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Short Communication

Studies on Triacylglycerol Acylhydrolase during Metamorphosis of *Hellula undalis* (Fabricius)

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Hellula undalis cabbage head boror is the most important pest contributing to about 41 percent of total mortality of the plant during the pre-heading stage [1]. The biological role of lipase [EC. 3.1.1.3.] is to carry out the hydrolysis of lipids such as triacylglycerols resulting in the production of free fatty acids along with diacylglycerols, mono-acyl glycerols or glycerol [2]. The fat body has a fairly diverse structure located in many areas of an insect's body, including under the body surface and surrounding organs. The main tasks of the fat body are the synthesis, transport, accumulation and release of the main organic compounds [3]. The fat body is made up of five main types of cells, which vary in composition, size and function during the different stages of growth. The morphology of the tissue is the same within a given species, but there may be some differences between species [4]. Fat tissue in the abdominal cavity, thorax area, or head surrounds the organs located there and may form less-regular structures [5]. In insects, during their first phases of life, fat cells develop from embryonic progenitor cells. They continue to multiply through the mitotic process, and then differentiate, as we mentioned above. The resulting fat body cells constantly multiply by endo-replication [6]. Further growth of insects is possible due to the processes of molting and metamorphosis. The transformation of insect (metamorphosis) into the adult complete histolysis of the fat tissue cells in the larva and the production of new tissues for the pupa by remodeling Insects by remodeling previous cells into adult cells [7]. During metamorphosis in most holometabolous insects, the fat body undergoes significant changes in shape, size, and function in a process called fat body remodeling, which includes programmed cell death and cell dissociation of larval fat body cells and the formation of adult fat body tissues [8]. In holometabolous insects, fat body remodeling is drastic, presumably because of the nonfeeding pupal stage, Lipid droplet is a major lipid storage organelle in fat body cells, and triglyceride is the main component of stored lipids [9]. In insects that do not feed as adults, it is these energy stores that provide fuel for flight and reproduction [10]. Insects that feed as adults do not rely completely on energy stores built up during the larval stages. Nevertheless, an appreciable amount of stored energy reserves is usually carried over from the last larval stage, increased by intense feeding during early adult life, and later mobilized to fuel flight and reproduction [11].

The larvae of *H. undalis* were obtained from infested heads of cabbage from farms. The collected larvae were released on fresh cabbage heads and then placed in insect rearing cage at laboratory conditions of 12 h photoperiod, temperature 28 ± 2^0C and relative humidity 78 %. The insect rearing cage made of wireguaze on all sides except the top. Cage has 45 cm width, 45 cm length and 45 cm height. The cage has an access door at the front side. The young larvae were reared in insect rearing cage provided with fresh cabbage heads and monitored daily. Initially, the larva was yellowish gray. Average body length of full-grown larva was found to be 15 mm. Mature larva was gray in colour with five brownish purple longitudinal bands running the length. The head capsule is black. The body is sparsely covered with moderately long yellow or light brown hairs and tapers at both the anterior and posterior ends. There are five larval instars. Larval developmental period was found to be 16 days. The containers containing full grown larvae were then shifted to bigger ones provided with a bed of water dipped bed of cotton covered with a layer of filter paper for purpose of pupation. Pupae were individually placed in glass jars (15 cm in height and 7 cm in diameter) covered with muslin cloth until the immergence of the adults. These dishes were supplied daily with some drops of water for maintaining appropriate humidity. Pupal period was found to be 9 days. The pupa was yellowish-brown and measure about 9 mm long.

To maintain the culture, larvae were collected from cabbage fields and confined in glass jars in the laboratory and daily fresh cabbage was provided as food. The pupae were kept in a glass jar for adult emergence [12]. The eggs masses and different larval instars were placed in Petri dishes as well as in transparent containers of different sizes depending upon the size

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of the larvae. The containers containing fully mature larvae were then shifted to bigger ones provided with a bed of water-dipped bed of cotton covered with a layer of filter paper for purpose of pupation. The freshly emerged adults then shifted to insect rearing cages, which were provided with 10% sucrose solution to record their longevity. The full-grown caterpillars stops feeding and become sluggish and spin cocoon between leaves and at the entrance of the feeding tunnel whereas the other fully mature larvae hide itself in the soil and pupate there. Then it changes itself in to brown colored pupa. The segmentations of different body parts are seen clearly [13]. The pupation period of *H. undalis* was noted 9 days.

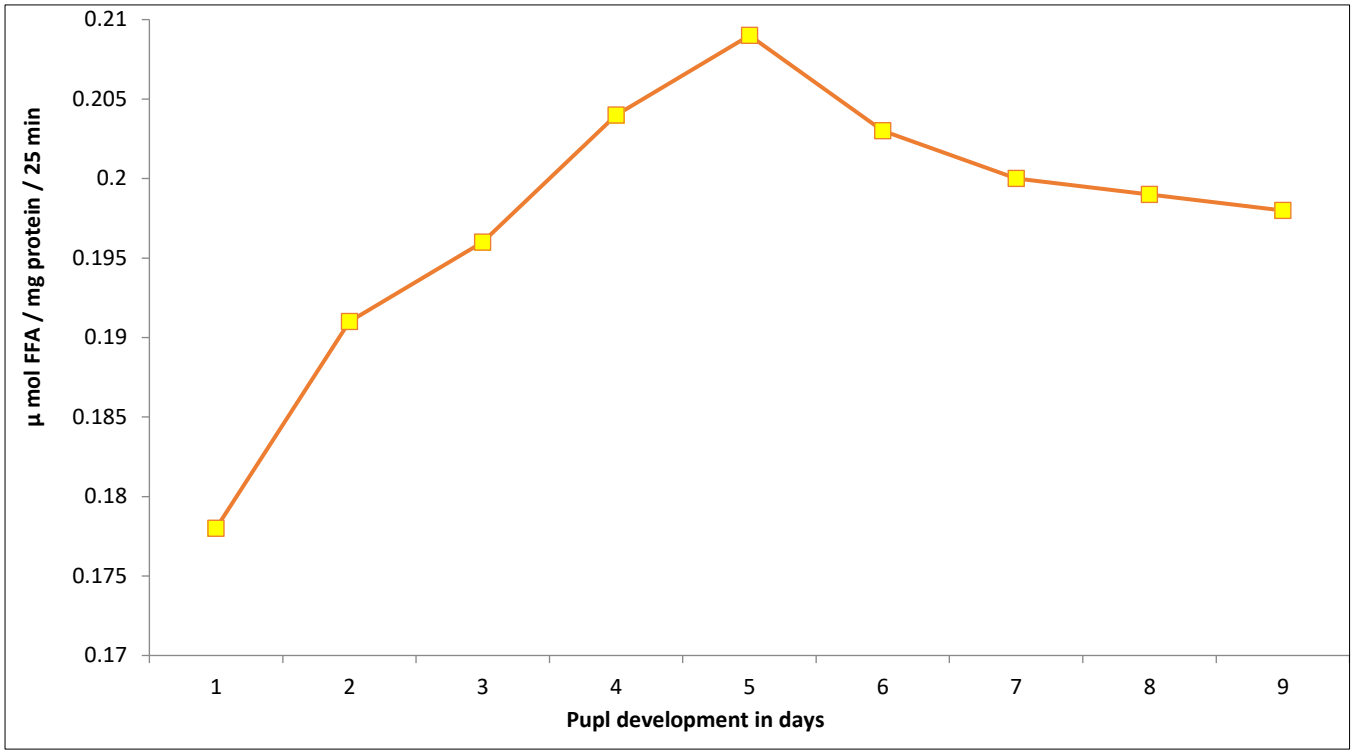

Fig 1 Triacylgycerol acylhydrolase activity during day pupal stages of *H. undalis*

Partial purification of triacylglycerol acylhydrolase will be attempted by ammonium sulphate precipitation method [14]. The desiccated ammonium sulphate will be used to ensure uniform and rapid dissolution. The day before use, ammonium sulphate will be placed over night in oven at 120 °C in a large beaker. Amount of powdered ammonium sulphate for 70 % saturation required 43.6 grams/ 100 ml. For 70% saturation, the desired amount 4.36 grams of powdered ammonium sulphate will be slowly added with rapid stirring on magnetic stirrer to the 10 ml (1%) homogenates of larvae, pupae, male moths and female moths, larval gut and larval fat body of *H. undalis*. Homogenates will be allowed to precipitate for 30 minutes at 4 ^{0}C with stirring. Precipitation will be recovered by centrifugation for about 30 minutes at 10000 rpm [15] and pellet was separated. The pellet will be re-suspended in a volume of phosphate buffer (suitable pH for larva, pupa, male moth, female moth, larval gut and larval fat body) equal to the volume of homogenates (10 ml) and then such partially purified enzyme (0.25 L) will be used to triacylglycerol acylhydrolase assay. total volume of 1.5 mL in glass stoppered conical flask [16] Transferred upper, clear 2 ml of chloroform phase and 1ml of colour reagent (Diphynilcabazone and diphynilcarcazid) was added. Plot graph of optical density [17].

The metamorphosis was completed in 9 days. The partial characterization of triacylglycerol acylhydrolase activity reveled optimum pH 7.8, incubation time 20 minutes, 1% enzyme concentration, substrate concentration 6 % and maximum activity at 37 °C optimum temperature and K_m value of pupal triacylglycerol acylhydrolase is 0.09614×10^{-2} mM pupa of *H. undalis.* The maximum lipase activity was observed in 5th day old pupa. Lipolytic activity gradually increased from 1st day to 5th day and decreased from 6th day to 9th day pupae of *H. undali.* Triacylgycerol acylhydrolase activity during day pupal stages of *H. undlis* shown in (Fig 1).

The maximum lipase activity was noted in 5-day old pupa and gradually increased from 1 to 5 day and decreased from 6 to 11-day pupae of *Earis vittella* [18]. The maximum lipase activity was observed in 5-day old pupa. liipolytic activity gradually increased from 1 to 5 day and decreased from 6 to 8-day pupae of *M. vitrata*. The Km value of pupal lipase is 0.095×10^{-2} mM. Lipolytic activity during pupal development of *M. vitrata* [19].

SUMMARY

Studies on triacylglycerol acylhydrolase activity during metamorphosis of *Hellula undalis* (Fab.). The partial characterization of triacylglycerol acylhydrolase activity reveled optimum pH 7.8, incubation time 20 minutes, 1% enzyme concentration, substrate concentration 6% and maximum activity at 37 °C optimum temperature and K_m value of pupal triacylglycerol acylhydrolase is 0.09614×10^{-2} mM pupa of *H. undalis.* The maximum lipase activity was observed in 5th day old pupa. Lipolytic activity gradually increased from 1st day to 5th day and decreased from 6th to 9th day pupae of *H. undalis*

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