International Journal of Food Science and Agriculture, 2026, 10(1), 88-95
https://www.hillpublisher.com/journals/jsfa/
ISSN Online: 2578-3475
ISSN Print: 2578-3467

Comparative Impacts of Clothianidin and Cypermethrin on *Apis mellifera* Survival, Behavior, and Colony Resource Storage

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How to cite this paper: Muhammad Asim Qaisrani, Muhammad Akhtar. (2026). Comparative Impacts of Clothianidin and Cypermethrin on *Apis mellifera* Survival, Behavior, and Colony Resource Storage. *International Journal of Food Science and Agriculture*, 10(1), 88-95.
DOI: 10.26855/ijfsa.2026.03.010

Received: January 26, 2026
Accepted: February 23, 2026
Published: March 28, 2026

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Abstract

Insecticide exposure poses significant risks to honeybee (*Apis mellifera*) survival and colony performance. This study assessed the effects of clothianidin and cypermethrin on worker mortality, behavior, foraging activity, and nectar and pollen storage. Clothianidin caused the highest worker mortality (78.33% at 96 h), whereas cypermethrin induced moderate mortality (38.33%), compared to 26.66% in controls. Behavioral impairments, including disorientation, impaired righting reflex, and flight instability, were most pronounced in clothianidin-treated bees (up to 70%), with cypermethrin producing moderate effects. Foraging activity declined by 38.0% in clothianidin-treated colonies and 18.7% under cypermethrin. Nectar and pollen storage were significantly reduced in treated colonies, with control colonies showing the highest levels (nectar: 3.85 kg; pollen: 2.18 kg), followed by cypermethrin and clothianidin. These results demonstrate that clothianidin severely disrupts honeybee survival and colony function, while cypermethrin exerts moderate but notable effects. The findings underscore the importance of evaluating insecticide impacts on pollinators to inform safer pest management practices and support ecosystem sustainability.

Keywords

Clothianidin; cypermethrin; *Apis mellifera*; worker mortality; behavioral impairment; foraging activity; nectar storage; pollen storage; colony performance; pollinator risk assessment

1. Introduction

Insect pollinators, including the western honeybee (*Apis mellifera* L.), contribute to the production of approximately 35% of global food crops [1]. The economic value of this ecosystem service is estimated at over €150 billion annually, highlighting a critical dependency between modern agriculture and pollinator health [2]. Despite their indispensability, honeybee populations have faced dramatic declines globally, characterized by high overwintering losses and Colony Collapse Disorder (CCD) [3]. While honeybee declines are multifactorial, exposure to synthetic insecticides, particularly neonicotinoids, has been shown to reduce colony performance and overwintering success, contributing to colony instability [4].

Among neonicotinoids, clothianidin is of concern due to its systemic nature and high persistence in soil and water, which can result in exposure to pollinators through plant tissues and hive resources [5]. Clothianidin acts as an agonist of nicotinic acetylcholine receptors (nAChRs) in insects, overstimulating the nervous system and leading to sublethal effects such as tremors, impaired coordination, and, at higher doses, mortality [6]. In contrast, Type II synthetic pyrethroids, including cypermethrin, have been reported to interfere with calcium/calmodulin-dependent signaling pathways in neural tissue, interfering with intracellular signaling pathways in neurons [7]. Although these chemicals possess distinct modes of action, both are frequently applied in the same agricultural landscapes, creating a potential risk of repeated exposure for non-target foragers.

The impact of these insecticides is not limited to acute lethality. Sublethal exposure, concentrations that do not cause immediate death, can trigger a systemic "cascade of failure" within the hive. Acute, sublethal exposure to clothianidin disrupts motor function and locomotor activity in worker honeybees, including altered movement patterns and postural control, as measured in a laboratory video-tracking assay [8]. Because *A. mellifera* relies on complex spatial navigation and the waggle dance to communicate resource locations, even small impairments in flight or motor function could plausibly disrupt foraging efficiency [9]. Exposure to sublethal doses of neonicotinoids can cause honeybee foragers to become disoriented and fail to return to the hive, which could contribute to a decline in the worker population and potentially impair colony functioning [10].

Furthermore, the foraging efficiency of a colony is a key determinant of its nutritional security. Honeybees must navigate vast distances to collect nectar and pollen, which serve as the essential carbohydrate and protein sources for brood rearing and overwintering [11]. Insecticide exposure has been shown to reduce the frequency of foraging trips and the volume of resources returned to the hive [12]. Over time, this disruption in the "foraging flux" results in the depletion of food stores. A colony with insufficient nectar and pollen storage faces increased susceptibility to pathogens and environmental stressors, potentially leading to functional starvation of the hive, even when the pesticide does not reach a lethal systemic dose [13, 14].

Despite the extensive body of work on individual pesticide toxicity, there is a lack of comparative data that tracks the progression from individual mortality to colony-level resource loss under standardized field conditions. Most toxicological frameworks rely on laboratory-based LD_{50} values, which fail to account for the social dynamics and environmental pressures of a working apiary [15]. This study aims to bridge this gap by evaluating the comparative effects of clothianidin and cypermethrin on *A. mellifera*. By integrating data on acute mortality, behavioral impairment, foraging frequency, and total resource storage, this research provides a holistic assessment of how distinct insecticide classes compromise the functional equilibrium and long-term viability of honeybee colonies in agroecosystems.

2. Materials and Methods

2.1 Experimental Colonies and Setup

The study was conducted using healthy, queen-right *A. mellifera* colonies, each comprising approximately 10,000–12,000 adult workers and headed by sister queens to minimize genetic variability. Colonies were maintained under standard apicultural practices and were screened for *Varroa destructor* and Nosema spp. before experimentation. All experimental procedures were designed to minimize stress to the bees and followed standard ethical practices for apiculture research.

2.2 Insecticides and Treatment Application

The study evaluated the effects of two insecticides, clothianidin and cypermethrin, on honeybee colonies. For mortality assessments, clothianidin was applied at 10 µg per bee, while cypermethrin was applied at 15 µg per bee. Behavioral assessments used lower concentrations, with clothianidin at 5 µg/L and cypermethrin at 50 µg/L. For foraging activity, bees were exposed to clothianidin at 0.1 mg/L and cypermethrin at 0.05 mg/L. Control colonies received an untreated 50% (w/v) sucrose solution or distilled water. Commercial formulations were also included at field-recommended doses: Arrivo® 10EC (FMC) at 330 mL per acre and Telsta® 20SC (Rudolf Group) at 200 mL per acre.

2.3 Effect of Insecticides on Worker Mortality of *Apis mellifera*

Mortality of *A. mellifera* workers was evaluated under controlled field conditions. Twelve healthy colonies, each containing approximately 10,000-12,000 workers and headed by a sister queen, were randomly assigned to three treatments: control (untreated), clothianidin (10 µg/bee), and cypermethrin (15 µg/bee), with four colonies per treatment. Insecticides were administered via sugar syrup feeders, ensuring uniform exposure across colonies. Worker mortality was recorded at 24, 48, 72, and 96 hours post-exposure by manually counting dead bees at the hive entrance.

Percent mortality was estimated based on the initial worker population of each colony and averaged across replicates. Observations were performed daily between 08:00 and 11:00 h, corresponding to peak foraging activity, to reduce variability in worker activity patterns. Data were expressed as mean ± standard deviation. Statistical differences between treatments at each time point were assessed using one-way ANOVA, followed by Tukey's HSD post hoc test at a 5% significance level. All analyses were conducted using IBM SPSS Statistics 20.

2.4 Assessment of Behavioral Impairment in *Apis mellifera*

Behavioral impairment in *A. mellifera* was evaluated using nine colonies, with three colonies assigned to each treatment: control, clothianidin, and cypermethrin. To minimize genetic variability, each colony was headed by a sister queen and maintained at a population of 10,000-12,000 workers. From each colony, 20 workers were randomly sampled, resulting in a total of 60 bees per treatment. Sublethal doses were administered via 50% (w/v) sucrose syrup, with Clothianidin applied at 5 µg/L and Cypermethrin at 50 µg/L, representing concentrations considered field-realistic. Control colonies received untreated syrup. Behavioral responses were recorded 24 hours post-exposure under climate-controlled laboratory conditions (25 °C, 60% relative humidity). Bees were assessed for disorientation, indicated by a lack of coordinated movement; impaired righting reflex, defined as the inability to upright within 10 seconds; and flight instability, observed as erratic flight or failure to take off. Data were expressed as the percentage of affected bees per colony. Statistical analyses were performed using R and IBM SPSS 20, with one-way ANOVA followed by Tukey's HSD post-hoc test to determine significant differences ($p < 0.05$). All experimental procedures followed standard apicultural ethical guidelines and were designed to minimize stress to the colonies.

2.5 Foraging Activity Assessment

Six healthy honeybee (*A. mellifera*) colonies were selected and standardized to ensure similar colony strength. Colonies were randomly assigned to one of three treatments: control, clothianidin, or cypermethrin. For the insecticide treatments, clothianidin was applied at 0.1 mg/L and cypermethrin at 0.05 mg/L, corresponding to their field-recommended doses. Treatments were applied by carefully spraying the hive entrance and interior surfaces to ensure uniform exposure while minimizing direct disturbance to the bees. Control colonies were sprayed with distilled water in the same manner. All applications were performed in the early morning under calm weather conditions to reduce environmental confounding effects.

Foraging activity was monitored 30 minutes after insecticide application, during the peak foraging period (08:00–11:00 h). A foraging trip was defined as a worker bee leaving the hive, collecting nectar or pollen, and returning. The number of foraging trips was recorded for each colony over a 30-minute observation period using a tally counter.

For each treatment, the mean number of foraging trips per colony was calculated. Percent reduction relative to control was determined using the formula:

$$\%\text{Reduction} = \frac{\text{Control mean} - \text{Treatment mean}}{\text{Control mean}} \times 100$$

All colonies were monitored carefully to minimize stress or mortality. Observations were conducted non-invasively, adhering to standard apicultural practices to ensure the welfare of the bees.

2.6 Effect of Insecticides on Nectar and Pollen Storage

Nine healthy, queen-right *A. mellifera* colonies, each containing approximately 10,000-12,000 adult workers and headed by sister queens, were selected and screened for *Varroa destructor* and *Nosema* spp. Frames were installed in each colony, and measurements were taken 28 days after frame installation to allow full colony acclimation and storage activity. Colonies were randomly assigned to three treatment groups ($n = 3$ per group): control (50 mL 50% sucrose), clothianidin (20 µg/colony), or cypermethrin (25 µg/colony). Treatments were administered via top-bottle feeders, and full consumption within 4 h likely ensured exposure of the foraging population. Nectar and pollen stores were measured by carefully weighing the frames in kilograms after clearing bees with a soft-bristle brush. Net changes in storage were calculated for each colony. Each colony served as a biological replicate, providing three replicates per treatment. Environmental conditions, including temperature and floral availability, were monitored to ensure uniformity across colonies. Data were analyzed using IBM SPSS Statistics 20 and verified in Origin. Differences among treatments were assessed using one-way ANOVA followed by Tukey's HSD test, with significance set at $p < 0.05$.

3. Results

3.1 Effect of Insecticides on the Mortality of *Apis mellifera*

Mortality of A. mellifera workers increased over time following insecticide exposure. *mellifera* workers over time (Fig. 1). At 24 hours, mortality was 3.33% in the control, 11.66% in clothianidin-treated workers, and 8.33% in cypermethrin-treated workers. By 48 hours, mortality rose to 6.66% in control, 25.00% in clothianidin, and 15.00% in cypermethrin. At 72 hours, clothianidin caused 40.00% worker mortality, cypermethrin 25.00%, and the control 16.66%. By 96 hours, clothianidin induced the highest mortality (78.33%), followed by cypermethrin (38.33%) and control (26.66%). These results indicate that clothianidin is highly toxic to *A. mellifera* workers, producing rapid and pronounced mortality, whereas cypermethrin has a moderate effect.

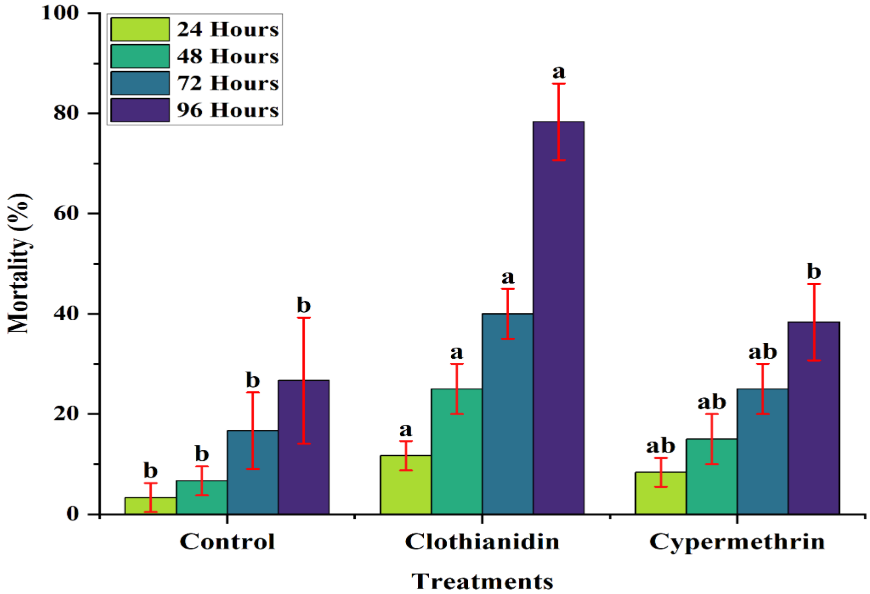

Figure 1. Cumulative mortality (%) of *Apis mellifera* workers over time following exposure to insecticides.

Notes. Values represent mean ± standard deviation (SD). Different letters indicate statistically significant differences among treatments at each time point (Tukey's HSD test, $p < 0.05$).

3.2 Effect of Insecticides on *Apis mellifera* Forager Workers on Behavioral Impairment

Exposure to insecticides significantly affected the neurological and flight behaviour of *A. mellifera* workers compared to the control ($p < 0.05$). Disorientation was minimal in control bees (3.33%) but markedly increased after exposure to clothianidin (65%) and cypermethrin (50%), with clothianidin causing the highest level of disorientation. Similarly, the impaired righting reflex was significantly elevated in clothianidin-treated bees (70%), followed by cypermethrin (30%), while control bees showed only 1.67% impairment. Flight instability was also substantially affected, with clothianidin and cypermethrin causing 55% and 45% instability, respectively, compared to 3.33% in the control group. Statistical analysis confirmed that all insecticide treatments significantly differed from the control ($p < 0.05$), and clothianidin consistently induced the strongest behavioral disruptions.

Table 1. Behavioral impairment of *A. mellifera* workers 24 hours post-exposure

Treatment	Disorientation (%)	Impaired Righting Reflex (%)	Flight Instability (%)
Control	3.33±2.89c	1.67±2.89c	3.33±2.89b
Clothianidin	65.00±5.00a	70.00±5.00a	55.00±5.00a
Cypermethrin	50.00±5.00b	30.00±5.00b	45.00±5.00a

Notes. Data are expressed as mean ± standard deviation. Different letters within the same column indicate significant differences between treatments (Tukey's HSD, $p < 0.05$).

3.3 Effect of Insecticides on Foraging Activity of *Apis mellifera* Workers

The foraging activity of *A. mellifera* workers was significantly affected by insecticide treatments. Control colonies exhibited the highest mean foraging trips per day (42.83), serving as the baseline. Colonies exposed to clothianidin experienced the greatest reduction, with a mean of 26.66 trips per day, representing a 38.0% decrease relative to control (🔴 Strong ↓↓↓). Colonies treated with cypermethrin had a mean of 34.83 trips per day, corresponding to an 18.7% decrease (🟡 Moderate ↓↓) compared to the control (Table 2). These findings suggest that clothianidin exerts a stronger inhibitory effect on honeybee foraging activity than cypermethrin at field-recommended doses.

Table 2. Effect of clothianidin and cypermethrin on foraging activity of *Apis mellifera* workers

Treatment	Mean Foraging Trips/Day	% Reduction vs Control	Effect on Foraging
Control	42.83	–	–
Clothianidin	26.66	38.0	🔴 Strong ↓↓↓
Cypermethrin	34.83	18.7	🟡Moderate↓↓

Notes. Data are expressed as mean ± standard deviation. Different letters within the same column indicate significant differences between treatments (Tukey's HSD, $p < 0.05$).

3.4 Effect of Insecticides on Nectar and Pollen Storage in *Apis mellifera* Colonies

The application of insecticides significantly affected nectar and pollen storage in honeybee colonies. Colonies in the control group stored the highest amount of nectar (3.85 kg/colony) and pollen (2.18 kg/colony). Exposure to clothianidin reduced nectar storage to 2.10 kg/colony and pollen storage to 1.20 kg/colony. Colonies treated with cypermethrin showed intermediate values for nectar (2.45 kg/colony) and pollen storage (1.38 kg/colony).

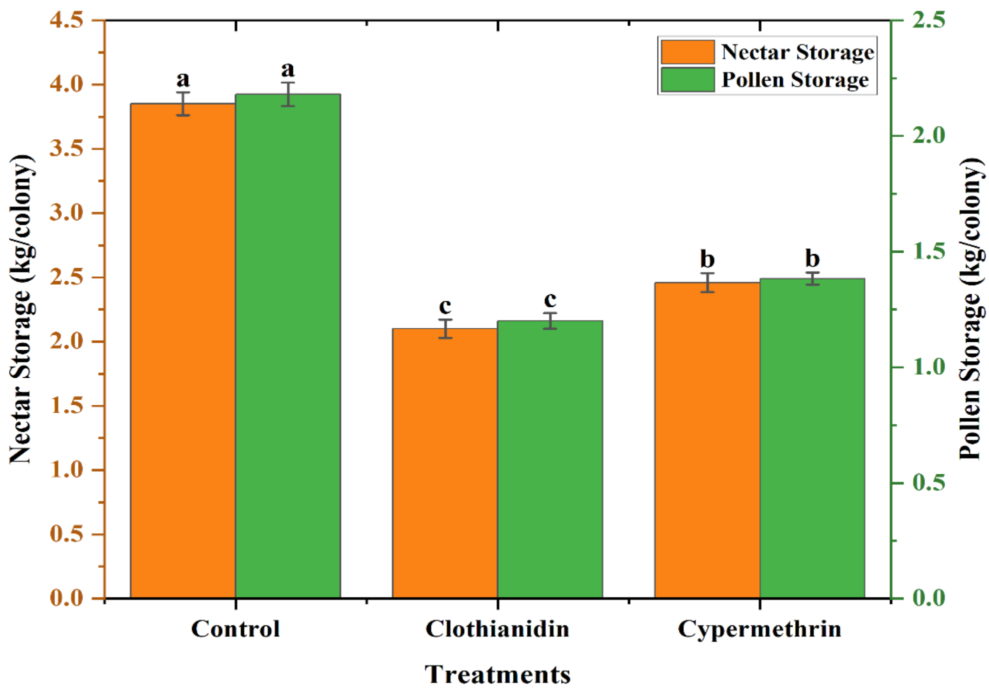

Figure 2. Nectar and pollen storage (kg/colony) in *Apis mellifera* under control, clothianidin, and cypermethrin.

Notes. Bars show means ± SD; different letters indicate significant differences (one-way ANOVA, Tukey's HSD, $p < 0.05$).

4. Discussion

The present study demonstrates that exposure to insecticides, specifically clothianidin and cypermethrin, has profound impacts on honeybee (*A. mellifera*) survival, behavior, foraging activity, and overall colony performance.

Mortality data clearly indicate that clothianidin is highly toxic to worker bees, reaching 78.33% at 96 hours post-exposure, which reflects its acute lethal potential. These findings are consistent with numerous previous studies showing that neonicotinoids reduce honeybee survival and interfere with critical behaviors such as orientation, homing, and navigational efficiency [10, 13, 16, 17]. Such impairments are particularly detrimental to colony functioning, as they compromise the ability of foragers to locate floral resources and return to the hive, thereby affecting both individual and collective colony health.

Cypermethrin, a pyrethroid, also caused significant mortality, though to a lesser extent than clothianidin. Pyrethroids are known to act on voltage-gated sodium channels in neurons, leading to persistent depolarization that disrupts neural signaling and motor coordination and behavior, resulting in moderate mortality and reduced functional efficiency in honeybees [18-21]. While pyrethroids generally produce less acute mortality than neonicotinoids, their sublethal effects, particularly on neuromuscular function, can severely compromise foraging performance and colony maintenance.

Behavioral impairments were evident in both insecticide treatments, with significant increases in disorientation, impaired righting reflex, and flight instability compared to control colonies [22, 23]. Bees exposed to clothianidin exhibited the most pronounced behavioral deficits, consistent with reports of neonicotinoid-induced disruption of flight dynamics and homing ability [10, 17, 22]. Such disruptions may be attributed to the agonistic effect of neonicotinoids on nicotinic acetylcholine receptors in the insect central nervous system, which alters synaptic transmission and affects neural processes underlying orientation and coordinated motor activity [23]. Cypermethrin-treated bees also exhibited impaired locomotion and flight instability, supporting evidence that pyrethroids interfere with voltage-gated sodium channels, leading to neuromuscular disruption [18]. Impairments in motor coordination and flight are critical sublethal endpoints, as they directly affect the ability of bees to forage efficiently, perform recruitment dances, and return resources to the colony [10, 17, 22].

"Foraging activity was reduced under exposure to both insecticides. Clothianidin-treated bees exhibited a 38% reduction in foraging trips, whereas cypermethrin-treated bees showed an 18.7% reduction. This decline in foraging efficiency is consistent with previous reports that neonicotinoid exposure impairs recruitment behavior and orientation, thereby disrupting collective foraging [22]. Sublethal effects on foraging not only reduce nectar and pollen intake but can also interfere with information transfer between workers, diminishing the colony's ability to exploit floral resources effectively. Such reductions may lead to broader colony-level consequences, including diminished resource accumulation and compromised brood provisioning, potentially exacerbated by increased susceptibility to pathogens under pesticide stress [26].

Consistent with reductions in foraging activity, nectar and pollen storage were also significantly affected in insecticide-treated colonies. Control colonies maintained the highest levels of stored resources, whereas clothianidin-treated colonies exhibited the lowest accumulation, with cypermethrin-treated colonies showing intermediate levels. Decreased storage of nectar and pollen may have long-term implications for colony growth, brood development, and overwintering success [13, 21, 23, 24, 27]. In addition, reduced resource availability within the colony can increase competition among workers and exacerbate stress, making colonies more susceptible to other environmental pressures and pathogens.

Comparative analyses indicate that neonicotinoids generally produce stronger sublethal effects than pyrethroids, though both insecticide classes impose substantial stress on honeybee colonies. Moreover, additive and synergistic effects have been reported when multiple insecticides co-occur in the environment, amplifying the adverse consequences for colony survival, behavior, and resource acquisition [24, 26, 28]. At field-recommended doses, both clothianidin and cypermethrin altered honeybee behavior, impaired foraging efficiency, and reduced colony-level resource storage, highlighting the vulnerability of pollinators to commonly applied agricultural chemicals [10, 23]. The present results underscore the necessity of considering sublethal endpoints, such as disorientation, impaired locomotion, reduced foraging, and diminished resource storage, alongside mortality when evaluating the risks of insecticides to pollinator health.

5. Conclusion

This study demonstrates that exposure to insecticides significantly affects *A. mellifera* workers, impacting mortality, behavior, foraging activity, and colony-level resource storage. Clothianidin was highly toxic, causing rapid mortality and behavioral impairments, and substantial reductions in foraging and nectar/pollen storage. Cypermethrin also affected honeybee survival and behavior, though to a moderate extent compared with clothianidin. Sublethal effects, including disorientation, impaired righting reflex, and flight instability, were observed in both insecticide treatments, highlighting the broader ecological risks beyond direct mortality. Reduced foraging activity and compromised nectar and pollen stores indicate that even field-recommended doses of insecticides can impair colony performance and productivity. These findings underscore the urgent need for careful management of insecticide use and consideration of pollinator health in agricultural practices to maintain sustainable pollination services and colony viability.

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