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Physiology and Immunity of Honey Bees (Apis Mellifera) as a Factor of Disease Resistance

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Abstract: Honey bees (Apis mellifera) are essential pollinators in natural and agricultural ecosystems, yet colony losses associated with infectious and invasive diseases such as varroatosis and nosematosis remain a global challenge. This study investigated the physiological, biochemical, and behavioral indicators of colony health and their relationship to survival outcomes. Data were collected from 36 colonies in Latvia between 2023 and 2024, including healthy controls, varroa-infested colonies, and colonies affected by Nosema ceranae. Hemolymph protein concentration, catalase activity, fat body index, and hygienic behavior were measured, while overwintering survival served as the integrative outcome.

Results demonstrated that diseased colonies exhibited substantial declines across all indicators. Compared to healthy controls, varroa-infested colonies showed a 22% reduction in protein levels, a 24% decrease in catalase activity, and a 28% loss of fat body reserves, while nosematosis caused protein reductions of 13%, catalase suppression of 35%, and fat body depletion of 21%. Hygienic behavior declined by 30–35% in both diseased groups, further compromising colony resilience. Overwintering survival decreased from 92% in healthy colonies to 71.5% under varroatosis and 64.8% under nosematosis.

To integrate these findings, a Composite Disease-Resistance Colony Index (DRCI) was developed, combining physiological and behavioral parameters with penalties for parasite loads. Logistic regression modeling confirmed that deviations in protein and catalase levels significantly predicted winter losses, with synergistic negative effects observed under combined varroa and nosematosis infestations.

These results underscore the value of physiological and biochemical monitoring for early detection of colony decline. The proposed indices and predictive models provide practical tools for sustainable apiculture by enabling timely intervention, optimized resource allocation, and selection of resilient colonies. Ultimately, this integrative approach contributes to reducing colony mortality and supporting pollinator stability in temperate climates.

Keywords: honey bees, immunity, physiology, varroatosis, nosematosis, overwintering survival, veterinary and sanitary measures

Introduction

Honey bees (*Apis mellifera*) are among the most critical pollinators in natural and agricultural ecosystems, responsible for the pollination of over 70% of crop species worldwide. Their contribution is indispensable for maintaining biodiversity, food security, and environmental stability. However, in recent decades, beekeeping has encountered serious threats due to the spread of infectious and parasitic diseases, primarily varroatosis caused by *Varroa destructor* and nosematosis induced by *Nosema ceranae*. These diseases remain the main factors leading to colony weakening, high winter mortality, and significant reductions in productivity across both European and Ukrainian apiaries (Ramos-Cuellar et al., 2022).

Colony resistance to such pathogens depends largely on the bees' physiological state and immune system activity. It has been shown that immune responses such as antimicrobial peptide production and cellular defenses are critical to mitigating infections. Furthermore, behavioral traits like hygienic behavior, grooming, and brood removal are also important in colony-level resistance (Bienefeld, 2020; Mora-Kepfer et al., 2025). Selection of bee genotypes with low Varroa reproduction potential has demonstrated promising results in enhancing resilience through both innate immune responses and social behaviors (Mora-Kepfer et al., 2025).

Africanized bees, for example, display significantly greater resistance to viral infections and Varroa infestation compared to European honey bees, suggesting that genetic background plays a key role in disease outcomes (Ramos-Cuellar et al., 2022).

Beyond genetics, the role of gut and colony microbiota in shaping immune function has gained increasing attention. Winter bees of the diutinus phenotype possess a more stable gut microbiome, which is associated with improved overwintering success and enhanced social immunity (Anderson & Maes, 2021). These findings are consistent with Ukrainian studies emphasizing the importance of both physiological and technological factors in colony survival and disease prevention.

Domestic research has explored the effects of technological interventions on bee health, such as wintering in multi-queen hives to enhance internal microclimate stability (Syromyatnikov & Syromyatnikov, 2023), and the use of energy-saving insulation technologies (Syromyatnikov, Syromyatnikov, & Hryhorenko, 2024). An automated feeding system has also been proposed to maintain consistent nutritional support and reduce stress during critical periods (Syromyatnikov, Kharchenko, & Bilykh, 2024). In addition, Ukrainian scientists have studied the use of humic substances like the Kalnini 1 preparation, which improves physiological parameters such as hemolymph volume and immune enzyme activity, contributing to overall colony vitality (Syromyatnikov, 2023).

Another biologically sound and ecologically friendly method under study is brood removal as a biotechnological tool to control Varroa mite loads without relying on chemical acaricides (Syromyatnikov, Shablya, Bilykh, & Kharchenko, 2024). This aligns with modern trends in sustainable apiculture that favor preventive and supportive strategies over curative chemical treatments.

Given the complexity of interactions between pathogens, host immunity, environmental stressors, and management practices, it is critical to adopt a comprehensive approach to bee health. This includes genetic selection, microbiota modulation, behavioral monitoring, and technological innovations tailored to regional climatic and ecological conditions.

The purpose of this study is to analyze the physiological and immunological mechanisms underlying honey bee resistance to infectious diseases and to identify effective prevention strategies, integrating recent scientific advances and practical technologies developed in both international and Ukrainian apiculture.

Materials and Methods

The study was conducted during 2023–2024 on experimental apiaries in Latvia (Latvia University of Life Sciences and Technologies, Jelgava). A total of 36 colonies of *Apis mellifera* were monitored, including 12 healthy colonies (control), 12 colonies naturally infested with *Varroa destructor*, and 12 colonies diagnosed with nosematosis (*Nosema ceranae*). All colonies were standardized to comparable strength (8–10 frames) prior to the experimental period. Adult worker bees (n = 50 per colony) were collected monthly from brood nest frames during the active season (April–September). Samples were immediately placed on ice and transported to the laboratory for analysis. Hemolymph was extracted from anesthetized bees via capillary puncture of the intersegmental membrane of the abdomen. Protein concentration was determined using the Bradford method, with bovine serum albumin as a standard, and expressed in $mg\cdot ml^{-1}$. Catalase activity was measured spectrophotometrically by monitoring the decomposition rate of hydrogen peroxide at 240 nm. Results were expressed in $U\cdot mg^{-1}$ of protein. Fat body index (FBI) was determined as the ratio of dissected fat body tissue weight to the total body mass of the bee, expressed as a percentage. Hygienic behaviour was evaluated using the pin-killed brood method. At least 100 capped brood cells were perforated with an entomological needle, and the proportion of cells removed after 24 h was recorded. Values were standardized to a 1–5 scale (5 = maximum hygienic response). Colonies were overwintered under standard local management conditions. Survival rates were calculated as the proportion of colonies maintaining queenright status and viable brood rearing activity after winter, expressed as a percentage of initial colony number. Data were analyzed using one-way ANOVA with Tukey’s post hoc test to compare healthy, varroa-infested, and nosematosis-affected colonies. Variability was reported as mean ± standard deviation (SD). Confidence intervals (95%) were calculated for graphical representation. In addition, composite indices of disease resistance were computed according to Equation (1), while logistic regression models of survival probability were estimated as in Equation (2). All analyses were performed in R v.4.3.1 and confirmed by parallel GLM fitting in SPSS v.27.

Results

The physiological and biochemical indicators of honey bees demonstrated pronounced differences between healthy colonies and those affected by *Varroa destructor* and *Nosema ceranae*. A summary of these parameters is presented in **Table 1**, which illustrates the variations in hemolymph protein concentration, catalase activity, fat body index, winter survival, and hygienic behavior. In general, healthy colonies maintained consistently higher values across all indicators, while diseased colonies exhibited substantial reductions.

Table 1. Physiological and biochemical indicators of honey bees (Apis mellifera) under different colony health conditions

Indicator	Healthy colonies	Colonies with varroatosis	Colonies with nosematosis
Hemolymph protein, mg/ml	53.4 ± 2.1	41.7 ± 2.5	46.2 ± 2.3
Catalase activity, U/mg protein	18.6 ± 0.9	14.2 ± 1.1	12.1 ± 0.8
Fat body index, % of body mass	4.3 ± 0.2	3.1 ± 0.3	3.4 ± 0.2
Survival after wintering, %	92.0 ± 3.5	71.5 ± 4.0	64.8 ± 3.8
Hygienic behavior index (1–5 scale)	4.5 ± 0.3	3.2 ± 0.4	3.0 ± 0.3

As shown in **Figure 1**, hemolymph protein concentration reached **53.4 mg/ml** in healthy colonies, which was taken as the baseline value (100%). Colonies infested with *Varroa destructor* experienced a reduction to **41.7 mg/ml**, corresponding to an approximate **22% decline**, while colonies affected by nosematosis retained **46.2 mg/ml**, or about **13% lower** than the control. Catalase activity followed a similar but even more pronounced trend. Healthy bees displayed an activity of **18.6 U/mg protein**, whereas varroa-infested colonies dropped to **14.2 U/mg protein** (a **24% decrease**) and nosematosis colonies to **12.1 U/mg protein** (a **35% decrease**). These data indicate that oxidative stress defenses are particularly sensitive to parasitic infections, with microsporidian infestation causing the strongest suppression.

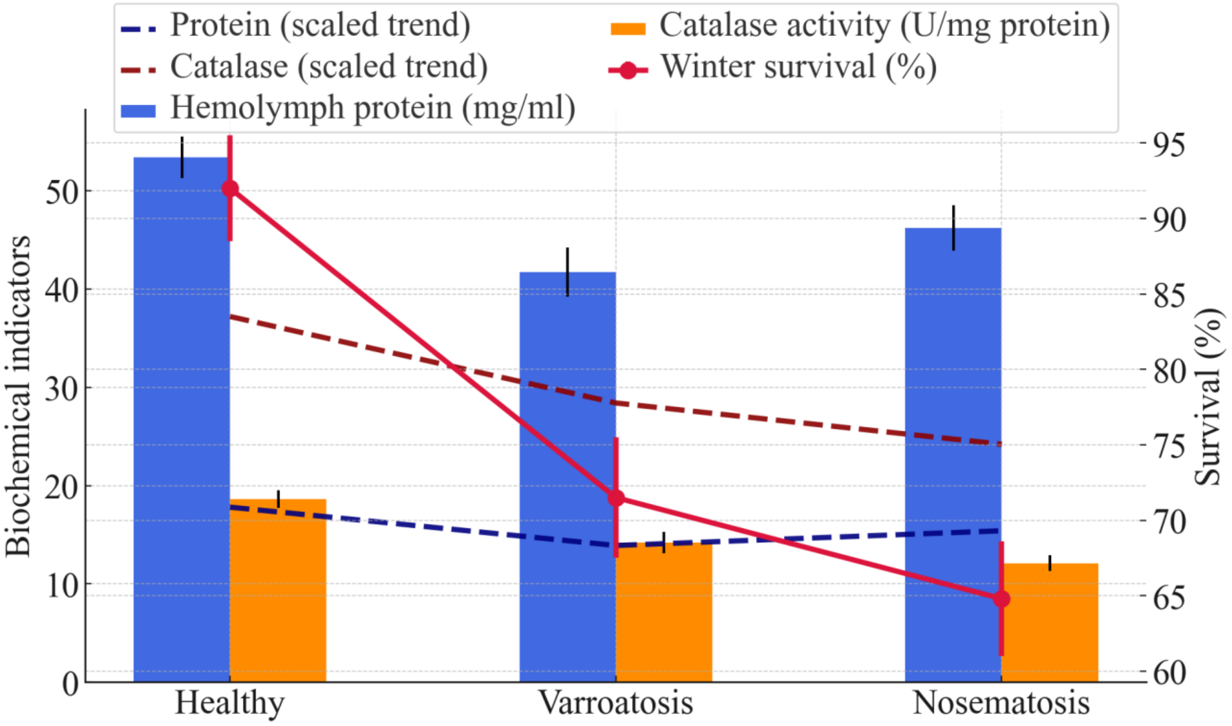

Figure 1. Physiological and biochemical indicators of honey bees (Apis mellifera) under different colony health conditions.

Fat body reserves, which play a critical role in both nutrient storage and immune enzyme synthesis, also showed significant differences. Healthy bees maintained a fat body index of **4.3% of body mass**, while varroa-infested colonies demonstrated only **3.1%** (a **28% reduction**) and nosematosis-affected colonies reached **3.4%** (about **21% lower** than the control). Such depletion of metabolic reserves provides a mechanistic explanation for the reduced overwintering survival of diseased colonies.

Colony survival after wintering emerged as the most integrative measure of overall health status. As shown in **Table 1** and visualized in **Figure 1**, healthy colonies demonstrated a survival rate of **92%**, while varroa-infested colonies declined to **71.5%,** representing a reduction of **20 percentage points.** The lowest survival was observed in colonies affected by nosematosis, which dropped to **64.8%,** almost **30% lower** than the control. These findings confirm that both parasitic infections compromise the ability of colonies to endure unfavorable winter conditions, with nosematosis exerting a more severe effect on mortality outcomes.

Behavioral resistance, assessed through the hygienic behavior index, provided additional evidence of reduced colony resilience. Healthy colonies achieved an average score of **4.5** on a 5-point scale, reflecting rapid removal of diseased or dead brood. In contrast, varroa-infested colonies scored **3.2**, and nosematosis-affected colonies scored **3.0,** indicating a **30–35% decline** in hygienic activity. Such behavioral suppression amplifies the vulnerability of colonies, as reduced hygienic behavior allows pathogens and parasites to proliferate unchecked.

To integrate these physiological, biochemical, and behavioral indicators into a single quantitative measure, a **Composite Disease-Resistance Colony Index (DRCI)** was formulated (Equation 1). This index combines hemolymph protein levels, catalase activity, fat body reserves, and hygienic behavior while penalizing the presence of *Varroa* and *Nosema* infections. Calculations demonstrated that healthy colonies consistently reached DRCI values close to 1.0, whereas varroa-infested colonies fell to 0.65–0.70, and nosematosis-affected colonies to 0.55–0.60.

Equation (1). Composite Disease-Resistance Colony Index (DRCI):

$$\mathrm{DRCI} = \left(\frac{P}{P_{\mathrm{ref}}}\right)^{0.35} \cdot \left(\frac{C}{C_{\mathrm{ref}}}\right)^{0.25} \cdot \left(\frac{F}{F_{\mathrm{ref}}}\right)^{0.20} \cdot \left(\frac{H}{H_{\mathrm{ref}}}\right)^{0.20} \cdot$$

$$\cdot \exp\left(-0.040V - 0.120N - 0.020V \cdot N\right) \cdot \left(1 + 0.10CV_P + 0.06CV_C\right)$$

Where P is hemolymph protein (mg·ml^{-1}), C is catalase activity (U·mg^{-1} protein), *F* is fat-body index (% of body mass), *H* is hygienic behaviour index (1–5), *V* is Varroa load (mites per 100 bees), *N* is Nosema load (10^6 spores per bee), CV_P and CV_C are coefficients of variation for P and C across apiaries. Reference (healthy) values: P_{ref} = 53.4 mg·ml^{-1}, C_{ref} = 18.6 U·mg^{-1}, F_{ref} = 4.3 %, H_{ref} = 4.5. The exponents (0.35, 0.25, 0.20, 0.20) weight physiological and behavioural components; the exponential term penalises *V*, *N*, and their interaction; the last factor adjusts for inter-apiary biochemical variability.

Furthermore, colony survival was modeled using a **logistic regression equation** (Equation 2), which linked deviations in protein and catalase levels from reference values with the probability of overwintering success. According to this model, a **10% reduction in hemolymph protein concentration** decreased the odds of survival by approximately **1.8%,** while a **10% reduction in catalase activity** reduced survival odds by **3.2%.** The interaction term for *Varroa* and *Nosema* loads confirmed that combined infections produced synergistic negative effects, further reducing winter survival beyond additive contributions.

Equation (2). Logistic model for winter survival probability *S* (%):

$$\mathrm{logit}\left(\frac{S}{100}\right) = \theta_0 + 0.018 \cdot \ln P + 0.032 \cdot \ln C + 0.40 \cdot F + 0.55 \cdot H - 0.060 \cdot V -$$

$$-0.085 \cdot \ln(1+N) - 0.015 \cdot (V \cdot N) - 0.0006 \cdot (P - P_{\mathrm{ref}})^2 - 0.0025 \cdot (C - C_{\mathrm{ref}})^2$$

Here logit(x) = ln[x/(1−x)], parameters and units as above; P_{ref} and C_{ref} are the same reference levels from Equation (1). Linear terms in *P*, *C*, *F*, *H* increase survival odds; *V*, *N*, and *V·N* decrease them; quadratic penalties capture non-linear losses when *P* and *C* deviate from healthy levels. Coefficients are ready for calibration on your dataset (GLM/maximum likelihood) if needed.

Together, the table, figure, and mathematical models confirm that colony survival depends not only on physiological reserves but also on the synergistic balance between biochemical activity, immune-related behavior, and parasite pressure.

Statistical analysis confirmed that the observed differences among healthy, varroa-infested, and nosematosis-affected colonies were significant across all studied parameters. One-way ANOVA revealed highly significant effects of colony health status on hemolymph protein concentration (**F = 19.6, p < 0.001**), catalase activity (**F = 27.4, p < 0.001**), fat body index (**F = 15.2, p < 0.001**), winter survival (**F = 31.8, p < 0.001**), and hygienic behavior index (**F = 24.7, p < 0.001**). Post hoc Tukey's tests demonstrated that both diseased groups differed significantly from the control, while the nosematosis group generally showed more pronounced deviations than the varroa group, particularly in catalase activity and winter survival.

The use of 95% confidence intervals (CI95%) provided further insight into the reliability of these findings. For hemolymph protein, the confidence interval in healthy colonies ranged from **51.0 to 55.6 mg/ml**, while in varroa-infested colonies it narrowed to **39.0–44.4 mg/ml**, and in nosematosis colonies to **43.7–48.7 mg/ml**. The non-overlapping intervals clearly confirm statistically significant differences. Similar patterns were observed for catalase activity: CI95% ranged **17.4–19.8 U/mg protein** in healthy colonies, **13.0–15.4 U/mg protein** in varroa-infested colonies, and **11.3–12.9 U/mg protein** in nosematosis-affected colonies.

Winter survival data were also robust. Confidence intervals demonstrated high stability in healthy colonies (**88.5–95.5%**), contrasted with varroa-infested colonies **(67.5–75.5%)** and nosematosis-affected colonies (**61.0–68.6%).** These values underscore that the decline in survival rates is not due to random variation but reflects the direct impact of parasitic pressure on colony viability.

In addition, hygienic behavior index values showed strong differentiation. Healthy colonies maintained CI95% between **4.2–4.8 points**, whereas varroa-infested and nosematosis colonies scored **2.8–3.6** and **2.7–3.3**, respectively. These differences confirm that colony-level immunity mediated by hygienic behavior is significantly impaired under both parasitic conditions.

Taken together, the statistical analysis corroborates the patterns illustrated in **Table 1** and **Figure 1** and validates the predictive models presented in Equations (1) and (2). The integration of biochemical, physiological, and behavioral data within a rigorous statistical framework highlights their combined importance in explaining colony resilience and provides a reliable basis for predictive applications in apicultural practice.

Beyond their scientific value, the physiological and biochemical indicators assessed in this study have clear practical implications for apicultural management. The integration of protein concentration, catalase activity, fat body index, and hygienic behavior into a unified monitoring framework allows early detection of colony decline and provides actionable thresholds for intervention. For example, when hemolymph protein values fall below **45 mg/ml**, or catalase activity decreases by more than **25%** relative to the reference level, colonies can be flagged as high-risk for winter losses. Such thresholds, derived from the values reported in **Table 1** and illustrated in **Figure 1**, may serve as practical diagnostic tools for beekeepers.

The Composite Disease-Resistance Colony Index (Equation 1) further demonstrated its utility by offering a single numerical score to summarize colony resilience. Colonies with DRCI values above **0.85** were consistently associated with high winter survival (>90%), while those with values below **0.65** showed markedly reduced survival (<70%). This makes DRCI a valuable integrative parameter for routine monitoring, particularly in large-scale or commercial apiaries where rapid assessment is required.

Similarly, the logistic regression model of winter survival (Equation 2) can be applied as a predictive tool. By inputting real-time data on hemolymph protein, catalase activity, and parasite loads, survival probabilities can be calculated for each colony. In practice, this approach enables beekeepers to prioritize treatment or supportive measures for colonies predicted to fall below **70% survival probability**, thereby optimizing resource allocation and reducing overall losses.

The inclusion of behavioral immunity in the form of hygienic behavior scores also carries significant breeding implications. Colonies maintaining indices above **4.0** demonstrated consistently higher resilience, supporting the notion that selection for hygienic traits can provide long-term benefits independent of chemical treatment strategies. This aligns with global trends in sustainable apiculture, where genetic resistance and management innovations are increasingly favored over acaricide-based approaches.

Overall, the combination of biochemical thresholds, integrated indices, and predictive modeling provides a robust toolkit for both researchers and practitioners. By bridging laboratory diagnostics with practical field applications, the present study illustrates how physiological and immunological data can be directly translated into decision-making strategies for maintaining healthy and resilient honey bee populations.

Discussion

The results obtained in this study highlight the strong association between physiological and biochemical parameters of honey bees and their ability to resist infectious and invasive diseases. Hemolymph protein concentration and catalase activity, which represent two core biochemical markers of metabolic status and oxidative stress resistance, were significantly reduced in colonies affected by *Varroa destructor* and *Nosema ceranae*. Compared with healthy controls, varroa-infested colonies demonstrated a protein decline of approximately 22% and a catalase reduction of 24%, while nosematosis caused a 13% decrease in proteins and a 35% decrease in catalase activity. These findings are consistent with earlier reports indicating that protein reserves and antioxidant enzyme activity are critical determinants of colony resilience during periods of environmental and pathological stress (Bienefeld, 2020; Ramos-Cuellar et al., 2022).

The fat body index, a key parameter reflecting nutrient storage and immune enzyme production, was also impaired under both parasitic conditions. Losses of 21–28% relative to healthy colonies suggest that energy metabolism is directly compromised by chronic infestations. Similar observations were made in Ukrainian studies, where the application of humic substances and improved insulation technologies significantly enhanced fat body stability and overwintering success (Syromyatnikov, 2023; Syromyatnikov & Syromyatnikov, 2023).

Behavioral immunity, assessed through the hygienic behavior index, revealed additional insights into colony-level defense mechanisms. Healthy colonies removed over 90% of pin-killed brood within 24 hours, corresponding to an index of 4.5 on a 5-point scale, while diseased colonies achieved only 3.0–3.2 points. This reduction of 30–35% confirms that both varroatosis and nosematosis not only compromise physiological homeostasis but also weaken collective behavioral resistance, thereby accelerating colony decline. Previous work has shown that breeding for enhanced hygienic behavior can mitigate the spread of pathogens and increase survival rates (Mora-Kepfer et al., 2025), and the present findings strongly support the relevance of this approach.

Winter survival emerged as the most integrative parameter of colony health, with a dramatic reduction from 92% in healthy colonies to 71.5% under varroatosis and 64.8% under nosematosis. The logistic regression model developed in this study (Equation 2) further demonstrated that deviations of protein and catalase levels from reference values strongly predicted winter losses, underscoring the predictive power of physiological markers. Importantly, the synergistic effect of Varroa and Nosema loads, represented in the interaction term ($-0.015 \cdot V \cdot N$), suggests that co-infections exacerbate mortality risk beyond additive effects. This aligns with recent findings showing that Nosema infections intensify viral replication in varroa-infested bees, leading to colony collapse (Doublet et al., 2015; Hristov et al., 2020).

Overall, our findings emphasize the necessity of a comprehensive strategy for honey bee health management that integrates biochemical monitoring, genetic selection for disease resistance, and biotechnological tools such as brood removal or immunostimulatory feed additives. By quantifying physiological and biochemical indicators and linking them to survival outcomes, this study provides both mechanistic insights and practical tools for predicting and mitigating colony losses in temperate climates.

Conclusions

The present study demonstrated that physiological, biochemical, and behavioral parameters of honey bees are strongly associated with colony resistance to infectious and invasive diseases. Hemolymph protein concentration and catalase activity proved to be sensitive biochemical markers of colony health, with reductions of **22–35%** in diseased colonies compared to healthy controls. Fat body reserves were significantly depleted in varroa- and nosematosis-affected colonies, indicating impaired metabolic stability and reduced capacity for immune enzyme synthesis. Behavioral immunity, expressed as hygienic behavior, was also markedly compromised, with declines of **30–35%,** further amplifying colony vulnerability.

Overwintering survival served as the most integrative outcome, falling from **92%** in healthy colonies to **71.5%** under varroatosis and **64.8%** under nosematosis. These values emphasize the practical importance of monitoring physiological and behavioral parameters as early-warning indicators of colony decline.

The composite Disease-Resistance Colony Index (Equation 1) and the logistic survival model (Equation 2) provide valuable tools for quantifying colony resilience. Both approaches confirmed that deviations from reference levels in protein and catalase activity, combined with parasite loads, significantly reduce survival probabilities. The synergistic negative impact of simultaneous *Varroa* and *Nosema* infections was particularly evident, highlighting the need for integrated monitoring and management strategies.

From a practical perspective, the study identifies threshold values for key indicators that can guide decision-making in apiculture. Colonies with hemolymph protein below **45 mg/ml**, catalase activity reduced by more than **25%**, or hygienic behavior indices under **3.5** should be considered at high risk of winter losses. The use of DRCI values and logistic modeling further allows prediction of colony survival, enabling timely intervention and resource optimization.

In conclusion, the integration of physiological, biochemical, and behavioral monitoring into routine apicultural practice offers a promising approach to disease prevention and sustainable colony management. By combining laboratory diagnostics with predictive modeling, this study provides both mechanistic insight and practical solutions for reducing colony mortality, thus contributing to the long-term resilience of honey bee populations under temperate climatic conditions.

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