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Effect of honeybee venom and Egyptian propolis on the honeybee (*Apis mellifera* L.) health in vivo

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Abstract

Background: Honeybees are one of the most important pollinators in the world, and their products are nowadays included in most anticancer, antiallergic, antimicrobial drugs and are included in cosmetic treatments. In the present study, honeybee venom and Egyptian ethanolic propolis extract (EP) were focused to test their effect on health and some genes for honeybee workers (defensin2, abaecin, hymenoptaecin, vitellogenin, and juvenile hormone esterase).

Results: Honeybee venom and Egyptian propolis extract (EP) were used as supplements in the nutrition with different concentrations in Varroa mites-infected colonies to measure the colonies' activities after treatment. The immune-related genes and antimicrobial peptides (AMPs) were evaluated by using qRT-PCR. Treated colonies with HBV and EP showed up-regulation of immune and immune-related genes' expressions and increased the life span, activities and their density of bee workers. The data illustrated that the highest gene expression fold of juvenile hormone esterase was detected in the treated colonies with Egyptian ethanolic propolis extract (EP), while the highest vitellogenin expression fold in treated colonies was with honeybee venom. The up-regulation of antimicrobial peptides occurred in colonies with both treatments.

Conclusion: The findings suggest that honeybee venom and Egyptian ethanolic propolis extract (EP) could be used as potential supplements, even at the lowest concentration to develop the immunity of worker bees to increase their efficiency and prevent loss of honeybee colonies due to several diseases closely associated with Varroa mites' infections that cause sudden death.

Keywords: Honeybee venom, Propolis, Antimicrobial peptides, Vitellogenin, Juvenile hormone

Background

Honeybees (*Apis mellifera* L.) are significant pollinators, and the importance of honeybee products (venom, wax, royal jelly, propolis, honey, and pollen grains) has been demonstrated in previous research that used pollen grains and royal jelly to treat colonies (Wang et al. 2014).

Honeybee viruses, such as deformed wing virus (DWV), kakugo virus (KV), *Varroa destructor* virus-1 (VDV-1), black queen cell virus (BQCV), recombinant virus (VDV-1/DWV), acute bee paralysis virus (ABPV), slow bee paralysis virus (SBPV) and others, are one of the main drivers of colony losses. Several of these viruses are vectored by mite parasites, such as *Varroa destructor* (De Grandi-Hoffman and Chen 2015; Beaurepaire et al. 2017), and typically affect all stages of honeybee development. Some Egyptian apiaries plague with *Nosema* sp. and viral infections, which reduce honeybee immunity (Abd-El-Samie et al. 2021). Honeybee viruses participated in colony collapse disorder all over the world; the viruses are detected using

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RT-PCR (Cox-Foster et al. 2007; Abd-El-Samie et al. 2021). To fight against invading microbes, insects rely on their immunity. The generation of immune effectors, antimicrobial peptides (AMPs), is a key component of humoral immunity (Lemaitre and Hoffmann 2007). AMP; abaecin is a key immune effector of honey bee involved in the response to infection by multiple parasites (Evans and Pettis 2005; Evans et al. 2006) and has been shown to have significant heritable variation in its expression (Decanini et al. 2007). Defensin comes in two varieties: Defensin I, which is thought to have a role in social immunity, and Defensin 2, which is more likely to play a role in individual immunity (Ilyasov et al. 2013). The toll and immune deficiency (IMD) pathways are two different signal transduction cascades that produce AMPs. AMP can be activated for a brief period and transported to the infection site (Lemaitre and Hoffmann 2007; Schlüns and Crozier 2007). In terms of activity potency, AMPs are comparable to antibiotics, and they can be used to build antifungal and antibacterial medications (Mahlapuu et al. 2016). The transcriptional activation of immunity-related genes (IMRGs) is not only regulated by microbial infection, but also influenced by insect hormones; hormone levels are tightly regulated by multiple internal and external factors and juvenile hormone (JH) function relates to the regulation of the yolk protein vitellogenin (Vg) (Corona et al. 2007; Pandey and Bloch 2015). The use of antifungal and antibiotic medications to treat honeybee diseases causes suppression of their immune and the appearance of pathogens that are stable to them addition pollution of honeybee products. The solution to this challenge is to improve honeybee immunity by increasing the level of AMPs expression in the honeybees themselves (Casteels et al. 1993). The expression levels of three critical immune genes encoding the antimicrobial peptides abaecin, defensin 2, and hymenoptaecin were evaluated to acquire insight into the immunological mechanisms involved in resistance to these parasites. As a result, we resorted to using the most significant natural products, honey bee venom (HBV) and Egyptian propolis ethanolic extract (EP), to raise the honeybee's immunity. The composition and the activities of honeybee venom and propolis were approved previously and characterized by advanced techniques (Tanuwidjaja et al. 2021; Ghallab et al. 2021). This study aimed to determine the effect of honeybee venom and EP as supplements in the nutrition on the regulation of honey bee AMPs gene expression, increase honeybee resistance to pathogens, reduce sudden bee death that causes colony collapse disorder, and determine the efficacy of honeybee venom and EP treatments on the secretion of juvenile hormone and vitellogenin secretion.

Methods

Egyptian ethanolic extract propolis preparation (EP)

The propolis was collected from the apiary of El-Dokki Honeybee Research Department, Plant Protection Research Institute, Agriculture Research Center, Giza, Egypt; during the spring to autumn season of 2019 after collection, propolis was kept in the freezer at −20 °C immediately to crush easily. 100 g of crushed frozen propolis was dissolved in 1 L of ethanol 80% and left in dark place until use (Ghallab et al. 2021).

Honeybee venom collection

Honeybee colony almost density 12,000 by electrical stimulation was used to collect 1 gm of venom according to Benton protocol with some modifications (Benton et al. 1963). Thus the modifications were the electrical apparatus was powered by 18 V to honeybee sting the glass with plastic foil of apparatus for 10 s; then, the honeybee venom was collected dried at 27 °C for 35 min and then kept frozen at −20 °C until use.

Colony selection and management

In winter season from February 2019, the apiary formed from 21 asymptomatic infested with Varroa mites' colonies (*Apis mellifera* L.) with poor propolis in colonies at Honeybee Research Department, Plant Protection Research Institute, was selected for the experiment. All colonies with the same strength were used in this investigation (4 frames per colony consist of one honey and pollen + 3 frames sealed and unsealed brood). Their queens were replaced with 4-month-old new Carniolan queen hybrid. The total area of a sealed and unsealed of both sides of frames was measured by inch square weekly during the experimental period using the Langstroth frame.

Twenty-one honeybee colonies were divided into 3 colonies for control, and eighteen honeybee colonies were divided into 2 groups for treatments with honeybee venom and Egyptian ethanolic propolis extract; each group was divided into 3 subgroups for each concentration of treatments. The concentrations of the EP treatment were 1, 3, and 5 g/L and the concentrations of honeybee venom were 0.25, 0.5, and 1 g/L. Both treatments were added in sugar syrup only; the honey bee venom was directly dissolved in the sugar solution yielding concentrations of 0.025, 0.05, and 0.1%. After extracting the propolis with alcohol, three different concentrations of propolis were taken; 10, 30, and 50 ml of the extract, equivalent to 1, 3, and 5 g of raw propolis, respectively, was directly added to the sugar solutions, yielding concentrations of 0.1, 0.3, and 0.5%, respectively. Feeding with different concentrations of treatments continued until November 2019, and all colonies were fed once a month.

Honeybee samples collection

Adult honeybee workers' samples were collected randomly from untreated and treated colonies, and 10 honeybees' workers were collected from inside of each colony; a total of 210 bees from all colonies were kept at − 20 °C until RNA extraction.

RNA extraction

Total RNA was extracted from 5 adult workers of honeybees using BIOZOL-BIOFLUX (Catalog No. 10760055-1) following the manufacturer's instructions. RNA quality and quantity were verified using NanoDrop 2000 (Thermo Scientific, USA). The cDNAs were synthesized from the extracted RNAs.

Reverse transcription and oligonucleotides synthesis

The cDNA synthesis was performed using Applied Biosystem kit (Cat. No. 10400745). A total of 5 immune genes of honeybees (Defensin2, Abaecin, Hymenoptaecin, Juvenile hormone esterase, and Vitellogenin) were measured, and B-actin using as a reference gene for normalization (Cunha et al. 2005). RT-PCR was performed using specific primer pairs listed in (Table 1) and was synthesized by Thermo Fisher Scientific (Invitrogen).

Conventional RT-PCR

RT-PCRs were run in the thermal cycle Techne Gene Amp. (PCR system FGENO2TD), and the thermal profile used for amplification of target cDNA by using Go Taq® Green PCR Master (2X) # A9281, according to Promega's manufacturer's. The thermal conditions began with an initial denaturation at 95 °C for 3 min, followed by 35 cycles of 95 °C for 45 s, 50 °C, and 72 °C for 1 min, then the final extension step at 72 °C for 7 min, for all genes. PCR products were analyzed by gel electrophoresis on a 1.5% agarose gel following ethidium bromide staining (Sigma-Aldrich, E7637) and visualized by UV transilluminator (CUVP upland A, USA). ExoSAP-IT (Affymetrix, USA) kit was used to purify the PCR products following the manufacturer's instruction (Cat. No. 75001.1.EA).

Sequencing of PCR products

Sequencing reactions were performed using BigDye Terminator v3.1 Cycle Sequencing (Applied Biosystems, CA, USA) and analyzed using 3130XL genetic analyzer (Applied Biosystems, CA, USA). Sequence data were collected and analyzed using BioEdit software version 7.0.0 (Hall 1999) to confirm the identification of the amplified genes.

Gene expression

The reactions were occurred by using SYBR™ Green PCR Master Mix (2X) kit (Cat. No. 4309155) as follows: 5 µl of Master Mix, 1 µl of each primer forward and 1 µl of each primer reverse (10 µM), 1 µl of cDNA and 2 µl of nuclease water with total volume 10 µl for each reaction for the detected genes. The instrument was Applied Biosystems 7500 SDS v1.5.1 Real-Time PCR System-Thermo Fisher Scientific. The thermal conditions began with initial activation: 50 °C for 2 min and 95 °C for 15 min, then following 40 cycles: 94 °C for 15 s, 50 °C for 30 s, and 72 °C for 30 s.

Melting curve analysis was performed following PCR amplification. The raw threshold cycle (Ct) values of selected gene transcripts were normalized to the Ct values of β-actin. Relative expression levels were calculated using the $2^{-\Delta\Delta Ct}$ method (Livak et al. 2001). Data were presented as mean ± SE. P values were calculated by t test. Values of $P > 0.05$ were considered statistically significant.

Table 1 List of used primers in the study

The primer	Sequences (5′-3′)	Position on the genome	Accession number	References
Actin (Fwd.)	TGCCAACACTGTCCTTTCTG	208	AB023025	Cunha et al. (2005)
Actin (Rev.)	AGAATTGACCCACCAATCCA	344		
Vitellogenin (Fwd.)	TTCTGATAAAGGCGTTGCT	173	NM_001011578	Corona et al. (2007)
Vitellogenin (Rev.)	GTTTTCTCCATCTTTGCTAAAGTCA	914		
JH esterase (Fwd.)	GTTTACGTGCCGGCAGATAG	511	NM_001011563.1	Bomtorin et al. (2014)
JH esterase (Rev.)	TTCTGAAACCCATCGCAACG	737		
Abaecin (Fwd.)	CAG CAT TCG CAT ACG TAC CA	6	AF442147.1	Schlüns and Crozier (2007)
Abaecin (Rev.)	GAC CAG GAA ACG TTG GAA AC	77		
Defensin (Fwd.)	TGT CGG CCT TCT CTT CAT GG	88	NM001011616.2	Schlüns and Crozier (2007)
Defensin (Rev.)	TGA CCT CCA GCT TTA CCC AAA	288		
Hymenoptaecin (Fwd.)	ATT CAT GGC ATC GTG AAC AA	2982	XM003251652.4	Schlüns and Crozier (2007)
Hymenoptaecin (Rev.)	CTG TGG TGG AGT TGT TGG TG	3122		

Statistical analysis

Results were expressed as the mean ± standard error (SE). Statistical significance between different samples of untreated and treated honeybee colonies was analyzed using one-way ANOVA. Statistical significance was defined as $P < 0.01$ and $P < 0.001$.

Results

Colony selection and management

Twenty-one colonies were observed weekly every month from the beginning of therapy in February 2019 until November 2019. The number of Varroa mites in treated colonies and sealed–unsealed broods reduced month after month, compared to untreated colonies. The overall average brood density of all honeybee venom treated colonies was 1860, 2644, and 3564 inches for HBV concentrations of 0.25, 0.5, and 1.0 g/L, respectively, while it was 1686, 2341, and 3182 inches for all treated colonies with Egyptian ethanolic propolis extract (EP) concentrations of 1, 3, and 5 g/L, respectively. The brood in honeybee venom treated colonies was found to be higher than in Egyptian ethanolic propolis extract (EP) treated colonies. Therefore, the hive was raised to a second round, while untreated colonies remained the same (Fig. 1).

PCR products of immune genes

The PCR products with the expected fragments of 71 base pair (bp) fragment for defensin2, 48 bp for abaecin, 112 bp for hymenoptaecin, 290 bp for juvenile hormone esterase, and 790 bp for vitellogenin were generated from asymptomatic honeybees and visualized on 1.5% agarose gel electrophoresis (Fig. 2). The obtained PCR fragments were recovered and sequenced. The nucleotide sequences of the fore-mentioned immune and immune-related genes under study were submitted to GenBank and subjected to a homology search using BLASTX which confirm their identification Additional file 1: Tables and Figs. S1–S5.

Gene expression

The studied honeybee genes were successfully amplified in all cases, and the specificity of the amplified products was confirmed by single peaks in the melting curve analysis. Expression levels of defensin, abaecin, hymenoptaecin, juvenile hormone esterase, and vitellogenin in untreated and treated honeybee colonies were compared. In general, the genes' expressions increased in both treatments in honeybee colonies than untreated colonies. In Egyptian ethanolic propolis extract (EP) treatment, non-significant differences were obtained for defensin2 in the treated colonies with 1.0 g/L of propolis, the hymenoptaecin was slightly down-regulated, and abaecin gene was slightly increased than untreated colonies. Defensin2 and hymenoptaecin levels increased significantly in 3 g/L EP and then declined in 5 g/L EP (Fig. 3). On the other hand, the level of vitellogenin increased as EP concentrations increased. In comparison with EP treatment concentrations, vitellogenin was up-regulated in honeybee venom (HBV) treatment concentrations, especially at 1.0 g/L. Moreover, juvenile hormone esterase increased in EP than honeybee venom treatments and it was steadily up-regulated as honeybee venom treatment concentrations increased (Fig. 4a, b). The antimicrobial peptide, abaecin expression folds were the same in 0.5 and 1.0 g/L honeybee venom treatments, but the expression folds of defensin2 and hymenoptaecin were lower in 0.5 g/L honeybee venom treatments than in other concentrations of honeybee venom treatments (Fig. 5). The highest concentration of honeybee venom (HBV) treatment (1 g/L) caused the highest rate of gene expression folds of Vg and abaecin by 3.94, 1.79, respectively, while the lowest concentration of honeybee venom treatment (0.25 g/L) caused the highest

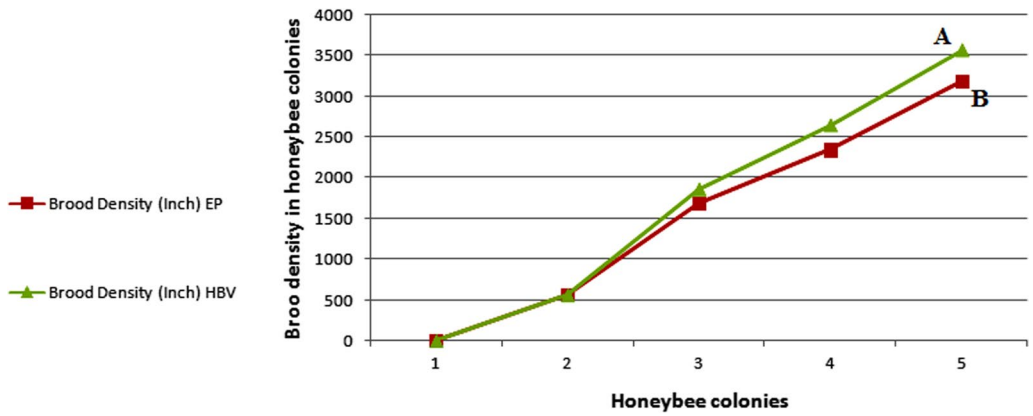

Fig. 1 The average of brood density in honeybee colonies showed in line A: the treated colonies with honeybee venom (HBV) concentrations more than line B: the treated colonies with Egyptian ethanolic propolis extract (EP)

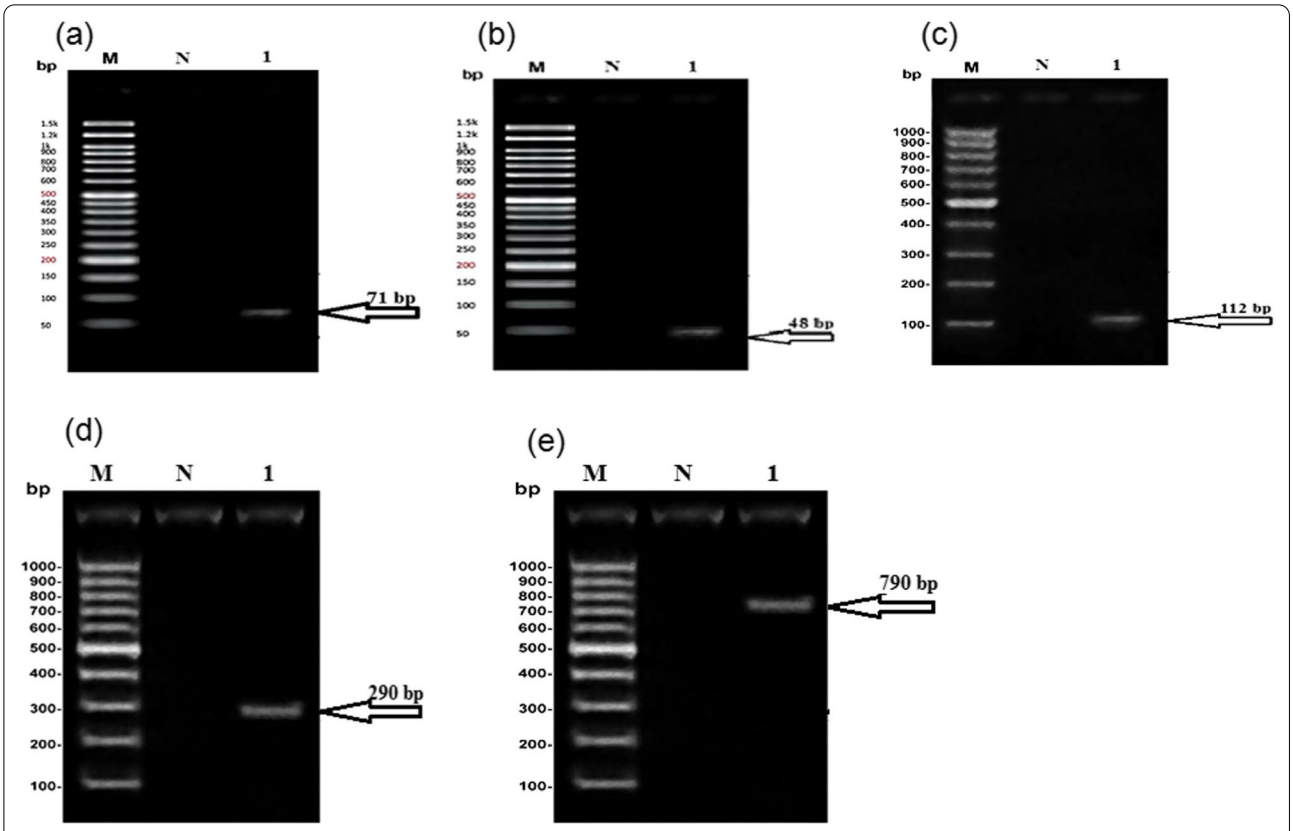

Fig. 2 Ethidium bromide-stained agarose gel electrophoresis of PCR-amplified fragments using specific primers represents: **a** defensin2 shows size 71 bp, **b** abaecin shows size 48 bp, **c** hymenoptaecin shows size 112 bp, **d** juvenile hormone esterase shows size 290 bp, and **e** vitellogenin shows size 790 bp. Lane M: molecular size marker (GeneDireX®), Lane N: negative control

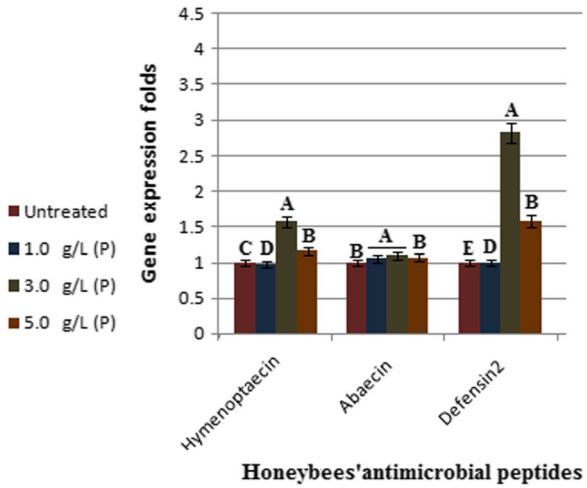

Fig. 3 The effect of Egyptian propolis ethanolic extract (EP) in different concentrations on the expression folds of antimicrobial peptides for honeybee with ± SE performed in triplicate

rate of gene expression folds of defensin2 and hymenoptaecin by 3.34 and 1.96 folds, respectively. In addition, propolis treatment's highest concentration (5 g/L) increased the expression of JHE by 3.63 folds, while propolis treatment with concentration (3 g/L) increased the gene expression folds of defensin2 and hymenoptaecin by 2.83 and 1.53 folds, respectively, but had no effect on abaecin.

Discussion

This is the first trial to use honeybee venom (HBV) and Egyptian ethanolic propolis extract (EP) in honeybee colonies as a diet with sugar syrup. In the winter, honeybee venom from winter workers did not include toxins and the allergen Api m 12, commonly known as vitellogenin, was absent (Danneels et al. 2015). In addition, colonies with queens descended from crosses between high-propolis-producing colonies showed significantly increased brood and worker bee longevity (Nicodemo et al. 2014). In this study, the results indicated rearing of the brood density in both the honeybee venom and EP treatments, suggesting that honeybee immunity had improved and they were better able to tolerate diseases. Honeybee venom is high in proteins and enzymes (Tanuwidjaja

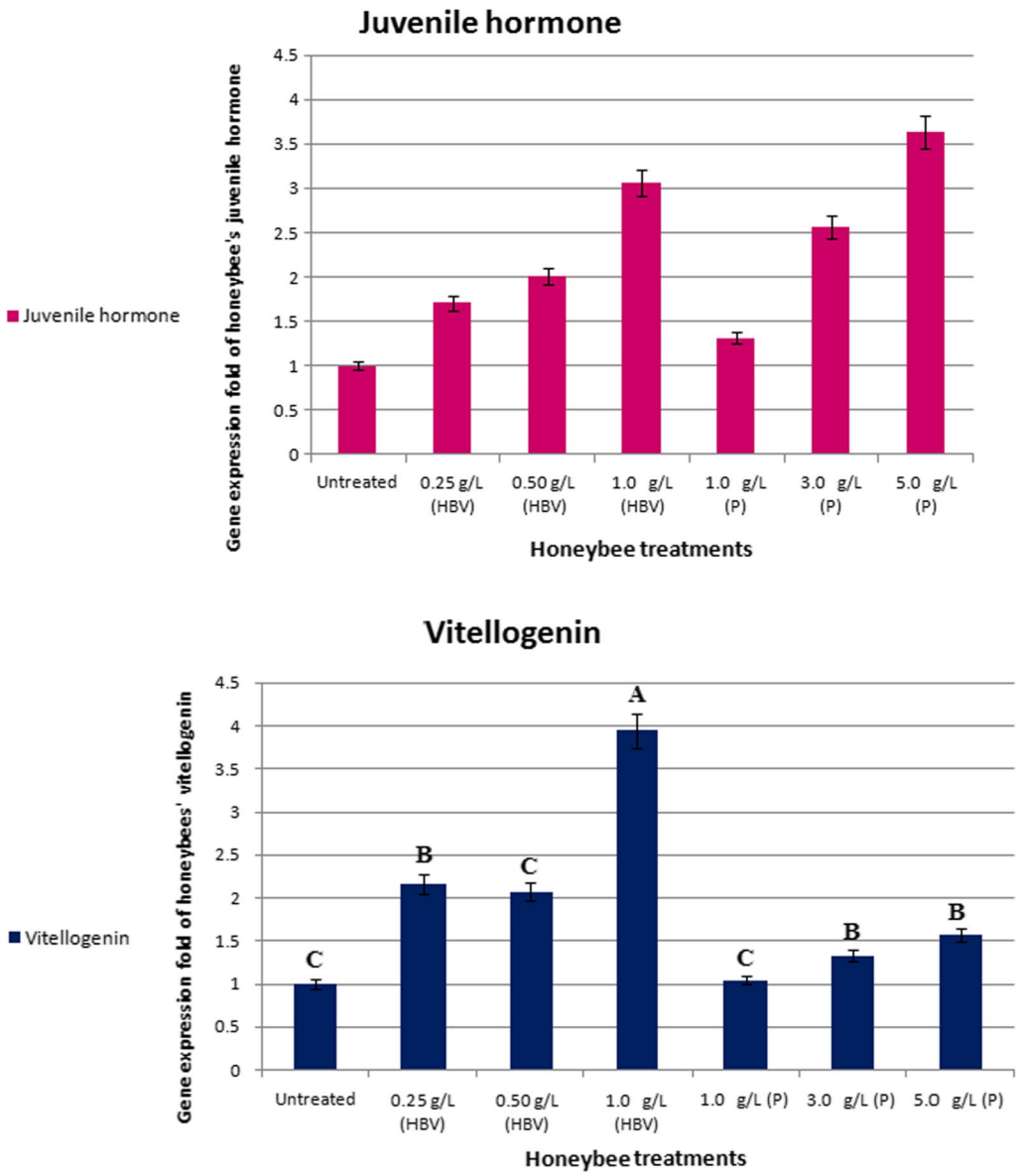

Fig. 4 **a** The effect of honeybee venom (HBV) and Egyptian propolis ethanolic extract (EP) in different concentrations on the expression folds of juvenile hormone esterase (JHE) for honeybee with ± SE performed in triplicate. **b** The effect of honeybee venom (HBV) and Egyptian propolis ethanolic extract (EP) in different concentrations on the expression folds of vitellogenin (vg) for honeybee with ± SE performed in triplicate

et al. 2021), and propolis contains many flavonoids, terpenoids, esters, proteins, enzymes, and other components (Asgharpour et al. 2020; Ghallab et al. 2021). This is in agreement with previous researchers who reported that diet is crucial to an individual's and the colony's appropriate development (Bryś et al. 2021). Precocious foraging is linked to a protein and lipid deficiency (Toth and Robinson 2005), which accelerates the aging process and leads to a decrease in the colony population. Many pathogens infect honeybees and suppress their immune systems, including the deformed wing virus (DWV), neonicotinoids and microsporidian parasites of the genus *Nosema* sp.(Pluta and Sokol 2021), and one of the most important parasite is *Varroa destructor.* Recently, Ramsey et al. (2019) suggested that Varroa were exploiting the fat body which is an essential tissue to proper immune function. According to our observations, Varroa mites decreased in both honeybee venom and EP supplemental food-treated colonies. This could be explained by some venom on the cuticle of honeybees may be due

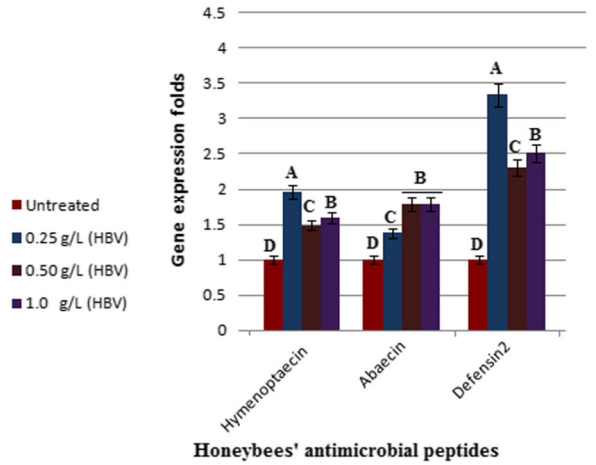

Fig. 5 The effect of honeybee venom (HBV) in different concentrations on the expression folds of antimicrobial peptides for honeybee with ± SE performed in triplicate

to self-grooming movement, which is thought to be an immune fortification of social insects and EP in honeybee colonies as a diet with sugar syrup. In addition, propolis' chemical compositions, which included several volatile components (Asgharpour et al. 2020; Ghallab et al. 2021), may help to resist Varroa mites permanently, as seen in propolis-treated colonies. So, in the colonies natural protein nutritional supplements should be supplemented with additional additives to improve Life especially during the winter when blooms and pollen are few because malnutrition weakens honeybees' immune systems and makes workers more susceptible to infection (Wang et al. 2014; Bryś et al. 2021). Consequently, due to Varroa mites reduction in both honeybee venom (HBV) and Egyptian ethanolic propolis extract (EP) supplemental food-treated colonies, related virus titers may be dropped in the treated colonies, indicating that EP and honeybee venom had indirect antiviral properties, as previously reported (Baracchi et al. 2011; Borba et al. 2015; Tanuwidjaja et al. 2021). Obtained findings are consistent with Pusceddu et al. (2021) who reported that propolis high in phenols was administered to brood cells to alter reproducing parasites and that propolis in the hive can have direct impacts on brood infections (Simone-Finstrom 2017), with a good effect on honeybees and the potential to improve Varroa mite mortality. In comparison with parasitized bees from untreated cells, Damiani et al. (2010) found out that the phenotype of mite-infested bees emerging from propolis-treated cells is associated with a decreased DWV load. As a result, propolis may act as an indirect inhibitor of mite-induced viral multiplication (Annoscia et al. 2019). In addition, honeybee venom contains an apidaecin peptide which is produced by the venom glands (Van Vaerenbergh et al. 2013). The expression of apidaecin by venom apparatus epithelial cells may protect individual honey bees from infections. Alternatively, its presence in the venom could play a role in the hive's social immunity (Baracchi and Turillazzi 2010; Van Vaerenbergh et al. 2013). Moreover, honeybee venom's major component is a melittin, which inhibits the infectivity of a wide range of viruses (Memariani et al. 2020). Moreover, the present research focused on increasing honeybee immunity, which is expressed in antimicrobial peptides (defensin2, abaecin, and hymenoptaecin) and related immune genes [vitellogenin (Vg) and juvenile hormone esterase (JHE)]. JHE or JHE-like genes have not been documented to be directly involved in the immune response in insects or crustaceans; nonetheless, rising evidence has indicated the presence of hormonal immunity regulation in insects (Zhu et al. 2018).

In the present study, the over-expression of the investigated antimicrobial peptides (AMPs) defensin2, hymenoptaecin, and abaecin in treated colonies with EP and HBV was detected by RT-qPCR gene expression, the key findings of this study were immunological, and immune-related gene expression was up-regulated in treated colonies compared to untreated colonies. According to previous studies (Abd-El-Samie et al. 2021) different honeybee viruses had spread widely among the apiaries globally and the reproduction of these viruses resulted in the reduction of vitellogenin (Dainat et al. 2012). Because this protein (Vg) has pleiotropic effects (Amdam et al. 2007; Antúnez et al. 2013), they discovered that when the titer of viral, fungal, or pathogens increased in the colonies, the rate of Vg and AMPs decreased, leading to colony collapse disorder (Antúnez et al. 2009; Chaimanee et al. 2012), although Vg damages the microorganism cell wall and has anti-microbial activity (Park et al. 2018). The level of Vg and AMPs increased with increasing treatment concentrations of honeybee venom and EP in our data; however, it was found out that even the low concentration of honeybee venom (0.25 g/L) could increase the expression folds of target genes. This is most likely due to melittin, which is associated with the insect's immune system (Baracchi et al. 2011). Additionally, propolis treatment increased the expression folds of JHE and Vg gradually with increasing the concentration of EP treatment, while the best effect of EP concentrations was 3 g/L for AMPs, but did not effect on abaecin, which is similar to Borba et al. (2015). Simone-Finstrom et al. (2009) imply that propolis activates the cellular immune system rather than the humoral immune system. Additionally, honeybees with propolis in their colonies have greater immunity and health than those without propolis (Simon-Finstrom et al. 2017). Propolis stimulates high-level expression of the immune system response in bees

challenged with microorganisms (Turcatto et al. 2018). Only 0.1 percent propolis fed in a pollen substitute diet greatly increases activation of antimicrobial peptide genes (defensin-1, abaecin, hymenoptaecin, and apidaecin) in bees infected with *Escherichia coli.* So, applying propolis to inside of hives allowed to a lower investment in immune function by reducing immune gene expression in uninfected adult worker bees (Simone-Finstrom et al. 2009; Borba et al. 2015). On the other hand, immune genes (Lyzozyme-2 and -3, Defensin-1), were up-regulated by pollen feeding in healthy bees (Alaux et al. 2010). The present study indicated that supplemental honeybee venom treated colonies had higher brood density and activity than propolis-treated colonies as well as untreated colonies. Honeybee venom went much beyond the traditional stereotype of predator defense, suggesting that the diverse nesting biology of honeybee species may be linked to the employment of venom in a social immunity context (Baracchi et al. 2011). Assuming that the venom gland is the only source of some cuticular peptides and that bees are unable to select which peptides spread selectively on the cuticles, differences in cuticular profiles may be influenced by the length of time these compounds can remain on the insect bodies and the frequency with which venom is applied (Baracchi et al. 2011). Indeed, bee venom is present on the cuticle of adult bees, and on comb wax, it may act as a social antiseptic device (Baracchi and Turillazzi 2010). Honeybee venom is utilized as self-defense since it contains various antibacterial toxins, including melittin, PLA2, adolpanin, dopamine, and hyaluronidase (Park et al. 2014), which can boost honeybee immunity.

Obtained results revealed that JHE was gradually elevated by both supplemental treatments of honeybee venom and EP. Its titer was increased with each increase in treatment concentration, especially the highest concentration (5 g/L) of EP gave the highest expression folds of JHE. Insect metamorphosis is only triggered when JH is metabolized by juvenile JHE (Bomtorin et al. 2014; Li et al. 2021). JH promotes metamorphosis and caste differentiation in honeybee larvae and is a very significant hormone in honey bees. JH hastens development (Pandey et al. 2020), and this is what was observed in this study due to the overexpression of JHE. The JH route was found to be usually down-regulated throughout larval development, indicating that its activity is hostile to the ecdysteroid pathway. Nonetheless, in drones, the genes involved in JH synthesis had a higher expression. Providing the supplement nutrition propolis in the sugar syrup as a diet may explain the increase in JHE in the honeybee body and density due to the acceleration of larval growth (Huang et al. 2014). The ability of the juvenile hormone to impact the expression of several ecdysteroids signaling genes suggests that crosstalk between the two hormones may be significant in the bee brain and behavior regulation (Pandey and Bloch 2015). Finally, the use of a large amount of either treatment of honeybee venom and EP may cause a defect in honey bee colonies as reported previously (BenVau and Nieh 2017; Aurori et al. 2021), and it may be costly for beekeepers, so using the smallest amounts and concentrations of both treatments, especially since the low concentrations produced satisfactory results, is recommended.

Conclusions

It could be concluded that supplementing honeybee nutrition with Egyptian ethanolic propolis extract (EP) and honeybee venom as well as a treatment in honeybee colonies can improve immunological response, honeybee density, and activity. Thus, saving honeybees from death and unexpected colony collapse by employing natural therapies in the smallest quantity and at the lowest cost can be done and also protecting beekeepers from annual losses due to Varroa mites' infestation, honeybee viruses, and other diseases.

Abbreviations

HBV: Honeybee venom; EP: Egyptian ethanolic propolis extract; AMPs: Antimicrobial peptides; DWV: Deformed wing virus; KV: Kakugo virus; VDV-1: *Varroa destructor* virus-1; BQCV: Black queen cell virus; VDV-1/DWV: Recombinant virus (*Varroa destructor* virus-1/deformed wing virus); ABPV: Acute bee paralysis virus; SBPV: Slow bee paralysis virus; IMD: Immune deficiency; IMRGs: Immunity-related genes; JH: Juvenile hormone; Vg: Vitellogenin; RNA: Ribonucleic acid; RT-PCR: Reverse transcriptase-polymerase chain reaction; Gm: Gram; g/L: Gram/Liter; µM: Micromolar; µl: Microliter; Ct: Threshold cycle; SE: Standard error; Bp: Base pair; PLA2: Phospholipase 2; JHE: Juvenile hormone esterase; MF: Methyl farnesoate.

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s41938-022-00580-0.

Additional file 1. **Tables (S1–S5)**. Results of homology search of Defensin, Abaecin, Hymenoptaecin, Juvenile hormone esterase, and Vitellogenin PCR amplified fragments revealed by Gene bank database, respectively. **Figs. (S1–S5)**. Results of DNA sequence analysis of Defensin, Abaecin, Hymenoptaecin, Juvenile hormone esterase, and Vitellogenin PCR amplified fragment, respectively from the asymptomatic honeybees (using forward primer) using ABI PRISM model 310 DNA automated sequencer.

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This study was conducted in the Molecular Biology laboratory, Department of Entomology, Faculty of Science, Cairo University.

Author contributions

HS carried out the experiments, all authors designed the study, EM supervised the work and wrote the manuscript with their input. EM and HS analyzed the data. All authors read and approved the final manuscript.

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Availability of data and materials
The datasets generated and analyzed during the current study are available in the Supplementary materials.

Declarations

Ethics approval and consent to participate
Not applicable.

Consent for publication
Not applicable.

Competing interests
The authors declare that they have no competing interests.

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