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EFFECT OF SUB-LETHAL CONCENTRATIONS OF LUFENURON ON GROWTH, DEVELOPMENT AND REPRODUCTIVE PERFORMANCE OF *TRIBOLIUM CASTANEUM* (HERBST) (COLEOPTERA: TENEBRIONIDAE)

Arora M.S.[1], Salokhe S. G.[2*], Mukherjee S. N.[3]

[1]N. Wadia College, Pune.
[2]A. M. College, Hadapsar, Pune
[3]National Chemical Laboratory, Pune
**Corresponding Author, e-mail id: shailajasalokhe@yahoo.co.in*

ABSTRACT : The effect of the chitin synthesis inhibitor lufenuron on the various developmental stages of red flour beetle *Tribolium castaneum* was determined by exposing them to different sub-lethal concentrations (LC_{10}, LC_{20} and LC_{40}) of lufenuron through diet for 24 hrs. There was a dose dependent effect on the larval weight, time taken for pupation, and adult emergence, percentage pupation and percentage adult emergence. When two day old larvae were fed on sub-lethal concentrations through diet a small proportion of pupal –adult intermediates were observed at LC_{20} and LC_{40}. Adults emerging from the larvae fed on diet containing LC_{10}, LC_{20} and LC_{40} of lufenuron did not show any variation in the fecundity and hatchability of eggs from that of control. The fecundity of adults fed with sub-lethal concentration of lufenuron (obtained against two day old larvae through diet) was not affected, however, percentage hatching and survival was affected. Interestingly there was a reversal of the effect within ten days of treatment with respect to percentage hatching and survival. When eggs were exposed to treated diet hatching was not affected. The present data suggest that lufenuron even at sub-lethal concentrations has a very good larvicidal and ovicidal activity in *T. castaneum.*

Key words: Lufenuron, Sub-lethal effects, *Tribolium castaneum.*

INTRODUCTION

Insecticides with novel modes of action such as chitin synthesis inhibitors disrupt cuticle formation by inhibiting the synthesis, polymerization or deposition of chitin in insect eggs or larvae (Cohen, 1987). Suppression of chitin deposition in treated insect often causes high mortality during moulting when the procuticle is subjected to the stresses of ecdysis and cuticular expansion (Dean et. al. 1998). There are very few studies on the effect of sub-lethal concentrations of IGRs on insect pests. Radwan et al. (1978) studied the effect of sub-lethal doses of Dimilin on the reproductive performance of Spodoptera lituralis Boisduval for three consecutive generations. Biddinger and Hull (1999) reported the sub-lethal effect of several IGRs on the tufted apple bud moth Platynota idaeusalis. Recently, Salokhe et al. (2003) studied the sub-lethal effects of flufenoxuron on the growth, development and reproductive performance of Tribolium castaneum (Herbst). These studies are important for the assessment of overall ecological impact since non- target species in the vicinity of the treated area often receive sub-lethal doses.

Lufenuron (Match®) an acylurea insect growth regulator interferes with chitin biosynthesis. Like other benzoylphenylureas it acts mainly by ingestion (Anonymous 1997). Buholzer et al.(1992) observed that lufenuron ingested pest larvae ceased feeding, stopped growing and finally died. Similar exposure did not affect adults. Lufenuron was found to be more selective control product than traditional organophosphorous sprays (Whiting et. al. 2000).

Further, it has been reported that lufenuron is suitable for integrated pest management (IPM) programs because of its long residual action and safety to adult beneficial insects, mites and spiders (Anonymous 1998). Lufenuron has been reported to be effective against number of serious pests of fruit crops such as *Epiphyas postvittana,* the light brown apple moth (Whiting *et al*. 2000). The effect of lufenuron on potato tuber moth *Phthorimaea operculella* (Zeller) eggs was studied by Emmanuel *et al*. (2000). Lufenuron was found to be effective against many pests of horticulture (Buholzer *et al.*1992). However sub-lethal effects of lufenuron on stored product pests have not been reported so far.

The present endeavor was to study and investigate the effect of sub-lethal doses (LC_{10}, LC_{20} and LC_{40}) of lufenuron on the various stages in the life cycle of *Tribolium castaneum* with respect to growth, development and reproductive end points and to throw light on its possible use for control of stored product pests in view of available reports.

MATERIALS AND METHODS

Maintenance of *T. castaneum* Culture:

A stock culture of *T. castaneum* was maintained on a diet containing wheat flour and 5% Brewers yeast, at 29±1ºC and 60 % relative humidity. Eggs were collected by sieving (sieve number 40) diet infested with adults. Newly emerged adults were obtained by collecting pupae and monitoring them for adult emergence. A stock solution of lufenuron (2.5□l in 50 ml acetone) was prepared. Different volumes of lufenuron from stock solution were thoroughly incorporated into diet. The treated flour was kept at room temperature for twenty four hours, for complete evaporation of the solvent before use in the experiments. Determination of LC_{50} through diet was carried out by releasing two day old larvae of *T. castaneum* in diet treated with various concentrations of lufenuron. Acetone mixed diet was used as control. The control and experimental units were kept in a cooling incubator at 30˚C before and after the treatment. For each concentration tested sets of five replicates of twenty larvae each were taken. The mortality count was taken after seven days. Subsequently, the sub-lethal doses (LC_{10}, LC_{20} and LC_{40}) were deduced by extrapolation from the regression analysis.

Effect on larval development:

Effect of sub-lethal concentrations (LC_{10}, LC_{20} and LC_{40}) of lufenuron through diet on survival and metamorphosis of the larvae was examined by releasing two day old larvae (20 larvae per replicate and three replicates per treatment) in the treated diet. Acetone mixed diet was used as control. After 24 hours, the larvae were transferred to normal diet. On seventh day after the start of the experiment, the larvae were weighed, ten at a time and their survival was recorded. Once pupation had begun in any treatment, observations were made every day for adult emergence. Percentage pupation, time taken for pupation, percent adult emergence and time taken for adult emergence were recorded. Regression analysis was performed to determine dose dependent effects.

Effect on fecundity and fertility of adults:

To study the effect of sub-lethal concentrations, LC_{10}, LC_{20} and LC_{40} of lufenuron (as obtained for two day old larvae) through diet, on adult's reproductive potential, the newly formed pupae were isolated and sexed (Sokoloff, 2001). Two days after adult emergence male and female were kept in separate vials containing diet treated with sub-lethal concentrations of lufenuron for 48h. Then they were transferred to normal diet for mating and egg laying. Acetone treated diet was used as control. The crosses were performed as follows:

Untreated females × untreated males
Treated males × untreated females
Treated females × untreated males
Treated males × treated females

All experiments were replicated five times. Eggs were collected after 48h to record fecundity, fertility and survival of larvae. Data was analyzed by one way ANOVA.

Effect on hatching of eggs:

Effect of sub-lethal concentrations (LC_{10}, LC_{20}, LC_{40}, as obtained for two day old larvae) of lufenuron through diet on the hatchability of eggs was determined by placing ten eggs in treated diet and recording hatching of eggs every day, till hatching in the control was completed. Acetone treated diet was used as control. All experiments were replicated five times. Data was analyzed by one way ANOVA.

RESULTS

Sub-lethal concentrations of lufenuron for 2-day old larvae of *T. castaneum* deduced from the regression equation (Y= 3.563X - 7.4876) by extrapolation of the regression analysis were LC_{50}-0.0175%; LC_{40}-0.0137%; LC_{20}-0.00937%; LC_{10}-0.006879% (Fig.1).

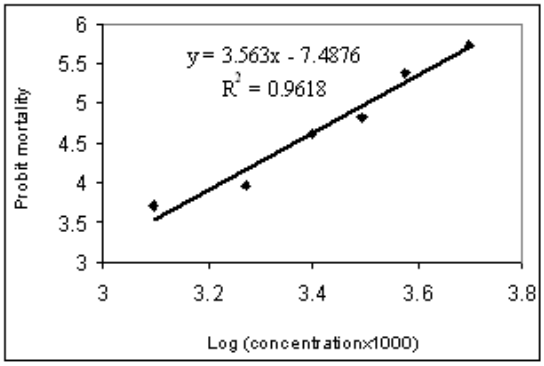


Fig.1: Regression graph of dose-response for lufenuron on mortality of second instar larvae of *T. castaneum.*

Effects on growth and development:

Dietary treatment of *T. castaneum* larvae with sublethal concentrations (LC_{10}, LC_{20}, LC_{40}) of lufenuron significantly reduced the larval weight on 7th day of their growth period compared to the control (Table 1, Fig. 2) in a dose-dependent manner. There was significant increase in time taken for pupation and adult emergence compared with the control (Table 1, Fig. 3). A significant decrease in percent pupation and percent adult emergence were observed in a dose-dependent manner (Table 1, Fig. 4). At LC_{20} and LC_{40} concentrations moulting of pupa into adult was affected, resulting in the development of pupal-adult intermediates (Fig.9, 10). Further, it was found that, number of eggs laid by adults developed from larvae treated with sub-lethal concentrations of lufenuron was not significantly different from that of control. Also, percent hatching of such eggs and percent survival of the larvae was not affected (data not presented).

Effects on fecundity and fertility:

Fecundity of *T. castaneum* adults fed on the diet mixed with sub-lethal concentrations (LC_{10}, LC_{20}, LC_{40} as obtained for larvae) of lufenuron was not significantly different from that of the adults fed on the normal diet (Table 2a). However, as the time progressed percentage hatching (fertility) of eggs was increased except in pairs where only males were treated but there was no effect of concentration of lufenuron on percent hatching (Table 2b,c, d). Also it was found that for all concentrations tested, percentage survival of the larvae was initially reduced except in the pairs where only males were treated. Further it was observed that as time progressed, percentage survival of the larvae was increased and recovered to that of control within ten days (Fig. 6a, b, c).

Table-1. Trendline for graph showing dose-response for lufenuron on time taken for pupation of *T. castaneum* larvae. A Equation for trendline 1.

Dose	%larval survival X±S. E.	Larval weight (mg)(10larvae) X±S. E.	% pupation X±S. E.	Time taken for pupation X±S. E.	% adult emergence X±S. E.	Time taken for adult emergence X±S. E	%PAI X±S. E.
0.00(Control)	98±0.447	0.00388 ±0.000217	96±0.54	19.2±0.83	96±0.54	24.8±0.83	0.00
LC_{10} (0.006879%)	90±0.707	0.00270 ±0.00015	92±0.83	22.6±1.14	92±0.83	27.2±0.83	0.00
LC_{20} (0.0093%)	82±0.836	0.00258 ±0.00013	72±0.54	23.6±1.14	64±0.54	29.6±1.51	2.0
LC_{40} (0.0137%)	51.6±1.14	0.00178 ±0.00018	54±1.14	26.6±1.51	46±1.51	31.8±1.30	4.6

One-Way ANOVA on columns selected between Col(A1) to Col(A8):
F = 30.47526
p = 1.88343E-10
At the 0.05 level ,the means are significantly different.

SUMMARY

Groups	*Count*	*Sum*	*Average*	*Variance*
Column 1	4	0.02987	0.007468	3.28E-05
Column 2	4	321	80.25	422.9167
Column 3	4	0.01094	0.002735	7.49E-07
Column 4	4	314	78.5	377
Column 5	4	92	23	9.306667
Column 6	4	298	74.5	563.6667
Column 7	4	113.4	28.35	9.13
Column 8	4	6.6	1.65	4.756667

ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	36979.57849	7	5282.797	30.47526	1.88E-10	2.422631
Within Groups	4160.330101	24	173.3471			
Total	41139.90859	31				

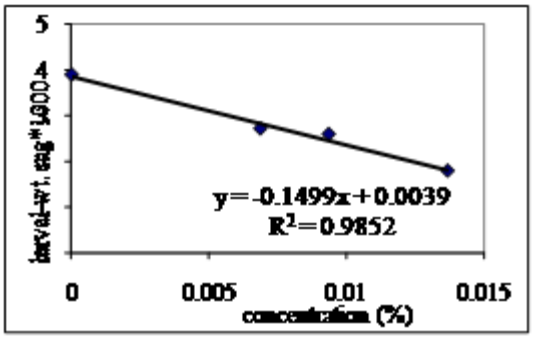


Fig.2: Regression graph of dose-response for lufenuron on weight of the larvae of *T.castaneum.*

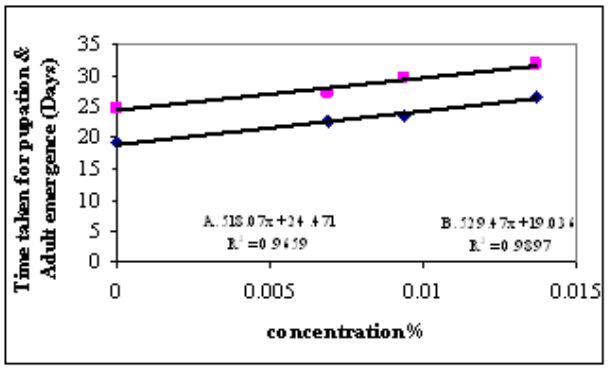


Fig.3: Regression graph of dose-response for lufenuron on time taken for pupation and adult emergence of *T. castaneum* larvae

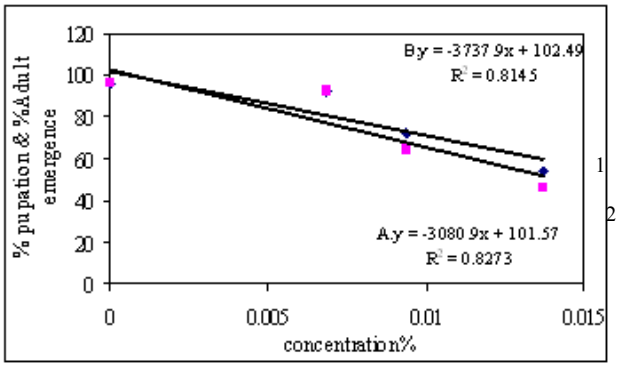


Fig.4: Regression graph of dose-response for lufenuron on %pupation and % adult emergence of *T. castaneum* larvae.

Dietary treatment of sub-lethal dose of lufenuron to adults resulted in mortality of F1 generation to a greater extent. Few larvae became black and shriveled, failed to shed the old cuticle and died while hatching after 2-3 days (Fig. 7). Later on hatchability of eggs and survival of the larvae increased with progress in time after the treatment and recovered to its untreated level (Table 2, Fig.6a, b, c).

Table 2a: Treated male X Treated female

Dose	Fecundity X± S. E.					% Hatching as on 4th day X ± S. E.				
	2days	4days	6days	8days	10days	2days	4days	6days	8days	10days
Control	20±4.9	18.4±3.3	21.12±4.3	20.4±3.6	20.4±2.4	97.92±2.7	98.02±2.27	97.32±2.5	97.90±2.7	98.16±3.8
LC_{10}	17.6±6.1	20.4±5.4	19.0±6.5	21.8±3.7	20.8±3.4	89.26±6.6	88.26±3.6	93.24±2.3	97.64±3.3	98.74±2.8
LC_{20}	18.4±2.8	19.4±3.5	18.0±6.7	17.6±3.5	19.4±3.2	87.54±5.9	86.14±9.0	90.44±3.9	93.55±6.6	100±0.0
LC_{40}	19.4±4.2	19.6±3.7	23.0±4.8	17.4±4.4	20.6±3.8	80.12±7.5	84.58±7.9	89.74±2.9	94.9±3.5	97.0±4.2

Table 2a: One-Way ANOVA on columns selected between 2d →10d:

For Fecundity				For % Hatching			
Data	**Mean**	**Variance**	**N**	**Data**	**Mean**	**Variance**	**N**
2days	18.85	1.13	4	2days	88.71	53.42787	4
4days	19.45	0.67667	4	4days	89.25	36.458	4
6days	20.28	4.98027	4	6days	92.685	11.83477	4
8days	19.3	4.65333	4	8days	95.9975	4.50403	4
10d	20.3	0.38667	4	10days	98.475	1.5569	4
F = 0.68527 p = 0.61312 **At the 0.05 level, the means are NOT significantly different.**				F = 3.31799 p = 0.03897 **At the 0.05 level, the means are significantly different.**			

Table 2b: One-Way ANOVA on rows selected between Control → LC_{40}:

For Fecundity				For % Hatching			
Data	**Mean**	**Variance**	**N**	**Data**	**Mean**	**Variance**	**N**
Control	20.064	1.02848	5	Control	97.864	0.10308	5
LC_{10}	19.92	2.692	5	LC_{10}	93.428	22.51852	5
LC_{20}	18.56	0.668	5	LC_{20}	91.534	30.49538	5
LC_{40}	18.92	1.892	5	LC_{40}	89.268	49.34732	5
F = 1.74368 p = 0.19841 **At the 0.05 level, the means are NOT significantly different.**				F = 2.5971 p = 0.08831 **At the 0.05 level, the means are NOT significantly different.**			

Table2 b: Treated male X Untreated female

Dose	Fecundity X± S. E.					% Hatching as on 4th day X ± S. E.				
	2days	4days	6days	8days	10days	2days	4days	6days	8days	10days
Control	20±4.9	18.4±3.3	21.12±4.3	20.4±3.6	20.4±2.4	97.92±2.7	98.02±2.27	97.32±2.5	97.90±2.7	98.16±3.8
LC_{10}	18.8±1.9	19.0±4.4	20.0±2.4	18.8±2.0	19.4±3.4	98.75±2.6	99.16±1.8	100.0±0.0	98.04±2.6	98.32±3.8
LC_{20}	19.4±2.07	19.6±3.04	19.8±2.5	19.8±3.9	20.6±2.5	97.9±2.7	100.0±0	98.03±2.7	98.25±2.3	100±0.0
LC_{40}	18.6±2.4	19.6±2.9	19.8±2.7	20.8±2.8	21.0±3.9	99.09±2.04	98.17±2.5	97.69±3.17	98.3±3.7	98.4±3.4

Table 2b: One-Way ANOVA on columns selected between 2d →10d:

For Fecundity				For % Hatching			
Data	**Mean**	**Variance**	**N**	**Data**	**Mean**	**Variance**	**N**
2days	19.2	0.4	4	2days	98.415	0.35937	4
4days	19.15	0.33	4	4days	98.8375	0.85643	4
6days	20.18	0.4016	4	6days	98.26	1.42967	4
8days	19.95	0.75667	4	8days	98.1225	0.03469	4
10days	20.35	0.46333	4	10days	98.695	0.76197	4
F = 2.64952 p = 0.07455 **At the 0.05 level, the means are NOT significantly different.**				F = 0.51347 p = 0.72696 **At the 0.05 level, the means are NOT significantly different.**			

Table 2b: One-Way ANOVA on rows selected between Control → LC_{40}:

For Fecundity				For % Hatching			
Data	**Mean**	**Variance**	**N**	**Data**	**Mean**	**Variance**	**N**
Control	19.98	1.32427	4	Control	97.864	0.10308	5
LC_{10}	19.3	0.28	4	LC_{10}	98.854	0.59138	5
LC_{20}	19.85	0.27667	4	LC_{20}	98.836	1.14473	5
LC_{40}	19.75	0.97	4	LC_{40}	98.31	0.25315	5
F = 0.48976 p = 0.69588 **At the 0.05 level, the means are NOT significantly different.**				F = 2.1481 p = 0.1342 **At the 0.05 level, the means are NOT significantly different.**			

Table2c: Treated female X Untreated male

Dose	Fecundity X± S. E.					% Hatching as on 4th day X ± S. E.				
	2days	4days	6days	8dsys	10days	2days	4days	6days	8days	10days
Control	20±4.9	18.4±3.3	21.12±4.3	20.4±3.6	20.4±2.4	97.92±2.7	98.02±2.27	97.32±2.5	97.90±2.7	98.16±3.8
LC_{10}	19.0±2.3	20.2±2.5	19.2±1.4	19.0±2.5	19.0±2.5	88.6±1.9	92.45±4.9	97.0±2.6	99.0±2.0	100±0
LC_{20}	18.0±1.5	20.2±3.9	20.4±3.4	20.6±6.0	16.4±2.7	87.7±2.1	91.6±3.1	91.14±2.1	97.8±3.1	98.2±2.4
LC_{40}	20.6±2.8	21.2±2.4	21.6±3.8	16.4±2.7	18.4±2.8	75.79±3.8	89.4±2.3	89.6±2.2	96.0±2.3	97.84±3.0

Table 2c: One-Way ANOVA on columns selected between 2d →10d:

For Fecundity				For % Hatching			
Data	**Mean**	**Variance**	**N**	**Data**	**Mean**	**Variance**	**N**
2days	19.4	1.30667	4	2d	87.5025	82.31682	4
4days	20	1.36	4	4d	92.8675	13.45089	4
6days	20.58	1.0896	4	6d	93.765	15.78037	4
8days	19.1	3.74667	4	8d	97.675	1.5425	4
10days	18.55	2.75667	4	10d	98.55	0.9604	4
F = 1.21131 p = 0.34691 **At the 0.05 level, the means are NOT significantly different.**				F = 3.40802 p = 0.03583 **At the 0.05 level, the means are significantly different.**			

Table 2c: One-Way ANOVA on rows selected between Control → LC_{40}:

For Fecundity				For % Hatching			
Data	**Mean**	**Variance**	**N**	**Data**	**Mean**	**Variance**	**N**
Control	20.064	1.02848	5	Control	97.864	0.10308	5
LC_{10}	19.28	0.272	5	LC_{10}	95.41	22.9055	5
LC_{20}	19.12	3.412	5	LC_{20}	93.288	20.79372	5
LC_{40}	19.64	4.808	5	LC_{40}	89.726	74.88358	5
F = 0.36958 p = 0.77599 **At the 0.05 level, the means are NOT significantly different.**				F = 2.00371 p = 0.15408 **At the 0.05 level, the means are NOT significantly different.**			

Effects on hatching of eggs:
When freshly laid eggs of *T. castaneum* were kept in diet with various sub-lethal concentrations of lufenuron it was found that the percentage hatching of eggs was not affected (Table 3).

Table 3 Effect of sub-lethal concentrations of lufenuron through diet on hatching of eggs of *T. castaneum*

Dose of Lufenuron (ppm)	% eggs hatched (X±S. E.) 2days	3days	4days
Control (0.00)	12±5.7	90±9.4	100±0.0
LC_{10} (0.006879)	10±5.7	90±7.0	100±0.0
LC_{20} (0.0093)	10±5.0	88±8.3	98±4.4
LC_{40} (0.0137)	12±5.7	92±8.3	100±0.0

Table 3a: One-Way ANOVA on columns selected between 2d →10d:

Between days				**Between Concentration**			
Data	**Mean**	**Variance**	**N**	**Data**	**Mean**	**Variance**	**N**
2days	11	1.33333	4	Control	67.33333	2321.33333	3
3days	90	2.66667	4	LC_{10}	66.66667	2433.33333	3
4days	99.5	1	4	LC_{20}	65.33333	2321.33333	3
				LC_{40}	68	2368	3
F = 5665.4 p = 1.12133 x 10^{14} **At the 0.05 level, the means are significantly different.**				F = 0.00165 p = 0.9999 **At the 0.05 level, the means are NOT significantly different.**			

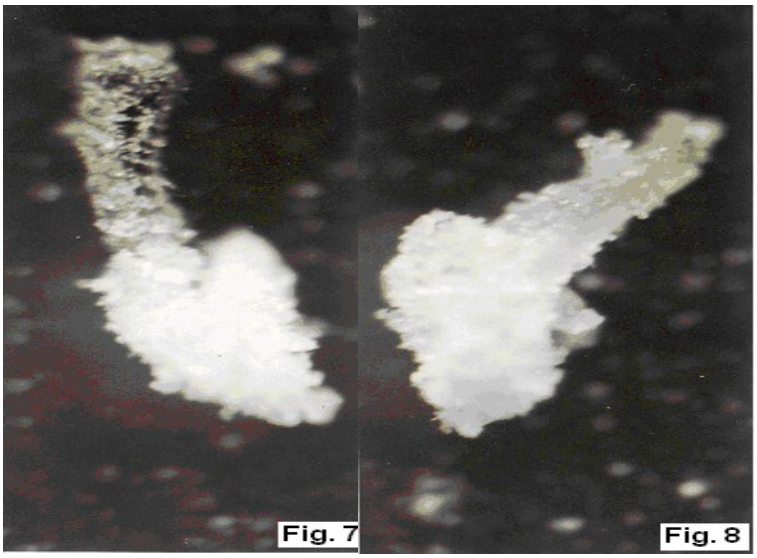


Fig.7: Photograph showing dead larva hatched from the egg laid by the *T. castaneum* adults treated with sublethal concentration of lufenuron.
Fig.8: Photograph showing normal larva hatched from the eggs laid by the *T. castaneum* adults.

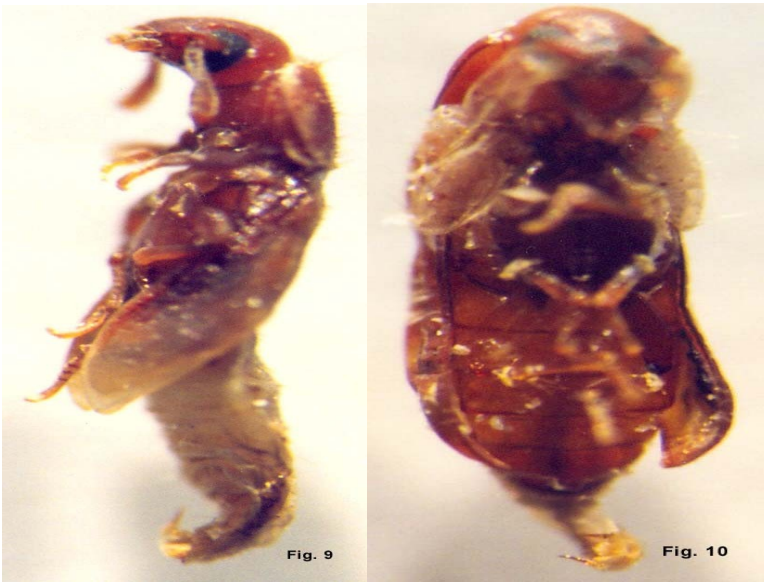


Fig.9: Pupal adult intermediates developed from *T. castaneum* larvae treated with sublethal concentrations of lufenuron.
Fig.10:Pupal adult intermediates developed from *T. castaneum* larvae treated with sublethal concentrations of lufenuron.

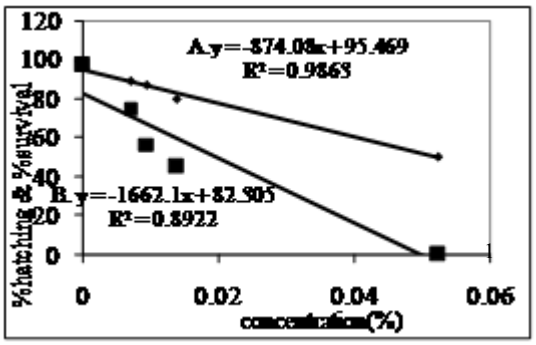
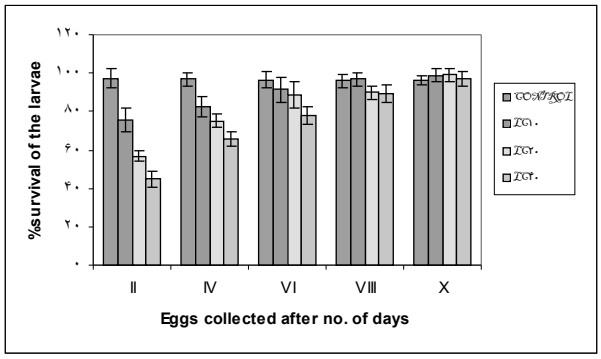
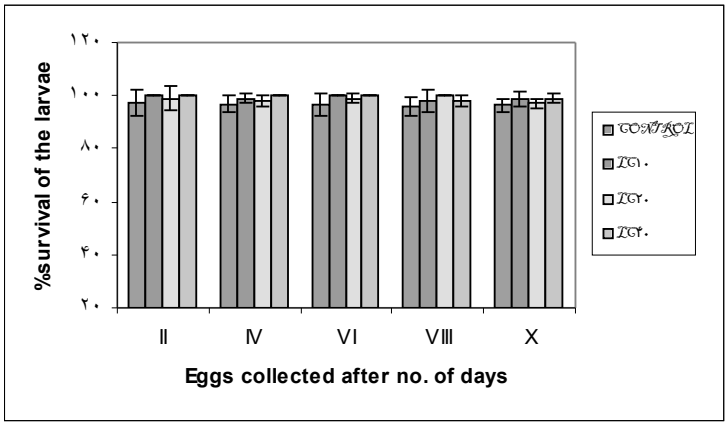


Fig.5: Regression graph of dose-response for lufenuron on %hatching and % survival of *T. castaneum* eggs.

a) Treated female X Treated male[2]



b) Treated male X Untreated female



c) Treated female X Untreated male

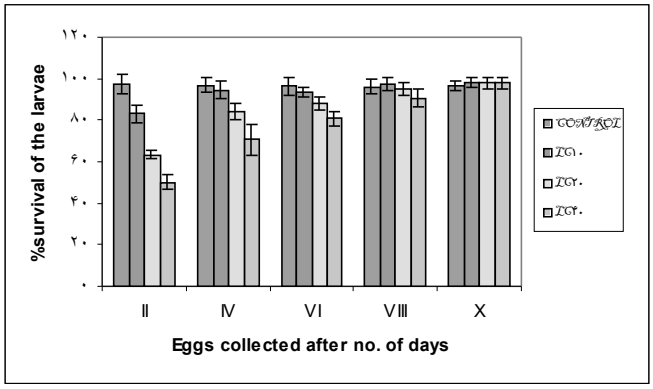


Fig.6: Effect of sublethal concentration of lufenuron on fertility of *T. castaneum* eggs.

DISCUSSION

Sub-lethal dose of lufenuron (LC_{10}, LC_{20} and LC_{40}) incorporated in the diet and fed for 24 hr to two day old larvae of *T. castaneum* were shown to affect their growth and development. Growth retardation was reflected by lower larval weight and delay in pupation and adult emergence. The pupae and adult developed from treated larvae failed to shed their cuticle, which lead to deformity or death. Also, abnormal stage like pupal-adult intermediate was observed. Developmental abnormalities were similar to those found with the use of flufenoxuron in *T. casteneum* by Salokhe et. al (2003) and hydroprene in *T. castaneum* and *T. confusum* by Arthur (2001). However, larval-pupal intermediates were not observed in the present study. Formation of intermediates and deformed adults at higher doses similar to those found with the use of JH analogues suggest that lufenuron may influence reproduction by causing hormonal imbalance (Bull, 1986; Deecher *et al*. 1990a, 1990b). It was observed that fecundity and hatchability of eggs in adults developed from treated larvae was not significantly different from that of control. When eggs were kept in lufenuron treated diet their hatching percentage was not affected as was observed in flufenoxuron on *T. castaneum* by Salokhe et. al.(2003). This is due to the fact that lufenuron has no contact action as observed by Anonymus (1997). Further, it was found that fecundity of *T. castaneum* adults fed on sub-lethal dose of lufenuron was not affected, but initially there was reduction in percentage of egg hatch. In fact, the hatching percentage was dose dependent.

Similar, observations have been reported by Ioriatti (1993) in lufenuron residue ingestion by leafroller species pest of Italian apples. Ovicidal effect have also been reported by Ascher *et al*. (1986) in the females of *Carpophilus hemipterus* (L) who laid sterile eggs after exposure to hexaflumuron. Elek and Longstaff (1994) found that the fecundity of *T. castaneum* and *O. surinamensis,* recovered almost to their untreated levels after two weeks of exposure to BPUs followed by two weeks on untreated wheat. Inhibition of eggs hatch also has been detected by Leonard *et al*. (1987) in *Musca domestica* (L) fed on diet treated with hexaflumuron and by Horowitz *et al.* (1992) in *Earias insulana* (Boisduval) after prolonged exposure to hexaflumuron. According to the Retnakaran and Wright (1987) the ovicidal effect through adult was due to inhibition of chitin formation of the embryo, which usually dies inside the eggs shell as a fully formed larva. The eggs laid by untreated females crossed with males treated with sub-lethal dose of lufenuron, did not differ in their hatching percentage and survival from that of control. Thus, it suggests that males were unable to transfer the amount of lufenuron required to inhibit egg hatch. Similar findings were reported by Moore *et al*. (1978) in diflubenzuron treated boll weevil where male transferred diflubenzuron to female by physical contact rather than during copulation. Failure of eggs laid by treated adults to hatch and mortality of the larvae after hatching clearly indicates the ovicidal and larvaecidal effect of lufenuron on *T. castaneum*. These findings suggest that, lufenuron can possibly be used in the management of this stored grain pest. Further research, under field condition would be needed to verify these findings. In the class of insect growth regulators, the chitin synthesis inhibitors are better controlling agents than JH analogs against stored product insect pests (Kramer et al. 1979). Benzoylphenylurea show low toxicity to mammals and are widely used in agricultural practice. The Spanish government has established MRL for several BPU in vegetables ranging from 0.01 and 0.5 ppm. (Lopez-Lopez et al. 2004). There is further scope to set the MRL values for these compounds in food grains.

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