LIST OF CHANGES AND ANSWERS TO REVIEWERS

Ms. Ref. No.: molecules-4498453

Title: *Piper fuligineum* (Piperaceae) essential oil on Euschistus heros: lethal and sublethal toxicities and selectivity to an egg parasitoid used in applied biological control

Dear Editor,
Thank you for your careful review of our manuscript and for your comments. The manuscript has been revised according to your suggestions, as detailed below. The corrections have been highlighted in red text.

Responses to reviewers

Reviewer 2

Reviewer 2: The LC50 and LC90 values in Table 2 should be carefully checked.

There is an obvious inconsistency between the text and Table 2. For example, the LC50 value of 1-day-old eggs is reported as about 1,100 mg L^{-1} in the text, but Table 2 shows 0.11. This may be a calculation or decimal error. The authors should carefully check all LC values based on the original data and confirm that the units are correct in the text, tables, and figures.

Response: We thank the Reviewer for this correction in the table. The values in the table were originally in percentage and were changed to mg L^{-1} to align with the results presented in the text. Please confirm the lines 155-159.

Reviewer 2: The identification of 1-naphthalenol needs further confirmation.

The authors reported that 1-naphthalenol was one of the major compounds (9.88%) in EOPF. However, this compound is unusual as a major component in Piper essential oils. The authors should clarify whether this compound was confirmed by retention index and/or standard compounds, and discuss whether it could be related to analytical artifacts during extraction or GC-MS analysis.

Response: We thank the reviewer for this important observation. In this study, the chemical constituents of EOPF were identified by comparing their mass spectra with those available in commercial spectral libraries, as described in the manuscript. Using this approach, 1-naphthalenol was identified and represented 9.88% of the total identified

constituents. Therefore, it was considered one of the main constituents detected in the essential oil.

Importantly, our results do not indicate that 1-naphthalenol originated from contamination during the extraction procedure or from analytical artifacts related to the GC-MS analysis.

The presence of different chemical constituents, including compounds that are not commonly reported as major components of *Piper* essential oils, may be related to the marked chemical variability observed in this genus. This variability can be influenced by several factors, such as geographic location, soil and climate conditions, seasonality, plant developmental stage, genetic background, cultivation practices, and post-harvest conditions. Therefore, the chemical profile found in this study may reflect the specific environmental and biological conditions under which the plant material was grown.

This point has been clarified in the revised manuscript.

Reviewer 2: The chemical standardization of EOPF should be discussed.

Essential oils are natural products with high variation depending on plant origin, extraction method, and environmental conditions. Although the authors identified several major compounds, the manuscript does not discuss how EOPF can be standardized as a botanical pesticide. I suggest the authors discuss possible chemical markers and the relationship between chemical composition and insecticidal activity.

The practical application value of EOPF needs further discussion.

The insecticidal activity was mainly evaluated under laboratory conditions using relatively high concentrations. Whether these concentrations are realistic for field application should discuss. Some information about field efficacy, persistence, formulation, and possible phytotoxicity to soybean plants would improve the manuscript.

Response: We appreciate the assistance in improving the discussion. These suggestions were added in lines 339–362, as were the additional references in lines 612–627.

Reviewer 2: The conclusion about IPM compatibility should be reconsidered.

The evaluation of EOPF effects on T. podisi is an important part of this study. However, the results showed that EOPF negatively affected parasitism and adult emergence, especially at higher concentrations. In addition to toxicity, the authors should discuss the strong repellency observed in free-choice tests. Avoidance behavior of parasitoids may reduce the efficiency of biological control in IPM

programs. Therefore, EOPF should be considered as a potential botanical insecticide candidate, but its compatibility with biological control requires further evaluation.

Response: We have rewritten the conclusion. Please check lines 494–498.

Reviewer 2: Table 1 in the submitted manuscript appears incomplete. Please provide the complete table.

Response: The current table presents 19 constituents and their retention indices, as well as the percentage of each constituent found in the essential oil. Please check lines 97–99.

Reviewer 2: Chemical names should be consistent throughout the manuscript. For example, "caryophyllene" and "Cariofilene" should be corrected.

Response: We thank the reviewer for the correction. We made the change to Table 1 (lines 97–99).

Reviewer 2: The description of parasitoid bioassay in Section 4.3.3.2 needs language correction for clarity.

Response: We have rewritten section 4.3.3.2 in lines 446–463.

Reviewer 2: The typo in the abstract "40.00 mg L^{-1}" should be corrected to "40.000 mg L^{-1}".

Response: We thank the reviewer for the correction. We made the change to the line 31.

Reviewer 2: Please check Figure 3. The positive control (Engeo Pleno™ S) appears missing in some panels.

Response: We re-examined Figure 3 and observed that the Engeo Pleno™ S control is present in all four panels (lines 172-178).

Reviewer 2: "Egg sensitivity" (Line 256-257) should be changed to "egg susceptibility".

Response: The expression “egg sensitivity" has been replaced with the standard entomological term “egg susceptibility” in line 256-257.

Reviewer 2: The reference format should be checked carefully according to the requirements of Molecules.

Response: We verified the references to comply with Molecules' standards (lines 517-643).