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Study of proteases activity during larval development of *Chilo partellus* (Swinhoe).

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Abstract
Chilopartellus is one of the most serious maize stem borer.The larval developmental period is of 28 days. The acidic, Neural and alkaline proteases activity during larval development of *Chilopartellus* have been studied. The partial characterization of acidic alkaline and neutral proteases during larval development of *Chilo partellus*has been also studied.The Sharp increase in acidic, alkaline, neutral proteases activity was noted on the onset of each instar. Then gradual decrease in enzyme activity was observed at the end of each instar. The physiological significance of proteases during larval development of *Chilopartellus* is discussed.

Keywords: Proteases, Larval development, *Chilopartellus*.

INTRODUCTION

The maize stem borer *Chilopartellus* (Lepidoptera-Crambidae) is very serious pest in India. In early stages of crop growth this insect larvae attacks the leaves and central shoot. The larvae feed and damage the inner vascular tissue of stem to form elongated tunnel leading to great loss in yield.

The newly hatched larva of *Chilopartellus*is tiny, cylindrical and pale brown in colour. The first instar larvae feed on young tender leaves near the base of whorl. From the second instar onwards the larvae feeds and damage inner vascular tissue of the stem to form elongated tunnel. The larva of *Chilopartellus*is caterpillar type having mandibulate type of mouthparts, three pairs of jointed thoracic appendages with claws and five pairs of prolegs. The larval developmental period is of 28 days with five instars which lasts for 4, 6, 7, 6 and 5 days respectively. The moulting is observed in 4, 10, 17, 23 and 28-day larvae. The larval growth was computed from mean time of egg hatching to the pupal state.

Within the egg as a whole there is no growth. Only a kind of inner growth occurs in the egg representing a transformation of stored yolk components into active protoplasm. The growth during insect development is restricted to the larval development and during this feeding period there will be deposited all the mass necessary for final adult (1). In insects the larval form is highly variable in structure and is adapted for life in different environments. The entire postembryonic development of insects is punctuated by moults during which the old cuticle is replaced by a new one. The food requirements for normal growth and development are essential. A number of substances, particularly amino acids and vitamins, are essential for any development. The balance between different constituents is also important (2). Dipteran larvae are known to accumulate lipids, glycogens and proteins during development (3). The reasons for storing these constituents in larva is fairly obvious, this material later on can be used during metamorphosis.

Proteins provide chief structural elements of muscles, glands and other tissues. A certain amount of protein is stored in fat of body and much is deaminated or converted into carbohydrate or fat body and used for energy production (3). Insect larvae depend on uptake and utilization of exogenous proteins for both growth and development. The ingested proteins must be broken down into amino acids before being absorbed. The proteolytic enzymes are responsible for the degradation of proteins (4).

The developing insect larva, in contrast to egg and pupa, depends on a continuous supply of food of energy production and growth. The major mechanism underlying the growth in larval development is protein synthesis, which is of course directly related to both amino acids and proteins in diet.

Few studies have been carried out in proteases during larval development of various insects.Purification and characterization of trypsin like proteinase from midgut of larva hornet *Vespa orientalis*studied by Hagenmaier (5). He reported that, hornet protease in homologous with the other serine proteases. According to experimental results the pH profile, temperature activity, the mode of action as shown by inhibitors and the cleavage specificity on β-chain of insulin are all quiet similar to mammalian trypsin. Ahmad *et al.* (6) studied alkaline protease in the larvae of the armyworm, *Spodopteralitura.* He reported that the alkaline proteases activity in the gut was found to increase with the onset of pupation. Eguchi and Iwamoto (7) studied the alkaline proteases in the midgut tissue and digestive fluid of silkworm, *Bombyxmori*. According to their results, the proteolytic enzymes in the alimentary canal care predominantly localized in the gut contents. The proteases, however, are somewhat different in thermo stability and in other properties of enzymes. The bound form of tissue proteases may be a source of digestive fluid protease. Proteolytic activity in the digestive fluid of larvae of *Trichoplusiani.*was investigated by Pritchett *et al.* (8). They observed both tryptic and chymotryptic activities in the digestive fluid of *Trichoplusiani*. larvae. Baker (9) studied the properties of midgut proteases in larvae of *Attagenusmegatoma*. According to him the midgut protease activity exhibited high temperature and alkaline pH optima. The total protease levels declined in starved condition but increased after 48 hr of feeding. Characterization of an acidic

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proteinase from the posterior midgut of *Rhodniusprolixusstal*was carried out by Houseman &Downe (10). They reported that the *Rhodniusprolixusstal*contained Cathepsin D in the posterior midgut to breakdown ingested blood proteins. The presence of Cathepsin B and lysosomalcarboxypeptidase B, which have also been detected in the posterior midgut of *R. prolixus*and other blood sucking Hemiptera.Christeller*et al.* (11) studied the partial purification and characterization of the major midgut proteases of grass grub larvae, *Costelytrazealandica*. They reported that trypsin can be considered the major target in attempts to interfere with protein digestion. Meenakshisundaram&Gujar (12) worked on alkaline proteases from some Lepidopteran larvae. They reported that the lepidopteran larvae possess alkaline proteases in the midgut region which are almost having similar properties with respect to the optimum conditions of pH, temperature, time and differing in their substrate and inhibitor specificity which probably make the insect to thrive on certain selected host plants utilizing different plant proteins qualitatively and survival of herbivore insect.

However the information on proteases in larval development of *Chilopartellus* is rather scanty. There exists a lacuna in the field of proteases during larval development of *Chilopartellus.* Therefore present study attempts to provide information on proteases during larval development of *Chilopartellus,*which may be useful in controlling this most serious pest of maize.The results are discussed in regards to changes undergone during larval development of *Chilopartellus.*

MATERIAL AND METHOD

The culture of *Chilopartellus* was maintained in the laboratory on natural food of cut pieces of fresh maize stems. Egg masses were put in tender maize shoots for hatching. The young larvae were trapped in tender maize shoots. The later instars were inserted in cut pieces of fresh maize stems. The pupae were collected and kept in glass jar lined with white paper at its inner side for emergence of moths. The eggs were laid in bunches and larvae hatched within six days.

Larval stages for study

The newly hatched larva of *Chilopartellus*is tiny, cylindrical and pale brown in colour. The first instar larvae feed on young tender leaves near the base of whorl. From the second instar onwards the larvae feeds and damage inner vascular tissue of the stem to form elongated tunnel. The larva of *Chilopartellus*is caterpillar type having mandibulate type of mouthparts, three pairs of jointed thoracic appendages with claws and five pairs of prolegs. The larval developmental period is of 28 days with five instars which lasts for 4, 6, 7, 6 and 5 days respectively. The moulting is observed in 4, 10, 17, 23 and 28-day larvae. The larval growth was computed from mean time of egg hatching to the pupal state. In case of larval developmental stages from 1-day to 28-day were taken for study of proteases activity.

The larvae were isolated, cleaned with distilled water weighed and homogenized in chilled water. The homogenates were diluted with chilled water so as to get various concentrations for proteases activity.

METHOD

In order to perform the experimental part of present investigation the biochemical assay of CathepsinBlikev and cathepsin D like enzymes was carried out according to method of Mycek(13). The biochemical assay of Trypsin like and Chymotrypsin like enzymes was carried out according to method of Rick (14). The biochemical assay of neutral protease wascarried out according to method of Wilkes and Prescott (15).

RESULTS

Acidic protease activity

Cathepsin D like enzyme activity

Change in the Cathepsin D like enzyme activity of *Chilopartellus* during larval growth were shown in Fig.1. Gradual increase in enzyme activity from 1 to 2-day larvae and decrease from 2 to 4-day larvae was observed. After 4-day it remains constant upto 5-day larvae. Sharp increase in enzyme activity from 5-day to 6-day, sharp decrease from 6-day to 7-day and gradual decrease from 7-day to 10-day larva was observed. After 10-day slight increase in enzyme activity from 1- to 11-day larvae and decrease from 11 to 17-day larvae was observed. Increase in enzyme activity from 17 to 20-day larvae and decrease from 20 to 23-day larvae was observed. From 23 to 28-day larvae the enzyme activity was almost constant. Maximum activity was observed in 6-day and minimum in 20-day larvae.

Cathepsin B like enzyme activity

Change in the CathepsinB like enzyme activity of *Chilopartellus* during larval growth were shown in Fig.1.Changes in Cathepsin B like enzyme activity of *Chilopartellus*during larval growth were shown in Fig.1 Gradual increase in enzyme activity from 1 to 2-day and decrease from 2 to 4-day larvae was observed. Sharp increase in enzyme activity from 4 to 6-day, sharp fall from 6 to 7-day and gradual decrease from 7 to 10-day larvae was observed. Sharp increase in enzyme activity from 10 to 12-day larvae, sharp fall from 12 to 13-day larvae was observed and then it remained almost constant up to 17-day larvae. Slow increase from 17 to 19-day larvae, slow decrease from 19 to 23-day larvae, slow and low increase from 23 to 24-day larvae and sharp fall from 24 to 25-day larvae was observed. Then it remained constant upto 28-day larvae. Maximum activity was observed in 6-day and minimum in 28-day larvae.

Neutral protease activity

Changes in neutral protease enzyme activity of *Chilopartellus*during larval growth were shown in Fig. 2. Gradual increase in enzyme activity from 1 to 2-day and sharp decrease from 2 to 3-day larvae was observed after 3-day the enzyme activity remained constant up to 4-day larvae. Sharp increase in enzyme activity from 4-day and sharp decrease from 6 to 7-day larvae was observed. After 7-day the slow decrease in enzyme activity was observed up to 13-day larvae. Gradual increase in enzyme activity from 13 to 15-day larvae, decrease from 15 to 17-day larvae, gradual increase from 17 to 20-day larvae and decrease from 20 to 23-day larvae was observed. Gradual increase in enzyme activity from 23 to 24-day larvae and gradual decrease from 24 to 27-day larvae was observed. From 27-day it remained constant upto 28-day larvae. Maximum activity was observed in 6-day and minimum in 28-day larvae.

Alkaline protease activity

Chymotrypsin like enzyme activity

Changes in Chymotrypsin like enzyme activity of *Chilopartellus*during larval development were shown in fig. 3.Gradual increase in enzyme activity from 1 to 3-day and decrease from 3 to 4-day larvae was observed. Sharp increase in enzyme activity from 4 to 6-day larvae and sharp decrease from 6 to 10-day larvae was observed. Gradual increase in enzyme activity from 10 to 15-day larvae, gradual decrease from 15 to 17-day larvae, gradual increase from 17 to 19-day larvae and gradual decrease from 19 to 20-day larvae was observed. The enzyme activity remained almost constant up to 23-day larvae. Gradual increase in enzyme activity from 23 to 25-day larvae and gradual decrease from 25 to 28-day larvae was observed. Maximum activity was observed in 6-day and minimum in 22-day larvae.

Trypsin like enzyme activity

Changes in Trypsin like enzyme activity of *Chilopartellus*during larval growth were shown in Fig. 3.Steady decrease in enzyme activity from 1 to 4-day larvae, sharp increase from 4 to 6-day larvae and sharp decrease from 6 to 10-day larvae was observed. Gradual increase in enzyme activity from 10 to 16-day, gradual decrease from 16 to 17-day, gradual increase from 17 to 22-day larvae and gradual decrease from 22 to 23-day larvae was observed. Gradual increase in enzyme activity from 23 to 24-day larvae and gradual decrease from 24 to 28-day larvae was observed. Maximum activity was observed in 6-day and minimum in 28-day larvae.

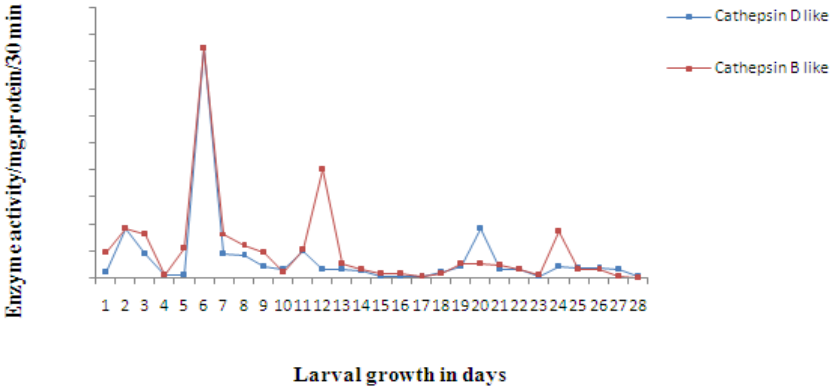

Fig 1. Acidic protease activity during larval development of Chilopartellus (Swinhoe).

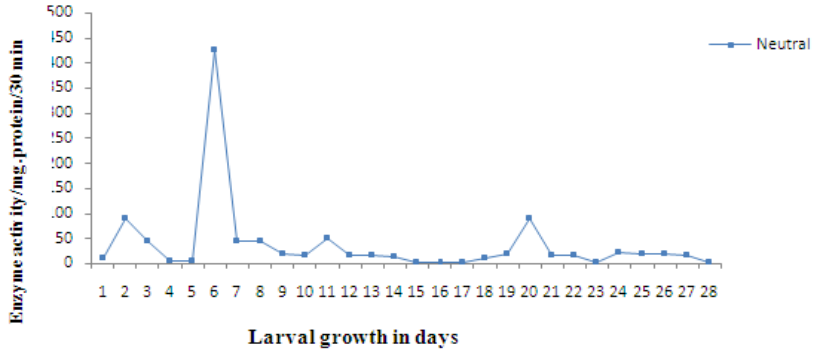

Fig 2.Neutral protease activity during larval development of Chilo partellus (Swinhoe).

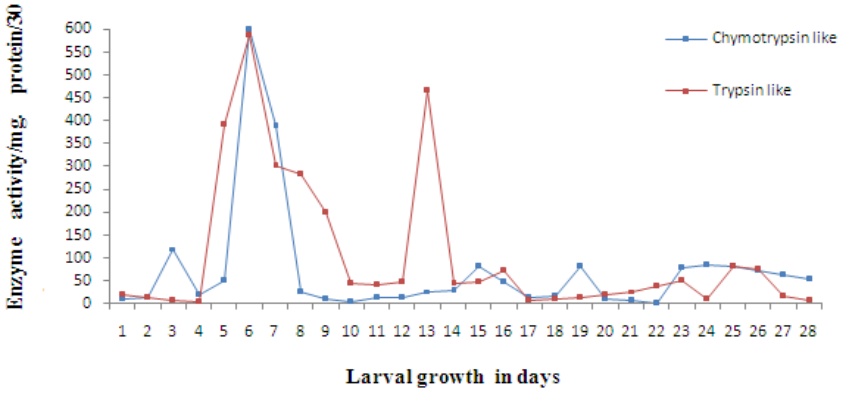

Fig 3.Alkaline protease activity during larval development of Chilopartellus (Swinhoe).

DISCUSSION

Larval Proteases

The occurrence of different digestive enzyme in the alimentary canal of insects in frequently said to depend mainly on the chemical composition of the diet ingested by the animals. Insect proteases are important enzymes existing freely in the lumen or bound to the microvillar membrane thatproteolyze different kinds of proteins in a variety of insect species (16),(17).

Houseman &Downe (10) studied the characterization of an acidic proteinase from the posterior midgut of *Rhodniusprolixusstal*and stated that Cathepsin D in the posterior midgut involve in breakdown of ingested blood proteins. The presence of Cathepsin D as an extra cellular digestive proteinase is consistent with the presence of Cathepsin B and lysosomalcarboxypeptidaseB, that have also been detected in the posterior midgut of *R. prolixus*and other blood sucking Hemiptera. Housemen & Down (18) identified these enzymes in the midgut of these insects. Although the list of proteinases is not complete, those that have been characterized represent all the proteinases essential for the breakdown of ingested blood. Endopeptidasehydrolyze protein to smaller components that in turn are digested to free amino acids by exoproteinase.

Comparison between the levels of aspartic & cysteine proteinase of the larval midgut of *Callosobruchusmacullatus and Zabrotessubfasciatus*(BOH) was studied by Silva &Filho (19). Ahmad *et al.* (6) investigated alkaline proteases in the larvae of the armyworm, *Spodopteralitura.* The alkaline proteases activity in the gut of *Spodopteralitura*was found to increase with the development of larvae and decreased with the onset of pupation fasting of the fifth instar larvae cuased to a slight increase in protease activity at 4 hr which decline consistently on further starvation.

It is evident from the changes in protease activity and protein concentration that the protein concentration appears to influence the protease activity. During 7 to 9-day the larvae become voracious feeders. This presumably accounts for the enhanced protein concentration in the gut. The protein concentration false after 9thday as the larvae gradually becomes pupae and gives up food consumption. Such changes in gut proteases activity during development have also been demonstrated in larvae of *Bombyxmori*. The protease activity of *Galleria mollonella*(20) also varied markedly with the developmental stages having a sharp rise just before ecdysis.

Starvation profoundly affects the protease activity. There was a slight increase in protease activity during first 4 hr of starvation, which consistently declined on further starvation as also shown earlier with *Bombyxmori*(21). At 4 hr, there was a very marked decrease in protein concentration of gut fluid, presumably, due to proteolysis, which account for the marked increase in specific activity.

Changes in protein concentration and protease activity during further starvation also suggest a relationship between them. It is of interest to mention here that feeding of the larvae with high protein diet causes a marked stimulation in protease activity (22). The proteolytic activity of several insects decreases during starvation and increases again on refeeding (23); (24); (25), (26); (27) also suggests the influence of the protein on gut proteolytic activity.

According to Eguchi& Iwamoto (7) the protease in the midgut tissue and digestive fluid increase with increasing feeding from 1 to 9-day of fifth instar. The results suggest that the secretion of protease occur in response to feeding. As inferred by Dadd (23) from experimental results in *Tenebrio*, the synthesis and discharge of the midgut proteases may be interdependent, since little enzyme is accumulated in the epithelial tissue when the total midgut enzyme is greatly increased and tissue protease may reasonably be considered an index of the rate of synthesis of enzyme in the secretary cells.

Baker (28) has studied midgutprotelytic activity in the black carpet beetle, *Attagenusmegatoma.* The control of proteolyic digestive enzyme in the larvae of *A. megatoma* was not regulated by the amount of food being consumed but rather by the amount of protein present in midgut. When starved larvae of the black carpet beetle, *Attagenusmegatoma,* were fed selected diets, increases in poteolytic trypsin and chymotrypsin activity correlated with total midgut protein and not with the amount of food consumed. These results support a secretagogue control mechanism for protease synthesis in larvae of *A. megatoma.*

Garcia *et al.* (29) studied the protease secretion in the intestine of fifth instar larvae of *Rhodniusprolixus*. In the protein fed larvae the protein content and the specific protease activity increased. These findings exclude neurosecretory control & indicate that ingested protein stimulate the proteolytic activity of *Rhodniusprolixus*midgut.

According to Lehane (30) the basis of the secretagoue mechanism is that digestive enzymes are produced in direct proportion to the quantities of amino acids available for their synthesis and that is a consequence of the quantities of amino acids released from the food during digestion.

Baker (9) reported that the total protease level declined in staved larvae but increased after 48 hr of feeding. These *in vitro* studies indicate that midgut homogenates of *A. megatoma*possess total protease activity with an optimum pH range in the alkaline region similar to that of any other insect species. Protease activity in *A. megatoma*larvae starved for extended periods declined to immeasurable levels. When these larvae were given a food source, feeding occurs immediately, and guts were nearly full after 24 hr. However no protease activity was detected until 48 hr. Such delays between the onset of feeding and a measurable increase in protease activity in insects are common (26) and have been used to argu for a distinct synthesis of enzyme I response to ingested food rather than activation of a zymogen already present in gut lumen.

Meenakshisundaram&Gujar (12) studied on alkaline proteases in Lepidoptera larvae. It was observed that the initial protein peaks had very low protease activity. The protease activity however showed rising trend and then reached at the peak. It is evident from above results that the alkaline midgut region of the test insect is conductive to proteases to degrade efficiently the proteinacious substrate present in the food to drive the required amino acids after digestion for the growth and development.

In the present investigation gradual increase in proteases activity of *Chilopartellus*from 1 to 2-day larva suggest the degradation of yolk proteins and ingested proteins after hatching for growth of larva. Gradual decrease in enzyme activity from 4 to 4-day larvae suggests depletion of yolk proteins. Sharp increase in enzyme activity from 4 to 6-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 10 to 12-day larvae indicates the active feeding stage of larva and degradation of

proteins. Fall in enzyme activity from 12 to 13-day larvae indicates quiescent stage of larvae in which degradation of protein is low and synthesis and accumulation of proteins may be high. After 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 larvae 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 24-day larvae indicates slow feeding larval stage with very very low protein catabolism. Decrease in enzyme activity from 24 to 28-day larvae indicates the protein synthesis and accumulation of proteins in the haemolymph and fat body in prepupal stage.

The decline in the enzyme activity in 4, 10, 17, 23 and 28-day larva indicates first, second, third and fourth larval-larval moults and fith larval-pupal moult respectively.Our results are in agreement with Ahmad *et al.*(6); Fujii& Kato (21);; Ishaaya*et al*. (22); Dadd (23); House (24); Engelmann (25), (26); Janda& Krieg (27); Eguchi& Iwamoto (7); Baker (9), (28) and Meenakshisundaram&Gujar (12).

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