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Research Article

# Early Behavioral Indicators of Mortality Risk in Pyrethroid-Exposed Bees Using Explainable Artificial Intelligence

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**Abstract**

Pollinator populations, which play a critical role in maintaining global ecosystem health, have been experiencing marked declines worldwide due to widespread pesticide usage. However, early behavioral indicators of lethal stress induced by chemical exposure remain insufficiently characterized, largely because conventional ecotoxicological assessments predominantly focus on mortality-based endpoints. In this study, we evaluated the potential to predict mortality risk at an early stage using behavioral markers, based on 1.506 behavioral observation records collected from seven bee species exposed to lambda-cyhalothrin. To this end, we implemented explainable artificial intelligence models, including Random Forest, XGBoost, and LightGBM, and interpreted the model outputs using SHAP analysis. Among these models, Random Forest and XGBoost demonstrated the strongest performance in distinguishing high mortality risk, achieving an accuracy of 0.873 on an independent test dataset. SHAP-based model interpretation revealed a temporal behavioral progression associated with elevated mortality risk: cramps and apathy emerged as early warning indicators (2–4-hour window), uncoordinated movement represented the intermediate phase, and the dorsal recumbent position characterized the terminal collapse stage. These findings demonstrate that behavioral early-warning signals of lethal pesticide stress can be reliably detected prior to mortality and highlight the potential of explainable artificial intelligence as a robust decision-support tool for pollinator health monitoring and pesticide risk assessment.

**Keywords:** Behavioral biomarkers, Explainable AI, Pollinator health, Pyrethroid ecotoxicity, Sublethal effects

## Introduction

Animal pollinators are essential biotic agents that support the reproductive processes of flowering plants, thereby sustaining ecosystem functioning and enhancing agricultural productivity. Among them, bees represent one of the most widespread and efficient pollinator groups globally. With more than 20.000 recognized species, bees contribute to the reproductive success of nearly 90% of flowering plant species and significantly enhance the yield of numerous agricultural crops [1]. Approximately one-third of global food production directly depends on bee-mediated pollination [2], and the annual economic value of these pollination services is estimated to exceed 200 billion USD [3,4]. Despite their ecological and economic importance, the pollination services provided by bees are facing increasing global threats. A growing body of evidence indicates a persistent and rapid decline in pollinator populations, particularly among bee species, driven by intensified anthropogenic pressures [5,6]. Although multiple interacting drivers contribute to these declines -including habitat loss, climate change, parasites, and pathogens- widespread pesticide use in agricultural landscapes has emerged as a pervasive and acute stressor. Exposure to neurotoxic agrochemicals has been shown to severely impair bee navigation, foraging behavior, immune function, and reproductive biology, thereby accelerating colony collapse and contributing to the decline of wild bee populations [5,7].

Pesticide exposure, one of the most significant threats to bee health, remains insufficiently integrated into comprehensive environmental management strategies. Current regulatory frameworks continue to rely predominantly on acute, mortality-based endpoints, which hinders the incorporation of early behavioral warning signals into risk assessment processes. Yet, exposure to sublethal pesticide doses can disrupt navigation, social organization, and colony-level functioning well before mortality or colony collapse becomes apparent [8]. Michelangeli et al.[6] emphasized that such sublethal effects may play a critical, yet frequently overlooked, role in long-term population declines and argued for the more systematic integration of behavioral indicators into ecological risk evaluation. However, quantitative and temporally explicit analyses of behavioral progression under toxic stress remain limited. Most existing studies focus on static behavioral markers or isolated reaction patterns, making it difficult to capture the sequential and emergent neuro-motor disruptions induced by chemical exposure.

In this context, the early detection of pesticide-induced ecological impacts not only increases the sensitivity of environmental risk assessment frameworks but also substantially improves the timing and effectiveness of interventions aimed at safeguarding pollinator health. To overcome the limitations of traditional observational approaches and systematically analyze early behavioral signals, data-driven computational methods such as Artificial Intelligence (AI) and Machine Learning (ML) have increasingly been integrated into ecotoxicological research in recent years [9,10]. These approaches not only enhance predictive performance but also possess the capacity to learn multivariate behavioral patterns and model their temporal progression under toxic stress [11]. However, the inherently "black-box" nature of many ML algorithms -where decision-making processes are not directly interpretable- poses challenges for biologically grounded inference and limits their reliability for regulatory decision-makers. To address this issue, Explainable Artificial Intelligence (XAI) techniques, particularly SHAP (SHapley Additive exPlanations) and LIME (Local Interpretable Model-agnostic Explanations), provide model-agnostic and model-specific interpretability frameworks that elucidate not only what the model predicts but also why and how [7]. By enabling transparent attribution of relative feature contributions to model outcomes, XAI approaches allow pesticide-related behavioral disruptions to be interpreted in a mechanistically meaningful way. Thus, XAI establishes a new methodological axis in ecotoxicology by coupling predictive accuracy with biological interpretability, which is critical for the early identification of environmental stress signals.

This study introduces an innovative modeling framework that integrates XAI approaches into ecotoxicology to enable the early detection of behavioral stress responses induced by pesticide exposure. We analyzed a dataset comprising 1.506 individual behavioral observations representing lambda-cyhalothrin exposure across seven bee species -*Andrena vaga, Bombus terrestris, Colletes cunicularius, Osmia bicornis, Osmia cornuta, Megachile rotundata,* and *Apis mellifera*- using powerful tree-based machine learning algorithms, including Random Forest, XGBoost, and LightGBM. Following mortality risk classification, model decision pathways were interpreted using SHAP analyses, which allowed us to explicitly quantify the directionality and relative importance of behavioral indicators contributing to elevated mortality risk. This integrative approach not only achieved high predictive accuracy but also enabled the characterization of the behavioral progression underlying pyrethroid-induced lethality.

# Material and Methods

## Ethical Statement

This study did not involve any procedures requiring ethical approval.

## Data Source and Experimental Context

This study is based on the re-analysis of an open-access dataset comprising 1.506 individual behavioral observations that document the time-resolved effects of lambda-cyhalothrin exposure on seven bee species: *Andrena vaga*, *Bombus terrestris*, *Colletes cunicularius*, *Osmia bicornis*, *Osmia cornuta*, *Megachile rotundata*, and *Apis mellifera*. The data were originally collected by Jütte et al.[12] under standardized cage test conditions, with behavioral and mortality assessments recorded at 2, 4, 24, 48, 72, and 96 hours post-exposure. Detailed experimental procedures, including rearing conditions, exposure protocols, and scoring criteria, are described comprehensively in the original publication. The dataset used in this study is publicly available through the OpenAgrar Repository: *https://www.openagrar.de/receive/openagrar_mods_00092232*.

## Mortality Rate and Behavioral Variable Encoding

At each observation time point, colony-level mortality was calculated using the number of dead and surviving individuals. Specifically, the mortality rate was obtained by dividing the number of dead individuals by the total number of individuals observed (dead + alive).

To assess the ability of behavioral indicators to predict mortality outcomes, this continuous mortality measure was converted into a binary risk variable based on toxicological decision thresholds:

- Low Risk: Mortality <30%
- High Risk: Mortality ≥30%

This threshold was selected to represent biologically meaningful colony-level stress while still enabling early detection of behavioral deterioration preceding terminal mortality, consistent with growing emphasis on sublethal and functional endpoints in ecotoxicological risk assessment.

For each individual, seven behavioral indicators reflecting the neuro-motor progression of pesticide-induced decline (moribund, cramps, apathy, uncoordinated, restless, dorsal, and vertigo) were evaluated. Following the scoring procedure described by Jütte et al.[12], these variables were treated as numerical features in the analysis. In cases where behavioral scoring captured intensity rather than simple presence/absence, the quantitative grading was retained in the modeling process. This allowed the relationship between behavioral progression and mortality risk to be evaluated in terms of both occurrence and severity.

### Data Preprocessing

Prior to analysis, the dataset was processed to prevent bias during model training. Missing values in the behavioral variables were imputed using a median-based approach that preserves interspecific variance structure. To prevent differences in measurement scales from disproportionately influencing model decisions, all explanatory variables were standardized using z-transformation (mean = 0, standard deviation = 1). To ensure an objective evaluation of model performance, the dataset was partitioned into training (75%) and testing (25%) subsets using a stratified sampling strategy that maintains both class proportions and representation across species. This approach was specifically selected to avoid artificially inflating or diminishing the model's discriminative ability in cases where the high-mortality class contains comparatively fewer observations.

### Machine Learning Algorithms and Hyperparameter Optimization

To evaluate the extent to which behavioral indicators can predict high mortality risk, three ensemble tree-based machine learning algorithms were applied. Random Forest constructs multiple decision trees on bootstrap-resampled subsets of the training data and aggregates their predictions through majority voting, thereby reducing overfitting and improving generalization performance [13]. XGBoost employs a gradient boosting framework that iteratively refines decision trees to minimize residual errors, and incorporates L1/L2 regularization and parallel computation, enabling high accuracy and efficiency in large and imbalanced datasets [14]. LightGBM further enhances computational efficiency by converting continuous variables into histogram bins and applying a leaf-wise tree growth strategy, which facilitates fast and stable learning in high-dimensional feature spaces [15].

Hyperparameter optimization for each model was conducted using stratified 5-fold cross-validation to maximize predictive performance. To maintain sensitivity, particularly for the high-mortality class, class imbalance was addressed using algorithm-specific weighing strategies: class_weight = 'balanced' for Random Forest, scale_pos_weight = (neg/pos) for XGBoost, and class_weight = {0:1, 1:k} for LightGBM. The key hyperparameters used in the final models are summarized in *Table 1*.

### Model Explainability

To examine the direction and magnitude of each behavioral indicator's contribution to model decisions, SHAP analysis was conducted on the XGBoost model. The SHAP beeswarm plot visualized both the sign (positive or negative influence) and the relative effect size of each behavior on mortality risk, thereby revealing a coherent behavioral progression from early to intermediate and terminal stages of collapse in accordance with the model's internal decision structure.

***Table 1.*** *Machine learning models used in this study and their key hyperparameters*

| Model | Hyperparameters |
|---|---|
| Random Forest | n_estimators=500, max_depth=None, class_weight='balanced' |
| XGBoost | n_estimators=600, learning_rate=0.05, max_depth=4, scale_pos_weight=(neg/pos) |
| LightGBM | num_leaves=31, learning_rate=0.05, class_weight={0:1, 1:k} |

### Performance Evaluation

Multiple evaluation metrics were used to comprehensively assess model performance. While accuracy indicates the overall proportion of correctly classified observations, it is not sufficient on its own when class distributions are imbalanced. Therefore, performance was primarily interpreted through Precision, Recall, and F1-score, which more accurately represent the model's ability to distinguish the high-mortality class. Precision reflects the proportion of individuals predicted as high mortality that were correctly classified, whereas Recall represents the proportion of actual high-mortality individuals that were successfully identified by the model. The F1-score, calculated as the harmonic mean of Precision and Recall, is particularly appropriate for imbalanced datasets. In addition, Support values were reported to indicate the number of instances in each class, allowing these metrics to be contextualized relative to class prevalence. This evaluation strategy prioritizes the reliable early detection of high mortality risk, rather than solely maximizing overall accuracy.

### Computational Environment and Libraries

All analyses were conducted in Python 3.11 using Google Colab. Data processing and management were performed with pandas and NumPy, and visualizations with Matplotlib. The Random Forest model was implemented using scikit-learn, while gradient boosting classifiers were trained with XGBoost and LightGBM. Model explainability was assessed using the SHAP library to identify the contribution of behavioral predictors to mortality risk. All code and analytical steps were executed in a fully reproducible workflow.

## Results

*Table 2* summarizes the performance metrics of the classification models developed to predict high mortality

***Table 2.*** *Early warning classification performance of Random Forest, XGBoost, and LightGBM models based on behavioral predictors*

| Model | Accuracy | Precision (High) | Recall (High) | F1 (High) |
|---|---|---|---|---|
| Random Forest | 0.873 | 0.538 | 0.778 | 0.636 |
| XGBoost | 0.873 | 0.545 | 0.667 | 0.600 |
| LightGBM | 0.762 | 0.333 | 0.667 | 0.444 |

risk based on behavioral response variables. The Random Forest model achieved the highest overall accuracy (87.3%), with a recall of 0.778 for the high-mortality class, indicating strong sensitivity in identifying high-risk cages. XGBoost yielded a similar overall accuracy (87.3%), with a recall of 0.667 and an F1-score of 0.600 for the high-mortality class. In comparison, LightGBM demonstrated lower performance in distinguishing the high-mortality class (F1 = 0.444). These results indicate that sublethal behavioral alterations contain detectable signals associated with increased mortality risk, supporting the potential of behavior-based approaches for early warning assessment.

The confusion matrices shown in *Fig 1-A,B,C* provide a detailed comparison of the models' ability to detect the high-mortality class (y = 1). The Random Forest model correctly classified 77.8% of high-mortality cases (7 out of 9), indicating strong sensitivity in identifying high-risk cages. XGBoost correctly identified 66.7% of high-mortality cases (6 out of 9). In contrast, LightGBM also identified 66.7% of high-mortality cases but produced a higher number of false positives in the low-mortality class. For the low-mortality class (y = 0), both Random Forest and XGBoost maintained high classification accuracy (RF: 48/54; XGB: 49/54), whereas LightGBM showed reduced performance (42/54). These results reflect higher true-positive detection in the high-mortality class for Random Forest and XGBoost compared to LightGBM.

SHAP analysis was performed to interpret model outputs and identify the behavioral patterns associated with high mortality risk *(Fig 2)*. The SHAP distribution plots revealed not only the relative importance of each behavioral variable but also the direction of their contribution to mortality classification. Higher values of

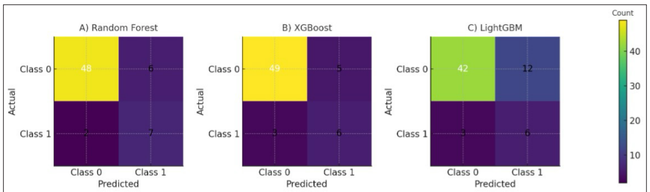


**Fig 1.** Confusion matrices for the three classification models: A- Random Forest, B- XGBoost, and C- LightGBM, illustrating classification performance for the high-mortality (Class 1) and low-mortality (Class 0) groups

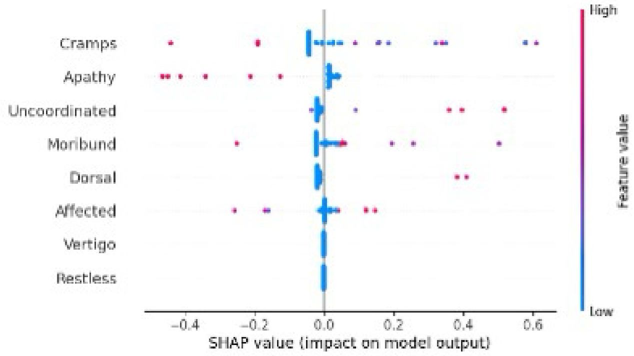


**Fig 2.** SHAP beeswarm plot illustrating the contributions of behavioral variables to the classification of high-mortality cases

cramps and apathy were predominantly associated with negative SHAP values, indicating that the increase of these behaviors contributed to model predictions of high mortality risk during earlier neuromuscular impairment stages. In contrast, higher values of uncoordinated and dorsal were concentrated in the positive SHAP region, reflecting their stronger association with later-stage loss of motor coordination and postural control. For the moribund variable, SHAP contributions appeared across both positive and negative regions, consistent with its occurrence during rapidly transitioning terminal phases. Taken together, the SHAP patterns indicate a sequential progression of behavioral decline associated with elevated mortality risk, transitioning from cramps → loss of coordination → dorsal posturing → moribund state.

# Discussion

Our classification models demonstrated high sensitivity in detecting impending colony-level mortality based on sublethal behavioral alterations. Among them, the Random Forest model achieved the strongest performance, correctly identifying the majority of high-mortality cases (recall = 0.778; accuracy = 87.3%). XGBoost showed comparable overall accuracy (87.3%) with moderately lower sensitivity (recall = 0.667), while LightGBM yielded lower discriminative performance for the high-mortality class (accuracy = 76.2%; F1 = 0.444). Notably, both Random Forest and XGBoost maintained low false-negative rates, minimizing the likelihood of failing to detect cages experiencing severe toxic stress. In the context of early warning systems, prioritizing sensitivity over precision is a strategically appropriate trade-off, as the consequences of overlooking a high-risk colony are substantially greater than issuing a false alert. These results suggest that subtle yet consistent behavioral signals emitted during the early stages of stress can be decoded by machine learning models to provide actionable early risk detection. Accordingly, the behavioral patterns captured in this study may serve as valuable indicators for timely intervention in managed colonies.

The SHAP explainability analysis revealed a clear sequential structure in the behavioral signature associated with lethal pesticide stress. Higher values of cramps and apathy were associated with negative SHAP contributions, indicating their prominence during early stages of neuromuscular impairment. In contrast, elevated uncoordinated and dorsal scores contributed positively to mortality predictions, reflecting later-stage collapse characterized by loss of motor coordination and postural control. The moribund behavior showed mixed SHAP contributions across both positive and negative regions, consistent with its occurrence during rapidly transitioning terminal phases. Together, these patterns indicate a cascading progression of neuromuscular decline: early muscle spasms and reduced activity → loss of coordination → failure to maintain dorsal posture → terminal moribund state. This staged behavioral collapse aligns with known acute neurotoxic responses reported in bees and bumblebees, where pesticide exposure initially disrupts coordination and elevates agitation, followed by reduced mobility and postural failure under sustained stress [16]. Our machine-learning-derived behavioral signature therefore reflects established toxicodynamic processes, but presents them within a unified, temporally ordered framework. The identification of this structured progression is a novel contribution, suggesting that monitoring the sequence of behavioral anomalies may provide richer diagnostic insight than evaluating behaviors as isolated symptoms. Future work could validate this cascade across additional species and stressors, yet the present findings already advance our understanding of how pesticide-induced neurotoxicity unfolds behaviorally in pollinator systems.

From an ecological perspective, these findings are particularly significant. Insect pollinators function as keystone species, supporting approximately 75% of global crops and 88% of wild flowering plant species [17]. Declines in bee populations therefore pose direct risks to global food security and ecosystem resilience. Recent research has identified contemporary pesticide use -especially neonicotinoids and pyrethroids- as a major driver of these declines [8]. For example, Guzman et al. reported that increasing neonicotinoid and pyrethroid use across the United States was associated with substantial reductions in wild bee occupancy, exceeding 40% in some groups [17]. Similarly, Dicks et al.[18] emphasized that pollinator conservation is essential for ecosystem stability and human well-being, noting that pesticides contribute to pollinator declines not only via acute toxicity but also through indirect sublethal effects. Within this context, our behavioral prediction framework has clear conservation relevance: it enables the detection of lethal pesticide impacts before colony collapse becomes visible. Such early warning capability may provide beekeepers and ecologists with a practical decision window in which to relocate colonies, adjust pesticide application timing, or implement mitigation measures to preserve pollination services. These results therefore reinforce the growing scientific consensus that sublethal behavioral indicators should be integrated into pollinator health monitoring systems. As Ulrich et al.[19] argue, social insects such as bees are keystone species in terrestrial ecosystems, and AI-enabled behavioral monitoring holds considerable potential for ecological protection and restoration. By providing a measurable link between environmental stressors and colony outcomes, our findings contribute to this emerging direction and support proactive pollinator conservation strategies.

From a practical standpoint, these findings highlight pathways for implementing behavior-based monitoring in pollinator health assessment. Ongoing work in Precision Apiculture already leverages non-invasive sensing and machine learning to track colony conditions. For example, acoustic monitoring systems can automatically classify hive states [20], and computer vision platforms have been developed to continuously record behavioral activity over extended periods [19]. Likewise, Hossain and Baer introduced an "Electronic Bee Veterinarian" framework that uses temperature sensors and predictive modeling to alert beekeepers to colony stress -whether thermal, pathological, or pesticide-related- several days in advance [21]. The behavior scoring approach developed in this study could be integrated into such frameworks. A practical monitoring protocol could combine internal or external hive cameras with computer vision pipelines to quantify key behavioral metrics such as muscle spasms, mobility patterns, and postural stability. These quantified metrics could then be supplied to the machine learning classifier to generate early risk alerts. Such alerts may prompt targeted inspections or mitigation measures, including modifying pesticide application, providing supplemental nutrition, or relocating colonies to safer foraging environments. Importantly, integrating multiple data streams -such as temperature, acoustic activity, and behavioral indicators- will likely yield the most robust early warning systems.

Although this study focused on a pyrethroid insecticide, which is among the most widely used neurotoxic compounds in agricultural systems, the proposed behavior-based early warning framework is not inherently chemical-specific. While behavioral trajectories may vary across pesticides with different modes of action, core manifestations of neurotoxic stress such as impaired coordination, postural instability, and reduced activity are expected to represent convergent functional endpoints, supporting the broader applicability of the approach.

In summary, this study demonstrates that machine learning can transform subtle behavioral disruptions into interpretable and actionable indicators of lethal stress. This advances pollinator monitoring beyond passive observation and toward a new generation of digital, responsive, and preventative colony health management.

# Declarations

**Availability of Data and Materials:** The dataset used in this study is publicly available on OpenAgrar at https://www.openagrar.de/receive/openagrar_mods_00092232.

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**Ethical Statement:** This study did not involve any procedures requiring ethical approval.

**Competing Interest:** The author declares that there are no competing interest.

**Declaration of Generative Artificial Intelligence (AI):** The author declare that the article, tables and figures were not written/ created by AI and AI-assisted Technologies.

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