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Changes in lipase activity during larval development of *Earias vittella* (Fabricius)

Patil TC[1], Gejage RM[2] and Bodare RD[3]

[1]Research student, Dept. of Zoology, SGM College, Karad, Dist. Satara-415410 (MS), India.
[2] Research guide, Dept. of Zoology, KRP Kanya Mahavidyalaya, Islampur, Sangli-415 409.
[3]Research co-guide PG. Dept. of Zoology, S. G. M. College, Karad, Satara-415 124
Email: tejashricjpatil@gmail.com, Email: ramesh.gejage@yahoo.com

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ABSTRACT

Changes in triacylglycerol lipase activity during larval development of *E.vittella* have been attempted. The larval developmental period was found to be 17 days. The maximum triacylglycerol lipase activity was observed in 11-day old larvae of *E. vittella*. The triacylglycerol lipase activity revealed optimum pH 8, substrate concentration 5% and K_m value was found to be 0.14273×10^{-2} mM. The specific activity of 5th day, 11th day and 17th day larvae was found to be 13.625, 22.838, and 16.435 µmol free fatty acids/mg protein/25minutes respectively. The gradual increase in triacylglycerol lipase activity noted from 5-day old larvae to 11-day old larvae and gradual decrease from 11-day old larvae to 17-day old larvae. The mean and standard deviation of larval triacylglycerol lipase was 18.893 and 2.5007 respectively. The physiological role of triacylglycerol lipase during larval development of *E. vittella* is discussed.

Key words: Triacylglycerol lipase, insect, larva, *E.vittella* (F.).

INTRODUCTION

The okra fruit and shoot borer, *E. vittella* is a pest of okra, *Abelmoschus esculen*tus (L. Moench) in India. Okra has great economic importance because of its nutritional value. India rank first in the world with 3.5 million tonnes of okra produced over 0.35 million hectors land. It is called lady's finger in England and bhindi in India (Tripathi *et al.,* 2011). The okra fruit contains some essential mineral salts such as calcium, magnesium, potassium; iron and vitamin C. Okra fruit has anti-diabetic property (Amin, 2011). In insect triacylglycerol lipase have essential role to hydrolyse triacylglycerol. A few studies have been carried on *E. vittella* which is pest of okra (Roqaya, 2000 and Tripathi *et al.,* 2011). Many workers have noted in detail the various aspects of triacylglycerol lipase in insect species (Pawar *et al.,* 2014; Roudsari *et al.,* 2014; Chamani *et al.,* 2015; Gaikwad and Bhavane, 2016; Ranjbar *et al.*, 2015; Marepally and Benarjee, 2016). However,the information about the triacylglycerol lipase during larval development of *E. vittella* is

rather scanty. In the present work, an attempt has been made to estimate the triacylglycerol lipase activity during larval development of *E. vittella* which mainly concerns with release of energy for their active larval growth.

MATERIAL AND METHODS.

The culture of *E. vittella* was maintained in the laboratory on natural food of okra fruits (Roqaya, 2000). The larvae from 5th to 17th day old were used for study. Partial purification of triacylglycerol lipase was attempted by ammonium sulphate precipitation method (Dawson *et al.,* 1969). Precipitation was recovred by centrifugation for about 30 minutes and pellet was separated. The pellet was re-supended in a volume of phosphate buffer (pH8) equal to the volume of homogenate and then such partially purified enzyme (0.25 ml) was used to lipase assay. Lipase assay contains 0.25ml partially purified larval lipase enzyme, 1ml of phosphate buffer pH 8 and 0.25ml of substrate (Hayase and Tappel, 1970) in total volume of 1.5ml. The liberated fatty acids were measured calorimetrically (Itaya, 1977). The absorbance was read at 540nm. Protein estimation (Lowry *et al*., 1951) method included 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. Immediately, 0.5 ml reagent II was added rapidly performing the total volume of 5.5 ml. Reagent I contained 2 % Na_2CO_3 in 0.1N NaOH, 1 % sodium tartarate in distilled water and 0.5 % $CuSO_4$ in distilled water. Reagent II included 1 part of Folin and ciocateu's reagent (phenol reagent) [2N] and 1 part of water. After 30 minutes of incubation reading was taken calorimetrically at 750 nm. Reagent I and Reagent II were prepared freshly just before experiment.

RESULTS AND DISCUSSION

The larval developmental period was found to be 17 days. The maximum triacylglycerol lipase activity was observed in 11-day old larvae of *E. vittella*. The triacylglycerol lipase activity revealed optimum pH 8, substrate concentration 5% and K_m value was found to be 0.14273 $\times 10^{-2}$ mM. The specific activity of 5th day, 11th day and 17th day larvae was found to be 13.625, 22.838, and 16.435 µmol free fatty acids/mg protein/25minutes respectively. The gradual increase in triacylglycerol lipase activity noted from 5-day old larvae to 11-day old larvae and gradual decrease from 11-day old larvae to 17-day old larvae. The triacylglycerol lipase activity of 5-day old larvae was 40.35 % less than 11-day larvae. The triacylglycerol lipase of 17-day larva was 28.04 % less than 11-day larva. Changes in triacylglycerol lipase activity during larval development of *E.vittella* are shown in fig 1.

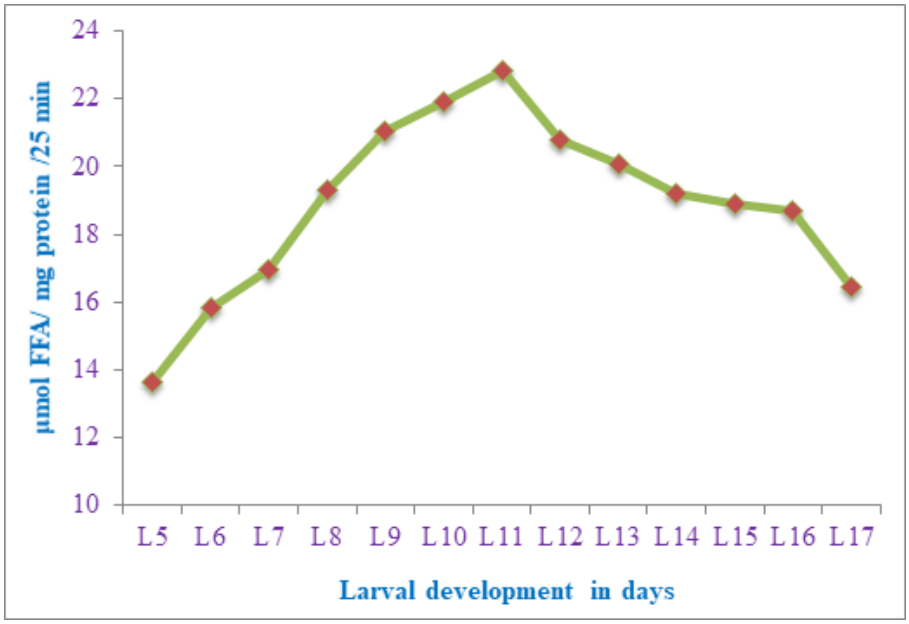

Fig 1.Changes in triacylglycerol lipase activity during larval development of *E. vittella.*

DISCUSSION

Larval midgut lipase enzyme in larva of *Tramea virgina* shows peak activity at pH 7 and temperature 37^{0}C (Tembhare and Muthal, 1992). Phospolipase activity from salivary gland of tobacco hornworm, *Manduca sexta* reveals optimum pH range 8-10, reaction time 30 min., optimum temperature 18-38 ^{0}C (Tunaz and Stanley, 2004). Lipase activity of gypsy moth larval midgut tissue shows K_m value 0.310 mM and V_{max} value 1.47 U /mg protein maximum pH 8.2 temperature at 37^{0}C (Mrdakovic *et al.,* 2008). The activity of lipase was 0.486 µmol /min /mg protein and 0.27µmol /min / mg protein in midgut and salivary glands of *Chilo supprressalis.* The optimum temperature for lipase activity was 37^{0}C in midgut and salivary glands.V_{max} of midgut and salivary glands were 0.5 and 0.35 µmol /min /mg protein respectively and K_m value of midgut and salivary gland were 15 and 19 mM respectively in *C. suprressalis* (Zibaee *et al.,* 2008). TG hydrolase activity of larval fat body of *Manduca sexta* increases as larva grew to the last instar and then decreased to minimum during pupa formation (Arrese *et al.,* 2010). Lipase activity in gut homogenate of *Helicoverpa armigera* wasstudied at pH 6.8, temperature 37 ^{0}C and time of incubation 30 min (Sarate *et al*., 2012). Larval lipase of *Pieris brassicae* shows optimum pH 11 optimum temperature 30^{0}C (Ziabee, 2012). Purified lipase had the highest activity at pH 10 and temperature 35-40^{0}C and specific activity of 5.6 µmol/min /mg protein in *Naranga aenescens.* The third instar larva shows highest activity V_{max} value is 8.64 and K_m value is 28.4 ±2.97 (Zibaee and Fazeli-Dinab, 2012). In larval midgut of *Galleria mellonella* lipase activity studied at pH 6.5 and at temperature 20-25^{0}C with 10min incubation (Alipour *et al.,* 2013). The larval muscle lipase activity from *Helicoverpa armigera* was increases from 7th day to 10th larvae and then decreases from 10th day 15thday (Pawar *et al.,* 2014). It was found that optimum pH of digestive soluble and membrane bound lipases in the gut of *Bacterocera oleae* larvae obtained 4 and 6 soluble lipase and 6 for membrane bound lipase and optimum temperature 50^{0}C and 35^{0}C respectively (Roudsari *et al.,* 2014). Digestive lipase enzyme form larval midgut of *Papillo polytespolytes* reveals lipase activity was optimum at pH 7.8, period of enzyme incubation was 15 min, and optimum temperature 40 ^{0}C, specific activity of larval lipase enzyme was 9.3474µg palmitic acid/ µg protein /hr (Gaikwad and Bhavane, 2015). Purified lipase activity in the larval midgut of *Ectomyelois ceratoniae* shows an enzyme with specific activity of 0.4U mg^{-1} protein, highest activity at pH 7 and temperature of 30 ^{0}C (Ranjbar *et al.*, 2015). Highest lipase activity was observed in the midgut of carob moth, *E. ceratoniae* larvae reared on artificial diet at 37^{0}C temperature (Teimouri, *et al.,* 2015). The highest enzyme activity was observed in 6th instar larvae of *H. armigera,* the highest enzyme activity was observed at 40 ^{0}C temperature and at optimum pH 9.5 (Chamani *et al.,* 2016). Lipase activity from larvae of *Antheraea mylittadrury* revealed 7.87µmol /min/mg of specific activity and highest lipase activity at pH 8 and temperature 37^{0}C (Marepally and Benarjee, 2016). In present study, maximum activity of triacyglycerol lipase from larva at pH 8 indicates the presence of alkaline lipase in the larvae of *E. vittella.* The tertiary structure of enzyme held together due to ionic and hydrogen bonds. These bonds occur because of attraction between the oppositely charged groups on the amino acids that made up the enzyme protein. The increasing and decreasing the pH of solution around the enzyme may alters the tertiary structure of enzyme hence further increase or decrease in pH decreased the enzyme activity. The maximum activity at 5% substrate concentration indicates maximum substrate concentration for larval triacylglycerol lipase of *E. vittella*. 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 0.14273 $\times 10^{-2}$ mM indicates more affinity of enzyme with substrate. The main source of energy during larval growth is lipid and lipolytic acivity is instrumental in release of energy. In present study, the increased triacylglycerol lipase activity in 5 to 11-days of larval development of *E. vittella* 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 triacylglycerol lipase of 5th and 11th day larvae of *E. vittella* was found to be 13.625 and 22.838 µg free fatty acids/mg protein /25minutes respectively. The decrease in enzyme activity from 11 to 17-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 11th day larvae indicates most active larval stage that requires more energy for structural components and growth. The specific activity of triacylglycerol lipase of 17th day larvae of *E. vittella* was found to be 16.435 µg free fatty acids/mg protein /25minutes. The presence of triacylglycerol lipase in larvae, the proportion of triacylglycerol may vary with the physiological state of insect. The later larval stage fat bodies may reserve fat in the form of triacylglycerides which may be utilized for energy and histogenesis in metamorphosis of *E. vittella*. The mean and standard deviation of larval triacylglycerol lipase was 18.893 and 2.5007 respectively. One-way analysis of variance (ANOVA) between larval and male adult moths $p \leq 0.028$ and $F > 5.40$ indicates true hypothesis with significant differences ($p \leq 0.05$ and $F > 1$) in triacylglycerol lipase during larval and male adult moth development of *E. vittella.* Similar findings were reported by above authors.

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