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Research Article

Assessment of the Trophic Effects of Selected Insecticides on Colony Strength and Honey Quality in *Apis mellifera* L.

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ABSTRACT

Honey bees (*Apis mellifera* L.), vital agricultural pollinators, face growing threats from insecticide exposure, highlighting the need to understand their trophic-level impacts for sustainable apiculture and crop production. The present study aimed to evaluate the effects of three insecticides viz., flonicamid, pyriproxyfen, and nitenpyram, on colony strength and honey quality. A sunflower crop was cultivated at four distinct locations, where twelve honey bee colonies were established (three per site). Each colony was either exposed to one of the insecticides or maintained as a pesticide-free control. Standardized protocols were employed to assess adult bee population, brood area, honey yield, and honey quality parameters. The lowest adult bee population ($11,874 \pm 512.95$ bees/colony), brood area (807.14 ± 19.65 cm²), and honey yield (3.44 ± 0.19 Kg/colony) were observed in pyriproxyfen-treated colonies. In contrast, control colonies exhibited the highest values for adult bee population ($20,499 \pm 487.31$ bees/colony), brood area (1297.8 ± 9.73 cm²), and honey yield (8.24 ± 0.22 kg/colony). Colonies exposed to nitenpyram showed intermediate effects across all measured parameters. A similar trend was observed in honey quality, with pyriproxyfen-treated colonies producing honey with the lowest enzymatic and antioxidant profiles, followed by flonicamid and nitenpyram. These findings reveal that insecticide exposure disrupts colony health and honey quality, emphasizing the need to avoid pyriproxyfen and flonicamid during crop bloom and to carefully time nitenpyram applications. Pollinator-safe pest management is essential for sustainable agriculture and food security.

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Introduction

Pollinators play a crucial role in the reproduction of wild plants, enhancement of agricultural productivity, food security, and overall ecosystem health (Requier et al.,

2023). Among them, honey bees (*Apis mellifera*), the most widely managed pollinators, are indispensable for both crop pollination and the production of commercially valuable hive products (Papa et al., 2022).

However, the global decline in honey bee populations poses a significant threat to the sustainability of these essential ecosystem services (Turley et al., 2022). This decline is attributed to multiple interacting factors, including habitat loss, monoculture practices, pathogens, parasites, pesticide exposure, and environmental pollution (Trinkl et al., 2020; Manzoor et al., 2025).

The health and productivity of *A. mellifera* colonies are largely determined by colony strength (Delaplane et al., 2013a). Key indicators of colony performance include adult bee population, brood area, and honey yield (Islam et al., 2024). Adult bees are critical for colony maintenance, foraging, brood care, and hive defense. Research has shown a significant positive correlation between colony size and honey yield; larger colonies (approximately 24,500 bees) tend to produce more honey than smaller ones (Kumari et al., 2023). Similarly, the brood area, the comb space occupied by developing bees, is a strong predictor of future colony productivity (Delaplane et al., 2013b; Singh et al., 2023); as it enables colonies to better utilize nectar flows, thereby increasing honey production.

The widespread use of insecticides in modern agriculture, particularly for managing phloem-feeding and lepidopteran pests, has become a cornerstone of pest control (Böhme et al., 2018). Active ingredients such as Flonicamid, Pymetrozine, and Emamectin benzoate disrupt target pests by inhibiting feeding, blocking stylet penetration, or inducing neurotoxic paralysis (Sarkar et al., 2022; Akram et al., 2024). However, sub-lethal exposure to these chemicals in honey bees has been associated with impaired olfactory learning, disoriented homing behavior, and reduced brood development, ultimately affecting colony strength indicators such as adult population, brood area, and honey yield (Goulson et al., 2015; Siviter et al., 2024).

Among exposure routes, trophic exposure is particularly insidious and biologically significant. This occurs when bees consume contaminated nectar, pollen, water, or guttation droplets (Sanchez-Bayo and Goka, 2014). Systemic insecticides like Flonicamid, Pymetrozine, and Emamectin benzoate are absorbed and translocated within plant tissues, eventually reaching floral resources collected by foragers. Once introduced into the hive, these residues permeate the colony's trophic network, affecting larvae, nurse bees, and the queen through chronic sub-lethal exposure (Sanchez-Bayo and Goka, 2014). This can disrupt larval development, queen

fecundity, and worker behavior, leading to reduced adult populations, diminished brood areas, and lower honey production (Tsvetkov et al., 2017). Field studies confirmed that long-term, low-level insecticide exposure can suppress immune function, impair neural processing, and reduce colony resilience (Wood et al., 2019).

Numerous studies have examined the trophic effects of insecticides on colony strength under varying field conditions. Neonicotinoids, organophosphates, and pyrethroids have been widely implicated. For instance, Imidacloprid and Thiamethoxam impair foraging behavior and thermoregulation, resulting in reduced brood development and honey yield (Tsvetkov et al., 2017; Wood et al., 2019). Organophosphates such as acephate and chlorpyrifos are highly toxic, significantly decreasing adult populations and brood areas (Ali et al., 2021). Pyrethroids like Lambda-cyhalothrin have also been associated with increased mortality and diminished foraging efficiency (Boily et al., 2013). Although laboratory and semi-field studies have provided mechanistic insights into insecticide toxicity, field-based evaluations are essential for understanding real-world exposure and trophic impacts.

This study aims to evaluate the trophic effects of three commonly used insecticides viz., Flonicamid, Pyriproxyfen, and Nitenpyram on key colony strength parameters of *A. mellifera* under field conditions, as well as their impact on honey quality under laboratory conditions. The central hypothesis is that these insecticides, through trophic transfer via foraging or trophallaxis, negatively affect colony performance by reducing adult bee population, brood development, and honey production, while also compromising honey quality. It is anticipated that the magnitude of sub-lethal and chronic toxicity will vary among the tested insecticides. Findings from this study will contribute to identifying pollinator-safe pest management strategies and provide a scientific basis for minimizing risks to honey bees through informed agrochemical use.

Materials and Methods

Experimental setup and insecticide treatments

The study was conducted at the honey bee research area of the Islamia University of Bahawalpur. Twelve healthy and vigorous colonies of *Apis mellifera*, each with an estimated population of 15,000 bees, were selected and divided into four treatment groups. To evaluate the impact of insecticide application on sunflower crops and

honey bee colonies, sunflower was sown at four different sites, each comprising a 500 m² block. Insecticide treatments were applied during the flowering stage at recommended doses following standard protocols, while control plots received no insecticide application.

To prevent overlap in foraging areas and minimize interference among colonies, experimental plots were separated by more than 3 km, exceeding the typical foraging range of honey bees (1.5-2.5 Km) as reported by Abou-Shaara (2014) and Steffan-Dewenter and Kuhn (2003). Furthermore, *A. mellifera* tends to forage preferentially on the most suitable and proximate floral resources (Seeley et al., 1991), supporting the decision to maintain adequate spatial separation between plots.

Three insecticide treatments were evaluated in this study to assess their impact on colony strength:

Treatment T1 = Ulala® (Flonicamid 50% DF), manufactured by ICI, applied at the recommended dose of 150 g/ha (Kodandaram et al., 2017).

Treatment T2 = Matoi® (Pyrifluquinazon 20% WG), a product of Jaffer, applied at 400 g/ha as recommended by Jahnavi et al. (2023).

Treatment T3 = Knockout® (Nitenpyram 25% SP), developed by Syngenta, applied at a dose of 247 g/ha according to Asif et al. (2016).

Treatment T0 = Control (no insecticide application) used as a baseline for comparison.

To facilitate foraging, three honey bee colonies were placed in each sunflower field starting from the 7th day after insecticide application. Data were collected to assess the trophic-level effects of these insecticides on key parameters of honey bee colony performance.

Adult bee population

The trophic effects of various insecticides on the adult bee population were evaluated under field conditions, following the method of Delaplane et al. (2013a), with modifications suitable for insecticide exposure assessment. All frames from each hive were inspected on both sides to assess bee coverage and colony strength. Comparisons were made among three insecticide-treated colonies and one untreated control colony. To estimate the adult bee population, a standard sample of 300 worker bees was collected from each treatment group and weighed to determine the average weight of an individual bee. Subsequently, each frame was weighed with bees present and then reweighed after the bees were gently brushed off. The difference in weight

was used to estimate the total number of adult bees, using the previously calculated average bee weight (Delaplane et al., 2013a; Guzman-Novoa et al., 2024).

Brood area measurement

The brood area was assessed on the central three frames from each replicate and treatment group using a modified grid method. The grid consisted of square units, each measuring 25 cm² (Amir and Peveling, 2004). The sealed brood area on each frame was measured and marked using the grid (Ismail et al., 2006). The arrangement of the grid over the brood frames created a pattern that allowed for accurate visualization and quantification of the brood-covered area. The number of empty cells was subtracted, and the average brood coverage was calculated (Tarpay et al., 2013; Pettis et al., 2016). The final brood area was determined by multiplying the number of brood frames by the average percentage of brood coverage per frame, following the methodology described by Lee et al. (2019).

Honey production

After the completion of the experiments, honey production data were collected to compare the output of the bee colonies. Honey was extracted from all colonies using a honey extractor, following standard honey extraction procedures. The production data were then subjected to statistical analysis (Aziz et al., 2015) to determine the effects of various insecticides on honey yield. This comparison aimed to evaluate the impact of different insecticidal treatments on the productivity of the bee colonies.

Honey quality

At the end of the experiment, honey was extracted, and 250 g samples were collected from all treatments, following the protocol of Islam et al. (2024). The analysis was conducted by separating the control and treatment groups. Honey samples stored at room temperature were used to assess pH, moisture, and sugar content, while enzymatic analyses were performed using samples maintained at elevated temperatures. All samples were stored at 4°C until analysis.

For pH determination, distilled water was mixed with the honey, and measurements were taken using a digital pH meter (Dr. Meter pH-100). Moisture content, diastase activity, pH, and ash content were determined according to the standard methods described by Bogdanov et al. (2002). Electrical conductivity, fructose content, total phenolic content, and oxygen radical absorbance capacity (ORAC) were measured using the standard

protocols outlined by Aazza et al. (2014). Flavonoid content was determined following the method described by Popova et al. (2005).

Statistical analysis

All statistical analyses and data visualizations were performed using R software (version 4.4.0; R Core Team, 2024). Field data related to colony strength parameters, including adult bee population, sealed brood area, and honey yield, were analyzed using a Completely Randomized Design (CRD). Treatment means were compared using the Least Significant Difference (LSD) test at a 5% significance level ($p < 0.05$).

For laboratory data, including pH, moisture content, ash content, diastase activity, electrical conductivity, fructose, total phenolic content, flavonoid content, and oxygen radical absorbance capacity (ORAC), a CRD was also employed. Treatment means were compared using the LSD test at a 1% significance level ($p < 0.01$) to ensure higher specificity and sensitivity in detecting differences among insecticide treatments.

All graphical representations were generated using the ggplot2 package in R to clearly and effectively illustrate treatment effects and support interpretation of the statistical results.

Results

Adult bee population

A statistically significant difference was observed in the adult bee population among colonies exposed to different insecticide treatments under field conditions ($F_{(3,8)} = 87.3$, $P < 0.05$). The coefficient of variation (CV = 4.45) indicated high precision and consistency across replications. The highest adult bee population was recorded in the control group ($20,499 \pm 487.31$ bees/hive), followed by colonies treated with Nitenpyram ($16,496 \pm 290.76$ bees/hive), suggesting relatively better colony strength in the absence of chemical exposure. Colonies treated with Flonicamid exhibited moderate adult bee populations ($13,678 \pm 248.53$ bees/hive), while the lowest population was observed in colonies exposed to Pyriproxyfen ($11,874 \pm 512.95$ bees/hive), as illustrated in Figure 1.

Brood area

A highly significant difference in sealed brood area was observed among colonies exposed to different insecticide treatments ($F_{(3,32)} = 175$, $P < 0.05$). The coefficient of variation (CV = 4.42%) indicated high experimental precision and minimal data variability.

Colonies in the control group exhibited the largest brood area (1297.8 ± 9.73 cm²), suggesting optimal colony development in the absence of insecticidal stress. Among the insecticide treatments, Nitenpyram resulted in a relatively larger brood area (1176.1 ± 13.31 cm²), followed by Flonicamid (1039.9 ± 18.85 cm²). In contrast, Pyriproxyfen had the most adverse impact on brood development, yielding the smallest brood area (807.14 ± 19.65 cm²), as illustrated in Figure 2.

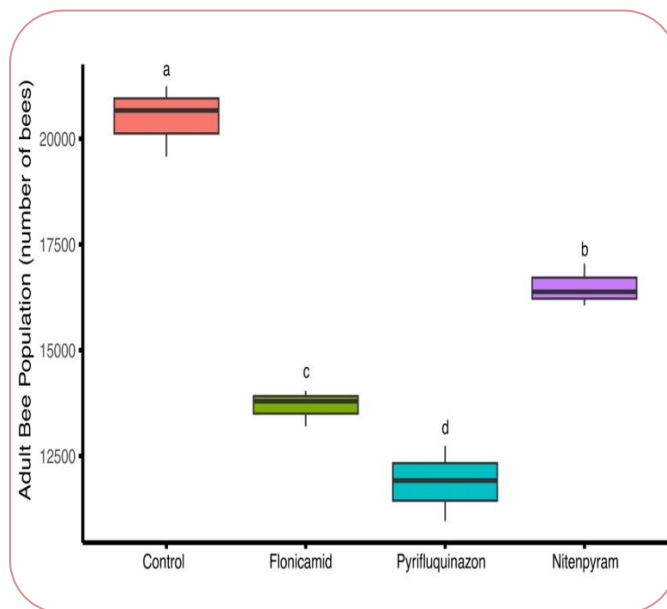


Figure 1. Trophic effects of different insecticides on adult honey bee populations compared to the control group.

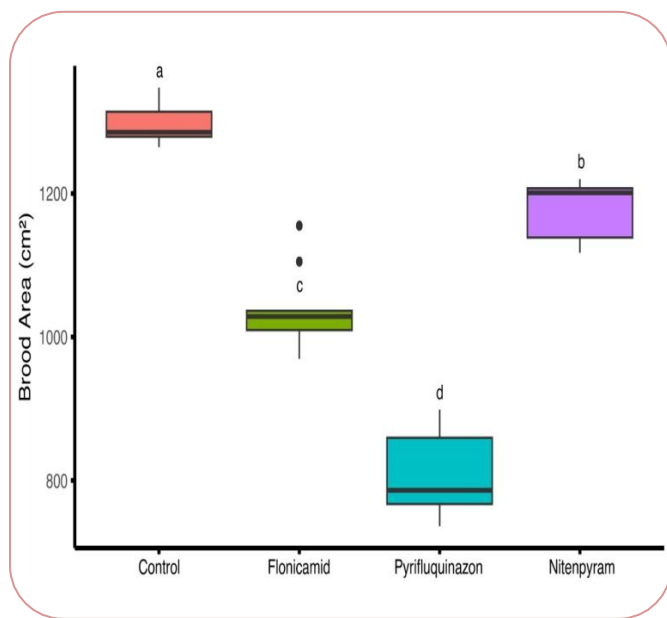


Figure 2. Trophic effects of different insecticides on honey bee brood area compared to the control group.

Honey production

A highly significant difference in honey production was observed among colonies treated with different insecticides ($F_{(3,8)} = 115$, $P < 0.05$). The coefficient of variation ($CV = 5.82\%$) indicated good experimental precision. Colonies in the control group produced the highest honey yield (8.24 ± 0.22 Kg/hive), reflecting optimal foraging efficiency and nectar conversion in the absence of chemical stress. Among the insecticide treatments, Nitenpyram resulted in a moderate honey yield (6.38 ± 0.17 Kg/hive). In contrast, Flonicamid and Pyriproxyfen significantly reduced honey production, yielding 4.85 ± 0.19 Kg/hive and 3.44 ± 0.19 Kg/hive, respectively. These findings suggest that exposure to Pyriproxyfen and Flonicamid may impair foraging behavior and overall colony productivity, as illustrated in Figure 3.

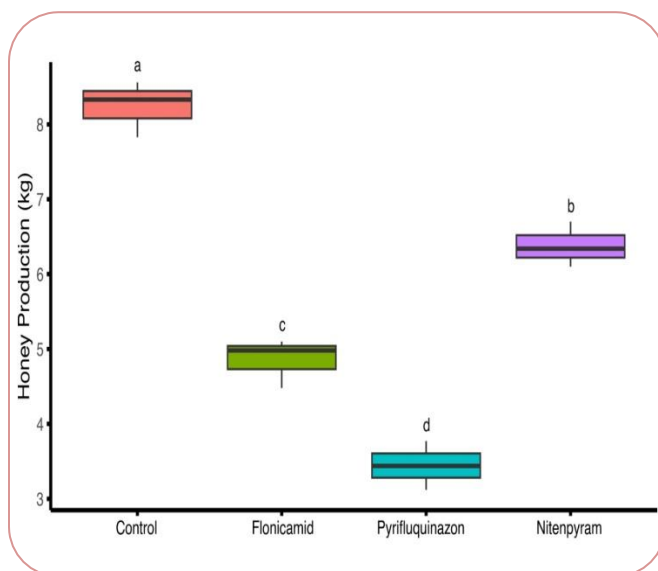


Figure 3. Trophic effects of different insecticides on honey production compared to the control group.

Honey quality

The trophic effects of three insecticides, Flonicamid, Pyriproxyfen, and Nitenpyram, on honey quality were evaluated in comparison to a control group without pesticide exposure. Honey samples were analyzed after an eight-week feeding period, and significant differences were observed across all measured parameters.

The pH of honey was highest in Flonicamid-treated colonies (4.17 ± 0.02), followed by Nitenpyram (4.07 ± 0.05), Pyriproxyfen (3.97 ± 0.09), and lowest in the Control (3.82 ± 0.09). Diastase activity, indicative of enzymatic content, was greatest in the Control group

(10.22 ± 0.19 units/g), followed by Nitenpyram (8.14 ± 0.10 units/g), Flonicamid (7.18 ± 0.16 units/g), and lowest in Pyriproxyfen (6.24 ± 0.28 units/g).

Regarding moisture content, Pyriproxyfen-treated honey exhibited the highest levels ($20.38 \pm 0.17\%$), followed by Flonicamid ($19.91 \pm 0.09\%$), Nitenpyram ($19.32 \pm 0.05\%$), and the Control ($18.77 \pm 0.17\%$). Ash content, which reflects mineral residue, was also highest in Pyriproxyfen ($0.95 \pm 0.06\%$), moderate in Flonicamid ($0.62 \pm 0.03\%$) and Nitenpyram ($0.55 \pm 0.02\%$), and lowest in the Control ($0.36 \pm 0.03\%$).

Electrical conductivity, an indicator of ionizable mineral content, was highest in Nitenpyram (0.94 ± 0.02 mS/cm), followed by Flonicamid (0.83 ± 0.01 mS/cm), Pyriproxyfen (0.74 ± 0.03 mS/cm), and the Control (0.59 ± 0.01 mS/cm).

In terms of fructose content, the Control honey had the highest concentration (396.21 ± 2.64 mg/Kg), followed by Pyriproxyfen (352.33 ± 4.66 mg/Kg), Flonicamid (320.56 ± 7.43 mg/Kg), and the lowest in Nitenpyram (307.45 ± 3.21 mg/Kg).

The total phenolic content, representing antioxidant potential, was highest in the Control (60.50 ± 0.58 mg GAE/100 g), followed by Nitenpyram (40.17 ± 1.78 mg GAE/100 g), Flonicamid (28.52 ± 0.49 mg GAE/100 g), and lowest in Pyriproxyfen (15.40 ± 0.57 mg GAE/100 g). Similarly, flavonoid content was highest in the Control (19.51 ± 0.38 mg QE/100 g), followed by Nitenpyram (18.61 ± 0.07 mg QE/100 g), Flonicamid (18.05 ± 0.07 mg QE/100 g), and Pyriproxyfen (17.60 ± 0.07 mg QE/100 g). Finally, the oxygen radical absorbance capacity, reflecting antioxidant activity, was greatest in the Control (36.44 ± 0.28 μ mol TE/g), followed by Nitenpyram (32.38 ± 0.17 μ mol TE/g), Flonicamid (30.94 ± 0.49 μ mol TE/g), and lowest in Pyriproxyfen (27.99 ± 0.34 μ mol TE/g) (Table 1).

Discussion

The trophic impact of insecticides on honey bee colonies extends beyond population dynamics and brood development, influencing both the physicochemical and biochemical properties of honey. In the present study, field exposure to Flonicamid, Pyriproxyfen, and Nitenpyram significantly reduced adult bee populations and brood areas compared to the control. These findings corroborate previous research demonstrating that even field-realistic exposure levels to these insecticides can adversely affect colony health and honey quality.

Table 1. Trophic impact of three insecticides (Flonicamid, Pyriproxyfen, and Nitenpyram) on honey quality parameters compared to the control group (no pesticide application).

Treatments	pH	Diastase activity (Schade units/g)	% Moisture contents (w/w)	% Ash contents (w/w)	Electrical conductivity (mS/cm)	Fructose contents (mg/kg)	Phenolic contents (mg GAE/kg)	Flavonoid contents (mg QE/kg)	ORAC (μ mol TE/g)
Control	4.17 $\pm$ 0.02a	10.22 $\pm$ 0.19a	18.77 $\pm$ 0.17c	0.36 $\pm$ 0.03c	0.59 $\pm$ 0.01d	396.21 $\pm$ 2.64a	60.50 $\pm$ 0.58a	19.51 $\pm$ 0.38a	36.44 $\pm$ 0.28a
Flonicamid	3.97 $\pm$ 0.09ab	7.18 $\pm$ 0.16c	19.91 $\pm$ 0.09ab	0.62 $\pm$ 0.03b	0.83 $\pm$ 0.01b	320.56 $\pm$ 7.43c	28.52 $\pm$ 0.49c	18.05 $\pm$ 0.07bc	30.94 $\pm$ 0.49b
Pyriproxyfen	3.82 $\pm$ 0.09b	6.24 $\pm$ 0.28d	19.32 $\pm$ 0.05bc	0.95 $\pm$ 0.06a	0.74 $\pm$ 0.03c	352.33 $\pm$ 4.66b	15.40 $\pm$ 0.57d	17.60 $\pm$ 0.07c	27.99 $\pm$ 0.34c
Nitenpyram	4.07 $\pm$ 0.05ab	8.14 $\pm$ 0.10b	20.38 $\pm$ 0.17a	0.55 $\pm$ 0.02b	0.94 $\pm$ 0.02a	307.45 $\pm$ 3.21c	40.17 $\pm$ 1.78b	18.61 $\pm$ 0.07ab	32.38 $\pm$ 0.17b
F value	4.38	75.1	29.6	47	66.3	66.3	359	17.1	106
P value	0.0422	< 0.001	0.0001	< 0.001	< 0.001	< 0.001	< 0.001	0.0008	< 0.001

Values are presented as Mean $\pm$ SE. Different letters (a, b, c, d) indicate statistically significant differences according to LSD at the 0.01 level.

Insecticide exposure is known to compromise adult bee longevity and overall colony size by disrupting metabolic and neurological pathways. For instance, Tosi et al. (2017) demonstrated that neonicotinoids impair immune and detoxification systems in worker bees, leading to premature mortality. This is consistent with our observation, where the lowest adult bee population was recorded in colonies treated with Pyriproxyfen (11,874 $\pm$ 512.95 bees/hive), indicating a likely disruption of critical physiological systems. Similarly, Woodcock et al. (2017) reported reduced worker bee populations and colony growth due to long-term pesticide exposure under field conditions. Our findings align with this trend, as colonies treated with Flonicamid and Pyriproxyfen exhibited significantly lower adult populations (13,678 $\pm$ 248.53 and 11,874 $\pm$ 512.95 bees/hive, respectively) and brood areas, indicating impaired colony development. In contrast, the control group had the highest adult bee population (20,499 $\pm$ 487.31 bees/hive), reinforcing the detrimental effects of chemical stress on colony vitality.

Pesticide exposure can also impair foraging

efficiency and nectar-processing behavior, ultimately reducing honey yields. Gill et al. (2012) reported that bees exposed to sub-lethal concentrations of neonicotinoids showed significant reductions in pollen and nectar foraging. Similarly, Siede et al. (2018) observed lower honey yields in colonies subjected to pesticide treatments. Our study confirmed these trends, with colonies treated with Pyriproxyfen and Flonicamid producing significantly less honey compared to the control and Nitenpyram treatments, suggesting compromised foraging and nectar metabolism. The relatively better performance of Nitenpyram-treated colonies may be attributed to its short residual persistence and lower trophic toxicity (Akayama and Minamida, 1999; Tomizawa and Casida, 2005).

Honey quality parameters, such as pH, diastase activity, moisture content, ash content, and electrical conductivity, are important indicators of both environmental conditions and colony health. Di Pasquale et al. (2013) reported reduced diastase activity and increased moisture in honey under pesticide stress, suggesting impaired enzyme secretion and inefficient

nectar dehydration. Similarly, Mulugeta and Tadese (2017) observed significant variations in ash content and electrical conductivity in honey from pesticide-exposed colonies. Our findings support these results, with significant alterations in honey parameters observed in insecticide-treated colonies, particularly in the Pyriproxyfen and Flonicamid groups.

The sugar and antioxidant profiles of honey may also be negatively affected by insecticide exposure. Gregorc et al. (2018) found that colonies treated with neonicotinoids produced honey with lower concentrations of flavonoids and phenolic compounds due to reduced foraging and disrupted metabolic processes. Likewise, Alaux et al. (2010) reported altered antioxidant enzyme activities and reduced phenolic retention in honey from pesticide-exposed bees. Our results are consistent with these findings, as colonies exposed to Pyriproxyfen and Flonicamid exhibited decreased levels of fructose, total phenolic content, flavonoids, and oxygen radical absorbance capacity (ORAC), indicating trophic suppression of metabolic pathways involved in honey production.

Conclusion

In conclusion, the insecticides Pyrifluquinazon, Flonicamid and Nitenpyram exerted significant trophic effects on *A. mellifera* colonies and the physicochemical and biochemical quality of honey. The most severe impacts were observed in colonies exposed to Pyrifluquinazon and Flonicamid, which showed reduced adult bee populations, smaller brood areas, and decreased honey production compared to the control. Although Nitenpyram caused moderate side effects, it performed better than the other two insecticides in most evaluated parameters.

Exposure to these insecticides also led to notable alterations in honey quality, including reduced diastase activity, total phenolic and flavonoid content, and ORAC values, alongside increased ash and moisture content. These changes indicate impaired bee metabolism and reduced foraging efficiency resulting from chemical stress. It is therefore recommended to avoid the application of insecticides such as Pyrifluquinazon and Flonicamid on blooming crops. Although Nitenpyram appeared less harmful at its trophic level, its application should be carefully timed to minimize bee exposure. Overall, the adoption of integrated, pollinator-friendly pest management strategies, coupled with improved coordination between beekeepers and farmers, is essential to protect honey bee health, sustain pollination services, and ensure the production of high-quality honey.

Authors' Contributions

BA, MAA, SUI, AM, MS, designed and conceived the study idea; BA completed the experiments. SUI, MJS, MIA, MUY analysed the data and performed visualizations and statistical data analysis; BA, MAA, SUI, wrote the original draft; AM, MS, SUI, MIA, reviewed and edited the manuscript; MJS, MIA, and MUY reviewed the manuscript and provided funds; BA and MAA provided the resources and supervision; All authors made valuable revisions and edited the manuscript and approved the final version.

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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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