

Effect of Chlorfenapyr (sub-lethal concentration) on Development, Growth and Reproductive Performance of *Tribolium castaneum* (Herbst) (Coleoptera: Tenebrionidae)

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Abstract-The insecticide Chlorfenapyr that inhibits Adenosine Tri Phosphate (ATP) production in cellular structure of insects was first registered in the USA for control of termites, cockroaches and ants under the trade name Phantom. In the present work Chlorfenapyr 5% SL (INTREPID™) was used to observe the effect on the *Tribolium castaneum* development, as well as growth. The sub-lethal concentrations of Chlorfenapyr were given to first instar larvae of *Tribolium castaneum* through diet of wheat flour for 24 hours. There was effect on development and growth of *Tribolium castaneum* life cycle by dose dependent manner. There was reduction in larval weight, % pupation and time taken for pupation. Similarly there was reduction % adult emergence and time taken for adult emergence. Adult reproductive potential, fecundity and egg hatchability was also affected. The adult emerged from LC₂₀ concentration laid 76% non-viable eggs and the larvae which were emerged from viable eggs survived for 9 to 10 days. The adult emerged from LC₄₀ concentration laid 87% non viable eggs and the larvae which were emerged from viable eggs survived for 6-7 days.

When adults of different ages 2, 3, 4 day old, treated with Chlorfenapyr through diet, exhibited trans-ovario-ovicidal activity. Which were reflected by production of non-viable eggs. Fecundity of treated adults was reduced in dose dependent manner. In the Morphometric studies sub-lethal doses of Chlorfenapyr (LC₂₀ and LC₄₀ concentrations obtained via food) when given topically to the newly emerged adults, showed varied length of the life cycle stages of *Tribolium castaneum*.

The newly formed pupae when treated with concentrations higher than LC₂₀ and LC₄₀ i.e. 0.2, 0.4 ppm of Chlorfenapyr showed the morphological deformities in emerging adults. It was in the form of reduced wings and incomplete shedding of pupal case. When female and male of *Tribolium castaneum* both were treated topically by the sub-lethal concentration of Chlorfenapyr, showed drastic difference as compared with, when only female or male were treated.

Key words: Biological parameter, Chlorfenapyr, Egg hatching, Sub-lethal concentration, *Tribolium castaneum*.

I. INTRODUCTION

For the health of whole Nation Food security is very vital, moreover, for its independence and honour among the community of Nations in the world. According to World Food Summit (WFS) declaration, more than 800 million people mainly in developing countries do not have enough food to meet their basic nutritional needs. Shortage in

food grain production can lead to harmony upsetting in the world. Therefore insect pests are the major concern. A poor post-harvesting technology has reduced 35% of crop annually. The synthetic pesticides reduce these losses by constituting an efficient method. Man has struggled to protect his crops from evasion by insects, microbial pathogens and other pests, over the centuries. Almost any kind of stored grains are subjected to attack by insects, which are highly specialized and in most cases are of small size and have high reproductive potential, which make them easily concealed in grains and carried to many parts of the world. Approximately one third of the global food production is destroyed annually by field and storage pests. *Tribolium castaneum* (Herbst), commonly called Red flour beetle, is wide spread stored grain insect [1]. It is cosmopolitan pest which primarily attacks milled grain products and is of great economic importance. It is affinity for raw stored grains, milling facilities, food warehouses [2]. Both adult and larvae feed on grain dust and broken grains but not undamaged whole grains. Although, it develops well on broken grain kernels or processed grain products [3], damages flour and bakery, bran, cereals, and dried fruits. Quality and quantity of stored products are adversely affected by *Tribolium castaneum* [4]. Furthermore, *Tribolium castaneum* secretes lethal chemicals [5], which directly attacks on the grains and as a result grains are contaminated mainly due to hydroxyl quinone [6]. The beetle has a stable carbolic smell due to benzoquinones secretion [7], which passes from the contaminating grain to the flour and may have carcinogenic effects [8]. *Tribolium castaneum* is originally much more pesticide resistant than other stored product insects and this resistance can rapidly increase more than 10 fold. Its short development cycle and easiness of laboratory cultures maintain on a simple medium has made this species a popular choice as a model organism for studying pesticide effects [9].

The insecticidal pyrole Chlorfenapyr (INTREPID™) is new compound which was initially registered in USA to control termites, cockroaches and ants. Its activity is based on the mitochondrial effect. Its mode of action is totally different from conventional pesticides. It functions to uncouple oxidative phosphorylation of ATP production and loss of energy leading to cell dysfunction and subsequent death of organism [10]. Insect metabolic processes are influenced by Chlorfenapyr insecticides due to which they are in demand against many field pests to protect vegetables like cabbage, chillies and cotton plant. Present studies had undertaken to evaluate the effect of Chlorfenapyr on, Biological and Morphometric parameters of different developmental stages like adult, egg, larvae, and pupae of *Tribolium castaneum* to suggest suitable management options.

II. MATERIAL AND METHODS

A. Maintenance of culture of *Tribolium castaneum*

Tribolium castaneum culture was maintained whole wheat flour which was 95% and Brewer's yeast was 5% by weight, at $29 \pm 1^{\circ}\text{C}$ and 60% relative humidity. Eggs were collected every two days from cultures, by sieving (sieve number 40) diet infested with adults. Newly emerged adult were obtained by collecting pupae and monitoring them for adult emergence.

B. Studies of dose response on first instar:

The preparation of stock solution of Chlorfenapyr was carried out. Different volumes of Chlorfenapyr from stock solution were made and then thoroughly incorporated into diet. For the complete evaporation of solvent from treated flour, was kept at room temperature, before experiment was started. The LC_{20} and LC_{40} values of Chlorfenapyr through diet was determined by releasing neonates of *Tribolium castaneum* in diet treated with various concentrations of insecticide. Acetone mixed diet was used as control. The tested sets of five replicates with 10 first instar larvae for each concentration were taken. After 7 days interval the mortality was recorded. By regression analysis, the values of LC_{20} and LC_{40} were extrapolated.

C. Effect on larval Development and growth of *T. castaneum*:

The insecticide Chlorfenapyr, its effect of sub-lethal concentrations (LC_{20} and LC_{40}) through diet on larval development growth and morphological end points such as percentage pupation, time taken for pupation, percent adult emergence and time taken for adult emergence was examined. Examination was done by introducing 10 neonates of *Tribolium castaneum* in Chlorfenapyr in treated diet. The tested sets of five replicates were prepared. Acetone mixed diet was used as control. The larvae were transferred to normal diet after 24 hours. These larvae ten at a time, were weighed on seventh day after the start of the experiment.

Observations were made every day, once pupation had begun in any of the treatment, for percentage pupation, time taken for pupation, percent adult emergence and time taken for adult emergence. To analyze the data for significance regression analysis and one way ANOVA was carried out.

D. Effect on Fertility and Fecundity of Adults *T.castaneum*:

The fresh white newly formed pupae were isolated from the culture. These pupae were observed daily for adult emergence. The newly emerged adults were sexed. In the separate glass vials containing diet treated with LC₂₀ and LC₄₀ of Chlorfenapyr, the two day, three day and four day old, adults were kept in a pair. Acetone treated diet was used as control. Observations were made for their reproductive potential and egg hatchability. All experiments were replicated five times. By ANOVA, the obtained data was analysed and the results were compared by using Students- t test.

E. Effect on egg hatchability of *T.castaneum*:

The hatchability of eggs was determined by placing twenty eggs in the Chlorfenapyr (LC₂₀ and LC₄₀) through diet, for the observations of effect of insecticide. Acetone treated diet was used as control. Hatching of eggs was recorded every day, till hatching in the control was completed. All the experiments were replicated five times. Data was analysed by two ways ANOVA. Regression analysis was performed to determine dose-dependent relationship.

F. Effects of topical treatment of Chlorfenapyr on Morphometric measurements and analysis of different life cycle stages of *T. castaneum*

The sub-lethal dose (LC₂₀ and LC₄₀ of Chlorfenapyr as obtained for one day old larvae through diet), its 1µl was given topically to each the of female and male adults on the ventral surface between the mesothoracic and metathoracic legs by using a Hamilton micro-syringe. Distilled water treated adults were kept as a control. The eggs of these topically treated pair were collected and measurements were taken using digital Vernier calliper (MITUTOYO make). The measurements of eggs, neonates, 7th, 10th, 15th day old larvae, pupae, adults were recorded and analysed by ANOVA and Z-test. All the experiments were replicated for five times.

G. Effects of topical application on Pupae

By collecting last instar larvae and monitoring them for pupation, newly fresh white pupae were obtained. These pupae were treated from its ventral surface by using Hamilton micro-syringe, with different concentrations of Chlorfenapyr to find out LC₂₀ and LC₄₀ concentrations. The dispensing volume of solution at any concentration was always 1µl per pupae and control pupae treated similarly with 1µl of distilled water alone. After 24 hours of treatment, the treated and control pupae were transferred to normal diet. Mortality was recorded by observing % adult emergence. LC₂₀ and LC₄₀ values were deducted by extrapolation of probit mortality. At LC₂₀ and LC₄₀ treated pupae showed mortality but the development was normal. Therefore higher doses (0.2, 0.4 ppm) of Chlorfenapyr were applied to observe the effect on development of *Tribolium castaneum*. At these concentrations, observations were made on % adult emergence, time taken for adult emergence, abnormal adults, and pupal-adult intermediates and percent normal adults.

H. Effect of Topical application on adults

The pupae were isolated and sexed according to [11], and kept separately for emergence of adults, from subcultures of beetles. The dose response was determined by applying 1µl various concentrations of Chlorfenapyr to the ventral surface of the newly emerged, fresh, adults, in-between the mesothoracic and metathoracic legs using a Hamilton micro syringe. Adults treated similarly with 1µl of distilled water alone were used as controls. The treated and control adults were transferred to normal diet, after 24 hours of treatment. Mortality records were noted. The extrapolation of probit mortality was used to derive LC₂₀ and LC₄₀ values of Chlorfenapyr. The newly emerged female and male adults were treated with LC₂₀ and LC₄₀ of Chlorfenapyr and their crosses were performed as follows:

1. Normal male × Normal female
2. Treated male × Normal female
3. Treated female × Normal male
4. Treated male × Treated female

Five replicates per concentration and a pair of adults per replicate were prepared. After 24 hours of the treatment normal diet was provided to above adults. Observations were made on adults fertility, reproductive

potential and ovi-position. All experiments were replicated five times. Eggs were collected daily up to the seventh day after the treatment. Thus fecundity and fertility was recorded. This data was analyzed by one way ANOVA.

III. RESULTS:

A. *Effect on larval Development and growth of T. castaneum:*

The sub-lethal concentrations of Chlorfenapyr via food to newly hatched larvae of *Tribolium castaneum* derived from the regression equation were $LC_{20} = 0.0094\text{ppm}$, and $LC_{40} = 0.0256\text{ppm}$ (Fig. A.1). Dietary treatment of *Tribolium castaneum* larvae with sub-lethal concentrations of Chlorfenapyr (LC_{20} and LC_{40}) significantly reduced the larval weight on seventh, tenth and fifteenth day in dose dependent manner. Similarly pupae and adults weight was also reduced as compared to the control in dose dependent manner (Fig.A.2). A significant reduction in larval survival (%), pupal formation (%) and adult emergence (%) were observed with increase in concentration of Chlorfenapyr (Table A.1, Fig.A.3). Furthermore adults emerged from larvae fed on diet at LC_{20} and LC_{40} concentrations, laid non viable eggs (76% and 87% respectively). At LC_{20} , larvae which emerged from viable eggs died immediately or survived up to 9-10 days. Similarly at LC_{40} which emerged from viable eggs survived up to 6-7 days of larval stage.

B. *Effect on Fertility and Fecundity of Adults T.castaneum:*

When the number of eggs laid by treated two, three, four day old adults were compared with control, using ANOVA test. The student t-test for equality of average was also found to reduce in dose dependent manner (Table B.1) (Where, $t_{18}, 0.05 = 2.101$). It was observed that, the average fertility rate was reduced.

C. *Morphometric Measurements of different life cycle stages of T.castaneum:*

The sub-lethal concentration of Chlorfenapyr effect on different stages of *Tribolium castaneum* viz eggs, neonates, 7th, 10th, 15th day old larvae, newly formed pupae and adult were compared with control. The length of all stages were analysed by ANOVA and Z test the value (Z, $0.05=1.64$)(Table C-1), in which results showed all the values were significant. The length of each of the developing stages was reduced in dose dependent manner as compared to control. (Table C1) (Fig-C1-C10)

D. *Effect of topical application on Pupae of Tribolium castaneum*

The LC_{20} and LC_{40} values for topical application of newly emerged adults deduced from extrapolation of probit log analysis were $LC_{20} = 0.029\text{ ppm}$ and $LC_{40} = 0.101\text{ ppm}$ (Fig D1) at these concentrations, mortality was recorded with no abnormalities of emerging adult. High dose of Chlorfenapyr 0.2 and 0.4 ppm treatment to newly formed pupae results into 63.33% and 86.66% mortality respectively during pupal stage itself. Newly formed pupae, when treated with higher concentrations of 0.2 & 0.4 ppm of Chlorfenapyr the emerging adults had morphological deformities. They were with reduced wings and unable to free themselves from pupal cuticle (FigD-2, 3). There was delay in adult emergence with increasing concentration of Chlorfenapyr as compared with control (Table D-1).

E. *Effect of topical application on adults of Tribolium castaneum*

The LC_{20} and LC_{40} values for topical application of newly emerged adults deduced from extrapolation of probit log analysis were $LC_{20} = 0.05\text{ ppm}$ and $LC_{40} = 0.127\text{ ppm}$ (Fig E1). When either sex adults of *Tribolium castaneum* were treated topically with sub-lethal concentration of Chlorfenapyr there was reduction in fecundity as compared to that of control, (Table E1 and E2). The fecundity of normal females when crossed with treated males was less in both the concentrations respectively than that of control (Fig. E2). When only females treated by sub-lethal concentration of Chlorfenapyr crossed with normal males, number of eggs laid was lesser than that of the pair in which the normal females and treated males were crossed (Fig. E3). Fecundity was reduced when both sexes *Tribolium castaneum* were treated compared to the pair in which any one sex was treated, ($t_{12}, 0.05=2.179$) (Table, E1 and E2) (Fig. E4). In LC_{20} concentration, the average number of eggs laid by pair in which treated female crossed with normal male were more than that of both the sexes were treated (Table E3 and E4). At LC_{40} concentration, the average number of eggs laid by a pair with treated female was not equal to the pair in which only males were treated. The increasing concentrations of insecticide Chlorfenapyr decrease the reproductive potential of adults, irrespective of gender.

IV. Discussion

Laboratory evaluation of sub-lethal concentrations LC₂₀ and LC₄₀ of Chlorfenapyr on different stages of *Tribolium castaneum* revealed that it affects the development and growth of the larvae. First instar of *Tribolium castaneum* treated with sub lethal concentration of Chlorfenapyr through diet showed retardation in growth by lowering the larval weight on 7th, 10th, 15th day development. Similar observations of reduction in weight of the larvae of *Tribolium castaneum* due to Flufenoxuron treatment was recorded [12]. The adult emergence was delayed by 5 to 6 days in LC₂₀ and by 19 to 20 days in LC₄₀ as compared to control. The adults emerged from both the concentrations laid non viable eggs, (76% for LC₂₀ and 87% for LC₄₀). Hatching from the remaining viable eggs resulted in 9 to 10 days larval survival in LC₂₀ and 6 to 7 days of larval survival in LC₄₀. Hatching failure of eggs laid by treated adults and the rise in larval mortality immediately after hatching and reduction in moulting stage formation was found in treated doses. This clearly indicates the ovicidal and larvaecidal effect of Chlorfenapyr on *Tribolium Castaneum*.

There was variability in response of adults *Tribolium castaneum* of different ages with respect to fecundity when fed on a diet treated with sub-lethal concentration of Chlorfenapyr. Our observations of dose dependent variability in fecundity was in agreement with those observed in lufenuron treated *T. Castaneum* [13]. All specific age adults like two, three, four day old adults showed a dose dependent effect. Therefore due to Chlorfenapyr insecticide the number of eggs laid by treated adults was reduced as compared to control.

Survival of *Tribolium castaneum* from the first instar larvae to adult, through each life cycle stage was definitely affected by Chlorfenapyr. This was irrespective of media of application, like via food or via topical application. The topical application as well as through food treatment of Chlorfenapyr produced marked decline in the number of viable eggs. Reproductive potential of *Tribolium castaneum*, indicated distinct variation due to Chlorfenapyr. Similar observations have been reported in the activity of 3 insect growth regulators for control of tortricids present in Tortino Region [14]. Ovicidal effect have also been reported in the females of *Carpophilys hemipterus* (L) it laid sterile eggs after exposure to benzoylphenylureas [15]. Thus we observed that the treatment of Chlorfenapyr, not only interfere with the normal development of *Tribolium castaneum*, but also affect the reproductive potential of the adults. Chlorfenapyr hampers the supply of energy which needed for all the metabolic activity, thus resulting in growth inhibition. It means this compound uncouples oxidative phosphorylation at the mitochondria, resulting in disruption of ATP production [16], and ultimately showed the effect on development and growth. The effect was on length and moulting of the life cycle stages as well. Larval instars treated by Chlorfenapyr, were found as growth inhibited. This is reflected by reduction in weight gain. It has also latent effect on egg hatchability. Similar observations were found in *Spodoptera Littoralis* (Lepidoptera: Noctuidae) of Lufenuron [17]. Therefore in the Morphometric measurements showed the length reduced due to Chlorfenapyr as compare to control.

In the present study the inability of the adults to free themselves from pupal covering, could be due to weakening of elytra by the Chlorfenapyr residue, as well as the wing deformities found in the topical application on pupae, similar observations were reported on, both *Tribolium castaneum* and *Tribolium confusum* on exposure to hydroprene [18]. The reduction in fecundity of treated females and reproductive potentials of males by topical treatment of sub-lethal concentrations of Chlorfenapyr, results in egg sterilization in treated females through disruption of oogenesis and by interference with spermatogenesis in males. Similar observations were made on Flufenoxuron treated *Tribolium castaneum* [11]. The present study reveals that sub-lethal concentration of Chlorfenapyr exhibit transovarial ovicidal activity. Chlorfenapyr affects insect through ingestion or by contact. Chlorfenapyr activates and uncouples oxidative phosphorylation by disrupting mitochondria. Therefore insects die as they are not able to create their own energy. The larvae or adults either would die or fail to develop further and fail to reproduce [10]. Thus Chlorfenapyr can be used in IPM programme to control the population of *Tribolium castaneum*.

V. REFERENCES

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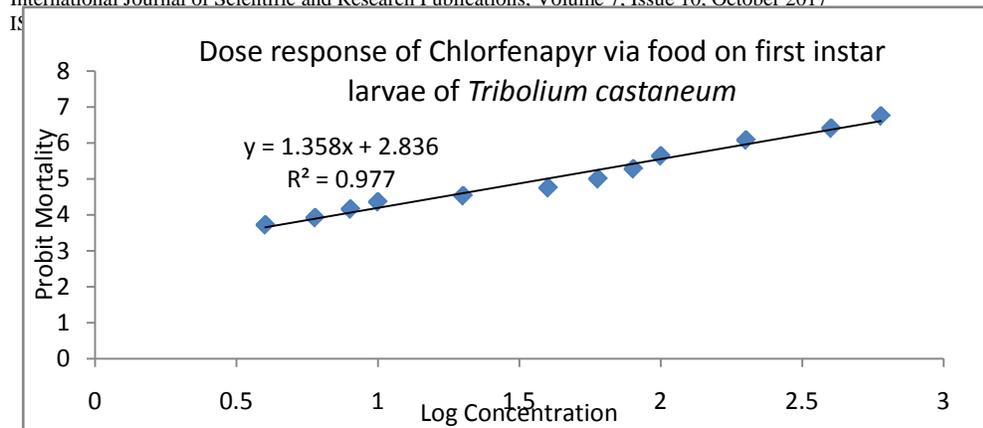


Fig.A.1 Regression graph of dose response of Chlorfenapyr via food on first instar larvae of *T.castaneum*

Table A-1 Effect of sub lethal concentration of Chlorfenapyr on development and growth of *T.castaneum*

Dose	% Larval survival $X \pm SE(X)$	% pupation $X \pm SE(X)$	Pupal Weight	pupation (Days) $X \pm SE(X)$	% adult emergence $X \pm SE(X)$	Adult weight	Time taken for adult emergence (Days) $X \pm SE(X)$
0. 00	94 \pm 1.56	92 \pm 1.47	31.4 \pm 2.77	18 \pm 1.24	90 \pm 1.24	26 \pm 4.02	25 \pm 1.2
LC ₂₀	74 \pm 0.96 *	68 \pm 1.92 *	27.3 \pm 1.61	24.2 \pm 1.03	64 \pm 2.0 *	18 \pm 1.52	30.2 \pm 1.03
LC ₄₀	56 \pm 0.96 *	52 \pm 1.47 *	20.9 \pm 1.78	30.2 \pm 2.96	50 \pm 1.75 *	13.86 \pm 1.89	44.2 \pm 2.38

* Significance at 5 % level of significance.

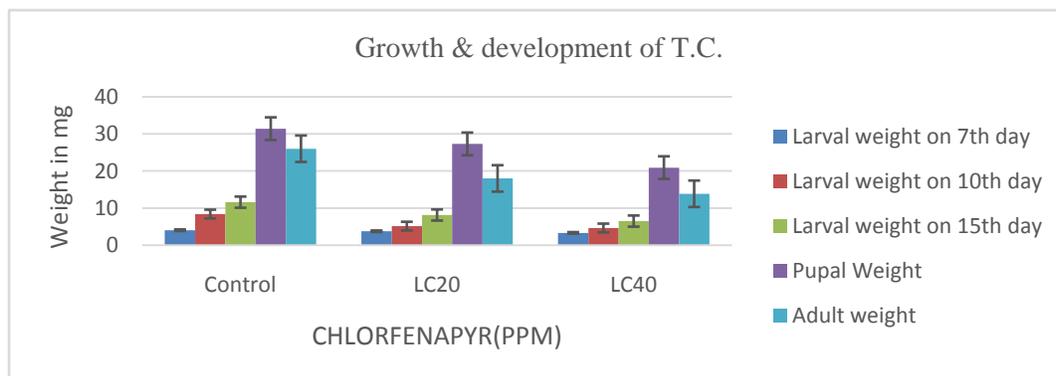


Fig.A.2 Effect of sub-lethal concentrations of Chlorfenapyr on development and growth of *Tribolium castaneum* comparing weight in mg.

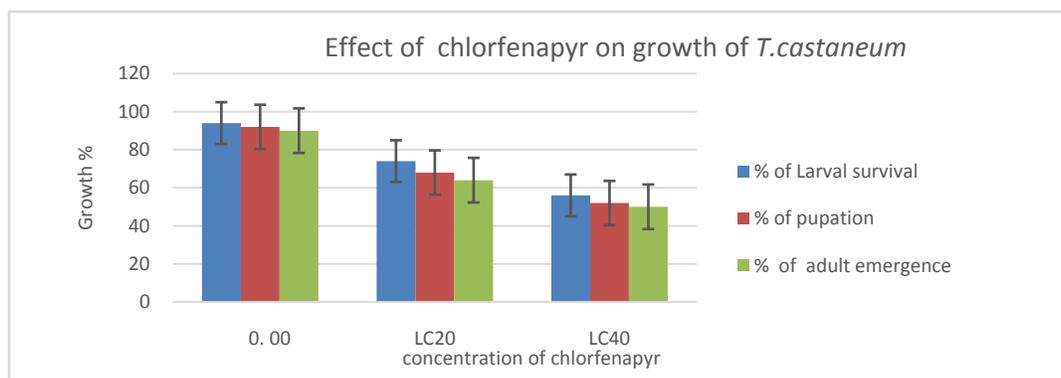


Fig.A.3 Average survival rate of larvae, Pupae and adult of *Tribolium castaneum* in control and Chlorfenapyr treated (LC₂₀ and LC₄₀).

Table B.1 Effect of sub-lethal concentrations of Chlorfenapyr on fertility and fecundity of adults of different ages of *T. castaneum*

μ_1 = average no, of eggs laid by insect(Control)

μ_2 = average no, of eggs laid by insect(LC₂₀)

μ_3 = average no, of eggs laid by insect(LC₄₀)

To test	Different age of insects		
	2 days old adult	3 days old adult	4 days old adult
Ho: $\mu_1 = \mu_2$ against H1: $\mu_1 > \mu_2$	2.546818	7.223649	14.21557
Ho: $\mu_1 = \mu_3$ against H1: $\mu_1 > \mu_3$	3.925679	10.47855	18.3553
Ho: $\mu_2 = \mu_3$ against H1: $\mu_2 > \mu_3$	2.13809	3.628247	8.142857
Conclusion	$\mu_1 > \mu_2 > \mu_3$	$\mu_1 > \mu_2 > \mu_3$	$\mu_1 > \mu_2 > \mu_3$

$t_{18,0.05} = 2.101$

All values are significant.

Table C.1 Morphological measurement of different stages of *Tribolium castaneum* control and Chlorfenapyr treated (LC₂₀, LC₄₀ via Food)

μ_1 = average length for control

μ_2 = average length for LC₂₀

μ_3 = average length for LC₄₀

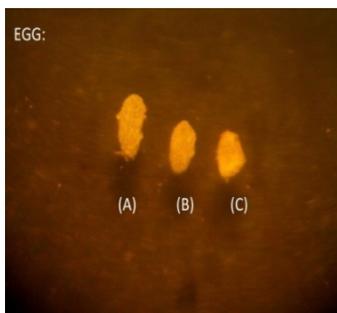
Average Length	Different stages of <i>Tribolium castaneum</i>						
	Eggs	1st instar Larvae (2Day old)	7 day Larvae	10 day Larvae	15 day Larvae	Pupae Length	Adult Length
$\mu_1 - \mu_2$	25.87652	26.50293	8.048299	48.62107	181.5159	12.42298	14.61627
$\mu_1 - \mu_3$	34.4424	43.28499	59.425	112.273	49.68933	22.68223	29.08403
$\mu_2 - \mu_3$	8.127949	23.11146	-0.4505	9.540551	10.67078	6.997114	14.95668
Conclusion	$\mu_1 > \mu_2 > \mu_3$	$\mu_1 > \mu_2 > \mu_3$	$\mu_1 > \mu_2,$ $\mu_1 > \mu_3,$ $\mu_2 = \mu_3$	$\mu_1 > \mu_2 > \mu_3$			

$Z_{0.05} = 1.64$

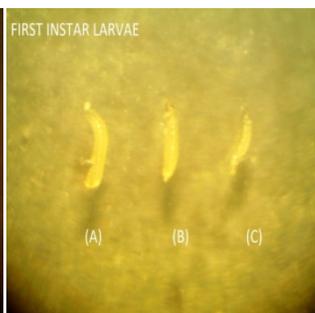
All values are significant except ($\mu_2 - \mu_3$) for 7 days Larvae.

Comparative figures of lengths *T. castaneum*, treated by via food dose given topically to newly emerge adult, figures of F2 generation.

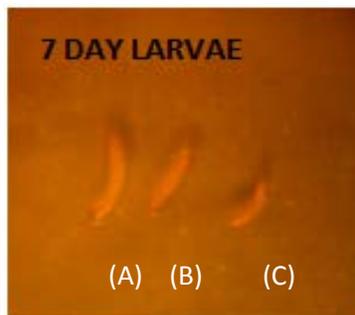
I- Egg



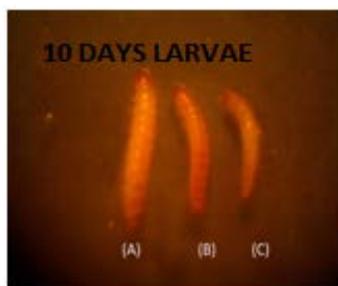
II-First Instar Larvae



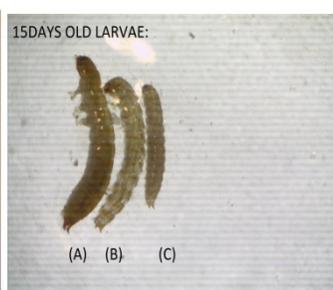
III-7th Day Larvae



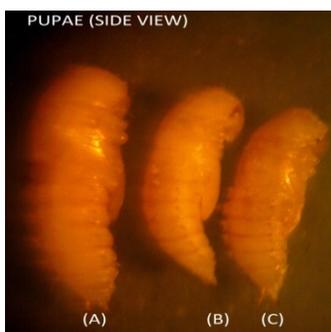
IV- 10th Day Larvae



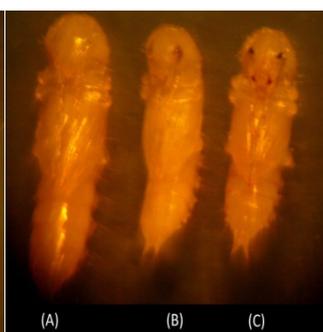
V- 15th Day Larvae



VI- Pupae (Side view)



VII-Pupae (Ventral view)



VIII- Adult

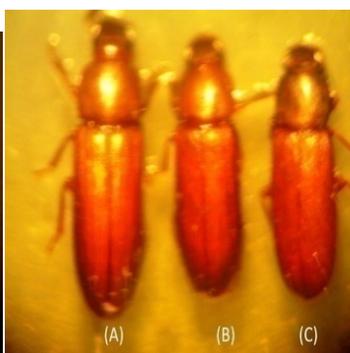


Figure C-1 (I to VIII) –Variation in lengths of life cycle stages of *Tribolium castaneum* due to Chlorfenapyr treatment.

(A) -Control,

(B) -LC₂₀,

(C) -LC₄₀

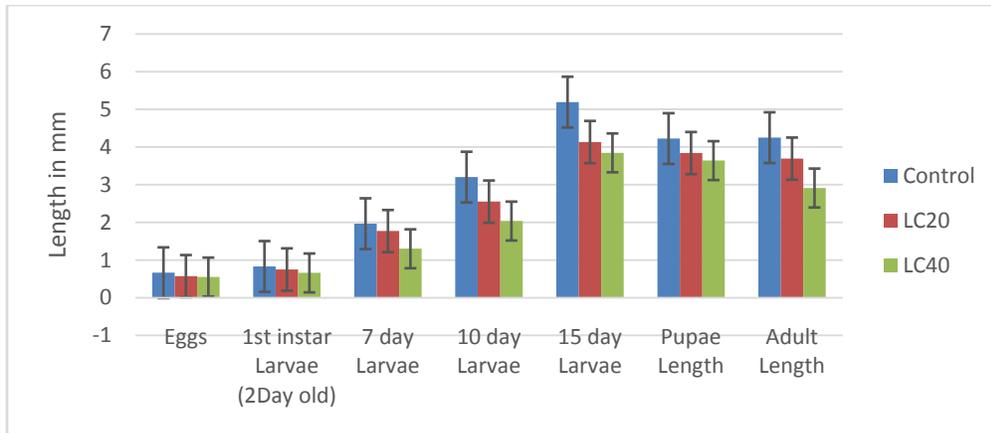


Figure.C-9 Comparative Lengths of life cycle stages of *Tribolium castaneum* of Control and Chlorfenapyr treated (LC₂₀, and LC₄₀).

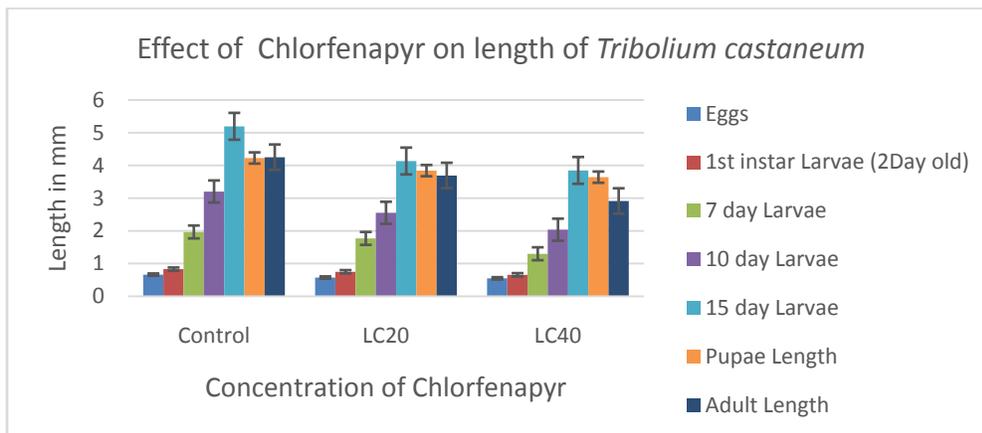


Fig-C10 Comparative Lengths of life cycle stages of *Tribolium castaneum* of Control and Chlorfenapyr treated (LC₂₀, and LC₄₀).

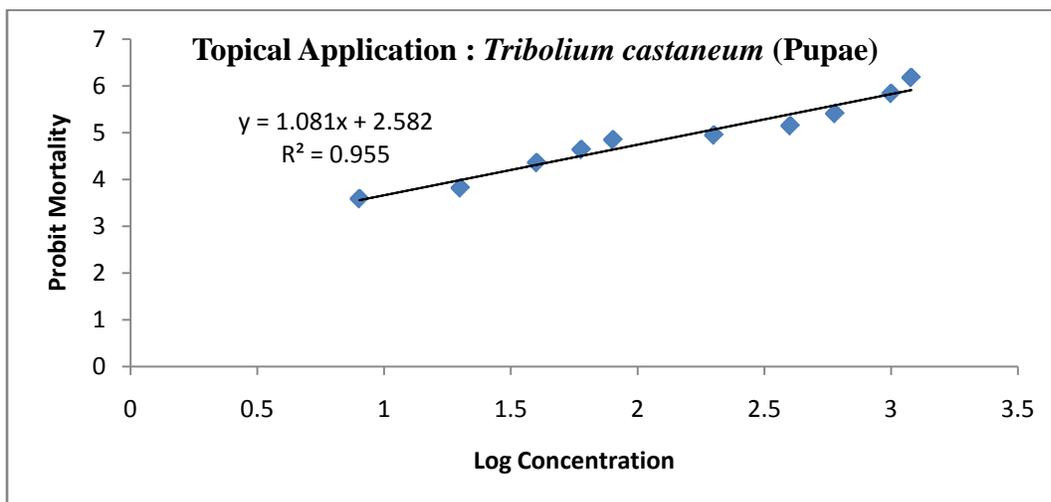


Figure : D-1 Regression graph of dose response of Chlorfenapyr via Topical application on Pupae

Tribolium castaneum

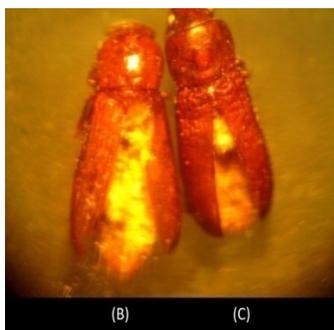


Fig D, 2 Abnormal wings formed in adult due to topical treatment of pupae (at 0.2 ppm concentration)

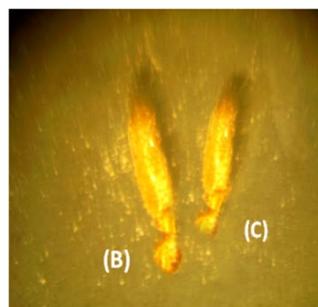


Fig D, 3- Pupal-adult intermediate with incomplete shedding of pupal case due to topical treatment of pupae (at 0.4 ppm concentration)

Table D1 – Effect of Topical application on Pupae

Concentration (ppm)	Control	LC ₂₀	LC ₄₀	0.2	0.4
% Mortality	0	20	40	63.33	86.66
Time taken for adult emergence (in days)	3-4	6-7	7-8	9-10	11-12
Normal adults out of survived (%)	100%	100%	100%	27.27%	0%
Abnormal adults out of survived (%)	0	0	0	72.70%	100%

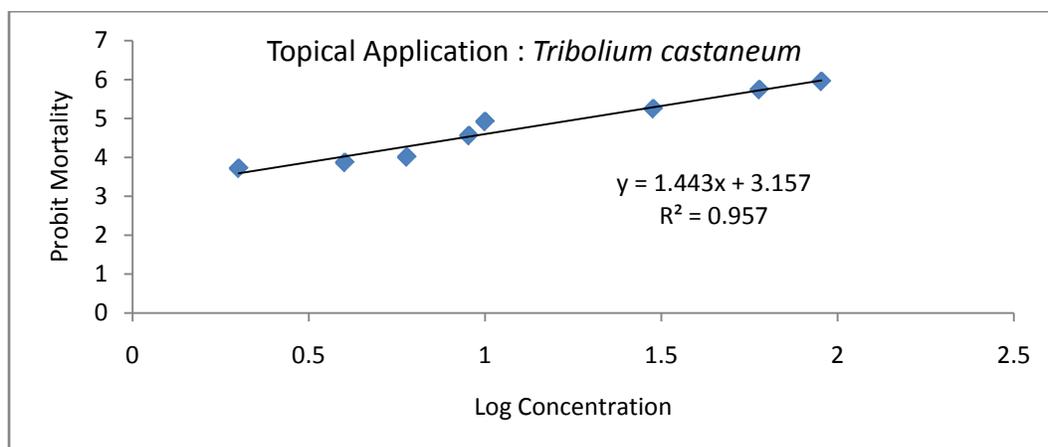


Fig. : E-1 Regression graph of dose response of Chlorfenapyr via Topical Application on adult *Tribolium castaneum*

Table : E-1 Effect of Topical application of Chlorfenapyr on fecundity of adult *Tribolium castaneum* :

Chlorfenapyr Dose in ppm	Normal Male X Normal Female (μ_1)	Treated Male X Normal female (μ_2)	Treated female X Normal male (μ_3)	Treated Male X Treated female (μ_4)
LC ₂₀	71.2±1.77904	40.6 ± 1.4026	24.6 ± 1.0545	17.4 ± 1.5476
LC ₄₀	71.2±1.77904	30.4 ± 2.1292	21.2 ± 2.2081	10.8 ± 3.4233

μ_1 = average no, of eggs laid by Normal male X Normal female

μ_2 = average no, of eggs laid by Treated Male X Normal female

μ_3 = average no, of eggs laid by Treated female X Normal male

μ_4 = average no, of eggs laid by Treated Male X Treated female

Table : E-2 ANOVA Conclusion

To test	Egg laying by LC ₂₀ treated pair	Egg laying by LC ₄₀ treated pair
Ho: $\mu_1=\mu_2$ against H1: $\mu_1 > \mu_2$	4.719436	6.04461
Ho: $\mu_1=\mu_3$ against H1: $\mu_1 > \mu_3$	6.592873	8.257228
Ho: $\mu_1=\mu_4$ against H1: $\mu_1 > \mu_4$	9.2014	10.38912
Ho: $\mu_2=\mu_3$ against H1: $\mu_2 > \mu_3$	2.862014	1.985047
Ho: $\mu_2=\mu_4$ against H1: $\mu_2 > \mu_4$	5.896611	4.543013
Ho: $\mu_3=\mu_4$ against H1: $\mu_3 > \mu_4$	1.488417	3.335802
Conclusion	$\mu_1 > \mu_2 > \mu_3$, $\mu_1 > \mu_4$, $\mu_2 > \mu_4$, $\mu_3 = \mu_4$	$\mu_1 > \mu_2 > \mu_4$, $\mu_2 > \mu_4$, $\mu_3 > \mu_4$, $\mu_2 = \mu_3$

$$t_{12,0.05} = 2.179$$

All values are significant except for hypothesis Ho: $\mu_3=\mu_4$ against H1: $\mu_3 > \mu_4$ for egg laying for LC₂₀ and Ho: $\mu_2=\mu_3$ against H1: $\mu_2 > \mu_3$ for egg laying for LC₄₀.

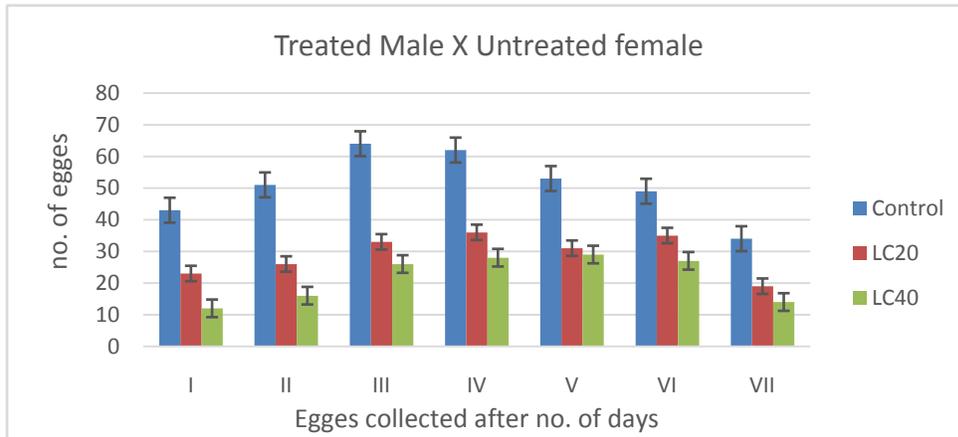


Fig. E2 Fecundity of treated Male X untreated Female of *T.castaneum*

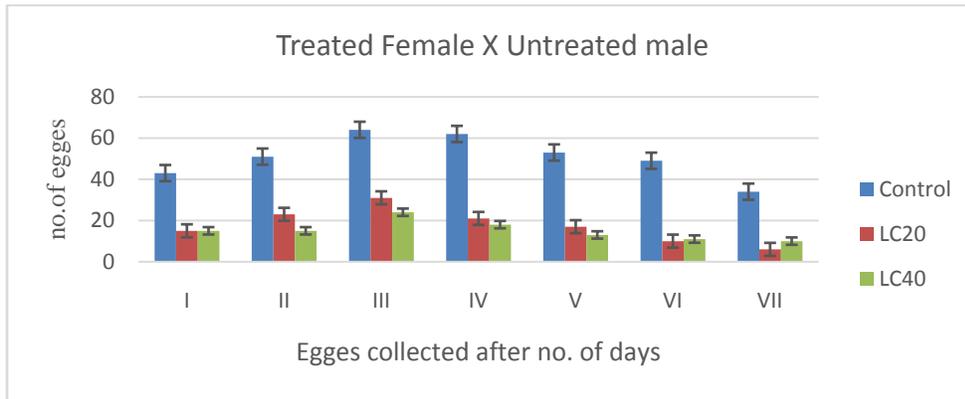


Fig. E3 Fecundity of treated Female X untreated Male of *T.castaneum*

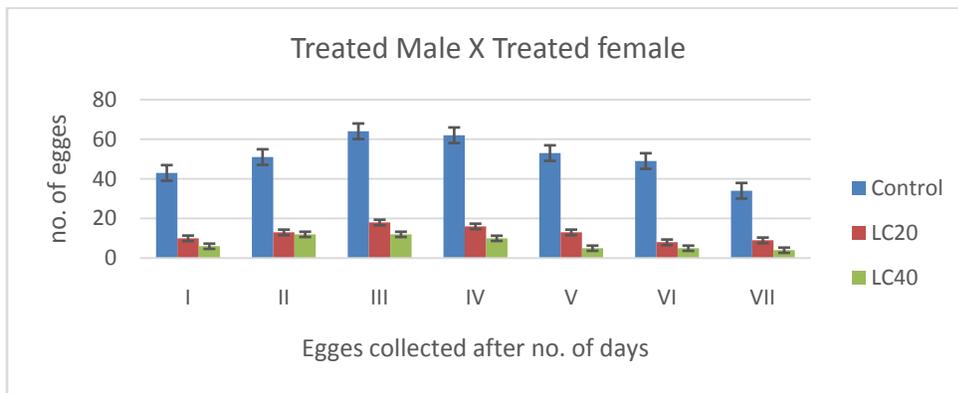


Fig. E4 Fecundity of treated Female X treated Male of *T.castaneum*