2. Calculation of LD_{50} or LC_{50}

The following steps are taken in calculating the lethal dose of insecticide which is capable of killing 50 per cent of the test population.

(i) Prepare different concentrations of test insecticide and release a known number of dated insects of same age in each concentration. Adequate number of replicates and a control treatment (solvent spray) be taken to minimise the error. After a fixed duration note down the mortality of insects in the treatment as well as in control. Moribund insects be counted as dead. It is desirable to try a standard preparation at several concentrations simultaneously on the same test insect to evaluate relative potency of test preparation with respect to standard preparation.

(ii) Find out the log value of the concentrations of insecticides used with the help of log table. This is denoted as x. Write down the value of actual concentration used in the column one and of x in column two of the Table 1.

(iii) Write down the number of insects released for bioassay at a particular concentration in column three of the table. It is denoted by n. With the help of the data on mortality of test insects calculate the percentage of mortality. The percentage be corrected by applying the Abbotts formula

$$\frac{T-C}{100-C} \times 100$$

Where T = % mortality in the treatment
C = % mortality in the control.

(iv) With the help of the data of corrected mortality empirical probit value be obtained from the probit values given in any standard book (e.g. Table No. 1 in Finney's book entitled probit analysis). These are written in column number six.

(v) Now plot the point on a graph keeping the data on log concentration of insecticides on the horizontal line i.e. on x axis while corresponding data on empirical probit on vertical line i.e. y axis (Fig.1). These plotted points will be irregular and on different heights on the graphs.

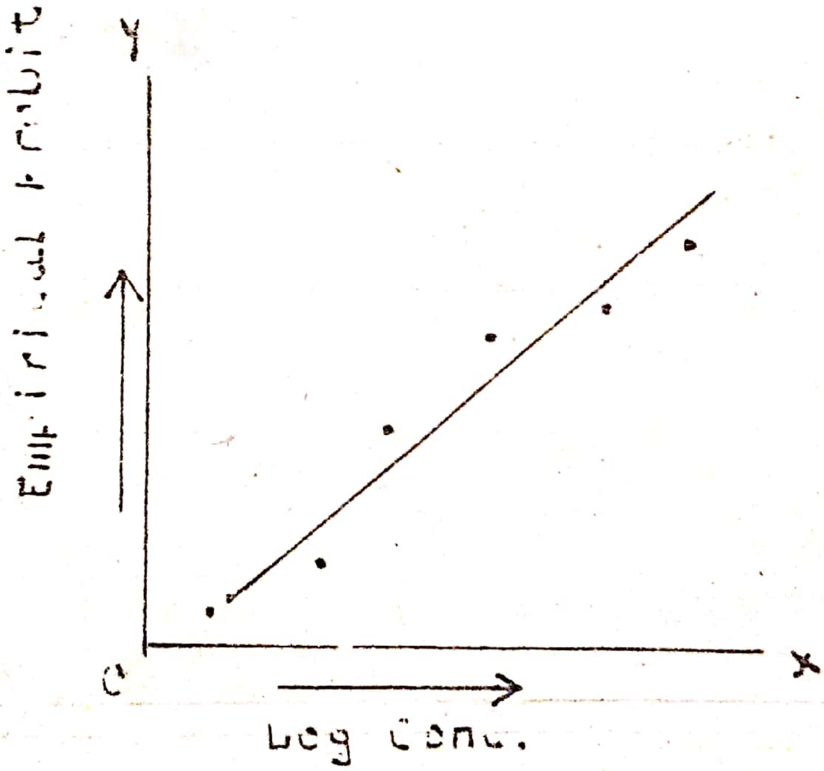

Fig.1 – Points on Probit Graph.

(vi) Assess the position of the points on the graph with care and draw an 'Eye fit' line. While fitting the 'Eye fit' line care should be taken that the line passes closely through the points corresponding to 20% to 80% mortality and about half the plotted points are above and rest are below the eye fit line. Having drawn the eye fit line the next step is to find out the expected probit by shifting the actual points plotted on the graph on the eye fit line itself. The expected probit value is denoted by capital Y and it should be written in column seven.

(vii) Using the value of expected probit corresponding with natural percentage of mortality (i.e. per cent mortality in control) find out the weighting coefficient with the help of the Table (Table II of Finny's book). The weighting coefficient is denoted by 'w'. Its value be written in column eight.

(viii) With the help of data on expected probit and corrected percentage of mortality find out the value of working probit (Table IV of Finney's book). The value of the working probit are denoted as 'y' and are written in column nine.

(ix) In column ten of the table the values of n w be written. They are evidently obtained by multiplying values of n from column three and value of w from column eight.

(x) In column eleven, twelve, thirteen, fourteen and fifteen the following corresponding values be written after calculating them with the help of the data already obtained

Column		Data to be calculated		Mode of Calculation
11	–	nwx	–	(value in column 3) x (value in column 8)
12	–	nwy	–	(column 3) x (column 8) x (column 9)
13	–	nwx^2	–	(column 3) x (column 8) x $(\text{column } 2)^2$
14	–	nwy^2	–	(column 3) x (column 8) x $(\text{column } 9)^2$
15	–	nwxy	–	(column 3) x (column 8) x (column 2) x (column 9)

(xi) Calculate the value of $\bar{x}$ and $\bar{y}$. These are the mean values to be calculated as under:

$$\bar{x} = \frac{Snwx}{Snw} \quad \text{i.e.} \quad \frac{\text{Total of column 11}}{\text{total of column 10}}$$

$$\bar{y} = \frac{Snwy}{Snw} \quad \text{i.e.} \quad \frac{\text{Total of column 12}}{\text{total of column 10}}$$

(xii) Calculate the value of regression coefficient b by the help of following formula:

$$b = \frac{Sxy}{Sxx} \quad \text{Where}$$

$$Sxy = Snwxy - \frac{(Snwx)\ (Snwy)}{(Snw)}$$

i.e. total of column 15 – $\frac{\text{total of column 11 x total of column 12}}{\text{total of column 10}}$

$$Sxx = Snwx^2 - \frac{(Snwx)^2}{Snw}$$

i.e. total of column 13 – $\frac{(\text{total of column 11})^2}{(\text{total of column 10})}$

(xiii) Apply regression equation which is as under:-

$$Y = \bar{y} + b\ (x - \bar{x})$$

Where Y = probit of 50% mortality (From Table No. 1 of Finney's book)

$\bar{y}$ = means (see xi)

b = regression coefficient (see xii)

x = log concentration (unknown)

$\bar{x}$ = means (see xi)

(xiv) As all the values except x are known in the formula $Y = \bar{y} + b(x-\bar{x})$, the value of x can be calculated. Take the antilog of the value of x which would be the concentration of insecticide capable of killing 50 per cent population of insects i.e. it is LC_{50}.

Table 1: Data sheet for Probit Analysis

1	2	3	4	5
Dose or concentration	Log. of Dose/ conc.	Total number of insects	Percentage mortality	Corrected percentage mortality
	x	n		

6	7	8	9	10
Empirical Probit	Expected Probit	Weighting coefficient	Working Probit	
	Y	w	y	nw

11	12	13	14	15
nwx	nwy	nwx^2	nwy^2	nwxy

In order to avoid the negative values of log with transforming the concentration of insecticide into log, the concentration is generally multiplied with 10 or multiple of 10. In such cases the value of x after taking antilog is, therefore, divided with same value with which the concentration was multiplied.

The above test procedure is valid if the mortality in control is small (say less than 20%) and is based on sufficient number of test insects in the control batch. Further there should be no heterogenity of test batches of insects applied to different concentrations of insecticides.

3. **Why bioassay is necessary?**

(i) Bioassay helps in ascertaining the potency of the pesticide used.

(ii) Relative toxicity of different pesticides can be ascertained.

(iii) Insect resistance to different insecticides can be studied.

(iv) Micro–quantities of insecticidal residues retained by the plants can be detected.

(v) Formation of toxic metabolities produced from the insecticides can also be assured.

(vi) New and promising toxicants can be screened.

(vii) New formulations can be developed.

4. **Why LC_{50}/LD_{50} is taken as criterion for comparing the relative toxicities of toxicants?**

In an experiment if the percentage of mortality of an insect is plotted against logarithm of the corresponding concentration/dose, a sigmoid curve is obtained (Fig. 2). The curve indicates that mortality will not increase significantly by increasing the concentration/dose of the toxicant of lower and higher levels of kill. A careful examination of the graph shows that concentration/dose giving 100% kill and zero kill can not be read out with this graph. Further the corresponding concentration/dose giving 1 to 5 percent and 95 or more than 95 per cent kill also can not be worked out with accuracy. The reason being that in both these situations the graph becomes more or less parallel to the x-axis (abscissa) and even a major change in concentration/dose will not produce an effect on the mortality. On the other hand the graph is steepest in the region of 50 per cent response as a result the slightest change in the concentration/dose will produce a large change in the mortality. Thus this concentration/dose LC_{50} or LD_{50} is the most sensitive and reliable index of toxicity. It is due to this reason that LC_{50} or LD_{50} is adopted as the standard for comparing the relative toxicity of insecticides.

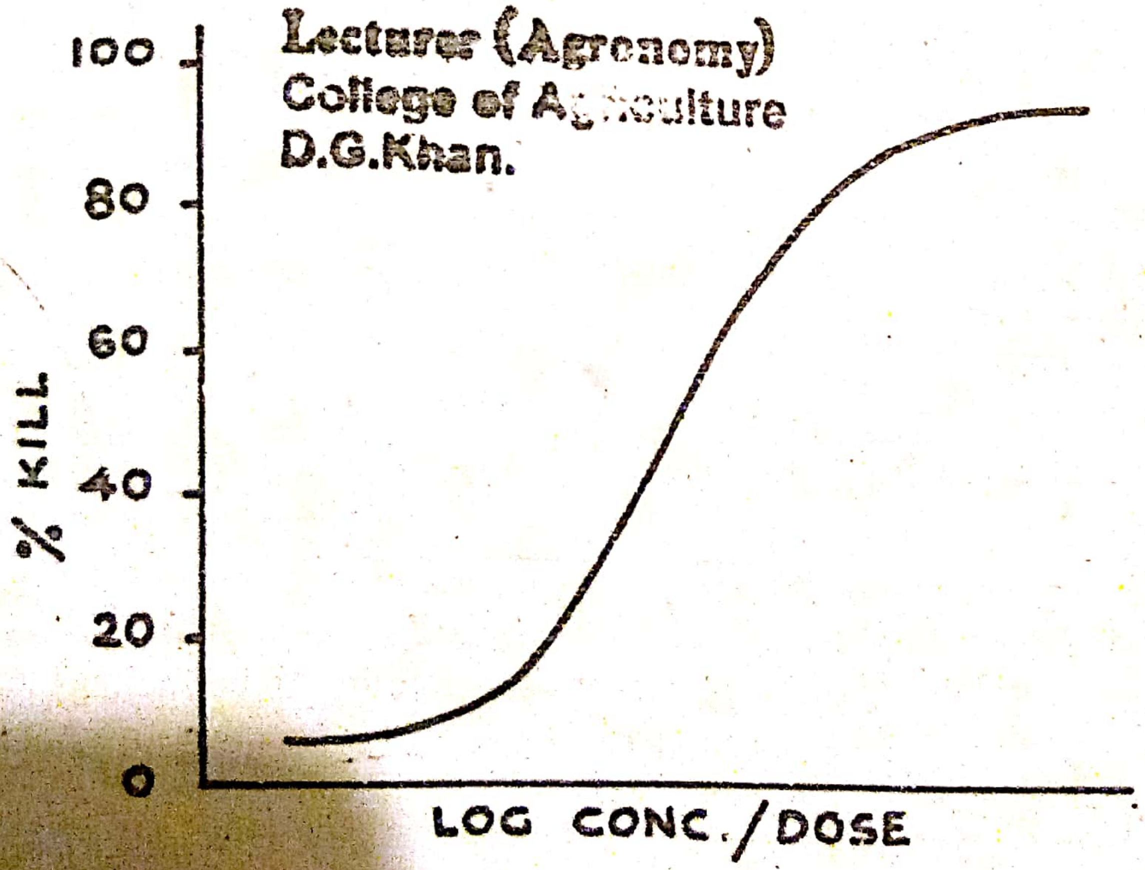

Fig.2 – Dose Response Curve