SUMMARY AND CONCLUSIONS
SUMMARY AND CONCLUSIONS

Detoxifying enzymes, Carboxylesterase, GST and Monoxygenases are mainly responsible for the insecticide resistance in *H. armigera* which is a notorious cotton pest distributed widely over Africa, Asia, Australia and Europe. Because of its wide host range and nature of damage, it has attained the status of national pest, causing enormous crop losses of US$ 300-600 million on cotton and pulses (King, 1994). Resistance to synthetic pyrethroid insecticide has been a major cause of failure to control the cotton bollworm in Australia (Gunning *et al.*, 1984), in Thailand and Turkey (Ahmad and McCaffery, 1988) in India (Dhingra *et al.*, 1988; McCaffery *et al.*, 1989) and in Indonesia (McCaffery *et al.*, 1991). At this juncture, to identify the insecticide resistance mechanism, it is essential to have the information on carboxylesterase from midgut of *H. armigera* with regard to its purification, characterisation and properties.

The spectrophotometric enzyme assay in the American bollworm, *H. armigera* collected from Akola region was studied during two cropping seasons 1998-1999 and 1999–2000. Low levels of pyrethroid resistance in the starting season were observed while from December to March cypermethrin and fenvalerate resistance trend was in increasing pattern in both the seasons with little fluctuations. The reverse pattern of GST activity in general was followed in both the cropping seasons where as increased levels of GST at the starting of seasons and decreased levels at the later half of season were observed. Thus the monthwise studies related to resistance revealed that high esterase activity coincided with the low glutathione S-transferase levels and vice versa, and pyrethroid resistance frequenceties of cypermethrin and fenvalerate were observed to be present throughout the year which is related to esterase activity.

Carboxylesterase and glutathione S-transferase activity towards α-naphthyl acetate and CDNB respectively was detected in various stages of life cycle of
*H. armigera* and was found highest in the 2 days old fifth instar larvae. Both enzymes were purified and in the column chromatography, maximum carboxylesterase and glutathione S-transferase activity were found in bound fraction. Also the studies revealed that the glutathione S-transferase showed a single isozyme after coomassie blue staining of molecular weight 30 kd and nine carboxylesterase isozymes showed a molecular weight range of 19 to 100 kd after substrate staining. The carboxylesterase isozymes having molecular weight ranging from 64 to 100 kd were found only in field collected resistant *H. armigera* strain. Therefore these isozymes are responsible for insecticide resistance.

The ion exchange chromatography using DEAE-Sephadex was the best one among salt precipitation, gel filtration, electrophoresis, dialysis etc. While studying enzyme parameters, carboxylesterases showed maximum enzyme activity at its optimum pH 7.6, temperature 47°C and substrate concentration 0.3 mM. Another important and useful factor for studying the nature and structure of enzyme is the effect of reducing and denaturing agents. There is no effect of 10% SDS and 1 mM 2 mercaptethanol on carboxylesterase activity. But above this concentration the carboxylesterase activity get reduced.

**CONCLUSIONS**

1) In Akola region *Helicoverpa armigera* populations have developed substantial levels of resistance against pyrethroids (cypermethrin and Fenvalerate), cyclodiene (endosulfan) and organophosphate (profenophos) insecticides.

2) In both the years (1998-1999 and 1999-2000) low levels of pyrethroid resistance were observed in the starting of season from August to November, while from December to March, the resistance trend was high.
3) The endosulfan resistance profiles were similar during 1988 to 2000. The frequency was low at the starting of season and at the end of season and increased with slight variations in October, November, December and January.

4) Profenophos is the best synergist with cypermethrin showing suppression of cypermethrin resistance indicating the predominance of esterase mediated mechanism of resistance in Akola population of *H. armigera*.

5) The enzyme assay data from 1998 to 2000 indicated the high esterase levels were generally coincided with low GST activity and vice versa showing esterase mediated pyrethroid resistance in *H. armigera*.

6) Highest midgut carboxylesterase activity showed high catalytic activity than carboxylesterase from whole body, haemolymph, cuticle, fat bodies indicating predominant metabolic function of midgut. Similar GST activity was found to be highest in fat bodies of *H. armigera* indicating the major site of metabolism of organophosphate insecticides.

7) Increased carboxylesterase and GST titres of each moult during larval development stages from 3rd to mid 5th instar was correlated with the increased rate of food consumption and larval growth.

8) DEAE-Sephadex is the best anion exchanger for the purification of carboxylesterase from midgut of *H. armigera* showing highest enzyme activity on the basis of its substrate and inhibitor specificities, than purification by DEAE-Cellulose ion exchange chromatography, gel filtration and electrophoresis.

9) Electrophoretic pattern of carboxylesterase showed maximum 9 isozymes having molecular weight ranging from 17 kd to 100 kd. 34 kd and 45 kd carboxylesterase bands are common with slight variations in susceptible as well as resistant strain
of H. armigera. The extra bands ranging from 64 kd to 100 kd are responsible for insecticide resistance in H. armigera. Hence the resistant population showed maximum isozyme bands than susceptible H. armigera population.

10) Heat and acid treatment of carboxylesterase showed thermostability and relatively acid stability indicating glycoprotein nature. It's treatment with sodium metaperiodate resulted in complete loss of enzyme activity suggesting the presence of carbohydrate moieties.

11) The carboxylesterase activity was found to be maximum at its optimum pH 7.6 with optimum substrate concentration of 0.3 mM as the gut pH of H. armigera is alkaline. The substrate affinity was maximum at its optimum temperature 47° C with enzyme quantity 10 µl. It indicated that the enzyme esterase has highest affinity for α-naphthyl acetate at these conditions to give product α-naphthol.

12) In-vitro treatment by carbaryl and paraoxon with carboxylesterase in H. armigera at 10 µM and 1 µM respectively resulted in best inhibition reaction. They have been implicated in detoxification of carbamate and organophosphate. The presence of serine containing esteratic site was confirmed by the fact that the esterase activity was inhibited by organophosphate compounds.

13) Absence of cystein was confirmed in the active site of carboxylesterase as there is no effect of 5 % SDS and 1mM 2 mercaptoethanol on carboxylesterase activity.