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Development of resistance in *Tribolium castaneum*, Herbst (Coleoptera: Tenebrionidae) towards deltamethrin in laboratory

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Abstract **-** A strain of Tribolium castaneum (Herbst) was developed for resistance against deltamethrin, after six generation of selection from a laboratory susceptible strain, through topical application method. The selection was initiated at the dose 0.0004, which was increased during successive generations' upto 0.026 in sixth generation. The resistance ratio of selected strain was determined, and it was found to be 370.5 fold in sixth generation as compared to susceptible strain.

Index Terms*: Selection, resistance, deltamethrin, Tribolium castaneum*

I. INTRODUCTION

Tribolium castaneum Herbst (Coleoptera: Tenebrionidae) also known as the red flour beetle is a common, worldwide pest of stored product infesting many commodities. The pest contributes to the maximum spoilage of the stored grains at larval as well as the adult stages. Besides being abundant in the granaries they are also familiar sights in the households. The fact that flour beetle had been found well preserved in the Pharaonic tombs of the sixth dynasty, 2500 B.C., suggest that these beetles probably become pest as man learned to store seeds. In Hindu literature they have been referred as"Ghun". Infestation by these beetles results in an unappealing smell due to the secretion of benzoquinones from abdominal glands. This widely distributed pest species is extremely facile and tactable genetic model.

As a consequence of repeated chemical treatments, many cases of insecticide resistance have been detected in the genus Tribolium around the world. The first record date back to the end of 1959s and the first half of 1960s (Anonymous, 1958; Kumar and Morrison, 1965). In India the first report of pesticide resistance from a storage insect pest came in 1971, when T. castaneum was found to have developed resistance against malathion (Bhatia et al., 1971) and p, p' DDT (Bhatia, 1971).

The red flour beetle has developed resistance against almost all the insecticides commonly used against it such as phosphine, methyl bromide, organophosphates, pyrethroids and insect growth regulators (Anisur-Rahman and Shahjahan, 2000; Champ and Dyte, 1976; Collins, 1998; Dhaliwal and Chawla, 1995; El-Lakwah et al., 1996; Horowitz et al., 1998; Pacheco et al., 1994; Pimental et al., 2007; Werner, 1997; Zettler and Arthur, 1997)

Synthetic pyrethroid are being extensively used in controlling insect pests because of their characteristics like quick knock down, broad spectrum activity and low mammalian toxicity. In India pyrethroids were introduced in 1980 for the control of number of field pests (Bengston et al., 1983, Ramzan and Chahal, 1987). Resistance towards some commonly used synthetic pyrethroids, viz., permethrin, cypermethrin, deltamethrin and fenvalerate has already been reported (Dhingra et al., 1988; Mc Caffery et al., 1989, Saxena et al., 1989, 1992; Armes et al., 1992; ; Sinha, S. R. and Saxena, J. D. 1999; Padhee et al., 2002). The purpose of this study was to investigate the development of resistance in Tribolium castaneum towards a synthetic pyrethroid deltamethrin through bioassay method in laboratory.

II. MATERIAL & METHOD

A. Rearing of the test insects

The selection of deltamethrin resistant strain of T. castaneum was initiated with a population of composite nature, collected from two different regions of Agra (Cant and Dayalbagh). These field populations were mixed together in a common jar for rearing. Insects were reared on wheat flour containing 5% brewer's yeast at 30±2º C and 70% Rh as per the WHO standard method. The progeny of this culture was designated as parental strain.On emergence of appreciable number of adults, insects were sieved out and used for bioassay tests.

B. Selection Procedure

The selection of resistance was done by topical application method. The base line susceptibility to deltamethrin of the parental strain was evaluated by bioassay. On the basis of bioassay tests of the parental strain, the dose which killed 60-70 percent population was chosen for applying selection pressure. For obtaining the first generation the adults (300-400) of the parental strain were treated with this dose of insecticide. Then after 48 hrs live insects transferred to fresh medium for rearing. The concentration of insecticide was increased in the subsequent generations for rearing second, third, fourth, fifth and sixth

generation respectively. Bioassay tests were conducted in each successive generation in order to monitor the increase in resistance level. Thus, the selection for the deltamethrin - resistant strain was carried out for six generations. The susceptible strain (parental strain) was simultaneously maintained without any insecticidal exposure, for comparison.

C. Insecticide and Method of Bioassay

Technical grade deltamethrin (98.10%), obtained from Tagros Chemicals India Ltd., was used for the experiments. Bioassay of both susceptible and selected strains of T. castaneum was done by topical application method. Six to eight graded concentrations of the insecticide were prepared in acetone and applied at the rate of 0.5µl to each adult insect on the ventral surface of the mid thorax with the help of a micropipette (0.5-10µl). Each concentration replicated three times with 10 insects in each replicate. In control insects were treated with acetone only. The insects after the treatment with different concentrations of insecticide along with the control were kept at 30 ± 2^0 C and 70% Rh.. Mortality counts were taken after 48 hr of treatment and the data were subjected to probit analysis (Finney, 1972).

D. Statistical Analysis

Mortality data from insecticide treated strains were corrected for control mortality by Abbott's formula (Abbott's, 1925) transformed in logits and analysed by INDOSTAT (Software). Relative susceptibility of population to chemical was estimated by resistance ratio (RR = LC50 of Resistant strain/ LC50 Susceptible strain)

III. RESULTS AND DISCUSSION

The selection of deltamethrin-resistant strain of T. castaneum was initiated with the parental population. The susceptibility of parental population was determined through bioassay tests. The LC50 to deltamethrin of parental population was 0.00019%. Bioassay was carried out in order to ascertain the base-line toxicity of parental strains to deltamethrin.

The selection of insects for resistance was initiated by ascertaining the dose which gave 70% mortality of susceptible individuals in each generation. The survivors of each selection pressure were reared in fresh wheat flour medium .The details of dosages on insecticidal pressure used in different generations have been given in Table 1

Table 1: Doses of deltamethrin used in successive generations of selection of T. castaneum

Generation	LC_{70}
Parental	**0.0004**
First	**0.0005**
Second	**0.003**
Third	**0.007**
Fourth	**0.014**
Fifth	**0.026**

The increased resistant level in the selected strain was measured in each successive generation through bioassay tests. The results of the bioassay tests performed after successive generations showed that the LC50 value increased from 0.0002 % in the parental generation to 0.0006, 0.001, 0.003, 0.003, 0.0068, and 0.074 % in the first, second, third, fourth, fifth, and sixth generation respectively (Table 2, fig-1). The data showed that there was a progressive increase in the level of resistance to deltamethrin as was evident from the fact that the concentration of insecticide given as selection dose, increased from 0.0004 % for first generation to 0.026 % for sixth generation. The results showed that resistance at LC50 and its concurrent ratio increased in the order of > 6 in second >17 in third and fourth >34 in fifth >370.5 folds in sixth generation.

Table 2 -Toxicity of deltamethrin to the adults of *Tribolium castaneum* in the successive generations

Generation	Degrees of freedom	Heterogeneity χ^2	S. E. of b	Regression equation	LC_{50} (%)	Fiducial limits	Resistance ratio
P1	5	4.1356	0.1167	Y=10.1764+ 1.3959072x	**0.0002**	0.0002-0.0002	-
F1	6	8.4844	0.1285	Y=7.7646 + 0.8635396x	**0.0006**	0.0004-0.0009	**3**
F2	6	7.0091	0.1014	Y=8.1795+ 1.0928895x	**0.0012**	0.001 - 0.0015	**6**
F3	6	4.7559	0.1293	Y=8.1157+ 1.2600802x	**0.0034**	0.0027-0.0042	**17**
F4	7	12.1093	0.0866	Y=6.8378 + 0.7431464x	**0.0034**	0.0025-0.0045	**17**
F5	7	9.1103	0.0833	Y=6.9874 + 0.9163402x	**0.0068**	0.0053-0.0086	**34**
F6	7	4.4980	0.0699	Y=5.7296 + 0.6454653x	**0.0741**	0.0524-0.1047	**370.5**

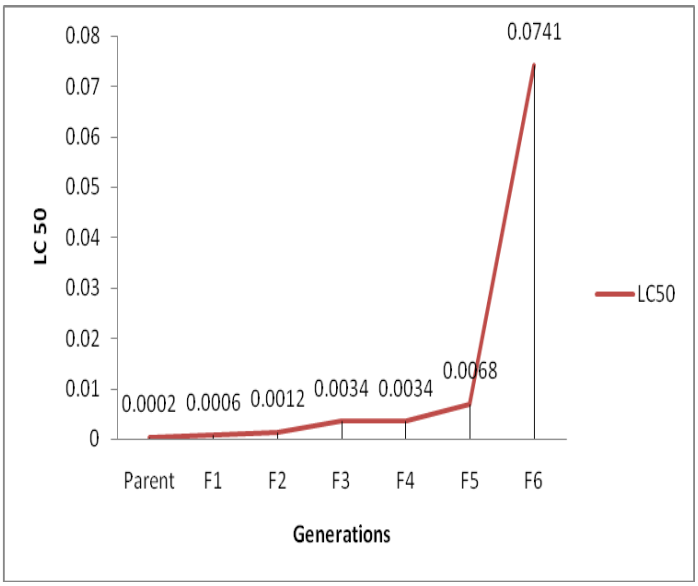

Fig 1: Showing increase in LC50 value in successive generations.

Thus the present investigation showed that topical method was appropriate to assess the high degree of resistance to deltamethrin in T .castaneum.

There are very few reports on laboratory selection for resistance to deltamethrin and other synthetic pyrethroids in the stored grain pests. Selection of S. oryzae in the laboratory over 25 generations using permethrin and deltamethrin resulted in resistance levels of x256 and x98, respectively (Heather, 1986). Misra (1995) selected a fenvalerate-resistant strain of T. castaneum in the laboratory having >210 fold resistance through seven generations of selection. It appeared from the above reports that in S. oryzae, a 98 – fold resistance to deltamethrin was acquired after 25 generations. Saxena and Sinha (1999) reported a high degree of resistance 891.94 fold to deltamethrin in Tribolium castaneum through treated flour method in six generations of selection. while in the present investigation with T. castaneum, 370.5 fold resistance was attained in six generations by topical application method for selection.

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