

Reviewer 1.

This work aimed to identify genes potentially associated with etofenprox resistance in *Sogatella furcifera*. The authors performed a bioassay to identify the most etofenprox-resistant field population. Once the field population with the highest sensitivity to etofenprox was identified, the respective procedures were carried out to investigate molecular mechanisms of resistance such as voltage-gated sodium channel mutations by complete sodium channel sequencing and identifying candidate resistance genes. The sensitive population was subdivided to expose the organisms to two doses of etofenprox and subsequently, by RNA-seq, differences in gene expression in general were determined, and DGE for CYP, CCE, CP, ABC, UGT and GST gene families. Potential mutant sites in the nucleotide sequence the sodium channel that produce resistance to pesticide in insect were located, but changes were not observed WBPH. Differences in gene expression in general were observed. The 25-ppm dose showed significant differences with NT, while NT did not differ in gene expression with 100 ppm. The greatest differences in DEG were observed in organisms exposed to 25 ppm of etofenprox.

Comments:

The study is very interesting and presents very valuable information for understanding the molecular mechanisms of resistance through detoxification. There are one element that I think is very important to review and clarify, is regarding plasticity. I think it is important to mention in the introduction whether the authors consider it as a mechanism of resistance to insecticides and especially how they define plasticity for the study, since it seems to me that it can confuse the reader interested in the evolution of plasticity as a characteristic or byproduct of natural selection (see DeWitt and Scheiner 2004, phenotypical plasticity, functional and conceptual approaches, Oxford University Press).

Response:

We sincerely appreciate your time, thoughtful comments, and valuable suggestions, which will improve the quality and clarity of our manuscript. Based on your valuable comments and suggestions we have revised our manuscript using the track changes tool.

We appreciate your insightful comment regarding the need to clarify the use of plasticity in the context of our study. In response, we have revised the introduction to define plasticity as it relates to our findings explicitly. In this study, we use plasticity to refer to the reversible, dynamic changes in gene expression that occur in response to environmental stimuli, such as insecticide exposure. These changes do not involve permanent genetic mutations but reflect an organism's ability to adapt metabolically through altered expression of detoxification-related genes, such as CYPs, GSTs, and UGTs.

To prevent confusion, we have distinguished this functional plasticity from the evolutionary concept of plasticity as a heritable trait shaped by natural selection, as discussed in DeWitt and Scheiner (2004). The revised introduction and result sections (Kindly see lines 94-100 and 302-360) now clearly delineate this context, ensuring it aligns with the study's focus on metabolic resistance mechanisms rather than evolutionary plasticity.

Comments:

It is also important to ensure that the complementary tables and figures are available. I did not find access to them and the information could not be checked.

Response:

When submitting the manuscript draft, we uploaded supplementary data as a separate file, but this problem probably occurred due to a technical error or our mistake. We have now ensured that all complementary tables and figures are included as supplementary files with the submission. These materials contain detailed data on various analysis related to the manuscript. We have also cross-referenced these materials within the main manuscript text to improve accessibility and ensure readers can easily locate and interpret the additional information.

Below are some specific observations

Comment: Line 82, I think a few lines are needed to say why it is important to demonstrate resistance to etofenprox

Response:

Thank you so much for your valuable suggestion. We have added the suggested information on the revised manuscript from lines 84-86.

Comment: 95, Is it correct that the last three insecticides have 1B associated with them? If so, what do the numbers and letters in parentheses mean? Also, check that there is congruence between the figure caption and the information given in the text, in the current presentation they are not completely congruent.

Response:

Thank you for pointing this out. The classification (1A and 1B) represents the insecticide's mode of action (MoA) group as per the Insecticide Resistance Action Committee (IRAC) guidelines. **1A** refers to carbamates, which inhibit acetylcholinesterase. In comparison, **1B** refers to organophosphates, which also act as acetylcholinesterase inhibitors but belong to a different chemical class. Please see lines 106-109 of the revised manuscript. Thank you. Furthermore, in this section (2.1) of the results, we have only mentioned and discussed the tables, not the figures.

Comment: 00-101. What result does this article show "no WBPH-susceptible strains to etofenprox?", I am not clear on the relevance of the idea, it seems out of place. Perhaps the idea is "this result does not allow evaluating resistance since it does not include lines with different susceptibility to etofenprox."

Response:

Thank you so much for your meticulous point. We have revised the sentence to avoid any confusion. Please see lines 113-114 of the revised manuscript.

Comment: 104, delete "further"

Response:

Deleted, thank you. Please see line 118 of the revised manuscript.

Comment: 119, I suggest that you indicate what the numbers and literals in parentheses in the first column mean.

Response:

Thank you for your detailed advice, we believe that it would enhance the clarity of our manuscript and will make it reader-friendly. We added the relevant information as a comment to **Table 1**. Please see lines 134-136 of the revised manuscript.

Comment: 121, add the probability of Chi-square, and explain what SR means

Response:

Thank you so much for pointing out these important points. We have edited **Table 2** as you advised. Please see line 138 of the revised manuscript.

Comment: 154-158- Please review this sentence, is a bit confusing. The sentence "To ensure the reliability of RNA-seq data for gene expression analysis, sequencing was performed on three distinct WBPH populations..." seems somewhat inconsistent with the goals of estimating resistance and molecular mechanisms of resistance. Is the dose assay really intended to ensure that the RNA-seq information is good or true? Or did the authors use RNA-seq to explore molecular mechanisms of resistance, for example gene overexpression? I assume they expected the level of expression to correlate positively with etofenprox doses. I consider that reliability of the RNA-seq was assessed by the three biological replicates.

Response:

We have revised the sentence to avoid confusion. Kindly see lines 168-172 of the revised manuscript. Thank you.

204-206, It is the same information or the same data, so the idea is confusing or ambiguous.

Response:

We have revised the sentence to avoid confusion. Kindly see lines 215-229 of the revised manuscript. Thank you.

Comment: 251, what is MA plot

Response:

We have added the information regarding the MA plot in the revised manuscript. Kindly see lines 266-274 of the revised manuscript. Thank you.

Comment: 280, I find it very interesting to include the perspective of plasticity. But it causes me some uncertainty and I have doubts, since no background is presented in the introduction. Phenotypic plasticity, which in a strict sense has been defined as the capacity of a genotype to express different phenotypes depending on an environmental gradient (DeWitt and Scheiner 2004). Here the differential expression of genes in three subpopulations was studied, however it has not been said whether the populations subjected to different doses have the same genotype. It is advisable to briefly explain the perspective of plasticity used in this study, so that the reader can assess the study in the general context of the study of phenotypic plasticity.

Response:

We have included the suggested information regarding the plasticity in both the introduction and result sections of the manuscript and we hope that it will enhance the clarity of our manuscript and will make it reader-friendly. Kindly see lines 94-100 and 302-360 of the revised manuscript. Thank you.

Comment: 424, Please indicate the relationship between etofenprox and sulfoxaflor...

Response:

Thank you for your comment. We accept that there is no direct relationship between etofenprox and sulfoxaflor. Therefore, in the revised manuscript we have revised the sentence to clarify that the consistency lies in the observed sublethal effects on WBPH rather than a relationship between the two insecticides. Kindly see lines 474-478 of the revised manuscript.

Comment: 457, I suggest that the authors discuss further why 25 ppm differs from NT, and why NT and 100 ppm does not differ, those results seem very strange or counterintuitive to me.

Response:

We can only make assumptions because we haven't yet found any explicit references for this part. We guess that if the reaction threshold is exceeded, metabolism is not activated.

At 25 ppm, it is a concentration sufficient to detect and react to foreign substances and activate metabolism, such as detoxification enzymes. However, at a high concentration of about 100 ppm, it is difficult to activate this system, so this phenomenon seems to occur.

Further research is ongoing, and a more comprehensive review will be possible once we understand more about detoxification enzyme activation and the entire detoxification system.

Kindly see lines 508-517 of the revised manuscript.