IN VITRO EVALUATION OF OXIDATIVE STRESS BIOMARKERS IN HONEY BEE (*Apis mellifera*) LARVAE FOLLOWING AMINOL® 806 (2,4-D HERBICIDE) EXPOSURE

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INTRODUCTION: The decline in honey bee populations can lead to environmental and economic losses, which have already been recorded worldwide. Several factors may contribute to this decline, including pathogens, climate change, a decrease in the diversity of bee forage plants, and pesticide exposure. 2,4-D is a pesticide widely used to control broadleaf weeds and is the third most utilized pesticide in Brazil. Despite its extensive use, its effects on bee health remain poorly understood, as it is primarily a herbicide. OBJECTIVE: This study aimed to evaluate the effects of acute dietary exposure to a commercial 2,4-D formulation (AMINOL® 806) on antioxidant enzyme activities in in vitro-reared honey bee larvae. MATERIALS AND METHODS: In vitro-reared bee larvae were subjected to single acute dietary exposures to different doses of 2,4-D (5, 10, and 25 µg a.i./larva) on day 4. Larvae from each test group (n = 6 per group) that were alive on day 7 were used to determine catalase (CAT) and superoxide dismutase (SOD) enzyme activities, as well as thiobarbituric acid reactive substances (TBARS) content. Values were standardized by total protein content and expressed as percentages relative to the control mean. 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: 2,4-D exposure induced dose-dependent antioxidant responses in bee larvae. The intermediate dose (10 µg a.i./larva) significantly increased SOD activity (253.87 ± 11.02%), while the highest dose (25 µg a.i./larva) elevated both SOD (457.15 ± 47.08%) and CAT (332.1 ± 60.21%) activities, indicating an enhanced defense against oxidative stress. Larvae exposed to the highest 2,4-D concentration (25 µg a.i./larva) also showed significantly reduced TBARS levels compared to controls (57.19 ± 2.495%), indicating suppressed lipid peroxidation. Our findings reveal that dietary exposure to the commercial 2,4-D formulation AMINOL® 806 triggers oxidative stress in in vitro-reared honey bee larvae. Given that the larval stage represents a critical developmental period for bees, pesticides affecting this stage require thorough investigation to understand their potential colony-level consequences.

Keywords: Apicultural toxicology; Larval toxicity; Pesticide