The red flour beetle, *Tribolium castaneum* (Herbst) belongs to family Tenebrionidae. This widely distributed pest species is extremely facile and tactable genetic model, and represent the most speciose of all animals’ orders. In addition to its phylogenetic position, *Tribolium* is predisposed to become the second insect model system because of its easy husbandry and suitability for both molecular and genetic analysis and give strong competition to the traditional *Drosophila* based genetic model.

Little is known about resistance mechanism and their inheritance in agriculturally important pests. Information about the genetic basis of resistance can facilitates efforts to detect and monitor resistance, to assess the risk of resistance, to model the evolution of resistance and to delay resistance development in pests. The present research work was carried out with *T. castaneum* by developing deltamethrin resistant strains in laboratory and then understanding the underlying mechanism of insecticide resistance. This work was divided into three main parts:

1. Biology, cytogenetic and isozyme patterns
2. Development of pyrethroid resistance
3. Inheritance studies

**1. Biology, Cytogenetic and Isozyme Patterns**

Adults of *Tribolium castaneum* were collected from two different areas of the city of Agra, Dayalbagh and Cantonment area and pooled together to have a heterogeneous population with large variability. These adults were identified according to the identification proposed by British Museum of Natural History (Freeman, 1990). The pooled heterogeneous strains were cultured in the standard wheat flour medium fortified with yeast at 30±2°C and 70% Relative humidity. On emergence of appreciable number of adults, insects were sieved out and used either for experiments or rearing the next generation. Subsequent generations of experimental strain were maintained in similar manner. The progeny thus reared in the laboratory was designated as parental strain (P1) with which the selection for resistance study was initiated and their cytogenetic and nonspecific esterase patterns were also standardized.

In cytogenetic analysis the karyotype of *T. castaneum*, showed a diploid number of 2n = 20 chromosome and sex determining mechanism was of “parachute type” due to the X and y chromosome (9A + Xyp). C- banded preparations were also made to
identify the heterochromatised regions, showed positive staining in centromeric regions of all autosomes. In sex chromosomes only Xp chromosome indicated heterochromatised block. Nucleolar organizing regions were also utilized as cytogenetic marker.

In isozyme studies three functional zones of nonspecific esterase activity have been detected in the adults of *Tribolium castaneum*. EST-1 is highly anodal while EST-3 is cathodal. The relative mobility of EST-1 to EST-3 was 0.953, 0.8, 0.69, 0.58, 0.26, 0.18 and 0.14. EST-1 is highly active in comparison to other zones. This pattern of nonspecific esterase is used as a standard pattern of *T. castaneum* adult in further studies with resistant population for comparative analysis.

2. Development of Pyrethroid Resistance

The selection of insects for resistance was initiated by ascertaining the dose which gave about 70% mortality of susceptible individuals in each generation by topical application method. The increased resistant level in the selected strain was measured through bioassay tests which were conducted in each successive generation in order to monitor the increase in resistance level. The results showed progressive increase in the resistance factor in successive generations of selection were x3 in the first, x6 in the second, x17 in third and fourth, x34 in fifth and then x370.5 in sixth generation. The strain thus developed by this method was designated as Deltamethrin-Resistant (R) strain. Further selection after six generation was discontinued since higher concentration of the insecticide posed problems in delivering applications.

In case of resistance population the chromosomal aberrations were grouped under various generations and it varied from polyploidy, dicentric, triradial, ring formation, bridge, breaks, wooly appearance, elongation, clumping, reciprocal translocation, interarm interchange, somatic reduction and corrosive effect.

The percentage of abnormal cells to be 8, 11, 13.33, 19, 38.66 and 73.66 in RTF₁, RTF₂, RTF₃, RTF₄, RTF₅, and RTF₆ generations respectively. From the observation, it is evident that the percentage of abnormal cells having chromosomal abnormality has been noticed to be increased with increased concentration of dose of the pesticide in different generations of resistant *Tribolium*. The increased trend in the percentage of aberrant cells and in the number of CAs per generation was similar as the results
shown in bioassay at different resistant generations. In all resistant generations, significantly higher effects were seen in highly resistant generation (RTF₆).

The native PAGE results indicate that the difference in esterase activity between resistant and susceptible strains is caused by high intensity of EST-1a and EST-1b bands. These bands were more intense in the RTF₆ strains. The activity of esterase isozyme in RTF₆ strain of *Tribolium* was more than that of the susceptible strain. The difference in intensity between populations indicates a quantitative mechanism of resistance involving over-production of the same enzyme that is present in susceptible insects. This is in accordance with the results of bioassay in which resistance ratio was greater in RTF₆ strain. This conclusion is supported by results of chromosomal investigation where increased abnormalities in chromosomes were observed in resistant strain.

With cytogenetical and isozymal markers some morphological mutants at pupal stage were also observed in RTF₆ generation. When compared to normal pupa they showed shiny transparent white skin, extra pair of antennae, enlargement of body segments, reduced size of elytra. Although the adults of RTF₆ generation were very good in flying ability in comparison to susceptible strains. They fly high than the susceptible strain.

The investigation on the correlation between the resistance ratio, chromosomal abnormality ratios and increase activity of esterase isozyme revealed the interrelation between these three different studies during the course of resistance in *Tribolium* and confirms the development of resistance in laboratory.

### 3. Inheritance studies

Studies on the inheritance of resistance to deltamethrin in the laboratory selected resistant strain of *T. castaneum* were also undertaken by making genetic crosses. These crosses were made between the susceptible (S) and resistant (R) individuals. In order to have unmated fresh adults for various crosses, the pupae were utilized for segregation of males and females.

Resistance to synthetic pyrethroid deltamethrin of the F₁ progenies of R x S and S x R crosses had fallen in the mid parent range (D of LC₅₀ for these crosses 0.895 and
0.847 respectively). When D value is compared between two different crossings in each strain, the difference is small. The value of SF X RM was less than RF X SM which indicated that the female was responsible for the resistance. This indicates that resistance was autosomal and inherited by incompletely dominant or partially dominant gene.

Data on enzymatic studies in inheritance analysis also supports the results of toxicity analysis that the resistance in *T. castaneum* against deltamethrin in the present investigation was derived by incomplete dominant genes. Morphological changes in pupa also showed that these genes are also associated with the phenotypic expressions responsible for body size and cuticle. Result of the present study revealed that inheritance of resistance to deltamethrin in *Tribolium castaneum* was derived by incomplete dominant genes and no sex linkage.

**Conclusion**

In conclusion (Fig. ) the present work demonstrates that the resistance towards deltamethrin in red flour beetle *Tribolium castaneum*, was developed successfully in laboratory after six generations of selection and supported by chromosomal and isozymal studies. Inheritance studies with resistant strains indicate incomplete dominant traits mechanism. Data from reciprocal crosses confirms the absence of maternal effects on resistant strains so the resistance is linked autosomally. Phenotypic mutants also support the resistance.
FUTURE PERSPECTIVES

Future work will focus on to identify genes coding for deltamethrin resistance. Results of our toxicology and non specific esterase profiling from the resistant *Tribolium* strain indicate that the ability of *Tribolium* to survive deltamethrin treatment is associated with enhanced esterase activity. Enhanced activity of esterases in insects has been found to contribute to resistance to several insecticide classes including the Ops (Mouches *et al.*, 1987; Gunning *et al.*, 1997; Zhu and Gao 1998; Pasteur *et al.*, 2001; Rossiter *et al.*, 2001; Zhou *et al.*, 2004; A. Y. Li *et al.*, 2007). However, it is still not clear whether the genetic basis of this esterase-mediated resistance is the result of multiple gene copies (i.e., gene amplification), or if the overproduction is caused by a change in the sequence of regulatory DNA outside the protein coding region that leads to increased protein synthesis.

As in our study we have also found chromosomal aberrations in different generations of resistant *Tribolium* during the course of selection and phenotypic mutants so further studies related to chromosome linkage to resistant genes will also provide better understanding of mechanism of resistance towards deltamethrin in this beetle.