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Estimation of total Proteins in larvae of *Tribolium castaneum* (Coleoptera: Tenebrionidae) exposed to Lufenuron

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ABSTRACT
The first instar larvae of *Tribolium castaneum* were treated with sub-lethal concentrations (LC20 and LC40) of Lufenuron through the culture medium for 2days, 4days, 8days and 16days to investigate total soluble protein content in the larval tissues during development. It was found that for all concentrations tested, there was a significant reduction in total soluble protein content of the treated larvae as compared to that of control. At LC20 and LC40, there was a progressive decrease in the total soluble protein as a function of increase in age of the larvae. Thus sub-lethal concentrations of Lufenuron alter the total soluble protein content of *Tribolium castaneum* larvae during development there by resulting in developmental abnormalities as observed earlier by Salokhe et al.,(2010).

Introduction

Insect growth regulators are (IGRs) gaining Importance nowadays over the conventional neurotoxic chemical pesticides used in food stores against insect pests, mainly because they have low mammalian toxicity and degrade rapidly in the environment (Antognini, 1972; Eisler, 1992). Benzoylureas are an entirely different class of insecticides that act as insect growth regulators (IGRs), Lufenuron (Match) is newly introduced synthetic Insect Growth Regulator used as a crop protection product (pesticide) worldwide for variety of crops..

The principal effect of IGRs is to disturb development and metamorphosis and their action is therefore, much slower than that of conventional insecticides. These compounds claim to be safe for natural enemies and provide an alternative to conventional insecticides because lethal and sub lethal effects of the latter are usually considered highly risky to beneficial species (Ruberson et al. 1998).

The benzoyl phenyl urea (BPU) compounds form a group of IGRs, were found to inhibit chitin synthesis in insects **(**Post and Vincent, 1973**;** Post et al., 1974; Deul et al., 1978; Gijswijt et al., 1979) and effective larvicides (Mulder and Gijswijt, 1973**;** Ishaaya and Casida, 1974; Grosscurt, *1978;* Hammock and Quistad, 1981) at low doses (Loschiavo,1976; Mian and Mulla, 1982a, b; Eisa et al., 1984; Elek,1994). Generally, BPUs do not kill adult insects but prevent juvenile stages from completing their development (Dales etal. , 1994**).** Lufenuron, an acylurea IGR, interferes with biosynthesis of chitin, a vital and almost indestructible part of the insect exoskeleton. Chitin is a polymer of N-acetyl-D-glucosamine bound with the protein. Cross-linking of chitin and protein to form cuticle, plays a crucial role in the development of insects. Suppression of chitin deposition in treated insects often causes mortality during molting when the procuticle is subjected to the stress of ecdysis and cuticular expansion (Dean et al., 1998). Among the changes which occur in the physiology of insects, those involving chitin and protein should yield the most information since they are very closely associated with growth and reproduction processes (Muzzarelli, 1977). It was found that benzoyl phenyl ureas affect a cascade event involved in chitin biosynthesis **(**Post et al., 1974; Marks and Sowa, 1974**).** The disturbance of the formation of cuticular tissue by benzoyl phenyl ureas led to biochemical studies on the possible effects on the chitin and protein constituents of the cuticle. Effect of sub-lethal concentration of Lufenuron on biological parameters (survival and metamorphosis) of *A. aegypti* larvae was reported for the first time by Salokhe et al., (2010). *Tribolium* infestation is a major worldwide problem in stored products and food factories, resulting in both contamination and substantial economic damage (Mondal, 1994**; Mondal and Port, 1994**). The present work aims to determine the effectiveness of Lufenuron on the development of red flour beetle, *Tribolium castaneum*.

Materials and Methods

Maintenance of culture

A stock culture of *T.castaneum* was maintained on a diet containing wheat flour and 5% Brewer's yeast, at 29 to 30 degrees and 60% relative humidity. Culture was maintained in plastic bottles with required aeration. Eggs were collected by sieving (sieve no 40) the diet infested with adults. Larvae were maintained in Petri dishes and fed wheat flour + 5% Brewer's Yeast. Each larval stage was maintained in separate Petri dish so as to facilitate easy harvest of required stages for bioassay

Protein extraction and estimation

1st instar Larvae of Tribolium castaneum were introduced in control and treated (with sub lethal concentration of Lufenuron, LC20- (0.01ppm) and LC40(0.001PPM), an acetone mixed diet was used as control. Three replicates of 250 larvae each were made for each concentration. Twenty four hours after the treatment, 200 larvae of 1st instars and 150 larvae were weighed and homogenized in protein extraction buffer (1 mg/4ml) containing 5 mM Tris-HCl (pH 8.6), 0.1 ml Tris, 1 ml SDS, and 0.1% Triton X-100, followed by centrifugation at 10,000 rpm for 15min at 4ºC for 20min. Protein content of the supernatant was determined by the Lowry method (1951). The entire experiment was repeated thrice on different occasions.

Results:

It was observed that in Lufenuron treated larvae of *Tribolium castaneum* there was reduction in protein content as compared to that of control (Table-1). Further it was found that the reduction in protein was dependent on period of exposure and concentration of toxicant (dose). In 1st instar larvae,

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treatment with LC20 and LC40 of Lufenuron resulted in decreased total protein content as compared to that of control, there was significant difference in reduction of protein content of LC20 and LC40 treated larvae. At LC20 -33.8 %reduction was noted on 2nd day of exposure , which gradually increased in reduction to -56.51 % on 4th day, -83.92 % on 8th day and -118.96 % on 16th day of exposure. At LC40 the reduction in protein is observed -17.28% on 2nd day, -54.83 % on 4th day, -74.57 % on 8th day and -94.54 % on 16th day of exposure(fig I,II and III). Alteration in the percentage of total soluble protein content was significant in both the exposure periods and the doses of treatment at Lc20 and Lc40.

Table. 1. Total soluble protein content and percent change values in larvae of Tribolium castaneum(n=3)

Exposure period	2 Days	4 Days	8Days	16 Days
Control	950	960	1030	1070
LC20	710	580	560	490
S.D.	±0.04	±0.31	±0.62	±0.14
% Change	-33.80	-65.51	-83.92	-118.96
LC40	810	620	590	550
S.D.	±0.01	±0.52	±0.05	±0.33
% Change	-17.28	-54.83	-74.57	-94.54

Figure I.

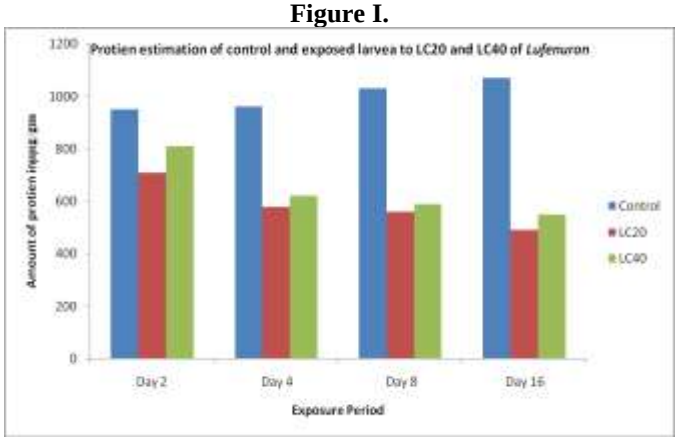


Figure II.

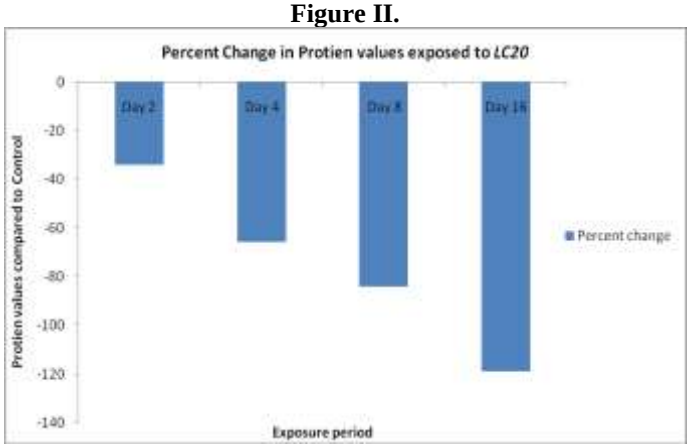
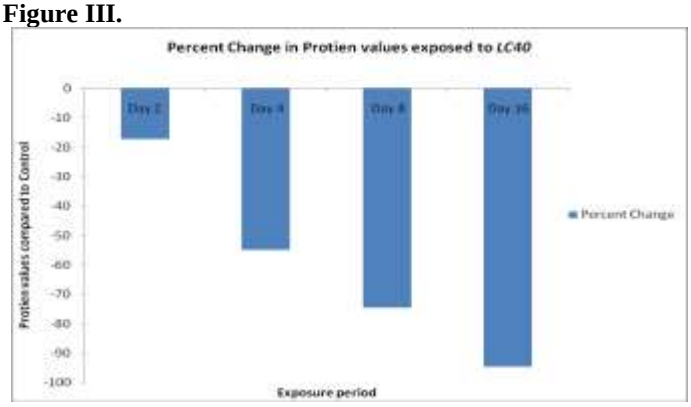


Figure III.



Discussions:

Sub-lethal concentrations of Lufenuron alter total soluble protein contents of 1st instars larvae of *Tribolium castaneum***.** Reduction in the chitin content of the II and IV instars larvae was in proportion with the concentration of Lufenuron on A.aegypti as reported by Salokhe et al., (2013). Further they observed variation in protein:chitin due to treatment with the sub-lethal concentrations of Lufenuron. Quantitative analysis of protein revealed that there was decrease in the total soluble proteins in *Spodoptera littoralis* larvae treated with Flufenoxuron (Sammour et al., 1996). Similar observations were made in house fly larvae treated with TH6040 (Ishaaya and Casida, 1974). Since cross-linking of chitin and protein to form cuticle plays important role in the development of insect, variation in the ratio in Lufenuron treated larvae of *A. aegypti* affected growth and development as reported earlier by Salokhe et al.,(2010**).**

These are the stress proteins which play a major role in protection and maintenance of many fundamental cellular functions (Fink, 1999; Naideau et al., 2001). Observed expression of these proteins probably provides protection against insect growth regulatory stress at LC20 and LC40 of Lufenuron as deficiency of such proteins was found to interfere with cuticle formation (Marcu et al., 1998). Decrease in the quantity of these proteins in Lufenuron treated larvae possibly resulted in the development of abnormal adults as reported earlier (Salokhe et al., 2010). However, more specific analysis would be required to identify these proteins and there by conclude on the effect of such changes on development of larvae.

The observations made in the present work can be attributed to the observations and reasons quoted by the above Scientists. The abnormal development of larvae and reduction in protein contents in 1st instar larvae of T. castaneum are the results of prevention of formation of protein /chitin leading to abnormal development of larvae followed by prolonged metamorphosis and death of most larvae without undergoing moulting.

Conclusion:

The effect of Lufenuron on developing larvae of *T.casteneum* is observed to be in the form of reduction of total protein content in 1st instar larvae treated with LC20 and LC40 for a specific period during experimentation. It can be concluded that Lufenuron prevents the Protein formation in larvae where Proteins play a major role in metamorphosis of insects for undergoing moulting. Hence Lufenuron use to control Insects seems to be effective in protection of stored food grains.

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