Identification of Biomarkers for Immuno-Ecotoxicity in PAMP-challenged *Tribolium castaneum* larvae using Transcriptome Analyses

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Background

Climate change, invasive species and habitat fragmentation pose major challenges for the stability of ecosystems. It is assumed that in stressed ecosystems, pathogens in particular exert strong pressure on populations[1]. In addition, the organisms in the environment are exposed to a large number of directly or diffusively released environmental pollutants such as pesticides or drugs. The ecotoxicological hazard and risk assessment of chemicals, which aim to assess the impact of chemicals in the environment, currently do not include the identification of effects regarding the immunocompetence of organisms[2]. However, studies indicate that chemicals can induce various effects on immune related parameters[3].

Immune challenge development

Currently, there are no reliable methods to identify immuno-ecotoxic modes of action (MoA) in the activated immune system of environmental organisms. The present project aims at the identification of reliable markers for the MoA of immunotoxic chemicals in *Tribolium castaneum* larvae. In a first step, a suitable immune challenge method was established via microinjection of Pathogen Associated Molecular Patterns (PAMPs) into larvae to induce an immune system activation:

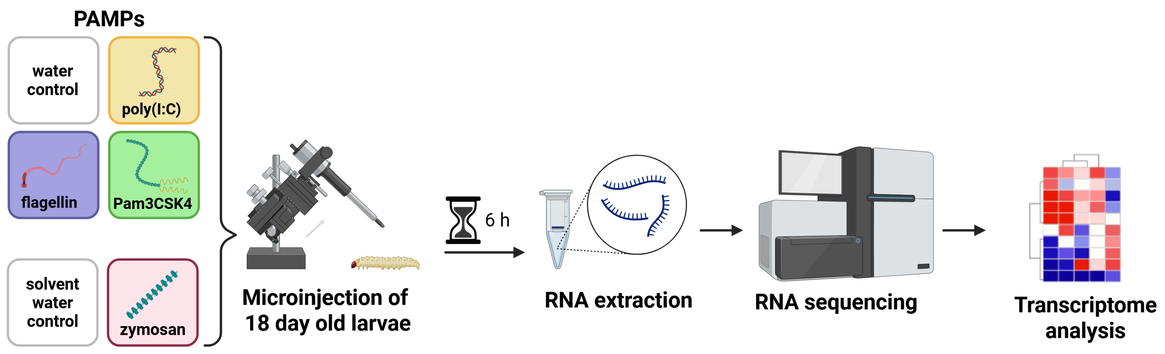

Figure 1: Scheme of the immune challenge experiment performed to identify a PAMP that induces an immune reaction in Tribolium castaneum *larvae (created in BioRender).*

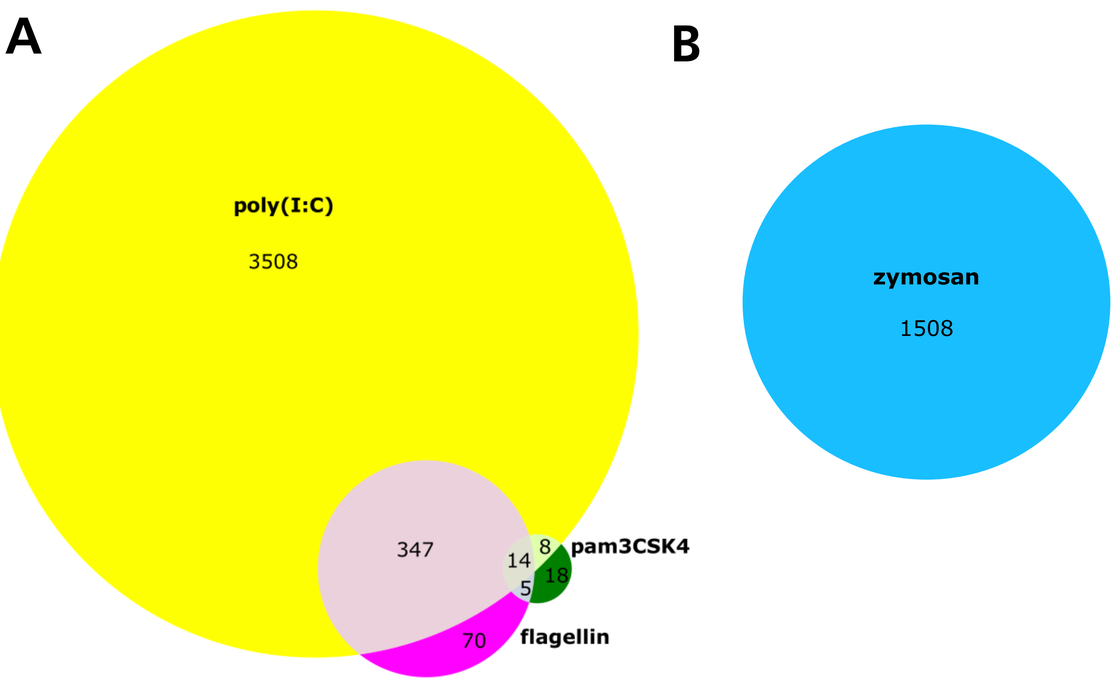

Figure 2: Venn diagrams showing the significantly (padj < 0.05) differentially expressed genes (DEGs) of the different PAMP-injection treatments A: poly(I:C), flagellin, Pam3CSK4 in comparison to water control, B: zymosan in comparison to solvent control.

→ All PAMPs induced **differential gene expression**
→ The highest DEG numbers were found for **poly(I:C)** and **zymosan**

Next step:
Development of a pipeline for **overrepresentation analysis** of affected biological functions

Exposure method development

To investigate the influence of chemicals on immune system activation, immune challenge will be combined with previous chemical exposure. To ensure chemical uptake by larvae, exposure of 13 days old larvae via food for 5 days was tested using fipronil as model substance.

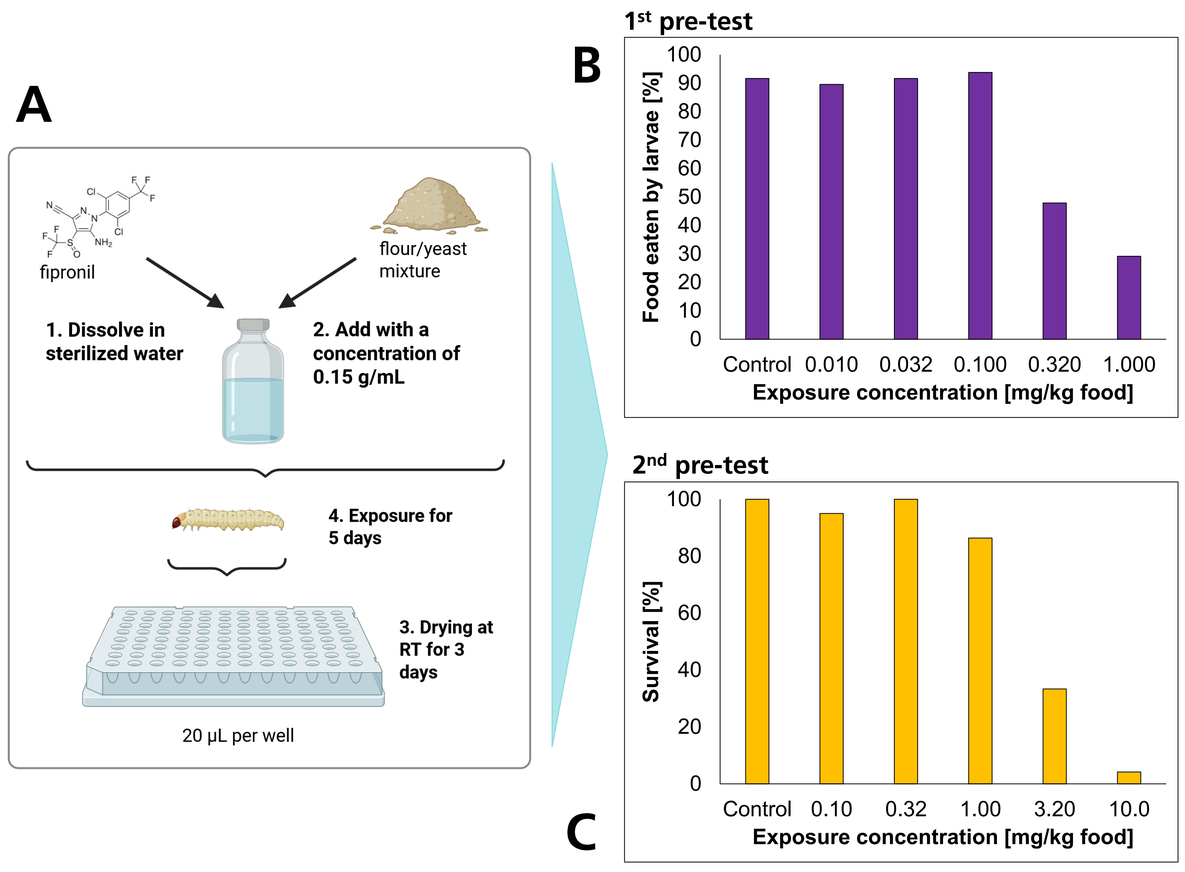

Figure 3: A: Scheme of the test set-up for chemical exposure of Tribolium castaneum *larvae (created in BioRender), B: Results of 1st pre-test (food consumption), C: Results of 2nd pre-test (mortality).*

Fipronil exposure via food resulted in reduced feeding (1st pre-test) and mortality (2nd pre-test).

→ **Exposure method is suitable for main testing**

Outlook

In the main tests, the chemical exposure will be combined with the immune challenge. As exposure chemicals, substances with known MoA will be used to distinguish between immunomodulation and other MoA. However, no chemicals are known that are specifically designed to primarily interfere with the insect immune system. For this reason, chemicals are used that affect the vertebrate immune system and whose targets are also found in insects, e. g. mTOR inhibitors (rapamycin, everolimus).

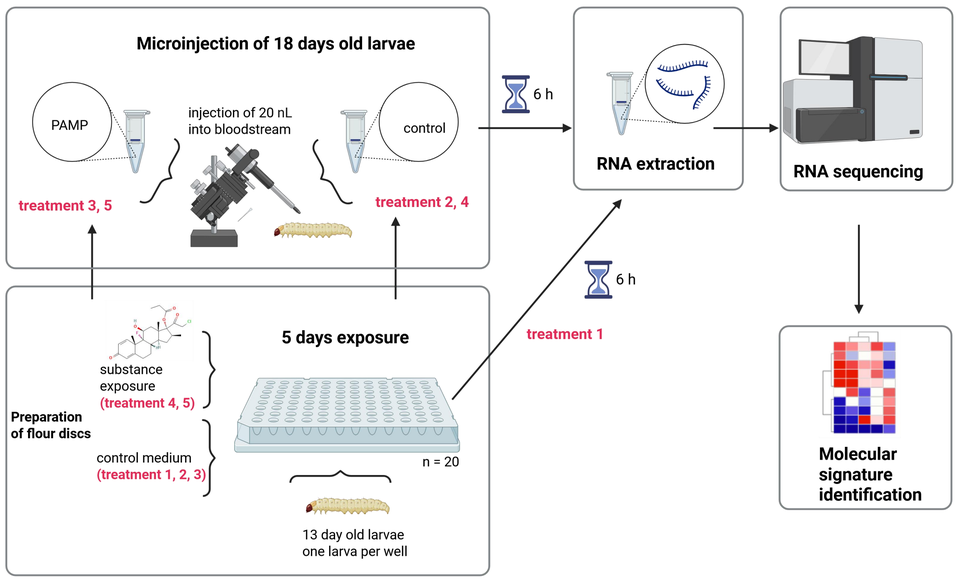

Figure 4: Scheme of the main test set-up which combines chemical exposure and immune challenge of Tribolium castaneum *larvae (created in BioRender).*

[1]Vicente-Santos, Amanda; Willink, Beatriz; Nowak, Kacy; Civitello, David J.; Gillespie, Thomas R. (2023): Host-pathogen interactions under pressure: A review and meta-analysis of stress-mediated effects on disease dynamics. In: Ecology letters 26 (11), S. 2003–2020. DOI: 10.1111/ele.14319.
[2]Segner, Helmut; Wenger, Michael; Möller, Anja Maria; Köllner, Bernd; Casanova-Nakayama, Ayako (2011): Immunotoxic effects of environmental toxicants in fish - how to assess them? In: Environmental science and pollution research international 19 (7), S. 2465–2476. DOI: 10.1007/s11356-012-0978-x.
[3]Fabrizio Lisi, Marcel Amichot, Nicolas Desneux, Jean-Luc Gatti, Raul Narciso C. Guedes, Francesco Nazzi, Francesco Pennacchio, Agatino Russo, Francisco Sánchez-Bayo, Xingeng Wang, Lucia Zappalà, Antonio Biondi, Pesticide immunotoxicity on insects – Are agroecosystems at risk?, Science of The Total Environment, Volume 951, 2024, 175467, ISSN 0048-9697, https://doi.org/10.1016/j.scitotenv.2024.175467.