

Partial characterization of lipase in male moth of *Maruca vitrata* (Fabricius) (Lepidoptera, Pyralidae)

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ABSTRACT: Partial characterization of lipase in male insect moth of *Maruca vitrata* revealed pH 7.6, temperature 37 °C, incubation time 25 minutes, substrate concentration (5%), enzyme concentration (1%) and K_m value 0.0993×10^{-2} mM. Maximum lipase activity noted in 4th day insect moth of *M. vitrata*. The adult longevity of insect moth was found to be seven days. The physiological significance of lipase in male insect moth of *M. vitrata* has discussed. © 2025 Association for Advancement of Entomology

KEY WORDS: Enzyme kinetics, hydrolases, lepidoptera adult, spotted pod borer

The bean pod borer, *Maruca vitrata* (F.) (Lepidoptera, Pyralidae), is one of the most serious pests of cowpea, *Vigna unguiculata* (Taylor, 1967). It is one of the serious pests and occurs during flowering and pod formation stages causing huge losses (Pappu *et al.*, 2010). In insects, lipids are involved in several physiological functions like molting during larval and adult development (Kawooya and Law, 1988). Triacylglycerol lipase hydrolyzes certain positions of triacyl glyceride while different phospholipases act on different ester bonds within phospholipids (Zibae *et al.*, 2008). However, the information about the partial characterization of lipase in male insect moth of *M. vitrata* is rather scanty. In the present work, partial characterization of lipase in male insect moth of *M. vitrata* has been discussed.

The larvae from infested pods were kept in cowpea pods in insect rearing cage. Tiny larvae were transferred to cowpea pods in glass tubes and covered with muslin cloth. A full-grown larva bore large exit holes, leave the pea pods and pupates underneath the pods. The pupae were collected immediately after pupation and were kept in glass jar having 15cm in height and 7cm in diameter covered with muslin cloth until the immergence of the adults. The pupation period of *M. vitrata* was noted as 8 days. Newly emerged male and female moths of *M. vitrata* were kept as 10 pairs in round glass jar. Adults were placed in rounded glass jar, having 12cm height and 30cm in diameter and covered with muslin cloth until the deposition of eggs. Adults were fed with sucrose (10%) solution. Pea pods were placed in rounded glass jar as an

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oviposition site and removed daily (Sharma, 1998). Ammonium sulphate precipitation method was used for purification of lipase (Dawson *et al.*, 1969). For uniform and rapid dissolution, the desiccated ammonium sulphate is used. Ammonium sulphate was placed over night in oven at 120°C in a glass beaker. For 70 per cent saturation required ammonium sulphate was 43.6g 100⁻¹ ml. Homogenate was allowed to precipitate for 30 minutes at 4°C with stirring. Centrifugation used for recovered precipitation about 30 minutes and pellet was separated. Lipase assay included 0.25mL enzyme, 0.25mL substrate (olive oil) and one mL phosphate buffer. Reaction was stopped by two mL Cu-TEA reagent; 10mL of chloroform was used as solvent. Then 2mL of chloroform phase was taken in stoppered test tube and 1mL of colour reagent was added (Hayase and Tappel, 1970). Liberated fatty acids were measured by using calorimeter. The absorbance was read at 540nm (Itaya, 1977).

The survival of male moth of *M. vitrata* was found to be 7 days. Partial characterization of lipase in male insect moth of *M. vitrata* revealed pH 7.6, temperature 37°C, incubation time 25 minutes, substrate concentration (5%), enzyme concentration (1%) and K_m value 0.0993×10^{-2} mM. Maximum lipase activity noted in 4th day insect moth of *M. vitrata*.

Effect of pH on lipase activity: In present work, maximum lipase activity in male insect moth at pH 7.6 indicated the presence of alkaline lipase in the male insect moth of *M. vitrata* (Fig. 1). Highest lipase activity was observed at optimum pH 9.5 in male of *Helicoverpa armigera* (Chamani *et al.*, 2016). Lipase activity in *Callosobruchus maculatus* studied at pH 8 (Silva *et al.*, 2017). Lipase activity in host specific *Bemisia tabaci* G., studied at pH 8 (Anokhe *et al.*, 2018). Effect of pH on lipase activity in butterfly, *Junonia atlites* and *Junonia almanac* studied at pH 6.8 (Saptarini *et al.*, 2021).

Effect of enzyme concentration on lipase activity: In present study, maximum lipase activity in male insect moth at one per cent enzyme concentration suggest rate of release of fatty acid

was optimum at one per cent enzyme concentration. Increased enzyme concentration showed more rate of reaction after certain concentration further increase in enzyme concentration never increased the reaction rate (Fig. 2). The maximum lipase activity of the enzyme noted at one per cent enzyme concentration in male of *M. separata* (Pol and Salunkhe, 2001). Biochemical studies of lipase in *Callosobruchus maculatus* (Coleoptera, Chrysomelidae) determined at enzyme concentration (2 %) (Silva *et al.*, 2017).

Effect of temperature on lipase activity: In the study, maximum lipase activity was at 37°C, indicating the optimum temperature for enzymatic reaction (Fig. 3). This result suggest the rate of enzyme reaction increased with increase in temperature, within the temperature range in which the enzyme was stable and retained full activity and this happen up to a certain temperature called as optimum temperature. Highest lipase activity studies on male *H. armigera* showed 40°C as its optimum temperature (Chamani *et al.*, 2016). Biochemical studies of lipase in *Callosobruchus maculatus* (Coleoptera: Chrysomelidae) determined 37 °C as its optimum temperature (Silva *et al.*, 2017). Lipase activity of butterfly, *Junonia atlites* higher than *J. almanac* 37°C as its optimum temperature (Saptarini *et al.*, 2021).

Effect of time on lipase activity: The maximum lipase at 25 minutes of incubation time suggested enzyme required 25 minutes for optimum activity (Fig. 4). Lipase activity of *Tribolium confusum* was noted at incubation time 30 minutes (Barra *et al.*, 2015). Digestive lipase of dung beetle, *Chironitis arrowi* was reported 10 minutes as its incubation time (Gaikwad and Bhawane, 2015). Biochemical studies of lipase in *Callosobruchus maculatus* (Coleoptera, Chrysomelidae) studied indicated 30 minutes as its incubation time (Silva *et al.*, 2017).

Effect of substrate concentration on lipase activity: In the present work, maximum lipase activity was at 5 per cent substrate concentration, suggesting it as the optimum substrate concentration. The K_m value 0.0993×10^{-2} mM indicated more affinity of enzyme with the substrate (Fig. 4).

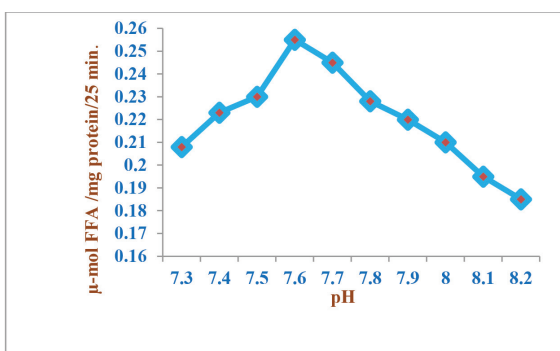


Fig. 1 Effect of pH on lipase activity in male moth in *M. vitrata*

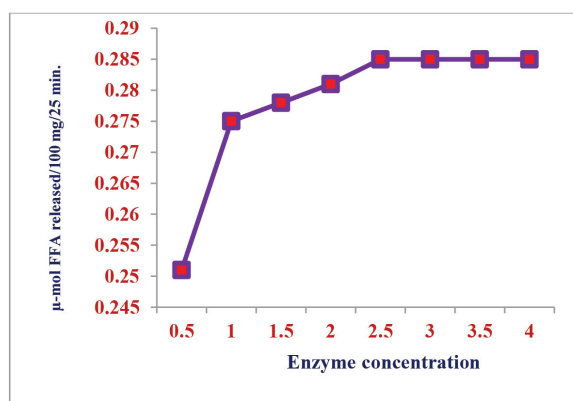


Fig. 2 Effect of enzyme concentration on lipase in male moth of *M. vitrata*

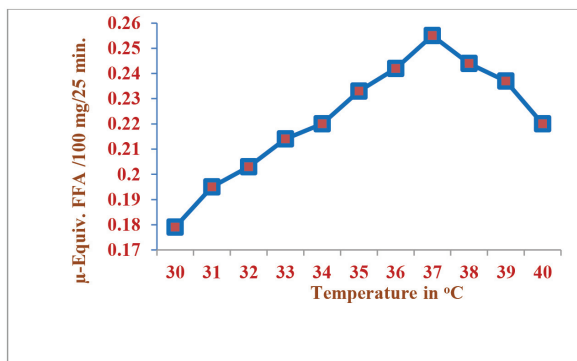


Fig. 3 Effect of temperature on lipase activity in male moth of *M. vitrata*

Maximum lipase activity of phospholipase B in male of *Culex pipiens fatigans* showed $0.8 \mu\text{mol mL}^{-1}$, as the optimum substrate concentration (Rao and Subrahmanyam, 1969). In male of *M. separata* the maximum lipase activity was noted at 5 per cent substrate concentration (Pol and Salunkhe, 2001).

In present study, the increased in lipase activity in male insect moth of *M. vitrata* suggested utilization

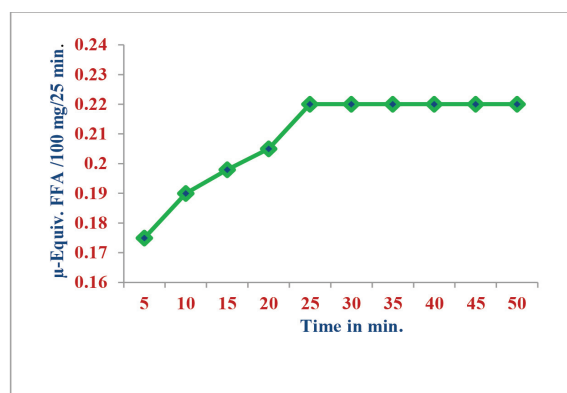


Fig. 4 Effect of incubation time on lipase activity in male moth of *M. vitrata*

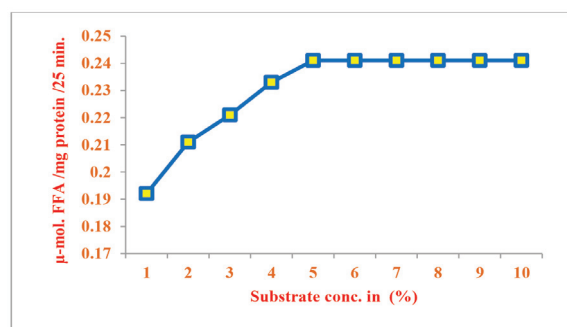


Fig. 5 Effect of substrate concentration on lipase activity in male moth of *M. vitrata*

lipid during the active flight in search of female moth for mating. This increased lipase activity may be concerned with structural components and sperms production. In insects, including male adults, lipase activity can indeed vary and may be higher compared to other developmental stages or female insects. Lipase enzyme in insect is involved in lipid digestion and metabolism. The variation in lipase activity among different developmental stages of male moths can be attributed to differences in nutritional requirements, reproductive physiology, and overall metabolic demands. The maximum lipase activity in 4-day adult male of *M. vitrata* indicates most active stage of moth and energy derived from catabolism of triglycerides. The main source of energy during the male adult development is lipid and lipolytic activity.

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