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Quantitative estimation of total cholesterol level in different tissue of parasitized bug, *Leptocoris augur* Fabr, a pest of Kusum tree, *Schleichera oleosa* lour

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Abstract
Leptocoris augur is a pest of kusum plant (*Schleichera oleosa*), which in turn is a host of lac insect. The bug is parasitized by a mermithid nematode, *Hexamermis vishwakarma*, which naturally checks rapid built-up of bug population.
The present work reports the results of cholesterol content in different tissue such as Gonads (i.e., testes and ovaries), muscles and haemolymph of parasitized bugs. The total extracted cholesterol was estimated by the method of Annino (1976) and Frings, *etal*, (1972).
The concentration of total cholesterol in control bug (*L. augur*) was very high in the haemolymph as compared to that of the parasitized bugs. The cholesterol content of fat body and muscles of *L. augur* (host) was diminished by *H. vishwakarma* infections. This increasing trend in cholesterol level was observed before the emergence of parasitic juvenile from the host body in female and male bugs. The cholesterol level in haemolymph increased by the mermithid parasitism in reproductive organs of both the sexes. But, in gonads the average level of cholesterol was also found to be depleted as the parasitism advances as compared to control one.

Keywords: *Leptocoris augur*, *Hexamermis vishwakarma*, cholesterol content, Parasitized

Introduction
Leptocoris augur is a pest of kusum plant (*Schleichera oleosa*), which in turn is a host of lac insect. This bug is a gregarious feeder and by its de-sapping habit, viability of the seed is lost (Dhiman and Gulati, 1986) [2]. It is parasitized by a mermithid nematode, *Hexamermis vishwakarma*, which naturally checks rapid built-up of bug population (Dhiman and Kumkum, 2006) [3], (Kumkum 2021).
Lipid are important source of energy for insects. These are obtained from the food and some are synthesized by insect also from the parasitized host bug's muscles, gonads and haemolymph, large quantity of amino acids, lipids and carbohydrates are taken up by the parasitic juvenile of *H. vishwakarma*.
H. vishwakarma. Not only utilize fat body proteins but also the lipid content of the fat as a result of which fat bodies disappears from the haemolymph of *L. augur.* The present work reports the results of cholesterol content in different tissue such as gonads (i.e., testes and ovaries), muscles and haemolymph of parasitized bugs.

Materials and Methods
Total Cholesterol was estimated by the one step method of Wybenga, *et. al*., (1970). Suitable aliquots of lipid extract were transferred to Test (T) in test tubes. 3.0ml of Cholesterol reagent was subsequently added to blank (B), standard (S) & test (T) test tube and it was shaken vigorously for some time. Later, in standard (S) test tube 0.015 ml of working Cholesterol standard, (200mg%) was added. Mixed well and kept the tubes immediately in the boiling water bath exactly for 90 seconds (1½ minutes). These were cooled immediately to room temperature under running tap water. O.D. of standard (S) and test (T) against blank (B) on a Colorimeter with a yellow green filter or on a Spectophotometer at 560 nm was measured. Quantitative determination of cholesterol biochemical's in muscles was made by using a Commercial Kit obtained from Span-Diagnostics Ltd. Company (Surat). Bovine Serums albumin was obtained from Sigma Chemical Company (St. Louis, Mo., U.S.A.). All others chemicals, reagents and solvents were of highest purity and were procured from S. Merk, S.D. Fine Chemicals and Qualigens (Mumbai). The extraction of lipid was done as per the method Folch, *et al*, (1957) and estimation was done according to the procedure outlined by Barnes and Blackstock (1973). The total extracted cholesterol was estimated by the method of Annino (1976) and Frings, *et al.*, (1972) [4]. The O.D. of standard(S) and Test (T) sample was measured on a colorimeter with a green filter against the reagent blank at 540 nm on photo-colorimeter.

Results
The results of analysis are presented in Table-1, 2 and 3. The total lipid in *L. augur* are phospholipids, cholesterol and small amount of high-density lipoprotein cholesterol. In control bugs, the average cholesterol level in haemolymph was estimated as 087.5 ± 14.06 mg/dl in female and 83.3 ± 13.94 mg/dl male. Whereas in 5

days old parasitized female and male bugs, it was 100.0 ± 12.909 mg/dl and 87.5 ± 14.068 mg/dl, in 10 days parasitized bug, the quantity of cholesterol gradually increased as 101.0 ± 9.12 mg/dl in female and 104.1 ± 11.93 mg/dl, in male bug. In 15 days, parasitized female and male bug, it was 104.1 ± 7.68 mg/dl and 116.1 ± 5.27 mg/dl. This increasing trend in cholesterol level was observed before the emergence of parasitic juvenile from the host body *i.e* 104.1 ± 10.03 mg/dl in female and 108.3 ± 8.33 mg/dl in male bugs (Table- 1). The cholesterol level in haemolymph increased by the mermithid parasitism in reproductive organs of both the sexes. But, in gonads the average level of cholesterol was also found to be depleted as the parasitism advances as compared to control one. Lipids are essential structural components of the cell membrane and cuticle and they provide a rich source of metabolic energy. In control bugs it was estimated as 100.0 ± 9.12 mg/dl in ovaries and 41.60 ± 8.33 mg/dl in testes. The average cholesterol level in parasitized ovaries was seen 87.5 ± 10.70 mg/dl, and in testes 37.5 ± 5.590 mg/dl after 5th day of parasitizatism. It gradually decreased as 75 ± 11.18 mg/dl in ovaries and 25.0 ± 5.50 mg/dl in testes after 10 days of parasitization, in 15 days, it was 41.66 ± 10.540 mg/dl in ovaries and 16.0 ± 2.635 mg/dl in testes. Before emergence of parasitic juvenile falls to minimum as 47.91 ± 12.25 mg/dl in ovaries and 10.83 ±1.054 mg/dl in testes respectively (Table- 2). The average cholesterol level in control bugs was estimated as 62.5 ± 10.70 mg/dl in female and 27.08 ± 5.01 mg/dl in male muscles (Table - 3), whereas in 5 days parasitized host bugs, it was as 54.1 ± 11.93 mg/dl in female muscles and 25.0 ± 5.59 mg/dl in male muscles; after 10 days, the quantity of cholesterol was further decreased significantly as 43.8 ± 10.03 mg/dl in female muscles and 22.91 ± 5.96 mg/dl in male muscles. In 15 days, parasitized female and male bug, it was 25.0 ± 5.590 mg/dl and 11.25 ± 1.25 mg/dl. This decreasing trend (quantitatively) in cholesterol level was observed before the emergence of parasitic juvenile too, *i.e.*, 12.9 ± 2.61 mg/dl in female muscles and 5.41 ± 1.50 mg/dl in female muscles.

Table 1: Average cholesterol level in haemolymph (mg/dl) of Control and Parasitized bugs (*L. augur*) in laboratory.

S. No.	Days of Parasitization	Sex (Female)	Sex (Male)
		(♀)	(♂)
		X ± SX	X ± SX
1.	Control	087.5 ± 14.06	083.3 ± 13.94
2.	5th day	100 .0 ± 12.90	087.5 ± 14.06
3.	10th day	101.0 ± 09.12	104.1 ± 11.93
4.	15th day	104.1 ± 07.68	116.6 ± 05.27
5.	Just before emergence	104.5 ± 10.03	108.3 ± 08.33

Average has been taken of 10 observations of each one.
Abbreviation- X = Average mean
SX = Standard error

Table 2: Average cholesterol level in gonads (mg/dl) of Control and Parasitized bugs (***L. augur***) in laboratory.

S. No.	Days of Parasitization	Sex(Female)	Sex (Male)
		(♀)	(♂)
		X ± SX	X ± SX
1.	Control	100.00 ± 09.12	41.60 ± 8.33
2.	5th day	087.50 ± 10.70	37.50 ± 5.59
3.	10th day	075.00 ± 11.18	25.00 ± 5.50
4.	15th day	041.66 ± 10.54	16.60 ± 2.63
5.	Just before emergence	12.90± 02.61	10.83 ± 1.05

Average has been taken of 10 observations of each one.
Abbreviation- X = Average mean
SX = Standard error

Table 3: Average cholesterol level in muscles (mg/dl) of Control and Parasitized bugs (***L. augur***) in laboratory.

S. No.	Days of Parasitization	Sex (Female)	Sex (Male)
		(♀)	(♂)
		X ± SX	X ± SX
1.	Control	62.5± 10.70	27.08 ± 05.01
2.	5th day	54.10±11.93	25.00 ± 05.59
3.	10th day	43.80±10.03	22.91± 05.96
4.	15th day	25.00±05.59	11.25± 01.25
5.	Just before emergence	12.90± 02.61	05.41 ± 01.50

Average has been taken of 10 observations of each one.
Abbreviation- X = Average mean
SX = Standard error

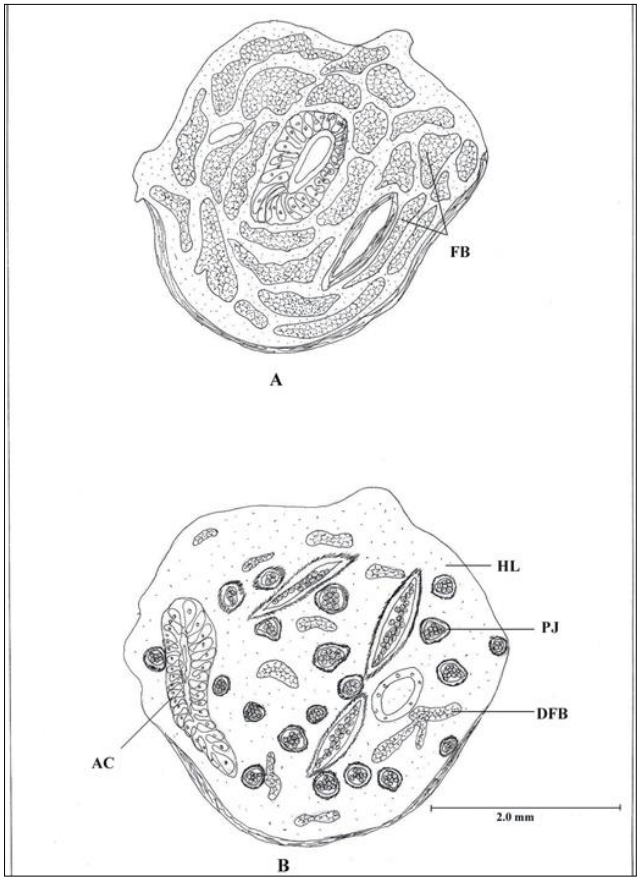

Fig 1: a. t.s. through abdomen of healthy *L*. augur b. parasitized *L.* augur
Abbreviation – fb- fat bodies, hl – haemolymph, pj- parasitic juvenile dfe- degenerated fat body, ac- alimentary canal

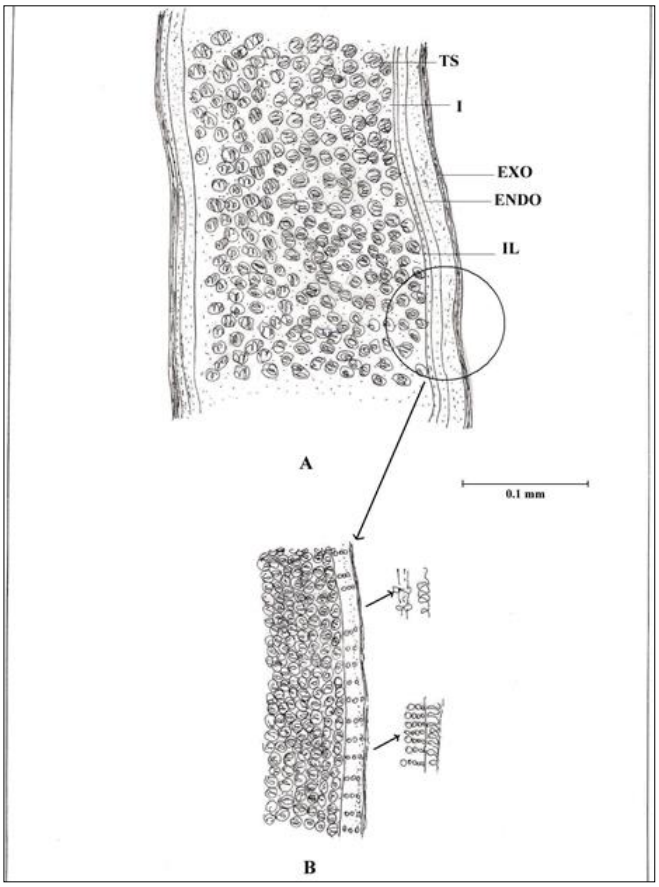

Fig 2: Absorption of nutrients through body wall of *Hexamermis Vishwakarma* Dhiman Abbreviation-Intestine, EXO- Exocutical, TS- Trophosome, IL- Intestinal lumen

Discussion

The concentration of total lipid in control bug (*L. augur*) was very high in the haemolymph as compared to that of the parasitized bugs, but the average level of cholesterol appears to be increased. During the middle of the first reproductive cycle, the female generally has higher cholesterol level of the haemolymph. Cholesterol in control bugs is related to its utilization in the synthesis, steroid metabolites and regulators, or it is used for structural components of growing cells and tissues, as suggested by Robbins, *et al.*, (1971) [12]. This is in close agreement with the findings of Rutherford, *et al.*, (1976) [15] and Rao, *et. al*., (1992) [11].

The cholesterol level in haemolymph increased by the mermithid parasitism in reproductive organs of both the sexes. This was due to the fact that the parasitic juveniles might digest the protein lipid and secreting hydrolytic enzymes. Mermithid parasitism had induced changes in the host metabolism.

The lipid content of fat body and muscles of *L. migratoria* (host) was diminished by *Mermis nigrescens* infections as indicated earlier by Justum and Goldsworthy (1974) [7].

Fuchs (1915) [15], Schvester (1950; 1957) [16]; Ruhm (1956) [13]; Nickle (1971 [9]; Poinar and Caylor (1974) [10], reported that bark beetles infected with *Parasitylenchus* and *Neoparasitylenchs* are also known to show aberrant gallery construction, reduced longevity, reduced flight activity, reduced fat body and sterilization.

H. vishwakarma not only utilize fat body proteins but also the lipid content of the fat as a result of which fat bodies disappears from the haemolymph of *L. augur*. The fatty acid composition of the host haemolymph is not significantly changed by *H. vishwakarma* parasitism in *L. augur* of both the sexes but the levels of cholesterol appear to be increased. Rutherford and Webster (1976) [15] also observed similar changes. According to Gordon and Webster 1971 [6], female locust hosts generally contain more nutrients than males and nymphs, and that developing *Mermis nigrescens* remain longer, grow large, and are more likely to become female in female locusts. This implies that emergence from the host may be influenced by the availability of a limiting nutrient or by the buildup of a toxic product in the haemocoel. Heavy infection of *L. augur* increased the uric acid level of the haemolymph five times more that of the control. Thus, *H. vishwakarma* infections diminish the efficiency of excretory system.

All the parasitized bugs are incapable of taking flight. A bluish mark develops on the abdominal sternite either towards right or left of the median body axis. Feeding requirements of the host bug greatly increases and it devotes more time to feeding. Moreover, no copulation and oviposition has been observed in parasitized bugs. Finally, after the emergence of parasitic juvenile, the host bug gets tired and dies soon. Since, parasitic juvenile of *H. vishwakarma* first takes nutrients from haemocoelamic fluid, hence, there is quantitative loss in this vital body fluid and then fat bodies are consumed. Flight muscles as well as other thoracic muscles are devoured by the parasitic nematode. Super- parasitism results in the destruction of posterior part of alimentary canal. Testes gets greatly reduced, vasa-deferentia dissolved and no connection is left between male gonads and ejaculatory duct. Thus, male bugs are sterilized.

In female, maturation of ovum in ovary is greatly affected and hence ovaries have been recorded smaller. Moreover, oviducts are also damaged. Muscles of external genitalia have also been observed destroyed. Parasitization in nymphal instars inhibits the ecdysis. It seems that parasitic juveniles produce some substance which inhibits the actively and secretion of prothoracic glands which secretes moulting hormone or ecdysone. Thus, parasitism is fatal for either sex and brings sterility and 100% mortality of this bug.

Conclusion

The concentration of total cholesterol in control bug (*L. augur*) was very high in the haemolymph as compared to that of the parasitized bugs. The cholesterol content of fat body and muscles of *L. augur* (host) was diminished by *H. vishwakarma* infections (Fig. 1 and Fig. 2).

This increasing trend in cholesterol level was observed before the emergence of parasitic juvenile from the host body in female and male bugs. The cholesterol level in haemolymph increased by the mermithid parasitism in reproductive organs of both the sexes. But, in gonads the average level of cholesterol was also found to be depleted as the parasitism advances as compared to control one. Due to parasitism is fatal for *Leptocoris augur* (both sex) and brings sterility and 100% mortality of this bug.

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