e-ISSN: 2455-667X



Annals of Natural Sciences (Peer Reviewed and UGC Approved International Journal) Vol. 3(3), September 2017: 103-113 Journal's URL: http://www.crsdindia.com/ans.html Email: crsdindia@gmail.com

Annals of Natural Sciences

ORIGINAL ARTICLE

Evaluation of Reproductive Ability under Stress of Insect Growth Regulators in Antigastra catalaunalis Duponchel

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ABSTRACT

The insect growth regulator, a fourth generation insecticide, accidentally came in the existence in the Laboratory of Philips, Duphar, The Netherlands, while preparing the herbicides. First insect growth regulator synthesized, was diflubenzuron, which belong Benzoyl phenyl urea group. Later, different groups of insect growth regulators, having chitin biosynthesis inhibiting property, were identified.

Key words: Insect growth regulator, Fecundity, Hatchability

Received: 4th August 2017, Revised: 22nd August 2017, Accepted: 24th August 2017 © 2017 Council of Research & Sustainable Development, India

How to cite this article:

Verma V.K. (2017): Evaluation of Reproductive Ability under Stress of Insect Growth Regulators in *Antigastra catalaunalis* Duponchel. Annals of Natural Sciences, Vol. 3[3]: September, 2017: 103-113.

INTRODUCTION

The different groups of insect growth regulators though differ in their chemical structure and mode of action, but have a common characteristic, i.e., they exhibit lethal action in juvenile stages and sterility in sexually mature adults, thus the pest population declines very rapidly. Besides, they also inhibit the food consumption and growth of individuals, which survive sub lethal treatments. This becomes an additional benefit in the field of pest management as surviving pest will consume less food, causing least injury to agroecosystem. The suppression of pest population by the use of insect growth regulators has already been achieved by many workers. (Flint *et.al*, 1978; Zepp *et.al.*, 1979; Hopkins *et.al.*, 1982; Velcheva, 1983; Lecheva, 1985; Sharma, 1993; Moraschini, 1998;Zhong *et.al.*, 2001; Nakano and Romano, 2002) etc.

A perusal of the literature shows that the efficacy of the fourth generation insecticides depends upon the mode of their entry, and stage of the life-cycle to which these are applied. Some are more effective when applied to adults or larvae by feeding method than when applied to adults or larvae by residue film method. The insect growth regulators induce variable biological effects: these are not equally or identically effective, some are more effective than others in the same species and their influence may be sex oriented. Different species exhibit different response to the same fourth generation insecticides.

MATERIALS AND METHODS

Test Insect: *Antigartra catalaunalis* Duponchel. **Systematic Position:**

- Phylum-Arthropoda
- Class-Insecta

- > Order-Lepidoptera
- Family-Pyralidae
- ➢ Genus-Antigastra
- ➢ Species-catalaunalis

SOURCES:

Male and Female, *Antigastra catalaunalis* Dup. were collected in second week of July, 2009 from sesame field. Their large population and swarms may be seen during rainy season (July-September). To collect the larvae the sesame crop was inspected time to time.

LABORATORY STOCK OF THE INSECT:

The insect was reared and maintained in the laboratory in order to ensure regular supply of the insect and its developmental stages during whole tenure of the present investigation as described below. To begin with, the stock was established with the help of field collected moths. These moths were maintained on 10 per cent sugar solution in glass chimneys with tender sesame leaves (Sesamum indicum). Eggs obtained from them were kept as such for hatching. Larvae hatched from eggs were transferred on tender sesame leaves in petridishes (15 cm dia) and reared on them till pupation. The food supply to larvae was renewed twice a day in view of evaporation of water, which proceeds fast when leaves are detached from plants. The sesame leaves were treated with KM_nO₄ solution for five minutes followed by washing in running water. These leaves were dried under shade and provided to the experimental larvae. The larval period lasted for about 15.25 days. All possible precautions were taken to save larvae from bacterial and fungal infections. The first and second instars were reared in pertidishes but from third instar to pupations they were reared in pneumatic troughs (25 cm dia.) in small groups. When larvae acquired full growth and stopped feeding, they were transferred in separate pneumatic troughs having 6 inches thick moist soil layer on their bottoms. The larvae pupated in leaves made coverings. Pupae, thus obtained were kept as such for eclosion. Moths emerged from pupae were reared in pneumatic troughs as described above. In this way the progeny of moths of succeeding generations were reared generation after generation continuously till the tenure of the investigation. The laboratory reared insects and larvae were maintained throughout the tenure of investigation into the Department of Zoology, D.V. (P.G.) College, Orai, Jalaun by the technique described above with slight modifications as when found necessary.

INSECT GROWTH REGULATORS USED AND APPLICATION:

The following fourth generation insecticides whose efficacy as insecticides has already been proved in different crop pests employed against *Antigastra catalaunalis* in this investigation-Novaluron and Chlorfluazuron

The different concentrations of insect growth regulators mentioned above were applied against *A. catalaunalis*. The concentrations considered in this work included 0.0001, 0.001, 0.01, 0.10, 0.50 and 1.00 per cent. These concentrations were obtained by dissolving the desired quantity of insect growth regulator in acetone or methanol.

The insect was treated with different concentrations of insect growth regulators used in this investigation by two methods namely- Adult feeding method and Residue film method. Details of both methods are mentioned below:

(I) RESIDUE FILM METHOD (RFM):

In this method of treatment 1 to 2hr old adults were exposes to a thin film of residue of a concentration of a particular insect growth regulator. For obtaining the thin film of the chemical as residue, about 10 ml of a concentration of a chemical was poured in a petridish (10 cm dia.) and the petridish was tilted in different ways to spread the chemical on the whole floor area of the petridish and its raised periphery. Thereafter, the petridish was kept in the air for the evaporation of the solvent. This led to the formation of a thin film of a concentration of a insect growth regulator in the petridish as residue. Adults

were left in petridishes having thin film of the insect growth regulator for 24 hours. The petridishes were covered by thin muslin cloth to prevent the escape of the adults. Such treated adults were employed in the different experiments as described later on. This method of treatment will be designated as RFM in the text from here onwards.

(II) ADULT FEEDING METHOD (AFM):

In this method of treatment a concentration of a particular insect growth regulator was mixed in 20 per cent sugar solution which was supplied to adults for feeding. From here onwards this method of treatment will be referred as AFM in the text.

The reproductive ability is examined as per standard laboratory methods and guidelines. The data obtained from the studies were subjected to statistical analysis. Various statistical techniques mentioned below have been applied to study the nature and relationship between variables to know the reliability and precision test the significant difference between the observed and corresponding expected values and to predict the estimated values of effectiveness for a given value of concentration.

RESULTS AND DISCUSSION

Effect of Novaluron on Reproduction of Antigastra catalaunalis Dup. under A.F.M.:

The sexual maturity of the adult, in response to earlier treatment of parent moths by adult feeding method with any concentration of the novaluron was delayed (P<0.05). In this respect, the concentrations from 0.0001% to 1.0 per cent affected the preoviposition period (3.02 to 3.08 days) identically (P>0.05) with less prolonging effect as compared to any of the concentrations from 0.0001% to 1.0% which delayed this period more but identically. The different concentrations of this insect growth regulator applied earlier to adults, shortened the oviposition period markedly (P<0.05 or 0.01) but on the basis of the statistical analysis considering their curtailing effect on the oviposition period, these concentrations could be arranged as 0.0001% or 0.001% or 0.01% or 0.10% or 0.50% or 1.0 per cent.

The treatment of parent moths with any concentration of the novaluron by the adult feeding method, reduced the fecundity significantly (P<0.01). The concentrations from 0.0001% to 0.01% exerting almost identical effect on the fecundity (221.2 to 226.2 eggs/female, P>0.05) caused less decline in the fecundity as compared any of the other strengths 0.10% to 1% (P<0.01). Among the concentrations (0.001% to 1%), the fecundity, varying from 77.4 to 166.2 eggs/female and declining with higher concentration, differed strongly with them (P<0.01). The treatment of the insect by the A.F.M., reduced the percentage of hatching/female as compared the untreated condition (P<0.05). The percentage of hatching, varying from 33.3 to 86.2 per cent and decreasing with the increasing concentration under the A.F.M. differed significantly from concentration to concentration (X²-P<0.05).

Every concentration of the novaluron applied by the A.F.M., prolongs the incubation period (P<0.05 in case of .0001%, .001% and .01% concentrations and P<0.01 in case of 0.10 to 1.00% concentrations). The concentrations, 0.0001% and .001% affected the incubation period identically (3.96 to 4.00 days; P<0.05), causing less delay in the incubation period as compared other remaining concentrations (0.01% to 1.0%) among which the egg stage, varying from 4.55 days to 7.26 days and delaying with the advancing concentration, differed from concentration to concentration significantly (P<0.05).

The reduction in the fecundity of the insect under the A.F.M. varied from 36.52 to 78.26 per cent among different concentrations of the novaluron but it was affected identically by the concentrations, 0.0001%, 0.001% and 0.01% of course to less extent as compare that induced by any concentration from 0.10 to 1.00% among which varying from 53.32 to 78.26 per cent and increasing with the advancing concentration, differed with them (P<0.05). Likewise, under the A.F.M. the net sterility, varying from 5.26 to 63.41 per cent

and increasing with the rise in the concentration differed with the concentrations of the novaluron applied to parent moths by feeding method (P<0.005). The per cent control over the reproduction under the influence of different concentrations of the novaluron under A.F.M., varying from 39.88 to 92.03 per cent and increasing with the advancing concentration depended on the strength of the novaluron applied (P<0.05).

Effect of Novaluron on Reproduction of Antigastra catalaunalis Dup. under R.F.M.:

The preoviposition period was affected by every concentration of the novaluron as residue film (P<0.05). In response to adults treatment with residue films of different concentrations of the novaluron the preoviposition period (3.20 to 3.84 days) was affected identically by 0.0001%, 0.001% and 0.01% concentrations (P<0.05) and likewise, it was also affected alike by 0.10, 0.50 and 1.00% concentrations, of course, with more prolongation. As regards the influence of the novaluron residue film on the oviposition period, the duration of egg laying was affected by every concentration of the novaluron and this period, varying from 2.68 to 9.27 days among residue films of different concentrations and decreasing with the increasing concentration, differed significantly with the residue film concentrations (P<0.05).

The fecundity (eggs/female) was reduced by residue film of any concentration of the novaluron applied to the adult (P<0.05). The fecundity varied from 110.8 to 225.1 eggs/female in response to treatment with residue film of different concentrations and appearing to decrease with the advancing concentration but the concentrations from 0.0001% to 0.01% caused almost identical reduction in fecundity (P<0.05) but the fecundity, varying from 110.80 to 198.30 eggs/female in response to treatment with residue films of 0.10% to 1.00% concentrations of the novaluron and tending to be indirectly proportional to the concentration differed significantly with the concentrations. Any of these concentrations. As regards of residue films of different concentrations of novaluron, the fertility i.e., the per cent eggs hatched/female, varying from 38.4 to 86.5 per cent and decreasing with the increasing concentration, differed significantly with the residue films of different concentrations of the novaluron (P<0.05).

The residue film of every concentration of the novaluron increased the duration of the egg significantly (P<0.05). The incubation period, varying from 3.85 to 6.66 days among the direct proportionality to the concentration. But statistical analysis showed that concentrations, 0.0001% and 0.001% affected the oviposition period identically (P>0.05) and concentrations from 0.01% to 1.00% differed from 0.0001% or 0.01% concentrations in affecting this period. Among these concentrations, the oviposition period, varying from 2.74 to 8.23 days and decreasing with the increasing concentration, differed significantly from concentration to concentration (P<0.05).

Under residue film method, the per cent redution in the fecundity and the per cent sterility, varying from 36.77 to 69.88 per cent and from 4.95 to 57.8 per cent respectively and exhibiting direct proportionality to the concentration, differed significantly with the concentration of the novaluron (P<0.05). Further, as regards the effect of novaluron under this method of treatment on the per cent control of the reproduction it, varying from 39.90 to 86.88 per cent and exhibiting direct proportionality to the concentration, depended significantly on the concentration (P<0.05).

Effect of Chlorfluazuron on Reproduction of Antigastra catalaunalis under A.F.M.:

The treatment of adults by feeding method with any concentration of the chlorfluazuron prolonged the pre-oviposition period significantly (P<.05). In response to earlier treatment of parent adults with different concentrations of this fourth generation insecticide, the above mentioned period varied from 2.44 to 3.02 days and appeared to increase with the rise in the concentration but as per statistical analysis, the concentrations from 0.0001% to 0.10% influencing it identically, caused significantly less

prolongation in it as compared the 0.50% and 1.00% concentrations which also prolonged it identically. Further, every concentration of this insect growth regulator applied to the adults by feeding method also affected the oviposition period and in response to different concentrations of the chlorfluazuron applied as above, this period, varying from 3.56 to 8.94 days and reducing with the advancing concentration, depended on the concentration of this insect growth regulator (P<0.05).

	(Values are means ± S.E.)			
Mode of treatment	Concentration %	Pre-oviposition period (days)	Oviposition period (days)	
AFM	.0001	3.02±0.12	8.14±0.23	
	.001	3.04±0.14	8.00±0.12	
	.01	3.06±0.14	7.12±0.22	
	.10	3.06±0.26	4.36±0.24	
	.50	3.07±0.22	3.32±0.32	
	1.00	3.08±0.15	1.46±0.26	
RFM	.0001	3.20±0.24	9.27±0.26	
	.001	3.12±0.23	8.96±0.18	
	.01	3.05±0.14	8.23±0.17	
	.10	3.72±0.13	7.06±0.19	
	.50	3.76±0.14	5.11±0.11	
	1.00	3.84±0.18	2.68±0.19	
	Control	1.26±0.24	9.46±0.33	

Table 1: Effect of Novaluron reproductive periods in *Antigastra catalaunalis* Dup.

Table 2 : Effect of Chlorfluazuron on	reproductive	periods in <i>Anti</i>	<i>aastra catalaunalis</i> Dup
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	(Values are means ± S.E.)			
Mode of treatment	Concentration %	Pre-oviposition period (days)	Oviposition period (days)	
AFM	.0001	2.44±0.12	8.94±0.15	
	.001	2.46±0.12	8.56±0.14	
	.01	2.56±0.12	7.56±0.12	
	.10	2.60±0.13	6.96±0.16	
	.50	2.89±0.12	5.12±0.11	
	1.00	3.02±0.14	3.56±0.14	
RFM	.0001	2.20±.12	8.94±0.14	
	.001	2.25±0.12	8.60±0.11	
	.01	2.31±0.12	7.84±0.11	
	.10	2.40±0.11	6.56±0.11	
	.50	2.54±0.12	5.60±0.16	
	1.00	3.00±0.14	3.65±0.14	
	Control	1.26±0.24	9.46±0.36	

Every concentration of the chlorfluazuron applied to parent adults reduced the fecundity and fertility both significantly (P<0.05) and in response to its different concentrations applied to adults by feeding method, these two varying from 108.6 to 262.3 eggs/ female and from 40 to 78.7 per cent respectively and exhibiting indirect proportionality to the

concentration, differed from concentration to concentration (P<0.05) (Table-16). However, under such treatment, all concentrations were not effective in changing the incubation period; only 0.50% and 1.00% concentrations prolonged this period significantly (P<0.05). The latter was found more effective than the former one (P<0.05). In response to parent's treatment with different concentrations of the chlorfluazuron the reduction in fecundity, net sterility and control over reproduction, varying from 26.32 to 69.66 per cent, from 13.52 to 65.93 per cent and from 36.30 to 86.87 per cent respectively and all decreasing with the advancing concentration, differed significantly with different concentrations (P<0.05).

Effect of Chlorfluazuron on Reproduction of *Antigastra catalaunalis* Dup. Under R.F.M.:

The residue film of any concentration of the chlorfluazuron applied to the female delayed the sexual maturity significantly (P<0.05) and in response to the females' treatment with residue films of different concentrations of this insect growth regulator, the preoviposition period, varying from 2.20 to 3.00 days, tended to prolong with the advancing concentration but the statistical analysis revealed that the concentrations from 0.0001% to 0.50% exerting identical influence, causes significantly less prolongation in pre-oviposition period as compared the 1.0 per cent concentration (P<0.05) (Table-14). Similarly, the oviposition period was also affected by every concentration of this insecticide applied as residue film to the female (P<0.05) and in response to the female's treatment with residue films of different strengths of this insecticide, the oviposition period, varied from 3.65 to 8.94 days and exhibiting direct proportionality to the concentration, differed significantly with different concentrations (P<0.05).

(Values are means ± S.E.)				
Mode of treatment	Concentration %	No. eggs laid by a female	No. of eggs hatched	% hatched
AFM	.0001	226.3±4.64	195.07±0.14	86.2
	.001	224.8±5.32	191.52±0.12	85.2
	.01	221.2±3.72	184.03±0.14	83.2
	.10	1.66.2±5.47	113.51±0.12	68.3
	.50	119.9±4.75	77.09±0.14	64.3
	1.00	77.4±6.84	25.77±0.12	33.3
RFM	.0001	225.1±4.42	194.71±0.14	86.5
	.001	226.6±4.34	193.96±0.18	85.6
	.01	222.0±3.62	178.48±0.12	80.4
	.10	198.32±5.32	133.46±0.12	67.3
	.50	143.6±2.26	86.44 ±0.11	60.2
	1.00	110.8±4.12	42.54 ±0.14	38.4
	Control	356.0±0.92	324.9±1.14	91.0

Table 3: Effect of Novaluron on fecundity and fertility in Antigastra catalaunalis Dup

The residue film of every concentration of the chlorfluazuron applied to the female, reduced considerably her fecundity and fertility (P<0.05) and in response to the female's treatment with residue films of different concentrations of the insect growth regulator, these two, varying from 110.3 to 257.4 eggs/female and from 34.4 to 78.8 per cent respectively and tending to decrease with the advancing concentration, depended significantly on the concentration of the insecticide.

However, the residue films of the concentrations from 0.0001% to 0.10% did not affect the incubation period significantly but the 0.50% and 1.0% residue film concentrations prolonged this period (3.70 to 4.06 days) significantly, the former being more effective (P<0.05).

Further, the reduction in fecundity, net sterility and control over reproduction, varying from 27.72 to 69.02 per cent, from 13.40 to 62.20 per cent and from 37.41 to 88.30 per cent respectively among the residue films of different concentrations and increasing with the advancing concentrations, depended significantly on the strength of the residue film of this insect growth regulator (P<0.05).

(Values are means ± S.E.)				
Mode of treatment	Concentration %	No. eggs laid by a female	No. eggs hatched	% hatching
	.0001	262.3+4.63	206.4+3.42	78.7
	.001	248.3+3.24	176.8+5.10	71.2
AFM	.01	230.6+6.14	150.6+4.22	65.3
	.10	212.4+4.26	128.3+6.10	60.4
	.50	170.5+3.33	71.1+2.42	45.2
	1.00	108.6+2.22	43.3+1.42	40.0
	.0001	257.4+2.12	202.8+3.44	78.8
	.001	2.45.4+3.23	175.2+5.56	71.4
	.01	201.3+4.46	133.5+4.40	66.3
RFM	.10	182.5+2.35	113.7+5.10	62.3
	.50	143.6+5.26	62.2+3.43	43.3
	1.00	110.3+3.30	37.9+2.10	34.4
	Control	356.0+0.92	324.9+1.14	91.0

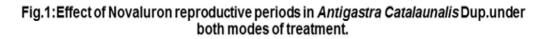
Table 4: Effect of Chlorfluazuron on fecundity and fertility in Antigastra catalaunalis Dup.

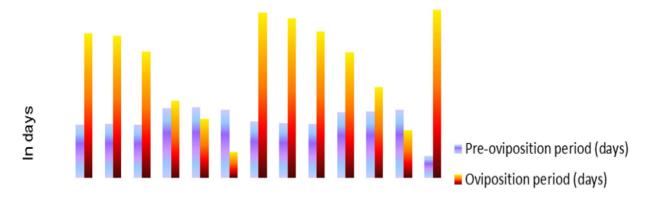
Table 5: Effect of Novaluron on fecundity and fertility in Antigastra catalaunalis Dup.

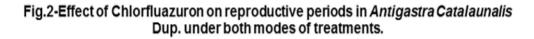
(Values are means ± S.E.)			
Mode of treatment	Concentration (%)	Incubation period (days)	
AFM	.0001	3.96±0.14	
	.001	4.00±0.18	
	.01	4.55±0.12	
	.10	5.46±0.12	
	.50	6.50±0.11	
	1.00	7.26±0.14	
RFM	.0001	3.85±0.14	
	.001	3.96±0.12	
	.01	4.30±0.14	
	.10	5.30±0.12	
	.50	6.34±0.14	
	1.00	6.66±0.12	
	Control	3.06±0.42	

	(Values are means ± S.E.)			
Mode of treatment	Concentration (%)	Incubation period (days)		
AFM	.0001	3.13±0.16		
	.001	3.26±0.14		
	.01	3.30±0.12		
	.10	3.34±0.11		
	.50	3.75±0.13		
	1.00	4.72±0.14		
RFM	.0001	.3.10±0.12		
	.001	3.19±0.12		
	.01	3.40±0.11		
	.10	3.46±0.12		
	.50	3.70±0.14		
	1.00	4.06±0.42		
	Control	3.06±0.42		

Table 6: Effect of Chlorfluazuron on fecundity and fertility in Antigastra catalaunalis Dup.







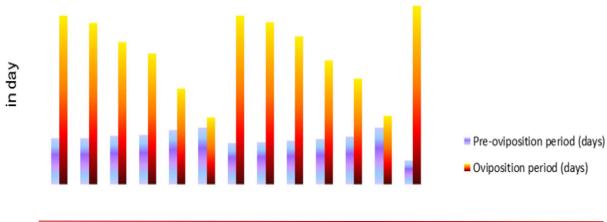


Fig.3-Effect of Novaluron on fecundity and fertility *in Antigastra catalaunadis* Dup.under both modes of treatment.

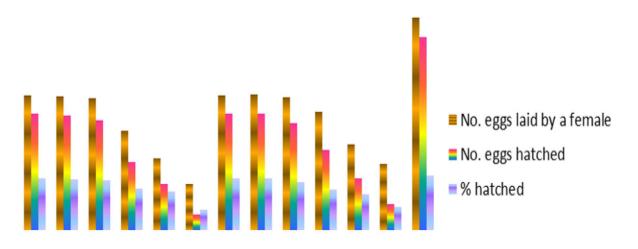
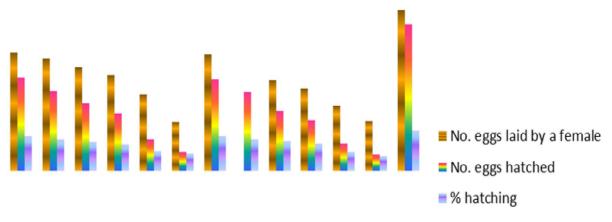
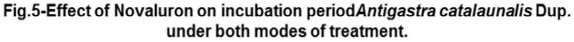


Fig.4-Effect of Chlorfluazuron on fecundity and fertility in Antigastra catalaunalis Dup. under both modes of treatment.





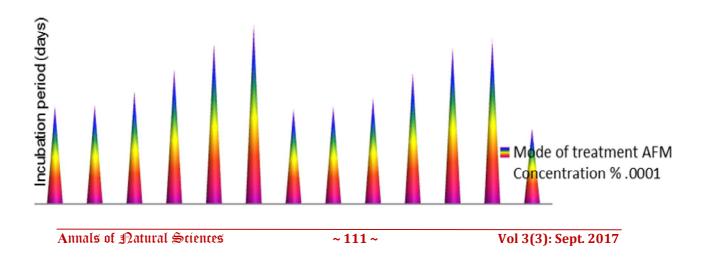
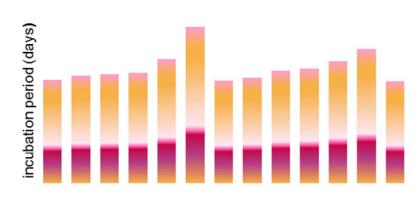


Fig.6-Effect of Chlorfluazuron on incubation period in Antigastra catalaunalis Dup. in both modes of treatment.



Mode of treatment AFM

- Mode of treatment AFM Concentration% .001
- Mode of treatment AFM Concentration% .01
- Mode of treatment AFM Concentration% .10
- Mode of treatment AFM Concentration% .50
- Mode of treatment AFM Concentration% 1.00
- Mode of treatment RFM Concentration% .0001
- Mode of treatment RFM Concentration% .001
- Mode of treatment RFM Concentration% .01

The literature reveals that among lepidopterous insects, the oral administrations of insect growth regulators in adults has led to the adverse influences on the reproduction, i.e., it leads to the sterility with different levels of success (Flint *et.al.*, 1968 a & b; Sharma, 1993, Gupta *et.al.* 2005., and Gupta and Khattri 2012). Our results also indicate that when both insecticides are administered orally in adults, they are able to induce sterility which exhibits direct proportionality to their concentration. However, the effective concentration which causes more than forty per cent sterility differs from insecticide to insecticide. Chlorfluazuron exerts such influence at 0.0001 per cent level and novaluron does not acquire it even at the latter level. However, at one percent concentration both four insect growth regulators are able to cause very high sterility but not cent per cent. At this concentration, the sterility inducing potential differs between them and on this basis; both can be arranged as novaluron (83.73%) and Chlorfluazuron (72.53%) in descending order. However, in case of both insect growth regulators, there is a progressive increase in the sterility with the advancing concentrations which decrease the fecundity and fertility accordingly. As per our results, at one per cent concentration of a fourth generation insecticide which induces very high sterility and longevity of the female Antigastra catalaunalis is very much reduced but contrary to this, in Prodenia litura, the induction of complete or very high sterility does not affect the longevity of the female. Further, in cabbage looper moths fed on 0.0001 per cent diflubenzuron and penfluron, only partial and low sterility is acquired. In *P.ricini* also, fourth generation insecticides are able to induce similar sterility at 0.0001% concentration. The both fourth generation insecticides screened under this investigation are able to control the reproduction in Antigastra catalaunalis to the extent of about 93 to 97.44 per cent at one per cent concentration and in this respect, at one per cent concentration the novaluron is found more effective fourth generation insecticide and chlorfluazuron which exert similar influence in controlling the reproduction, is the less effective one under the adult feeding method.

When insect growth regulators are applied as their residue film they affect preoviposition and oviposition periods with tendencies corresponding to those under oral

administration method. As the residue film, every concentration of both insect growth regulators exerts influence on the preoviposition and oviposition periods. Further, barring the residue films of 0.0001% and 0.001% concentrations of chlorfluazuron the residue films of other concentrations of this insecticide cause prolongation in the incubation period which generally increase with the increase in the concentration of the residue film of insect growth regulator. Further, as the residue film, every concentration of both insect growth regulators is able to reduce the fecundity and fertility; chlorfluazuron usually exhibits indirect proportionality to the concentration of the residue film of both insect growth regulators employed in this investigation. Depending on their potential for causing the reduction in the fecundity and fertility, the residue films of different concentrations of fourth generation insecticides increase the sterility proportionately and accordingly, affect the control over the reproduction in Antigastra catalaunalis leaving chlorfluazuron the residue films of 0.0001 to 0.50 per cent concentrations of novaluron cause sterility much below (40%) which may be reckoned as partial sterility but the residue film of one per cent concentration of both insect growth regulators causes more than fifty per cent sterility; at this concentration of the residue film chlorfluazuron induces more than sixty per cent sterility.

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