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Triacylglycerol Acylhydrolase Activity during Larval Development of *Hellula undalis* [Fabricius]

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ABSTRACT
The cabbage borer, Hellulla undalis (F.) is Pest cabbage. Changes in activity of triacylglycerol lipase during larval development of H. undalis have been studiedAlmost16 days period was found to be developmental of larval life. The maximum triacylglycerol lipase activity was observed in 10-day old larvae of H. undalis. The triacylglycerol lipase activity manifested optimum pH,7.7 incubation time was 20 minutes, 37 ^{0}C temperature, 1% concentration of enzyme, 6% substrate concentration and value of Km was found to be 0.0938 ×10^{-2} mM.The specific activity of 4th day, 10th day and 16th day larvae was found to be 0.1904, 0.2518 and 0.2047µmol free fatty acids/mg protein/20minutes respectively. Triacylglycerol lipase activity gradual increase from 4-day old larvae to 10-day old larvae and gradual decrease from 10-day old larvae to 16-day old larvae was noted. The mean and standard deviation of larval triacylglycerol lipase was 0.219 and 0.017 respectively. The triacylglycerol lipase activity of 4-day old larvae was 25 % less than 10-day larvae. The triacylglycerol lipase of 16-day larvae was 19 % less than 10-day larvae. The physiological role of triacylglycerol lipase during larval development of H. undalisis discussed.
Keywords: *Triacylglycerolacyl hydrolase,insect, larva, H. undalis (F.).*

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INTRODUCTION

The Cabbage head borer, *H. undalis* is a pest of cabbage, Brassica *oleracea* var. *capitata* (Linn.) in India. Cabbageis the most common winter vegetable. It is grown worldwide due to it's palatability and taste in addition to antibacterial, antioxidant anti-inflammatory properties and It is used as salad, boiled and dehydrated vegetable as well as in cooked curries and pickles. Cabbage main edible part is head and it is good source of amino acids, protein (1.6%), vitamins (B, C and A), also reach in minerals, calcium, iron, magnesium, sulphur, phosphorous and potassium. It contains 2.4% fat, 0.2%, carbohydrate [1].In insect triacylglycerol lipase have essential role to hydrolase triacylglycerol. A few studies have been carried on *H.undalis* which is pest of cabbage [2-6]. Many workers have noted in detail the various aspects of triacylglycerol lipasein different insect species [7-11]. However, the information about the triacylglycerol lipase during larval development of *H.undalis* is rather scanty. In the present work, an attempt has-been made to estimate the triacylglycerol lipase activity during larval development of *H.undalis* which mainly concerns with release of energy for their active larval growth.

MATERIAL AND METHODS

The life cycle of *H.undalis*in the laboratory was maintained on natural food of cabbage [3]. The larvae were placed in petridishes. The fully mature larvae shifted to big containers provided with cotton bed with water-dipped bed and it covered with filter paper layer for pupation. In insect rearing cages, freshly emerged adults were shifted and nectary solution of 10% sucrose provided for feeding and longevity of adult. Newly hatched tiny larvae were transferred to growing heads of cabbage placed in glass tubes and covered with muslin cloth and larvae were taken for study of triacylglycerol lipase activity. Partial purification of triacylglycerol lipase was carried by ammonium sulphate precipitation method [12]. The desiccated ammonium sulphate was used to ensure uniform and rapid dissolution. Ammonium sulphate was placed day before use overnight in oven at 120 ^{0}C in a large beaker. Powdered amount of ammonium sulphate for 70 % saturation required 43.6 grams/ 100 mL. The desired amount 4.36 grams of powdered ammonium sulphate were slowly added with rapid stirring on magnetic stirrer to the 10 mL (1%)

homogenate. Homogenate was allowed to precipitate at 4 ^{0}C with stirring for 30 minutes. Precipitation was recovered by centrifugation for 30 minute and separated the pellet. The re-suspended pellet in a phosphate buffer (pH7.8) equal to the volume of homogenate and then purified enzyme (0.25 mL) used to triacylglycerol lipase assay. Enzyme assay contains 1mL of phosphate buffer pH 7.8, 0.25mL of substrate and 0.25mL partially purified larval lipase enzyme [13] total1.5mL. volume. The incubation was carried out 20 minutes at 37^{0}C temperature in glass stoppered conical flask in metallic shaker. with Cu-TEA reagent (2 mL) Stopped reaction. Flask twenty times shaken and chloroform was added exactly 10 mL after 15min. The contents were vigorously shaken and kept for the separation of organic and aqueous phases. After 5 min in centrifuge tube transferred5 mL of chloroform phase. Then added 2 mL of water was without mixing and the tubes centrifuge for few minutes. The removed carefully upper water layer and chloroform phase exactly 2 mL of was taken in another stoppered test tube. Then colour reagent 1 mL was added. At the end measured liberated fatty acids calorimetrically method [14]. Absorbance read was 540 nm. Protein estimation [15] method included 0.5 mL partially purified enzyme, 4.5 mL of reagent I mixed well and allowed to stand for incubation 10 minutes at room temperature. Immediately, reagent II 0.5 mL was added rapidly performing the total volume of 5.5 mL. I Reagent contained 2 % Na_2CO_3 in 0.1N NaOH, 1 % sodium Tartarate in distilled water and 0.5 % $CuSO_4$ in distilled water. II Reagent included 1 part of water and 1 part of Folin and ciocateu's reagent (phenol reagent) [2N]. After 30 minutes of incubation reading was taken calorimetrically at 750 nm. Reagent II and Reagent I were prepared freshly just before experiment.

RESULTS AND DISCUSSION

The larval developmental period was found to be 16 days. The maximum triacylglycerol lipase activity was observed in 10-day old larvae of *H.undalis*. The triacylglycerol lipase activity manifested optimum temperature 37 ^{0}C, optimum pH 7.7, incubation time 20 minutes, 6% substrate concentration 1%, enzyme concentration and K_m value was found to be 9.38 ×10^{-2}mM. The specific activity of 4th day, 10th day and 16th day larvae was found to be 0.190, 0.251, and 0.204µmol free fatty acids/mg protein/20minutes respectively. The gradual increase in triacylglycerol lipase activity noted from 4-day old larvae to 10-day old larvae and gradual decrease from 10-day old larvae to 16-day old larvae. The mean and standard deviation of larval triacylglycerol lipase was 0.219 and 0.0175respectively. Triacylglycerol lipase activity during larval development of *H.undalis* is shown in fig. 1

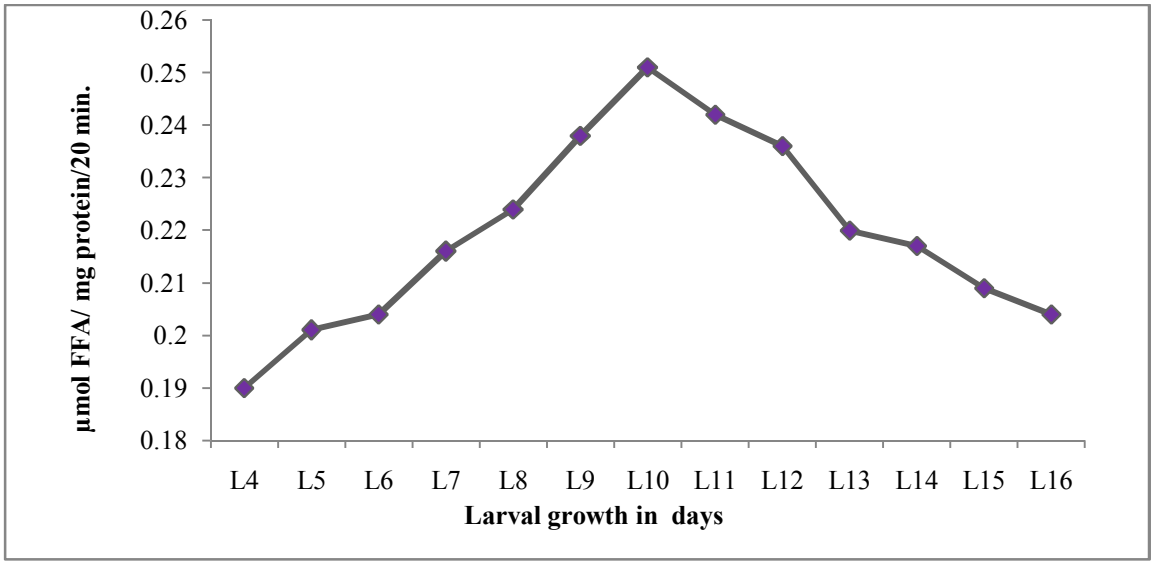

Fig1.Triacylglycerol lipase activity during larval development of *H.undalis.*

DISCUSSION

The maximum activity was noted at pH 7.8, time 30 minutes, 1% enzyme concentration, 37 ^{o}C temperature in eggs of *Leucinodes orbonalis* (Guenee). The gradual increase in Lipase activity was observed from 1-2 day eggs of *L.orbonalis* [16].Partial characterization of lipase revealed at pH 7.8, incubation 30 minutes 1% enzyme concentration, substrate concentration 5%and 37^{o}Ctemperature in the larvae of *L. orbonalis.*The gradual increase in lipase activity was found from 1–8-day larvae of *L. orbonalis*[17]. Larval gut lipase activity was maximum at pH 7.3 and gradual increase in larval gut lipase activity noted from 6–8-day larvae of *L.orbonalis*. Laraval fat body was1.3 times more than gut in 8day

larvae *L. orbonalis*[18]. Larval triacylglycerol ester hydrolase activity of *P.demoleus* reveled pH 7.8 and lipase activity of 9 day control larvae of *P.demoleus* was 1.2384, 1.1333 and 1.0625 times less compare to 9 day fluoride, cobalt and zinc respectively treated larvae[19]. Lipase activity was maximum at 1% enzyme concentration, 37^{o}C temperature and 6% substrate concentration inlarval fat body, muscle, gut, and heamolymph of *P.demoleus.* The gradual increase in larval tissue, larval fat body, gut, muscle and heamolymph lipase activity noted from 6 day larvae to 9 day larvae of *P.demoleus*[20]. Lipase activity was maximum at pH 7.4 in larval heamolymph of *P.demoleus.* The lipase activity of 9 day larval heamolymph was 2. 2748 fold more as compare to 14 day larval heamolymph of *P. demoleus* [21].The protein content of 1st day female adult was 5.32 % less than 3rd day female adult of *E. virtella*[22].Lipase activity was increased with potassium, calcium and decreased with copper and iorn in 11th day larva of *E. virtella*[23].The maximum lipase activity of *E. vittella* was noted in 5th day in pupae [24].Lipase Activity was maximum at pH 7.7, 1 % enzyme concentration, temperature 37^{o}C, 5% substrate concentration and Km value 0.18086 ×10^{-2} mM. The maximum lipase activity was observed on 8 day female adult of *E.vittella*[25]. The larval mid gut lipase activity is highest at pH 6.5 and temperature 37^{o}Cobserved in *Rhynchophorus palmarum.* Lipase showed that the highest activity at pH 6.5[7]. Lipase activity was maximum at pH 8, substrate concentration 5%, temperature37 ^{o}C and Km value 0.142273x10^{-2}in the larvae of *E. vittella*. The maximum lipase activity was noted in 11th day larvae of *E.vittella*[26].Lipase activity was maximum at pH 7.8 in male math of *E. vittella.* Lipase activity of 1st day male moth was 38.06 % less than 7 day male moth of *E. vittella* [27].Lipase activity was maximum at pH 7.8 and Km value. 0.13x10^{-2} and 0.20 x10^{-2} respectively for male and female moth of *E.vittella* Lipase activity was maximum in 7day male moth and 8 day female moth of *E.vittella*[28].Lipase activity was maximum at pH 7.7 and pH 7.4 respectively in fat body and heamolymph of larval *P. demoleus.* Larval heamolymph and fat body activity was noted from 6 to 14 day larvae of *P.demoleus* [29].Lipase activity was maximum at pH 8.1, and gradual increase lipase activity noted from 1 to 5 day and decrease from 5 to 9 day in the female moth of *Helicoverpa armigera*[30].In present study, maximum activity of triacyglycerol lipase from larva at pH 7.7 indicates the presence of alkaline lipase in the larvae of *H. undalis.* The maximum activity at 5% substrate concentration indicates maximum substrate concentration for larval lipase of *H. undalis.* This result also suggests that at the saturation of enzyme, further addition of substrate molecules never increase the reaction velocity any more. The Michaelis Menten constant calculated from the Lineweaver-Burk plot the K_m value 9.38 ×10^{-2} mM indicates more affinity of enzyme with substrate. The main source of energy during larval growth is lipid and lipolytic activity is instrumental in release of energy. In present study, the increased lipase activity in 4 to 10-day of larvae of *H. undalis* 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 10th day larvae of *H. undalis* was found to be 0.190 and 0.251µmolfree fatty acids (FFA)/ mg protein/ 20minutes respectively. The decrease in enzyme activity from 10 to 16-days indicates storage of lipids for the further development of larvae 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 10th day larvae indicates most active larval stage that requires more energy for structural components and growth. The specific activity of triacylglycerol lipase of 16th day larvae of *H. undalis* was found to be 0.204µmol free fatty acids/mg protein /20minutes. 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 triacyl glycerides which may be utilized for energy and histogenesis in metamorphosis of *H. undalis.* The mean and standard deviation of larval triacylglycerol lipase was 0.219 and 0.017 respectively. Similar findings were reported by above authors.

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