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### Biological and biochemical effects of lufenuron (IgR) on growth, development and reproductive performance of *Tribolium castaneum* (Herbst) (Coleoptera: Tenebrionidae) (Adults)

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#### ABSTRACT

The effect of the chitin synthesis inhibitor lufenuron on the various developmental stages of red flour beetle *Tribolium castaneum* adults was determined by exposing them to different sub-lethal concentrations (LC<sub>10</sub>, LC<sub>20</sub> and LC<sub>40</sub>) of lufenuron through diet for 24 hrs on 1 day old adults. 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<sub>20</sub> and LC<sub>40</sub>. Adults emerging from the larvae fed on diet containing LC<sub>10</sub>, LC<sub>20</sub> and LC<sub>40</sub> of lufenuron showed minor 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 1 day old adults through diet) was affected, also, 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. We observed similar results with larval and adult stages therefore present data suggest that lufenuron even at sub-lethal concentrations has a very good larvicidal and ovicidal activity in *T. Castaneum*.

**Keywords:** *Tribolium castaneum*, lufenuron, sub-lethal effects.

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## INTRODUCTION

Insect growth regulating (IGR) compounds are emerging as safer alternative Chemical insecticides as they inhibit development of immature and reduce adult emergence instead of being toxic to its target. Benzoylphenylureas are the class of IGR insecticides that act by inhibition of chitin Synthesis and so interfere with the information of insect cuticle. Suppression of chitin Deposition in treated insect often causes high mortality during moulting[15] . The recently introduced benzoylphenylureas, Lufenuron (Match) is an IGR with Proven wide activity against many of horticultural and domestic pests. We studied sub-lethal Effects of flufenoxuron on the growth, development and reproductive performance of *Tribolium castaneum* (Herbst)[4]. Previous researches Observed that lufenuron ingested pest larve ceased feeding, stopped growing and finally died. 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. Lufenuron has been reported to be effective against number of serious pests of fruit crops. However sub-lethal effects of Lufenuron on stored product pests have not been reported so far. The present Endeavour were to study and investigate the effect of sub-lethal doses of Lufenuron on the various stages in the life cycle of *Tribolium castaneum* with respect to Growth, development, reproductive end points and also biochemical effects to throw light on its possible use for control of stored product pests in view of available reports[3] .

### Review of Literature

There are very few studies on the effect of sub-lethal Concentrations of IGRs on insect pest's . Previous Research shows the effect of sub-lethal doses of Dimilin on the reproductive performance of *Spodoptera lituralis* Boisduval for three consecutive generations[7][19] .The sub – Lethal effect of several IGRs on the tufted apple bud moth *Platynota idaeusalis*[1][12]. Lufenuron has been reported to be effective against number of serious pests of fruit crops such as *Epiphyas postvittana* , the light brown apple moth. The effect of Lufenuron on potato tuber moth *Phthorimaea operculella* (Zeller) eggs were Studied .Lufenuron were found to be effective against many pests Of horticulture found that Lufenuron residue Ingestion by leafroller species present on Italian apples did not affect female fecundity but *Carpophilus hemipterus* (L) (Coleoptera : Nitidulidae) laid sterile eggs for 12-14 days after Adults were exposed to hexaflumuron for 24 hrs[9][11]. Found that the fecundity of *Tribolium castaneum* and *O. surinamensis*, recovered Almost to their untreated levels after two weeks of exposure to 1mg kg of BPU's Followed by two weeks on untreated wheat . Inhibition of eggs hatch also has been Detected in house fly. *Musca domestica* (L)(Diptera: Muscidae) fed on diet treated With hexaflumuron and *Earias insulana* (Boisduval) (Lepidoptera: Noctuidae) after prolonged exposure to hexaflumuron[2][17]. This ovicidal effect through adult has been reported to Be due to inhibition of chitin formation of the embryo, which usually Dies inside the eggs shell as a fully formed larva.

## **Objectives**

- a. To evaluate dose response study against different age of larvae(Salokhe et al.) and adults of T.castaneum with various methods (residual,topical,through food).
- b. To study effect of sub-lethal concentrations of Lufenuron on fertility of T.castaneum adults.

## **MATERIALS AND METHODS**

### **MAINTENANCE OF T.castaneum culture:**

A stock culture of the T.castaneum were maintained on a diet containing wheat Flour and 5% Brewers yeast at 29+1 c and 60% relative humidity .Eggs were collected Collected by sieving (sieve number 40) diet infested with adults. Newly emerged Adults were obtained by collecting pupae and monitoring them for adult Emergence .

### **Preparation of different doses of Lufenuron (5.4% ec):**

Lufenuron (1ppm) were prepared.Different volumes of Lufenuron from stock Solution were used for experiments .

#### **a. Determination of Dose Response for larvae and adults:**

- a. **Toxicity through diet:** Lufenuron of different concentrations were thoroughly incorporated into diet. Determination of LC50 through diet were carried out by newly emerged adults of T.castaneum in diet treated with various concentrations of leufenuron.Acetone mixed with the diet were used as control.The control and experimental units were kept in a cooling incubator at 30 Degree C before and after the treatment.For each concentration tested sets of 5 replicates of 10 adults each were taken.The mortality count were taken after 24 hrs.Subsequently,the sub lethal doses(LC10,LC20 AND LC40) were deduced by extrapolation from the regression line obtained by probit analysis.
- b. **Residual film method:** A film of lufenuron of different concentrations were prepared on petriplates(2cm diameter) and newly emerged adults of T.castaneum were released in it.The mortality count were taken after 24 hours.Subsequently,Tthe sublethal doses(LC10,LC20 and LC40) were deduced by extrapolation from the regression line obtained by probit analysis.
- c. **Toxicity by tropical application:** Lufenuron of different concentrations are applied tropically with Hamilton syringe on ventral side of newly emerged adults of T.castaneum.The mortality count were taken after 24 hrs. Subsequently,Tthe sublethal doses(LC10,LC20 and LC40) were deduced by extrapolation from the regression line obtained by probit analysis.

**b. To study effect of sublethal concentrations of lufenuron on fecundity and fertility of T.castaneuem.**

## RESULTS

Sub-lethal concentrations of lufenuron for 1-day old adults of T. castaneum deduced from the regression equation ( $Y= 3.563X - 7.4876$ ) by extrapolation of the probit analysis were  $LC_{50}$ -0.0175%;  $LC_{40}$ -0.0137%;  $LC_{20}$ -0.00937%;  $LC_{10}$ -0.006879% (Table1).(Please note nearly same results were obtained when experiments were performed by Salokhe et al. on larvae)[21].

### 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 adult weight on 7<sup>th</sup> 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. 2). At  $LC_{20}$  and  $LC_{40}$  concentrations moulting of pupa into adult was affected, resulting in the development of pupal-adult intermediates. 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[6]. 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 significantly different from that of the adults fed on the normal diet.

Dietary treatment of sub-lethal dose of lufenuron to adults resulted in large mortality. Few adults became black and shriveled, died after 2-3 days (Fig. 1).



Fig. 1



Fig. 2

Table 1

Dose	%larval survival X±S. E.	Larval weight (mg)(10larvae) X±S. E.	%pupation	Time taken for the pupation	%adult emergence	Time taken for adult emergence	%PAI
0.00	98±0.447	0.00388±0.000217	96±0.54	19.2±0.83	96±0.54	24.8±0.83	0.00
LC <sub>10</sub> (0.006879%)	90±0.707	0.00270±0.000158	92±0.83	22.6±1.14	92±0.83	27.2±0.83	0.00
LC <sub>20</sub> (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 <sub>40</sub> (0.0137%)	51.6±1.1 4	0.00178±0.00018	54±1.14	26.6±1.51	46±1.51	31.8±1.30	4.6

### DISCUSSION

Sub-lethal dose of lufenuron (LC<sub>10</sub>, LC<sub>20</sub> and LC<sub>40</sub>) incorporated in the diet and fed for 24 hrs to 1 day old adults of *T. castaneum* were shown to affect growth, development. Growth retardation was reflected by lower adults weight. At sub-lethal doses pupae and adult developed from treated larvae failed to shed their cuticle, which lead to death or deformity in them. Along with the above effect abnormal stage like pupal-adult intermediate was also observed[5]. Developmental abnormalities were similar to those found with the use of flufenoxuron treatment in *T.* on both *T. castaneum* and *T. confusum* on exposure to

hydroprene[21]. However, larval-pupal intermediates as observed in previous research on this subject were not observed in the present study. Formation of intermediates as well as 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 we found that fecundity and hatchability of eggs in case of adults developed from treated larvae was not significantly different from that of control (data not presented). Also, when eggs were kept in lufenuron treated diet their hatching percentage was not affected as was observed in case of flufenoxuron on *T. castaneum*[21]. This is due to the fact that lufenuron has no contact action unlike flufenoxuron. 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 (Table 2). Similar, observations have been reported previously where in they found that lufenuron residue ingestion by leafroller species present on Italian apples did not affect female fecundity but reduced the percentage egg hatch[8]. Ovicidal effect through adult, have also been reported for other insect species. Previously reported females of *Carpophilus hemipterus* (L) (Coleoptera: Nitidulidae) laid sterile eggs for 12-14 days after adults were exposed to hexaflumuron for 24 hrs. Previous researches found that the fecundity of *T. castaneum* and *O. surinamensis*, recovered almost to their untreated levels after two weeks of exposure to  $1\text{mg kg}^{-1}$  of BPU followed by two weeks on untreated wheat. Inhibition of eggs hatch also has been detected in house fly, *Musca domestica* (L)(Diptera: Muscidae) fed on diet treated with hexaflumuron and *Earias insulana* (Boisduval) (Lepidoptera: Noctuidae) after prolonged exposure to hexaflumuron[16][23]. This ovicidal effect through adult has been reported to be due to inhibition of chitin formation of the embryo, which usually dies inside the eggs shell as a fully formed larva[13]. When males treated with sub-lethal dose of lufenuron were crossed with untreated females, their hatching percentage and survival was similar to that of control. Thus, our data possibly suggest that male beetles were unable to transfer the amount of lufenuron required to inhibit egg hatch[20]. Similar findings were reported by in diflubenzuron treated boll weevil where male transferred diflubenzuron to female by physical contact rather than during copulation, the treated males mated to untreated females were unable to transfer sufficient amount to affect egg hatch[18]. 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*[14]. These findings suggests 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 (IGRs), comparatively the chitin synthesis inhibitors (CSIs) are better controlling agents than juvenile hormone analogs (JHAs) against stored product insect pest populations[22]. Benzoylphenylurea show low toxicity to mammals, and they are widely used in agricultural practice. The Spanish government has established MRL for several BPU compounds in vegetables ranging between 0.01 and 0.5 ppm. There is further scope to set the MRL values for these compounds in food grains.

## CONCLUSION

We can thus conclude that lufenuron even at sub-lethal concentrations has a very good larvicidal and ovicidal activity in *T. Castaneum*. Moreover lufenuron doses to adults have significant effects on growth and development of adults and their fertility as well.

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