American Journal of Biology and Natural Sciences
https://biojournals.us/index.php/AJBNS
ISSN: 2997-7185

The Efficacy of Aqueous and Alcoholic Extracts of Yas Plant (Eriobotrya Japonica) Leaves on Mortality of Larval and Adult Stages of Rusty Flour Beetle (Tribolium Castaneum)

Zainab M. Abbas, Fatima Kareem Shandookh
Wasit University, College of Science, Department of Biology, Wasit, Iraq

Received: 2024, 15, Apr
Accepted: 2025, 21, May
Published: 2025, 19, Jun

Annotation: The increasing demand for sustainable pest management strategies has led to a growing interest in the development and application of natural bioactive compounds as alternatives to synthetic insecticides. This study evaluates the insecticidal potential of aqueous and alcoholic leaf extracts derived from Eriobotrya japonica (commonly known as Yas plant or loquat) against both larval and adult stages of the rusty flour beetle, Tribolium castaneum —a major pest of stored grains and cereal products. The investigation employed probit analysis , using two complementary statistical approaches: Finney's method assuming a lognormal distribution , and least squares probit regression based on a normal distribution , to estimate lethal dose thresholds (LD_{10}, LD_{50}, LD_{90}, LD_{100}) across two exposure durations (24 and 48 hours).

Results revealed that adult beetles exposed for 48 hours exhibited the highest sensitivity to the stimulus, with an LD_{50} value of 4.7367 units (95% confidence

interval: 3.9561–5.8424). In contrast, larvae showed significantly greater tolerance , particularly after 24-hour exposure, where mortality rates did not increase appreciably even at higher concentrations. Notably, extending the exposure period to 48 hours did not markedly enhance larval mortality, suggesting intrinsic physiological resistance mechanisms such as reduced cuticle permeability, enhanced detoxification pathways, or sequestration of toxic compounds.

Model fit was statistically acceptable across all experimental groups, as evidenced by non-significant chi-square tests and high p-values (>0.8) , indicating strong agreement between observed and expected responses. Both analytical methods yielded consistent results, reinforcing the reliability of the estimated lethal doses.

These findings demonstrate that Eriobotrya japonica leaf extracts exhibit time- and dose-dependent toxicity , particularly effective against adult T. castaneum . The observed differential susceptibility among developmental stages underscores the importance of tailoring application strategies based on life cycle dynamics. Given biodegradability low mammalian toxicity and demonstrated efficacy E. japonica holds promise as a botanical insecticide in integrated pest management (IPM) programs aimed at reducing reliance on chemical pesticides.

This study contributes to the expanding body of research on plant-derived insecticides provides a quantitative framework for evaluating biological responses to external stimuli offering

practical insights for sustainable pest control in agricultural and storages systems.

Keywords: Eriobotrya japonica loquat, botanical insecticide, Tribolium castaneum, probit analysis, LD_{50}, stored product pests, biological control, integrated pest management.

1. Introduction

Insect pest pose significants threats to agricultural productivity food security and ecosystem balance globally. Among them, *Tribolium castaneum* (Herbst) commonly known as the rusty flour beetle a cosmopolitan pest that infests stored grains and cereal products [1]. The development of eco-friendly alternatives to synthetic chemical pesticides have become increasingly important due to environmental concerns and the growing problem of insect resistance [2]

Botanical-based insecticides have emerged as promising tools in sustainable pest management [3]. *Eriobotrya japonica* (Thunb.) Lindl., locally referred to as Yas plants is traditionally used for its medicinal properties and contains bioactive compounds such as flavonoids, alkaloids, and terpenoids [4]. However, its efficacy as an insecticide especially against stored-grain pests like *T. castaneum* remain underexplored.

Probit analysis is a standard statistical method used in toxicological studies to model the relationship between stimulus dosage and the probability of mortality or growth inhibition [5]. It allows estimation of lethal doses (e.g., LD_{50}, LD_{90}), which are crucial for evaluating the potency of bioactive agents [6]. Two complementary methods was used in this study:

- ✓ Finney's Method: Assumes a lognormal distribution of responses.
- ✓ Least Squares Probit Regression Based on a normal distribution model.

Understanding the differential susceptibility among life stages (adult vs. larva) and exposure durations (24 vs. 48 hours) is essential for developing targeted pest control strategies [7]. Several studies have shown that the developmental stage significantly influences susceptibility to toxins, with adults often being more vulnerable than immature forms [8].

This research aims to:

- ✓ Evaluate the insecticidal activity of aqueous and alcoholic leaf extracts of E. japonica
- ✓ Estimate lethal dose thresholds using probit analysis.
- ✓ Compare susceptibility among life stages and exposure durations.
- ✓ Assess model fit and reliability for accurate interpretation of results.

Such insights provide a quantitative framework for assessing beetle response to external stimuli, with potential applications in integrated pest management and toxicological research [9].

1.1 Background and Significance

The Order Coleoptera includes some of the most diverse and ecologically important insect species. While many beetles play beneficial roles in ecosystems such as aiding decomposition and pollination others are notorious agricultural pests [1]. Effective management of these pests requires a deep understanding of their biology and responses to environmental stressors [2].

Traditional pest control strategies heavily rely on synthetic chemical insecticides. However

overuse of these chemicals has led to severe consequences including environmental contamination non-target organism toxicity, and the emergence of resistant pest populations [7]. In response there has been a global shift toward exploring safer and more sustainable alternatives, particularly those derived from natural sources [3].

Botanical insecticides offer a promising solution due to their biodegradability low mammalian toxicity and multi-target mechanisms of action, which reduce the likelihood of resistance development [3]. Plants such as *Azadirachta indica* (neem) *Chenopodium ambrosioides* and *Eriobotrya japonica* have demonstrated insecticidal activity through various modes of action, including feeding deterrence, growth disruption, and neurotoxic effects [4].

Among these *Eriobotrya japonica* , commonly known as loquat or Yas plant, has been widely studied for its medicinal properties, including antimicrobial, antioxidant, and anti-inflammatory activities [4]. However, its potential as an insecticidal agent remains largely unexplored, particularly against economically important pests like *Tribolium castaneum*

Tribolium castaneum is a major pest of stored grain commodities worldwide. Its ability to thrive in dry storage conditions and reproduce rapidly makes it a persistent threat to food security [1]. Control measures typically involve fumigant and contact insecticides; however resistance to conventional insecticid been reported in several regions necessitating alternative approaches [8].

Probit analysis provid a robust statistical framework for evaluating dos-response relationships in biological systems. Originally developed by Bliss (1934) this method transforms binomial response data into a continuous scale, allowing for precise estimation of effective or lethal doses required to achieve a defined level of effect (e.g., LD_{50}, LD_{90}) [6] Finney's method, which assumes a lognormal distribution of responses, is one of the most widely used approaches in entomology and toxicology [5].

In contrast least squares probit regression offers an alternative approach assuming a normal distribution of responses Comparing results from both models enhances confidence in finding and supports better decision-making in pest control programs [6].

Several studies have successfully applied probit modeling to evaluate the efficacy of botanical insecticid against pests such as mosquito aphids and stored product insects [10]. These studies highlight the importance of standardized methodologies and careful interpretation of model outputs to ensure reliable conclusions

1.2 Objectives

This research aims to:

- Estimate lethal dose thresholds (LD_{10}, LD_{50}, LD_{90}, LD_{100} for *Eriobotrya japonica* leaf extracts affecting *Tribolium castaneum* at different developmental stage
- Compare sensitivity between adult beetles and larvae
- Evaluate differences in response based on exposure duration (24 vs. 48 hour)
- Assess model fit using chi-square statistics and p-values.
- Provide a foundation for future applications in pest control and toxicological research.

By systematically analyzing the dose-dependent effect of *E. japonica* extracts across life stages and exposure times, this study contributes to the growing body of knowledge on botanical insecticides and their role in sustainable agriculture and pest management.

2. Materials and Methods

2.1 Experimental Design and Sample Preparation

This study investigated the effects of a stimulus on adult beetles (*Coleoptera*) and their larval stage over two exposure durations (24 and 48 hours). The experimental design followed standard

procedures for insect bioassays as described by Robertson et al. [11], with modifications to accommodate both developmental stages and time-dependent responses.

Each beetle cohort was exposed to five increasing concentrations of the stimulus, with each group containing 18 individuals (N = 18) per dose level. Mortality or growth inhibition was recorded after 24 and 48 hours of exposure. The response was calculated as a percentage of affected individuals relative to the total number per dose group.

The actual percent response data were then subjected to probit transformation and analyzed using two statistical methods:

- ✓ Finney's method, which assumes a lognormal distribution of responses.
- ✓ Least squares probit regression, based on a normal distribution model.

All analyses were conducted using Excel-based tools and probit analysis software packages designed for biological assays [12].

This dual-stage and dual-duration approach allowed for comparative assessment of sensitivity across life cycles and time points, providing insights into optimal application strategies in pest control settings [13].

2.2 Plant Material and Extraction Procedure

Fresh leaves of *Eriobotrya japonica* (Yas plant) were collected from local farms during the growing season, authenticated by a taxonomist, shade-dried, and ground into fine powder. Two types of extracts were prepared:

Aqueous Extract:

- ✓ Powdered leaves (50 g) were boiled in 500 mL distilled water for 30 minutes.
- ✓ The solution was filtered using Whatman No. 1 filter paper.
- ✓ The filtrate was concentrated under reduced pressure using a rotary evaporator at 40°C.
- ✓ Stock solutions were prepared at 10% concentration and serial dilutions (0.1%, 0.5%, 1.0%, 2.0%, and 5.0%) were made for bioassay experiments.

Alcoholic Extract:

- ✓ Powdered leaves were macerated in 70% ethanol (v/v) for 72 hours at room temperature.
- ✓ The mixture was filtered and the solvent was removed under vacuum using a rotary evaporator.
- ✓ Residual extract was dissolved in sterile distilled water to prepare stock solutions.
- ✓ Serial dilutions were similarly prepared for testing.

All extracts were stored in dark glass vials at 4°C until use to prevent degradation of bioactive compounds [14].

2.3 Insect Culture and Bioassay Conditions

Adults and larvae of *Tribolium castaneum* (Herbst), commonly known as the rusty flour beetle, were obtained from a laboratory-reared culture maintained under controlled conditions: 28 ± 2°C temperature, 65% relative humidity, and a 12-hour light/dark photoperiod.

Healthy, active individuals were selected for the experiment. Each cohort (N = 18 per dose) was placed in Petri dishes lined with filter paper and treated with different concentrations of the aqueous and alcoholic leaf extracts. Controls received only distilled water or ethanol without plant material.

Exposure periods were set at 24 and 48 hours. After each time interval, mortality was recorded by

observing lack of movement upon probing with a fine brush. Larvae showing no response to mechanical stimulation were considered dead [15].

Mortality percentages were calculated as follows:

Percent Mortality=(Total Number ExposedNumber of Dead Individuals)×100

All experiments were repeated three times to ensure reproducibility.

2.4 Probit Analysis: Finney's Method (Lognormal Distribution)

Finney method is a widely used approach in toxicological study to estimate lethal doses (LDs) such as LD_{50}, LD_{90}, and LD_{100} [16]. This method involves transforming the observed mortality percentages into probit values using standard tables or computational algorithms [17].In this study the logarithm base 10 of the stimulus concentration was plotted against the corresponding probit-transformed response percentages. A linear regression line was fitted to the data points, from which key parameters were derived:

- Beta (β): Slope of the regression line, indicating the steepness of the dose response curve
- Intercept: The value of the regression line when log(dose) = 0
- Confidence intervals (CI): Estimated at $\alpha = 0.001$ to ensure high precision in LD estimation
- Lethal dose thresholds (e.g., LD_{10}, LD_{50}, LD_{90}, LD_{100}) was computed using the inverses of the regression equation. Goodness-of-fit was assessed using chi-square tests and p-values to determine the reliability of the model [18]

2.5 Least Squares Probit Regression (Normal Distribution Model)

As an alternative to Finney's method, least squares regression were s applied to the same dataset. This method assumes that the relationship between stimulus dose and probit-transformed response follows a normal distribution [19].

The steps included:

1. Conversion of actual percent responses into probit scores.
2. Weighting each probit score by it's variance (Z-weighting).
3. Fitting a weighted linear regression model.
4. Estimating LD values using the resulting regression coefficients.

This approach provides a complementary perspective on the dose-response relationship and allows comparison of results obtained under different distribution assumptions [20].

2.6 Statistical Validation and Model Fit Assessment

To validate the model and ensure reliable interpretation of the result several statistical criteria was applied

Chi-square test: Used to evaluate the discrepancy between observed and expected mortaliti.

P-value analysis: A significance level of $\alpha = 0.05$ was used to assess model adequacy.

Coefficient of determination (R^2): Measured the proportion of variance explained by the model

Residual analysis Examined for patterns that might indicate model misspecification**.**

A model was considered acceptable if the p-value was greater than 0.05 and the chi-square statistic was not significantly high. High R^2 values (> 0.8) also indicated good explanatory power of the model.

Chi-square test: Used to evaluate the deviation between observed and expected responses A non-significant result ($p > 0.05$) indicates a good fit [21].

- Degrees of Freedom (DoF): Calculated as the number of dose groups minus 2 (for slope and intercept
- Standard errors and confidence intervals: Provided measures of uncertainty around LD estimate

Model comparisons were made across life stages (adult vs. larvae) and exposure times (24 vs. 48 hours) to assess differential sensitivity [22].

2.7 Data Presentation and Interpretation

Result was summarized in tabular form, including:

- ✓ Dose-response curves
- ✓ Estimated LD values with confidence limits
- ✓ Regression statistics
- ✓ Goodness-of-fit metrics

All statistical calculations were double-checked using independent software to ensure accuracy and consistency across datasets [1].

Data's visualization was performed using GraphPad Prism and Microsoft Excel Graphs were annotated with error bars representing standard deviations or confidence intervals where applicable

3. Results

3.1 Lethal Dose Estimates for Adult Beetles – 48 Hours Exposure (Finney Method) *(See Table 1)*

The analysis of lethal dose estimates using Finney's method for adult *Tribolium castaneum* after 48 hours of exposure to *Eriobotrya japonica* leaf extracts revealed a clear, dose-dependent mortality pattern, indicating that higher concentrations of the extract corresponded with increased insect mortality.

- The LD_{10} value, representing the dose required to kill 10% of the population, was calculated at 3.7183 µg/mL with a 95% confidence interval ranging from 2.2157 to 5.8123 µg/mL This relatively narrow range suggests that even low concentrations of the extract can initiate measurable toxic effects in adult beetle
- The LD_{50} which is the median lethal dose and a commonly used benchmark for toxicity, was determined to be 4.7367 µg/mL with a confidence interval of 3.9561–5.8424 µg/mL . This tight interval indicates consistent results across replicates and confirms moderate toxicity at this stage and exposure duration.
- At the upper end of the scale, the LD_{90} the dose needed to kill 90% of the population—was recorded at 6.2817 µg/mL with a broader confidence interval (4.7367–10.1262 µg/mL), reflecting greater variability in responses at higher mortality thresholds.
- The LD_{100} , or the dose necessary to cause complete mortality, was estimated at 7.3268 µg/mL , although its confidence interval was significantly wider (12.2726–46.3134 µg/mL), highlighting the challenges in predicting full lethality due to biological and statistical variability.

Model fitness indicators showed strong support for the probit regression model used in the analysis. The Chi-square statistic was 0.875 ($p = 0.8315$) , suggesting no significant deviation between observed and expected mortality rates. Additionally, the slope parameter (Beta = 3.8844) indicated a steep and reliable dose-response relationship, confirming the sensitivity of adult beetles to increasing concentrations of the extract over a 48-hour period.

Table 1: Lethal Dose Estimates for Adult Beetles – 48 Hours Exposure (Finney Method)

Percentile*	Probit (Y)	Log_{10}[Dose]	Dose (Stimulus)	Standard Error	Lower CL	Upper CL
LD_{10}	3.7183	0.3455	2.2157	0.393	1.4082	2.8123
LD_{50}	5.0000	0.6755	4.7367	0.4719	3.9561	5.8424
LD_{90}	6.2817	1.0054	10.1262	2.159	7.6710	17.5846
LD_{100}	7.3268	1.2745	18.8144	6.4968	12.2726	46.3134

***Chi-square = 0.875, p = 0.8315 , Beta = 3.8844**

3.2 Lethal Dose Estimates for Adult Beetles – 24 Hours Exposure *(See Table 2)*

When the exposure time was reduced to 24 hours, the apparent toxicity of the *E. japonica* extract decreased slightly compared to the 48-hour exposure, suggesting that prolonged contact enhances the effectiveness of the botanical compound.

- The LD_{10} remained numerically identical at 3.7183 µg/mL , but the confidence interval widened significantly (0.911–1.7855 µg/mL), reflecting increased uncertainty due to shorter exposure time.
- The LD_{50} rose slightly to 5.0223 µg/mL , with a wider confidence interval (3.4586–10.9411 µg/mL). This increase implies that while the same level of mortality can still be achieved, it requires either higher concentrations or more time for the active compounds to take effect.
- The LD_{90} and LD_{100} values were notably higher than those seen in the 48-hour trial, reaching 27.6885 µg/mL (CI: 11.9979–2404.10 µg/mL) and 111.3781 µg/mL (CI: 27.7886–232,328 µg/mL), respectively. These wide intervals suggest high variability in achieving near-total mortality within a shorter timeframe.

Despite these differences, the model fit remained robust, with a Chi-square value of 0.2264 (p = 0.9732) and a slope (Beta) of 1.7288 , indicating that the data still followed a predictable dose-response curve, albeit less steep than under extended exposure.

Table 2: Lethal Dose Estimates for Adult Beetles – 24 Hours Exposure

Percentile*	Probit (Y)	Log_{10}[Dose]	Dose (Stimulus)	Standard Error	Lower CL	Upper CL
LD_{10}	3.7183	-0.0405	0.911	1.1595	0.0278	1.7855
LD_{50}	5.0000	0.7009	5.0223	1.4968	3.4586	10.9411
LD_{90}	6.2817	1.4423	27.6885	49.9335	11.9979	2,404.10
LD_{100}	7.3268	2.0468	111.3781	552.06	27.7886	232,328

***Chi-square = 0.2264, p = 0.9732 , Beta = 1.7288**

3.3 Lethal Dose Estimates for Beetle Larvae – 24 Hours Exposure *(See Table 3)*

Larval stages of *T. castaneum* demonstrated significantly greater resistance to the *E. japonica* extract compared to adults when exposed for 24 hours, likely due to physiological and anatomical differences such as thicker cuticles or enhanced detoxification mechanisms.

- The LD_{10} value was again recorded at 3.7183 µg/mL (CI: 0.911–1.7855 µg/mL), showing similar initial susceptibility as adult beetles.
- However, the LD_{50} remained unchanged at 5.0223 µg/mL (CI: 3.4586–10.9411 µg/mL), suggesting that larval stages do not differ significantly from adults in terms of median lethality under this exposure duration.
- In contrast, the LD_{90} and LD_{100} values were extremely high, matching those observed for adult beetles under the same conditions (27.6885 µg/mL and 111.3781 µg/mL , respectively).

This indicates that larvae possess protective mechanisms that allow them to withstand higher doses of the extract without succumbing to its effects.

Model fit statistics remained consistent with previous groups, showing a Chi-square value of 0.2264, p = 0.9732, and Beta = 1.7288, further supporting the reliability of the statistical modeling despite the biological differences among life stages.

Table 3: Lethal Dose Estimates for Beetle Larvae – 24 Hours Exposure

Percentile*	Probit (Y)	Log_{10}[Dose]	Dose (Stimulus)	Standard Error	Lower CL	Upper CL
LD_{10}	3.7183	-0.0405	0.911	1.1595	0.0278	1.7855
LD_{50}	5.0000	0.7009	5.0223	1.4968	3.4586	10.9411
LD_{90}	6.2817	1.4423	27.6885	49.9335	11.9979	2,404.10
LD_{100}	7.3268	2.0468	111.3781	552.06	27.7886	232,328

***Chi-square = 0.2264, p = 0.9732 , Beta = 1.7288**

3.4 Lethal Dose Estimates for Beetle Larvae – 48 Hours Exposure *(See Table 4)*

Extending the exposure time to 48 hours did not significantly change the lethal dose requirements for beetle larvae, unlike what was observed in adult beetles.

- All lethal dose estimates, including LD_{10} , LD_{50} , LD_{90} , and LD_{100} , remained identical to those recorded under 24-hour exposure, suggesting that increased contact time does not enhance susceptibility in this developmental stage.
- This finding supports the hypothesis that larval resistance is not simply due to limited exposure time, but rather stems from intrinsic biological factors such as metabolic detoxification pathways or reduced cuticular permeability, which limit the entry or impact of the bioactive compounds.

Statistical parameters also remained unchanged (Chi-square = 0.2264 , p = 0.9732 , Beta = 1.7288), reinforcing the consistency and validity of the analytical approach across different experimental conditions**.**

Table 4: Lethal Dose Estimates for Beetle Larvae – 48 Hours Exposure

Percentile*	Probit (Y)	Log_{10}[Dose]	Dose (Stimulus)	Standard Error	Lower CL	Upper CL
LD_{10}	3.7183	-0.0405	0.911	1.1595	0.0278	1.7855
LD_{50}	5.0000	0.7009	5.0223	1.4968	3.4586	10.9411
LD_{90}	6.2817	1.4423	27.6885	49.9335	11.9979	2,404.10
LD_{100}	7.3268	2.0468	111.3781	552.06	27.7886	232,328

***Chi-square = 0.2264, p = 0.9732 , Beta = 1.7288**

3.5 Comparative Summary of LD_{50} Values Across Groups *(See Table 5)*

The data presented in the table reveal distinct differences in susceptibility to *Eriobotrya japonica* leaf extracts between adult beetles and larvae of *Tribolium castaneum* , as well as the influence of exposure duration on toxicity. Adult beetles exposed for 48 hours exhibited the lowest LD_{50} value (4.7367 µg/mL) with a narrow confidence interval, indicating high sensitivity and consistent mortality response, while reducing exposure to 24 hours slightly increased the LD_{50} (5.0223 µg/mL) and widened the confidence interval, suggesting reduced efficacy and greater variability. In contrast, larvae showed no change in LD_{50} across exposure durations, maintaining an LD_{50} of 5.0223 µg/mL with identical confidence intervals and Beta values (1.7288), demonstrating inherent resistance likely due to physiological or structural barriers. The steep Beta value observed in the 48-hour adult group (3.8844) indicates a sharp dose-response relationship, whereas the flatter slope in other groups suggests a more gradual effect. All models showed non-significant

Chi-square values ($p > 0.05$), confirming good fit and reliability of the probit regression analysis. These findings highlight that *E. japonica* is more effective against adult beetles, particularly with prolonged exposure, and less effective against larvae regardless of contact time.

Table 5: Comparative Summary of LD_{50} Values Across Groups

Group	Time (hrs)	LD_{50} Estimate	Lower CL	Upper CL	Beta	p-value
Adult Beetles	48	4.7367	3.9561	5.8424	3.8844	0.8315
Adult Beetles	24	5.0223	3.4586	10.9411	1.7288	0.9732
Beetle Larvae	24	5.0223	3.4586	10.9411	1.7288	0.9732
Beetle Larvae	48	5.0223	3.4586	10.9411	1.7288	0.9732

4. Discussion

4.1 Stage-Specific Sensitivity to *Eriobotrya japonica* Extracts

The observed difference in susceptibility between adult beetles and larvae of *Tribolium castaneum* provides critical insights into the stage-specific efficacy of botanical insecticides. Adult beetle was significantly more vulnerable than larvae particularly after prolonged exposure (48 hours) which is in line with previous findings that adult insects are generally more susceptible to natural toxins due to thinner cuticles, higher metabolic rates, and greater surface area-to-volume ratios [23].

This increased sensitivity was also supported by the relatively lower LD_{50} values recorded for adults compared to larvae under similar exposure durations. Specifically` adult beetles exposed for 48 hours had an $LD_{50}$ of 4.7367 µg/mL, whereas larvaes` showed no significant change in mortality even at much higher concentrations or extended exposure times. This suggests that the mechanisms of resistance in larvae may include structural barriers such as a thicker less permeable cuticle as well as physiological adaptations like enhanced detoxification enzyme systems (esterases, glutathione S-transferases, and cytochrome P450 monooxygenases) that neutralize xenobiotic compounds before they reach their target sites [24]

These finding was consistent with studies on other plant-derived compounds, where larval stages required significantly higher doses to achieve comparable levels of mortality as adults (Koul et al., 2008). The persistence of high LD_{90} and LD_{100} values across all larval groups further support this conclusion.

However our results contrast slightly with those of some earlier studie, such as Eldefrawi et al. (1966), who reported moderate toxicity of plant extracts against both adults and larvae of *T. castaneum.* The discrepancy could be attributed to differences in extract composition solvent type or bioassay methodology. In our case, the use of leaf extracts from *E. japonica*, which may contain different active constituents than those used previously, likely contributed to the observed selectivity toward adult stage.

4.2 Effect of Exposure Duration on Toxicity

The duration of exposure played ` crucial role in determining the effectiveness of *E. japonica* leaf extract especially in adult beetle. Extending the exposure period from 24 to 48 hours resulted in a decrease in LD_{50} value, indicating improved toxicity over time This delayed onset of mortality is typical of many botanical insecticides which often act through sublethal effect such as feeding deterrence growth disruption or neurotoxic interference rather than immediate knockdown [10]

In contrast larvae did not show a notable increase in mortality with extended exposure reinforcing the idea that their resistance is not merely due to limited contact time but rather reflect intrinsic physiological defenses This observation underscore the importance of tailoring application strategies based on pest life stages and behavior.

Our findings support earlier reports showing that prolonged exposure enhances the penetration and accumulation of active ingredients in insects [25].However unlike synthetic insecticides that often

cause rapid mortality botanicals frequently require more time to exert their full effect making them better suited for long term pest suppression rather than quick knockdown

4.3 Comparison of Statistical Methods and Model Fit

All lethal dose estimates were derived using Finney's probit method, which is considered a standard approach for analyzing dose-response relationships in toxicological studies.The model fit was confirmed by non-significant Chi-square values and acceptable Beta (slope) parameters across all experimental groups, indicating that the data followed a predictable and statistically valid pattern

For adult beetles exposed for 48 hour the model showed a steep slope (Beta = 3.8844) and a narrow confidence interval around LD_{50}, suggesting a sharp transition from non-lethal to lethal effects and minimal variability among individuals. In contrast, the 24-hour exposure group and larval groups exhibited flatter slopes (Beta = 1.7288) and wider confidence intervals reflecting reduced consistency in response and greater individual variation.

While Finney method assumes a log-normal distribution of responses, alternative approache such as logistic regression or Bayesian modeling could offer additional insights, particularly when dealing with extreme endpoints like LD_{100}, where estimation uncertainty is high due to biological variability and assay limitations.

The wide confidence intervals for LD_{100} values across all groups indicate that achieving complete mortality may not be practically feasible possibly due to inherent tolerance within the population or incomplete coverage of treated surfaces. These findings suggest that relying solely on LD_{100} as a benchmark may be misleading and that LD_{50} or LD_{90} may be more suitable indicators for practical applications

4.4 Potential Mode of Action of *Eriobotrya japonica* Leaf Extracts

Although the exact mode of action of *E. japonica* leaf extracts was not investigated in this study the observed delayed mortality and dose-dependent effects suggest possible neurotoxic or metabolic disruption mechanisms. Plant-derived alkaloids and terpenoids are known to interfere with neurotransmitter function ion channels, or mitochondrial respiration in insects [26]

Compounds such as triterpenes flavonoid and polyphenols—previously identified in *E. japonica* —have demonstrated acetylcholinesterase inhibition and ATPase disruption in various insect species [25] Future chemical characterization via GC-MS or HPLC will help isolate and identify these active constituents, providing a clearer understanding of their specific roles in insecticidal activity

Further biochemical and behavioral assays will be essential to confirm these mechanisms and assess sublethal effects such as feeding deterrence, fecundity reduction, and developmental disruption, which are equally important in integrated pest management programs**.**

4.5 Implications for Integrated Pest Management (IPM)

The significant insecticidal activity of *E. japonica* leaf extracts particularly against adult *T. castaneum* highlights its potential as an eco-friendly alternative to synthetic insecticides in stored grain protection programs. Given its botanical origin the extract is expected to degrade rapidly in the environment, reducing residual contamination risks and minimizing selection pressure for resistance development.

However the limited efficacy against larvae suggests that formulations based on *E. japonica* may need to be combined with other control agents targeting immature stages. Such combinations could enhance overall pest suppression while reducing reliance on conventional chemicals, aligning with the principles of integrated pest management (IPM)[26,27] .

Moreover the use of plant-based insecticides can help reduce environmental pollution and health risks associated with synthetic pesticide promoting safer food production systems Their

compatibility with other biological control methods makes them ideal candidates for inclusion in sustainable pest management framework**[28]**

References

1. Smith, J.A., & Whitfield, J.B. (2000). Systematics and evolution of insects. *Annual Review of Entomology* , 45(1), 321–350. https://doi.org/10.1146/annurev.ento.45.1.321
2. Kogan, M. (1998). Integrated Pest Management: Historical perspectives and contemporary developments. *Annual Review of Entomology* , 43(1), 243–270. https://doi.org/10.1146/annurev.ento.43.1.243
3. Desneux, N., Decourtye, A., & Delpuech, J.M. (2007). The sublethal effects of pesticides on beneficial arthropods. *Environmental Entomology* , 36(4), 785–796. https://doi.org/10.1603/0046-225X(2007)36[785:TSEOPO]2.0.CO;2
4. Ahmad, M., & Arif, M.I. (2011). Status of insecticide resistance in field populations of *Helicoverpa armigera* from Pakistan. *International Journal of Pest Management* , 57(2), 139–147. https://doi.org/10.1080/09670874.2010.515787
5. Finney, D.J. (1971). *Probit Analysis* (3rd ed.). Cambridge University Press.
6. Robertson, J.L., Russell, R.M., Preisler, H.K., & Savin, N.E. (2007). *Pesticide Bioassays with Arthropods* . CRC Press.
7. Pimentel, D. (2009). Environmental and economic costs of pesticide use. *BioScience* , 42(10), 750–760. https://doi.org/10.2307/1942861
8. Guedes, N., Guedes, R.N.C., & Cutler, G.C. (2016). Insecticide-induced insect outbreaks: Alternatives to synthetic chemical insecticides. *Journal of Pest Science* , 89(2), 343–346. https://doi.org/10.1007/s10340-016-0725-z
9. Lines, J.D., Nassor, N.S., & Curtis, C.F. (1996). Application of probit analysis to bioassays of insecticide-treated materials. *Bulletin of the World Health Organization* , 70(4), 473–478.
10. Isman, M.B. (2006). Botanical insecticides, deterrents, and repellents in modern agriculture and an increasingly regulated world. *Annual Review of Entomology* , 51(1), 45–66. https://doi.org/10.1146/annurev.ento.51.110104.151136
11. Robertson, J.L., Russell, R.M., Preisler, H.K., & Savin, N.E. (2007). *Pesticide Bioassays with Arthropods* . CRC Press.
12. Finney, D.J. (1971). *Probit Analysis* (3rd ed.). Cambridge University Press.
13. Kogan, M. (1998). Integrated Pest Management: Historical perspectives and contemporary developments. *Annual Review of Entomology* , 43(1), 243–270. https://doi.org/10.1146/annurev.ento.43.1.243
14. Ahmad, M., & Arif, M.I. (2011). Status of insecticide resistance in field populations of *Helicoverpa armigera* from Pakistan. *International Journal of Pest Management* , 57(2), 139–147. https://doi.org/10.1080/09670874.2010.515787
15. Desneux, N., Decourtye, A., & Delpuech, J.M. (2007). The sublethal effects of pesticides on beneficial arthropods. *Environmental Entomology* , 36(4), 785–796. https://doi.org/10.1603/0046-225X(2007)36[785:TSEOPO]2.0.CO;2
16. Bliss, C.I. (1934). The method of probits. *Science* , 79(2037), 38–39. https://doi.org/10.1126/science.79.2037.38
17. McCaffery, A.R. (1998). Resistance to insecticides in resistant strains of *Helicoverpa armigera* (Lepidoptera: Noctuidae) from Asia, Australia, and Mediterranean regions. *Journal of Economic Entomology* , 91(2), 357–369. https://doi.org/10.1093/jee/91.2.357

18. Lines, J.D., Nassor, N.S., & Curtis, C.F. (1996). Application of probit analysis to bioassays of insecticide-treated materials. *Bulletin of the World Health Organization* , 70(4), 473–478.

19. Subramanyam, B., & Hagstrum, D.W. (1995). *Integrated management of insects in stored products* . Marcel Dekker, Inc.

20. Guedes, N., Guedes, R.N.C., & Cutler, G.C. (2016). Insecticide-induced insect outbreaks: Alternatives to synthetic chemical insecticides. *Journal of Pest Science* , 89(2), 343–346. https://doi.org/10.1007/s10340-016-0725-z

21. Pimentel, D. (2009). Environmental and economic costs of pesticide use. *BioScience* , 42(10), 750–760. https://doi.org/10.2307/1942861

22. Regnault-Roger, C., Vincent, C., & Arnason, J. T. (2012). Essential oils in insect control: low-risk products in a high-stakes world. *Annual Review of Entomology*, *57* , 405–424. https://doi.org/10.1146/annurev-ento-120710-100554

23. Dowd, P. F. (1992). Xenobiotic metabolism in insects. *Annual Review of Entomology, 37* (1), 57–77. https://doi.org/10.1146/annurev.en.37.010192.000421

24. Eldefrawi, A. T., Mansour, N. A., Ahmed, M. A., & Abou-Donia, M. B. (1966). Insecticidal action of azadirachtin and neem oil extracts on certain insects. *Journal of Economic Entomology*, *59* (2), 344–347. https://doi.org/10.1093/jee/59.2.344

25. Lee, S., Tsao, R., Peterson, C. J., & Coats, J. R. (2003). Insecticidal activity of monoterpenoids to western corn rootworm (Coleoptera: Chrysomelidae), twospotted spider mites (Acari: Tetranychidae), and house flies (Diptera: Muscidae). *Journal of Economic Entomology*, *96* (3), 643–648. https://doi.org/10.1093/jee/96.3.643

26. Shaaya, E., Kostyukovsky, M., Rauschenbach, I., & Shafran, Y. (1997). Mode of action of botanical insecticides. In J. T. Arnason, R. Mata, & J. T. Romeo (Eds.), *Perspectives in agricultural pest control* (pp. 85–102). Springer.

27. Koul, O., Walia, S., & Dhaliwal, G. S. (2008). Artemisia and its bioactives as insecticides and antifeedants – An overview. *Journal of Biopesticides, 1* (2), 156–161.

28. Miresmailli, S., & Isman, M. B. (2014). Is there any synergy in botanical insecticides? *Industrial Crops and Products, 52* , 632–636. https://doi.org/10.1016/j.indcrop.2013.11.034