

Research Article

Comparative effects of antibiotics and bee venom on toxicity and immune-related gene expression in honey bee (*Apis mellifera* L) larvae reared *in vitro*

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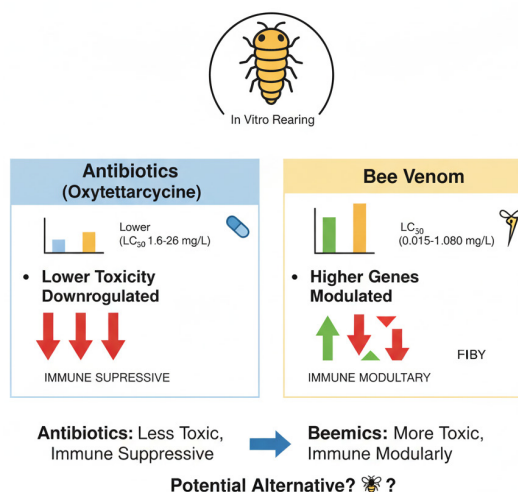
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ABSTRACT

Honey bees (*Apis mellifera*) play a critical role in pollination and global food security, yet their populations are increasingly threatened by pathogens, pesticides, and management practices. Understanding how commonly used apicultural treatments affect larval development and immunity is therefore essential. *In vitro* larval rearing provides a controlled environment to investigate the physiological and molecular responses of honey bee larvae to such stressors. In this study, the toxicological effects of oxytetracycline (antibiotic) and bee venom were compared by determining LC₅₀ values and assessing the expression of immune-related genes. LC₅₀ analysis revealed that bee venom exhibited higher toxicity than the antibiotic, with values ranging from 0.015 to 1.080 mg/L over six days, whereas antibiotic treatments showed LC₅₀ values between 1.617 and 2.663 mg/L. The LC₅₀ value represents the concentration required to cause 50% mortality, with lower values indicating higher toxicity. Gene expression profiling (RT-qPCR) demonstrated that antibiotic exposure significantly downregulated several immune-associated genes, including *Loc406090* (–9.67 fold), *Loc406102* (–4.14 fold), and *Loc406109* (–3.51 fold) ($P < 0.05$), compared to controls. In contrast, bee venom treatment led to both up- and down-regulation patterns, with a notable downregulation of *Loc406103* and *Loc413113* ($P < 0.05$). These findings suggest that although bee venom is more acutely toxic at higher concentrations, it modulates immune gene expression differently from antibiotics, potentially enhancing certain immune pathways. The results highlight the dual impact of antibiotics and bee venom on honey bee health and underscore the importance of further research into bee products as potential alternatives to antibiotics in apiculture.

GRAPHICAL ABSTRACT

Comparative Effects of Antibiotics and Bee Venom on Honey Bee Larval Health



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1. Introduction

Honey bees (*Apis mellifera*) are among the most important and economically valuable pollinators worldwide, contributing not only to agricultural productivity but also to the stability of natural ecosystems (Kabakcı *et al.*, 2024; Rader *et al.*, 2014; Ollerton *et al.*, 2011). Their pollination services enhance seed quality, fruit set, and crop yield, and it is estimated that approximately one-third of global food production depends directly or indirectly on insect pollination (Klein *et al.*, 2007). Recent global assessments have highlighted a significant decline in pollinator populations, driven by multiple interacting stressors including pesticide exposure, habitat loss, climate change, and infectious diseases (Potts *et al.*, 2020; IPBES, 2016). These pressures threaten not only ecosystem functioning but also long-term food security, emphasizing the urgent need to better understand factors affecting honey bee health at early developmental stages.

Among these agents, antibiotics occupy a central position due to their long-standing use in the control of bacterial brood diseases, particularly American foulbrood caused by *Paenibacillus larvae* (Reybroeck *et al.*, 2012). Oxytetracycline remains one of the most widely applied antibiotics in apicultural practice, although its use is increasingly restricted in many regions due to concerns related to residue accumulation, antimicrobial resistance, and unintended effects on bee physiology (Dai *et al.*, 2021). Oxytetracycline acts by inhibiting bacterial protein synthesis through interference with aminoacyl-tRNA binding at the ribosomal A site; however, this broad-spectrum activity may also affect non-target microbial communities associated with the honey bee gut. Disruption of the larval and adult gut microbiota has been shown to impair nutrient metabolism, immune competence, and resistance to opportunistic pathogens (Raymann *et al.*, 2018; Sagili *et al.*, 2018).

Increasing evidence indicates that repeated or chronic exposure to antibiotics such as oxytetracycline may suppress immune-related gene expression and alter innate immune signaling pathways in insects, including honey bees (Bulson *et al.*, 2021; Son *et al.*, 2007). Such immunological effects are particularly concerning at the larval stage, where immune system development and metabolic programming occur concurrently. Consequently, evaluating the toxicological and immunological consequences of antibiotic exposure at early developmental stages is essential for understanding their long-term implications for colony health.

In contrast to synthetic antibiotics, bee venom has attracted growing interest as a natural product with reported antimicrobial and immunomodulatory properties. Bee venom is a complex mixture of bioactive components, including melittin, phospholipase A₂, and apamin, which can exert cytotoxic, antimicrobial, and immune-modulating effects depending on dose and biological context (Dickel *et al.*, 2022). Experimental studies in vertebrate systems have demonstrated that bee venom can modulate immune responses, for example by altering immunoglobulin profiles and inflammatory signaling pathways (Ortiz-Alvarado *et al.*, 2020). However, despite its pharmacological potential, the effects of bee venom on honey bee larvae—particularly at the level of immune-related gene expression—remain poorly characterized.

Importantly, while bee venom is often discussed as a potential alternative to conventional antibiotics, its intrinsic toxicity raises critical questions regarding dose-dependent safety and developmental stage-specific responses in honey bees. Comparative studies that evaluate both toxicity and immune modulation are therefore required to assess whether bee venom could realistically complement or partially replace antibiotic use in apiculture.

Based on this background, the present study aimed to provide a comparative assessment of oxytetracycline and bee venom in *Apis mellifera* larvae reared under *in vitro* conditions. Specifically, we evaluated (i) larval toxicity by determining LC₅₀ values over the first six days of development and (ii) dose-dependent changes in immune-related gene expression using RT-qPCR. By directly contrasting the toxicological and immunological profiles of these two treatments, this study seeks to clarify their relative impacts on honey bee larval health and to contribute evidence-based insights into the potential role of bee-derived products as alternatives or supplements to antibiotics in apiculture.

Table 1.

Composition of the larval diet used for *in vitro* rearing of *Apis mellifera* larvae.

Component	Percentage (%)
Royal jelly	53
D-glucose	6
D-fructose	6
Yeast extract	1
Distilled water	34

2. Material and Methods

2.1 Material

2.1.1 Preparation of larval the diet

A stock larval diet was prepared following previously established *in vitro* rearing protocols (Kabakcı and Akdeniz, 2020; Kaftanoğlu *et al.*, 2010). The diet consisted of royal jelly, D-glucose, D-fructose, yeast extract, and distilled water (Table 1). All components were weighed gravimetrically, homogenized thoroughly to ensure uniform consistency, aliquoted into sterile containers, and stored at −20°C until use. Prior to feeding, diet aliquots were thawed at room temperature and gently mixed to avoid phase separation.

2.1.2 Preparation of antibiotics and bee venom solutions

For antibiotic treatments, a stock solution of oxytetracycline was prepared by dissolving 1 g of oxytetracycline (Sigma-Aldrich, USA) in 9 mL of ultrapure water, yielding a final stock concentration of 100 mg/mL. For bee venom treatments, 1 g of lyophilized bee venom (commercial source) was similarly dissolved in 9 mL of ultrapure water and homogenized until complete dissolution.

Working solutions were freshly prepared prior to each feeding event by diluting the stock solutions with larval diet at a 1:1 (w/w) ratio. Serial dilutions were then performed using the larval diet as diluent to obtain the final exposure concentrations used in the experiments (expressed as gm/L or mg/L, as indicated). All concentrations reported in this study refer to the final concentration administered to the larvae.

2.2 Method

2.2.1 Bee venom production and characterization

Bee venom was collected from 25 honey bee (*Apis mellifera*) colonies maintained at the Muş Alparslan University apiary (Muş, Türkiye). A commercially available electric venom collector delivering 12–18 V was used to stimulate venom secretion. The venom dried on a glass surface was carefully scraped, pooled, and stored at −20°C until analysis (İlhan *et al.*, 2024; Kabakcı and Akdeniz, 2020).

The relative composition of major venom components (melittin, phospholipase A₂, and apamin) was determined using high-performance liquid chromatography (HPLC) as described previously (İlhan *et al.*, 2024). This compositional analysis was conducted to characterize the venom used in the toxicity assays and not as a quantitative exposure variable. The percentage composition of bee venom components is summarized in Table 2.

2.2.2 Larval source and grafting procedure

Larvae were obtained from a single healthy colony containing eight frames to minimize genetic and environmental variability. To synchronize larval age, the queen was confined on an empty frame for

Table 2.

Contains % amounts in 1g bee venom of the bee venom content obtained from honey bees in this study.

Bee venom	Apamine %	Phospholipase A2 %	Mellitin %
1 gr	2.5	11	60

24 h to allow egg laying. Newly hatched, one-day-old larvae of uniform age were grafted under sterile conditions into Petri dishes for *in vitro* rearing, following established protocols (Dai *et al.*, 2021; Kabakcı and Akdeniz, 2020).

2.2.3 Incubation conditions

Larvae were maintained in a controlled incubator (Mikrolab, Türkiye) at $34 \pm 1^\circ\text{C}$ and 90–95% relative humidity, conditions optimized for honey bee larval development (Kabakcı and Akdeniz, 2020).

2.2.4 Feeding regime and experimental treatments

Larvae were fed daily with freshly prepared diets supplemented with predefined concentrations of either oxytetracycline or bee venom. All larvae received identical feeding volumes to ensure that observed effects were attributable to treatment concentration rather than nutritional differences. Mortality was recorded daily by counting live and dead larvae, and these data were used to calculate LC_{50} values.

Before the onset of defecation (day 6), larvae were collected and stored at -20°C for molecular analyses. On day 6, Kimwipes tissue paper (Kimberly-Clark, USA) was placed in Petri dishes to facilitate defecation. After a 24 h defecation period, larvae were transferred to sterile 24-well culture plates (Nest Biotechnology, China) and maintained until adult emergence. Adult bees were sampled at day 20 post-grafting and stored at -20°C for downstream analyses (Kabakcı and Akdeniz, 2020).

2.2.5 Statistical analysis

All statistical analyses were performed using SPSS software version 16.0 (SPSS Inc., Chicago, IL, USA). Median lethal concentration (LC_{50}) values were estimated by probit analysis with 95% confidence intervals, following U.S. EPA guidelines (EPA, 1993). Probit model assumptions were verified prior to analysis.

Differences among treatment groups were assessed using one-way analysis of variance (ANOVA), followed by Tukey's post hoc multiple comparison test. A significance threshold of $P < 0.05$ was applied throughout.

For gene expression analyses, relative mRNA levels were calculated using the $2^{-\Delta\Delta\text{Ct}}$ method, with β -actin as the internal reference gene. Each treatment group consisted of three independent biological replicates ($n = 3$ larvae or pupae per group), and each qPCR reaction was performed in technical triplicate. Results are presented as mean $\pm$ standard deviation (SD).

Data visualization and reproducible statistical graphics were generated using Python 3.10, employing the libraries matplotlib (v3.8.0), seaborn (v0.13.0), and pandas (v2.1.0). Heatmaps and clustered heatmaps were used to illustrate relative gene expression patterns and similarities among treatments.

2.2.6 RNA isolation, cDNA synthesis, and quantitative real-time PCR (qRT-PCR)

Total RNA was extracted from larvae and pupae using Wizol™ Reagent (Wizbio, Republic of Korea) according to the manufacturer's instructions. RNA concentration and purity were assessed spectrophotometrically, and only samples with acceptable purity ratios were used for downstream analyses. cDNA synthesis was performed using the High Capacity cDNA Synthesis Kit with RNase Inhibitor (ABT, Turkey).

Quantitative real-time PCR was conducted using a Rotor-Gene system (Corbett, USA) with SYBR-Green chemistry. Primer efficiencies were confirmed to be within acceptable ranges based on previous validation studies (Powell *et al.*, 2022; Li *et al.*, 2017). Relative expression levels of immune-related genes (LOC406090, LOC406102, LOC406103, LOC406109, LOC725087, LOC413113, LOC726999) were calculated using β -actin as the reference gene. Primer sequences are provided in Table 3.

3. Results

3.1 LC_{50} toxicity analysis

Prior to toxicity assays, the compositional profile of bee venom was characterized to ensure consistency of the tested material. HPLC

Table 3.

Primer sequences of the genes used in this study.

Gene	Forward (5'-3')	Reverse (5'-3')
β -actin	CTCTTCTTCGGATTGGTGGG	TCATCACITTTAGCGACGGT
LOC406090	CTCTTCTTCGGATTGGTGGG	TCATCACITTTAGCGACGGT
LOC406102	TAGCAACACGAAGCAAGCC	GTAGCAAGTGATGGACGGTTC
LOC406103	TCAAGACACCGTAGTCGCAA	CTATTTCGTTTCATCACGACGG
LOC406109	GAAGGAAACGGAATGAAGGG	GCATTTCATGTAGCACATTAGC
LOC725087	TTCTTTGCTGCTGTTTCG	CTCTCGTCATTATTTCGCT
LOC413113	ACCATATTCAAACGCAGTACC	TAGTCTGTAGAGGGCAGGGTT
LOC726999	GAACATGACTCGAACGCATTG	TTCTCGCTGTCGTTGGATG

analysis confirmed that bee venom consisted predominantly of melittin (~60%), phospholipase A_2 (~11%), and apamin (~2.5%), consistent with previous reports (İlhan *et al.*, 2024). This characterization was performed to describe venom composition rather than to quantify exposure variability.

As shown in Fig. 1, oxytetracycline treatments exhibited relatively higher LC_{50} values throughout the six-day feeding period, increasing gradually from Day 1 (1.617 M) to Day 6 (2.663 mg/L). In contrast, bee venom treatments resulted in substantially lower LC_{50} values, with the lowest value observed on Day 2 (0.015 mg/L). LC_{50} estimates were calculated using probit analysis and are presented with 95% confidence intervals.

Statistical analysis revealed significant differences in LC_{50} values across days for both treatments ($P < 0.05$). Bee venom LC_{50} values ranged from 0.015 to 1.080 mg/L, whereas oxytetracycline LC_{50} values ranged from 1.617 to 2.663 mg/L. These results indicate a higher acute toxicity of bee venom under *in vitro* conditions; however, the observed values reflect short-term exposure in a controlled system and do not directly represent field-level exposure scenarios.

All LC_{50} values were estimated using probit analysis based on concentration-mortality data collected across the experimental period. Dose-response relationships were modeled by fitting a generalized linear model with a probit link function, using log-transformed concentrations as the independent variable. Model adequacy was assessed through goodness-of-fit statistics and residual diagnostics. The median lethal concentrations (LC_{50}) were calculated along with their corresponding 95% confidence intervals, derived from the fitted model parameters. All estimates are provided in the supplementary dataset and summarized in the figure legends (Supplementary data).

3.2 Immune-related gene expression in larvae

3.2.1 Overall effects of antibiotics and bee venom

As illustrated in Fig. 2, exposure to oxytetracycline resulted in a consistent down-regulation of all analyzed immune-related genes relative to the control group. The most pronounced reductions were observed for Loc406090 (−9.67 fold), Loc406102 (−4.14 fold), and Loc406109 (−3.50 fold), all of which were statistically significant ($P < 0.05$). These values represent relative fold regulation calculated using the $2^{-\Delta\Delta\text{Ct}}$ method and indicate the direction and magnitude of transcriptional responses rather than absolute transcript abundance.

In contrast, bee venom exposure produced more variable and gene-specific responses. While several genes showed minor and non-significant changes, Loc406103 and Loc413113 were significantly down-regulated ($P < 0.05$). This pattern suggests that antibiotic treatment elicited a broad immunosuppressive transcriptional response, whereas bee venom affected a narrower subset of immune-related genes.

3.2.2 Dose-dependent effects of antibiotics

Gene expression profiles across different oxytetracycline concentrations are summarized in Fig. 3. A non-linear dose-response pattern was observed, with the most pronounced transcriptional

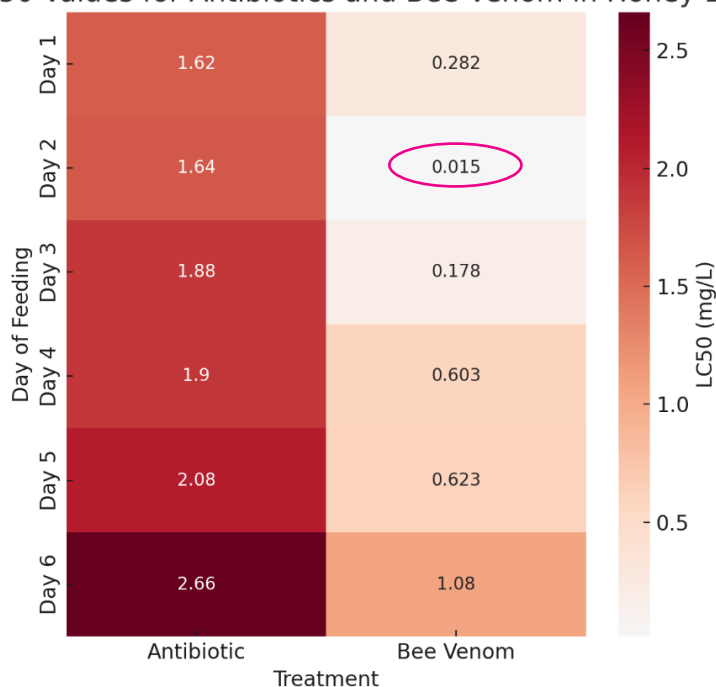
Heatmap of LC₅₀ Values for Antibiotics and Bee Venom in Honey Bee Larvae

Fig. 1. Heatmap of LC₅₀ values (mg/L) for antibiotics and bee venom across six feeding days in *Apis mellifera* larvae. Red indicates higher LC₅₀ values, while blue indicates lower LC₅₀ values. LC₅₀ estimates were obtained by probit analysis and are presented with 95% confidence intervals.

Heatmap of Gene Expression in Larvae Treated with Antibiotics and Bee Venom

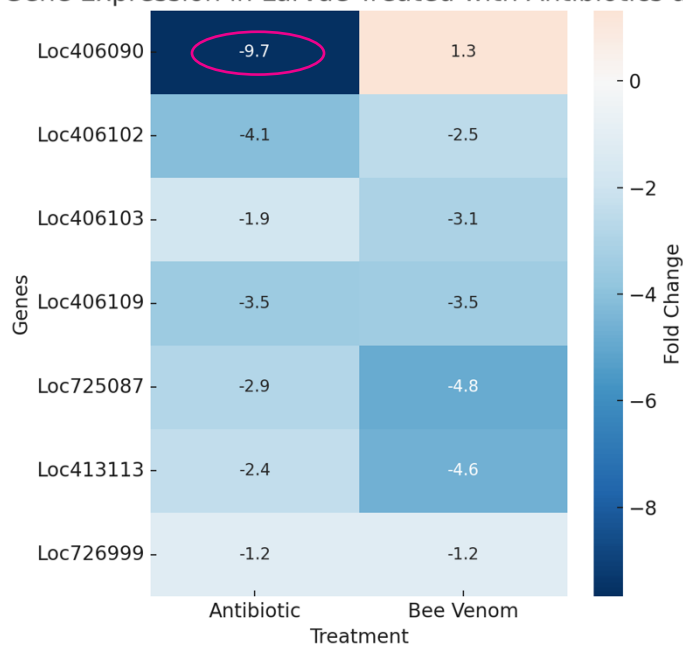


Fig. 2. Heatmap of immune-related gene expression (fold regulation) in *Apis mellifera* larvae treated with antibiotics and bee venom under *in vitro* conditions. Red indicates up-regulation and blue indicates down-regulation relative to the control group ($2^{-\Delta\Delta Ct}$ method). Antibiotic treatment caused strong down-regulation across most genes, with the most pronounced effect in *Loc406090* (-9.67 fold, $P < 0.05$), whereas bee venom significantly down-regulated *Loc406103* and *Loc413113* ($P < 0.05$).

suppression occurring at the lowest tested concentration (0.0001 mg/L). At this dose, *Loc406090* (-1175 fold) and *Loc413113* (-514 fold) exhibited marked down-regulation.

Although these fold-change values are numerically large, they primarily reflect strong relative differences arising from low baseline expression levels and $\Delta\Delta Ct$ -based normalization. To facilitate interpretation, gene expression patterns were additionally visualized using standardized heatmaps and log-scaled color gradients. At

higher concentrations (0.01–0.1 mg/L), gene expression changes were generally attenuated, and in some cases mild up-regulation was observed, suggesting partial transcriptional compensation.

3.2.3 Dose-dependent effects of bee venom

The transcriptional responses of larvae to increasing bee venom doses are shown in Fig. 4. At the highest concentration (0.1 mg/L), *Loc406090*

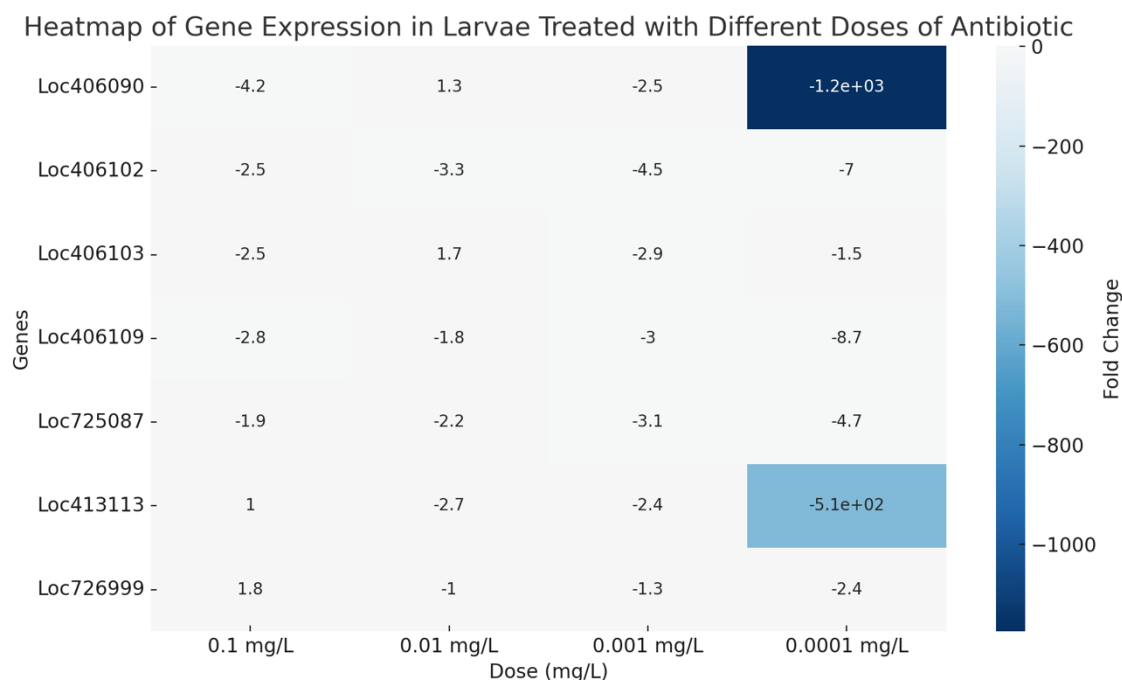


Fig. 3. Heatmap of fold regulation values for immune-related genes in *Apis mellifera* larvae treated with different doses of antibiotic under *in vitro* conditions. Red indicates up-regulation and blue indicates down-regulation relative to the control group ($2^{-\Delta\Delta C_t}$ method). Strong down-regulation was observed at the lowest tested dose (0.0001 mg/L), especially in *Loc406090* (−1175 fold) and *Loc413113* (−514 fold). Moderate effects were seen at higher doses, where some genes such as *Loc406090* and *Loc406103* showed mild up-regulation at 0.01 mg/L.

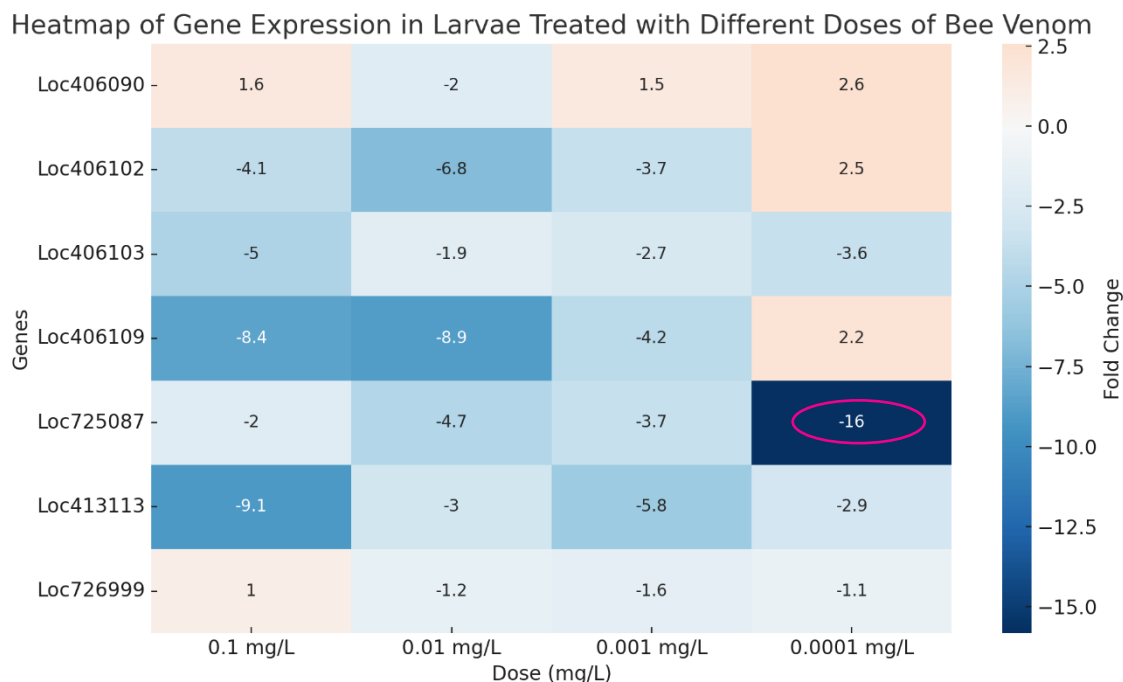


Fig. 4. Heatmap of immune-related gene expression (fold regulation) in *Apis mellifera* larvae treated with different doses of bee venom under *in vitro* conditions. Red indicates up-regulation and blue indicates down-regulation relative to the control group ($2^{-\Delta\Delta C_t}$ method). At 0.1 mg/L, mild up-regulation was observed in *Loc406090* (+1.58 fold) and *Loc726999* (+1.04 fold, n.s.), whereas strong down-regulation occurred in *Loc406103* (−4.97 fold, $P < 0.05$) and *Loc413113* (−9.10 fold, $P < 0.05$).

and *Loc726999* exhibited mild, non-significant up-regulation, whereas *Loc406103* (−4.97 fold) and *Loc413113* (−9.10 fold) were significantly down-regulated ($P < 0.05$). Intermediate doses (0.01–0.001 mg/L) produced mixed expression patterns, with alternating up- and down-regulation depending on the gene.

Overall, bee venom induced dose-sensitive and gene-specific transcriptional responses, with fewer extreme fold changes compared to oxytetracycline exposure at low concentrations. This suggests that bee

venom modulates immune gene expression in a more targeted manner under *in vitro* conditions.

3.3 Immune-related gene expression in pupae

T Gene expression patterns in pupae exposed to bee venom are presented in Figs. 5 and 6. At the highest concentration tested (0.1 mg/L), all analyzed genes were strongly up-regulated, with particularly high

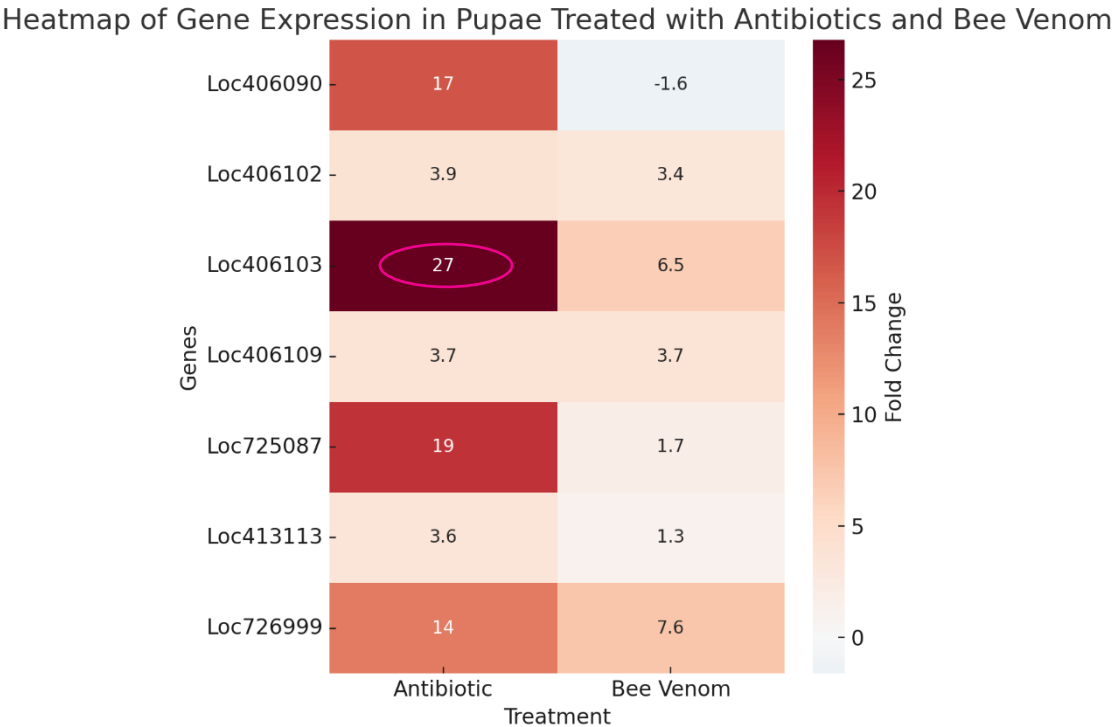


Fig. 5. Heatmap of immune-related gene expression (fold regulation) in *Apis mellifera* pupae treated with different doses of bee venom under *in vitro* conditions. Red indicates up-regulation and blue indicates down-regulation relative to the control group ($2^{-\Delta\Delta Ct}$ method). At 0.1 mg/L, all genes were strongly up-regulated, particularly *Loc725087*, *Loc406103*, and *Loc726999*.

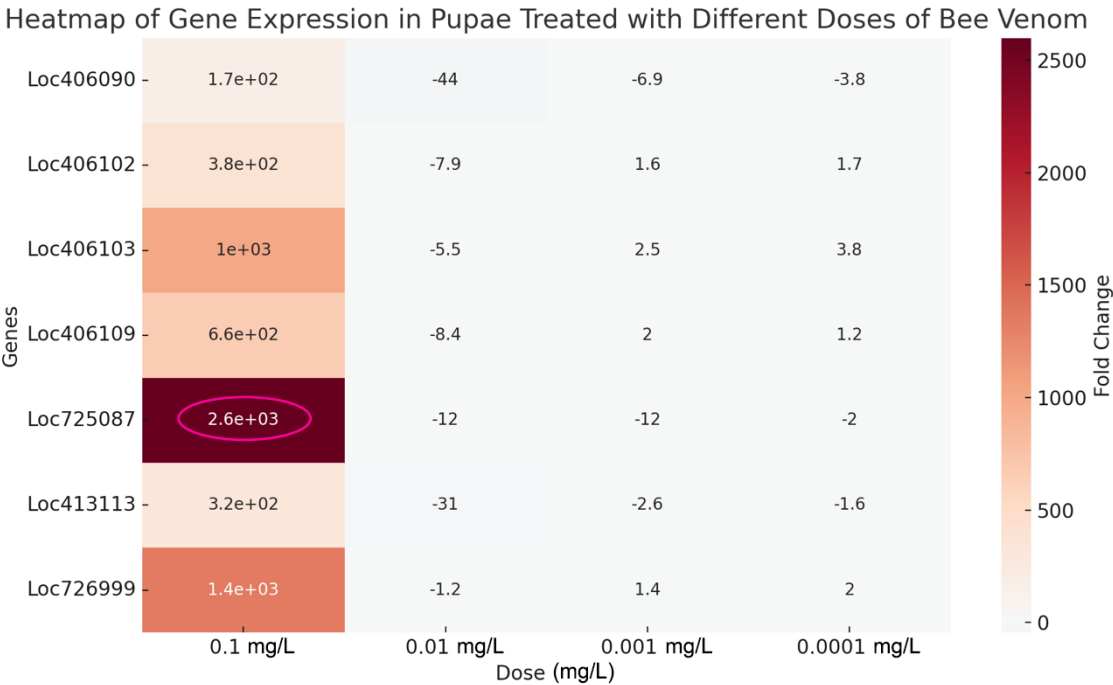


Fig. 6. Heatmap of immune-related gene expression (fold regulation) in *Apis mellifera* pupae across different doses of bee venom under *in vitro* conditions. Red indicates up-regulation and blue indicates down-regulation relative to the control group ($2^{-\Delta\Delta Ct}$ method). At 0.01 mg/L, down-regulation was dominant, especially in *Loc406090* and *Loc413113*, while lower doses (0.001–0.0001 mg/L) produced mixed patterns.

fold regulation observed for *Loc725087* (+2599 fold), *Loc406103* (+1004 fold), and *Loc726999* (+1354 fold). These large fold-change values likely reflect heightened transcriptional responsiveness during the pupal stage, where developmental remodeling and stress sensitivity are increased.

At 0.01 mg/L, most genes were down-regulated, with *Loc406090* (–44.42 fold) and *Loc413113* (–30.52 fold) showing the strongest suppression. Lower concentrations (0.001–0.0001 mg/L) produced

mixed responses, characterized by modest up-regulation in some loci and down-regulation in others.

Collectively, these results indicate that bee venom exerts stage-specific and dose-dependent effects on immune-related gene expression, with pupae displaying greater transcriptional variability than larvae. The observed patterns emphasize relative regulatory shifts rather than absolute expression levels.

4. Discussion

The present study provides a comparative evaluation of the toxicological and immunological effects of oxytetracycline and bee venom on honey bee (*Apis mellifera*) larvae and pupae reared under *in vitro* conditions. While antibiotics remain a common tool for controlling bacterial diseases in apiculture, their use is increasingly questioned due to concerns related to microbiota disruption, antimicrobial resistance, and unintended physiological effects (Powell *et al.*, 2021; Raymann *et al.*, 2017). In contrast, bee venom has been proposed as a biologically derived alternative with reported antimicrobial and immunomodulatory properties (Son *et al.*, 2007). Our results highlight that both treatments exert distinct and context-dependent effects, emphasizing the need for careful interpretation of toxicity and immune-related gene expression data.

4.1 Toxicological implications

The LC₅₀ analysis demonstrated that bee venom exhibited lower LC₅₀ values than oxytetracycline throughout the six-day exposure period, indicating higher acute toxicity under *in vitro* conditions. These LC₅₀ estimates, derived from probit analysis and accompanied by 95% confidence intervals, reflect short-term exposure in a controlled experimental system and should not be directly extrapolated to field-level exposure scenarios. The higher toxicity of bee venom is likely associated with its major bioactive components, particularly melittin and phospholipase A₂, which are known to disrupt cellular membranes and induce cytotoxic effects at elevated concentrations (Nakajima *et al.*, 1986).

By contrast, oxytetracycline exhibited higher LC₅₀ values, suggesting lower acute toxicity in larvae. However, lower acute toxicity does not necessarily imply biological safety, as antibiotics may exert chronic and sublethal effects that are not captured by LC₅₀ metrics alone. Previous studies have shown that antibiotic exposure can disrupt gut microbial communities and compromise immune competence in honey bees (Raymann *et al.*, 2017; Li *et al.*, 2017). Therefore, toxicity assessments should be interpreted alongside molecular and immunological endpoints, as performed in the present study.

4.2 Immune-related gene expression in larvae

Gene expression analyses revealed that oxytetracycline exposure resulted in a broad down-regulation of immune-related genes in larvae. This generalized suppression suggests that antibiotic exposure may interfere with innate immune regulation rather than targeting specific immune pathways. Similar transcriptional suppression of antimicrobial peptides and immune signaling genes has been reported following antibiotic treatment in honey bees and other insects (Li *et al.*, 2017; Sarvari *et al.*, 2020).

In contrast, bee venom exposure induced more gene-specific transcriptional responses, with significant down-regulation observed primarily for Loc406103 and Loc413113. While these genes are annotated as immune-related, their precise functional roles in honey bee immunity remain incompletely characterized, and therefore mechanistic interpretations should be made cautiously. Rather than indicating immune enhancement or suppression *per se*, the observed changes likely reflect stress-responsive modulation of transcription in response to venom components.

Notably, extreme fold-change values observed at low oxytetracycline concentrations (e.g., -1175 fold) raised concerns regarding biological plausibility. These values should be interpreted as relative transcriptional shifts derived from $\Delta\Delta C_t$ -based normalization, which can amplify apparent fold changes when baseline expression levels are low. Accordingly, the present study emphasizes the direction and consistency of gene regulation rather than absolute fold magnitude, an approach commonly adopted in comparative gene expression studies.

4.3 Stage-specific responses in pupae

Pupal gene expression analyses revealed markedly different response patterns compared to larvae. At high bee venom concentrations,

most immune-related genes were strongly up-regulated, whereas intermediate concentrations resulted in pronounced down-regulation. This stage-specific divergence likely reflects developmental differences in metabolic activity, tissue remodeling, and stress sensitivity between larvae and pupae.

The strong transcriptional activation observed at the highest dose may represent a generalized stress or compensatory response rather than targeted immune stimulation. Given that pupal development involves extensive cellular reorganization, transcriptional variability during this stage may be inherently higher, potentially amplifying fold-change estimates. Similar stage-dependent sensitivity to xenobiotics has been reported in other insect systems (Yokoyama *et al.*, 2019), supporting the notion that developmental context is critical when interpreting toxicogenomic data.

4.4 Biological relevance and practical considerations

Collectively, the results indicate a fundamental trade-off between the two treatments. Antibiotics such as oxytetracycline exhibited lower acute toxicity but induced broad suppression of immune-related gene expression, which may increase vulnerability to pathogens and compromise long-term colony health. Bee venom, on the other hand, displayed higher acute toxicity but elicited more selective and stage-dependent transcriptional responses.

While bee venom is often discussed as a potential alternative to antibiotics, the present findings suggest that its application would require careful dose optimization and strict consideration of developmental stage. The narrow margin between sublethal and toxic concentrations underscores the importance of cautious interpretation and further validation. At present, bee venom should not be viewed as a direct replacement for antibiotics, but rather as a candidate for exploratory research into complementary or integrative disease management strategies.

4.5 Limitations and future directions

This study was conducted under *in vitro* conditions, which allow controlled manipulation of exposure variables but do not fully capture the complexity of colony-level interactions, such as social immunity, microbiota exchange, and environmental stressors (Tang *et al.*, 2025). Accordingly, the observed gene expression patterns should be interpreted as indicative rather than definitive predictors of in-hive responses.

Furthermore, the functional roles of several analyzed loci (e.g., Loc406090, Loc413113) remain poorly resolved. Future studies integrating functional genomics approaches, such as RNA-seq or targeted gene knockdown, alongside microbiota profiling, would be valuable for elucidating the mechanistic basis of the observed transcriptional responses. Field-based trials will ultimately be required to determine whether the immunological effects observed under *in vitro* conditions translate into meaningful improvements in colony resilience.

5. Conclusions

This study provides a comparative evaluation of the toxicological and immunological effects of oxytetracycline and bee venom on *Apis mellifera* larvae reared under *in vitro* conditions. The results demonstrate that oxytetracycline, while exhibiting lower acute toxicity based on LC₅₀ estimates, induced a broad and consistent down-regulation of immune-related genes, suggesting a potential risk of immunosuppression during early developmental stages. Such transcriptional suppression may contribute to increased vulnerability to pathogens, particularly when antibiotic exposure occurs repeatedly or at sublethal levels.

In contrast, bee venom exhibited higher acute toxicity, as reflected by lower LC₅₀ values with associated confidence intervals, but elicited more selective, dose-dependent, and stage-specific effects on immune gene expression. Larval responses were characterized mainly by gene-specific down-regulation, whereas pupal stages displayed pronounced transcriptional variability, including strong up-regulation at higher doses. These findings indicate that bee venom modulates immune-

related transcription in a complex manner that depends on both concentration and developmental context.

Taken together, the present results highlight a fundamental trade-off between reduced acute toxicity and broader immunosuppressive effects in antibiotics, versus higher toxicity but more targeted transcriptional modulation in bee venom. Importantly, these outcomes were observed under controlled *in vitro* conditions and should therefore be interpreted within the limitations of this experimental system.

Rather than positioning bee venom as a direct replacement for antibiotics, this study underscores the need for cautious, evidence-based evaluation of bee-derived products as potential complementary tools in apiculture. Future research should focus on optimizing dose ranges, integrating functional and microbiome-based analyses, and validating these findings under field-relevant conditions to determine whether such approaches can contribute to sustainable and microbiota-friendly disease management strategies in honey bee colonies.

CRediT authorship contribution statement

Dilek Kabakcı: Designed and planned the study, created the dataset, was responsible for writing the article and interpreting the analyses.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

The data sets used and/or analyzed in the current study are available on request from the corresponding author.

Declaration of generative AI and AI-assisted technologies in the writing process

The authors confirm that there was no use of artificial intelligence (AI)-assisted technology for assisting in the writing or editing of the manuscript and no images were manipulated using AI.

Ethics approval

As non cephalopod invertebrates honey bee not subject to animal ethic approval at Republic of Turkey (ased on the 'Regulation on the Welfare and protection of Animal Used for Exsperimental and Other Scientific Purpose' published in the official Gazette dated December 13.2011 and numbered 28141).

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Supplementary data

Supplementary material to this article can be found online at https://dx.doi.org/10.25259/JKSUS_1832_2025.

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