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Research Article

Evaluation of Sub-Lethal Residual Effects of Bifenthrin on Health Biomarkers and Performance of *Apis mellifera* L.

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ABSTRACT

Apis mellifera L. face growing threats from sub-lethal pesticide exposure that disrupts their physiology and behavior, endangering colony survival and pollination efficiency. The present study evaluated the residual sub-lethal effects of bifenthrin on key health biomarkers, social interactions, and foraging behavior of worker honey bees under laboratory and semi-field conditions. Bees were exposed to four bifenthrin treatment levels along with a control group. Phenoloxidase (PO) activity, lifespan, antennation, trophallaxis, and foraging behavior were assessed using standard protocols and subjected to statistical analysis. PO activity was highest in the control group (26.64 ± 1.11 U/mg tissue) and lowest in the T1 treatment (8.90 ± 0.59 U/mg tissue). Lifespan declined from 43.00 ± 0.58 days (control) to 34.33 ± 0.33 days (T1). Similarly, antennation and trophallaxis frequencies decreased from 65.00 ± 4.93 and 45.00 ± 4.04 bees/event (control) to 37.00 ± 3.46 and 21.33 ± 3.18 bees/event (T1), respectively. Foraging activity, including incoming and pollen-carrying bees, also declined significantly, from 349.00 ± 17.58 to 237.00 ± 11.59 bees/5 min and from 121.67 ± 6.12 to 76.33 ± 5.93 bees/5 min, respectively. Strong positive correlations were observed among behavioral and physiological parameters, indicating their interdependence in maintaining colony performance. These findings demonstrate that residual sub-lethal exposure to bifenthrin significantly impairs immunity, longevity, social interaction, and foraging efficiency in *A. mellifera*, posing serious risks to colony health. Future studies should clarify the mechanisms of these impairments, employ diverse biomarkers, and develop pollinator-safe pest management strategies.

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Introduction

Honey bees (*Apis mellifera* L.) face a complex combination of interacting stressors, including

pathogens, parasites, inadequate nutrition, and pesticide exposure, all of which contribute to colony decline and collapse (Harwood and Dolezal, 2020). Among these,

sub-lethal exposure to insecticides, particularly neonicotinoids, organophosphates, and pyrethroids, impairs behavior, cognition, immunity, and longevity without causing immediate mortality (Bartling et al., 2024). Colony Collapse Disorder (CCD), characterized by worker bees abandoning the hive, has frequently been associated with such pesticide exposure (Quarles, 2008). Bifenthrin, a synthetic pyrethroid widely used in agriculture, acts by disrupting sodium channel function in the insect nervous system (Gammon et al., 2019; Bhardwaj et al., 2020). Due to its high chemical stability, bifenthrin is persistent in the environment and may remain active in soil, on plant surfaces, and within pollen and nectar long after application. This persistence results in potential residue exposure to pollinators (Ramasubramanian and Paramasivam, 2021). Bees can transport these residues into hives through contaminated nectar and pollen, leading to the contamination of wax and stored food (Sanchez-Bayo and Goka, 2014; Calatayud-Vernich et al., 2018). Although such exposure seldom causes immediate mortality, chronic sub-lethal exposure disrupts neurological and behavioral functions, affecting learning, foraging efficiency, brood care, and social coordination, ultimately reducing overall colony fitness (Wu et al., 2011; Adamczyk et al., 2025).

Health biomarkers provide an effective means to detect early or subtle effects of pesticide exposure before visible colony-level damage occurs (Badiou-Bénéteau et al., 2012; Adamczyk et al., 2025). Measurement of these biomarkers offers a sensitive indicator of sub-lethal toxic effects that might be overlooked by mortality-based assessments alone (Di Noi, 2023). The use of biomarkers enhances understanding of the impacts of chronic pesticide exposure on individual and colony health, thereby improving risk assessment and guiding intervention strategies (Adamczyk et al., 2025).

Phenoloxidase (PO) is an essential enzyme in the insect immune system that mediates melanization and encapsulation of pathogens. Its expression level serves as a key indicator of immune competence (Marieshwari et al., 2023). Exposure to sub-lethal doses of pesticides has been shown to inhibit PO activity in honey bees, rendering colonies more susceptible to infections (Bartling et al., 2024). Therefore, PO activity is considered a sensitive biomarker of immune suppression caused by chemical stressors (Kunat-Budzyńska et al., 2025).

Chronic insecticide exposure has also been associated

with behavioral impairments such as reduced foraging success, disrupted communication dances, and decreased worker lifespan (Sugumaran et al., 2024). Monitoring both longevity and biomarker responses provides a more comprehensive understanding of the long-term effects of sub-lethal exposures on colony survival (Calatayud-Vernich et al., 2018). Behavioral metrics directly link stress to colony performance, as shortened worker lifespans compromise colony resilience and productivity (Karsai and Wenzel, 1998). Long-term exposure to insecticides, even at sub-lethal concentrations, has repeatedly been linked to reduced lifespan through physiological stress, metabolic disruption, and cumulative cellular damage (Sugumaran et al., 2024).

Although the adverse effects of insecticides on honey bees are increasingly being investigated, the full range of sub-lethal consequences resulting from chronic exposure to contaminated wax, nectar, and pollen remains insufficiently explored. This gap limits our understanding of the impacts of residual pesticides (Sanchez-Bayo and Goka, 2014; Di Noi et al., 2021; Khoury et al., 2025) and constrains accurate assessment of bifenthrin's long-term risks under field conditions (Tosi et al., 2021; Phan et al., 2025).

Therefore, the present study aimed to evaluate the sub-lethal effects of residual bifenthrin exposure on the health and performance of *A. mellifera* by assessing key biomarkers such as PO activity, along with colony-level parameters including foraging activity, social interactions, and worker longevity. It was hypothesized that even residual bifenthrin levels would impair immune function, social behavior, and lifespan in worker bees. To comprehensively evaluate these effects, the study integrated physiological, behavioral, and molecular analyses. The findings are expected to contribute to the development of more sustainable frameworks for pesticide risk assessment and the protection of pollinator populations in agricultural ecosystems.

Materials and Methods

Experimental setup

To investigate the sub-lethal residual effects of bifenthrin on honey bees, four sub-lethal concentration levels and a control were prepared using the commercial formulation Bifenthrin 10 EC (Talstar®) (Balakrishnan et al., 2009). Serial dilutions of the recommended field dose (50 ml per 500 m²; equivalent to 1000 ml/ha) (Balakrishnan et al., 2009) were used to obtain the sub-

lethal treatments: one-half (T1 = 25 ml), one-quarter (T2 = 12.5 ml), one-tenth (T3 = 6.25 ml), and one-twentieth (T4 = 2.5 ml) of the field dose. A control treatment (T0) without any insecticide application was also included, making a total of five treatments.

To simulate oral exposure through contaminated nectar and pollen, sunflower (*Helianthus annuus* cv. Hysun-33) plants were grown to the flowering stage in five distinct field plots, each covering an area of 500 m² (Shurjeel et al., 2020). The plots were spatially separated by 3 km to minimize bee foraging overlap (Abou-Shaara, 2014; Ahmad et al., 2025). The bifenthrin solutions were applied to the respective plots using a knapsack sprayer, following standard field application protocols (Hussain et al., 2010). Spraying was carried out in the early morning (7:00-8:30 a.m.) under calm wind conditions (temperature 28 ± 2°C; relative humidity 60 ± 5%).

The experiment involved fifteen healthy *A. mellifera* colonies, each with an approximately equal population of 15,000 bees per hive. Colonies were randomly assigned to the five field plots (three colonies per treatment) 24 h after insecticide application to prevent direct contact exposure and ensure only residual exposure (Sanchez-Bayo and Goka, 2014).

Immune function (Phenoloxidase activity)

To assess PO activity as an immune biomarker, fifteen dead worker honey bees were randomly selected from each treatment hive (five bees per replicate) following the method of Islam et al. (2024). The thoraxes of the collected bees were dissected and prepared for biochemical analysis according to the procedure described by Saffari et al. (2010). Sampling for PO activity was conducted seven days after colony exposure to the treated plots to capture sub-lethal, residual contact, and oral exposure effects.

Thoracic tissues were homogenized, and 20 µl of the resulting supernatant was transferred into a cuvette. Subsequently, 505 µl of phosphate saline buffer (pH 7.4) and 675 µl of Milli-Q® water were added, and the mixture was gently mixed (López-Urbe et al., 2020). The solution was incubated at 37°C for 5 min. Following incubation, 300 µl of L-DOPA (L-3,4-dihydroxyphenylalanine) solution (2 mg/ml) was added as a substrate, and the change in absorbance was measured at 490 nm using a spectrophotometer at two time intervals: 0 and 10 min, as described by Islam et al. (2024).

Foraging activity

To assess the effects of various sub-lethal residual

exposures on the foraging activity of honey bees, field observations were conducted under stable weather conditions (temperature: 28-32°C; relative humidity: 60-70%). Observations were made twice weekly over an eight-week period. The parameters measured included the number of outgoing bees (Beekman et al., 2004), returning bees, and returning bees carrying pollen on their legs (Islam et al., 2024). Data were collected through visual inspection of the hives, with each observation lasting 5 min (Pernal and Currie, 2001). Observations were carried out between 8:00 a.m. and 10:00 a.m., a period known to correspond with peak foraging activity (Joshi and Joshi, 2010). This procedure also allowed for a uniform assessment of behavioral changes across treatment groups throughout the experimental period.

Social interaction

The social interactions within honey bee colonies following exposure to residual insecticide effects were evaluated by observing behaviors such as trophallaxis and antennation among worker bees. To record these interactions with high resolution and minimal disturbance to the colony, a high-definition GoPro Hero (CHDRB101-CN) camera was mounted on a selected frame inside the hive. A non-invasive, low-intensity light source was strategically positioned to provide adequate illumination without altering the bees' natural behavior or inducing stress (Goyret and Farina, 2003). The recorded videos were subsequently analyzed to quantify the duration of trophallaxis and the frequency of antennation among workers. Significant differences in social behavior were detected between colonies exposed to varying insecticide concentrations and the control colonies (Islam et al., 2024).

Worker bee longevity

When adult honey bees emerged from the brood cells, they were marked with a permanent color marker (POSCA PC-5M) to monitor their longevity, following the method described by Shurjeel et al. (2020). The exact time and date of emergence were recorded. A total of 15 newly emerged bees were marked each day for three consecutive days in each colony, using different colors on different days to distinguish between cohorts. To assess longevity, colonies were inspected daily at 10:00 a.m., and data were recorded for sixty days until no marked bees were observed (Islam et al., 2025). The collected data on worker bee longevity were then tabulated and analyzed following the procedure described by Manzoor et al. (2025).

Statistical analysis

Data on foraging activities were analyzed using a Randomized Complete Block Design whereas a Completely Randomized Design was applied for parameters related to social interactions. PO activity data were subjected to analysis of variance (ANOVA), followed by Duncan's post hoc test at the 0.01 significance level. In contrast, comparisons of foraging activity were conducted using the Least Significant Difference (LSD) post hoc test at the 0.05 significance level. All statistical analyses were performed in R software (R Core Team, version 4.2.2). The ANOVA and LSD tests were computed using the agricolae package. Plots and graphs, including treatment comparison plots, were generated using ggplot2 and the tidyverse package. Correlation analyses were conducted using the corrplot, RColorBrewer, and tibble packages within the same R environment.

Results

PO Activity

A statistically significant difference in PO activity was observed among the treatment groups [$F(4, 70) = 84.5$, $P < 0.001$]. The control group exhibited the highest PO activity (26.64 ± 1.11 U/mg tissue), indicating a strong baseline immune response under untreated conditions. Enzyme activity showed a progressive decline across the treated groups: T4 demonstrated a moderate reduction (17.55 ± 0.63 U/mg tissue), followed by T3 (14.12 ± 0.66 U/mg tissue) and T2 (11.89 ± 0.57 U/mg tissue). The lowest PO activity was recorded in T1 (8.90 ± 0.59 U/mg tissue), suggesting a pronounced inhibitory effect of the treatment on honey bee immune function. These trends are illustrated in Figure 1.

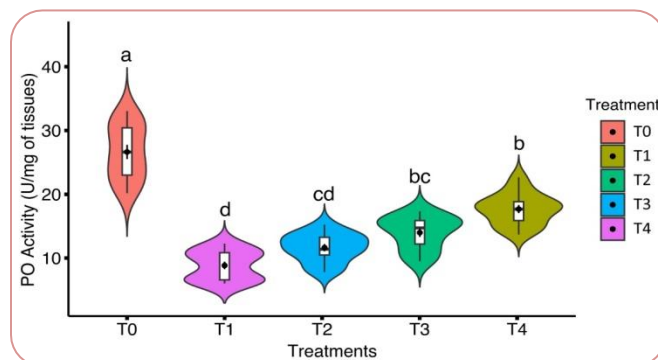


Figure 1. PO activity (U/mg tissue) in honey bees across different treatment groups. Data are presented as mean $\pm$ SE. Bars with different letters indicate statistically significant differences according to the LSD test at $P < 0.001$.

Adult bee lifespan

A statistically significant difference was observed in the lifespan of adult worker bees among the treatment groups [$F(4, 8) = 142.43$, $P < 0.05$], indicating a strong effect of chemical exposure on bee longevity. The control group (T0) exhibited the longest mean lifespan (43.00 ± 0.58 days), which was significantly higher than that of all treated groups. Bees in treatment T4 had the next highest lifespan (37.33 ± 0.33 days), followed by T3 (36.67 ± 0.33 days) and T2 (35.67 ± 0.33 days). The shortest lifespan was recorded in T1 (34.33 ± 0.33 days), suggesting that this treatment exerted the most detrimental impact on worker bee longevity, as illustrated in Figure 2.

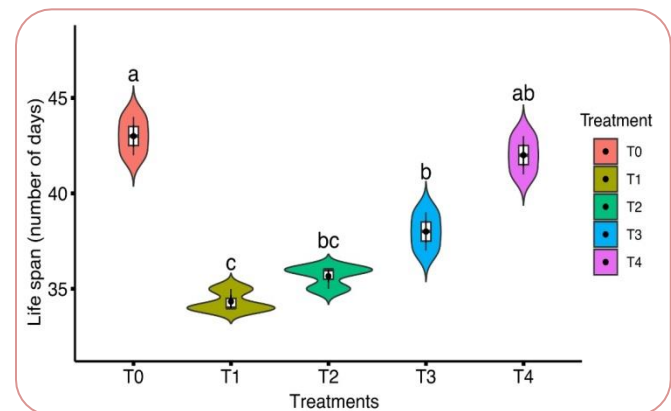


Figure 2. Effect of different treatments on the lifespan of worker bees (mean $\pm$ SE). Bars represent mean $\pm$ SE values. Columns with different letters indicate statistically significant differences according to the LSD test at $P < 0.05$.

Social interactions

A statistically significant difference was observed in antennation behavior among bees subjected to different treatments under laboratory conditions [$F(4, 10) = 8.35$, $P < 0.01$]. Bees in the control group (T0) exhibited the highest antennation frequency (65.00 ± 4.93 bees/event), indicating more active social interactions compared to the treated groups. In contrast, all treatment groups (T1-T4) showed a significant reduction in antennation behavior, with T1 bees displaying the lowest response (37.00 ± 3.46 bees/event), suggesting a suppressive effect of the treatments on antennal communication. No significant differences were detected among T1, T2, T3, and T4, which together formed a single homogeneous group, as illustrated in Figure 3.

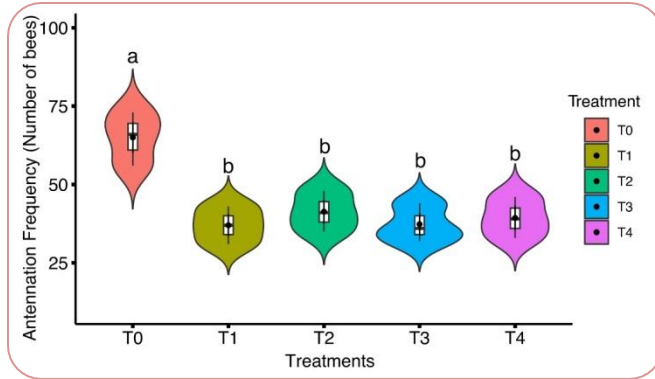


Figure 3. Effect of different treatments on the antennation behavior (Mean $\pm$ SE) of worker bees. Bars sharing the same letter are not significantly different according to the LSD test at $P < 0.05$.

The data indicate that the number of bees engaged in trophallaxis events varied significantly among the treatments. The control group (T0) exhibited the highest trophallaxis activity, with a mean of 45.00 ± 4.04 bees/event, reflecting enhanced social interaction in the absence of insecticide exposure. In contrast, the lowest number of trophallaxis events was observed in T1 (21.33 ± 3.18 bees/event), followed by T2 (23.33 ± 3.18 bees/event) and T3 (25.67 ± 3.76 bees/event), whereas T4 showed slightly higher activity (27.00 ± 4.16 bees/event). Statistical analysis (ANOVA) revealed a significant difference among treatments [$F(4,10) = 6.63, P = 0.0071$]. Further comparison using the LSD test showed that the control (T0) differed significantly from all insecticide-exposed treatments (T1-T4), which did not differ significantly from each other. These results suggest that sub-lethal exposure to insecticide residues suppresses trophallaxis behavior in worker bees, as shown in Figure 4.

Foraging efforts

The foraging activity of honey bees, including the number of incoming, outgoing, and pollen-carrying bees,

was significantly influenced by different treatments. The number of incoming bees was highest in the control group (T0) with a mean of 349.00 ± 17.58 bees per 5 min, followed by T4 (285.67 ± 13.92 bees per 5 min), while the lowest count was recorded in T1 (237.00 ± 11.59 bees per 5 min). The differences among treatments were statistically significant [$F(3, 44) = 250.69; P < 0.05$].

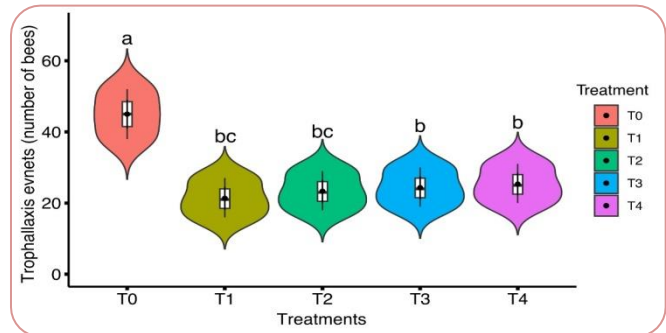


Figure 4. Effect of different treatments on trophallaxis behavior (mean $\pm$ SE) of worker bees. Bars sharing the same letter are not significantly different according to the LSD test at $P < 0.05$.

Similarly, the number of outgoing bees differed significantly among treatments [$F(3, 44) = 276.89; P < 0.05$], with the highest mean observed in the control group (325.00 ± 19.63 bees per 5 min), followed by T4 (250.00 ± 13.80 bees per 5 min), and the lowest in T1 (177.00 ± 13.32 bees per 5 min).

In the case of pollen-carrying bees, the control group (T0) again recorded the highest mean count (121.67 ± 6.12 bees per 5 min), followed by T4 (96.00 ± 4.58 bees per 5 min), whereas T1 showed the lowest value (76.33 ± 5.93 bees per 5 min). These differences were also statistically significant [$F(3, 44) = 118.54; P < 0.05$]. A comparative analysis of the mean values across treatments is presented in Table 1.

Table 1. Effect of different treatments on foraging activity parameters (mean $\pm$ SE) of worker bees, including incoming, outgoing, and pollen-carrying individuals.

Treatments	Incoming bees (Mean $\pm$ SE)	Outgoing bees (Mean $\pm$ SE)	Containing pollens (Mean $\pm$ SE)
T0	349.00 ± 17.58^a	325.00 ± 19.63^a	121.67 ± 6.12^a
T1	237.00 ± 11.59^e	177.00 ± 13.32^e	76.33 ± 5.93^d
T2	272.33 ± 11.26^c	191.00 ± 13.89^d	83.00 ± 6.08^c
T3	261.67 ± 13.59^d	225.00 ± 12.49^c	79.67 ± 7.62^{cd}
T4	285.67 ± 13.92^b	250.00 ± 13.80^b	96.00 ± 4.58^b
F (statistics)	250.69	276.89	118.54
P (value)	<0.05	<0.05	<0.05

Lower case letters in the end of mean $\pm$ SE are showing the significantly different according to LSD test at $P < 0.05$.

Correlation analysis of behavioral and physiological parameters

The Pearson correlation matrix (Figure 5) illustrates the strength and direction of linear relationships among various behavioral and physiological traits of honey bee workers, including lifespan, foraging activity, and social behaviors. The strongest positive correlation was observed between antennation and trophallaxis ($r = 0.99$), indicating an almost perfect linear relationship. This finding suggests that increased antennal interactions are closely associated with proportional increases in food-sharing activity. Both behaviors represent fundamental components of social cohesion in *A. mellifera*, and their strong interdependence may reflect shared regulatory mechanisms or similar responses to environmental stressors.

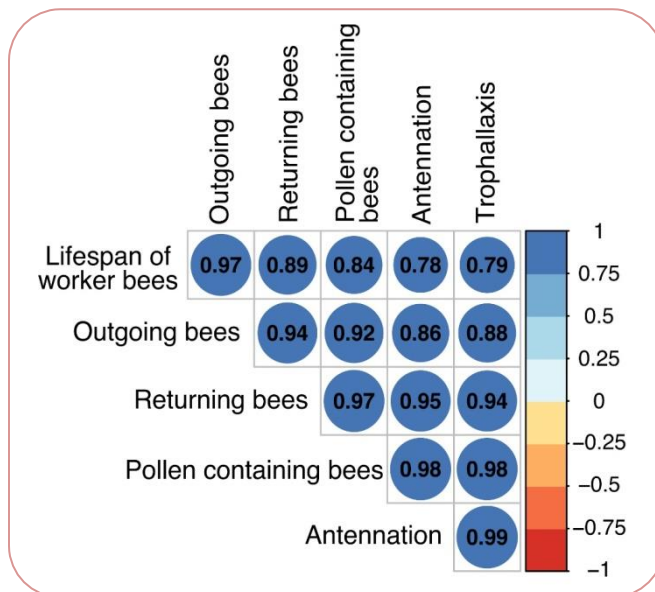


Figure 5. Correlation matrix plot showing pairwise Pearson correlation coefficients among the measured variables. Blue circles indicate positive correlations, while red circles represent negative correlations. The size and color intensity of each circle correspond to the strength of the correlation. Statistically significant correlations ($p < 0.05$) are indicated with asterisks.

Furthermore, worker lifespan was found to be highly correlated with the number of outgoing bees ($r = 0.97$), returning bees ($r = 0.89$), and pollen-collecting bees ($r = 0.84$), implying that colonies with longer-lived workers tend to exhibit greater foraging activity. Overall, the correlations were remarkably strong ($r > 0.78$), reflecting a tightly interconnected behavioral network.

These statistically significant relationships (presumably $p < 0.001$) highlight the potential of these parameters as reliable bioindicators of colony health. In particular, disruptions in key social behaviors such as trophallaxis and antennation could serve as early warning signs of declining colony vitality.

Discussion

This study provides clear evidence that residual, sub-lethal exposure to insecticides and varying treatment intensities significantly impair honey bee immune function, social behavior, foraging efficiency, and lifespan. These findings contribute to the growing body of literature emphasizing the multifaceted impacts of agrochemical stressors on pollinator health. Nevertheless, as in most field-based experiments, potential confounding variables, such as colony-scale variability, microclimatic differences, and nutritional inequalities among foraging plots, may have influenced the results and should be considered when interpreting the outcomes of the current study.

PO activity differed significantly among treatments. In the control group (26.6 ± 1.11 U/mg tissue), where no toxic stress was present, immune competence remained strong. PO activity showed a decreasing trend with increasing treatment intensity, reaching its lowest level in T1 (8.90 ± 0.59 U/mg tissue). This indicates a pronounced immunosuppressive effect, particularly under higher exposure levels. These findings are consistent with those of González-Santoyo and Córdoba-Aguilar (2012), who reported that PO is highly sensitive to environmental stressors and plays a central role in insect defense mechanisms. Similarly, Brandt et al. (2016) demonstrated that chronic exposure to neonicotinoids significantly reduces immune enzyme activities, thereby increasing susceptibility to pathogens. Although colony strength and age demographics were standardized, minor differences in immune responses could have arisen from natural genetic variability and pre-exposure colony health disparities.

A similar negative trend was observed in the lifespan of worker bees. The control group survived the longest (33.33 ± 0.33 days), whereas T1-treated bees had significantly shorter lifespans (25.00 ± 0.58 days). The reduced longevity observed under T1 and T2 treatments likely reflects increased physiological stress and metabolic trade-offs associated with detoxification processes. These findings are consistent with those of

Tosi et al. (2017), who reported that repeated exposure to sub-lethal pesticide doses markedly reduces worker lifespan, thereby compromising colony stability and resilience. Likewise, Wu et al. (2011) found that pesticide residues in stored pollen (beebread) were associated with reduced worker longevity and impaired brood development.

Insecticide exposure also disrupted social interactions, as evidenced by reduced antennation and trophallaxis frequencies. Bees in the control group exhibited the highest antennation frequency (65.00 ± 4.93 bees/event), whereas all treatment groups (T1-T4) showed significantly lower values, with T1 being the lowest (37.00 ± 3.46 bees/event). Similarly, trophallaxis was highest in the control group (45.00 ± 4.04 bees/event) and declined markedly among treated groups, particularly T1 (21.33 ± 3.18 bees/event). These observations align with previous reports indicating that sub-lethal pesticide exposure impairs communication and social behavior. For example, Graeff-Filho et al. (2025) and Williamson et al. (2014) demonstrated that antennation and trophallaxis rates in *A. mellifera* decreased following exposure to contact or oral pyrethroids and neonicotinoids, suggesting neurotoxic effects on sensory and motor coordination. Since these behaviors are essential for communication and food sharing (Naug, 2008), their disruption indicates weakened colony cohesion. Notably, there were no substantial differences among treated groups, suggesting that even the lowest level of exposure was sufficient to impair social cohesion.

Foraging activity was also reduced across all treatments in terms of incoming, outgoing, and pollen-carrying bees. The T1 group recorded the lowest foraging activity (e.g., 237.00 ± 11.59 bees/5 min for incoming bees), whereas the control group showed the highest (349.00 ± 17.58 bees/5 min). The consistent decline in these variables indicates organizational disruption in foraging motivation or capacity, likely due to neurotoxic effects of insecticides. Similar results were reported by Henry et al. (2012), who found that pesticide exposure impaired navigation and foraging behavior, potentially leading to nutritional deficiencies and compromised brood development. Likewise, Decourtye et al. (2004) showed that sub-lethal doses of imidacloprid impaired forager orientation and reduced the probability of returning to the hive, thereby weakening colony function. However, differences among plots due to environmental heterogeneity might also have

contributed to variability in foraging performance, underscoring the need for future studies to incorporate landscape-level standardization.

Correlation analysis revealed strong positive interrelationships between physiological and behavioral parameters. The near-perfect correlation between antennation and trophallaxis ($r = 0.99$) suggests that these social behaviors may be co-regulated or simultaneously disrupted by external stressors. Furthermore, strong positive correlations between lifespan and foraging parameters (Outgoing: $r = 0.97$; Returning: $r = 0.89$; Pollen: $r = 0.84$) indicate that longer-lived workers contribute more effectively to colony productivity. These associations highlight the importance of evaluating colony health holistically, as multiple indicators jointly reflect ecological stress. Further research involving larger sample sizes and multi-site replication is recommended to disentangle insecticide-specific effects from environmental variability and to better elucidate the sub-lethal impacts of bifenthrin on honey bee health.

Conclusion

The present study revealed that low-level residual exposure to bifenthrin causes severe deterioration in PO activity and performance indicators, namely social behavior, foraging efficiency, and worker bee longevity, in *A. mellifera*. Phenoloxidase activity was significantly reduced in all pesticide-exposed groups compared to the control, providing clear evidence of immune suppression under chemical stress. Worker longevity was also markedly decreased in treated colonies, indicating the physiological cost associated with detoxification and stress response mechanisms.

Antennation and trophallaxis behaviors were consistently impaired across all treated groups, with the greatest impairment observed in colonies exposed to higher bifenthrin concentrations. Similarly, foraging activity, including incoming, outgoing, and pollen-carrying bees, followed a comparable declining trend. The combined reduction in foraging efficiency and disruption of social communication suggests a compromised capacity of colonies to collect resources and sustain brood development.

Correlation analysis demonstrated positive associations among social behavior, foraging activity, and worker longevity, highlighting the interdependence of these traits in maintaining colony strength and productivity.

Therefore, it can be concluded that bifenthrin, even at sub-lethal residual levels, exerts substantial detrimental effects on *A. mellifera*. Its application should thus be carefully regulated and minimized to safeguard pollinator health.

Future studies should focus on elucidating the molecular and neurophysiological mechanisms underlying these impairments and exploring the interactions between immune suppression and behavioral alterations under field conditions. Long-term, multi-season investigations incorporating diverse biomarkers would enhance pesticide risk assessment frameworks and promote pollinator-safe pest management strategies, thereby ensuring honey bee survival and sustainable pollination services.

Authors' Contributions

MUY, MAA, and SUI conceived and designed the study; MUY, RA, and SUI conducted the experiments; MHK and SUI analyzed the data and performed statistical analysis and visualizations; MUY and SUI prepared the original draft of the manuscript; MUY, MAA, RA, and MS critically reviewed and revised the manuscript; MS and WJ provided funding support and contributed to manuscript review; MAA provided resources and overall supervision; All authors contributed to manuscript revisions, approved the final version, and agreed to be accountable for the content.

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Conflict of Interest

The authors declare no conflict of interest.

Sustainable Development Goals Targeted

SDG 2: Zero Hunger

SDG 12: Responsible Consumption and Production

SDG 15: Life on Land

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