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Lipolytic Activity During Larval Development of*Maruca Vitrata* (Fabricius)

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ABSTRACT
Larval lipolytic activity in M. vitrata has been studied. The larval growth duration was found to be 13 days. The optimum activity observed in 8th day larvae with pH 7.9. Lipase activity of 4th day, 8th day and 13th day larvae showed 0.238, 0.280, and 0.235µmol FFA/mg protein/25min. respectively. The increase in enzyme activity noted from 4 to 8-day larvae and maximum activity in 8-day larvae. The analysis of mean and standard deviation of larval lipase was 0.255 and 0.01430 respectively. The lipolytic activity of 4-day larva was 16.8% less than 8-day larva.
Keywords: *Lipaseactivity, larvae, M. vitrata(F.).*

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INTRODUCTION

A legume pod borer, *Maruc avitrata* Fabricius (Lepidoptera: Crambidae) is a pest of cowpea Asia[1].*M. vitrata* also causes considerable damage to lablab bean. It attacks on buds and pods. *M. vitrata* complete larval and pupal development inside the pod. This results in to poor pod formation and adverse effect on market value of green pods [2]. The loss due to pest is high in pulses. Pod borer is main barrier in increasing the productivity [3]. The legume pod boreris destructive during flowering [4]. Larva feeds on floral Parts like anthers, styles, and ovaries [5]. Flowering formation stage the attack is more and larva feeds on pods by webbing them [6]. In India damage has been found up to 51 per cent in pigeon pea crop [7]. The damage starts from 21 days after planting. Intensity is highest on flowers [8]. Larval period noted up to 14 days in cowpea [9-10]. However, the information about the lipase activity during larval development of *M. vitrata* rather scanty. In the present investigation, study on lipase activity during larval development of *M. vitrata* which mainly concerns with release of energy.

MATERIAL AND METHODS

*M. vitrat a*culture was maintained food of legume pods cow pea [6]. The larvae from infested pods were kept in cow peapods in
Insect rearing cage. Tiny larvae were transferred to cow pea podsplaced in glass tubes and covered with muslin cloth. Ammonium precipitation method use for partial purification of lipase [11]. For uniform and rapid dissolution, the desiccated ammonium sulphate is used. Ammonium sulphate was placed over night in oven at 120 ^{0}C in a glass beaker. For 70 % saturation required 43.6 grams/ 100 ml ammonium sulphate. Homogenate was allowed to precipitate for 30 minutes at 4 ^{0}C with stirring. Centrifugation use for recovered precipitation about 30 minutes and pellet was separated. [12]. Enzyme assay adopted included incubation period 25 minutes, 37^{0}C temperature, 2 ml Cu-TEA reagent. The flasks were shaken twenty times. After that10 ml of chloroform was added. Contents shake and kept for separation of aqueous and organic phase. Chloroform phase was transferred to centrifuge tube after 5 min. After 2 ml of water was added without mixing and the tubes were centrifuge for few minutes. Then 2 ml of chloroform phase was taken in stoppered test tube. After that 1 ml of colour reagent was added. At the end liberated fatty acids were measured calorimetrically [13].The absorbance was read at 540nm. For Protein estimation [14]0.5 ml partially purified enzyme, 4.5 ml of reagent I mixed well and allowed to stand for 10 minutes of incubation at room temperature. After that 0.5 ml reagent II was added rapidly performing the total volume of 5.5 ml. Reagent II included 1 part of Folin and ciocateu's reagent (phenol

reagent) [2N] and 1 part of water. Reading was taken calorimetrically at 750 nm after 30 min incubation. Reagent I and II were prepared freshly.

RESULTS AND DISCUSSION

The optimum lipase activity was noted in 13-day larvae with pH 7.9. The specific activity of 4th, 8th and 13th day larvae of *M. Vitrata* was recorded to be 0.238, 0.280, and 0.235µmolFFA/mg protein /25min. Increase in lipase activity noted from 4 to 13-day larvae and decrease from 8 to 13-day larvae. The lipase activity of 4-day larva was 16.8% less than 8-day larva and lipase activity of 13-day larva was 15% less than 8-day larva. The mean and standard deviation of larval lipase was 0.255 and 0.01430 respectively. Lipolytic activity during larval development of *M. vitrata* is shown in fig. 1.

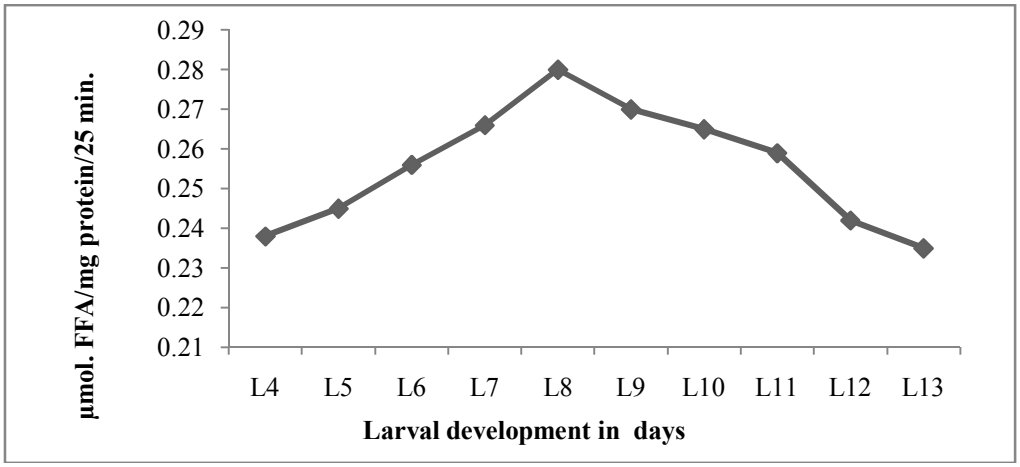

Fig 1. Lipolytic activity during larval development of *M.vitrata.*

Larval lipolytic activity increased from 6 to 8 day larval fat body of *L. orbonalis*[15-16]. Larval fat body lipase activity increased in *H. armigera* from 7 to 10 larvae [17]. Larval gut lipase activity of *H. armigera* revealed optimum pH 7.7[18]. Larval muscle lipase activity showed Km value 17.35mM [19].The lipolytic activity of 10% control larvae was 1.2 times more as compare to 10 day econeem treated larvae of *H.armigera.* [20].The lipase activity during development of *Mythimna separate* revealed maximum activity in 11, 6, 4 and 5 day in larvae, male pupae, male adult and female adult respectively[21].The maximum lipase activity was noted in 5-day female moth in *H. armigera*[22]. Purified lipase had the highest activity at pH 10 and temperature 35 ^{0}C and specific activity of 5.6 µmol FFA/min /mg protein in *Narangaaenescens.* The third instar larvae of *N*. aenescens showed highest activity V_{max} value is 8.64 and K_m value is 28.4 ±2.97 [24]. Purified lipase activity in the larval midgut of *Ectomyelois ceratoniae* showed highest activity at pH 7 and temperature of 30 ^{0}C [25].Highest lipase activity at 37^{0}C temperature observed in the midgut of carob moth, *E. Ceratoniae* [26].In present study, maximum activity of lipase from larva at pH 7.9 indicates the presence of alkaline lipase in the larvae of*M. vitrata.*The maximum activity at 5% substrate concentration indicates maximum substrate concentration for larval lipase of *M. vitrata*. This result also suggests that at the saturation of enzyme, further addition of substrate molecules never increase the reaction velocity any more. In enzyme substrate reaction, substrate molecules collides with enzyme molecules and increased substrate concentration noted increased the reaction rate further increased in substrate concentration did not affected the rate of reaction. The Michaelis Menten constant calculated from the Lineweaver-Burk plot the K_m value 9.56mM indicates more affinity of enzyme with substrate. Source of energy during larval growth is lipid.In present study, the increased lipase activity in 4 to 8-days of larval development of *M. vitrata* indicates early feeding period of larval development and such fast growing larvae required more energy for development of internal organs. This result indicates utilization of lipids for release of energy and supply of structural components to developing larvae. The specific activity of lipase of 4th and 8th day larvae of *M. vitrata* found to be 0.238 and 0.280µmol free fatty acids/mg protein /25minutes respectively. The decrease in activity from 8 to 13-days indicates storage of lipids for the further development of larva and larvae entering to pupal stage. This decrease in activity indicated slow feeding period of larvae and accumulation of lipid for pupal stage. The later stage larvae gradually stop feeding. Maximum lipase activity in 8th day larvae indicates most active larval stage that requires more energy for structural components and growth. The specific activity of lipase of 13th day larvae of *M. vitrata*was found to be 0.235µmol FFA/mg protein /25minutes. The presence of lipase in larvae, the proportion of triacylglycerol may vary with the physiological state of insect. The analysis of

mean and standard deviation of larval lipase was 0.255 and 0.01430. Similar findings were reported by above authors.

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