significant increase of 8% between 4 and 9°C. Similar studies carried out on ribonuclease and lysozyme show no apparent structural changes (≤ 0.2%, data not shown) within the same small, low temperature range.

This study thus indicates that temperature-dependent cell changes, even for small temperature alterations, cannot be altogether ignored. It is a common crystallographic practice to collect diffraction data at low temperature as it enhances the crystal’s lifetime in the beam and thereby increases the quality of the measurements. The data collected at different temperatures are often merged with the basic presumption that the structure remains largely unchanged and the data are isomorphous. Although true in most cases, this may not be universal as observed in the present case. Therefore, a degree of caution is necessary while merging data collected at different temperatures. These observations also underline the special features of the CaBP molecule wherein functional viability critically depends on large conformational changes.

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Role of carboxylesterase in relation to pyrethroid resistance towards *Helicoverpa armigera* (Hubner) (Lepidoptera: Noctuidae) in Tamil Nadu

*Helicoverpa armigera* is a serious pest of many crops in India including cotton. In India, large-scale failure to control this pest with insecticides was first recorded in the major cotton-growing region of Andhra Pradesh in 1987 (ref. 1). Synthetic pyrethroid is one of the latest chemical insecticides in the series of insecticide molecules released in the Indian market in 1982. Appearance of pyrethroid resistance in *H. armigera* has been reported in Andhra Pradesh1-3, Tamil Nadu4-5, Delhi, Punjab and Haryana6-9. Three types of pyrethroid resistance mechanisms were identified in *H. armigera*7, i.e. reduced cuticular penetration, decreased nerve sensitivity and enhanced metabolic detoxification. Among these, esterases-mediated metabolic detoxification plays an important role in the detoxification of pyrethroid insecticides in *H. armigera*10 and other insect pests11-12. However, the relationship between pyrethroid resistance and carboxylesterase in the Tamil Nadu populations was not known and this prompted the present investigation. This information could be relevant to management of *H. armigera* control programmes and might help in the development of strategies to overcome resistance to pyrethroids.

Four different populations of *H. armigera*, namely pyrethroid susceptible, fenvalerate-resistant, cypermethrin-resistant and deltamethrin-resistant were reared on semisynthetic diets13. The resistant individuals were collected from insecticidal selection pressure areas of Tamil Nadu under cotton cultivation. Pyrethroid-susceptible larvae were obtained from Tamil Nadu Agricultural University, Coimbatore. The F1 generation larvae were used for insecticide bioassay and carboxylesterase enzyme studies.

Bioassays were conducted on third instar larvae (30–40 mg weight/larva) using a procedure based on the standard *Heliothis* susceptibility test recommended by the Entomological Society of America14. Serial dilutions of technical grade pyrethroid insecticides (fenvalerate, cypermethrin and deltamethrin from Spic Science Foundation, Chennai) in analytical grade acetone were prepared and a 1.0 µl drop dispensed on to the thoracic dorsal of individual larvae using a Hamilton repeating dispenser fitted with a 50 µl syringe. Control larvae were treated with acetone alone. In each bioassay, 36 larvae were treated with different concentrations of insecticides. In assays including the synergist DEF was applied as 1.0 µl drops to the mesothorax 15–20 min prior to the insecticide treatment at a rate of 50 µg/larva. After treatment, each test larva was held individually at 25 ± 2°C with adequate

<table>
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<th>Unit cell parameters (Å)</th>
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then washed in ice-cold 20 mM phosphate buffer (pH 8.0) and homogenized (500 mg/2 ml buffer) using a glass homogenizer in the same buffer. The homogenate was centrifuged at 10,000 g at 4°C for 15 min. Supernatant was used for both carboxylesterase and its DEF inhibition assays. The carboxylesterase activity was spectrophotometrically assayed by the method of Van Asperen and Devonshire with suitable modifications. A standard reaction mixture, i.e., 100 µl of enzyme, 125 µl of α/β-naphthyl acetate substrate solution (1 mM) and 1.15 ml of 20 mM phosphate buffer (pH 8.0) was incubated for 30 min at room temperature. The reaction was stopped by the addition of 125 µl of coupling reagent (mixture of Fast blue B salt and SDS) and the absorbance was taken after 15 min at 605 nm using a spectrophotometer. The in vitro inhibition of DEF on carboxylesterase was evaluated by incubating the enzyme and the inhibitor, at the appropriate molar concentration (8.2 × 10^{-5}) for 10 min before the addition of substrate. The percentage inhibition was calculated by comparing the optical density (OD) of the inhibited samples with that of uninhibited samples. The amount of naphthol released by carboxylesterase was calculated using the standard graph. The amount of protein was measured by the method of Markwell et al.

The pyrethroid resistance levels of H. armigera are shown in Figure 1a. Compared with susceptible individual, the resistant individuals had high tolerance (fenthion 38-fold, cypermethrin 30-fold and deltamethrin 14-fold) to selected insecticides. This tolerance to insecticides could be attributed to the presence of high esterase activity in this insect. This fact was also supported by Ishaaya and Casida. In pyrethroid-resistant individuals, pre-treatment with the synergist DEF compound showed a considerable synergistic effect (2.1–5.2-fold), suggesting that carboxylesterase-mediated metabolic mechanism is most likely a component in the pyrethroid resistance of H. armigera populations in Tamil Nadu.

The carboxylesterase activity and its DEF inhibition of fifth instar larval midgut homogenates of susceptible and resistant individuals towards both α and β-naphthyl acetate as substrate was estimated (Figure 1b and c). These

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**Figure 1.** Resistance and synergistic levels (a), carboxylesterase activity (b) and DEF inhibition (c) on different populations of Helicoverpa armigera. Resistance Factor (RF) = LD_{90} of resistance individual/LD_{90} of susceptible individual. Synergistic Factor (SF) = LD_{90} without DEF/LD_{90} with DEF pre-treatment.

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food. Mortality was recorded 48 h after treatment. Larvae were regarded as dead if unable to move in a co-ordinated manner when prodded by a blunt probe. Control mortality was rare, but where necessary, corrections were made using Abbott's formula. Data were analysed by probit analysis.

The fifth instar larvae of H. armigera were dissected in 1.15% KCl solution and midguts split longitudinally, and their gut contents removed. They were
results revealed that pyrethroid-resistant individuals had higher amounts of carboxylesterase activity (1–9-fold) than susceptible individuals. These findings suggest that the carboxylesterase catalysing the hydrolysis of the two substrates was present at a higher rate in the resistant than susceptible individual. Between two substrates tested, α-naphthyl acetate showed higher enzyme activity than β-naphthyl acetate in both the individuals. In vitro inhibition of α- and β-naphthyl acetate hydrolysing carboxylesterase in susceptible and resistant larval midgut revealed that the DEF is a potent inhibitor for carboxylesterase and sensitivity of this enzyme was higher in resistant than susceptible individuals. This was earlier supported by Riskallah. However, the variation of inhibition among the different resistant populations of H. armigera may be due to the sensitivity of enzyme or resistance/susceptible status of the insect.

The wide variety of enhanced metabolic route found in the resistant population when compared with the susceptible population could be as a result of exposure to various insecticides used for controlling this pest in the field conditions. Thus, the results presented here clearly show that the levels of carboxylesterase enzyme are higher in the resistant individual when compared to the susceptible individual and these data obviously indicate the close correlation between pyrethroid resistance and enhanced levels of carboxylesterase activity. It can be concluded from this study that pyrethroid-resistant populations of H. armigera in Tamil Nadu have considerable levels of tolerance to pyrethroid insecticides and this tolerance could be due to the higher activity of carboxylesterase enzyme in this insect. However, further detailed studies on the seasonal changes of pyrethroid resistance as well as carboxylesterase activity in H. armigera in the Tamil Nadu ecosystem are being carried out.


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