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Spectrophotometric estimation of total Lipid of hosts along with propagating larval instars gut content of *Callosobruchus chinensis* Linn.

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Abstract- The estimation of total amount of Lipid of uninfected and infected hosts and larval instars gut content of *Callosobruchus chinensis* Linn. propagating on legumes like stored grains Gram (*Cicer arietinum*) and Moong (*Vigna radiata*). *Callosobruchus chinensis* Linn. (Coleoptera : Bruchidae) is a major stored grain pest of pulses of India. The pattern of variation in the different larval instars (I-IV) growing on these two hosts provide a unique gradual depletion of the total lipid content of the instar wise infected host as well as the larval gut content lipids.

Key words: Spectrophotometry, *Callosobruchus chinensis,* Lipid, Moong, Gram, Larval instars

INTRODUCTION

Callosobruchus chinensis Linn. is the pulse beetle. The pest (Coleoptera : Bruchidae) and its growth, development and metabolism mainly depends on its nutritional requirement and environmental condition. *Callosobruchus chinensis* Linn. is a pest of stored grains and are responsible for causing significant damage. The adult and grubs eat up the gram kennel and make a cavity. The concentration of biomolecules like protein, carbohydrate, lipids, amino acids, enzymes may vary during the life cycle of all organisms.

In order to strengthen the biochemical basis of host specificity in the pest has further been ascertained through quantitative estimation of total lipid through spectrophotometry or electrophoretic techniques which have been specially attempted in the present investigation.

Lipids serve as a source of metabolic energy and essential for structural components of cells. The lipid in the fat body is an energy reserve which can be mobilized rapidly during starvation, oogenesis, embryogenesis and moulting and is used to sustain continuous muscles actively.[1] Lipids are important constituents of cuticle and help in acylation of glucose-6- phosphate during chitin synthesis.[2]

Lipids are considered as an important group of compounds providing several biological functions as energy storage, cell membrane structure and signaling.[3]

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MATERIALS & METHODS

I- Preparation of Buffers for Spectrophotometric Estimation of biomolecules (Triglycerides):

(a). General Phosphate Buffer (0.1M)

It has following set of solutions:-

A- 0.2 M solution of monobasic sodium phosphate (27.6 g $NaH_2PO_4H_2O$ or 31.2g $NaH_2PO_42H_2O$ in 1000 ml)

B- 0.2M solution of dibasic solution phosphate (35.6 g $Na_2HPO_42H_2O$ or 53.60g $Na_2HPO_47H_2O$ or 71.6g of $Na_2HPO_412H_2O$ in 1000ml)

II - Estimation of Triglycerides by spectrophotometer:

The standard protocol of GPO-PAP method was used and the quantitative triglyceride in mg/dl was calculated at par with the standard kit of triglyceride through following equation. Simultaneously the blank was also used for standardizing the spectrophotometer instrument.

The GPO-PAP (Glycerol-3phosphate oxidase - phenol/4 chlorophenol aminophenazone) method for estimation of total triglycerides involves following 4 steps of reactions:-

1. Triglycerides + water – (enzyme esterase) → glycerol + carboxylic acid
2. Glycerol + ATP – (enzyme glycerol kinase) → glycerol-3-phosphate+ ADP
3. Glycerol – 3 – phosphate + oxygen - (enzyme glycerol-3-phosphate oxidase) → dihydroxyacetone phosphate + hydrogen peroxide
4. Hydrogen peroxide + 4 - aminophenazone + chloropheno – (enzyme peroxidase) → colored complex.

III- Reagent preparation for Triglycerides:

The contents of 1 bottle of L2 (Enzyme Reagent 2) was poured into 1 bottle of L1 (Enzyme Reagent). This working reagent is stable for at least 8 weeks when stored at 2-8°C. Upon storage the working reagent may develop a slight pink colour however this does not affect the performance of the reagent.

As much of working reagent may be made as and when desired by mixing together 4 parts of L1 (Enzyme reagent 1) & 1 part of L2 (Enzyme Reagent 2). Alternatively, 0.8ml of L1 and 0.2ml of L2 may also be used instead of 1 ml of the working reagent directly during the essay.

Test Sample Material:

The mixture of host samples (Gram & Moong) and the gut contents of different larval instar (I-IV) treated with phosphate buffer were used as test sample, pipetted into clean dry test tubes labeled as Blank (B), Standard (S) and Test (T).

The operational procedure was followed as per kit and the optical density of blank, standard and test were taken at 505nm wavelength at 37°C (RT).

Calculation formula:

$$Triglycerides\ in\ mg/\ dl = \frac{\text{Abs. T}}{\text{Abs. S}} \text{x} 200$$

OBSERVATION

In the present study significantly, lipids were identified during larvae growth. The body weights and diameter of larvae increased linearly with days, became larger during the course of development. The larvae may have gained fat to increase their weight during this period. Larval stage would deposit a large amount of fat to accumulate energy for later development in adults.

Estimation of Total Triglycerides

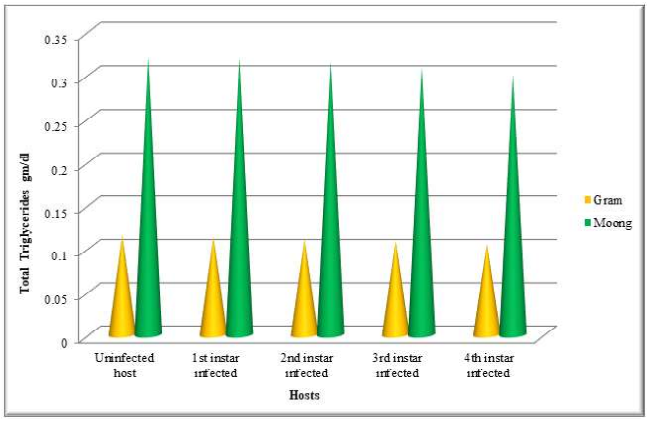
The results of estimation of total lipid by GPO/PAP method as reflected in Table 1 and Graph I A & B clearly establishes that Moong host contained more quantity of total triglyceride in mg/dl in uninfected state (0. 321) whereas, the value for Gram was 0.115 mg/dl.

The pattern of variation in the different larval instars (I-IV) growing on these two hosts provides a unique gradual depletion of the total lipid content of the instar wise infected host as well as the larval gut content lipids. For the host Moong the gradual depletion value of the total triglyceride ranged between 0.321 - 0.290 whereas for the larval instar gut content (I-IV), the value also reflected a declining range from 0.320 to 0.285 mg/dl. Similarly for the host-Gram, the gradual depletion values ranged between 0.115 - 0.104 whereas the gradual depletion value in the larval instar gut content ranged from 0.113 to 0.104.

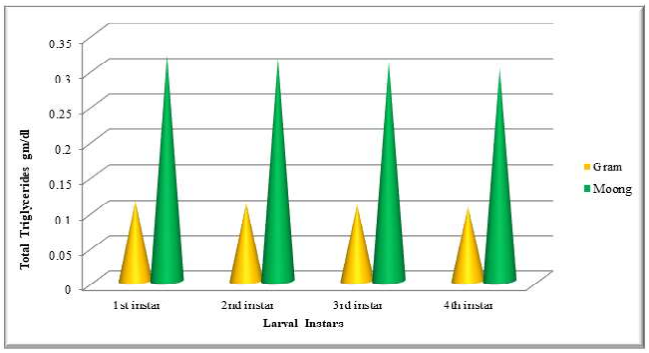
Although the difference range of depletion of host lipid was greater than that of the depletion range of gut content lipid of different larval instars thriving on the host, yet the growth of larval body did not show a significant dependency for the lipid on the host resource as per ANOVA result.

Table 1: Spectrophotometric estimation of Total Triglycerides (by GPO/PAP method) in mg/dl of uninfected & infected hosts - Gram & Moong along with propagating larval instars gut contents (I-IV) of beetle *C.chinensis L.*

Hosts	Total Triglycerides in gm/dl								
	Uninfected host	1st instar infected		2nd instar infected		3rd instar infected		4th instar infected	
		Host	Larva	Host	Larva	Host	Larva	Host	Larva
Gram	0.115	0.114	0.113	0.112	0.110	0.109	0.108	0.106	0.105
Moong	0.321	0.320	0.319	0.317	0.315	0.311	0.309	0.305	0.300



Graph I A : Spectrophotometric estimation of Total Triglycerides (GPO & PAP method) uninfected & infected hosts - Gram & Moong of Gram beetle *C.chinensis* L.



Graph I B : Spectrophotometric estimation of Total Triglycerides (GPO & PAP method) of larval instar gut content (I-IV) of Gram beetle *C.chinensis* L. from Gram & Moong

RESULT & DISCUSSION

The investigation covers the studies of spectrophotometric techniques for the quantitative estimation of lipids. The biochemical basis of host specificity in the pest has further been ascertained through quantitative estimation of the lipids through either spectrophotometric or electrophoretic techniques which have been specially attempted in the present investigation. The results of these quantitative biochemical assays furnished in the (Table 1 & Graph No. 1A & 1B) extend further strength to the findings of the biochemical investigation of host specificity in Gram beetle, *C.chinensis* L.

Stryer (Stryer's *Biochemistry*)[4] have extended the hypothesis that the quantity of the total lipids estimated from the gut content of the larval stages of the pest insects is directly proportional to the types of biomolecules consumed from the host source and their positive role in the metabolic activities. The Moong host in this investigation has been found to be rich in the biomolecular profile of monomeric nature which could have been easily displaced by the feeding larvae from the host source. The choiced by the molecules displaced from the host source played a catalytic role in the protein biosynthesis helpful in the overall morphogenetic processes and the lipids and cholesterol helpful in the fat body production, vitelo genesis and Gunaidol maturation as has been observed in the *Manduca* bugs (hemiptera). The gradual rise in the total lipid in the successive larval instar as estimated through the UV-spectrophotometer directly conveys the information that the larvae almost metabolically empowered in the Moong host over the Gram leading to better population growth. These findings have also been statistically examined and found to be significant through the applications of SD and ANOVA (Analysis of variance for multifactor experimental observations. Synthesising the concepts of the biochemical & biostatistical findings pertaining to this research work, it appears that the Gram beetle- *C.chinensis* L. has better preference to Moong over the Gram in terms of its host specificity. The detail information of spectrophotometric estimation of total of both host (uninfected & infected) and the gut content of the pest larval instar I-IV. It reflects the trend of gradual fall in the total Lipid. Almost trend has been obtained for the quantitative stimulation of total lipid (by GPO/PAP method) but the values of total lipids (triglycerides) in Gram host & the pest larvae I-IV growing on this host are substantially very low (Graphs I A). This might be considered as the constraints in the multiplication of the pest & its larval grown on this host while lipid contents of Moong & its dependent larval instars display a better range of estimated value which is a positive support for the pest multiplication (Graph I B).

REFERENCES

1. **Gilbert L. L. & Chino P. 1974.** Transport of lipids in insects. *J.Lipids Res*. **15:** 439-456.
2. **Wyatt G. R. & Pan M. L. 1978.** Insect plasma protein. *Am. Rav. Entamol*. **4:**779-817.
3. **Wymann M. P. and Schneiter R. 2008.** Lipid signaling in disease. Nature Reviews. *Molecular Cell Biology*. **9(2):**162-176.

4. **Stryer. 2003.** Biochemistry, IIIrd Edn, published by W.H.Freeman and company.

ADDITIONAL REFERENCES

5. **Chittenden. 1898.** Insect injurious to beans and peas. *Year book Agri. Dept,* U.S.A., p-168.

6. **Chino H. & Gilbert L. L. 1965**. Lipid release and transport in insect. *Biochem. Bio Phys. Acta*. **98:**94-110

7. **Chu X., Li M., Wang G., Wang K., Shang R., Wang Z., and Li L. 2020.** Evaluation of the low inclusion of full fatted *Hermetia illucens* larvae meal for layer chickens: Growth performance, nutrients digestibility, and gut health. *Front.Vet.Sci*. **7:**962.

8. **EI-Dakar M. A., Ramzy R. R., Ji H., and Plath M. 2020.** Bioaccumulation of residual omega 3 fatty acids from industrial *Schizochytrium* microalgal waste using black solider fly (*Hermetea illucens)* larvae. *J.Clean.Prod*. **268:**122288.

9. **Ewald N., Vidakovic A., Langeland M., Kiessling A., Samples S., and Lalander C. 2020.** Fatty acid composition of black solider fly larvae (*Hermetia illucens*) –possibilities and limitations for modifications through diet. *Waste management*. **102:**40-47.

10. **Harper S. H. 2000.** Physiology Chemistry, Oxford University, Press, London, U.K.

11. **Lawrence Gilbert I., Kostas Iatrou and Sarjeet S. Gill. 2005.** Comprehensive Molecular Insect Science: Biochemistry and Molecular Biology. *Elsevier Pregamon*. **IV:**I-513.

12. **Lehninger A. H. 2002.** *Basic Biochemistry* ELBS, paperback edition, Oxford, Great Braitan, New Delhi, India, pp 15-65.

13. **Reis A., Rudnitskaya A., Blackburn G. J., Fawzi N. M., Pitt A. R. and Spickett C. M. 2013.** A Comparison of five lipid extraction solvent systems for lipidomic studies of human LDL *Journal of Lipid Research,* **54 (7):**1812-1824.

14. **Van Handel E. 1985.** Rapid determination of total lipids in mosquitoes. *J. Aru. Mosq. Co Nrmol Assoc.,* **1(3):**302-304.

15. **Wyatt G. R. 1967.** The biochemistry of sugar and polysaccharides in insects. *Adv. Insect Physiol.* **4:**287-360.

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