Insecticidal effect of three IGRs, diflubenzuron, precocene (ii) and rakshak against *Papilio demoleus* (Linn.)

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Abstract

A laboratory bioassay was carried out to determine the LC$_{50}$ and relative toxicity of three insect growth regulators viz., Diflubenzuron (dimlin), Precocene II and Rakshak (azadirachtin) to control on the citrus butterfly *Papilio demoleus*. The bioassays studies revealed that Diflubenzuron showed the maximum efficacy (LC$_{50}$= 0.3456%) followed by Precocene II (LC$_{50}$= 0.5832%) and Rakshak (LC$_{50}$= 0.7525%). In terms of LC$_{50}$ values the order of toxicity was Diflubenzuron > Precocene II > Rakshak. The order of relative toxicity was observed as Diflubenzuron (LC$_{50}$= 1.687) > Precocene II (LC$_{50}$= 1.000) > Rakshak (LC$_{50}$= 0.775).

Keywords: Efficacy, *Papilio demoleus*, LC$_{50}$, insect growth regulators

Introduction

Insecticides are the most effective means of protecting crop against insects’ damage as they provide rapid control. The complex chemistry of pesticides, persistence in the environment, toxicity to animals and human beings and bioaccumulation- risks make pesticidal pollution as a critical problem. The OMRI approved active ingredient, azadirachtin, was registered as a reduced risk biopesticide by the U.S. EPA in 1985, and was soon registered and approved for pest control in organic systems (Organic Material Review Institute 2011) [11]. As an insect growth regulator azadirachtin has ecdysteroid and juvenile hormone properties (Aertz et al. 1997) [2], while also acting as a stomach poison and feeding deterrent. It has low mammalian toxicity, degrades rapidly in the environment, and shows little harm to beneficial insects (Lowery et al. 1993) [9]. Diflubenzuron also acts as an IGR, specifically, a chitin synthesis inhibitor towards insects. This chemical has become an important tool in management of grasshoppers, providing effective long term control if applied at the proper insect growth state (Latchininsky 2004) [8]. In addition, the minimal impact on natural enemies including damsel bugs, Nabidae, Coccinelidae and lace wings, Chrysopidae. [12, 13]

2. Materials and methods

2.1 Test insect

The lemon butterfly, *P. demoleus* is a key pest of citrus in India. It feeds voraciously on vegetative grow of citrus plants throughout the year. It is most destructive to citrus seedlings as well as new flushes.

2.2 Mass rearing of *Papilio demoleus* in the laboratory

Eggs and early larval instars were collected from the lemon nurseries and reared in the laboratory on fresh lemon leaves food was supplied daily in Environmental Chamber and maintained at 28±1° C temperature, 75-80% R.H. Third instar larvae of desired age groups were sorted out. The fully grown larvae were allowed to pupate on lemon leaves. Soon after emergence, adults were transferred on potted plants of lemon covered with a glass chimney for egg laying. The mouth of each chimney was covered with a muslin cloth secured with a rubber band. The cotton bolls soaked with 10% glucose solution were hanged with the help of a thread to provide food for adults. The eggs laid on leaves were removed from the slits of lemon leaf margins and were kept in petridishes for hatching. The newly hatched larvae were
transferred on soft, newly grown up leaves of lemon in petridishes with the help of camel hairbrush. The completely grown 3rd instar larvae were sorted out and placed in a separate glass dish at room temperature for the experiment.

2.3 Preparations of different concentrations of test compounds

The desired concentrations of Diflubenzuron, Precocene II and Rakshak were prepared as from the stock solution by diluting with desired amount of distilled water.

2.4 Testing of insect growth regulators

After conducting preliminary trial to find out the concentrations resulting in 20-80 per cent mortality of experimental insect’s bio-assay test by film method was done to work out the LC50 value. For the preparation of insecticidal films, both lids of Petridishes were sprayed with 1ml of each concentration of the formulation under the Potter’s tower at the constant pressure of 4lbs/sq. inch. The sprayed petridishes were gently shaken under an electric fan for about 10-15 minutes till the liquid of lid’s is evaporated leaving behind an uniform dry film of the formulation on glass surface. Thereafter, 30 3rd instar larvae starved for 6 hrs were exposed to insecticidal film of the formulation for 2 hrs. Following this the caterpillar were transferred to fresh tender leaves of lemon to clean petridishes.

2.5 Assessment of mortality and processing of data

The mortality counts of Papilio demoleus was recorded after 24 hours of treatment. The moribund insects were also counted as dead in the present experimentation. The percentage mortality was corrected for mortality in control formula as given below:

\[
\text{Corrected per cent mortality} = \frac{T-C}{100-C} \times 100
\]

Where,
\( T \) = observed mortality
\( C \) = mortality in control

2.6 Testing the significance of regression coefficient (b) of Probit on Log C

The testing of significance of regression coefficient has been done by ‘t’ test at a given degree of freedom (n-2), which is formulated as under:

\[
t = \frac{b}{S.E. of b}
\]

Where,
\( n \) = number of concentrations
\( b \) = regression coefficient at X

3. Result and discussion

3.1 Effect of Diflubenzuron

The probit analysis of insect growth regulator (table 1.) shows that regression coefficient of probit on log C was 1.5495812 per cent, which was highly significant at 1.0 per cent level of significance. This indicates that rate of increase of the probit corresponds with the increase in concentration and it was highly significant. The value of LC50 in this case was 0.3456 which indicates that 50.0 per cent mortality of P. demoleus will be obtained by using 0.3456 concentration of Diflubenzuron. Hughes et al. [3] and Karimzadeh et al. [5] were also reported same and introduced that hexaflumuron probably decreases chitin synthesis in endocuticle of various instars. The creation of larval-pupal intermediates and defective pupae has been reported in Spodoptera mauritia and H. armigera when treated with diflubenzuron. [4]

Table 1: Showing the toxicity of Diflubenzuron on 3rd instar larvae of Papilio demoleus

| No. | Dose     | Log dose | Subjects | Effected (%) | Emp. Probit | Expected Probit | c 2 | n * w | Working Probit |
|-----|----------|----------|----------|--------------|-------------|----------------|-----|-------|---------------|
| 1   | 2.00000  | 0.30103  | 30       | 27.857       | 6.466       | 89.040         | 0.448| 10.8127| 6.4322        |
| 2   | 1.75000  | 0.24304  | 30       | 26.786       | 6.242       | 87.154         | 0.122| 11.7952| 6.2353        |
| 3   | 1.50000  | 0.17609  | 30       | 24.643       | 5.921       | 84.709         | 0.153| 12.9169| 5.9154        |
| 4   | 1.25000  | 0.09691  | 30       | 23.571       | 5.792       | 81.443         | 0.164| 14.1975| 5.7870        |
| 5   | 1.00000  | 0.00000  | 30       | 21.429       | 5.566       | 76.902         | 0.506| 15.6465| 5.5558        |
| 6   | 0.75000  | 0.12494  | 30       | 20.357       | 5.463       | 70.228         | 0.081| 17.2259| 5.4625        |
| 7   | 0.50000  | 0.30103  | 30       | 17.143       | 5.180       | 59.583         | 0.074| 18.6940| 5.1796        |
| 8   | 0.25000  | 0.60206  | 30       | 13.929       | 4.911       | 40.111         | 0.498| 18.6674| 4.9129        |
|     |          |          |          | Total        |             | 2.045          |     |       |               |

\[ y = 5.7356 + 1.637866 x \]

3.2 Effect of Precocene (II)

Statistical analysis of the toxicity evaluation of Precocene II (Table2.) shows that the rate of increase of probit per unit increase of log C was 1.4548121 per cent and was found significant at 1.0 per cent level of significance. This indicates that the rate of increase of the probit with the increase in concentration was significant. The value of LC50 in this case was 0.5832 at 0.5832 per cent concentration of Precocene II. Kareem and Ahmad [6] evaluated the insecticidal effect of Precocene II against grasshopper.
3.3 Effect of rakshak

The toxicity of Rakshak was calculated on the basis of probit value (Table 3). The regression coefficient of probit on log C was 1.312931 which was highly significant at 0.01 per cent level of significance. This makes clear that probit in this case increase with the increase in concentration of the Rakshak. The value of LC50 was 0.7525 at 0.7525 per cent concentration of Rakshak. Azadirachtin causes growth disruption through its effect on ecdysteroid and juvenile hormone titres that may result in growth delay without mortality, or mortality from moulting aberrations at the larval or pupal stages [1]. Many investigations have found azadirachtin to cause toxicity at the pupal stage of development even when applications are upon early instar larvae [10]. In our investigation, either azadirachtin is causing delays in development without causing any mortality, or azadirachtin is delaying development and will cause mortality at the pupal stage of development.

Table 3: Showing the toxicity of Rakshak on 3rd instar larvae of *Papilio demoleus*

| Dose     | Log Dose | Subjects | Effect | Expected Probit | Chi square | n * w | Working Probit |
|----------|----------|----------|--------|-----------------|------------|-------|----------------|
| 2.00000  | 0.30103  | 30       | 24.643 | 5.921           | 71.754     | 1.598 | 16.9152        |
| 1.75000  | 0.24304  | 30       | 20.357 | 5.463           | 69.078     | 0.021 | 17.4422        |
| 1.50000  | 0.17609  | 30       | 19.286 | 5.366           | 65.859     | 0.033 | 17.9695        |
| 1.25000  | 0.09691  | 30       | 17.143 | 5.180           | 61.900     | 0.288 | 18.4711        |
| 1.00000  | 0.00000  | 30       | 15.000 | 5.000           | 56.883     | 0.579 | 18.8909        |
| 0.75000  | -0.12494 | 30       | 13.929 | 4.911           | 50.259     | 0.176 | 19.0983        |
| 0.50000  | -0.30103 | 30       | 11.786 | 4.728           | 40.953     | 0.034 | 18.7384        |
| 0.25000  | -0.60206 | 30       | 9.643  | 4.537           | 26.405     | 0.508 | 16.5020        |

3.4 Comparative toxicity against *Papilio demoleus*

The values of relative toxicity of different experimental insect growth regulators have been calculated by taking LC50 of Precocene II as unity (Table 4) Diflubenzuron showed their toxicity as 1.6875 as and more than Precocene II whereas the toxicity of Rakshak was 0.77501. In the present study, Diflubenzuron proved as most toxic amongst all insect growth regulators used against the larva of *P. demoleus* followed by the regulators of Precocene II and Rakshak respectively.

Table 4: Showing the relative toxicity, regression equation, LC50 and LC90 values of various insect growth regulators against *Papilio demoleus*

| S. No. | Insect growth regulators | Heterogeneity | Regression equation | LC50 | LC90 | Relative toxicity | Rank |
|--------|--------------------------|---------------|---------------------|------|------|------------------|------|
| 1      | Diflubenzuron            | 1.9428        | Y=5.7151+1.5495812x | 0.3456 | 2.3215 | 1.6875 | 1.9106 | I    |
| 2      | Precocene II             | 4.5163        | Y=5.3407+1.4548121x | 0.5382 | 4.4355 | 1.0000 | 1.0000 | II   |
| 3      | Rakshak                  | 3.2157        | Y=5.1622+1.3132931x | 0.7525 | 7.1215 | 0.77501 | 0.6228 | III  |

Y= Probit Kill
X= Log concentration

Various concentrations of Insect Growth Regulators were having a profound effect on larval reduction of *P. demoleus*. These research works can be of great importance for the farming community in many areas of the developing world. The major thrust of this work is its adaptability for use by small scale farmers plagued by the challenge of not being able to afford conventional pesticides on the market.

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