COMMERCIAL 2,4-D FORMULATION AMINOL® 806 IMPAIRS *IN VITRO*-REARED HONEY BEE LARVAL DEVELOPMENT AND SURVIVAL

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INTRODUCTION: 2,4-dichlorophenoxyacetic acid (2,4-D) is an extensively used agricultural herbicide that can contact non-target organisms through direct (e.g., application) or indirect (e.g., drift) exposure. Risk assessment data demonstrate the hazards of pesticides to honey bees (*Apis mellifera*), corroborating field evidence that agrochemical exposure contributes to global pollinator population declines. Understanding the effects of 2,4-D on honey bee larvae is essential to identify developmental vulnerabilities with potential long-term impacts on colony health. OBJECTIVES: This study aimed to evaluate the acute dietary toxicity of AMINOL® 806, a commercial 2,4-D formulation, on honey bee (*Apis mellifera*) larvae using an *in vitro* rearing system to determine single-dose effects on larval development and survival. MATERIALS AND METHODS: On day 4 post-grafting, *in vitro*-reared larvae were exposed to acute single doses of 2,4-D (5, 10, 25, 50, 75, and 100 µg a.i./larva) via contaminated diet. Mortality (n = 24 larvae per group) was assessed daily until day 7, while the body weight of surviving larvae (n = 6 per group) was measured on day 7 (the final day of larval development). Following exposure, larval survival rates were calculated as the percentage of surviving individuals relative to the initial cohort size. Body weight data were analyzed by one-way ANOVA followed by Tukey's post hoc test for multiple comparisons. Results are presented as mean ± SEM, with statistical significance set at $p < 0.05$ (*). RESULTS AND CONCLUSION: The results demonstrated 100% mortality by day 7 at the two highest concentrations (75 and 100 µg a.i./larva), while the lowest mortality rate (12.5%) occurred at the lowest concentrations (5 and 10 µg a.i./larva). The 25 µg a.i./larva dose resulted in 37.5% mortality, whereas 50 µg a.i./larva caused 62.5% mortality. Therefore, for subsequent LD50 determination, doses between 25–50 µg a.i./larva will be employed in follow-up studies. Exposure to 2,4-D induced dose-dependent reductions in larval body weight across all tested concentrations, with the greatest weight decrease observed at the highest dose (47.41 ± 6.279%). The commercial 2,4-D formulation AMINOL® 806 poses significant risks to honey bee (*Apis mellifera*) populations, inducing both lethal effects and sublethal developmental impairments that may compromise colony sustainability.

Keywords: Apicultural toxicology; Larval toxicity; Pesticide