



Effects of neonicotinoid pesticides on honeybee colony health and foraging behavior in agricultural landscapes of central Europe

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Abstract

Background: Neonicotinoid pesticides have been widely implicated in global pollinator declines, yet comprehensive field studies examining sublethal effects on colony-level parameters remain limited in Central European agricultural systems [12].

Objective: This study investigated the impact of three commonly used neonicotinoid compounds (imidacloprid, clothianidin, and thiamethoxam) on honeybee (*Apis mellifera*) colony health metrics, foraging efficiency, and behavioral patterns over a complete growing season [34].

Method: A randomized controlled field experiment was conducted across 24 apiaries in agricultural landscapes of Bavaria, Germany. Colonies (n=96) were assigned to four treatment groups: control, imidacloprid-exposed, clothianidin-exposed, and thiamethoxam-exposed. Data collection included weekly colony strength assessments, pollen trap analysis, RFID-tagged bee tracking, and residue testing of hive products. Statistical analyses employed mixed-effects models and survival analysis [56].

Key Results: Neonicotinoid-exposed colonies showed significant reductions in brood area (23-31% decrease, $p < 0.01$), adult bee population (18-27% decrease, $p < 0.05$), and foraging trip duration increases (34-42%, $p < 0.001$). Mortality rates were elevated by 15-22% in treated groups. Residue accumulation in wax and honey exceeded EU safety thresholds in 67% of exposed colonies [78].

Conclusion: Sublethal neonicotinoid exposure significantly impairs honeybee colony performance through multiple pathways including reduced foraging efficiency, impaired navigation, and decreased reproductive output. These findings support regulatory restrictions on neonicotinoid use in flowering crop systems and highlight the need for integrated pest management alternatives [9, 10].

Keywords: Neonicotinoids, *Apis mellifera*, colony collapse, foraging behavior, pesticide toxicity, pollinator conservation

Introduction

The global decline of pollinator populations represents one of the most pressing ecological challenges of the 21st century, with profound implications for agricultural productivity, ecosystem stability, and food security [1]. Among insect pollinators, the Western honeybee (*Apis mellifera*) serves as both a crucial agricultural asset and an indicator species for environmental health, making its population dynamics a focal point for conservation research and policy development [23]. Recent decades have witnessed alarming trends in honeybee colony losses, with annual mortality rates exceeding 30% in many regions, prompting intensive investigation into potential causative factors [4]. Neonicotinoid insecticides, introduced commercially in the 1990s, have become among the most widely applied pesticides globally due to their systemic action, broad-spectrum efficacy, and perceived lower mammalian toxicity compared to organophosphates [5]. These compounds act as agonists at nicotinic acetylcholine receptors in the insect nervous system, causing paralysis and death at lethal doses [6]. However, growing evidence suggests that sublethal exposures—concentrations insufficient to cause immediate mortality—may produce insidious effects on bee behavior, physiology, and colony-level performance that contribute to long-term population declines [78].

The mechanism by which neonicotinoids affect honeybees extends beyond simple neurotoxicity. Research has demonstrated impairments in learning and memory formation, critical for floral recognition and navigation [9]. Foraging bees exposed to field-realistic concentrations show

reduced proboscis extension response, impaired olfactory conditioning, and diminished ability to communicate resource locations through waggle dance precision [10, 11]. At the colony level, these individual-level deficits may cascade into reduced pollen and nectar collection, compromised thermoregulation, and decreased reproductive success through queen replacement failure and swarming inhibition [12].

Despite extensive laboratory studies documenting neonicotinoid effects on individual bees, field-based investigations examining colony-level outcomes under realistic exposure scenarios remain comparatively limited, particularly in Central European agricultural contexts where diverse cropping systems create complex exposure pathways [13, 14]. Previous field studies have yielded inconsistent results, with some reporting minimal colony-level impacts while others document substantial losses, potentially reflecting differences in exposure routes, landscape composition, and methodological approaches [15, 16].

This study addresses several critical knowledge gaps in current neonicotinoid-bee interaction research. First, we examine simultaneous exposure to three major neonicotinoid compounds rather than single-compound studies, reflecting real-world conditions where bees encounter pesticide mixtures [17]. Second, we employ longitudinal monitoring across an entire growing season to capture cumulative and delayed effects not visible in short-term experiments [18]. Third, we integrate multiple measurement scales from individual behavior (RFID

tracking) to colony demographics (brood area, adult population) to product contamination (residue analysis), providing a comprehensive assessment of neonicotinoid impacts^[19].

The specific objectives of this research were to: ^[1] quantify the effects of field-realistic neonicotinoid exposures on honeybee colony strength parameters including brood area, adult bee population, and stored resources; ^[2] assess changes in foraging behavior including trip frequency, duration, and load size using automated tracking technology; ^[3] measure accumulation patterns of neonicotinoid residues in hive products over time; and ^[4] evaluate relationships between residue levels and colony performance metrics. We hypothesized that neonicotinoid-exposed colonies would demonstrate reduced growth rates, altered foraging patterns, and elevated mortality compared to control colonies, with effects varying among compounds based on their chemical properties and persistence^[20].

Literature review

The theoretical framework for understanding neonicotinoid impacts on honeybees integrates toxicology, behavioral ecology, and social insect biology. At the molecular level, neonicotinoids bind irreversibly to nicotinic acetylcholine receptors (nAChRs) in the insect central nervous system, causing continuous neuronal stimulation that leads to paralysis and death at high concentrations^[21,22]. The selective toxicity to insects versus mammals arises from structural differences in receptor subtypes, though recent evidence suggests non-target organisms including beneficial insects experience significant effects at environmentally relevant concentrations^[23].

Sublethal effects operate through multiple physiological pathways. Neurobehavioral studies using electrophysiological recordings and calcium imaging have demonstrated that neonicotinoids disrupt synaptic transmission in mushroom bodies, brain regions critical for learning and memory in insects^[24]. Behavioral assays reveal impaired associative learning, with exposed bees showing reduced ability to link floral cues with rewards, potentially explaining observed decreases in foraging efficiency^[25]. Navigation capabilities are similarly compromised, with radio-frequency identification (RFID) tracking studies documenting increased homing failure rates and extended return times in exposed foragers^[26].

At the individual level, neonicotinoid exposure affects immune function, with transcriptomic analyses revealing downregulation of antimicrobial peptide genes and reduced expression of detoxification enzymes^[27]. This immunosuppression may increase susceptibility to pathogens including *Nosema* spp. microsporidia and deformed wing virus, creating synergistic stressors that amplify colony losses^[28]. Metabolic costs also increase, as bees must allocate energy toward detoxification processes involving cytochrome P450 enzymes, potentially reducing resources available for other physiological functions^[29].

Colony-level consequences emerge from the aggregation of individual effects within the superorganism structure of honeybee societies. Mathematical modeling suggests that relatively modest increases in forager mortality (15-20%) can destabilize colony population dynamics through positive feedback loops wherein reduced workforce diminishes resource acquisition, further weakening colony resilience^[30]. Empirical studies support these predictions, with multi-

year monitoring revealing that neonicotinoid-exposed colonies enter winter with smaller populations and reduced honey stores, increasing overwintering mortality risk^[31].

The exposure pathway complexity adds another dimension to neonicotinoid impacts. Bees encounter these compounds through multiple routes: direct contact with sprayed foliage, consumption of contaminated nectar and pollen, absorption through wax matrices, and trophallactic transfer within colonies^[32]. Systemic uptake by plants means that even seed treatments—initially promoted as minimizing non-target exposure—result in residue presence in floral tissues throughout the growing season^[33]. Landscape-scale studies demonstrate that exposure intensity varies with proximity to treated fields, crop rotation patterns, and availability of alternative forage sources^[34].

Previous field studies have produced heterogeneous results regarding colony-level impacts. Some large-scale trials in North America reported minimal differences between neonicotinoid-treated and control colonies in standard apicultural metrics, leading to industry arguments that laboratory findings lack ecological relevance^[35]. However, methodological critiques note that these studies often employed higher-than-field-realistic dosages, short observation periods, or inadequate replication, potentially masking subtle but biologically significant effects^[36]. European studies generally report stronger negative associations, possibly reflecting different agricultural practices, landscape compositions, or bee subspecies sensitivities^[37].

Meta-analyses synthesizing multiple studies conclude that neonicotinoids consistently reduce bee survival, reproduction, and foraging performance across experimental contexts, though effect sizes vary considerably^[38]. Compound-specific differences exist, with imidacloprid generally showing stronger effects than thiamethoxam at equivalent concentrations, potentially related to differential binding affinities and metabolic half-lives^[39]. Clothianidin occupies an intermediate position, with emerging evidence suggesting particular potency against bumblebees relative to honeybees^[40].

Research gaps persist in several areas. Long-term multi-generational effects remain poorly characterized, as most studies span single seasons despite potential carryover impacts through queen quality degradation or epigenetic modifications^[41]. Interactions with other stressors including varroa mites, nutritional stress, and climate extremes require more systematic investigation to understand cumulative risk^[42]. Finally, effectiveness of mitigation strategies such as buffer zones, flowering strip plantings, and integrated pest management protocols needs rigorous field validation to inform evidence-based policy^[43].

This study contributes to addressing these gaps by employing a comprehensive multi-scale approach across a full growing season in Central European agricultural landscapes, examining three major neonicotinoid compounds simultaneously, and integrating behavioral, demographic, and chemical measurements to elucidate mechanisms linking individual-level toxicity to colony-level outcomes.

Methodology

Research Design: This study employed a randomized controlled field experiment with four treatment groups and repeated measures over time. The experimental design

followed established protocols for pesticide ecotoxicology field trials while incorporating novel technological approaches for behavioral monitoring ^[44, 45].

Study Site and Duration: Research was conducted in Bavaria, Germany, across 24 agricultural sites representing typical Central European farming landscapes with mixed cropping systems including oilseed rape, maize, wheat, and sunflower. Sites were selected to ensure similar landscape composition within a 3km radius, minimum 5km separation between sites to prevent bee drift, and absence of commercial beekeeping operations within 2km. The study period spanned April through September 2024, covering the primary foraging season ^[46].

Sample Size and Allocation: Ninety-six healthy honeybee colonies (*Apis mellifera carnica*) were established in early spring 2024, with initial standardization ensuring comparable starting conditions: minimum 15,000 adult bees, 6-8 frames of sealed brood, adequate honey and pollen stores, and young queens (<6 months old). Colonies were randomly assigned to four treatment groups (n=24 per group): Control (no neonicotinoid exposure), Imidacloprid-exposed, Clothianidin-exposed, and Thiamethoxam-exposed. Randomization was stratified by site to ensure equal distribution across locations ^[47].

Treatment Application: Neonicotinoid treatments were applied following manufacturer recommendations for oilseed rape cultivation, the primary nectar source in the study region. Treated seeds contained: imidacloprid (600g active ingredient/100kg seed), clothianidin (450g AI/100kg seed), or thiamethoxam (400g AI/100kg seed). Control plots received untreated seeds. All sites followed identical agronomic practices excluding the variable of interest ^[48].

Data Collection Methods

Colony Strength Assessments: Conducted biweekly using standardized frame-counting methods. Measurements included: number of frames with sealed brood, open brood, eggs/larvae, adult bee population estimates (using grid sampling), and weight of stored honey and pollen ^[49].

Foraging Behavior Monitoring: Approximately 200 worker bees per colony were tagged with RFID microchips (0.4mg, 2.1mm diameter) glued to thoraxes. Automated readers at hive entrances recorded departure and arrival times, enabling calculation of trip frequency, duration, and daily activity patterns. Tags were applied to 5-day-old bees to ensure uniform age cohorts ^[50].

Pollen Trap Analysis: Modified pollen traps collected incoming pollen loads twice weekly. Samples were

weighed, identified to plant family using microscopic palynology, and analyzed for neonicotinoid residues using liquid chromatography-tandem mass spectrometry (LC-MS/MS) with detection limits of 0.1 µg/kg ^[51].

Hive Product Residue Testing: Wax comb samples (from brood areas) and honey samples were collected monthly and analyzed for neonicotinoid residues using validated extraction protocols ^[52].

Mortality Assessment: Dead bee counts were recorded from landing boards twice weekly, with subsamples preserved for pathogen screening via PCR diagnostics for common viruses and *Nosema* spp ^[53].

Statistical Analysis: Data were analyzed using R statistical software (version 4.3.1). Mixed-effects linear models accounted for repeated measures and nested data structure (bees within colonies within sites). Fixed effects included treatment, time, and their interaction; random effects included colony ID and site. Survival analysis (Cox proportional hazards) examined time-to-event outcomes including colony collapse. Post-hoc comparisons used Tukey's HSD test with $\alpha=0.05$. Power analysis indicated 80% power to detect 15% differences in primary outcomes with n=24 per group ^[54], ^[55].

Ethical Considerations: This study uses a simulated dataset created for academic training purposes. All procedures followed institutional animal care guidelines for invertebrate research. No endangered species were involved.

Results and Discussion

Colony Strength Parameters

Neonicotinoid exposure significantly affected multiple colony strength metrics throughout the growing season (Table 1). Brood area showed progressive divergence between treatment and control groups, with exposed colonies maintaining 23-31% less sealed brood by season end ($F_{3, 8} = 14.7$, $p < 0.001$). The imidacloprid group exhibited the strongest effect, with mean brood frames declining from 7.2 ± 0.3 in April to 4.8 ± 0.4 in September, compared to control maintenance at 6.9 ± 0.3 to 7.1 ± 0.3 frames.

Adult bee populations similarly declined in treated groups, though effects manifested later in the season (Figure 1). By August, neonicotinoid-exposed colonies contained 18-27% fewer bees than controls ($p < 0.05$), with clothianidin showing the largest population deficit. Honey storage was reduced by 15-22% in exposed colonies, potentially reflecting both reduced foraging efficiency and increased metabolic demands from detoxification processes.

Table 1: Colony Strength Metrics by Treatment Group (September 2024)

Parameter	Control	Imidacloprid	Clothianidin	Thiamethoxam	F-value	p-value
Sealed Brood (frames)	7.1 ± 0.3	4.8 ± 0.4	5.2 ± 0.3	5.6 ± 0.4	14.7	<0.001
Adult Bees (×1000)	32.4 ± 1.8	25.1 ± 1.6	23.7 ± 1.5	26.8 ± 1.7	11.2	<0.001
Honey Stores (kg)	18.6 ± 1.2	14.5 ± 1.1	14.8 ± 1.0	15.9 ± 1.3	8.9	0.002
Pollen Stores (frames)	3.8 ± 0.2	2.9 ± 0.3	2.7 ± 0.2	3.1 ± 0.3	9.4	0.001

Values represent mean±SE. Different superscript letters indicate significant differences (Tukey's HSD, $p<0.05$).

Foraging Behavior

RFID tracking revealed substantial alterations in foraging patterns among neonicotinoid-exposed bees (Table 2, Figure 2). Trip duration increased by 34-42% in treated groups ($p<0.001$), suggesting impaired navigation or reduced flight efficiency. Daily trip frequency decreased by 18-25%, resulting in substantially reduced total foraging time per bee. Load sizes (estimated from return weight measurements) were 12-19% smaller in exposed bees, compounding the reduction in colony-level resource acquisition.

Table 2: Foraging Behavior Metrics (August 2024 Average)

Metric	Control	Imidacloprid	Clothianidin	Thiamethoxam
Trip Duration (min)	42 ± 3	59 ± 4	56 ± 5	54 ± 4
Trips/Bee/Day	8.3 ± 0.6	6.2 ± 0.5	6.5 ± 0.6	6.8 ± 0.5
Load Size (mg)	18.4 ± 1.2	15.1 ± 1.0	14.8 ± 1.1	15.9 ± 1.0
Homing Failure (%)	8.2 ± 1.4	24.3 ± 2.1	27.1 ± 2.3	19.4 ± 1.8

Residue Accumulation

Neonicotinoid residues accumulated progressively in hive products throughout the season (Figure 3). By September, 67% of exposed colonies exceeded EU maximum residue limits (MRLs) for at least one compound in either wax or honey samples. Imidacloprid showed the highest accumulation in wax (mean: 12.4 µg/kg), while thiamethoxam predominated in honey samples (mean: 8.7 µg/kg). Residue levels correlated significantly with colony performance metrics: colonies with higher wax residues showed greater brood reduction ($r=-0.62$, $p<0.001$) and adult population decline ($r=-0.58$, $p<0.001$). This dose-response relationship strengthens causal inference regarding neonicotinoid effects.

Discussion

Our findings demonstrate that field-realistic neonicotinoid exposures produce substantial negative effects on honeybee colony health through multiple interacting pathways. The magnitude of observed effects—23-31% brood reduction and 18-27% population decline—exceeds thresholds considered sustainable for colony survival, particularly when compounded by additional stressors during overwintering^[57]. The compound-specific variation in effect strength (imidacloprid > clothianidin > thiamethoxam) aligns with differences in binding affinity to nAChR subtypes and metabolic persistence^[58]. Imidacloprid's longer half-life in plant tissues (40-60 days versus 20-30 days for thiamethoxam) likely contributes to prolonged exposure windows and greater cumulative impacts^[59]. Behavioral alterations provide mechanistic links between individual-level neurotoxicity and colony-level outcomes. Increased trip duration and reduced frequency directly translate to decreased resource acquisition, creating energetic deficits that manifest as reduced brood rearing and honey storage^[60]. Elevated homing failure represents irreversible workforce loss that colonies cannot easily compensate for given the specialized nature of foraging roles and age-related task allocation^[61]. The temporal pattern of intensifying effects suggests that

Notably, homing failure rates—bees departing but not returning within 24 hours—increased from 8% in controls to 19-27% in treated groups ($\chi^2=31.4$, $p<0.001$). This finding aligns with previous reports of neonicotinoid-induced navigation impairment and may partially explain population declines through irreversible forager loss^[56]. Temporal patterns showed that behavioral effects intensified over the season, with August measurements showing larger treatment-control differences than June measurements (treatment × time interaction: $F=4.3$, $p<0.01$), suggesting cumulative or delayed effects rather than acute toxicity alone.

sublethal impacts accumulate over time, potentially through progressive neural damage, immunosuppression, or metabolic exhaustion^[62]. This finding challenges short-term study designs that may underestimate chronic exposure risks and supports regulatory frameworks considering seasonal exposure durations rather than acute toxicity thresholds alone^[63]. Comparison with previous literature reveals consistency in direction but variability in magnitude of effects. Our observed 23-31% brood reduction exceeds the 10-15% reported in some North American field trials but aligns with European studies employing similar methodologies^[6465]. Discrepancies likely reflect differences in landscape context, bee subspecies, and exposure pathways rather than fundamental contradictions^[66]. Limitations include single-season duration preventing assessment of multi-generational effects, focus on one bee subspecies limiting generalizability, and inability to completely isolate neonicotinoid effects from background pesticide exposure in agricultural landscapes^[67]. Future research should incorporate multi-year monitoring, comparative studies across bee species, and evaluation of mitigation strategies such as flowering buffers and IPM protocols^[68]. Policy implications are clear: current regulatory approval processes emphasizing acute LD50 values inadequately capture sublethal chronic effects demonstrated here^[69]. Risk assessment frameworks should incorporate colony-level endpoints, behavioral metrics, and seasonal exposure modeling to better protect pollinator populations essential for agricultural sustainability^[70].

Conclusion

This study provides robust evidence that field-realistic neonicotinoid exposures significantly impair honeybee colony health through multiple pathways including reduced brood production, decreased adult populations, altered foraging behavior, and elevated mortality. Effects varied among compounds with imidacloprid showing strongest impacts, and intensified over the growing season suggesting cumulative toxicity. The findings have important implications for agricultural policy and pollinator conservation. Regulatory frameworks

relying primarily on acute toxicity metrics fail to capture the sublethal chronic effects documented here, potentially underestimating risks to pollinator populations. Evidence-based restrictions on neonicotinoid use in flowering crops, combined with promotion of integrated pest management alternatives, appear necessary to sustain pollinator services critical for food security.

Study limitations include single-season duration and focus on one geographic region and bee subspecies. Future research should examine multi-generational effects, interactions with other stressors including pathogens and climate extremes, and effectiveness of mitigation strategies. Long-term monitoring across diverse agricultural contexts will strengthen evidence bases for policy development.

Despite limitations, this study contributes valuable field-based evidence linking individual-level neonicotinoid neurotoxicity to colony-level population declines through quantifiable behavioral and demographic pathways. Such mechanistic understanding is essential for developing targeted conservation interventions and refining regulatory approaches to balance agricultural productivity with pollinator protection.

Ethical Statement

This study uses a simulated dataset created for academic training purposes. All content is original and newly written. No real-world identities, institutions, or published datasets are misrepresented. This article is intended for academic writing practice and legitimate research preparation only.

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