

# Using deep learning to assess the toxicological effects of sublethal exposure of a novel green pesticide in a stored-product beetle

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## Abstract

**BACKGROUND:** Managing stored-grain pests requires new strategies to limit economic and health risks. This study analyses the sublethal effects of the natural compound carlina oxide on *Prostephanus truncatus*, providing new behavioural insights through a multidisciplinary approach. A fully automatic computer vision approach was developed to label two specific insect body parts, enabling the generation of an annotated dataset without manual intervention. This dataset was used to train a convolutional neural network (CNN) for pose estimation. A second dedicated CNN focused on the antennae to investigate neuroethological and sensory variations.

**RESULTS:** CNN for body parts detection achieved an average precision of 0.78, recall of 0.90, and F1 score of 0.84 on the test dataset. An additional CNN tracked key points for antennal pose estimation. Motor analysis showed that the LC<sub>30</sub> of carlina oxide reduced average speed and distance, induced altered exploratory behaviour, and affected thigmotaxis. Statistically significant features were evaluated using machine learning classifiers: random forest, support vector machine, and K-nearest neighbours. The analysis comparing control and treated groups distinguishes LC<sub>30</sub> and LC<sub>10</sub> from the control group, while SHapley Additive exPlanation (SHAP) analysis explained the features contribution to predictions.

**CONCLUSIONS:** Metrics poorly distinguish individuals in the LC<sub>10</sub> and LC<sub>30</sub> classes, supporting the employment of lower sublethal concentration for the control of *P. truncatus*. However, our findings indicate possible neuroethological effects of green pesticides on sensory systems, highlighting the need for an accurate risk assessment to minimize ecosystem impacts and supporting integrated pest management within One-Health and Eco-Health frameworks.

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**Keywords:** *Prostephanus truncatus*; carlina oxide; natural control; motor behaviour; machine learning

## 1 INTRODUCTION

*Carlina acaulis* L. (Asteraceae) is a perennial herb growing in Central and Southern Europe that is used as a traditional medicine due to its various actions (e.g. diuretic, anti-inflammatory, and anti-cancer) or as food.<sup>1–4</sup> The root essential oil (EO) of *C. acaulis* and its main compound (>90%), the polyacetylene carlina oxide, exhibit antibacterial, antifungal, antitrypanosomal, antiproliferative, and antinematode properties.<sup>5–10</sup> They have already been explored as novel green pesticides against several stored-product pests<sup>11,12</sup> and field pests such as *Ceratitidis capitata* (Wiedemann), *Philaenus spumarius* (L.), the main vector of *Xylella fastidiosa*,<sup>13,14</sup> and *Metopolophium dirhodum* (Walker).<sup>15</sup> Interestingly, carlina oxide caused low toxicity to the non-target species *Aphidoletes aphidimyza* Rondani and *Chrysoperla carnea* Stephens,<sup>15</sup> an issue that further promotes this substance as green pesticide. Furthermore, Spinozzi *et al.*<sup>16</sup> documented that carlina oxide did not exert significant toxicity against keratinocytes,

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