Studies on lipolytic activity in muscle during larval development of *Helicoverpa armigera* (Hubner)

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ABSTRACT:

The larval muscle lipase revealed optimum pH 7.6, incubation time 20 minutes, temperature 37^0 C, enzyme concentration 1 %, substrate concentration 6 % and Michaelis-Menten constant 17.35mM. The lipase mainly hydrolyses the triglycerides to diglycerides and fatty acids. The lipolytic activity has been studied in the muscle of 7-day larva to 15-day larva. The gradual increase in muscle lipase activity was observed from 7-day larvae to 10-day larvae and decrease from 10-day larvae to 15-day larvae. The maximum lipase activity was observed in 10-day larvae. The physiological role of lipase in muscle during larval development of *H. armigera* (Hubner) has been reported in present paper.

INTRODUCTION:

Chick pea, *Cicer arietinum* (L) is one of the major grain legume crop grown as a sole or mixed crop in the Rice, Jowar, Sugarcane, Maize- based cropping system in India. Area under chick pea has shown a decreasing trend for the last two decades, as a result of increasing incidence insects (pod borer). Among the various constraints, incidence of gram pod borer, *Helicoverpa armigera* (Hubner) is the major cause of low

production in chick pea. The gram pod borer, *H. armigera* (Hubner) inflicted heavy crop losses from seedling to maturity but the losses reached at its peak when the pods appeared Deka *et al*. (1989). Farmers are unable to control this pest to desired level in spite of spending millions of dollars on pesticides. With the increasing pesticide utilizations, *H. armigera* is exhibiting resistance towards a wide range of insect killers McCaffery *et al*. (1991).

Lipase is an enzyme which is responsible for hydrolysis of triglyceride. Lipolytic enzymes are indispensable for the biological turnover of lipids. They are required as digestive enzymes in the transfer of lipid from one organism to another, that is from plant to animal and from animal to animal. Within the organisms, they are instrumental in the deposition and mobilization of the fat. They are also involved in the metabolism of intracellular lipids (Pol and Salunkhe 2002). Lipids are essential structural components of the cell membrane and cuticle, they provide rich source of metabolic energy for periods of sustained energy demand, they facilitate water conservation both by the formation of an impermeable cuticular barrier and by yielding metabolic water upon oxidation and they include important hormones and pheromones (Pol and Gejage 2002).

In the present investigation, an attempt has been taken to evaluate lipase activity in muscle during larval development of *H. armigera* which is mainly concerned with release of energy for their active life and structural components of larval growth.

MATERIALS AND METHODS:

The rearing of *H. armigera* (Hubner) was carried out in the laboratory on the natural food of chick pea, *C. arietinum* (L.). The larval developmental stages from 7-day to 15-day larvae were taken for study of lipase activity. For the enzyme preparation larval muscles were isolated under ice cold distilled water, weighed and homogenized using a ground glass mortar and pestle. The homogenate was centrifuged at 5000 rpm for 10 minutes. The homogenate was then diluted with cold distilled water so as to get desired concentration. Such homogenate was used for the assay of lipase activity (Hayase and Tapple 1970). The assay system contained 0.25 ml of 6 % olive oil dispersed in gum acacia; 1.0 ml of 0.2 M tris-maleate buffer pH 7.6 and 0.25 ml of 1 % (w/v) enzyme solution in a total volume of 1.5 ml. The incubations were carried out in a shaker with a continuous shaking for 20 minutes in glass stoppered conical flasks at

37 ^{0} C. The reaction was stopped with 2 ml of Cu-TEA reagent (1N acetic acid: 1M 2,2',2" trinitrilloethanol: 6.45% $Cu(NO_3)_2$, (1:9:10,v/v/v/). The colour was developed by the addition of 1 ml of 0.5 % solution of mixture of diphynyl carbazone and diphynylcarbazid (5:95 w/w) in methanol. At the end of the incubation the liberated fatty acids were measured colorimetrically (Itaya 1977).

RESULTS AND DISCUSSION:

Larval developmental period of *H. armigera* (Hubner) is of 15-days. The larval muscle lipase revealed optimum pH 7.6, incubation time 20 minutes, temperature 37^{0} C, enzyme concentration 1 %, substrate concentration 6 % and Michaelis-Menten constant 17.35mM. The lipolytic activity was studied in the muscle of 7-day larva to 15-day larva. The gradual increase in muscle lipase activity was observed from 7-day larvae to 10-day larvae and decrease from 10-day larvae to 15-day larvae. The maximum lipase activity was observed in 10-day larvae. Lipolytic activity in muscle during larval development of *H. armigera* (Hubner) is shown in figure 1.

The level of lipase activity was maximal at pH 7 to 8 in muscle of 6 day larvae (wandering stage) after which it declined in 7 day larvae of blowfly, *Calliphora erythrocephala* (Price 1975). All enzymes have an optimum pH range for their activities, which is often very narrow. The optimum pH does not depend only on the nature and ionic strength of the buffer, in general also depends on the temperature and substrate concentration (Bergmeyer 1983). Enzymes catalyzed reactions like all chemical reactions increase in rate with rises in temperature (Wiseman 1985). For many proteins, denaturation begins to occur at 45 to 50 ^{0}C and is severe at 55 ^{0}C (Bailey and Ollis 1986). The lipase activity was maximal at the broad pH 8.6, 1 % enzyme, 10 minutes of incubation time and increase in activity from 3-day to 4-day larval muscle of *C. rufifacies* (Pol and Sawant 1995). The optimal pH generally reflects the pH of the environment in which the enzyme normally functions. In the current study, optimum pH for muscle lipase activity was determined using Tris buffers of different pH. The enzyme activity increased with an initial increase in pH and optimum activity was noted at pH 7.6, suggesting alkaline nature of the enzyme. Incubation time 20 minutes was found to be optimum and at temperature 37 ^{0}C the activity was maximum. Sharp increase in rate of enzymatic reaction from 0.5 to 1% larval homogenate of *H. armigera* (Hubner) suggests maximum rate of release of fatty acids at 1 % enzyme concentration. Optimum substrate concentration was 6% and

Michaelis-Menten constant 17.35 mM. The findings noted herein indicates that extra digestive alkaline lipase (EC 3.1.1.3) exist in the larval muscle homogenate of *H. armigera* (Hubner) and due to this lipase diglycerol may be released from the muscle and served as important intermediate in the breakdown of triacylglycerol.

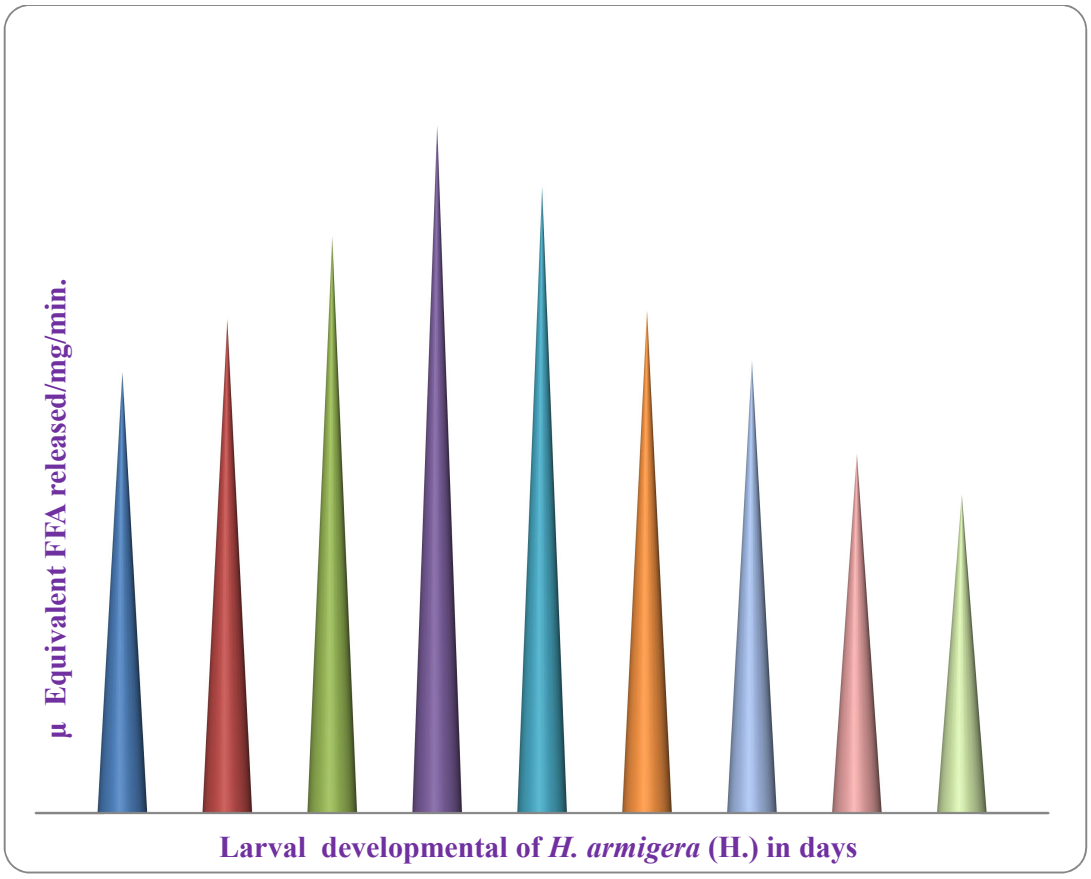

Fig.1: Liploytic activity in muscle during larval development of *H. armigera* (Hubner).

Muscles from *Hyalophora cecropia*, *Schistocerca gregaria* and a number of other species have lipases which act more rapidly on diacylglycerols and monoacylglycerols than on triacylglycerols (Crabtree and Newsholme 1972). Lipid is stored in the fat body in the form of triglyceride and transported to the muscles by the haemolymph in the form of diglyceride or free fatty acids and then the fat body must contain lipase activity (Candy and Kilby 1975). The lipid content of the muscle was 1.96 and 12.96% on fresh and dry weight basis respectively in the larvae of *Tenebrio molitor* (L.) (Coleoptera: Tenebrionidae) (Uner 1988). Lipolytic activity was increased from 3 to 4-day larval

muscle of *C. rufifacies* (Pol and Sawant, 1995). In general larval muscles are histolysed and adult muscles rebuilt in the pupa (Yadav 2004). In the present study, gradual increase in lipase activity from 7-day larval muscle to 10-day larval muscle of *H. armigera* (Hubner) indicates energy utilization in a series of moultings and energy required for the active life is supplied by triglyceride catabolism. Decrease in enzyme activity from 10-day larval muscle to 15-day larval muscle suggests the decreased rate of hydrolysis of lipid and gradual triglyceride accumulation which attains high concentration in prepupae. Maximum lipase activity in 10-day larval muscle of *H. armigera* (Hubner) indicates the possible mobilization of lipid in most active feeding larval stage which requires more energy and for structural components during larval growth. The minimum lipase activity of 7-day larval muscle as compared to 10-day larval muscle of *H. armigera* (Hubner) suggests histogenesis and early developmental period of larval development.

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