



International Journal of Research in Agronomy

E-ISSN: 2618-0618
P-ISSN: 2618-060X
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NAAS Rating (2025): 5.20
www.agronomyjournals.com
2025; 8(11): 693-695
Received: 13-09-2025
Accepted: 17-10-2025

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Acute toxicity of glyphosate to the Asian honey bee, *Apis cerana*: Evidence from topical and residue contact bioassays

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DOI: <https://www.doi.org/10.33545/2618060X.2025.v8.i11j.4251>

Abstract

Glyphosate is the most widely used broad-spectrum herbicide worldwide, targeting the plant-specific shikimate pathway but increasingly scrutinized for its potential effects on non-target pollinators. Acute toxicity of Glyphosate 41 SL (Roundup) to the Asian honey bee, *Apis cerana* was evaluated through LC₅₀ estimation using topical and residue contact bioassays. Topical application produced an LC₅₀ value of 6989 ppm, with wide fiducial limits (4623.06 - 10202.70 ppm) indicating that only relatively high concentrations result in 50% mortality under direct exposure. Residue contact exposure yielded a slightly higher LC₅₀ of 7932 ppm (fiducial limits: 4810.39-12810.17 ppm), reflecting reduced toxicity when bees encounter dried herbicide deposits rather than direct spray droplets. The difference between LC₅₀ values for the two exposure routes highlights the influence of application mode on acute lethality. Overall, the LC₅₀ estimates demonstrate low acute toxicity of Glyphosate 41 SL to *A. cerana*, while emphasizing the need to consider potential sublethal effects at lower, field-realistic concentrations.

Keywords: *Apis cerana*, glyphosate 41 SL, LC₅₀, acute toxicity, topical bioassay, residue contact, honey bee safety

Introduction

Honey bees play an indispensable role in natural and agricultural ecosystems through their pollination services, supporting the reproduction of wild flora and enhancing the yield and quality of numerous crops. In Asia, the Asian honey bee, *Apis cerana* Fabricius, is of particular ecological and economic significance due to its wide distribution, effective foraging behavior and adaptability to diverse environmental conditions. Beyond its importance as a managed pollinator, *A. cerana* contributes substantially to the productivity of fruit orchards, plantation crops and forest-based vegetation. However, increasing reliance on chemical weed and pest management practices in modern agriculture has heightened concerns regarding the unintended impacts of agrochemicals on bee health.

Glyphosate-based herbicides (GBHs), particularly formulations such as Glyphosate 41 SL (Roundup) represent the most widely used herbicides globally. Their popularity stems from their broad-spectrum weed control, cost effectiveness and perceived low toxicity to non-target organisms. Glyphosate acts by inhibiting 5-enolpyruvylshikimate-3-phosphate (EPSP) synthase in the shikimate pathway, a metabolic route present in plants, bacteria, and fungi but absent in animals. Owing to this plant-specific mode of action, glyphosate has historically been classified as safe for insects, including pollinators. However, an emerging body of research challenges this assumption, revealing that formulated GBHs may negatively affect honey bees even at sublethal doses. Surfactants, adjuvants, and other inert ingredients in commercial formulations can alter glyphosate bioavailability, enhance penetration, and contribute to toxicity beyond that of the active ingredient alone. Recent studies have demonstrated that exposure to GBHs impairs navigation and homing ability (Balbuena *et al.*, 2015) [3], disrupts the gut microbiome (Motta *et al.*, 2018) [9], and reduces larval survival and development (Dai *et al.*, 2018) [4]. Such effects raise concerns about the potential colony-level consequences of chronic or repeated exposure in agricultural landscapes.

Despite these findings, research on the impacts of glyphosate on *A. cerana* remains limited. Most toxicological assessments have focused on *A. mellifera*, even though *A. cerana* is the dominant pollinator in many parts of Asia and has been reported to exhibit higher sensitivity to certain pesticides (Abrol & Andotra, 2003)^[2]. Given the increasing use of GBHs across Asia and the proximity of treated fields to apiaries, it is crucial to generate species-specific toxicity data that accurately reflect the risk to *A. cerana* populations. To address this knowledge gap, the present study investigates the acute lethal toxicity of Glyphosate 41 SL to worker bees of *A. cerana* under controlled laboratory conditions. Two exposure pathways relevant to field scenarios were examined: topical application, representing direct spray exposure, and residue contact, simulating bee contact with glyphosate-contaminated foliage or surfaces. The study provides LC₁₀, LC₃₀, and LC₅₀ values for both bioassays and contributes essential baseline data for ecological risk assessment of GBHs on *A. cerana*. The findings are expected to support the development of pollinator-safe weed-management strategies and inform regulatory policies aimed at minimizing non-target impacts in agricultural ecosystems.

Material and Methods

The study was conducted using the commercial formulation Glyphosate 41 SL (Roundup®) which contains 41% w/v active ingredient. Stock solutions were prepared in distilled water and serially diluted to obtain the required concentrations, with Triton X-100 added at 1 mL/L as a surfactant. Forager bees of the Indian honey bees, *A. cerana* Fabricius, were collected in test tubes from healthy colonies maintained at the Apiary of the College of Forestry, Ponnampet, Keladi Shivappa Nayaka University of Agricultural and Horticultural Sciences, Shivamogga, Karnataka, India during morning hours when foraging activity was high. The collected bees were transferred to single-use bee rearing cups in the laboratory and allowed to acclimatize for approximately 1 h under controlled conditions of 25±2 °C and 65±5% relative humidity.

Bioassays were conducted following standard operating procedures outlined by OECD (OECD TG 214) and US EPA (US EPA OCSPP 850.3020) guidelines (OECD, 2017; EPA, 2017)^[10, 5]. For topical bioassay, the forager bees collected in the test tubes were anesthetized by chilling at 20 °C for two minutes, and transferred to single-use bee rearing cups (Evans *et al.*, 2009)^[6]. A 1µL droplet of the test solution was applied to the thoracic notum using a calibrated Hamilton® micro-applicator, while controls received distilled water. Residue contact bioassay was determined by applying 5 µL of the test solution onto Whatman No. 1 filter paper discs placed in Petri dishes and allowed them to dry for 1 h. fifteen bees per concentration were exposed with three replications for each of the twelve concentrations tested in both the bioassays. Bees were supplied with 50% sucrose solution and maintained at 25±2 °C and 50-70% RH. Mortality was recorded at 24, 48 and 72 h, and bees unable to right themselves were considered dead. Mortality data were corrected using Abbott's formula (Abbott, 1925)^[1] and dose-mortality relationships were analysed using probit analysis (Finney, 1971)^[7] to estimate LC₅₀ values along with 95% fiducial limits, slope, chi-square and regression parameters using statistical program SPSS version 16.0 software (IBM SPSS, Armonk, New York, USA)

Results and Discussion

The topical application of Glyphosate 41 SL produced a clear dose-dependent increase in mortality of *A. cerana* worker bees.

Probit analysis estimated an LC₅₀ value of 6989 ppm, with wide fiducial limits (4623.06-10202.70 ppm), suggesting relatively low acute toxicity when compared with commonly used insecticides. The corresponding LC₁₀ and LC₃₀ values were 1699 ppm and 3918 ppm, respectively. The slope of the probit line (12.14±0.17) indicated a steep dose-response relationship, while the regression equation ($Y = 2.09x - 8.02$) and chi-square value ($\chi^2 = 53.44$; $df = 10$) reflected an acceptable model fit. In the residue contact bioassay, mortality similarly increased with concentration, but slightly higher doses were required to elicit effects comparable to topical exposure. The LC₅₀ was calculated at 7932 ppm with fiducial limits of 4810.39-12810.17 ppm, while LC₁₀ and LC₃₀ values were 2166 ppm and 4663 ppm, respectively. The probit regression equation for this assay was $Y = 2.27x - 8.87$, with a slope of 11.98±0.19 and a chi-square value of 82.74 ($df = 10$). These results indicate that residue contact exposure is slightly less acute than direct topical deposition, consistent with expected differences in exposure intensity between the two pathways.

The present study provides quantitative evidence on the acute toxicity of Glyphosate 41 SL to *A. cerana* workers through topical and residue contact exposure pathways. Overall, the high LC₅₀ estimates of 6989 ppm (topical) and 7932 ppm (residue contact) indicate that Glyphosate 41 SL exhibits low acute lethality to *A. cerana* under laboratory conditions. These findings are consistent with previous reports suggesting that glyphosate, as a herbicide targeting the shikimate pathway, has limited direct toxicity to adult honey bees (Thompson *et al.*, 2014)^[11]. However, the broad fiducial limits associated with the LC₅₀ values reflect variability in bee responses and underscore the importance of cautious interpretation, particularly under field conditions where environmental factors may influence toxicity.

The slightly higher LC₅₀ observed in the residue contact assay compared with topical application aligns with expected differences in exposure intensity. Direct topical deposition simulates spray drift or accidental hit during herbicide application, representing a high-exposure scenario. In contrast, residue contact more closely mimics real-world foraging on contaminated substrates where exposure may be diluted or limited by cuticular barriers. Similar distinctions between topical and contact assays have been observed in honey bee toxicity evaluations for other herbicides and fungicides (Johnson *et al.*, 2010)^[8].

Although acute mortality was low, emerging literature suggests that GBHs may exert significant sublethal impacts on honey bees even at concentrations far below lethal thresholds. Studies have documented disruptions in navigation and homing ability due to altered neural signaling (Balbuena *et al.*, 2015)^[3], changes in gut microbiota affecting immunity and nutrient assimilation (Motta *et al.*, 2018)^[9], and reduced larval survival (Dai *et al.*, 2018)^[4]. These effects may collectively compromise colony performance and resilience. Thus, while the high LC₅₀ values observed in this study confirm that acute death is unlikely at field-realistic exposure levels, the potential for chronic or sublethal impacts should not be overlooked. The steep slopes (≈12) observed in both bioassays indicate a narrow margin between doses causing low and high mortality, suggesting that once a threshold concentration is surpassed, mortality may rise sharply. Although glyphosate is unlikely to reach such high concentrations in nectar, pollen, or surface residues under field-use scenarios, localized spray drift or improper application may pose transient risks.

Importantly, research on *A. cerana* remains limited compared with *A. mellifera*, despite evidence that the former may be more

vulnerable to certain agrochemicals (Abrol & Andotra, 2003) [2]. Species-specific differences in detoxification enzymes, foraging strategies, and cuticle properties may influence susceptibility to formulated herbicides. The present study contributes valuable baseline toxicity data for *A. cerana* but further research is necessary to assess chronic exposure, colony-level outcomes,

and interactions with other stressors such as pathogens, nutritional deficits, and co-occurring insecticides or fungicides. Overall, the study highlights the importance of evaluating both lethal and sublethal endpoints, as well as considering species-specific sensitivity, for a more accurate ecological risk assessment of GBHs in Asian agricultural landscapes.

Table 1: Dose-response mortality in *Apis cerana* to Glyphosate 41 SL at 72HAT

Particulars	Topical Application	Residue Contact
LC ₁₀ (Fiducial limits)	1699 ppm (513.68 -2914.57)	2166 (421.52 - 3851.27)
LC ₃₀ (Fiducial limits)	3918 ppm (2014.19 -5707.18)	4663 ppm (1960.73 -7096.99)
LC ₅₀ (Fiducial limits)	6989 ppm (4623.06 -10202.70)	7932 ppm (4810.39 - 12810.17)
Slope ± SE	12.14±0.17	11.98±0.19
Regression equation (Y =a+bx)	Y = 2.09x - 8.02	Y = 2.27x - 8.87
Chi-square (χ ²)	53.44	82.74
Degrees of freedom (df)	10	10

Conclusion

The present study demonstrates that Glyphosate 41 SL exhibits low acute toxicity to worker bees of *A. cerana* through both topical and residue contact exposure routes, with LC₅₀ values exceeding 6900 ppm in topical application and 7900 ppm in residue contact bioassays. Although direct lethality is minimal at field-realistic concentrations, the narrow dose-response slopes suggest that higher-than-typical exposure events could induce significant mortality. Moreover, given the growing evidence of sublethal effects of GBHs on honey bee behavior, physiology, and gut microbiota, reliance solely on acute mortality endpoints may underestimate real-world risks. The findings provide essential baseline toxicity data for *A. cerana*, a key pollinator in Asian cropping systems, and support the need for integrated risk assessments that incorporate chronic exposure, colony-level impacts, and interactions with other agricultural chemicals. Promoting judicious use of herbicides, minimizing spray drift, and adopting pollinator-safe weed management practices will help safeguard *A. cerana* populations and sustain pollination services within agricultural landscapes.

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