

1 **Propolis as a Natural Antimicrobial Defense against Honeybee Foulbrood Diseases: A**  
2 **Sustainable Approach to Colony Health and Pollination Security**

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23 **Abstract**

24 Honeybees are vital pollinators that contribute significantly in maintaining biodiversity and  
25 agricultural productivity by facilitating the pollination of flowering plants and trees. However,  
26 these insects are highly prone to a number of diseases that can adversely affect their resilience,  
27 colony vitality, and overall survival, resulting in considerable economic losses for beekeepers  
28 worldwide. The conventional use of antibiotics for disease control has led to the emergence of  
29 resistant bacterial strains and disruption of the honeybee gut microbiota, further rendering them  
30 prone to environmental conditions and exacerbating colony health decline. This review  
31 explores propolis as a natural, eco-friendly alternative for managing two major bacterial  
32 infections of honeybees including American foulbrood (AFB) and European foulbrood (EFB)  
33 caused by *Paenibacillus larvae* and *Melissococcus plutonius*, respectively. These serious  
34 bacterial diseases, which affect honey bee larvae, are leading to significant declines in honey  
35 bee populations. Propolis, a resinous material produced by bees from plant exudates, exhibits  
36 potent antimicrobial activity and plays a pivotal role in maintaining colony immunity and  
37 hygiene. The biological efficacy of propolis is largely determined by its chemical composition,  
38 which varies according to botanical and geographical origin. In this review we summarize the  
39 chemical diversity, antimicrobial mechanisms, and prophylactic potential of propolis,  
40 elucidating their effects on AFB and EFB diseases. The review underscores the significance of  
41 this naturally active substance produced by honeybees as a sustainable strategy for disease  
42 control in apiculture. The insights presented contribute to the development of antibiotic-free  
43 approaches to safeguard honeybee populations, enhance honey productivity, and support global  
44 pollination ecosystem stability.

45 **Keywords:** Foulbrood diseases, Honeybee, *Melissococcus plutonius*, *Paenibacillus larvae*,  
46 Propolis.

## 47 **1. Context**

48           The critical role of honeybees, specifically *Apis mellifera* (*A. mellifera*), in plant  
49           pollination highlights their ecological and economic significance. The benefits derived from  
50           pollination services rendered by honey bees frequently exceed the economic value of the  
51           products obtained from beehives. Nevertheless, the well-being and sustainability of honey bee  
52           populations face serious threats from numerous harmful pathogens, which pose considerable  
53           danger to these crucial insect species [1]. Propolis, a resinous substance collected by bees from  
54           plant sources, has gained attention for its potential role in enhancing bee health and serving as  
55           a natural biodefense against honeybee diseases [2]. Previous research has indicated that the  
56           propolis envelope within the nest acts as an antimicrobial layer and confers advantages to adult  
57           bees by diminishing the expression of constitutive immune genes [3]. Most importantly, the  
58           antimicrobial characteristics of propolis might help regulate pathogen levels and enhance the  
59           overall health of bee colony.

60           In recent decades, a diverse range of propolis samples collected from various  
61           geographical regions worldwide has been extensively studied for their pharmacological and  
62           biological properties. The biological and functional characteristics of propolis can be attributed  
63           to the presence of different chemical constituents, including flavonoids, phenolic compounds,  
64           and other bioactive compounds [4]. These constituents contribute to the diverse therapeutic  
65           effects of propolis and its potential applications in disease management. Propolis is recognized  
66           for its rich content of biologically active natural products, which exhibit potent antimicrobial,  
67           antioxidant, anti-inflammatory, and immunomodulatory properties. These properties make  
68           propolis a valuable natural remedy for inhibiting and controlling various bacterial, viral, and  
69           fungal diseases in honeybees [5].

70           Among the wide range of diseases studied in honey bees, AFB and EFB diseases caused  
71           by *Paenibacillus larvae* (*P. larvae*) and *Melissococcus plutonius* (*M. plutonius*),

72 respectively, are the two major bacterial diseases of honeybees that could negatively impact  
73 colony health and survival, resulting in a reduction of bee colonies and, consequently, a  
74 decrease in agricultural productivity [6]. The use of antibiotics as a control strategy for  
75 preventing and managing diseases, such as EFB and AFB can potentially lead to the emergence  
76 of antibiotic-resistant strains, posing long-term risks to colony health. While, by disrupting the  
77 core microbiota of honey bee guts, antibiotics may render bees more susceptible to  
78 opportunistic pathogens, ultimately increasing mortality rates. Moreover, antibiotics may only  
79 be effective against the vegetative cells of pathogens such as *P. larvae* and ineffective against  
80 their sporulated forms, further complicating disease management. While, the recurrence of  
81 symptoms following antibiotic treatment for non-spore-forming bacteria such as *M. plutonious*  
82 underscores the limitations of this approach in controlling honey bee diseases [7]. Additionally,  
83 the potential economic damage and human health risks associated with antibiotic residues in  
84 honeybee products emphasize the unsuitability of antibiotics as a therapeutic strategy for  
85 honeybee disease. These challenges highlight the need for sustainable and effective alternatives  
86 to antibiotic treatments to ensure the resilience of bee populations to bacterial diseases. Various  
87 natural biotherapeutic ingredients, such as probiotics, postbiotics, bacteriophages, antimicrobial  
88 peptides, bacteriocins, and propolis, have shown promise in combating honeybee pathogens, in  
89 addition to minimizing adverse effects on bee health and the environment [8].

90 The biological and functional characteristics of propolis are attributed to the presence  
91 of different chemical constituents, including flavonoids, phenolic compounds, and other  
92 bioactive compounds [9]. Owing to the vast antimicrobial efficacy of this natural resinous  
93 substance, it has been considered promising alternative to traditional antibiotic therapies,  
94 offering the advantages of being natural, sustainable, and environmentally friendly.

95

96

## 97 **2. Evidence Acquisition**

98 This review examines the significance of propolis in improving bee health and its  
99 potential as a natural remedy for the management of honey bee diseases. By investigating the  
100 therapeutic properties of propolis, we sought to highlight the crucial role of this natural  
101 substance in safeguarding honey bee populations and fostering sustainable beekeeping  
102 practices.

## 103 **3. Results**

### 104 **3.1 Honeybee Bacterial Diseases**

#### 105 **3.1.1 European foulbrood Disease**

106 EFB is a bacterial disease caused by *M. plutonius* which affects honeybee larvae before  
107 the cap stage. *M. plutonius* is morphologically similar to the secondary invader *Enterococcus*  
108 *faecalis* [10], and has been identified as the etiological agent of EFB disease. This disease is  
109 reported in most regions of the world where honeybees, especially *A. mellifera*, are reared and  
110 is among the list of diseases notifiable to the World Organization for Animal Health. Hives can  
111 often be infected with EFB but show no visible signs; however, sudden outbreaks of EFB can  
112 occur when the honey bee colony is under stress, which may be caused by hive movement,  
113 climatic conditions such as cool and wet weather, or poor nutrition.

114 EFB was initially identified in Australia in the late 1970s and has since become a global  
115 issue, significantly impacting the beekeeping industry worldwide. While this disease is  
116 primarily transmitted through the natural movement of bees, it is crucial to note that the spread  
117 also facilitated by the equipment utilized by beekeepers [1]. Understanding the distribution and  
118 spread of EFB involves a combination of factors including bee behavior, environmental  
119 conditions, and management practices. Therefore, prevention is considered the best approach  
120 for managing the spread of the disease among hives [1].

#### 121 **3.1.2 American Foulbrood Disease**

122 AFB caused by *P. larvae*, is a serious bacterial disease affecting honey bees larvae,  
123 leading to a substantial decrease in honeybee populations, and consequently, a loss to the  
124 beekeeping industry [1]. *P. larvae* first described in 1906, are gram-positive, rod-shaped, round-  
125 ended, spore-forming, facultative anaerobic bacteria that are responsible for AFB disease in  
126 honeybees. AFB can be transmitted between clinically-diseased colonies in a radius of one  
127 Kilometer when robber bees are absent, but over much longer distances when they are present  
128 [12].

### 129 **3.2 Management Strategies for the control of foulbrood diseases**

130 Effective management strategies are crucial for controlling foulbrood diseases in  
131 honeybee colonies and for preventing devastating losses. One key approach is the  
132 implementation of good hygiene practices, including regular inspections and the prompt  
133 removal of infected brood frames. This can help limit the spread of pathogens such as *P. larvae*  
134 and *M. plutonius* within the colony. Antibiotics such as oxytetracycline and tylosin are  
135 commonly used to treat foulbrood diseases; however, they are not a cure for AFB infection and  
136 may only inhibit the vegetative state of *P. larvae*, and most importantly the emergence of  
137 antimicrobial resistance has been alarming in this bacteria [13]. Hence, AFB disease poses a  
138 significant risk to bee health, and requires vigilance and proactive management strategies to  
139 mitigate its impact on bee colonies. Concerns surrounding the use of antibiotics in beekeeping,  
140 including the development of resistant strains and chemical residues in hive products, have led  
141 to restrictions on their use in many countries [14]. As a result, beekeepers are often left with  
142 no choice but to destroy the affected colonies when faced with these diseases.

143 Promoting genetic diversity within honeybee populations through selective breeding  
144 programs can enhance their resilience to different threatening biological agents [15]. Beekeepers  
145 can strengthen their colonies' natural defenses against foulbrood diseases by incorporating  
146 genetic diversity and disease resistance traits into breeding programs [8]. Overall, a

147 multifaceted approach that combines good hive management practices, natural treatments, and  
148 selective breeding for disease resistance could be the best strategy for effectively managing  
149 foulbrood diseases in honeybee colonies.

150 Among different naturally occurring antimicrobial agents, propolis has shown  
151 promising inhibitory effects against *P. larvae* and *M. plutonius*, making it a potential alternative  
152 to synthetic chemicals for disease control in beekeeping [15]. Studies have shown that by  
153 incorporating propolis-based products into hive management practices, beekeepers can  
154 maintain colony health, protect colonies from different diseases, while reducing their reliance  
155 on antibiotics. Propolis, with its diverse chemical composition and potent antibacterial  
156 properties, offers a natural and sustainable alternative for managing AFB and EFB in honeybee  
157 colonies. By understanding the chemical composition of propolis, including flavonoids and  
158 phenolic compounds with antimicrobial properties, researchers aim to elucidate how propolis  
159 interacts with pathogenic bacteria and enhances the immune response in bees. This knowledge  
160 is crucial for developing effective strategies for incorporating propolis into beekeeper disease  
161 management protocols.

### 162 **3.3. Propolis**

163 The word propolis as a Greek word is derived from the terms ‘Pro’ and ‘polis’ which  
164 means ‘in front of’ or ‘at the entrance to’ and ‘community’ or ‘city’, respectively [16].  
165 Therefore, propolis is considered a hive defensive substance, as bees utilize this waxy substance  
166 in the construction and maintenance of their hives owing to its waxy characteristics and  
167 mechanical properties.

168 The emphasis on propolis as a natural solution underscores its potential to provide a safe  
169 and sustainable alternative to antibiotics for controlling AFB and EFB in honeybee colonies.  
170 By harnessing the antibacterial effects of propolis, researchers have tried to offer beekeepers a  
171 viable option for managing these diseases, while minimizing the risks associated with antibiotic

172 use. Bees collect propolis from various plants found in diverse temperate climatic regions. The  
173 process of propolis production by honeybees involves a fascinating combination of salivary  
174 secretions, enzymes, and beeswax, along with various compounds obtained from plants and  
175 trees. The natural ingredients, such as pine gums, resins from poplar and palm trees, leaf buds,  
176 mucilage, and conifer secretions, contribute to the resinous and waxy properties of propolis  
177 [17]. Honey bees collect propolis from different plant sources and may acquire it during pollen  
178 digestion. This diverse range of botanical inputs enriches the chemical composition of propolis  
179 and enhances its antimicrobial properties [18]. Interestingly, the distribution of propolis in  
180 commercial hives differs from that in feral honeybee colonies. The smooth inner walls of  
181 commercial hives may not stimulate bees to deposit propolis as they would in natural settings.  
182 Nevertheless, both feral and managed colonies utilize propolis to seal holes and crevices in the  
183 nest, provide insulation, and defend themselves against intruders [19, 20]. This natural behavior  
184 of honey bees highlights the multifunctional role of propolis within the hive ecosystem,  
185 highlighting its significance in maintaining colony health and resilience. The intricate process  
186 of propolis collection and application underscores the resourcefulness and adaptability of  
187 honeybees in utilizing natural substances to protect their colonies and support their well-being.

188 The research highlighted in Figure 2 underscores the significant positive effects of  
189 propolis on bee colonies and their overall health. Propolis plays a crucial role in enhancing  
190 brood viability and longevity [21] and has been stated as collecting propolis by bees is their  
191 self-medicating ability [19], further emphasizing their importance in maintaining colony health.  
192 While, the studies of Saelao et al. [22] sheds light on how propolis contributes to stabilizing the  
193 honeybee microbiome, which serves as a social immune defense mechanism and supports  
194 microbial health within the colony. This stabilization of the microbiome is linked to the  
195 increased lifespan of pollinator insects, highlighting the intricate relationship between propolis,  
196 microbiome health, and bee longevity. Simone-Finstrom et al. [19] investigated the effects of

197 propolis supplementation on the gut microbiota of honey bees. Researchers found that propolis  
198 treatment led to changes in the abundance of certain bacterial taxa in the bee gut, resulting in a  
199 more balanced and diverse microbial community. This suggests that propolis may help promote  
200 a healthy microbiome in bees and enhance their resistance to pathogens. In a study conducted  
201 by Borba and his co-researchers [23], hives enriched with propolis were shown to reduce the  
202 microbial pathogen load and down-regulate the immune function. Furthermore, study  
203 demonstrated that propolis extracts have antimicrobial activity against key bee pathogens,  
204 including *P. larvae*, the causative agent of AFB [7]. These findings suggest that propolis can  
205 help regulate immune responses within bee colonies, potentially improving colony health and  
206 productivity. The collective evidence presented in these studies highlights the multifaceted  
207 benefits of propolis in bee colonies, ranging from enhancing immunity and microbiome stability  
208 to promoting longevity and productivity.

### 209 **3.3.1 Chemical composition of propolis**

210 Propolis is a complex natural substance that contains a diverse array of constituents  
211 including phenolic acids, flavonoids, terpenes, lipid-wax substances, beeswax, elements,  
212 vitamins, proteins, amino acids, and sugars [24]. Studies have classified propolis composition  
213 into two main categories: gross and detailed. Gross composition refers to the major constituents  
214 of propolis, such as total phenolic compounds, flavonoids, moisture, ashes, waxes, and other  
215 insoluble residues [25]. Analysis of different physical and chemical parameters in propolis is  
216 essential for standardizing and ensuring quality control of a propolis product. In contrast, the  
217 detailed composition refers to the individual constituents found in the propolis extracts.  
218 Analysis of the specific compounds present in propolis can provide insights into the bioactivity  
219 and potential therapeutic properties of this natural substance [25]. The diverse array of  
220 compounds found in propolis sheds light on the complexity and functionality of this natural  
221 substance.

222 The antimicrobial activity of propolis depends on the antimicrobial properties of its  
223 source plant resins [26], and the fact that colonies mix sources of resins creates an extremely  
224 complex mixture against which susceptible parasites and pathogens would have difficulty  
225 developing resistance. A previous study investigating the biological activity of single- and  
226 multiple-resin sources against stingless bee parasites and pathogens showed that, although some  
227 single-resin sources may be more effective against a single parasite or pathogen, mixed sources  
228 are considerably more effective because of the increased variety of chemical [26].

### 229 **3.3.2 Biological activity of propolis**

230 The biological activity of propolis is attributed to its complex chemical composition,  
231 and various compounds play a role in its diverse range of health benefits. Among the 80–300  
232 complex and varied mixtures present in propolis, less than 3% accounts for the active portion  
233 that is responsible for its biological activities [9]. Flavonoids are considered to be the major  
234 active biological substances in propolis, contributing to their antioxidant, anti-inflammatory,  
235 and anticancer properties. These compounds have been linked to immune-modulating effects  
236 and the ability to scavenge free radicals, thereby protecting cells from oxidative stress [27].

237 Propolis is highly recognized for its antioxidant properties, which are essential for  
238 protecting organisms from the oxidative damage caused by free radicals. Caffeic acid phenethyl  
239 ester (CAPE), a key component of propolis, is known for its antioxidant and anti-radical effects  
240 [28]. The antioxidant capacity of propolis is believed to enhance honeybee longevity by  
241 boosting its resistance to oxidative stress.

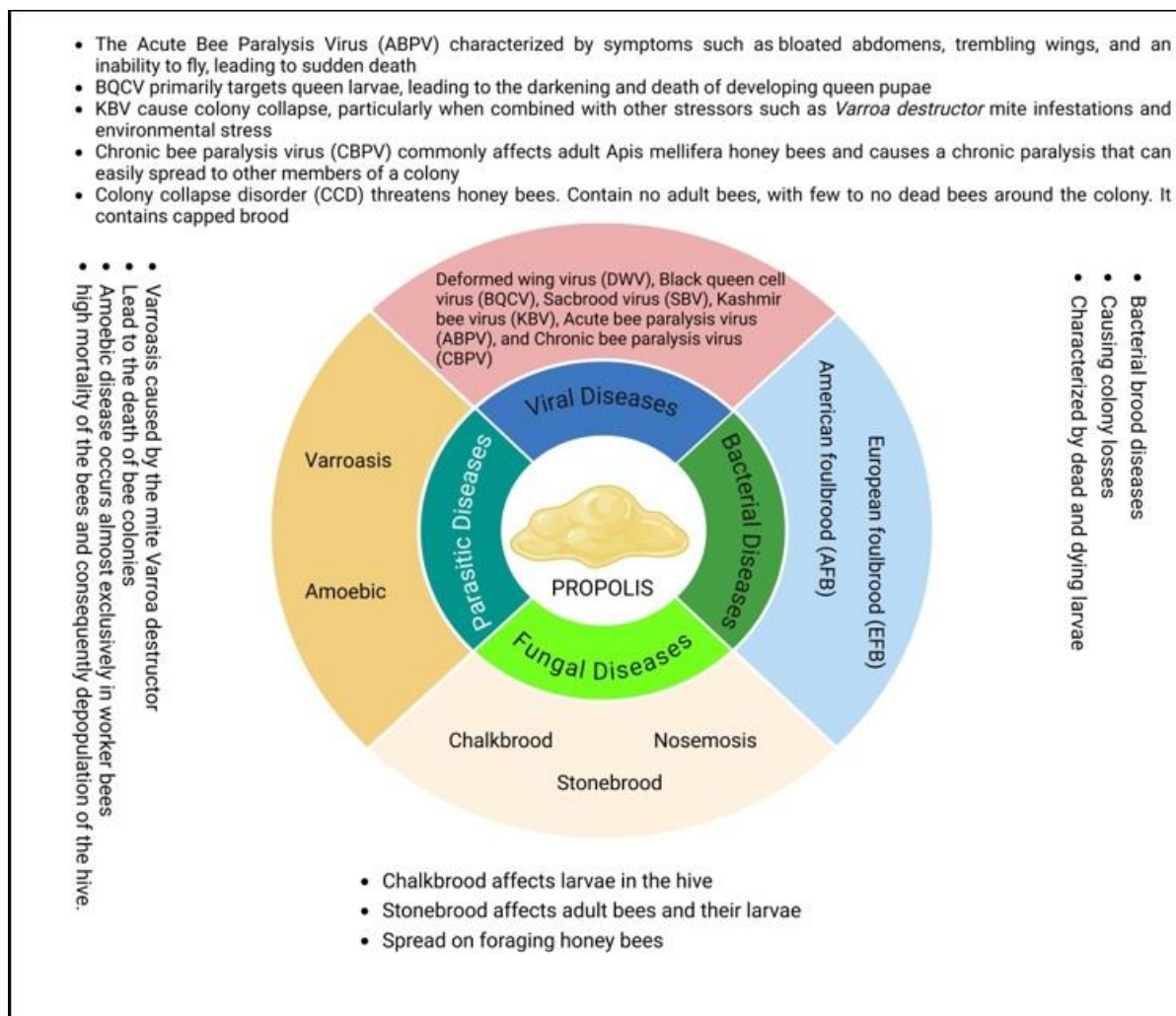
242 Propolis is not only beneficial for its antioxidant properties but also for its  
243 immunomodulatory effects, making it biologically significant to bee health. Bee immunity  
244 plays a critical role in protecting colonies from foulbrood diseases such as American foulbrood  
245 (AFB) and European foulbrood (EFB). The innate immune system of bees includes physical  
246 barriers, such as the cuticle and gut epithelium, as well as cellular defenses, such as

247 phagocytosis by hemocytes and antimicrobial peptide production. In contrast, the adaptive  
248 immune system of bees involves immune memory and specific recognition of pathogens.  
249 Studies have shown that propolis can enhance the host defense system and act as an indirect  
250 barrier for pathogen control [29]. Orsatti and Sforcin [30] found that propolis extracts and  
251 derivatives can boost immune responses by modulating Toll-like receptor (TLR) signaling. By  
252 enhancing TLR signaling, propolis may improve the immune response of bees when faced with  
253 pathogens, potentially aiding in combating foulbrood diseases and other infections that threaten  
254 bee colonies. Further research on the immunomodulatory effects of propolis on bee immunity  
255 in foulbrood diseases could provide valuable insights for beekeepers and researchers aiming to  
256 improve bee health and colony resilience.

### 257 **3.3.3 Antibacterial effects of propolis against honeybee diseases**

258 Studies have demonstrated that propolis can effectively eliminate various disease-  
259 causing agents in honeybee colonies, including bacterial, fungal and viral agents [31, 32].  
260 When supplemented with appropriate concentrations of propolis, honeybee colonies have been  
261 shown to exhibit lower bacterial loads and reduced expression of immune genes and  
262 antibacterial peptides, indicating enhanced immunity and resistance to infections [19].

263



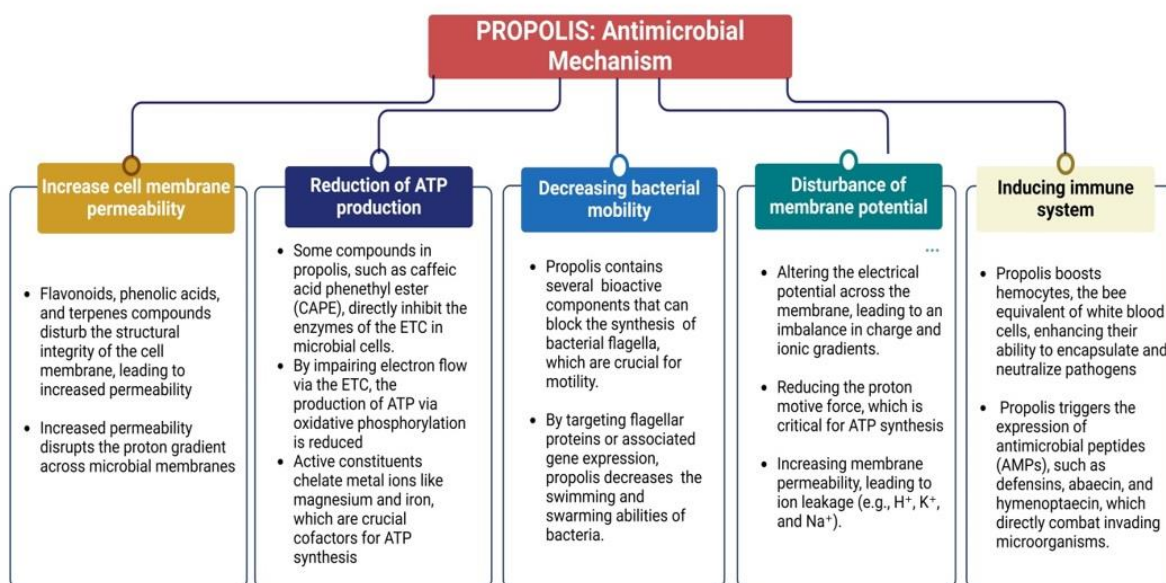
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265 **Figure 1.** Antagonistic action of propolis against different honeybee diseases

266 Several *in vitro* and field studies have demonstrated that propolis and its components  
 267 can reduce the bacterial, fungal, and viral pathogen loads (Figure 1). According to these  
 268 investigations, propolis has shown antimicrobial effects against *Paenibacillus larvae* [7, 9],  
 269 *Ascosphaera apis* [9], *Nosema* spp. [20], and impacts the ectoparasitic mite *Varroa destructor*  
 270 and viruses [33].

271 Despite significant differences in chemical composition, propolis sourced from different  
 272 geographic locations has been shown to exhibit similar antibacterial, antifungal, and antiviral  
 273 activities. These activities are attributed to the presence of active compounds, such as aromatic  
 274 compounds, caffeic acid, and flavonoids in propolis. In Figure 2, The pathways through which

275 propolis affects the permeability of microbial cell membranes, disrupts membrane potential,  
 276 inhibits adenosine triphosphate (ATP) production, and reduces bacterial mobility are illustrated.



277

278 **Figure 2.** Antimicrobial mechanism of propolis

279 **3.4 Propolis and Colony health**

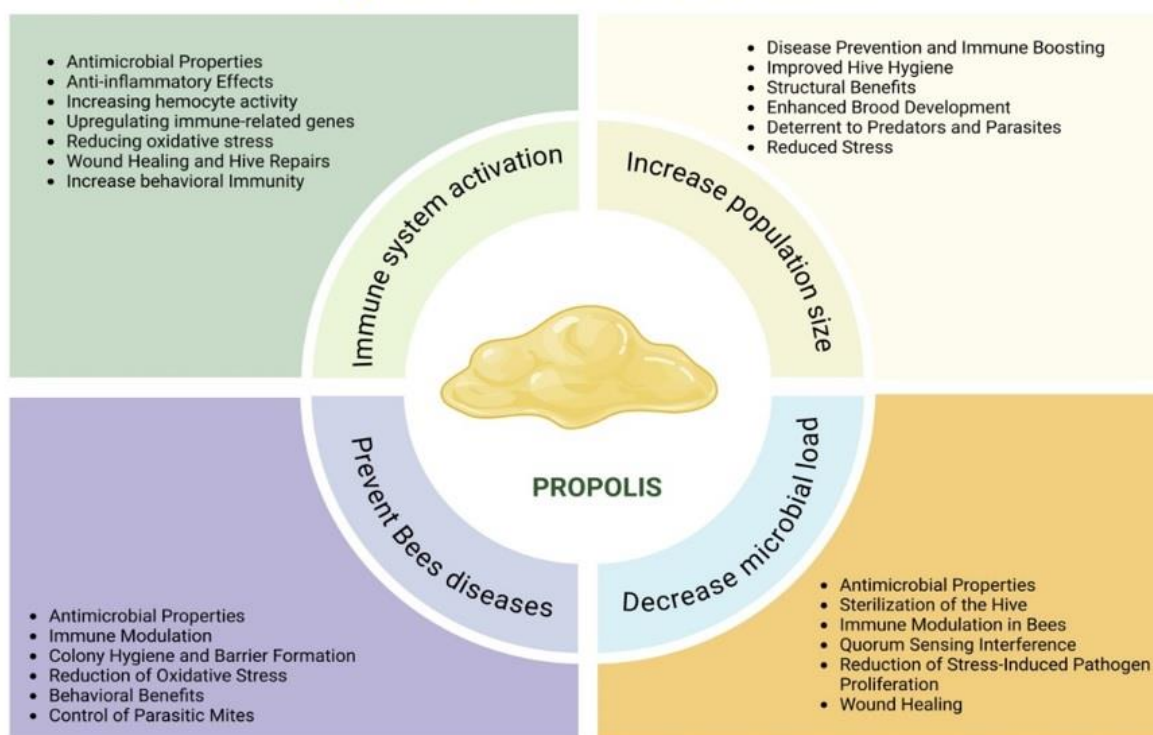
280 Honey bee colonies currently face significant annual mortality rates, primarily attributed  
 281 to pathogens, parasites, pesticides, and inadequate nutrition. It is commonly believed that  
 282 honeybees do not naturally ingest propolis; however, the administration of propolis extracts  
 283 may influence their health. Research has indicated that honey bees receiving propolis extracts  
 284 exhibit increased expression of antimicrobial peptides, such as defensin-1, abaecin,  
 285 hymenoptaecin, and apidaecin, in response to bacterial challenge [34]. This observation implies  
 286 that propolis intake may enhance the activation of the innate immune response of bees [35].  
 287 Additionally, when exposed to *Vairimorpha*, bees that were fed propolis demonstrated reduced  
 288 spore counts and improved survival rates [16]. Nevertheless, the comprehensive effects of  
 289 propolis consumption on honeybee health remain underexplored.

290 Propolis is considered to act as a protective barrier within the hive and has been linked  
 291 to the decreased expression of immune system genes [16]. Although honey bees possess

292 significantly fewer immune genes than numerous other insect species, they seem to offset this  
293 limitation through a distinctive array of defenses referred to as social immunity. This set of  
294 defenses serves to safeguard the plant against pathogens and pests. Such mechanisms include  
295 mechanical removal of parasites, expulsion of infected members, disposal of waste, and use of  
296 propolis for nest protection. Propolis potentially plays a role in alleviating some of these  
297 challenges [18].

298 To date, a number of benefits of propolis on bee health have been reported, including  
299 its positive impacts on bee colonies, such as increased brood viability and adult longevity. Bee  
300 colonies have also been observed to self-medicate by collecting propolis [36, 16]. The reduced  
301 expression of immune genes in these insects is linked to the presence of propolis in hives, which  
302 appears to function as an energy conservation strategy, as the presence of propolis may render  
303 the activation of these genes unnecessary by inhibiting microbial growth [34, 37].  
304 Overexpression of immune genes incurs costs for bees and can diminish the overall productivity  
305 of the colony. Additionally, the antimicrobial potential of different propolis extracts shows their  
306 possible role in the inhibition of different bacterial, viral, and fungal pathogens, thereby  
307 protecting bees against a number of diseases [35]. Studies have shown that honey bee colonies  
308 supplemented with appropriate concentrations of propolis exhibit lower viral levels and lower  
309 expression of individual immune genes and antibacterial peptides [37]. The overall health  
310 impact of propolis on honeybees is shown in Figure 3.

## Propolis: Benefits on Bees Health



311

312 **Figure 3.** A concise overview of the advantages of propolis imposed on the health of honeybees  
313 individually and their colonies.

314 It is showed that propolis is not only generally safe for consumption by honeybees but  
315 also has some apparent health-promoting effects on these insects. An important consideration  
316 when adding propolis to a bee diet is how it may affect the normal microflora in the bee guts.  
317 However, reports have shown that the honeybee microbiome is stabilized in the presence of  
318 propolis, which acts as a social immune defense and maintains colonial microbial health,  
319 consequently resulting in an increased life span of these pollinator insects [22].

### 320 3.5 Antibacterial effects of propolis against AFB and EFB disease causing agents

321 The antibacterial properties of propolis extracts sourced from various global regions were  
322 examined for their efficacy against *P. larvae* and *M. plutonious*. In the 1960s, Lindenfelser was  
323 the first to report the antibacterial activity of propolis against the etiological agent of AFB [38].

324 Subsequently, numerous studies have demonstrated the antibacterial effects of various types of  
 325 propolis against AFB and EFB (Table 1).

326 **Table 1.** Significant finding regarding effects of different propolis against AFB and EFB  
 327 diseases

Propolis Origin	Antagonistic effects	Extraction procedure	Country of Study	Technique	Significant finding	Ref.
Propolis envelop	AFB	NK	USA	Larval food antimicrobial assay	Propolis envelope protect the brood from bacterial pathogen infection, resulting in a lower colony-level infection load.	[3]
Ohio Propolis	EFB	Ethanollic extract	USA	Microdilution method	Demonstrated strong antibacterial properties of propolis against <i>M. plutonius</i> , at roughly the same concentration as antibiotic OTC (1.0 mg/mL).	[7]
Egyptian propolis	AFB and EFB	NK	Egypt	Agar petri dish	highly effect the growth of <i>P. larvae</i> and <i>M.plutonius</i> at the concentrations of 100 and 200 ppm, respectively	[39]
Propolis	AFB	Methanolic and acetonnitrile extract	different regions of USA	broth microdilution technique	Inhibited <i>P. larvae</i> growth in a dose dependent manner. 3-Acyl dihydroflavonols	[9]

					are active against the bee pathogens <i>P.</i> <i>larvae</i>	
Korean propolis	AFB	Raw propolis washed with alcohol and added to sugar syrup	South Korea	Studies conducted <i>in vivo</i> in bees	Decrease in the incidence of <i>P. larvae</i> in bees administered with propolis	[31]
Iranian propolis	EFB and AFB	Ethanollic extracts	Iran	HPLC, agar well diffusion assay, MIC	0.02 mg/mL of mixture of three Iranian propolis samples inhibited the growth of EFB and AFB	[40]
Green Brazilian / Minnesota propolis	AFB	Ethanollic Extracts	Brazil and USA	resistance assay method	It is predicted that the source of propolis is more important than the concentrations used. Propolis samples from Brazil were more effective than those from USA.	[41]
European propolis	AFB	Diethyl ether	Poland	tube dilution assay/ GC-MS analysis of extracts	Synergistic interaction among different compounds in propolis led to Higher anti- <i>P. larvae</i> activity	[42]
Romanian propolis	AFB	Ethanollic extracts	Romania	antimicrobial tests/ Folin-Ciocalteu	propolis antibacterial activity against the honeybee pathogen <i>P. larvae</i> is affected by	[43]

				colorimetric method	Interactions among its flavonoid's component	
Poplar Bulgarian propolis	AFB	Ethanol extract Ethyl acetate Petrol ether	Slovakia	Inhibition zone assay	pinocembrin, 3-O-acetyl pinobanksin, and the caffeate mixture were identified as the most active constituents in the propolis inhibiting <i>P. larvae</i>	[44]
Taiwanese green propolis	AFB	Ethanolic extract	Taiwan	HPLC/microdilution method	Due to structural difference among four different types of propolis, their interaction with the cell wall of gram-positive strains varies.	[45]
Turkish propolis	AFB	Ethanolic extract	Turkey and Greece	GC-MS analysis / disc diffusion method	Antimicrobial properties are more affected by kind of vegetation than geographic location	[46]
Yugoslavian propolis	AFB	Ethanol extract and aqueous extract	Yugoslavia	agar diffusion technique	5 and 10% (v/v) propolis extract inhibited the growth of <i>P. larvae</i>	[47]
Thailand propolis	AFB	Methanolic, Dichloromethane and hexane extract	Thailand	nuclear magnetic resonance/ agar well diffusion	Only methanolic extracts (100 mg/ml) inhibited <i>P. larvae</i> . Propolis through inhibition of enzymes	[48].

and microbroth involved in the DNA  
dilution assay repair pathway and  
malfunction of poly  
ADP ribose polymerase  
led to cell death

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328

329 Bastos et al. (41) investigated the antibacterial properties of several propolis samples  
330 collected from different regions of Brazil against *P. larvae* [41]. Their study revealed that the  
331 geographical source of propolis played a more significant role in determining the overall  
332 antibacterial activity than the concentrations tested. Specifically, propolis samples from Brazil  
333 exhibited greater effectiveness in inhibiting *P. larvae* growth than those from the USA. While,  
334 Isidorov and his co-researchers compared the antimicrobial properties of nine different  
335 European propolis samples derived from the resins of poplar, birch, and aspen plants against  
336 various strains of *P. larvae* [42]. According to their observations, despite differences in the  
337 chemical compositions of the studied propolis, all demonstrated the ability to inhibit *P. larvae*  
338 growth, with a minimum inhibitory concentration ranging from 7.8 to 62.4  $\mu\text{g mL}^{-1}$ . These  
339 researchers noted that, in addition to flavonoids, other phenolic compounds such as glycerides,  
340 phenylpropanoids, hydroxycinnamyl sesquiterpenols, and benzoates also played a role in  
341 influencing the strength of the antimicrobial activity observed against different *P. larvae*  
342 strains.

343 Borba et al. [3].investigated the protective effects of the propolis envelope as a natural  
344 defense mechanism against *P. larvae* in *A. mellifera* colonies. They discovered that the propolis  
345 envelope surrounding the colonies provided support to honeybees in combating the pathogen  
346 *P. larvae*. Colonies with a propolis envelope exhibited a significant decrease in clinical signs  
347 of AFB after two months of exposure to the pathogen [3]. These findings suggest that the  
348 propolis envelope acts as an antimicrobial barrier around the colony, safeguarding the brood

349 from bacterial infections and reducing the overall infection burden at the colony level. Collins  
350 et al. [49]. investigated the antimicrobial properties of CAPE that is abundantly found in  
351 propolis against *P. larvae*. Their findings indicated that CAPE effectively inhibited the growth  
352 of *P. larvae* by modulating intracellular oxidant and antioxidant levels. Consequently, they  
353 proposed that the presence of CAPE in propolis could be utilized to combat AFB [49]. Also,  
354 Kim et al. [40] explored the impact of varying concentrations of propolis (0.16, 0.3, and 0.6%)  
355 on different bee diseases. Their results suggested that administering propolis to bees could boost  
356 their immune response and effectively control AFB [49].

357 In a study conducted in Egypt in 2017, the antibacterial properties of varying  
358 concentrations of propolis against *M. plutonius* was investigated by a group of researchers.  
359 Their results revealed that propolis increased the percentage of healthy broods by 29.8%,  
360 indicating its potential effectiveness in combating bee diseases [39]. Furthermore, Murray and  
361 his colleagues [7] showed that both darkened and lightened brood comb and propolis extracts  
362 exhibited antimicrobial activity against *M. plutonius*, suggesting that propolis integrated into  
363 wax may contribute to its antimicrobial effects. While, Toutiaee et al. [32], reported synergistic  
364 antibacterial effects of four distinct Iranian propolis extracts against *M. plutonius* and *P. larvae*.  
365 Their results indicated that the combination of four different ethanolic propolis extracts  
366 significantly enhanced the antibacterial effects against these bacterial agents compared to mono  
367 propolis extracts, highlighting the potential synergistic benefits of combining different propolis  
368 extracts for improved antimicrobial efficacy in bee disease management.

#### 369 **4. Conclusions and future prospects**

370 As the global emphasis on sustainability increases, the significance of propolis in  
371 safeguarding honeybee health signifies its importance in environmentally friendly beekeeping  
372 practices and offers considerable potential for addressing ecological issues. It is becoming  
373 increasingly evident that this natural substance could serve as a valuable tool for mitigating the

374 impact of various biological threats faced by bee colonies. Propolis not only exhibits  
375 antimicrobial properties that can combat diseases but also enhances honeybee immune  
376 responses, population density, and overall activity levels. Despite these promising findings,  
377 further studies are necessary to assess the impact of intentionally adding propolis to honeybee  
378 colonies, the long-term effects of different types of propolis on colony health, and the dose  
379 effects of propolis against different diseases, particularly in commercial beekeeping operations  
380 where propolis enrichment could have significant implications. Moreover, treatments derived  
381 from propolis serve as natural substitutes for chemical antibiotics, aiding in the reduction of  
382 environmental pollution and fostering biodiversity.

383         Moreover, while propolis shows great promise for protecting honeybees, there is a need  
384 to carefully investigate its potential impact on the beneficial gut microbiome of bees. Disruption  
385 of the delicate balance of the bee gut microbiome could pose risks to bee health and survival,  
386 underscoring the importance of detailed research in this area. By addressing these knowledge  
387 gaps and conducting comprehensive studies on the effects of propolis in various beekeeping  
388 contexts, we can unlock the full potential of this natural substance to support sustainable  
389 beekeeping practices and ensure long-term health and viability of honeybee populations. The  
390 prospects of propolis in the beekeeping industry are bright, offering a holistic and eco-friendly  
391 approach to disease management and hive protection.

392         Propolis is an environmentally friendly natural substance that can play a vital role in  
393 promoting environmentally sustainable beekeeping by reducing reliance on synthetic  
394 antibiotics and chemical treatments. These natural substances of bee origin hold significant  
395 potential for combating foulbrood diseases and other threats to honeybee health. The inhibitory  
396 effects of propolis on *P. larvae* and *M. plutonius* highlight its importance in protecting bee  
397 colonies from these devastating diseases. Owing to the wide-spectrum antimicrobial potential  
398 of propolis, beekeepers can potentially reduce their reliance on conventional treatments and

399 adopt a more sustainable approach to hive protection. The integration of propolis-based  
400 strategies into modern apiculture practices could minimize the environmental footprint of  
401 disease management and safeguard pollinator populations critical for biodiversity and food  
402 security.

403         However, the diverse compositions of propolis present challenges in standardizing its  
404 effects, and ongoing research is essential to fully understand its long-term impact on colony  
405 health and productivity. Although studies have shown the potential of propolis as a natural  
406 strategy for modulating the bee microbiome and protecting honeybees from disease, further  
407 research is needed to fully understand the mechanisms underlying the interactions between  
408 propolis and the bee microbiome, and their long-term effects on bee health and the surrounding  
409 environment. Moreover, it is crucial to investigate the function of propolis as a social immune  
410 defense against parasites and pathogens, as well as its subtle indirect influences on individual  
411 immunity and detoxification enzymes, to enhance bee health.

412         Recognizing the potential health benefits of propolis for bees, some beekeepers have  
413 incorporated it into their pollen substitute formulations. Further research is essential to  
414 understand the long-term effects of propolis on colony health and productivity, which may  
415 encourage the beekeeping community to prioritize propolis collection that could contribute to  
416 improving resistance traits in bee colonies. As we strive to harness the potential of propolis for  
417 sustainable beekeeping practices, future research should focus on optimizing its use to enhance  
418 colony resilience and support environmental conservation efforts. It is crucial for researchers,  
419 policymakers, and beekeepers to collaborate effectively to utilize propolis as a fundamental  
420 element for sustainable beekeeping and environmental preservation.

#### 421 **Acknowledgements**

422 Not applicable

#### 423 **Author Contributions**

424 Study concept and design: N. M. and M. M.

425 Acquisition of data: N. M.

426 Analysis and interpretation of data: N. M.

427 Drafting of the manuscript: N. M. and M. B.

428 Critical revision of the manuscript for important intellectual content: N. M. and M. B.

429 Intellectual content: N. M. and M.B.

430 Administrative, technical, and material support: N. M., M. B., H.MM., M. M., and

### 431 **Conflict of interest**

432 The authors have no competing interests to declare relevant to the content of this article.

### 433 **Ethics Approval**

434 Not applicable

### 435 **Funding**

436 This research did not receive any grant from funding agencies in the public, commercial, or  
437 non-profit sectors.

### 438 **Data availability**

439 The authors confirm that the data supporting the findings of this study are available within the  
440 article.

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