



CHAPTER – SEVEN
GENERAL SUMMARY AND CONCLUDING
REMARKS

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1. General Summary :

From the study of general survey of the literature on the insect study it seems that scientists have been worked majority of insect diversity and control of economically important pests. Armyworm *Mythimna separata* is economically important polyphagous pest on graminaceous crops like maize, sorghum, sugarcane, rice, finger millet and wild grasses. In general are knowledge of the proteins and proteases study during development of *M. separata* is rather scanty. Hence in the present study efforts have been made to study the proteins and proteases during embryogenesis, larval growth, metamorphosis and adult development of the armyworm, *Mythimna separata*.

The embryogenesis period of *Mythimna separata* is of 5 days. During embryogenesis gradual increase in the amount of proteins from 1 to 4 day eggs indicates synthesis of new proteins which provides the structural components to the developing embryo.

The partial characterization of proteases during embryogenesis revealed the maximum activity at pH 3 (Cathepsin D like enzyme), pH 4.5 (Cathepsin B like enzyme), pH 7 (Neutral protease), pH 7.8 (Chymotrypsin like enzyme) and pH 8.2 (Trypsin like enzyme), temperature 37^oc, 30 min incubation time, 1% enzyme concentration and 2% substrate concentration (acidic and alkaline proteases) and 5% substrate concentration (Neutral protease).

The high proteases activity during early embryonic development suggests the active role of these enzymes in degradation of yolk proteins and supply of amino acids for protein anabolism during early

embryogenesis of *Mythimna separata*. The low enzyme activity during later period of embryogenesis indicates the decrease of catabolism role of these enzymes.

The larval growth period of *Mythimna separata* is of 25 days. During larval growth gradual increase in the amount of proteins from 1 to 17. day larvae (fourth instar) indicates synthesis of new proteins, which provides structural components to the somatic growth of developing larvae. After 17 days the protein content remained relatively constant upto 25 day larvae, which may be due to the less utilization of protein for the structural elements and storage of proteins in the fat body which are utilized during metamorphosis.

The partial characterization of proteases during larval growth revealed the maximum activity at pH 3.2 (cathepsin D like enzyme), pH 4.2 (cathepsin B like enzyme), pH 7 (Neutral protease), pH 7.9 (chymotrypsin like enzyme) and pH 8.1 (Trypsin like enzyme), temperature 37⁰c, 30 min incubation time, 1% enzyme concentration and 2% substrate concentration (acidic and alkaline proteases) and 5% substrate concentration (neutral protease).

Gradual increase in proteases activity of *Mythimna separata* from 1 to 2 day larva suggests the degradation of yolk proteins and ingested proteins after hatching for growth of larva. Gradual decrease in enzyme activity from 2 to 4 day larvae suggests depletion of yolk proteins. Sharp increase in enzyme activity from 4 to 9 day and high activity in 6 day larvae suggests the active feeding stage of the larva and degradation of proteins. Sharp fall in enzyme activity from 6 to 7 day and decrease from 7 to 10 day larvae suggests the low degradation of protein and synthesis of proteins. Gradual increase in enzyme activity from 12 to 13 day the enzyme activity remained constant up to 17 day indicates the larva enters in to the third moult and protein synthesis during this period. Slow but

low increase in enzyme activity from 17 to 20 day larvae indicates feeding stage of larva with protein catabolism. Decrease in enzyme activity from 20 to 23 day larvae indicates the synthesis and storage of proteins in the haemolymph and fat body. Slow but low increase in enzyme activity from 23 to 25 day larvae indicates slow feeding larval stage with very very low protein catabolism.

The metamorphosis period of *Mythimna separata* is of 10 day. During metamorphosis gradual decrease in the amount of proteins from 1 to 4 day pupae indicates the histolysis of most of the larval organs. Gradual increase in proteins from 4 to 6 day pupae indicates gradual increase in protein synthesis and utilization of such new proteins as structural components for development of imaginal organs of adult. After 6 day the amount of proteins remains constant which indicates completion of protein synthesis and gradual histogenesis. In later stages of metamorphosis the amount of proteins in female pupae was more as compared to male, indicates storage of proteins (vitellogenin) in the fat body which are utilized during oogenesis.

The partial characterization of proteases during metamorphosis revealed the maximum activity at pH 3.4 (cathepsin D like enzyme) pH 4.6 (cathepsin b like enzyme), pH 7 (Neutral protease), pH 7.7 (chymotrypsin like enzyme) and pH 8.4 (Trypsin like enzyme), temperature 37⁰c, 30 min incubation time, 1% enzyme concentration and 2% substrate concentration (acidic and alkaline proteases) and 5% substrate concentration (Neutral protease).

Gradual increase in protease activity during early metamorphosis from 1 to 6 day pupae of *Mythimna separata* suggests histolysis in which active role of these enzymes is degradation of proteins. Gradual decrease in protease activity during middle period of metamorphosis from 6 to 8 day pupae indicates histogenesis in which synthesis of protein occurs.

Gradual increase in enzyme activity from 8 to 10 day pupae suggests degradation of some proteins for the synthesis of secretory proteins in male and yolk proteins in female adult.

The adult developmental period of *Mythimna separata* is of 9 day. During adult development in case of fed male moth gradual increase in the amount of proteins from 1 to 3 day adults indicates the synthesis of proteins which are associated with reproductive functions like spermatogenesis and secretion of accessory glands. Decline in proteins from 3 to 6 day male adults indicates utilization of proteins during mating. Decrease in proteins from 6 to 9 day male adult may be due to inactive physiological state of male. In case of fed female moth gradual increase in proteins from 1 to 4 day adults indicates synthesis of yolk proteins (vitellogenin) in the fat body. which are afterword translocated to the developing oocytes decline in the protein from 4 to 7 day female adults suggests gradual oviposition. Decrease in proteins from 7 to 9 day female adults may be due to inactive physiological state of female.

The partial characterization of proteases during male adult development revealed the maximum activity at pH 3.1 (cathepsin D like enzyme), pH 4.3 (cathepsin B like enzyme), pH 7 (Neutral protease), pH 7.6 (chymotrypsin like enzyme) and pH 8.3 (trypsin like enzyme), temperature 37⁰c, 30 min incubation time, 1% enzyme concentration and 2% substrate concentration (acidic and alkaline proteases) and 5% substrate concentration (Neutral proteases).

The partial characterization of proteases during female adult development revealed the maximum activity at pH 3.3 (cathepsin D like enzyme) and pH 4.4 (cathepsin B like enzyme), pH 7 (Neutral protease), pH 7.8 (chymotrypsin like enzyme), pH 8.5 (trypsin like enzyme), temperature 37⁰c, 30 min incubation time, 1% onzyme concentration and

2% substrate concentration (acidic and alkaline proteases), 5% substrate concentration (Neutral proteases.)

During adult development in case of male maximum activity of acidic, neutral and alkaline proteases was observed in 1 day adults of *Mythimna separata*. This suggests degradation of stored proteins in the fat body and utilization of free amino acids for the synthesis of secretory proteins, which are released in the haemolymph and taken up by the accessory glands. Steady decrease in enzyme activity from 1 to 9 day adults suggests gradual decrease in protein degradation in the fat body minimum activity in 9 day adults indicates less role of proteases during late period of adult development.

In case of female gradual decrease in enzyme activity of acidic, neutral and alkaline proteases from 1 to 9 day female adults of *Mythimna separata* was observed. This suggests gradual degradation of stored proteins for the synthesis of yolk proteins in the fat body steady decrease in enzyme activity from 1 to 9 day adult indicates less role of proteases after egg laying in the fat body. Enzyme activity in 9 day adult indicates less role of proteases after egg laying in the late period of adult development.

While concluding the present work author is conscious of the fact that though this work is contributing to the basic information on proteins and kinetic properties of protease enzymes and their behaviour during different developmental stages of *Mythimna separata* much remains to be studied. So this, attempt is no way complete. Since armyworm *Mythimna separata* is economically important polyphagus pest of graminaceous crops and wild grasses, it is found in cultivated and uncultivated areas, the complete eradication of the pest is not possible. Hence the study and laboratory rearing of parasitoids of the armyworm as biological agents will help to control this most serious pest.

2. Concluding Remarks :

A) Study of proteins :

- a) During embryogenesis, larval growth and metamorphosis synthesis of new proteins is correlated with development of embryo, larva, and pupa which provides structural proteins.
- b) In male adults increased amount of protein is associated with the reproductive functions like spermatogenesis, secretion of accessory glands and internal development. While in later period of development of adults the protein content is decreased which indicates utilization of proteins for energy production. In later period this decline of protein is also related to the inactive physiological state of male.
- c) Increased amount of protein in female adults is due to the synthesis of yolk proteins in fat body. The decline of protein in the later period of development of female adults suggests gradual oviposition and further decline of proteins is related to the inactive physiological state of female.

B) Study of proteases activity :

- a) High activity of proteases during early period of embryogenesis is related to the degradation of yolk proteins and supply of amino acids for protein anabolism. The low activity of protease during later period of embryogenesis of *M. Separata* shows decreased catabolic role of enzyme.
- b) Decline in enzyme activity on 4, 10, 17 and 23 day of development of larva indicates first, second, third and fourth larval moults and fifth larval pupal moult respectively.

- c) Gradual decrease in protease activity on 6 to 8th day of metamorphosis indicates histogenesis in which protein synthesis occurs. Whereas gradual increase in protease activity from 8 to 10th day suggests degradation of some proteins in male and yolk proteins in female adults.
- d) In male steady decrease in enzyme activity from 1 to 9 day adults suggests gradual decrease in protein degradation in the fat body. Minimum activity in 9 day adults indicates less role of proteases during late period of adult development.

In female steady decrease in enzyme activity from 1 to 9 day adult indicates less role of proteases after egg laying in the fat body. Enzyme activity in 9 day adult indicates less role of proteases after egg laying in the late period of adult development.