OMICs- based biomarker selection for difenoconazole and metalaxyl toxicity in zebrafish embryos.

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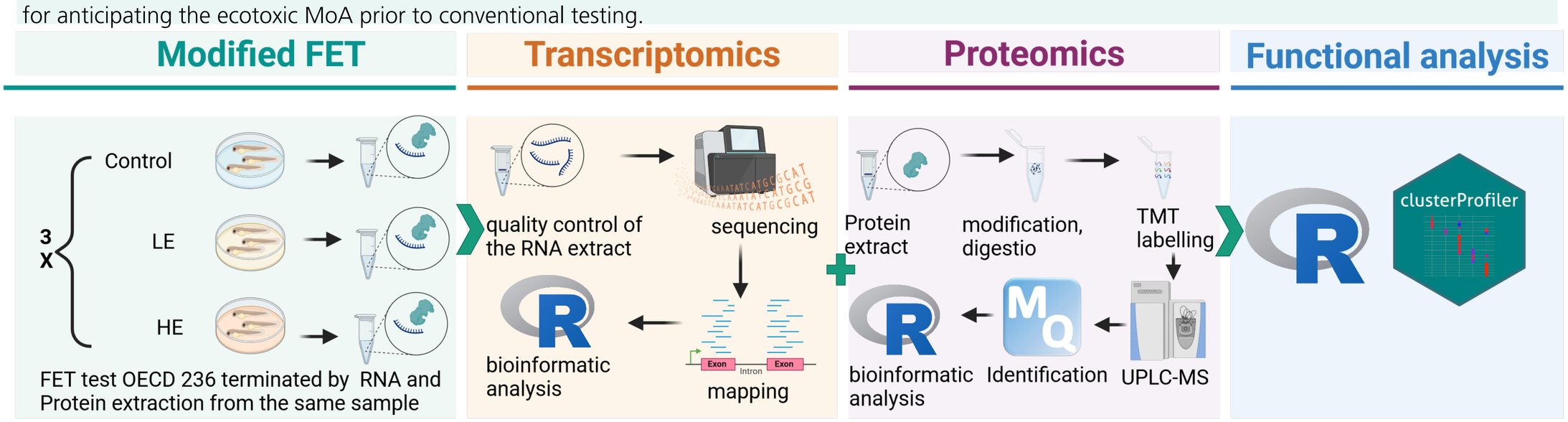
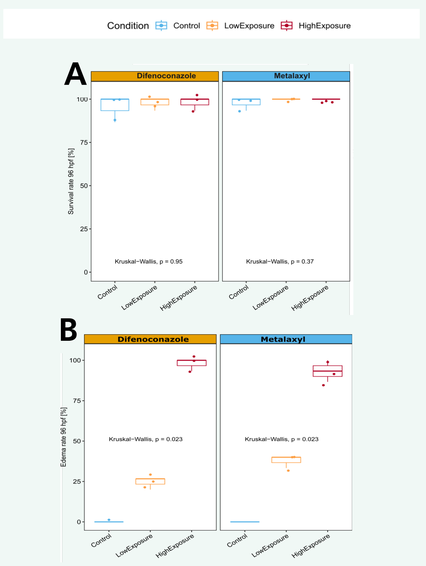
Background

A number of previous studies have examined the adverse effects of fungicides on non-target organisms inhabiting the ecosystem, and the findings of these studies highlighted the potential of fungicides to interfere with biological processes of organisms beyond fungi. The conventional testing strategies for evaluating ecotoxicity of chemical substances is increasingly time-consuming and costly. Moreover, conventional hazard assessment strategies offer limited insights into molecular and biological mechanisms underlying ecotoxicity. The integration of OMICs techniques in ecotoxicology can enhance our understanding of key events (KE) and key event relationships in an Adverse Outcome Pathways (AOPs). Our present study focuses on investigating early molecular responses in zebrafish embryos to the fungicides difenoconazole (sterol biosynthesis inhibitor) and metalaxyl (nucleic acid metabolism inhibitor), with the goal of identifying mode of action (MoA)-specific biomarker candidates, Which will potentially lead to the development of a predictive approach for anticipating the ecotoxic MoA prior to conventional testing.

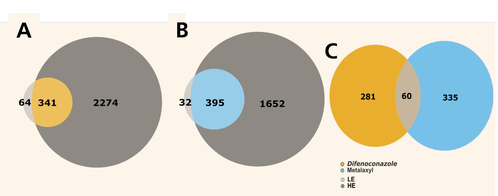
Methodology



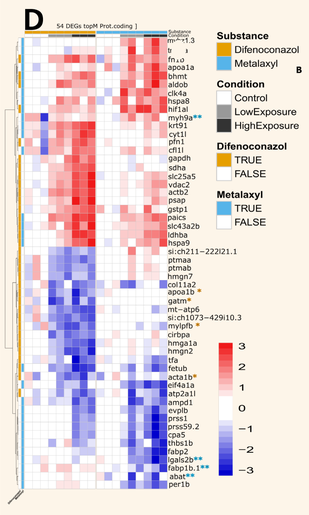
Results



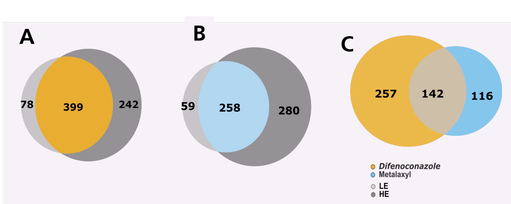
Physiological endpoints of zebrafish embryo toxicity test after 96 hours exposure to different concentrations of fungicides as compared to non-treated controls. **(A)** survival rate as an endpoint compared to corresponding controls. **(B)** rate of phenotypic malformations as an endpoint represented in yolk sac edema compared to corresponding controls.



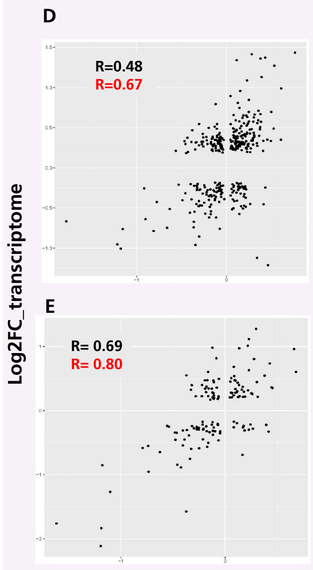
Transcriptome response in each condition for each fungicide. **(A and B)** and the overlap between the core DEG sets among the tested fungicides. **(C).**



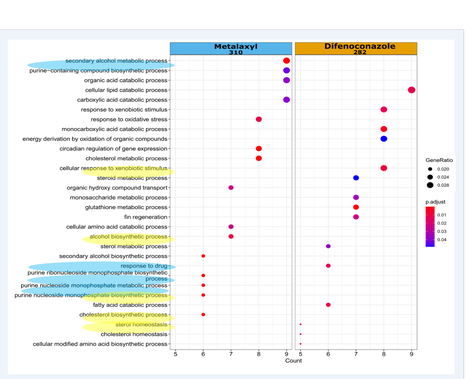
(D) Heatmap showing relative **gene expression patterns** of zebrafish embryos after exposure to sublethal concentrations of fungicides. The selected biomarkers are labelled with stars for difenoconazole (yellow) and metalaxyl (blue).



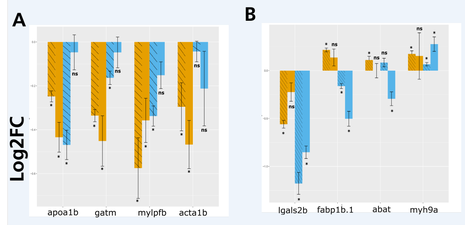
Proteome response in each condition for each fungicide. **(A & B)** and the overlap between the core DEP sets among the tested fungicides **(C).**



Transcriptome and proteome data correlation. For **(D)** difenoconazole and **(E)** metalaxyl. And for the core DEGs for each fungicide (labelled in red)



Overrepresented **biological processes** in GO terms from the core differentially expressed genes for each fungicide.



Bar plot showing the HE log2-fold change values of corresponding transcriptome and proteome for the selected **representative biomarker** candidates of **(A)** difenoconazole and **(B)** metalaxyl.

Conclusion

The most significant outcomes of this study were as follows: (1) molecular responses indicated concentration-dependent responses for both fungicides at both transcriptome and proteome levels. **(2)** ORA pointed out a number **of biological processes** influenced by the overrepresented gene sets, and some of the indicated biological processes were biologically related to the FRAC MoA of the corresponding tested fungicide. **(3)** Some DEG sets showed **substance-specific expression patterns**, from which potentially **substance-specific biomarker candidates** were selected for difenoconazole **(apoa1b, gatm, mylpfb and acta1b)** as well as metalaxyl **(lgals2b, abat, fabp1b.1 and myh9a). (4)** The **biological functions of the selected biomarker candidates** were also related to the FRAC MoA ofthe corresponding fungicide. **(5)** A correlation between the detected transcriptome and proteome was recorded.

OECD (2013), *Test No. 236: Fish Embryo Acute Toxicity (FET) Test*, OECD Guidelines for the Testing of Chemicals, Section 2, OECD Publishing, Paris,

Marghany, F., Ayobahan, S. U., Salinas, G., Schäfers, C., Hollert, H., & Eilebrecht, S. (2024). Transcriptomic and proteomic fingerprints induced by the fungicides difenoconazole and metalaxyl in zebrafish embryos. *Environmental Toxicology and Pharmacology*, *105*, 104348.

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