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# THE PERSISTANCE OF PETKOLIN RESIDUES MEASURED BY BIOLOGICAL METHOD

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The recovery of Petkolin residue on cotton plant leaves was determined by bioassay method. Emulsions of 1, 1.5, 2 and 3% Petkolin were sprayed on cotton leaves at the rate of 1 ml per leaf. Leaves from each concentration were picked 24 hr, 72 hr and 1 week after the treatment. Extractions were done in acetone and the extracts were tested against larvae of *Aedes aegypti* (L) according to WHO method. From 1, 1.5, 2 and 3% Petkolin emulsion sprays, respectively 7, 8, 10 and 11% of the sprayed Petkolin after 24 hr; 4, 4.3, 4.8 and 6% of the sprayed Petkolin after 72 hr and 2, 2.5, 2.9 and 3.3% of the sprayed Petkolin after 1 week was recovered. It was found that Petkolin is a short-lived insecticide and rapidly looses its residual effect.

Petkolin (petroleum-based insecticide[1]) tested in the laboratory[2–4] and in the field[5–8] has been found effective against a number of pests. Determination of its residual effect by spectrophotometric method has been done on cotton leaves.[9] In the present studies a bioassay method has been used to determine Petkolin residues on cotton leaves up to 1 week after the treatment with Petkolin emulsion. In this method mosquito larvae were confined in aqueous suspensions of extract of plants treated with Petkolin.

## Material and Method

Four different concentrations 1, 1.5, 2 and 3% emulsions of Petkolin (100% E.C.) were prepared in water by shaking to spray on the upper side of the cotton leaves in the field at 85–95°F and 70–80% R.H. Four different plants were selected one for each of the four different concentrations. Seventy-five cotton leaves almost of the same size were taken from the middle portion of each plant for the spray of each concentration. On each leaf 1 ml emulsion was carefully sprayed by a De-Vilbiss atomizer in order to minimize the wastage of emulsion. A fifth plant was kept as control on which no insecticide was sprayed. Twenty-five leaves from each plant were picked at random after 24 hr, 72 hr and one week respectively as post-treatment samples. At the same time 25 leaves from the control plant were also picked at random to prepare untreated extract for check. All the 25 leaves treated with one concentration were cut into small pieces (2 × 6 mm approx). with scissors and put into 125 ml of acetone in glass-stoppered flask (in order to prevent evaporation) for 24 hr for extraction. After 24 hr the material was filtered through Whatman filter paper No. 1. The filtrate was then evaporated to 30 ml over a hot plate and filtered again before testing. After filtration each paper was washed with 1 ml of pure acetone in order to extract any Petkolin residue left on the filter paper. These extracts were tested for toxicity to early 4th instar larvae of *Aedes aegypti* (L) reared in these Laboratories.[10] The method described in the WHO Test Kit was followed.[11] According to this 20 larvae were placed in each beaker containing 250 ml of water and aliquots of the extracts of leaves. After 24 hr mortality was observed. The tests were started from 1 ml quantity of 24 hr extract and was gradually increased to 1.5 ml of 72 hr extract and 3 ml of one week extract, in order to get atleast 10% mortality with lowest concentration i.e. 1% Petkolin residue extract. These volumes (1.0, 1.5 and 3 ml) for each concentration were decided after preliminary testing as standard for all the four concentrations because below this dose mortality was not appreciable. Testing experiments were repeated 10 times against the 4th instar mosquito larvae and the experiments were always run in duplicate thus making the total number of experiments as twenty. From the results obtained the average mortality of the 10 close readings was calculated and S.D. values were also calculated (Table 1).

*Preparation of Standard Curve.*—After the experiments were completed a standard curve was prepared to calibrate the method. Twenty five untreated leaves of cotton plants were selected from the middle portion of the plant when conditions of temperature and humidity were the same as during the experiments at the P.C.S.I.R. experimental field. These leaves were cut into pieces and placed in a stoppered flask-containing 125 ml of acetone for extraction. After 24 hr it was filtered and 1 ml of technical Petkolin (100%) was added to the filtrate which was then evaporated to 30 ml as in the case of treated extracts. Dilutions of this extract were prepared to contain 1–15 p.p.m. Petkolin. These dilutions were tested according to the method described above. From the statistically analysed dosage mortality data a standard curve was drawn on log paper (Fig. 1). The mortality in the control was not more than 3–5% at any count.

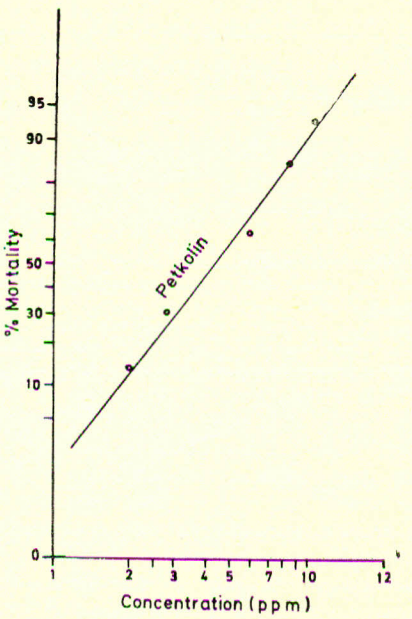


Fig. 1.—Standard dosage-mortality curve.

## Calculations

The amount of toxicant in p.p.m. in each treated sample against the mortality it yielded (Table 1) was directly read from the standard curve (Fig. 1). For example 1 ml of 24 hr Petkolin residue extract of cotton leaves treated into 1% emulsion of Petkolin gave 20% mortality of mosquito larvae (Table 1) which is equivalent to 2.5 p.p.m. in the standard curve. The actual amount of Petkolin in p.p.m. in the same quantity and in the same concentration (1 ml and 1%) of a standard test extract was calculated in the following way.

Amount of Petkolin in p.p.m. in a standard test extract

$$= \frac{A \times B}{C \times D} \times 1000000$$

Wher A, Actual amount of Petkolin used per 25 cotton leaves (ml); B, Amount of extract tested per beaker (250 ml water); C, Total amount of extract prepared (ml); and D, Amount of water per beaker (250 ml).

$$= \frac{0.25 \times 1}{30 \times 250} \times 1000000$$

= 33 p.p.m. (example from Table 1).

The per cent recoveries of Petkolin in all other test samples were calculated similarly (Table 1).

TABLE 1.—PER CENT RECOVERY OF PETKOLIN RESIDUES FROM COTTON PLANT LEAVES BY BIOASSAY METHOD 24, 72 HR AND ONE WEEK AFTER FIELD TREATMENT.

| Petkolin sprayed/25 leaves | | | Volume of extract | | | Average % mortality of mosquito larvae after 24 hr | | | Amount of Petkolin recovered (read from standard curve) as residue P.p.m. | | | Actual amount of sprayed Petkolin (calculated in a standard test) P.p.m. | | | Recovery of Petkolin (%) | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Emulsion | | | | Vol. tested 250 ml water (ml) | | | | | | | | | | | | | |
| Conc% | Volume (ml) | Actual volume of Petkolin (ml) | Final volume (ml) | 24 hr | 72 hr | One week | 24 hr | 72 hr | One week | 24 hr | 72 hr | One week | 24 hr | 72 hr | One week | 24 hr | 72 hr | One week |
| 1 | 25 | 0.25 | 30 | 1 | 1.5 | 3 | 20.0 | 14.5 | 2.5 | 2.1 | 2.0 | 33 | 50 | 50 | 100 | 7.5±0.92 | 4.4±0.42 | 2.0±0.16 |
| 1.5 | 25 | 0.37 | 30 | 1 | 1.5 | 3 | 45.0 | 40.0 | 35.5 | 4.0 | 3.5 | 3.7 | 49 | 74 | 148 | 8.2±0.57 | 4.8±0.49 | 2.4±0.36 |
| 2 | 25 | 0.5 | 30 | 1 | 1.5 | 3 | 77.0 | 64.0 | 68.3 | 7.0 | 5.2 | 5.9 | 66 | 100 | 200 | 10.4±2.16 | 5.3±0.77 | 2.9±0.05 |
| 3 | 25 | 0.75 | 30 | 1 | 1.5 | 3 | 96.0 | 94.0 | 91.6 | 11.0 | 10.1 | 10.05 | 100 | 150 | 300 | 11.0±1.22 | 6.8±0.40 | 3.3±0.09 |
| Untreated extract | | | 30 | 1 | 1.5 | 3 | 0.0 | 1.2 | 0.8 | — | — | — | — | — | — | — | — | — |

## Results and Discussion

The percentage recoveries of Petkolin residue from the cotton leaves by bioassay method are given in Table 1. The quantity of 1, 1.5, and 3 ml respectively from 1 day, 3 days, and 7 days extracts was tested according to the method referred above. From different mortalities obtained, the corresponding amounts of Petkolin in p.p.m. were directly read from the standard curve. The lowest sensitivity of Petkolin residue after 1 day, 3 days and 7 days extracts was respectively determined as 2.5, 2.1 and 2 p.p.m. while the highest sensitivity was found as 11, 10.1 and 10.05 p.p.m. respectively. The lowest percentage of Petkolin residue recovered from one day, three days and seven days extracts was 7.5%, 4.37% and 2% respectively while the highest amount of Petkolin was found to be 11%, 6.76% and 3.33% respectively.

It is apparent from the data that with respect to the different concentrations of Petkolin emulsions (1%, 1.5%, 32% and 3%) as well as different intervals of sampling (1 day, 3 days and 7 days) there was a decrease in the percentage recovery of Petkolin residue. It shows that an appreciable decrease in Petkolin residue was found in respect of sampling period. For example in 1% extract, recovery of Petkolin residue after one day was 7.5% and after three days it was 4.37% which is nearly half of 7.5% and after seven days it was 2% which is approximately half of 4.5%. More or less the same ratio of decrease in Petkolin residue was found in the case of 1.5%, 2% and 3% extracts.

It is therefore apparent that Petkolin is a short-lived insecticide and rapidly looses its residual effect which was determined to be 3.33% only of the sprayed Petkolin seven days after the treatment with as high as 3% Petkolin emulsion. Residual persistance of Petkolin up to seven days in the present studies was found in agreement with the results of Petkolin residue by spectrophotometric method reported by Ashrafi *et al.*[10]

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