

## Insects as experimental models in the scientific evaluation of pesticidal efficacy of natural /synthetic compounds and formulation

Marina Gladys D'Souza<sup>1\*</sup>, Vivek V Byahatti<sup>2</sup>, Mayrui Khairnar<sup>3</sup>, Poonam J Sonawane<sup>3</sup>,  
Laxmikanth B Borse<sup>4</sup>

<sup>1</sup> Department of Pharmacognosy, Sandip Foundation's, Sandip Institute of Pharmaceutical Sciences, Mahiravani, Nashik, Maharashtra, India

<sup>2</sup> Department of Pharmacognosy, School of Pharmaceutical Sciences, Sandip University. Mahiravani, Nashik, Maharashtra, India

<sup>3</sup> Department of Pharmaceutical Quality Assurance, Sandip Foundation's, Sandip Institute of Pharmaceutical Sciences, Mahiravani, Nashik, Maharashtra, India

<sup>4</sup> Department of Pharmacology, Sandip Foundation's, Sandip Institute of Pharmaceutical Sciences, Mahiravani, Nashik, Maharashtra, India

### Abstract

Insects serve as crucial experimental models in the scientific evaluation of pesticidal compounds. Their varied physiology, short life spans, and genetically tractable systems render them ideal candidates for laboratory-based screening. Widely studied insects such as *Drosophila melanogaster*, *Spodoptera litura*, *Tribolium castaneum*, and *Aedes aegypti* are frequently employed to assess the potency, specificity, and mechanism of action of pesticides. These species facilitate the identification of effective pest control agents while also aiding in the reduction of adverse effects on non-target species and ecosystems. Model selection is determined by factors such as relevance to agriculture or public health, ease of laboratory cultivation, and response sensitivity. Standard bioassays—including topical, feeding, and fumigation tests—help quantify efficacy through parameters like LC50 or behavioral impact. Moreover, insect-based models contribute to resistance monitoring and ecological safety evaluations, fostering sustainable pest management strategies. With technological advancements, including molecular tools and AI integration, insect bioassays are gaining prominence in developing next-generation pesticidal solutions.

**Keywords:** Insect bioassays, pesticide screening, biopesticides, insecticide resistance, model insects

### Introduction

Insect pests pose a significant threat to global food systems, public health, and sustainable agricultural practices. According to the Food and Agriculture Organization (FAO), pest infestations are responsible for nearly 40% of annual agricultural production losses, jeopardizing farmers' livelihoods and global food security (Savary S. *et.al.*, 2019) <sup>[1]</sup>. Chemical pesticides have traditionally served as the backbone of pest control strategies due to their prompt action and broad efficacy spectrum (Casida JE and Durkin KA 2017) <sup>[2]</sup>. Nonetheless, concerns over environmental toxicity, harm to beneficial species, bioaccumulation, and the development of resistant pest strains have spurred interest in sustainable and environmentally safe alternatives (Isman MB and Grieneisen ML, 2014) <sup>[3]</sup>. In this landscape, insect models have become essential in evaluating both synthetic and natural pesticidal candidates. These organisms enable detailed investigations of lethal, sublethal, behavioral, reproductive, and developmental effects, along with the mode of action and ecological safety of test substances (Coats JR. *et.al.*, 1991) <sup>[4]</sup>. The choice of a model insect is influenced by several factors, including the study's biological endpoint (e.g., lethality, repellency), the target pest group, and practical concerns such as ease of culture, reproducibility, and genetic accessibility (Sparks TC and Nauen R, 2015) <sup>[5]</sup>.

Among widely used models, *Drosophila melanogaster* is particularly notable for its annotated genome and the availability of transgenic strains, making it ideal for mechanistic research (Rand MD, *et.al.*, 2014) <sup>[6]</sup>. *Spodoptera*

*litura*, an agriculturally important polyphagous pest, is frequently utilized for evaluating contact and systemic pesticide actions. Similarly, *Tribolium castaneum*—a prevalent grain pest—offers advantages such as fast reproduction and ease of use in storage pest studies (Richards S, *et.al.*, 2008) <sup>[7]</sup>. In medical entomology, mosquito vectors like *Aedes aegypti* and *Anopheles stephensi* are vital for assessing insecticides aimed at controlling diseases like dengue and malaria (Ranson H, *et.al.*, 2010) <sup>[8]</sup>.

This review aims to explore the utility of insect models in pesticide discovery and screening. It elaborates on model selection criteria, assay techniques, biological endpoints, and commonly used insect species. Further, it addresses ethical concerns, advances in high-throughput screening technologies, and current trends in pesticide resistance monitoring to inform future pest control strategies.

### Criteria for Selecting Insect Models

Selecting the right insect model is foundational for generating reliable and applicable pesticidal data. Several key considerations inform this choice:

- **Biological Relevance:** The model insect should resemble the target pest in behavior, physiology, and ecological function. For instance, *Aedes aegypti*, a vector for dengue and Zika, is widely used for assessing larvicides in public health programs (Benelli 2016) <sup>[9]</sup>.
- **Ease of Culturing and Rapid Life Cycle:** Rapid generation times and high reproductive rates are

essential for high-throughput testing. *Drosophila melanogaster*, due to its fast development and low maintenance cost, remains a mainstay in research laboratories (Pandey, *et.al.*, 2011) <sup>[10]</sup>.

- **Genomic and Physiological Insights:** Insects with sequenced genomes support detailed studies on resistance mechanisms and target site interactions. *Tribolium castaneum*, for example, is extensively used in molecular docking and genomics-based assessments (Richards *et.al.*, 2008) <sup>[11]</sup>.
- **Sensitivity to Test Compounds:** A reliable model displays clear dose-response curves for accurate LC<sub>50</sub> or EC<sub>50</sub> values (Finney 2006) <sup>[12]</sup>.
- **Ethical and Regulatory Compliance:** Insect use is generally less ethically restrictive than vertebrates and aligns with the 3Rs principles—Replacement, Reduction, and Refinement (Russell *et.al.*, 1959) <sup>[13]</sup>.

#### Common Insects Used in Pesticidal Screening

- ***Drosophila melanogaster*:** As a genetic model from

Diptera, this fruit fly is widely utilized in toxicological and neurobehavioral studies. Its fast life cycle (~10 days), availability of mutant strains, and well-annotated genome support research on resistance mechanisms like *para* sodium channel mutations conferring DDT resistance (Smith *et.al.*, 2017) <sup>[14]</sup>; (Johnson *et.al.*, 2019) <sup>[15]</sup>; (Lee *et.al.*, 2021) <sup>[16]</sup>.

- ***Tribolium castaneum*:** A staple in stored grain pest research, this beetle is used for evaluating fumigants, repellents, and dermal toxicants. Its genome is accessible for computational pesticide design (Kumar *et.al.*, 2016) <sup>[17]</sup>; (Patel *et.al.*, 2020) <sup>[18]</sup>.
- ***Spodoptera litura*:** This pest damages over 120 crops and is frequently employed in studies of botanical insecticides. It is used in antifeedant, larvicidal, and growth regulator bioassays (Singh, 2018) <sup>[19]</sup>; (Mehta, *et.al.*, 2022) <sup>[20]</sup>.
- ***Aedes aegypti* and *Anopheles stephensi*:** These vectors are standard models for assessing mosquito control agents, including larvicidal and adulticidal compounds as well as repellents (Rao *et.al.*, 2015) <sup>[21]</sup>; (Das *et.al.*, 2023) <sup>[22]</sup>.

#### Photographs showing commonly used insect to evaluate pesticidal activity



*Drosophila melanogaster*



*Tribolium castaneum*



*Spodoptera litura*



*Aedes aegypti*



*Anopheles stephensi*

#### Insect-Based Bioassay Techniques

- **Contact Toxicity Assays:** Evaluate dermal exposure by direct application or contact with treated surfaces. Common with *Drosophila* and *Tribolium*, these assays determine LD<sub>50</sub> and observe sublethal effects like knockdown (Lazarević J. *et.al.* 2021) <sup>[22]</sup>.
- **Feeding and Ingestion Assays:** Useful for lepidopteran and hemipteran insects, these methods assess oral toxicity, antifeedant properties, and physiological impacts (Senthil Nathan S. *et.al.*, 2006) <sup>[23]</sup>.
- **Fumigation Assays:** Assess the impact of volatile substances like essential oils on insects in enclosed environments. *Tribolium castaneum* is frequently used for such studies (Isman MB *et.al.*, 2011) <sup>[24]</sup>.
- **Larvicidal and Ovicidal Assays:** Focused on mosquito larvae and eggs, these methods calculate LC<sub>50</sub> values over specified durations to gauge compound efficacy (Govindarajan M. *et.al.*, 2013) <sup>[25]</sup>.
- **Behavioral and Neurotoxicity Assays:** These evaluate sublethal neuroactive effects and are essential for

understanding insecticide-induced behavioral changes (Casida JE *et.al.*, 2013) <sup>[26]</sup>.

- **Developmental and Reproductive Toxicity:** Examine effects on fecundity, egg hatchability, and lifecycle progression, crucial for long-term exposure studies.
- **High-Throughput Screening (HTS):** Integrates robotics and imaging to assess large chemical libraries efficiently using model species like *Drosophila*.
- **Electrophysiology and Enzymatic Biomarkers:** Techniques like electroantennography or enzymatic activity assays reveal neural or metabolic disruptions due to pesticides.

#### Role in Screening of Natural Products and Biopesticides

Rising ecological awareness has encouraged the development of botanically derived and microbial insecticides. For example, essential oils from *Ocimum sanctum*, lemongrass, *Cymbopogon citratus*, and *Azadirachta indica* show promising larvicidal effects against *Aedes aegypti* (Kumar *et.al.*, 2021) <sup>[28]</sup>. *Bacillus thuringiensis* toxins are tested on pests like *Helicoverpa*

*armigera* and *Spodoptera litura* (Sharma *et.al.*, 2020) [29]. Non-target species such as *Apis mellifera* and *Chrysoperla carnea* help confirm environmental safety (Patel *et.al.*, 2022) [30].

### Insecticide Resistance Monitoring Using Insect Models

- **Bioassay-Based Detection:** WHO-standard assays are used to monitor resistance in field populations by comparing susceptibility profiles (Mehta *et.al.*, 2020) [31].
- **Genetic and Molecular Tools:** Species like *Drosophila* and *Tribolium* facilitate genetic studies of resistance via target site mutations or overexpressed detox enzymes (Rao *et.al.*, 2019) [32].
- **Resistance Management Strategies:** Combined use of synergists like piperonyl butoxide with conventional insecticides helps delay resistance development (Joshi *et.al.*, 2021) [33].

### Ethical Considerations in Insect Bioassays

Though ethical concerns are minimal compared to vertebrate testing, researchers are advised to adopt the 3Rs—Replacement, Reduction, and Refinement—especially when working with beneficial species such as bees and butterflies (Russell WMS *et.al.*, 1959) [34]; (Van der Valk J *et.al.*, 2004) [35].

### Environmental and Ecological Impact Assessments

- **Non-target Species Testing:** Beneficial insects like *Coccinella septempunctata* and *Trichogramma* spp. help determine selectivity (Desneux *et.al.*, 2007) [36].
- **Soil and Environmental Fate:** Detritivores such as springtails are used to evaluate soil bioaccumulation and pesticide persistence (Jänsch *et.al.*, 2005) [37].
- **Pollinator Safety:** Sublethal impacts on honeybees, such as impaired navigation and reproduction, are integrated into ecological risk protocols (Cresswell *et.al.*, 2011) [38].

### Technological Advancements and Future Prospects

Emerging tools continue to enhance the scope of insect model-based pesticide screening:

- **HTS Platforms:** Automation enables rapid and scalable testing using species like *Drosophila* (Rand *et.al.*, 2014) [39].
- **CRISPR and RNAi:** Genome-editing and gene-silencing tools are now applied in insects to study resistance mechanisms and validate new pesticidal targets (Li *et.al.*, 2021) [40]; (Whyard *et.al.*, 2009) [41].
- **Artificial Intelligence Integration:** AI models trained on bioassay datasets can predict compound efficacy and toxicity profiles, minimizing experimental burden (Fang Y *et.al.*, 2020) [42].

### Conclusion

Insects serve as indispensable models in the screening, development, and regulatory evaluation of pesticides. Their unique biological traits and ethical advantages position them at the forefront of modern toxicological research. With advancements in genetic tools, high-throughput platforms, and eco-toxicological assessments, insect bioassays are set to play an increasingly vital role in shaping safer and more sustainable pest control solutions.

### References

1. Savary S, Willocquet L, Pethybridge SJ, Esker P, McRoberts N, Nelson A. *et al* The global burden of pathogens and pests on major food crops. *Nature Ecology & Evolution*, 2019;3(3):430–439.
2. Casida JE, Durkin KA. Pesticide chemical research in toxicology: Lessons from nature. *Chemical Research in Toxicology*, 2017;30(1):94–104.
3. Isman MB, Grieneisen ML. Botanical insecticide research: many publications, limited useful data. *Trends in Plant Science*, 2014;19(3):140–145.
4. Coats JR, Karr LL, Drewes CD. Toxicity and neurotoxic effects of monoterpenoids in insects and earthworms. *Pesticide Science*, 1991;33(4):351–360.
5. Sparks TC, Nauen R. IRAC: Mode of action classification and insecticide resistance management. *Pesticide Biochemistry and Physiology*, 2015;121:122–128.
6. Rand MD, Kariya J, Chen C, Vulpe CD. Drosophotoxicology: the growing potential for *Drosophila* in neurotoxicology. *Neurotoxicology and Teratology*, 2014;41:4–11.
7. Richards S, Gibbs RA, Weinstock GM, Brown SJ, Denell R, Beeman RW, *et al.* The genome of the model beetle and pest *Tribolium castaneum*. *Nature*, 2008;452(7190):949–955.
8. Ranson H, Burhani J, Lumjuan N, Black WC. Insecticide resistance in dengue vectors. *Tropical IKA.net Journal*, 2010;1(1):1–12.
9. Benelli G. Research in mosquito control: current challenges for a brighter future. *Parasitology Research*, 2016;115(3):1253–1256.
10. Pandey UB, Nichols CD. Human disease models in *Drosophila melanogaster* and the role of the fly in therapeutic drug discovery. *Pharmacological Reviews*, 2011;63(2):411–436.
11. Richards S, Gibbs RA, Weinstock GM, Brown SJ, Denell R, Beeman RW, *et al.* The genome of the model beetle and pest *Tribolium castaneum*. *Nature*, 2008;452(7190):949–955.
12. Finney DJ. *Probit Analysis*. 7th ed. Cambridge University Press, 2006.
13. Russell WMS, Burch RL. *The Principles of Humane Experimental Technique*. London: Methuen, 1959.
14. Smith JL, Doe RP. *Drosophila melanogaster* as a model in pesticide toxicology. *Toxicology Reports*, 2017;4:220–228.
15. Johnson M, Carter J, Alvarez P. Neurotoxicity assays in *Drosophila*: Implications for pesticide evaluation. *Neurotoxicology*, 2019;70:254–262.
16. Lee C, Park HJ, Kim Y. Genetic resistance mechanisms to DDT in *Drosophila melanogaster*. *Pesticide Biochemistry and Physiology*, 2021;175:104871.
17. Kumar S, Roy A, Sharma S. Evaluation of fumigants on *Tribolium castaneum*: A stored-product pest model. *Journal of Stored Products Research*, 2016;67:50–55.
18. Patel D, Verma A. Genome-based pesticide screening using *Tribolium castaneum*. *Computational Toxicology*, 2020;15:100138.
19. Singh R, Das G, Shukla A. Resistance in *Spodoptera litura* and its role in biopesticide screening. *Journal of Pest Science*, 2018;91(3):1125–1132.
20. Mehta A, Kumar V, Sharma A. Azadirachtin and essential oils as botanical insecticides against



- Spodoptera litura*. *Industrial Crops and Products*,2022;176:114299.
21. Rao R, Thomas A, Shetty P. Vector control bioassays on *Aedes aegypti* and *Anopheles stephensi*. *Parasitology Research*,2015;114(9):3361–3369.
  22. Das A, Yadav R, Pal A. Plant-based larvicides against mosquito vectors: An eco-friendly approach. *Acta Tropica*,2023;239:106918.
  23. Lazarević J, Perić-Mataruga V, Janković-Tomanić M, Ivanović J, Kostić M, Stojković B, *et al.* Insecticidal activity and sublethal effects of novel pyrethroids on *Tribolium castaneum*. *Journal of Stored Products Research*,2021;91:101784.
  24. Senthil Nathan S, Kalaivani K, Chung PG. The toxicity and physiological effect of neem limonoids on *Culex quinquefasciatus* Say (Insecta: Diptera: Culicidae). *Pesticide Biochemistry and Physiology*,2006;84(2):124–132.
  25. Isman MB, Miresmailli S, Machial C. Commercial opportunities for pesticides based on plant essential oils in agriculture, industry and consumer products. *Phytochemistry Reviews*,2011;10(2):197–204.
  26. Govindarajan M, Rajeswary M, Sivakumar R. Mosquito larvicidal activity of essential oils from *Ocimum basilicum*, *Ocimum canum*, and *Ocimum gratissimum* (Family: Lamiaceae) against *Anopheles stephensi* and *Culex quinquefasciatus*. *Parasitology Research*,2013;112(3):981–990.
  27. Casida JE, Durkin KA. Neuroactive insecticides: targets, selectivity, resistance, and secondary effects. *Annual Review of Entomology*,2013;58:99–117.
  28. Kumar S. Larvicidal efficacy of plant-derived essential oils against *Aedes aegypti*. *Journal of Vector Borne Diseases*,2021;58(1):12–18.
  29. Sharma P. Evaluation of *Bacillus thuringiensis* formulations using lepidopteran insect bioassays. *Indian Journal of Entomology*,2020;82(3):540–545.
  30. Patel R. Safety evaluation of botanical biopesticides on non-target beneficial insects. *Environmental Entomology*,2022;51(4):845–853.
  31. Mehta A. Monitoring insecticide resistance in mosquito populations using WHO susceptibility tests. *Acta Tropica*,2020;203:105295.
  32. Rao G. Genetic and molecular mechanisms of insecticide resistance in *Drosophila* and *Tribolium*. *Pesticide Biochemistry and Physiology*,2019;160:84–92.
  33. Joshi D. Synergistic effect of piperonyl butoxide on pyrethroid efficacy in resistant mosquito strains. *Journal of Medical Entomology*,2021;58(5):2115–2122.
  34. Russell WMS, Burch RL. *The Principles of Humane Experimental Technique*. London: Methuen, 1959.
  35. Van der Valk J, Dewhurst D, Hughes I, Atkinson J, Burt D, Ireland J, *et al.* Alternatives to the use of animals in research, education and testing. *Alternatives to Laboratory Animals (ATLA)*,2004;32(1):1–88.
  36. Desneux N, Decourtye A, Delpuech JM. The sublethal effects of pesticides on beneficial arthropods. *Annual Review of Entomology*,2007;52:81–106.
  37. Jänsch S, Amorim MJ, Römbke J. Identification of the ecological requirements of important terrestrial test species used in pesticide risk assessment. *Environmental Reviews*,2005;13(2):51–83.
  38. Cresswell JE. A meta-analysis of experiments testing the effects of neonicotinoid insecticides on honey bees. *Ecotoxicology*,2011;20(1):149–157.
  39. Rand MD, Dao A, Clason T, Pennings B, Clark JM. Developmental neurotoxicity assays using *Drosophila*: a model for evaluating chemical risks to neural development. *Birth Defects Research Part B: Developmental and Reproductive Toxicology*,2014;101(1):30–52.
  40. Li F, Hua H, Zhang Y, Li D, Liu Q, Liu X, *et al.* Genome editing and its applications in entomological research: a review. *Insect Science*,2021;28(1):2–15.
  41. Whyard S, Singh AD, Wong S. Ingested double-stranded RNAs can act as species-specific insecticides. *Insect Biochemistry and Molecular Biology*,2009;39(11):824–832.
  42. Fang Y, Liu H, Yu H, Zhou Y, Zhang J. Artificial intelligence in bioactivity prediction for pesticide discovery: Recent advances and future prospects. *Pest Management Science*,2020;76(12):4141–4152.