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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 w 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

Different concentrations of IGR_S (Diflubenzuron, Precocene II and Rakshak) were prepared by adding desired quantity of distilled water. These IGRs have been registered in our country and are commercially available. For this purpose, the 10% stock solution was prepared for each test compound by the formula given below:

$$\text{Amount of test compound} = \frac{\text{Quantity of solution required X \% of solution desired}}{\text{Strength of formulation available}}$$

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 me work out the LC_{50} 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:

$$\text{Corrected per cent mortality} = \frac{T-C}{100-C} \text{X } 100$$

Where,
T = observed mortality
C = mortality in control

The mortality data recorded with different concentrations of IGRs formulations were subjected to probit analysis for determination of their LC_{50} values. [1]

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:

$$t = \frac{b}{\text{S.E. of b}}$$

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 LC_{50} 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 LC_{50} in this case was 0.5832 at 0.5832 per cent concentration of Precocene II. Karem and Ahmad [6] evaluated the insecticidal effect of Precocene II against grasshopper.

Table 2: Showing the toxicity of Precocene II on 3rd instar larvae of *Papilio demoleus*

No.	Dose	Log Dose	n	Effected	Probit	Expected	^{2}	n * w	Working Probit
1	2.00000	0.30103	30	26.786	6.242	79.502	1.762	14.8596	6.1683
2	1.75000	0.24304	30	23.571	5.792	76.892	0.048	15.6493	5.7905
3	1.50000	0.17609	30	20.357	5.463	73.661	0.521	16.4864	5.4552
4	1.25000	0.09691	30	19.286	5.366	69.563	0.395	17.3528	5.3611
5	1.00000	0.00000	30	17.143	5.180	64.197	0.649	18.1992	5.1748
6	0.75000	-0.12494	30	15.000	5.000	56.856	0.575	18.8925	4.9983
7	0.50000	-0.30103	30	13.929	4.911	46.156	0.001	19.0341	4.9104
8	0.25000	-0.60206	30	10.714	4.634	28.886	0.681	17.0488	4.6431
						Total	4.632		

y =5.3637 +1.528834 x

3.3 Effect of rakshak

The toxicity of Rakshak was calculated on the basis of probit value (Table3.) The regression coefficient of probit on log C was 1.3132931 which was highly significant at 1.0 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 LC_{50} 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 [2]. 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	Effected	Empirical probit	Expected probit	Chi square	n * w	Working probit
2.00000	0.30103	30	24.643	5.921	71.754	1.598	16.9152	5.8829
1.75000	0.24304	30	20.357	5.463	69.078	0.021	17.4422	5.4634
1.50000	0.17609	30	19.286	5.366	65.859	0.033	17.9695	5.3657
1.25000	0.09691	30	17.143	5.180	61.900	0.288	18.4711	5.1780
1.00000	0.00000	30	15.000	5.000	56.883	0.579	18.8909	4.9983
0.75000	-0.12494	30	13.929	4.911	50.259	0.176	19.0983	4.9105
0.50000	-0.30103	30	11.786	4.728	40.953	0.034	18.7384	4.7283
0.25000	-0.60206	30	9.643	4.537	26.405	0.508	16.5020	4.5446
					Total	3.238		

y =5.1734 +1.335909 x

3.4 Comparative toxicity against *Papilio demoleus*

The values of relative toxicity of different experimental insect growth regulators have been calculated by taking LC_{50} 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.

The LC_{90} values (Table 4.) in this case have also been calculated which resulted 2.3215, 4.4355 and 7.1215 for Diflubenzuron, Precocene II and Rakshak respectively. But these values are quite high to give 90.0 per cent kill of *P. demoleus*.

The overall efficacy of all the different insect growth regulators against *P. demoleus* was found in the following descending order:

Diflubenzuron (LC_{50}= 1.687) > Precocene II (LC_{50}= 1.000) > Rakshak (LC_{50}= 0.775)

Table 4: Showing the relative toxicity, regression equation, LC_{50} and LC_{90} values of various insect growth regulators against *Papilio demoleus*

S. No.	Insect growth regulators	Heterogeneity	Regression equation	LC_{50}	LC_{90}	Relative toxicity		Rank
						LC_{50}	LC_{90}	
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	0.5832	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
LC_{50}= Concentration calculated to give 50 per cent mortality
LC_{90}= Concentration calculated to give 90 per cent mortality

4. Conclusion

It is concluded that, these concentrations should enhance the management of lepidopterous pests in the vegetable agro ecosystems because they do not persist in the environment, have unique modes of action, low mammalian toxicity, and may be potentially compatible with natural enemies. These 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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