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Laboratory bioassay methods to assess the insecticide toxicity against insect pests-A review

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Abstract
Laboratory bioassays have become increasingly important because of their predictive value, generating comparative toxicity data on many chemicals in relatively short times at relatively low expense. The laboratory investigations provide a better understanding of insect-insecticide or insect-plant-insecticide interactions. It is a simple, versatile, easy and sensitive technique for determining toxicity of wide range of chemicals, which greatly facilitates the determination of the LD_{50}, LC_{50} or any other lethal concentration/dose. The objective of present reviews is to know the different laboratory bioassay techniques for evaluation of different insecticide against insect pests.

Keywords: Bioassay, insecticides, toxicity

1. Introduction
Chemical synthetic insecticides are still considered the mainstay of agricultural pest control. Although development of resistance against insecticides is a common phenomenon, recent advances in research and technology has renewed interest in this subject and resistance risk assessments have been developed for many species using different assay methods [1]. The principle of bioassay studies to evaluate the toxicity of insecticides with diverse mode of action to the same species under the same test conditions. Due to increasing uses of highly toxic insecticides to control destructive insect pests, it is important to analyze not only the technical and formulated materials but also minute quantities of their residues on/in plant and animal tissues [2]. In an effective bioassay, the indicator species should be sufficiently sensitive to detect even small amounts of insecticides and should express the response with increasing concentrations [3]. The toxic interactions of an insecticide with a biological system are dose dependent. The toxicity of an insecticide to an organism is usually expressed in terms of LD_{50} (lethal dose).This value represents the dose per unit weight lethal to 50% of the population of the organism [4]. The LD_{50} is commonly expressed as milligram per kilogram (mg/kg). In some cases, the LC_{50} (lethal concentration) is used to express the concentration of the insecticide in the external media that will kill half of the test population because the exact dose initially given to the insect cannot be determined [5]. Bioassay methods, on the other hand, are highly sensitive, simple in operation, easily adapted to the assay of newer insecticides and capable of detecting toxic metabolites. The main purpose of these bioassays is to select newer insecticides and their most appropriate doses that affect insects, as well as to test pest resistance [6] and the pesticide selectivity to natural enemies [7]. Important factors that influence bioassays are stage of the insects, choice of insecticide bioassay response, method of application, bioassay environment, diet, sample size, sampling, health of the organism and operator skill etc. In addition to choosing the correct bioassay technique, it is equally important that bioassay data be correlated with expected field efficacy of the pesticide [8]. Here the attempt was made to review the different laboratory bioassay methods available to evaluate the pesticide toxicity against insect pests.

2. Testing procedures
Batches of insects are exposed to dosages of an insecticide. The insects to be tested should be free from variations due to age, stage, sex, condition of nutrition, etc. With precise experimental conditions, batches as small as 10 to 20 individuals may be used and it is desirable to replicate the batches 3 to 5 times. The batches of insects should be so formed as to ensure that each batch is a random sample of the population. The dosages for testing should be spaced as evenly as possible over the mortality range and since the toxic effect is generally

related to logarithm of the dosage, rather than the dosage itself. The toxicity of an insecticide is usually determined using a dose response relationship. The animals available for testing are divided at random into several groups, usually six. One of these is used as the control group to be treated with solvent only. The other groups are treated with the test compound with doses usually in a geometric progression (i.e., 1, 2, 4, 6 or 1, 3, 9, 27 etc). After the insects are exposed to different concentrations of an insecticide, they may or may not be kept in another container having food depending upon the method employed. The mortality count is taken after an interval of time already calibrated at different time intervals and when it becomes constant at particular period. The period should be fixed an exposure period. Often a few insects die during an experiment from natural causes which not concerned with the insecticide use. The magnitude of this mortality should also be estimated. This can be done by exposing the batch of insects in control (where no insecticide is applied) exactly in the same manner as is done in exposure to insecticide. When there is natural mortality among the controls, the mortalities have to be corrected by Abhott's formula [9] as follows

$$\text{Corrected \% mortality} = \frac{\text{Percent survival in the untreated controls- Percent survival in the treated insects}}{\text{Percent survival in the treated insects}} \times 100$$

The control mortality should be less than 20%; otherwise, the corrected mortality will not be reliable. The mortality in control so obtained will affect the precision of the result.

3. Preparation of stock solutions

Technical grade (95-99% purity) insecticides are used for the laboratory tests. The active ingredient (a.i) of the insecticides varies so a 100 % stock solution is prepared using the correction factor (CF) below.

CF= 100% divided by % a.i of the insecticides.

For technical insecticide with 99.5 % a.i., CF= 100/99.5= 1.005

Given the CF, compute the weight of the technical insecticide needed for prepare desired volume and concentration using the formula: Concentration of insecticides x volume x CF

Eg. To prepare 2.5 ml of 10000 µg/ml stock solution, the weight of insecticide needed will be 10000 µg/ml x 2.5 ml x 1.005 = 25,125 µg or 25.125 mg or 0.025 g.

Weigh 0.025g of technical grade insecticide in a 6 ml screw cap vial using the analytical weighing balance and makeup the volume upto 2.5 ml with analytical grade acetone. After preparation secure the cap of the vials with parafilm to minimize evaporation. Store the prepared insecticide dilutions in a refrigerator (4 °C) or freezer (preferably -20 °C). Replace and dispose properly the tips after preparation of an insecticide.

4. Bioassay methods

Bioassay methods commonly employed for insecticide toxicity evaluation are topical application, potter's tower method, injection method, dipping method (leaf dip and larvae dip), contact or residual method, film method, etc.

4.1 Topical application

A commonly employed method is topical application, where the insecticide is dissolved in a relatively nontoxic and volatile solvent such as acetone and small, measured droplets are applied at a chosen location on the body surface on the thorax of individual third stage larvae with a operated micro applicator [1]. A motor driven topical applicator is available with micrometer-driven precision syringe. The advantage of this methods are, the high degree of precision and reproducibility that can be attained, large number of tests can be performed in a relatively short time, small number of insects (10-20) required per replication, simple and inexpensive equipment needed, small amount of toxicant and solvents used.

4.2 Potter's tower method

Uniform spraying or dusting on the body of insect can be done by means of potter's tower. Potter [10-11] designed a spray tower with a twin-fluid nozzle mounted centrally at the top of an open ended metal tube where the sprays falls vertically and deposits on horizontal plane. The topical application on entire insect body can be done by potter's tower by keeping the Petri dish containing known number of insects under the bottom part of the tower and spraying inside through nozzle fitted in the lower side by maintaining a particular pressure. This methodology simulates field exposure conditions and hence is informative for pest management. The technique has emerged as one of the most convenient methods of dispensing known amount of toxins accurately on insects. The major disadvantages of the method are the potter's tower method has a slow execution time and a high initial cost, due to the necessity of purchasing equipment.

4.2.1 Potter spray tower

The Potter spray tower has been named after C. Potter, who has developed this spray apparatus at Rothamsted Experimental Station, Harpenden, Herts, England [11]. The Potter tower is internationally recognized as the standard of reference for chemical spraying techniques in the laboratory. This type of apparatus is required for studying the biological effects of contact poisons on organisms. Potters spray tower is developed to prevent the operator exposure and contamination to the toxins/ pesticides.

4.2.2 Principle

It works on the principle of constant atmospheric pressure. Constant supply of 151b/sq.in through the input connection controlled by an on/off switch and exhaust valve. Fine adjustment is made by a sensitive needle valve on the left hand side of the instrument giving direct reading on the pressure gauge, or manometer (which is supplied as an extra). This pressure operates the air jack and nozzle head simultaneously.

4.2.3 Instrumentation

The tower is manufactured from an attractively finished high grade of stainless steel. Height of potter spray tower is 120 cm. Its standard sample reservoir capacity is 20 cc. Its operating pressure is 151b/sq.in. The tower contains quickly detachable atomizers and a pneumatically operated spray table, with all controls mounted conveniently at the front. This air operated spraying apparatus applies an even deposit of spray over a circular area of 9 cm diameter. The testing deposit of insecticide is to apply a spray solution or suspension to a Petri dish using a precision sprayer. This device delivers a known volume of spray in a frame mist that

settles on the surface to be treated. In tests with aphids or spider mites, a leaf substrate contained in a Petri dish can be sprayed by the spraying apparatus.

4.3 Injection method

In this technique the insecticide is directly injected into the insect body (thorax in insects) by hypodermic needle. The method employs a very fine stainless steel needle (27 or 30 gauge thickness and 0.41 or 0.30 mm in diameter) is used and the quantity of toxicant required is measured by micrometer. Small glass needles of 0.1-0.16 mm in diameter are used for injection in small insects. The insecticide is commonly dissolved in propylene glycol or peanut oil and injected intraperitoneally (into the body cavity). Care should be taken to avoid bleeding by the insect. This method is specifically employed to know the exact amount of toxicant needed inside the body of the insect [2].

4.4 Dipping method

This method is employed when topical application or injection methods are impractical, for example: with small plant-feeding insects, stored-product insects, housefly larvae, insect eggs, red spiders, etc. The insects are picked up with a pair of forceps and dipped into an aqueous solution of the chemical for short periods of time, which is either a suspension or an emulsion [12]. Insect immersion methods are convenient field based bioassays useful for extension and field workers. The methods are simple and are somewhat closer to field application of insecticides. In another dipping method the leaves are dipped in aqueous solution of insecticides for definite period in serial dilution of different concentrations. The leaves were carefully drained of excess solutions and air dried for an hr before being used [13]. After few minutes the known numbers of insects watch feed on the leaves in question are released and the mortality counts are done after a definite period. This method can also be employed against sucking insects which are not easily removed from the leaf surface. The leaves containing insects are dipped in insecticidal solution for a definite period and the population counts before and after definite time interval of dipping are made. This technique allows the product to be evenly distributed on the leaf surface and makes it possible to check whether or not the field doses are effective for the pest control [6].

4.5 Contact or Residual method

In this method, the formulated insecticide is diluted in a volatile solvent (acetone) and the insecticide solution is coated inside a glass vial. The solvent is allowed to evaporate by rotating the container so that the insecticide is spread evenly over the entire surface leaving a residual film. The dose is varied by the concentration f insecticide solution added to the vials [12]. Insects are released on to the treated surface and thus get exposed to residual film. Alternatively, the insecticide is applied evenly on to leaf, glass, filter paper, wood panel or other types of building materials and allowed to dry before exposing the insects to the residual deposits. For uniform application, equipment called Potter's tower is frequently used. The deposits are expressed as milligrams or grams of active ingredient per square meter (mg or g a.i./m^2). These techniques do not represent field situations and do not allow us to verify whether doses are being applied efficiently [7].

4.6 Film method

The technique involves insecticide solution is usually deposited on glass surface such as Petri dish, flask, vial, wide mouth jar etc. Petri dishs are most commonly used for evaluating insecticide efficacy. In this approach Petri dishes (5 cm diameter) are coated with one milliliter solution on their inner sides and the solution is allowed for uniform spreading in the Petri dish by swirling it gently and then allowing it dry up at room temperature. The target test insects are then released onto the film of the toxicant in the container. Thereafter; the known numbers of insects are exposed on for a period of 18-24 hours depending upon the recalculation [2, 14].

4.7 Fumigation method

The fumigation method is suitable for stored-product pests. The fumigation would be performed in a closed chamber at 30 ± 2 °C and 60 ± 5 % relative humidity. The insecticide is introduced into a sealed container along with the insects. Each fumigation test is replicated thrice or more along with control and after exposure the insects would be provided with small quantity of culture medium for a week and moved to recovery room. Adult mortality is recorded at different time intervals from the end of the exposure period [12].

4.8 Aqueous solution method

The principle of this method is to disperse the insecticide residue in a small amount of water miscible solvent (acetone or alcohol) into water as a solution or suspension. Then a small number of sensitive aquatic organisms such as mosquito larvae, micro crustaceans or fish are exposed to the solution. The amount of residue in a treated sample is obtained by comparing its toxicity to that of the standard. The amount of solvent in water should be as little as possible and its toxicity, if any, can be detected by control tests [2]. This method has the advantage of having the whole organism constantly in contact with the medium. Some have claimed uniform administration of the toxicant, but settling of the suspended material may occur. High sensitivity is thought to be the result of circulation and absorption of the toxicant through the gills or equivalent organs [15].

4.9 Photomigration method

Photomigration method is another aqueous solution method which was devised by Burchfield *et al* [16] using negative phototaxic response to larvae of *Aedes aegypti*. The insecticidal solution is carefully evaporated under gentle stream of air to dryness and the residue is redissolved in small amount of acetone and then 50 mL water is added. One to two hundred larvae are then confined behind a porus barrier in a glass trough containing above solution. After varying time period, the light is turned on and barrier is removed. Viable larvae rapidly migrate away from the light. After one minute exposure, a second barrier is kept in the trough and the larvae left behind are considered as dead. From a series of dilution T_{50} (The time required to inactivate 50 % of the test population) and LC_{50} are worked out.

The per cent mortality corresponding to each dose calculated from the number of insects released and the mortality observed after exposure period. The mortality in control if any will affect the precision of the result. In order to over this error, a correction is usually applied by using the Abott's formula.

The susceptibility of any population to a poison is assessed by constructing a dosage-mortality curve in which the dosage is plotted against the percentage mortality at a given period of time. Such a plot produces a sigmoid curve whose asymptotic approaches (infinite ends) at the regions of zero and 100% mortality are difficult to define without extensive testing. In probit transformation, the sigmoid curve is converted to a straight line by plotting the logarithm of the dosage against the probit value of percent mortality. This method of computation yields a straight line, which greatly facilitates the determination of the LC_{50}, LC_{90} or any other lethal concentration/dose.

5. Utility of bioassay

1. Bioassay is adopted to ascertain the potency of the chemicals as insecticides.
2. It helps to find out the property of synergism, potentiation and antagonism of a compound when used in a mixture with an insecticide.
3. Comparative or relative toxicity of insecticides is worked out on the basis of LC_{50} obtained as a result of bioassay. This gives an index for selecting promising insecticides for field trial against insect pests.
4. Bioassay helps in evaluation of insecticides for their safety to pollinators, predators and pathogens.
5. Bioassay can help the formulators in improving the effectiveness for their formulated products, through changes in solvent, spreader, emulsifier, stickers etc.
6. The quality of marketed insecticides can be checked through bioassay of samples collected and comparing them with standard.
7. The change in values of LC_{50} of an insecticide for an insect with the passage of time indicates variation in susceptibility which helps in detection of resistance if developed in the insect population. Cross resistance to other insecticides, use of synergists and mixed formulations to overcome resistance are also estimated through bioassay.
8. Formation of toxic metabolites, not quantitatively, due to use of insecticide can be determined by bioassay.
9. Through bioassay lethal time LT_{50} required to kill 50 % population to test animal. ED_{50} or EC_{50} i.e. the dose or concentration of chemical brings out sterility or other quantitative effects in 50 % population can also be worked out.
10. Bioassay can also be utilized for estimation of micro quantities i.e. residues of insecticides in different commodities in order to alarm the consumers from hazards associated.

5.1 Limitations

1. Requirement of most sensitive organism for particular toxicant.
2. Rearing, handling and maintaining of uniformity of test organism. There may be the complexity of rearing or assay method for a specific organism.
3. Sometimes there is susceptibility of particular organism to plant toxicity or extractives.
4. Standardization of observation time.
5. There may be great variation in results with the change in test organism.
6. Lack of specificity in general though there are some instances in which it is highly specific.
7. Does not tell about the quantities of the different toxic metabolites in case of residue determination.

6. Conclusion

The understanding of median lethal dose, lethal concentration and toxicity is very important for the better evaluation of the toxic of the particular pesticides. Bioassay can be a useful tool for the determination and study of different agricultural pesticides. It can be simple, swift, versatile and highly sensitive to a wide range of toxicants. Generally little or no expensive equipment or highly trained help is required. Although used to best advantage when the toxicant is known, bioassay can be used in some instances to identify toxicants.

7. References

1. Durmuşoğlu E, Hatipoğlu A, Gürkan MO, Moores G. Comparison of different bioassay methods for determining insecticide resistance in European Grapevine Moth, *Lobesia botrana* (Denis & Schiffermüller) (Lepidoptera: Tortricidae). Turkish Journal of Entomology. 2015; 39(3):271-276.
2. Sun YP. Bioassay-Insects. In: Principles, methods and general applications-volume 1 in Analytical methods for pesticides, plant growth regulators and food additives (Eds. Gunter Zweig), Published by Elsevier, 1963; 399-423.
3. Chhillar BS, Gulati R, Bhatnagar P. Bioassay. In: Agricultural Acarology. Published by Daya Publishing House, New Delhi, 2007, 220-230.
4. Finney DJ. Probit Analysis. 3rd ed. Cambridge, UK: Cambridge University Press, 1971.
5. Regupathy A, Dhamu KP. Statistics work book for insecticide toxicology, Softeck Computers, Coimbatore, Tamil Nadu, 2001, 1-206.
6. Siqueira HAA, Guedes RNC, Picanço MC. Insecticide resistance in populations of *Tuta absoluta* (Lepdoptera: Gelechiidae). Agricultural and Forest Entomology. 2000; 2:147-153.
7. Bacci L, Picanco MC, Barros EC, Rosado JF, Silva GA, Silva VF *et al*. Physiological selectivity of insecticides to wasps (Hymenoptera: Vespidae) preying on the diamondback moth. Sociobiology. 2009; 53:151-167.
8. Ball HJ. Insecticide resistance - a practical assessment. Bulletin of the Entomological Society of America. 1981; 27:261-262.
9. Abbott WS. A method of computing the effectiveness of an insecticide. Journal of Economic Entomology. 1925; 18:265-267.
10. Potter C. A laboratory spraying apparatus and technique for investigating the action of contact insecticides with some notes on suitable test insects. Annals of Applied Biology. 1941; 28:142-169.
11. Potter C. An improved laboratory apparatus for applying direct sprays and surface films with data on the electrostatic charge on atomized sprays. Annals of Applied Biology, 1952; 39:1-28.
12. Simon JY. The Toxicology and Biochemistry of Insecticides. CRC press, 2008, 1-276.
13. Immaraju JA, Morse JG, Brawne OL. Evaluation of three bioassay techniques for citrus thrips' resistance and correlation of the leaf dip method to field mortality. Journal of Agricultural Entomology. 1990; 1:17-27.
14. Birah A, Alpana BS, Mahapatro GK, Gupta P. Toxicity evaluation of emamectin benzoate against tobacco Caterpillar (spodoptera utura) by three different assay techniques. Indian Journal of Entomology. 2008; 70(3):200-205.
15. Dewey JE. Utility of bioassay in the determination of

pesticide residues. Agricultural and Food Chemistry. 1958; 6(4):274-281.
16. Burchfield HP, Hilchey JD, Storrs EE. An objective method for insecticide bio- assay based on photomigration of mosquito larvae. Contributions from Boyce Thompson Institute. 1952; 17:57-86.