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Spectrophotometric estimation of total protein of larval gut content of *Callosobruchus chinensis* Linn. infesting gram and moong

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Abstract : The estimation of total protein of the host and pest larval gut content of *Callosobruchus chinensis* Linn. infesting on legumes like stored grain Gram and Moong .These legumes contains rich protein in themselves which is very important component of the human dietary for their proper build up. The estimation of total protein is done by spectrophotometric (By Lowry's method) process. The pattern of the different larval instars (I-IV) stages growing on these two host provides the total protein content of the larval instars wise infected host and including the uninfected state of the host.

Keywords : Protein, Gram, Moong, Spectrophothmetric, *C. chinensis* L. Larval instars.

INTRODUCTION

The biochemical basis of the host specificity in the pest has been further ascertained through quantitative estimation of the protein through the spectrophotometric techniques which have been specially attempted in the present investigation[4,6,5] .The coloured compound of a solution has a distinct ability to absorb certain components of the electromagnetic spectrum with which it interacts except a visible colour which is being reflected. By calculating the amount of the light absorbed by a compound concentration of a compound can be determined precisely.

The gradual rise in the total protein 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 the better population growth. The population growth trend of the beetle during the laboratory culture on these two hosts has supported .The pattern of the different larval instars (I-IV) growing on these two host provides the total protein content of the larval instar wise infected host and simultaneous the larval gut content protein including the uninfected state of the two host.[7,1]

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MATERIALS AND METHODS

Preparation of Buffers for Spectrophotometric Estimation of biomolecules (Protein).

(A) General Phosphate Buffer(0.1m).

It has following set of solutions.

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

B:0.2M solution of dibasic solution phosphate(35.6g $NaHPO_42H_2O$ or 53.60g $NaHPO_47H_2O$ or 71.6g of $Na_2HPO_412H_2O$ in 1000ml)

Soluble Protein estimation by Lowry's method.

Lowry's method depend on quantitating the colour obtained from the reaction of protein with Folin-Ciocalteu reagent. The colour so formed is due to the reaction of the alkaline copper wit the protein and the reduction of *phosphomolybdate* and *phosphotungestate* by tyrosine and tryptophan present in the protein .The intensity of the colour depends on the amount of these acromatic acids (tyrosine and tryptophan) present and will thus vary for different proteins.

Reagents

(1)Solution A: 2% Na_2CO_3 in 0.1M NaOH. Dissolved 20g sodium carbonate and 4g sodium hydroxide in distilled water and volume is made to 1 litre.

(2)Solution B: (a) 1%$CuSO_4$.1% H_2O solution .1g of copper sulphate is dissolved in 100ml distilled water.

(b).2% Sodium potassium tartrate solution.2g of sodium pot.tartrate is dissolved in 100 ml distilled water.

Working solution of B: It was prepared fresh before use by mixing equal volume of solution B(a) and B(b).

(3)Solution C: Carbonate –Cu^{++} solution. Solution C is prepared by mixing Solution A and working solution of B. It was prepared fresh befors use by mixing 50 ml of solution A and 1 ml of working solution of B.The solution was discarded if precipitate was formed.

(4) Solution D: Folin-Ciocalteu reagent (IN). Equal volume of water was added if the reagent is of 2N concentration (!:!) before use and it was cold stored in dark amber coloured bottle.

Extraction of protein from host and pest samples.

0.5-1g of the host samples (Gram and Moong) were weighted and grinded well with a pestle and mortar in 5 to 10 ml of phosphate (buffer 0.1M Ph 7.5).It was centrifuged at 10,000 rpm for 10 mimutes to homogenize the mixture.Only the supernatant was used for protein estimation along with standard protein kit in spectrophotometer.

Estimation of protein of pest larvae.

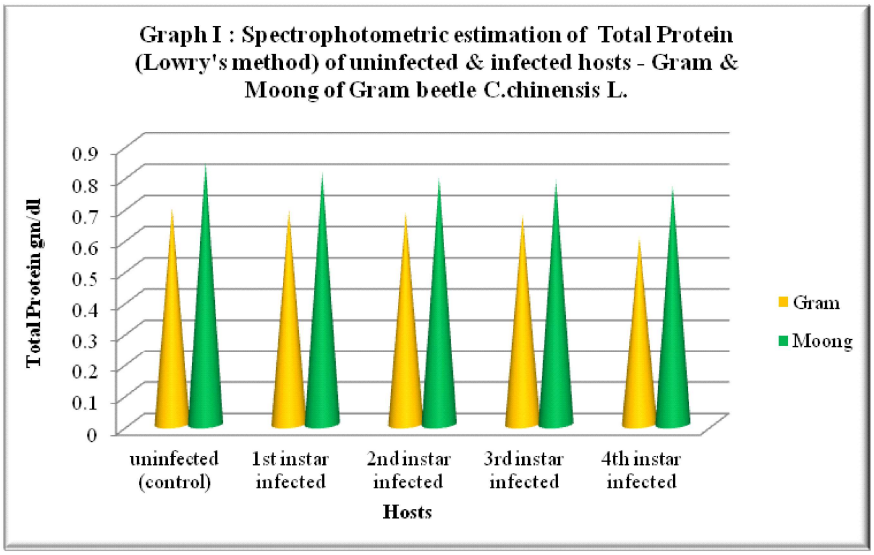
2gm of instar specific larvae (I-IV) of *C.chinensis* was taken by weight and mixed with 5ml phosphate buffer solution in a test tube. Further the larvae and the buffer were crused properly with the mortar and pestle to make the estimation solution. This solution was then homogenized well in ultracentrifuge at 10,000 rpm for 10 minutes. Only the supernatant solution was used along with standard protein kit in spectrophotometer. Simultaneously the blank was also used for standardizing the spectrophotometer instrument.

OBSERVATION

The quantitative estimation of the total protein were done by standard methods whose findings have been presented in **Table I** and **Graph I**

Table I: Spectrophotometric estimation of total protein (Lowry's method) in gm/dl of iuninfectedand infected host- Gram and Moong alongwith propagating larval instars I to IV of beetle *C.chinensis* L.

Hosts	Total protein in gm/dl								
	Uninfected Host	Ist Instar infected		IInd instar infected		IIIrd instar infected		IVth instar infected	
		Host	Larva	Host	Larva	Host	Larva	Host	Larva
Gram	0.695	0.690	0.217	0.682	0.229	0.671	0.251	0.602	0.266
Moong	0.843	0.811	0.235	0.792	0.258	0.785	0.279	0.766	0.291



The result of estimation of total protein by Lowry's method reflects which clearly establish the Moong host contained more quality of total protein in gm/dl in infected state (0.843) whereas the value of Gram was less (0.695 gm/dl).

The pattern of variation in the different larval instars (I-IV) growing on these two hosts also provide a gradual depletion of the total protein content of the larval instar wise infected host and simultaneous increase in the larval gut content protein. For the host Moong the gradual depletion value of the total protein ranged between 0.843-0.712 and for this host dependent pest larvae ,the elevation value of gut content protein ranged from 0.235-0.353. Similarly for the host Gram the gradual depletion values ranged between 0.695-0.553 and for this host dependent pest larvae, the elevation value ranged from 0.217-0.311.

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

RESULT AND DISCUSSION

The quantitative estimation of protein by Lowry's method ascertained the biochemical in vestigation of the host specificity in beetle *C.chinensis* L.[7,3] Stryer's Biochemistry[9] have extended the hypothesis that the quantity of the total protein estimated from the gut content of the larval instar of the pest insects is directly propotional to the types of the 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 the monomeric nature which could have been easily displaced by tefeeding larvae from the host source. [7,8,10]

The detailed information of spectrophotometric estimation of total protein (by Lowry's method) of both host and pest gut content of the larval instars (i-iv). It reflects the trend of the gradual fall in the total protein in gm/dl in the two host varieties linked with gradual rise in the total protein of larval instars(I-IV). Interestingly ,the value of the total protein content of a Moong (0.843gm/dl) is higher than that of Gram and the trend of rise in the protein content of larval instars reflected by the quantum difference between inter-larval total protein value in the Moong dependent pest larvae is also substantially greater than that of Gram.[1,2]

The quantitative estimation values of total protein in both Gram and Moong based larval development are pretty less than the value displaying significant correlation. Nevertheless the gradual rise in total protein of all the larval instar display denovo biosynthesis biomolecules in the larval gut even on the small amount of depletion of these substances from host source. Exhibition of better growth of larval instars of the pet *C.chinensis* L. On Moong host compared to gram is an extraordinary example of high growth output sponsored by low input of material and energy from host source.[8,10,2]

REFERENCES

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

2. **Guenther,W.C.,1964,** Analysis of Variance, Prentice Hall, Inc, Englewood diff.,N.J.pp-100-110.
3. **Harper,S.H.2000,** Physiology Chemistry, Oxford University, Press, London, U.K.
4. **Lawrence Gilbert I ,Kostas Iatrou and Sarjeet S . Gill 2005,** *Comprehensive Molecular Insect Science : Biochemistry and Molecular Biology*, Elsevier Pregamon, Vol –IV,pp-1-513.
5. **Lehninger, A.H., 2002,** *Basic Biochemistry* ELBS, paperback edition, Oxford, Great Britian, New Delhi, India, pp 15-65.
6. **Lipke, H and Franenkes,G.,1956**, Insect nutrition. Annu Rev. Ent -17-h4.
7. **Mathew,S.M.2008,** Principles of biochemistry Vth Ed WJUNG Publisher, Netherland.
8. **Rosenthal, G.A.(1981)**, Role of allelochemics in the specialization of trophic relationship between bruchus and legumes, Vol- 19: ed by V. labeyrie. Dr W.Junk Publishers. The Haque.pp,97-100.
9. **Stryer,2003,** Biochemistry, IIIrd Edn, published by W.H. Freeman and Company.
10. **Wyatt,G.R., 1961,** The biochemistry of haemolymph Ann.Ref.Ent.,6,7-102.

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