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Growth inhibitory effect of different insect growth regulators on citrus butterfly, *Papilio demoleus* Linn.

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Abstract
The morphogenetic effects of Insect Growth Regulators Diflubenzuron, Precocene II and Rakshak when applied by orally feeding method to third larvae of lemon butterfly, *P. demoleus* at different concentrations (1.0, 0.5, 0.25, 0.125, 0.06, 0.03, 0.015 and 0.007 per cent) resulted in to the larval-pupal intermediated at higher concentrations, deformed pupae (at medium and higher concentration and deformed adults (at medium and higher concentrations have been studied. These results indicate that IGRs have potential to be used to control *Papilio demoleus.*

Keywords: Morphogenetic effect, *Papilio demoleus*, insect growth regulators

1. Introduction
The lemon butterfly, *P. demoleus* is a key pest of citrus in India. It feeds voraciously on vegetative growth of citrus plants throughout the year. It is most destructive to citrus seedlings as well as new flushes. FIICCI, (2006) [1] and also because it shows rapid population growth under favorable circumstances Pathak *et al.* (2003) [10]. In recent times has expanded its range into new areas following the introduction and cultivation of citrus Matsumoto *et al.* (2002) [8]. Insect growth regulators IGRs are new generation insecticides and are capable of preventing normal development of insect pests. They may be regarded as eco-friendly agents in the sense they do not disturb the ecosystem. They do not kill the insects immediately and there is no quick knock down action. Since these compounds are specific in their action, hence, if they are used judiciously and at certain time then they will not kill the insect predators and parasites. Looking to the importance of IGRs, the moult inhibitors, neem based triterpenoid azadirachtin and anti-juvenile hormone compound like Precocene are included in the present experimentation.

2. Materials and methods
2.1 Test insect: *Papilio demoleus* Linn.
2.2 Mass Rearing of *Papilio demoleus* In the Laboratory:
Eggs and early larval instars were collected from the lemon nurseries and lemon plants 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 branch of 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 third 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 IGRs (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 Assessment of efficacy and processing of data

Efficacy of different IGR compounds used in the present research work was assessed on the morphological changes induced by Dimlin, Precocene II and Rakshak in larvae feeding on treated leaves in different concentrations of the test compounds. Results were compared with the control set of experiments.

3. Result and Discussion

The effects of IGR treatment against *Papilio demoleus* were evaluated as under:

3.1 Effect of Diflubenzuron

The detailed results, in respect of the effect of Diflubenzuron (Dimilin 25 W.P.) on the treated 3rd instar larvae of the insect, have been given in the table-1. It is event from the table that at higher dose of Diflubenzuron (1.0%) against freshly moulted 3rd instar larvae of test insect gave maximum mortality (73.33%), whereas it decreased at the lower concentrations and minimum larval mortality (10.0%) was recorded at the lowest concentration 0.007 per cent and the rest of the treatments behaved intermediary.

The larval-pupal intermediate (L-Pupal intermediates) were recovered at 1.0 and 0.50 per cent dosage and their percentage were 13.33and 6.66 per cent, respectively. Medium and lower concentrations of test formulation i.e. 0.250,0.125, 0.06, 0.03, 0.015 and 0.007 per cent result in such form (no L-P intermediates) as in case of control, pupal deformity was also noticed at higher concentration of 1.0 and 0.5 per cent and their value were 3.33 and 6.66 per cent, respectively.

As regards the pupation pattern in the treated larvae. It was observed that pupation at 1.0, 0.5, 0.25, 0.125, 0.06, 0.03, 0.015 and 0.007 per cent concentration of the test compound and percentage of such normal pupae were 13.33, 26.66, 50, 63.33, 70.00, 80.00, 86.66, and 90.00 percent respectively. Such normally pupated insects developed into deformed and normal adults. Deformed adults were recovered at the concentration of 0.25, 0.125, 0.06, 0.03, 0.015, and 0.007per cent and their respective percentage were 6.66, 6.66, 3.33, 6.66, 6.66, and 3.33, (Table1). It was noticed that normal adults emerged at the concentration of 1.0,0.50, 0.25, 0.125,0.06, 0.03, 0.015,and 0.07 per cent and their percentage were 13.33,26.66, 36.66, 50.00, 60.00,66.66, 70.00, and 80.00 respectively. Pupae and adults of the test insect over all efficacy of the diflubenzuron were evaluated in respect of percentage inhibition of adult emergence and maximum inhibition 86.66% was observed at highest concentration of 1.00 per cent. The lowest concentration 0.007% gave minimum inhibition (20.00%) and rest of the treatment behave intermediary and (6.66%) inhibition was observed in control set of treatment. Inhibition of chitin synthesis can be effective by having selective properties, efficacy on immature stages of pest and have ability to be used in Integrated Pest Management (IPM) programs Dhanapakiam and Sampoorani, (1997) [3]. Moser *et al.*, (1992) [9] and Hughes *et al*. (1982) [5] also reported some of these symptoms and introduced that hexaflumuron probably decreases chitin synthesis in endocuticle of various instars. The creation of larval-pupal intermediates and defective pupae have been reported in *Spodoptera mauritia*, Jagannadh *et al.* (1997) [6]

3.2 Effect of Precocene (II)

A critical observation of Precocene II in table- 2 shows that concentration of 1.0,0.50, 0.25, 0.125, 0.06, 0.03 and 0.015 per cent caused larval mortality to the extent of (average percentage) 60.00, 50.00, 40.00, 26.66, 20.00, 16.66 and 6.66 per cent respectively. The treated larvae, which did not change into dead larvae, larval-pupal intermediate, were further metamorphosed in deformed and normal pupal forms. Deformed pupae were found to restrict only to the concentrations of 1.0%, 0.50%, 0.06%, 0.03%, 0.015%, and actual percentage were calculated as 3.33% 6.66% 3.33% 3.33% 10.0%, and 13.33 per cent respectively. On the course of further development, treated larvae were found to pupate normally which did not become deformed. Such normal pupae were found to be developed in all the concentrations of Precocene. Maximum larvae pupated normally in 0.07% and 83.33 per cent respectively at the concentration mentioned, respectively. These values are at par the control. Minimum normal pupation was obtained at the higher concentration of 1.0 percent and its actual percentage of pupation was 26.66 per cent followed by 36.66, 60.00, 73.33, 76.66, 80.00 at the concentration of 0.50, 0.25, 0.125, 0.06 and 0.03 per cent respectively.

Whatever normal pupae recovered at various dosages mentioned in the table- 2 was found to transform in to three types of imaginal development. They are Pupal-adult intermediate (P-A), deformed adults and normal adults. As regards the P-A intermediates, they were recovered to the intermediary dosages of 0.50, 0.25, 0.125, and 0.06 per cent concentrations of Precocene and the actual percentage this form were 3.33, 13.33, 13.33 and 6.66 per cent respectively Deformed adult were developed from normally pupated pupae which were recovered at the dosages of 0.25, 0.125, and 0.06 per cent concentrations of the test compound. The actual percentages of adult development were 6.66, 10.00 & 6.66 percent respectively. Recovery of normal adults was noticed in all the concentrations of test compound. Minimum concentration of 0.007 was found to develop maximum percentage of normal adults the actual percentage was 86.00 per cent followed by 83.33 per cent at the lower concentration of 0.015 per cent. Minimum percentages of normal adults were found to recover at the concentration of 1.00 per cent and the actual percentage was 26.66 followed by 33.33 at the concentration of 0.50 per cent. More than 50% normal adults were found to develop at the concentrations of 0.125, 0.06 and 0.03 per cent and their actual percentages were 56.66, 63.00 and 76.66 per cent respectively. As regards the control, no morphogenetic or minimum effects like larval mortality, L-P intermediates, and deformed pupae-adult intermediates and deformed adults were noticed. Statistical analysis shows that data are statistically significant at the level 5% test of significance in the cases of L-P intermediates, normal pupae and normal adults. The data are non-significant in the case of deformed pupae and deformed adults. Thus Precocene II seems to induce partial sterility as is clear from the present investigation supported by the work of scientists Chakravorty *et al.* (1989) [2] and Taylor *et al.* (1992) [11].

3.3 Effect of Rakshak

It is amply documented from the result presented in table-3 that there is a definite positive relationship between the larval mortality and the dose of candidate compound used. The larval mortality was maximum (56.66%) at the dose of 1.00%, while minimum (10.00%) at 0.06% and it followed the decreasing trend with lowering the concentration of the test compound.

It is evident from the table (3) that maximum larval-pupal intermediate (16.66) was observed at highest concentration of 1.00 per cent; where as l-p intermediate could be recorded at 0.06 0.03 and 0.015 per cent concentrations. And at these concentrations larval-pupal intermediate recorded were 3.33, 6.66 and 6.66 per cent, respectively. Such form could not be recovered at concentration of 0.50, 0.25, 0.125, and 0.07 per cent. Treated larvae, which did not become died and l-p intermediates, were survived to develop further into normal and deformed pupae. As regards the deformed pupae, they were resulted in some dosages of Rakshak. The range of actual percentages deformed pupae was 3.33 and 6.66 at higher dose 1.0% 0.50% followed by 0.06, 0.03 and 0.015 per cent concentration 6.66, 6.66 and 3.33% deformed pupae. Similar results found in different crops and location such as Gujar and Mehrotra (1988) [4] and Kumar *et al*. (2015) [7].

Table 1: Juvenomimetic effect of Diflubenzuron on 3rd instar larva of *Papilio demoleus*

S. No.	Dosage of Dimlin (%)	Total no. of Larvae in 3 replication	Larval mortality (%)	Larval pupal intermediate (%)	Deformed pupae (%)	Normal pupae (%)	Pupal- Adult intermediate (%)	Deformed Adult (%)	Normal Adult (%)	Inhibition of adult emergence (%)
1	1.0	30	73.33 (8.59)	13.33 (3.72)	3.33 (1.96)	13.33 (3.72)	0.0 (0.0)	0.0 (0.0)	13.33 (3.72)	86.66 (9.33)
2	0.50	30	60.00 (7.78)	6.66 (2.67)	6.66 (2.67)	26.66 (5.21)	0.0 (0.0)	0.0 (0.0)	26.66 (5.21)	73.33 (8.59)
3	0.25	30	50.00 (7.11)	0.0 (0.0)	0.0 (0.0)	50.00 (7.11)	6.66 (2.67)	6.66 (2.67)	36.66 (6.09)	66.66 (8.19)
4	0.125	30	36.66 (6.09)	0.0 (0.0)	0.0 (0.0)	63.33 (7.99)	6.66 (2.67)	6.66 (2.67)	50.00 (7.11)	50.00 (7.11)
5	0.06	30	30.00 (5.52)	0.0 (0.0)	0.0 (0.0)	70.00 (8.39)	6.66 (2.67)	3.33 (1.96)	60.00 (7.78)	40.00 (6.36)
6	0.03	30	20.00 (4.53)	0.0 (0.0)	0.0 (0.0)	80.00 (8.97)	6.66 (2.67)	6.66 (2.67)	66.66 (8.19)	33.34 (5.82)
7	0.015	30	13.33 (3.72)	0.0 (0.0)	0.0 (0.0)	86.66 (9.33)	10.0 (3.24)	6.66 (2.67)	70.00 (8.39)	30.00 (5.52)
8	0.007	30	10.0 (3.24)	0.0 (0.0)	0.0 (0.0)	90.00 (9.51)	6.66 (2.67)	3.33 (1.96)	80.00 (8.97)	20.00 (4.53)
9	Control	30	6.67 (2.68)	0.0 (0.0)	0.0 (0.0)	93.37 (9.69)	0.0 (0.0)	0.0 (0.0)	93.37 (9.69)	6.66 (2.67)
C.D.at 5%			3.825	0.280	0.280	4.096	0.755	0.440	3.738	3.315

*Figures in parentheses are angular transformed value

Table 2: Juvenomimetic effect of Precocene II on 3rd instar larva of *Papilio demoleus*

S.N.	Dosage of Precocene (%)	Total no. of Larvae in 3 replication	Larval mortality (%)	Larval pupal intermediate (%)	Deformed pupae (%)	Normal pupae (%)	Pupal- Adult intermediate (%)	Deformed Adult (%)	Normal Adult (%)	Inhibition of adult emergence (%)
1	1.0	30	60.00 (7.78)	10.00 (3.24)	3.33 (1.96)	26.66 (5.21)	0.0 (0.0)	0.0 (0.0)	26.66 (5.21)	73.33 (8.59)
2	0.50	30	50.00 (7.11)	6.66 (2.67)	6.66 (2.67)	36.66 (6.09)	3.33 (1.96)	0.0 (0.0)	33.33 (5.82)	66.66 (8.19)
3	0.25	30	40.00 (6.36)	0.0 (0.0)	0.0 (0.0)	60.00 (7.78)	13.33 (3.72)	6.66 (2.67)	40.00 (6.36)	60.00 (7.78)
4	0.125	30	26.66 (5.21)	0.0 (0.0)	0.0 (0.0)	73.33 (8.59)	13.33 (3.72)	10.00 (3.24)	56.66 (7.56)	43.33 (2.19)
5	0.06	30	20.0 (4.53)	0.0 (0.0)	3.33 (1.96)	76.66 (8.78)	6.66 (2.67)	6.66 (2.67)	63.00 (7.97)	37.00 (6.12)
6	0.03	30	16.66 (4.14)	0.0 (0.0)	3.33 (1.96)	80.00 (8.97)	3.33 (1.96)	0.0 (0.0)	76.66 (8.78)	23.33 (4.88)
7	0.015	30	6.66 (2.67)	0.0 (0.0)	10.0 (3.24)	83.33 (9.15)	0.0 (0.0)	0.0 (0.0)	83.33 (9.15)	16.66 (4.14)
8	0.007	30	0.0 (0.0)	0.0 (0.0)	13.33 (3.72)	86.00 (9.30)	0.0 (0.0)	0.0 (0.0)	86.00 (9.30)	14.00 (3.80)
9	Control	30	6.67 (2.68)	0.0 (0.0)	0.0 (0.0)	93.77 (9.71)	0.0 (0.0)	0.0 (0.0)	93.37 (9.69)	6.63 (2.67)
C.D.at 5%			3.723	0.635	0.931	2.967	0.440	0.656	2.319	2.364

*Figures in parentheses are angular transformed value

Table 3: Juvenomimetic effect of Rakshak on 3rd instar larva of *Papilio demoleus*

S.N.	Dosage of Rakshak (%)	Total no. of Larvae in 3 replication	Larval mortality (%)	Larval pupal intermediate (%)	Deformed pupae (%)	Normal pupae (%)	Pupal- Adult intermediate (%)	Deformed Adult (%)	Normal Adult (%)	Inhibition of adult emergence (%)
1	1.0	30	56.66 (7.56)	16.66 (4.14)	3.33 (1.96)	33.33 (5.82)	0.0 (0.0)	0.0 (0.0)	33.33 (5.82)	66.66 (8.19)
2	0.50	30	43.33 (6.62)	0.0 (0.0)	6.66 (2.67)	50.00 (7.11)	6.66 (2.67)	3.33 (1.96)	40.00 (6.36)	60.00 (7.78)
3	0.25	30	36.66 (6.09)	0.0 (0.0)	0.0 (0.0)	63.33 (7.99)	0.0 (0.0)	0.0 (0.0)	63.33 (7.99)	56.66 (7.56)
4	0.125	30	23.33 (4.80)	0.0 (0.0)	0.0 (0.0)	76.66 (83.33)	10.00 (3.24)	13.33 (13.72)	53.33 (7.34)	46.66 (6.87)
5	0.06	30	10.0 (3.24)	3.33 (1.96)	6.66 (2.67)	80.00 (8.97)	3.33 (1.96)	0.0 (0.0)	76.66 (8.78)	23.34 (4.88)
6	0.03	30	0.0 (0.0)	6.66 (2.67)	6.66 (2.67)	86.66 (9.33)	0.0 (0.0)	6.66 (2.67)	80.00 (8.97)	20.00 (4.53)
7	0.015	30	0.0 (0.0)	6.66 (2.67)	3.33 (1.96)	90.00 (9.51)	3.33 (1.96)	0.0 (0.0)	86.66 (9.33)	13.34 (3.73)
8	0.007	30	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	93.37 (9.69)	3.33 (1.96)	0.0 (0.0)	90.00 (9.51)	10.00 (3.24)
9	Control	30	6.67 (2.67)	0.0 (0.0)	0.0 (0.0)	93.37 (9.69)	0.0 (0.0)	0.0 (0.0)	93.37 (9.69)	6.66 (2.67)
C.D.at 5%			1.552	0.402	0.440	2.935	1.145	0.261	2.426	2.037

*Figures in parentheses are angular transformed value

4. Conclusion

The important conclusion drawn from the present investigations made on three insect growth regulators namely, Diflubenzuron, Precocene and triterpenoid azadirachtin against *Papilio demoleus* in the form of various morphological deformities in larvae, pupae and adults are the pronounced effects of all the three IGRs. 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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