi as such signals, triggering adaptive growth responses [33,34]. Fourth, the observed species-specific stimulation strong in *Penicillium* spp. and *A. niger* but minimal in *A. ochraceus* suggests the involvement of taxon-specific metabolic capabilities or stress-sensing pathways [35,36].

This finding underscores the necessity of using adequate propolis concentrations to avoid unintended promotion of spoilage in food applications.

4.3 Species Specific Efficacy

Penicillium spp. was the most sensitive to CPE at high concentrations, while A. ochraceus was the most resistant across both propolis types Fig.4. These results align with previous studies showing that Libyan propolis effectively inhibits *A. ochraceus* at higher concentrations (68 mg/ml) but exhibits lower inhibition against *Penicillium* spp. [11], and that Misurata propolis exhibits differential activity against *Aspergillus* and *Penicillium* species [10]. The resistance of *A. ochraceus* may be linked to its ability to produce ochratoxin A, which can confer stress tolerance, as well as to structural features such as thicker cell walls or melanin [12, 37, 38]. Conversely, the higher β-glucan content in Penicillium cell walls may render them more susceptible to membrane-active phenolic compounds [13, 39].

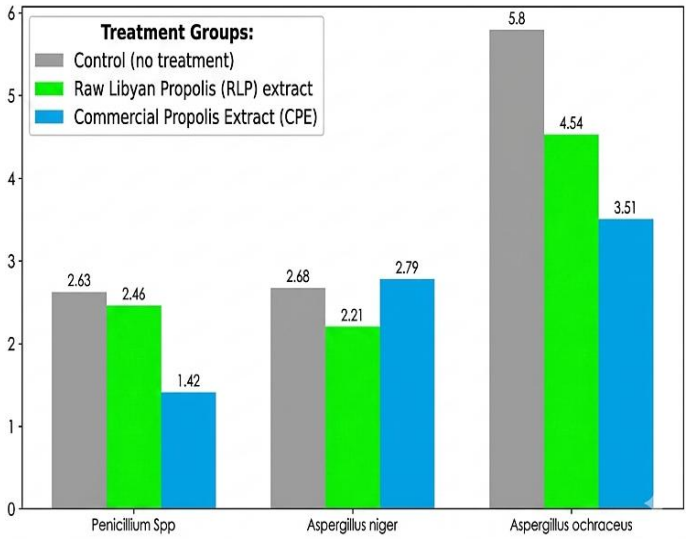

Fig. 4: Antifungal activity of Raw Libyan Propolis (RLP) and Commercial Propolis Extract (CPE) at 10 mg/ml against three fungal species. Values represent the Biomass Reduction.

4.4 Comparative Efficacy and Geographic Influence

RLP demonstrated superior activity against *A. niger* (64.7% inhibition at 25 mg/ml) compared to CPE (41.1% at 50 mg/ml). This is likely due to the unique chemical profile of Libyan propolis, which is rich in flavonoids (112.28 mg/g) and phenolic compounds (50.25 mg/g) and contains novel flavanonols and alkylresorcinols with potent bioactivity [7, 8, 18]. In contrast, CPE was more effective against *Penicillium* spp., reducing biomass to 1.42 g at 10 mg/ml compared with 2.46 g for RLP. This may reflect the concentrated flavone content and the standardized extraction process, which enhance the bioavailability of specific active compounds [17, 30].

These differences highlight the strong influence of geographic origin and botanical sources on propolis composition. Libyan propolis derives from a diverse Mediterranean–desert flora, including Pinus halepensis, Eucalyptus spp., and Acacia spp. [7], whereas commercial propolis is often sourced from poplar (Populus spp.) in Europe or Baccharis dracunculifolia in Brazil, resulting in distinct chemical profiles [4, 17]. The chemical diversity of Libyan propolis, previously documented by Siheri et al. [7], contributes to its unique antifungal properties.

A limitation of this study is the small number of replicates (n = 3 per treatment). While this is consistent with preliminary antifungal screening studies, it reduces statistical power. Future studies should expand the number of replicates to improve the reliability of mean comparisons

4.5 Implications

The species-specific efficacy patterns observed have practical implications for food preservation. RLP may be particularly suitable for controlling *A. niger* in fruits and vegetables, while CPE could be more effective against *Penicillium* spoilage in baked goods and dairy products [13]. The ability of both propolis types to inhibit *A. ochraceus* is promising for reducing ochratoxin A contamination [12]. However, the hormetic effect observed at low CPE concentrations emphasises the need for careful dose optimisation in practical applications.

A limitation of this study is that the raw Libyan propolis could not be traced to a single town or region, as it was pooled from multiple hives across the northern agricultural belt. Future studies should collect propolis from geo-referenced hives with precise location data to enable chemotypic comparisons.

A methodological limitation was the lack of a standardised inoculum (e.g., spore density or initial biomass) for the liquid biomass assays. While this does not invalidate the observed trends such as differential inhibition and low-concentration stimulation it may introduce variability that affects quantitative precision. Future studies should employ standardised inoculum protocols to improve reproducibility.

Future work should also include chemical profiling to identify active constituents in Libyan propolis, evaluate efficacy in real food matrices, and explore synergistic combinations with other natural preservatives [14]. Additionally, future studies should include *Penicillium* spp. in both liquid and solid media assays to enable consistent multi-species comparison. Until such data are available, the findings for *Penicillium* reported here should be considered preliminary.

5. Conclusion

This study demonstrated that both raw Libyan propolis (RLP) and commercial propolis extract (CPE) possess significant antifungal activity against *Aspergillus niger*, *Aspergillus ochraceus*, and *Penicillium* spp., with species-specific efficacy patterns. RLP showed superior activity against *A. niger*, while CPE was more effective against *Penicillium* spp. A biphasic dose–response was observed for RLP against *A. ochraceus*, with maximal inhibition at 10 mg mL^{-1}, whereas CPE provided stable inhibition across concentrations. Hormetic growth stimulation at low CPE concentrations highlights the importance of dose optimisation.

These results underscore the influence of propolis origin and standardisation on antifungal performance. Libyan propolis, with its unique chemical profile, represents a promising natural antifungal agent for food preservation and other applications. Further research is needed to characterise its active constituents, optimise application concentrations, and evaluate efficacy in complex food systems.

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7. References

[1] Simone-Finstrom, M., & Spivak, M. (2010). Propolis and bee health: The natural history and significance of resin use by honey bees. Apidologie, 41(3), 295–311. https://doi.org/10.1051/apido/2010016

[2] Zulhendri, F., Chandrasekaran, K., Kowacz, M., Ravalia, M., Kripal, K., Fearnley, J., & Perera, C. O. (2021). Antiviral, antibacterial, antifungal, and antiparasitic properties of propolis: A review. Foods, 10(6), 1360. https://doi.org/10.3390/foods10061360

[3] Wagh, V. D. (2013). Propolis: A wonder bees product and its pharmacological potentials. Advances in Pharmacological Sciences, 2013, 308249. https://doi.org/10.1155/2013/308249

[4] Bankova, V., Popova, M., & Trusheva, B. (2016). New emerging fields of application of propolis. Macedonian Journal of Chemistry and Chemical Engineering, 35(1), 1–11. https://doi.org/10.20450/mjcce.2016.864

[5] Anjum, S. I., Ullah, A., Khan, K. A., Attaullah, M., Khan, H., Ali, H., Bashir, M. A., Tahir, M., Ansari, M. J., Ghramh, H. A., & Adgaba, N. (2019). Composition and functional properties of propolis (bee glue): A review. Saudi Journal of Biological Sciences, 26(7), 1695–1703. https://doi.org/10.1016/j.sjbs.2018.08.013

[6] Siheri, W., Zhang, T., Ebiloma, G. U., Biddau, M., Woods, N., Hussain, M. Y., Clements, C. J., Fearnley, J., Edrada Ebel, R., Paget, T., Müller, S., Carter, K. C., Ferro, V. A., De Koning, H. P., & Watson, D. G. (2016). Chemical and antimicrobial profiling of propolis from different regions within Libya. PLoS ONE, 11(5), e0155355. https://doi.org/10.1371/journal.pone.0155355

[7] Siheri, W., Almutairi, S., & Watson, D. G. (2019). Propolis from Libya: Chemical composition and biological activities. In V. Bankova & M. Popova (Eds.), Propolis: Chemistry, biology and applications (pp. 45–62). Springer.

[8] Ali. M Alhaliba , Alauldin. O Alturshania, Adel. M Mlitan (2025). Chemical composition and antibacterial activity of Libyan propolis. Lebda Medical Journal; [LMJ]. https://lebmedj.elmergib.edu.ly/index.php/LMJ/article/view/259

[9] Przybyłek, I., & Karpiński, T. M. (2019). Antibacterial properties of propolis. Molecules, 24(11), 2047. https://doi.org/10.3390/molecules24112047

[10] Elsharkawy, E. R., & Aljabre, S. H. M. (2023). Antibacterial and

antifungal activity of the propolis growing in the basin of Mediterranean city Misurata, Libya. The Pharmaceutical and Chemical Journal, 10(4), 1–8.

[11] Amara, R. O., Khashur, M. M., Alabozidy, Z. A., & Rhuoma, A. A. (2025). Effectiveness of Libyan propolis against mold isolated from spoiled bread. Journal of Research in Agriculture and Food Sciences, 2(3), 232–240.

[12] EFSA Panel on Contaminants in the Food Chain (CONTAM), Schrenk, D., Bodin, L., Chipman, J. K., del Mazo, J., Grasl-Kraupp, B., Hogstrand, C., Hoogenboom, L. R., Leblanc, J. C., Nebbia, C. S., Nielsen, E., Ntzani, E., Petersen, A., Sand, S., Schwerdtle, T., Vleminckx, C., Mark, R., Wallace, H., & Alexander, J. (2020). Risk assessment of ochratoxin A in food. EFSA Journal, 18(5), e06113. https://doi.org/10.2903/j.efsa.2020.6113

[13] Saranraj, P., & Geetha, M. (2012). Microbial spoilage of bakery products and its control by preservatives. International Journal of Pharmaceutical and Biological Archives, 3(1), 38–48.

[14] Segueni, N., Boutaghane, N., Asma, S. T., Tas, N., Acaroz, U., Arslan-Acaroz, D., & Akkal, S. (2023). Review on propolis applications in food preservation and active packaging. Plants, 12(8), 1654. https://doi.org/10.3390/plants12081654

[15]Mesías, F. J., Martín, A., & Hernández, A. (2021). Consumers' growing appetite for natural foods: Perceptions towards the use of natural preservatives in fresh fruit. Food Research International, 150, 110749. https://doi.org/10.1016/j.foodres.2021.110749

[16] Wo´zniak, M.; Mrówczy´ nska, L.; Kwa´sniewska-Sip, P.;Wa´skiewicz, A.; Nowak, P.; Ratajczak, I. Effect of the solvent on propolis phenolic profile and its antifungal, antioxidant, and in vitro cytoprotective activity in human erythrocytes under oxidative stress.Molecules 2020, 25, 4266. [CrossRef]

[17] Kasote, D., Bankova, V., & Viljoen, A. M. (2022). Propolis: Chemical diversity and challenges in quality control. Phytochemistry Reviews, 21(6), 1887–1911. https://doi.org/10.1007/s11101-022-09816-1

[18] Pandey, D. K., Tripathi, N. N., Tripathi, R. D., & Dixit, S. N. (1982). Fungitoxic and phytotoxic properties of the essential oil of Hyptis suaveolens. Zeitschrift für Pflanzenkrankheiten und Pflanzenschutz, 89(6), 344–349.

[19] Eloff, J. N. Quantifying the antifungal activity of plant extracts against fungal growth in liquid culture. *South African Journal of Botany*,2019.126,234-240.

[20] Biswas, D., et al. Standardization of biomass quantification methods for antifungal susceptibility testing. *Journal of Microbiological Methods*,2020. 178, 106072.

[21] Logan A, Wolfe A, Williamson JC. Antifungal Resistance and the Role of New Therapeutic Agents. Curr Infect Dis Rep. 2022;24(9):105-116. doi: 10.1007/s11908-022-00782-5. Epub 2022 Jul 5. PMID: 35812838; PMCID: PMC9255453.

[22] Djenontin E, Lavergne RA, Morio F, Dannaoui E. Antifungal Resistance in Non-fumigatus Aspergillus Species. Mycoses. 2025 Apr;68(4):e70051. doi: 10.1111/myc.70051. PMID: 40219727; PMCID: PMC11992613.

[23] Heuer C, Leonard H, Nitzan N, Lavy-Alperovitch A, Massad-Ivanir N, Scheper T, Segal E. Antifungal Susceptibility Testing of Aspergillus niger on Silicon Microwells by Intensity-Based Reflectometric Interference Spectroscopy. ACS Infect Dis. 2020 Oct 9;6(10):2560-2566. doi: 10.1021/acsinfecdis.0c00234. Epub 2020 Sep 21. PMID: 32930571; PMCID: PMC7584364.

[24] Lobo-Vega R, Megías D, Río-Ropero A, Mato-López Á, Ashraph K, Manosalva J, Alcázar Fuoli L, Alastruey-Izquierdo A, Mellado E, Amich J. Establishing thresholds for azole tolerance and persistence in Aspergillus fumigatus to study their impact on voriconazole treatment in vivo. bioRxiv 2025.12.26.693403. doi: 10.64898/2025.12.26.693403.

[25] Calabrese EJ. Preconditioning is hormesis part I: Documentation, dose-response features and mechanistic foundations. Pharmacol Res. 2016 Aug; 110:242-264. doi: 10.1016/j.phrs.2015.12.021. Epub 2016 Jan 3. PMID: 26757428.

[26] López-Malo, A., Alzamora, S. M., & Palou, E. (2002). Aspergillus flavus dose–response curves to selected natural and synthetic antimicrobials. International Journal of Food Microbiology, 73(2-3), 213–218. https://doi.org/10.1016/S0168-1605(01)00639-0

[27] Barboráková, Z., Tančinová, D., Medo, J., Jakabová, S., Häubl, G., Jaunecker, G., Mašková, Z., & Labuda, R. (2025). Strain-Dependent Variability in Ochratoxin A Production by Aspergillus spp. Under Different In Vitro Cultivation Conditions. Microorganisms, 13(12), 2850. https://doi.org/10.3390/microorganisms13122850

[28] Berretta AA, Nascimento AP, Bueno PC, Vaz MM, Marchetti JM. Propolis standardized extract (EPP-AF®), an innovative chemically and biologically reproducible pharmaceutical compound for treating wounds. Int J Biol Sci. 2012;8(4):512-21. doi: 10.7150/ijbs.3641. Epub 2012 Mar 21. PMID: 22457606; PMCID: PMC3314192.

[29] Long N, Li F. Antifungal Mechanism of Natural Products Derived from Plants: A Review. Natural Product Communications. 2024;19(8). doi:10.1177/1934578X241271747

[30] Türkekul İ, Gülmez Y. Propolis: An enrichment material for mycelium development of oyster mushroom (Pleurotus ostreatus). Natural Resources. 2016;7(03):119–123.

[31] Dharma KS, Suryanti S, Widiastuti A. Hormesis in pathogenic and biocontrol fungi: From inhibition to stimulation. AGRIS. 2024.

[32] Garzon CD, Flores FJ. Detection and assessment of chemical hormesis on the radial growth in vitro of oomycetes and fungal plant pathogens. Dose-Response. 2013;11(3):361–373.

[33] Baur JA, et al. What is xenohormesis? American Journal of Pharmacology and Toxicology. 2008;3(1):152–159.

[34] Howitz KT, Sinclair DA. Xenohormesis: Sensing the chemical cues of other species. Cell. 2008;133(3):387–391.

[35] Calabrese EJ, Baldwin LA. Hormesis: A general biological phenomenon. Human and Experimental Toxicology. 2002;21(2):91–97.

[36] Garzon CD, Flores FJ. Hormesis in plant pathogens: A review of low dose stimulation of fungal and oomycete growth. Plant Disease. 2013;97(5):602–612.

[37] Wadhwa K, Kapoor N, Kaur H, Abu-Seer EA, Tariq M, Siddiqui S, Yadav VK, Niazi P, Kumar P, Alghamdi S. A Comprehensive Review of the Diversity of Fungal Secondary Metabolites and Their Emerging Applications in Healthcare and Environment. Mycobiology. 2024 Dec 3;52(6):335-387. doi: 10.1080/12298093.2024.2416736. PMID: 39845176; PMCID: PMC11749308

[38] Qin, Y., Xia, Y. Melanin in fungi: advances in structure, biosynthesis, regulation, and metabolic engineering. Microb Cell Fact 23, 334 (2024). https://doi.org/10.1186/s12934-024-02614-8

[39] Houbraken J, Samson RA. Phylogeny of Penicillium and the segregation of Trichocomaceae into three families. Stud Mycol. 2011 Nov 15;70(1):1-51. doi: 10.3114/sim.2011.70.01. PMID: 22308045; PMCID: PMC3233907.