AgriSustain-An International Journal

Agriculture

Review

Bioassays Methods for Evaluating Insecticide Toxicity: Principles, Methods, and Considerations

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Abstract

Bioassays are important tests used in toxicology to evaluate the toxicity of insecticides by observing their effects on living organisms. These tests measure how different factors such as chemical, biological or physiological conditions can impact test insects. Effective bioassays require selecting insects that are sensitive, easy to handle, and can be maintained under controlled conditions. Common bioassay methods include film application, topical application, Potter's tower method, injection, dipping, fumigation, aqueous solution, contact, and photo migration. Each method has its own advantages and is suited to different insect types, exposure routes, and research goals. Bioassays are instrumental in determining insecticide efficacy, studying synergistic effects, and ensuring safety of non-target species' safety. Despite their usefulness, bioassays face challenges like the need for sensitive organisms, consistent conditions, and possible variability in results. Understanding these methods and their limitations is crucial for improving pest management and ensuring environmental safety.

Keywords: Bioassay, insecticides, toxicity evaluation, pest management, insect response

OPEN ACCESS

CITATION

Tulasi B, Sake Manideep, Talapala Sai Kumar and Aswini R Bioassays Methods for Evaluating Insecticide Toxicity: Principles, Methods, and Considerations. *AgriSustain-an International Journal,* 2025, 03(1), 14-19.

ARTICLE INFORMATION

Received: October 2024
Revised: December 2024
Accepted: December 2024

DOI: **10.5281/zenodo.15094846**

Introduction

Bioassay is a combination of two words: bios (life) and assay (determination). Thus, bioassay refers to the determination of the relative toxicity of insecticides by studying their effects on living organisms. According to Finney (1952)[1], it is defined as the measurement of the potency of any stimulus-whether physical, chemical, biological or physiological-by observing the reactions it produces in living matter.

Basic Criteria for Test Insects in Bioassays

1. ***Availability:*** Using insects trapped from the field can be impractical for bioassay programs. Limited availability of insects throughout the year can be addressed by using artificial diets.
2. ***Food:*** While some test insects can be reared on plant-based food, artificial diets are often used to ensure a consistent supply.
3. ***Sensitivity:*** Test insects must be sensitive to the insecticidal response; examples include Drosophila, houseflies, and mosquito larvae.
4. ***Easy Handling:*** Test insects should be manageable and easy to handle carefully.

Bioassays are utilized to evaluate the effectiveness of chemicals as insecticides and to study interactions such as synergism, potentiation, and antagonism when combined with other substances. By determining LC50 values, bioassays help assess the relative toxicity of insecticides, guiding the selection of candidates for field trials against pests. They are also crucial for assessing the safety of insecticides on pollinators, natural predators, and beneficial pathogens.

Principle of bioassay

The fundamental principle of bioassays is to measure and compare the responses of insects exposed to treated samples with those exposed to a series of standard reference materials under the same conditions. These responses can include endpoints like knockdown, mortality, or specific effects such as photo-migration. With the growing use of highly toxic insecticides for pest management, it is important to analyze both the formulated and technical insecticides, as well as trace residues in plant and animal tissues.

For an effective bioassay, the indicator species must be highly sensitive to even minimal levels of insecticide and show a measurable reaction as concentrations rise. Toxic interactions between insecticides and biological systems are typically dose-dependent, with toxicity commonly measured by LD50, which represents the dose that kills 50% of the test population. In cases where the exact dose given to the insect is hard to determine, LC50 is used to express the concentration of insecticide in the environment that kills half of the test population.

Bioassay methods are appreciated for their sensitivity, ease of use, and flexibility. They are useful for evaluating new insecticides, determining optimal dosages, assessing the toxicity of insecticide metabolites, and testing pests for resistance to pesticides.

Key Factors Affecting Bioassays

Several important factors influence bioassays: the developmental stage of the insects affects their sensitivity, while the type of insecticide and application method influence their interactions with the test subjects. Environmental factors like temperature and humidity can alter results, and sample size impact's reliability. The health of the insects and the expertise of the operator are also crucial for achieving consistent and accurate results.

Insects used for testing should be uniform in age, developmental stage, sex, and nutritional status. With carefully controlled experimental conditions, batches of 10 to 30 individuals can be used, ideally with 3 to 5 replications. Each batch must be randomly selected from the population to ensure representativeness. Dosages should be evenly spaced across the mortality range, as toxicity is generally linked to the logarithm of the dosage rather than the dosage itself, making a dose-response relationship essential for determining toxicity.

Test animals are usually divided into several groups, typically six. One group serves as a control and is treated only with the solvent, while the other groups receive the test compound in doses that follow a geometric progression (e.g., 1, 2, 4, 8 or 1, 3, 9, 27). After exposure to different insecticide concentrations, the insects may be transferred to another container with food, depending on the method. Mortality counts are taken at set intervals until the rate stabilizes, although some insects may die naturally during the experiment for reasons unrelated to the insecticide.

$$\textbf{Corrected \% mortality} = \frac{\textbf{\% survival in the control - \% survival in the treatment}}{\textbf{\% survival in the treatment}} \times 100$$

Preparation of Insecticide Stock Solutions for Laboratory Tests

1. ***Correction Factor Method:*** Calculate the weight of insecticide needed using the formula: Weight = Concentration x Volume x CF. Weigh the insecticide, dissolve it in acetone to the desired volume, and store in a sealed vial.
2. ***Serial Dilution Method:*** Prepare a stock solution at the highest concentration. For each dilution, mix a measured volume of the stock with a solvent to achieve the desired lower concentration, then label and store each dilution.

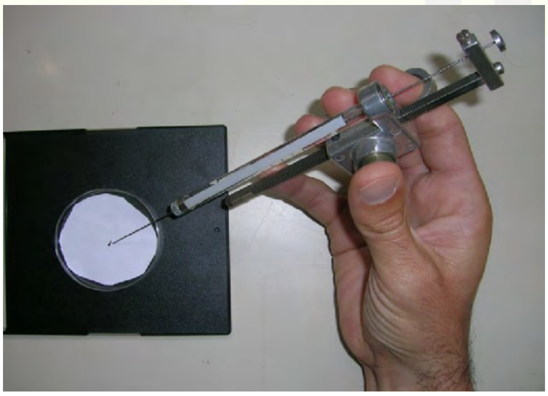
Factors Influencing Bioassays

Biological: While the selection of test insects for bioassays is based on their susceptibility to the toxicant, factors such as the insect's developmental stage, sex, and size also play a crucial role.

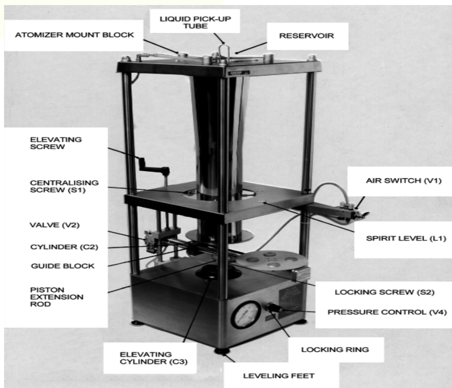
Physical and Chemical: Physical contamination with either toxic or non-toxic materials can interfere with the bioassay process. Chemically, some insecticides are more volatile and readily evaporate.

Bioassay methods

1. ***Film method:*** The insecticide solution is usually applied to a glass surface, with petri dishes being the most commonly used containers. A one-milliliter solution is spread evenly over the inner surface of the petri dish and allowed to dry. Once the solution has dried, the target insects are placed in the dish and exposed to the treated surface for 18-24 hours. A known number of insects are used to evaluate the insecticidal effect.
2. ***Topical application:*** This method is frequently used when the insecticide is dissolved in a relatively non-toxic and volatile solvent, such as acetone. Small, measured droplets of the solution are applied to a specific area of the body, typically the thorax of individual third-stage larvae, using a manually operated micro-applicator. Alternatively, a motor-driven topical applicator with a micrometer-controlled precision syringe can be used for accurate and consistent application of the insecticide droplets.



Topical Application



Potter's Tower Method

Advantages

This method provides a high level of precision, ensuring reliable and accurate results. It allows for a large number of tests to be conducted in a relatively short period, improving efficiency in experimental procedures. Furthermore, it requires only a small number of insects per replication (usually 10-20), making it suitable for studies with limited insect availability. The simplicity and cost-effectiveness of the necessary equipment enhance the method's practicality. Additionally, it uses only minimal quantities of toxicants and solvents, reducing both costs and environmental impact while maintaining safe handling conditions.

3. ***Potter's Tower Method:*** Uniform spraying or dusting on an insect's body can be achieved using Potter's tower. Potter designed a spray tower featuring a twin-fluid nozzle positioned centrally at the top of an open-ended metal tube. The sprays descend vertically and settle on a horizontal plane. To apply the treatment to the entire insect body, the Petri dish containing a known number of insects is placed beneath the tower, and the spray is applied through the nozzle at the bottom of the tower, using a controlled pressure. This method mimics field exposure conditions, making it valuable for pest management. The technique has become one of the most efficient methods for accurately dispensing a known amount of toxins onto insects.
4. ***Injection Method:*** In this technique, the insecticide is directly injected into the insect's body (usually the thorax) using a hypodermic needle. A very fine stainless-steel needle, typically 27- or 30-gauge (0.41 or 0.30 mm in diameter), is used for this purpose. For small insects, glass needles with diameters of 0.1-0.16 mm are employed. The insecticide is generally dissolved in propylene glycol or peanut oil and injected intraperitoneally (into the body cavity). Care must be taken to avoid causing bleeding in the insect. This method is particularly useful for determining the exact amount of toxicant inside the insect's body.
5. ***Dipping Method:*** In this approach, maggots are carefully picked up with forceps and dipped into an insecticidal solution. This immersion method is often used when topical application or injection is not feasible, making it particularly suitable for small plant-feeding insects where other methods might be impractical. The dipping method is a convenient and effective field bioassay technique, favored by extension workers and field personnel for its simplicity and ease of application in real-world settings.
6. ***Fumigation Method:*** This method is especially effective for controlling stored grain pests. Grains are subjected to fumigation with a specific insecticide preparation for a set duration. The fumigation occurs in a closed container

maintained at 30 ± 2 ˚C and 60 ± 5% relative humidity (RH). Each fumigation test, including controls, is replicated at least three times for reliability. After the exposure period, insects are transferred to a recovery room and given a small amount of culture medium for a week to assess their recovery and survival.

7. ***Aqueous Solution Method:*** In this method, the insecticide is mixed with a measured amount of water in a suitable container. Aquatic organisms, such as mosquito larvae, crustaceans, and fish, are then introduced into this solution. This approach ensures continuous and effective exposure of the entire organism to the treated water.
8. ***Contact or Residual Method:*** In this technique, the insecticide is diluted in a volatile solvent and coated inside a glass vial. The vial is rotated to allow the solvent to evaporate, leaving a thin residual film of insecticide on the inner surface. Insects are then placed into the treated vial and exposed to the residual insecticide. However, this method does not accurately simulate field conditions and does not verify the efficiency of the insecticide dose application.
9. ***Photo-migration Method:*** Developed by Burchfield et al., this method uses the negative phototactic response of Aedes aegypti larvae. The insecticidal solution is evaporated under a gentle stream of air, and the residue is redissolved in a small amount of acetone, with 50 mL of water added. One to two hundred larvae are confined behind a porous barrier in a glass trough containing this solution. After a set period, the light is turned on and the barrier is removed. Viable larvae quickly move away from the light. After one minute, a second barrier is placed in the trough, and larvae left behind are considered dead. The T50 (time required to inactivate 50% of the test population) and LC50 are determined from a series of dilutions.

Limitations

Testing requires identifying and using the most sensitive organism for a specific toxicant. Proper rearing, handling, and maintaining uniformity of the test organism are essential for reliable results. However, complexities may arise in the rearing process or assay methods for specific organisms. Standardizing observation times is crucial to reduce variability, but significant differences in results can still occur with changes in the test organism. While some methods may lack specificity, others achieve high specificity but may not account for the various toxic metabolites, especially in residue determination.

Conclusion

Bioassays are essential for evaluating the toxicity of insecticides across different insect species, aiding in the identification of effective chemicals and assessing their safety for non-target organisms. Each bioassay method has its own advantages and challenges, including the selection of suitable test insects and maintaining consistent conditions. Despite these limitations, bioassays are crucial in pest management as they offer valuable insights into insecticide potency, efficacy, and environmental impact. Enhancing these methods will further our understanding and application of insecticides in various ecological contexts.

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