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Short Communication

Studies on Lipolytic Activity During Larval and Pupal Development of *Maruca vitrata* (Fabricius)

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Spotted pod borer, *Maruca vitrata* (Fabricius) is serious pest on green legumes. Attack of the pest to crop starts right from pre flowering stage and lasts upto pod maturing stage. It forms web by using flower buds and flowers and feeding inside it [1]. *M. vitrata* causes heavy loss about 42 to 80% in cowpea [2]. In India cowpea is one of important leguminous plant. Cowpea is important crop cultivated by small scale farmers [3]. Lipases have a dynamic physiological role; catabolism of triacylglycerols that stored as depots of fat and those from nutritional lipids [4-5]. However, the information about lipase activity during larval and pupal development of *M. vitrata* rather is scanty. Hence, studies on lipolytic activity during larval and pupal development of *M. vitrata* have been undertaken.

Rearing of *M. vitrata* was attempted according to method of Sharma [13]. Pod borer, *M. vitrata* was reared in laboratory condition at temperature 28 ºC and 78 % humidity on their natural food cowpea pods. Larval stages from 4 to 13-day larvae and 1 to 7-day pupae were taken for study.

Lipase assay

Lipase activity was noted according to method of Hayase and Tappel [16]. Lipase assay system contains 0.25 mL of substrate; 0.25 mL partially purified larval and pupal enzyme and 1 mL of phosphate buffer pH 7.9 and pH 7.7 for larva and pupa respectively. The enzyme incubation was attempted at 37 °C temperature and 25 minutes of time in digital shaker. The reaction was stopped with 2 mL of Cu-TEA reagent and after15 minutes 10 mL chloroform was added. The contents were mixed well and allowed to separate aqueous and organic phases. After 15 minutes upper phase was removed and 5 mL chloroform phase was transferred to centrifuge tube. Then 2 mL of water was added without mixing and the tubes were centrifuge for few minutes. The upper water layer was removed carefully and exactly 2 mL of chloroform phase was taken in another stoppered test tube. Then 1 mL of colour reagent was added. At the end liberated fatty acids was measured calorimetrically. The absorbance was read at 540 nm. Lipase activity from larvae and pupae were expressed in terms of specific activity such as µmol of free fatty acids liberated / mg of protein /minute [15].

Preparation of standard cure for lipase assay

Molecular weight of oleic acid is 282.46. Placed 0.1 to 1.0 ml of standard 25 mM of oleic acid solutions and were diluted to 5 ml of chloroform (final 2.5 to 25 µmol of oleic acid). Centrifuge for 5 minutes at 1000 rpm at room temperature [6]. Transferred upper, clear 2 ml of chloroform phase and 1ml of colour reagent (Diphenylcarbazone and diphenylcarbazid) was added. Plot graph of optical density versus µmol of oleic acid [7]. The growth duration of larva and pupa was found to be 13 days and 7 days. The optimum activity observed in 8th day larvae with pH 7.9. Optimum activity was observed in 5th day old pupae with 7.7 pH. The increase in enzyme activity noted from 4 to 8-day larvae and 1 to 5-day pupae. The analysis of mean and standard deviation of larval lipase was 0.255 and 0.01430 respectively. K_m value of pupal lipase is 0.095×10^{-2}mM.

The lipase activity of *Helicoverpa armigera* shows maximum activity in 10% control larvae as compare to 10-day eco-neem treated larvae [8]. Lipolytic activity in *Naranga aenescens* revealed pH 10, temperature 35 °C and specific activity of 5.6 µmol FFA/min /mg protein [9]. Silkworm lipolytic activity during larval growth have been studied [10]. Maximum lipase activity revealed at 37 °C temperature in the midgut of carob moth, *Ectomyeleois ceratoniae* [11]. Lipolytic activity in *Cirrhinus reba* noted at optimum temperature 35 ºC [12]. Lipase activity in larvae of *Antheraea mylita* showed highest activity at 7.52 µmol/min/mg [13]. Purified lipase activity in the larvae of *Ectomyeleois ceratoniae* showed highest activity at pH 7 [14]. Pupal lipase enzyme activity in *Earias vittella* revealed at optimum pH 8 [15]. Maximum lipase activity in *Hellula undalis* noted at pH 7.7 [12].

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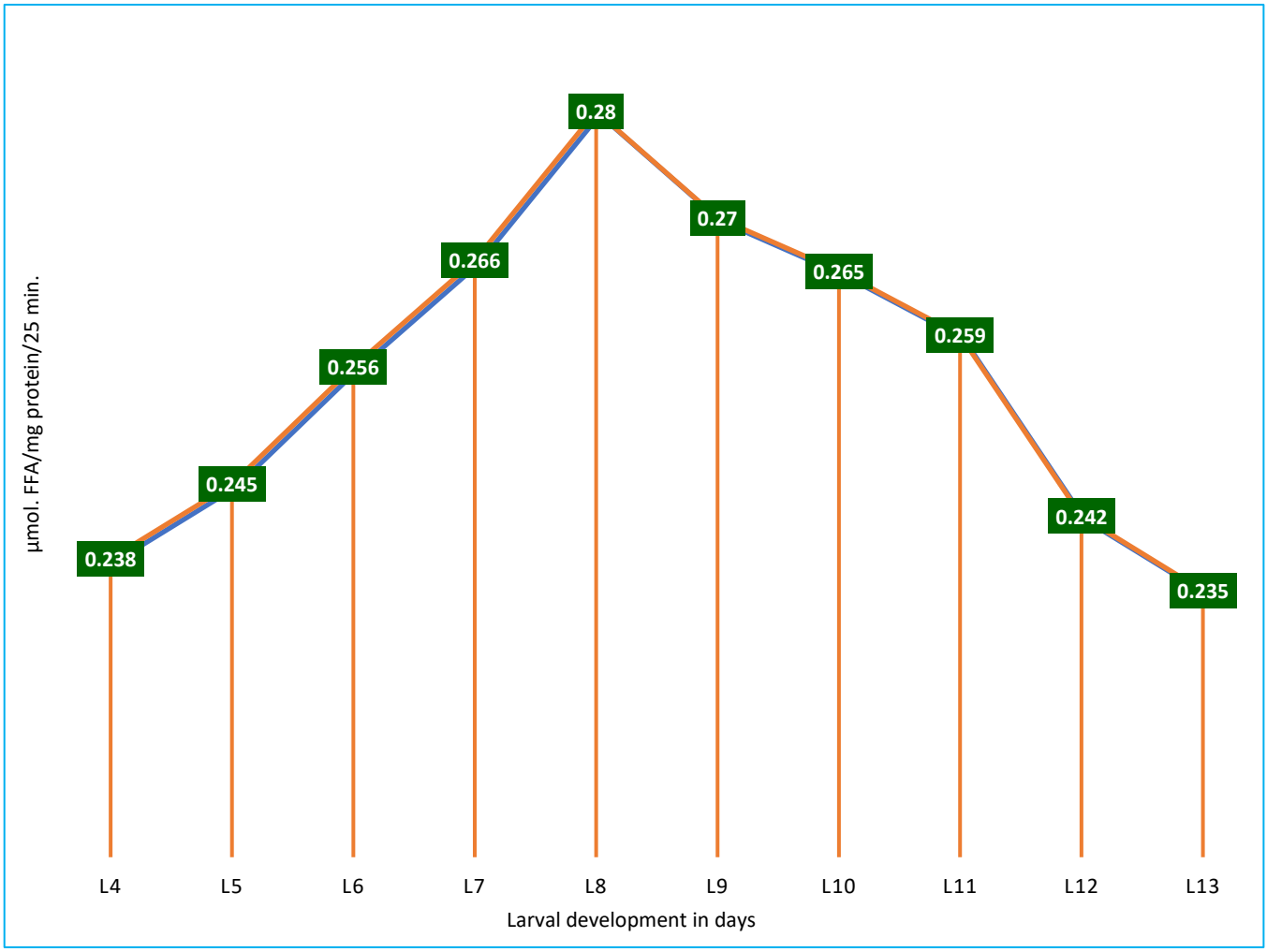

Fig 1 Studies on lipolytic activity during larval development of *M. vitrata*

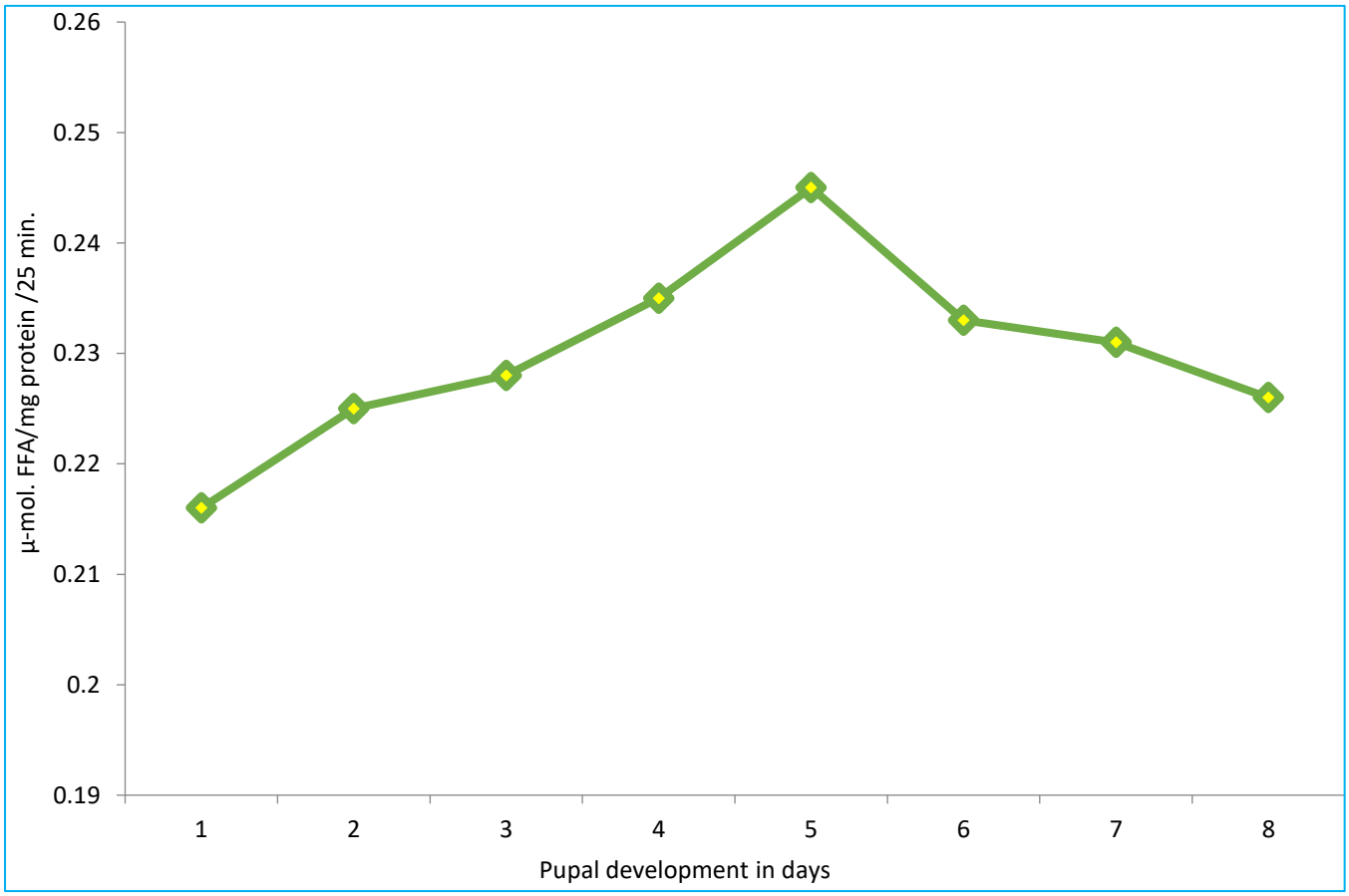

Fig 2 Studies on lipolytic activity during pupal development of *M. vitrata*

SUMMARY

In present study, maximum activity of lipase from larva at pH 7.9 indicates the lipase activate at alkaline pH in the larvae of *M. vitrata.* The increased lipase activity from 4 to 8-days of larval development of *M. vitrata* indicates fast growing larvae required more energy for development. This is useful for fulfilment of lipids for release of energy. The decrease in activity from 8 to 13-days indicates storage of lipids for the further development of larval and pupal stage. This decrease in activity indicated slowed feeding period of larvae and accumulation of lipid for pupal stage. Studies on lipolytic activity in larva and pupa of *M. vitrata* has been noted. The larval growth duration was found to be 13 days. The optimum activity observed in 8th day larvae with pH 7.9. Pupal growth duration was found to be 7 days. Optimum activity was observed in 5th day old pupae. The increase in enzyme activity noted from 4 to 8-day larvae and 1 to 5-day pupae of *M. vitrata*. The mean and standard deviation of larval lipase was 0.255 and 0.01430 respectively.

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