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(In The field of Agricultural Entomology)

Effects of imidacloprid and thiamethoxam nanoformulations on the survival and expression of antimicrobial genes of honeybees

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Abstract
Honey bees are among the primary pollinators of plants that encounter agricultural pesticides during foraging in fields and orchards. By the increasing demand for agricultural products, commercial pesticides, and nanoformulations severely apply to eradicate pests. Chronic and acute effects of pesticides, including neonicotinoids, have been well studied on honey bee activities. Still, due to their wide variety in synthesis, the assessment of nanoformulations of these pesticides on the survival and physiology, emphasizing the immune system of honey bees, has not been addressed. With this objective in mind, nanoformulations of imidacloprid and thiamethoxam were prepared. A comparative bioassay study of these formulations with their respective commercial formulations was conducted on young honey bees (1 to 3 days old). Before the bioassay assessment, the physical properties of synthetic nanoformulations were examined using Field Emission Scanning Electron Microscopy (FESEM), Fourier Transform Infrared Spectroscopy (FTIR), and X-ray Diffraction (XRD) analyses to confirm the loading of nanoparticles with the mentioned pesticides. The FESEM analysis revealed that the nanoformulations exhibited the lowest density and aggregation. In the FTIR analysis, characteristic peaks for nano-imidacloprid were observed at 3476.98, 3298.96, 29828.99, 1566.98, and within the 1300-1200 cm range. For nano-thiamethoxam, peaks appeared at 3286.96, 2886.96, 1586.64, and 1264.03 cm. XRD analysis results indicated approximate particle sizes for nano-imidacloprid and nano-thiamethoxam at 61.6 and 56 nanometers, respectively. At pH 5.5, the loading capacity of each pesticide (separately) into the studied carrier (sodium chitosan-alginate) showed a significant increase. At this pH, the release percentage of both pesticides from the carrier continued for up to 24 hours. Bioassay tests were conducted using two methods: oral and contact. The bioassay tests indicated reduced toxicity levels with nano-sized formulations of pesticides. Additionally, honeybee survival rates were examined in comparative treatments between commercial pesticide formulations and nano-formulations. It was determined that commercial formulations negatively impacted honeybee survival more than nano-formulations. Moreover, the expression levels of genes *abaecin* and *defensin2* upregulated with a reduction in pesticide concentration in honeybees. This indirectly suggests they could indicate interference within the honeybee's immune system.
Keywords: Cellular immunity, humoral immunity, antimicrobial peptides, Iranian honey bee, mortality