



DEPARTMENT
OF BIOCHEMISTRY

The Role of Nitric Oxide in Honeybee Immunity: Assessing the Impact of Acetamiprid Exposure

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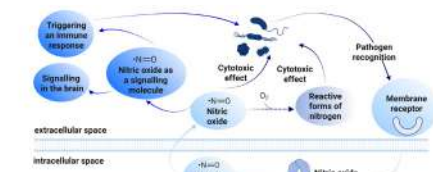
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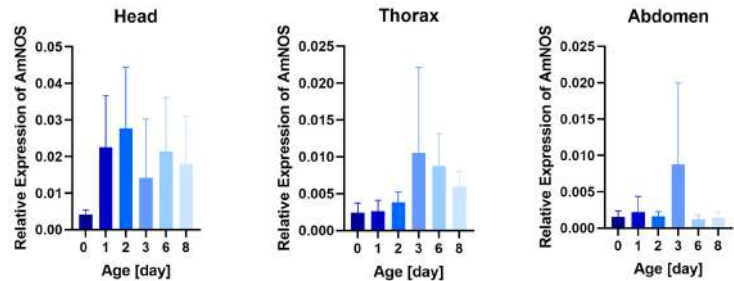
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Role of Nitric Oxide in Bee Immunity



In honey bees (*Apis mellifera*), NO is produced by an enzyme called NO synthase (AmNOS). During the immune response, NO serves as a signal molecule that activates further immune responses and also has a direct cytotoxic effect against pathogens. AmNOS most closely resembles vertebrate neuronal NO synthase, suggesting its presence in the bee head. However, the expression of AmNOS in the bee body and its regulation has not yet been described in detail.

Relative Expression of AmNOS in Physiologically Normal Bees



Physiological levels of AmNOS were measured in normal bees by determining relative gene expression using qPCR method. In the head, expression increased until day 3 and then declined; in the thorax, levels were generally low with a slight rise at day 3; and in the abdomen, expression was low with a transient day-3 peak before decreasing.

Acetamiprid

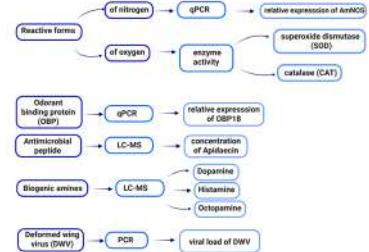


Neonicotinoids are a widely used class of insecticides that target the insect nervous system. Most have been banned in the EU because of their harmful effects on pollinators, but acetamiprid, the only neonicotinoid still permitted for outdoor use, remains widely applied. Even at low, sublethal doses, it can impair honey bee cognitive abilities (learning, memory, orientation), reduce reproductive success, and weaken immune defenses by altering antioxidant enzyme activity and antimicrobial peptide expression, ultimately compromising colony health.

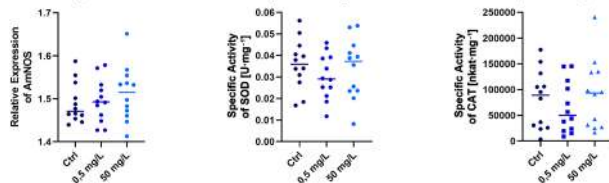
The Scheme of the Experiment



Methods

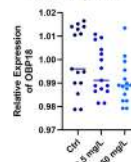


Reactive Nitrogen and Oxygen Species



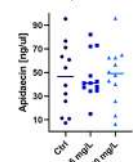
In acetamiprid-exposed bees, expression of AmNOS in bee heads remained unchanged. Activity of superoxide dismutase (SOD), which scavenges superoxide radicals ($2O_2^- + 2H^+ \rightarrow H_2O_2 + O_2$), showed no alterations. Activity of catalase (CAT), responsible for decomposing hydrogen peroxide to water ($2H_2O_2 \rightarrow 2H_2O + O_2$), also exhibited no significant changes. SOD and CAT activities were measured in the abdomens.

Odorant Binding Protein



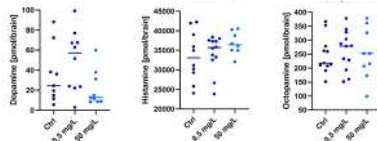
Odorant binding protein 18 (OBP18), involved in odor detection and chemical communication, suggested a trend towards decreased expression in the heads of bees after acetamiprid exposure.

Antimicrobial Peptides



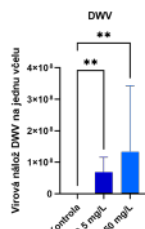
Apidaecin, an antimicrobial peptide involved in immune response, showed no significant changes in concentration in the hemolymph after acetamiprid exposure.

Biogenic Amines



Dopamine is linked to learning, memory, social interaction, and antioxidant capacity; histamine is involved in energy distribution, stress reaction, and olfaction; and octopamine is associated with light reception, mechanoreception, and venom activity. Their concentrations in the brains of bees showed no changes after acetamiprid exposure.

Deformed Wing Virus



Deformed wing virus (DWV), a widespread honey bee pathogen associated with wing deformities and colony losses, was measured in whole bees and showed significantly increased viral loads after exposure to 0.5 mg/L acetamiprid compared to controls. At 50 mg/L, viral loads were further elevated.

Conclusion

Acetamiprid exposure did not significantly affect AmNOS expression, antioxidant enzymes, odorant binding proteins, antimicrobial peptides, or biogenic amines. In contrast, deformed wing virus (DWV) loads were elevated, suggesting that acetamiprid may weaken antiviral defenses. Future work will include additional measurements to validate and extend these findings, with a focus on nitric-oxide-related pathways, including assessment of its key metabolites and related compounds.

Acknowledgements

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“In insect physiology, nitric oxide (NO) plays essential roles in molecular mechanisms underlying physiological processes, including immunity and stress responses. In honeybee immunity, NO acts as both a signalling and an effector molecule toxic to microorganisms. Exposure to neonicotinoid pesticides, such as acetamiprid, can disrupt NO metabolism, potentially affecting immune responses and neural function.

Bee population declines are linked to multiple environmental stressors, including pesticide exposure. Neonicotinoids impair immune function and behaviour, yet their precise effects on NO-related immune and neural functions remain unclear. As NO regulates immunity and neurotransmission, understanding how acetamiprid affects NO pathways is crucial for assessing pesticide-related risks.

Our current findings describe the relative gene expression of nitric oxide synthase gene expression (AmNOS) in physiologically normal honeybees. Using quantitative PCR, we assessed AmNOS expression in the head, thorax, and abdomen of bees aged 1, 2, 3, 6, and 8 days, reared under controlled laboratory conditions. AmNOS expression was highest in the head, where NO primarily functions in neuronal processes. Expression levels increased from day one, peaking in 3-day-old bees, likely reflecting early nervous system development associated with the transition to adult functionality. This increase aligns with elevated NO production, essential for its role as a signalling molecule in neural processes such as long-term memory formation.

Since NO plays a critical role in the nervous system, we extended our study to examine whether acetamiprid exposure disrupts NO regulation, leading to deficits in NO metabolism and associated physiological changes. To address this, we measured changes in gene expression and quantify antimicrobial proteins, oxidative and nitrative stress markers, and key immune pathway proteins. Additionally, biogenic amine levels were analysed to assess potential neurochemical disruptions.

Understanding how pesticides affect NO metabolism in bees can help beekeepers make informed decisions about hive management and pesticide exposure. This knowledge can contribute to developing targeted strategies for mitigating pesticide-induced stress, optimizing colony health, and supporting regulatory choices to reduce their impact on pollinators.

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