IJNRD.ORG ISSN : 2456-4184
INTERNATIONAL JOURNAL OF NOVEL RESEARCH AND DEVELOPMENT (IJNRD) | IJNRD.ORG
An International Open Access, Peer-reviewed, Refereed Journal

# Larval Trematodes Infecting with Fresh Water Molluscas Melania Tuberculata and Melania Scabra

**Chandore L.S.**

G.M.D. Arts, B W. Commerce & Science College Sinnar

**Sawant P.L.**

Associate professor

Arts, Commerce & Science Chhatrapati Shivaji College, Oomerga. Dist. Usmanabad

**Arjun shahu**

Assistant professor

Maratha Vidya Prasarak Samaj's

Shrimati Vimlaben Khimji Tejookaya Arts, Science & Commerce College

**Abstract:**

It is well documented that various developmental larval stages of diagenetic trematode parasites of vertebrates including humans and domestic animals such as sporocysts, radia and cercariae commonly develop in their intermediate aquatic snail hosts. In India, both common and rare forms of these trematode larvae have been reported from various freshwater snail species inhabiting different lentic and lotic habitats in different geographical regions. However, so far most studies have been done on their taxonomy, seasonal occurrence or infection and behaviour.

**Introduction:**

There are some environment aquatic indicators which perform behavior of snail. Aquatic indicator such as water salinity according to the localities of water resources. Physical indicator photo toxic, temperature shows the behavioral changes in the intermediate host snails related to the patency and normal activities of the snails.

Mostly snails are aquatic in natural habitat some time *Melania scabra* found on the terrestrial habitat. Respiration through gill lamdae is the main function of aquatic animals *Melania tuberculata* is sometime benthonic in nature so that they are merge in to the mud region it burrows the mud and receive the oxygen from this mud region but too much period they floating on the surface other water with some vegetation sub spectrum. According to the water BOD the snails are shows variation in their activities. The $O_2$ and $CO_2$ level depends on the contaminant particles of water it is the main factor for maintenance of respiratory system of snails. At the time of emerging cercariae

the snails are also changes their behavior changing movement and mostly show increasing gaseous bubbles around snails. From that number of radiae *cercariae* and *metacercariae* emerging from the infected snails.

**Material Method:**

In laboratory work snails are collected from various localities of water reservoir during the study period from June 2008 to 2010 at the beginning. This period snails are collected in the laboratory. After collection of snails first washout with tap water and dechlorinated. Infected snails are sorted with the help of morphological study and behavioral activities of the snails. There are two types of snails in present study *Melania tuberculata and Melania scabra*. The size of snail and color is mentioned for the identification of infected intermediate host snail. The batches of non-infected and infected snails are separated and observed under the laboratory inspections. The total periods are 7 days for observation of releasing cercariae and emerging cercariae from the infected host snails.

In this period there are also another species of host snail we have to study for compete the present study material *Melania tuberculata and Melania scabra*. All the collected snails comparatively studied by micro-observation with their morphology of shell, size of shell, color of shell and size of the snails. The data given with month wise early in the year for observation table.

In the laboratory experiments snails are detected and separate the tissue body in separate watch glasses. The experiments carried on this tissue material for estimation glycogen, proteins, lipids and nitrogenous waste matter. The tissue was dried in the thermostat oven adjusted at 65–70-degree Celsius in order to remove humus from tissue. The oven dried tissues were grinded in order to get prepared dry powdered form and then subjected to biochemical analysis.

**Estimation of glycogen**

The amount of glycogen biomolecules present in different body components or tissues was estimated by Anthron reagent method (De Zwann and Zandae 1972). The results are expressed in mg/ 100 mg in dry weight basis.

**Estimation of total lipid or fats**

The amount of total fats in both infected and non-infected snails body component are estimated with the help of Vaniline reagent method by Barnes and Blackstock (1973) and the results are expressed in terms of mg/100mg tissue on dry weight basis.

**Estimation of nitrogenous waste products.**

In the observation of glycogen and lipid simultaneously the nitrogenous waste products viz ammonia, urea and uric acid also determined quantitively in the same body tissue component of both infected and non-infected snail host *Melania tuberculata and Melania scabra*. The amount of ammonia was estimated by the titrimatic method determined by Hawk et.al (1965) the urea and uric acid was estimated by the Colorimetric method.

**Biochemical Content Analysis**

Table shows all types of content by comparatively infected and non-infected snails.

**Glycogen content**: In normal or non-infected snails least, glycogen was noticed in the mantle (i.e., 10.173 ± 123) and maximum in hepatopancreas (25.468 ± 1.444). in parasitized snail there decrease in the glycogen content of hepatopancreas during throughout the period of infections (15.155 ± 0.345) almost half of the content over non infected snails. Similar observation found in gonads of infected and non-infected snails.

**Total protein content:** Maximum amount of total protein content (47.145 ± 3.490) was present in non-infected snails. The infected snails find out decrease and shows alternation amount in all the tissues of organs of infected host snail due to larval trematode parasitic infection.

**Total lipids content:** In the laboratory observation total lipid contents also minimum as compared glycogen and protein in the infected and non-infected snails *Melania tuberculata and Melania scabra*. Minimum amount in muscles and maximum amount in hepatopancreas due to larval trematode infection.

Impact of larval trematode infection on nitrogenous waste product of different tissues of *M. tuberculata and M. scabra*

**Ammonia:** The ammonium level is minimum in foot in non-infected while infected shows maximum level.

**Urea:** amount of urea present in gonads (2.968 ± 0.020) and in the hepatopancreas (2.662 ± 0.041) of non-infected snails.

**Uric acid:** uric acid levels of all the tissues are low as compared with other types of nitrogenous waste of non-infected snails. Least amount of uric acid is noticed in foot muscles (0.319 ± 0.015) and maximum (1.0644 ± 0.0311) in hepatopancreas of normal snail during the biochemical investigations. The maximum amount of uric acid accumulation found in infected snails intermediate host *Melania tuberculata and Melania scabra*.

**Material Methods and Observations of Biochemical contents of Melania tuberculata and Melania scabra.**

The material collected from host snails are used for the determination of content of proteins, lipids, glycogen and ammonia, uric acid, urea. According to the various methods for identification and measurements of content of biomolecules by proper and the standard deviation method chemical dry weight with molecular level.

**Glycogen content:** the observation of glycogen content in infected and non-infected snail *Melania tuberculata and Melania scabra* properly noticed in mantle. Noninfected snail mantle (i.e. 11.173 ± 0.125) and maximum un hepatopancreas (25.478 ± 1.445). in parasitized snails there are decrease in the glycogen content of hepatopancreas during throughout the period of infection glycogen content is (13.672 ± 1.004) gonad (11.664 ± 8.00) non infected gonad (21.090 ± 2.200).

**Total protein content:** Maximum amount of protein content (45.136 +- 2.540) was present in the non-infected hepatopancreas and minimum amount in the mantle (22.110 +- 0.511) in normal non infected snails so the total protein content is double in non-infected snail than during patency period of infected snails due to larval parasitic infection.

**Total lipid content:** According to the laboratory observation infected and non-infected snails *Melania tuberculata* and *Melania scabra* total lipid content lowest in foot muscle (6.532+-6.20) and maximum (15.868+- 1.031) in hepatopancreas. Due to larval trematode infection, there are drastic changes found foot and mantle the total lipid contents table no. 9 and 10

**Impact of nitrogenous waste products of Melania tuberculata and Melania scabra tissues.**

Maximum nitrogenous waste products are found in infected snail tissue mantle and foot. There is continued increasing ammonium byproduct in the infected snails.

**Urea content:** In the observation period non-infected snail has maximum amount of urea in gonads and in hepatopancreas (2.777 ± 044). The infected snail shows fewer amounts in gonads and hepatopancreas but continuous accumulation occurs in rest fissure table fig. 1.1 and 1.2.

**Uric acid:** The uric acid level is less in the non-infected snail while more in the infected snails less amount of uric acid reported in the foot muscles (0.309 ± 0.014) and maximum (1.645 +-0.0311) in hepatopancreas of normal snail the biological investigations. In the infected uric acid accumulation declintissue due to trematode larval infection it absorbs all these material for metabolism.

**Table 1.1 Standard of protein by biuret method**

| Sr. No | B.S.A in ml | Distilled water in ml | Burette reagent in ml | Optical density |
|---|---|---|---|---|
| 1. | 0.1 | 0.9 | 2 ml | 0.08 |
| 2. | 0.8 | 0.8 | 2 ml | 0.15 |
| 3. | 0.7 | 0.7 | 2 ml | 0.25 |
| 4 | 0.6 | 0.6 | 2 ml | 0.32 |
| 5 | 0.5 | 0.5 | 2 ml | 0.40 |

*Source: Primary Data*

**Table 1.2 Amount of oxygen consumption during Initial phase of Melania tuberculata and Melania scabra**

| | **Amount of oxygen consumed in mg $O_2$/liter/hr./gm. body weight at different hours during Initial phase of the day** | | | | | |
|---|---|---|---|---|---|---|
| | 8:00 a.m. | 12:00 noon | 16:00 p.m. | 20:00 p.m. | 24:00 mid night | 4:00 a.m. |
| **Non-infected** | 0.0642 ± 0.0011 | 0.0638 ± 0.0020 | 0.0630 ± 0.0016 | 0.0632 ± 0.0017 | 0.0635 ± 0.0014 | 0.0640 ± 0.0013 |
| **Infected** | 0.0781 ± 0.0036 | 0.0778 ± 0.0021 | 0.0780 ± 0.0022 | 0.0784 ± 0.0034 | 0.0779 ± 0.0010 | 0.0755 ± 0.0042 |

*Source: Primary Data*

**Graph 1.1 oxygen consumption during Initial phase of Melania tuberculata and Melania scabra**

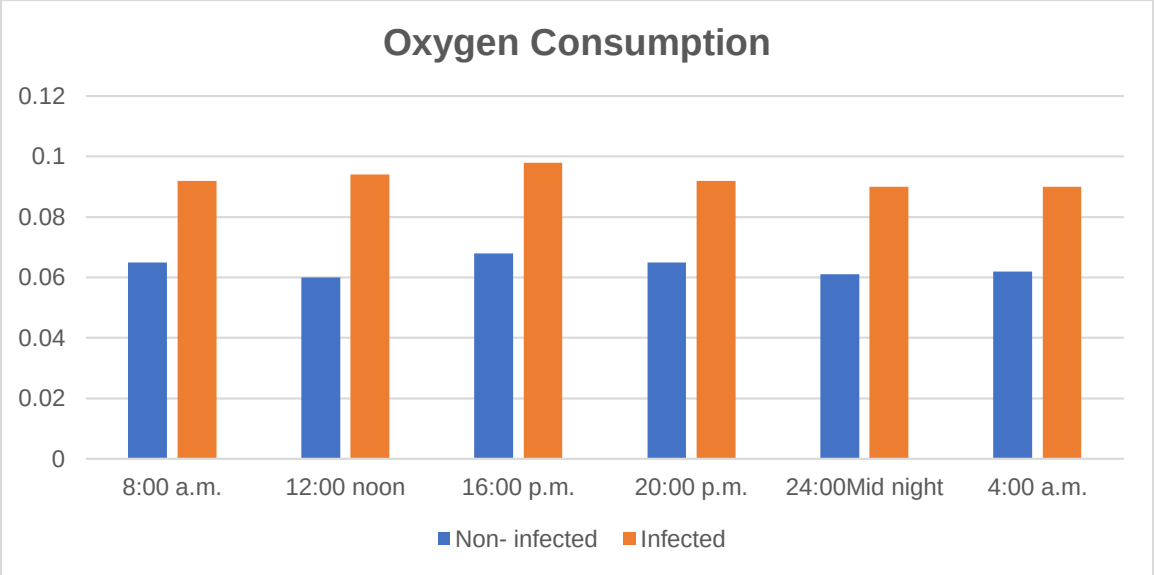



**Table 1.3 Amount of oxygen consumption during Peak phase of Infection of snail (Melania Scabra*)***

| Snail Category | Amount of oxygen consumed in mg $O_2$/liter/hr./gm. body weight at different hours during peak phase of the day | | | | | |
|---|---|---|---|---|---|---|
| **Time** | 8:00 a.m. | 12:00 noon | 16:00 p.m. | 20:00 p.m. | 24:00 Mid night | 4:00 a.m. |
| **Non-infected** | 0.0655 ± 0.0051 | 0.0650 ± 0.0090 | 0.0648 ± 0.0068 | 0.0635 ± 0.0012 | 0.0631 ± 0.0023 | 0.0632 ± 0.0036 |
| **Infected** | 0.0942 ± 0.0063 | 0.0944 ± 0.0032 | 0.0948 ± 0.0049 | 0.0972 ± 0.0009 | 0.0970 ± 0.0067 | 0.0910 ± 0.0078 |

*Source: Primary Data*

**Graph 1.2 oxygen consumption during Peak phase of Infection of snail (Melania Scabra*)***

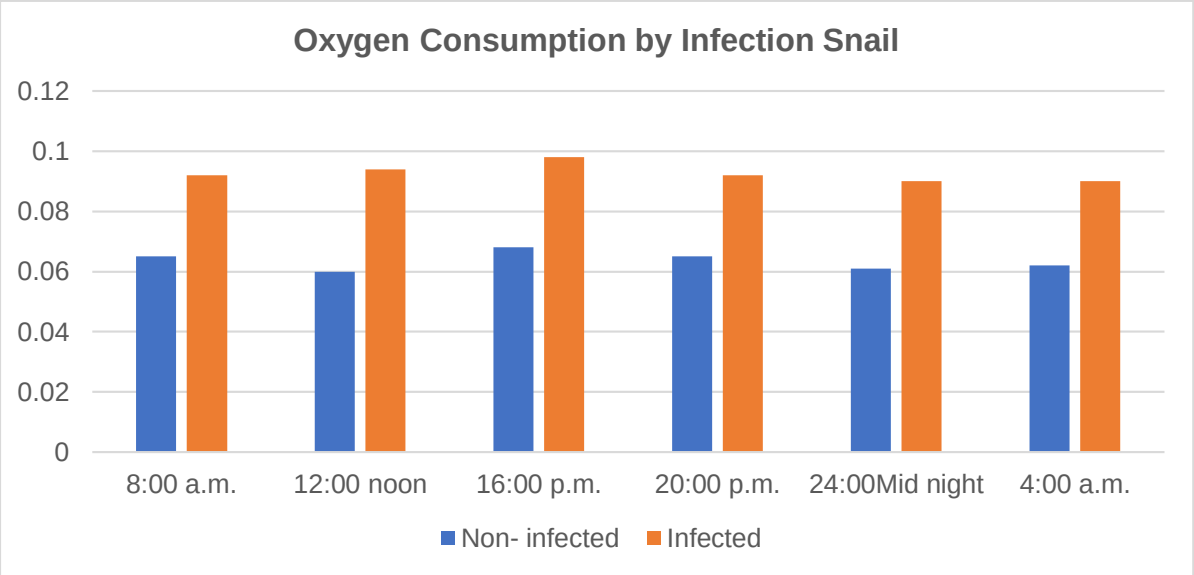



**Table 1.4 Amount of oxygen consumption during post phase of infection of snail Melania tuberculate**

| Snail Category | Amount of oxygen consumed in mg $O_2$/liter/hr./gm. body weight at different hours during post phase of the day | | | | | |
|---|---|---|---|---|---|---|
| **Time** | 8:00 a.m. | 12:00 noon | 16:00 p.m. | 20:00 p.m. | 24:00 mid night | 4:00 a.m. |
| **Non-infected** | 0.0672 ± 0.0042 | 0.0651 ± 0.0013 | 0.0641 ± 0.0071 | 0.0642 ± 0.0053 | 0.0640 ± 0.0080 | 0.0668 ± 0.0066 |
| **Infected** | 0.0751 ± 0.0063 | 0.0745 ± 0.0078 | 0.0740 ± 0.0055 | 0.0714 ± 0.0049 | 0.0704 ± 0.0049 | 0.0700 ± 0.0093 |

*Source: Primary Data*

**Graph 1.3 Oxygen consumption during post phase of infection of snail Melania tuberculate**

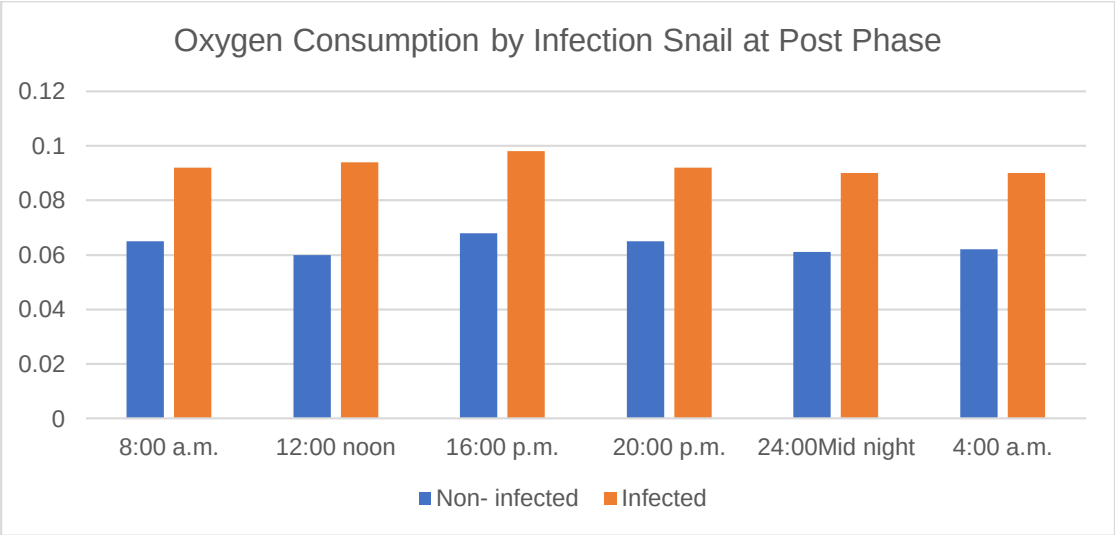



**Table 1.5 Amount of oxygen consumption during Initial phase of infected period of Melania tuberculata**

| Snail Category | Amount of oxygen consumed in mg $O_2$/liter/hr./gm. body weight at different hours during Initial phase of the day | | | | | |
|---|---|---|---|---|---|---|
| **Time** | 8:00 a.m. | 12:00 noon | 16:00 p.m. | 20:00 p.m. | 24:00 Mid night | 4:00 a.m. |
| **Non-infected** | 0.0642 ± 0.0011 | 0.0638 ± 0.0020 | 0.0630 ± 0.0016 | 0.0632 ± 0.0017 | 0.0635 ± 0.0014 | 0.0640 ± 0.0013 |
| **Infected** | 0.0781 ± 0.0036 | 0.0778 ± 0.0021 | 0.0780 ± 0.0022 | 0.0784 ± 0.0034 | 0.0779 ± 0.0010 | 0.0755 ± 0.0042 |

*Source: Primary Data*

**Graph 1.4 oxygen consumption during Initial phase of infected period of Melania tuberculate**

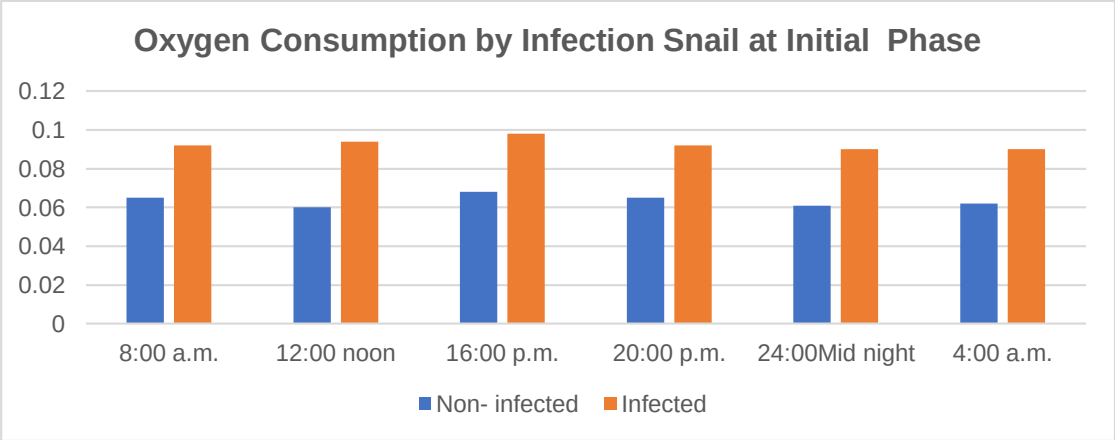


**Table 1.6 Amount of oxygen consumption during Peak phase of infection of snail (Melania tuberculate)**

| Snail Category | Amount of oxygen consumed in mg $O_2$/liter/hr./gm. body weight at different hours during peak phase of the day | | | | | |
|---|---|---|---|---|---|---|
| **Time** | 8:00 a.m. | 12:00 noon | 16:00 p.m. | 20:00 p.m. | 24:00 mid night | 4:00 a.m. |
| **Non-infected** | 0.062 ± 0.0011 | 0.068 ± 0.0020 | 0.060 ± 0.0016 | 0.062 ± 0.0017 | 0.065 ± 0.0014 | 0.060 ± 0.0013 |
| **Infected** | 0.071 ± 0.0036 | 0.078 ± 0.0021 | 0.070 ± 0.0022 | 0.074 ± 0.0034 | 0.0779 ± 0.0010 | 0.0755 ± 0.0042 |

*Source: Primary Data*

**Graph 1.5 oxygen consumption during Peak phase of infection of snail (Melania tuberculate)**

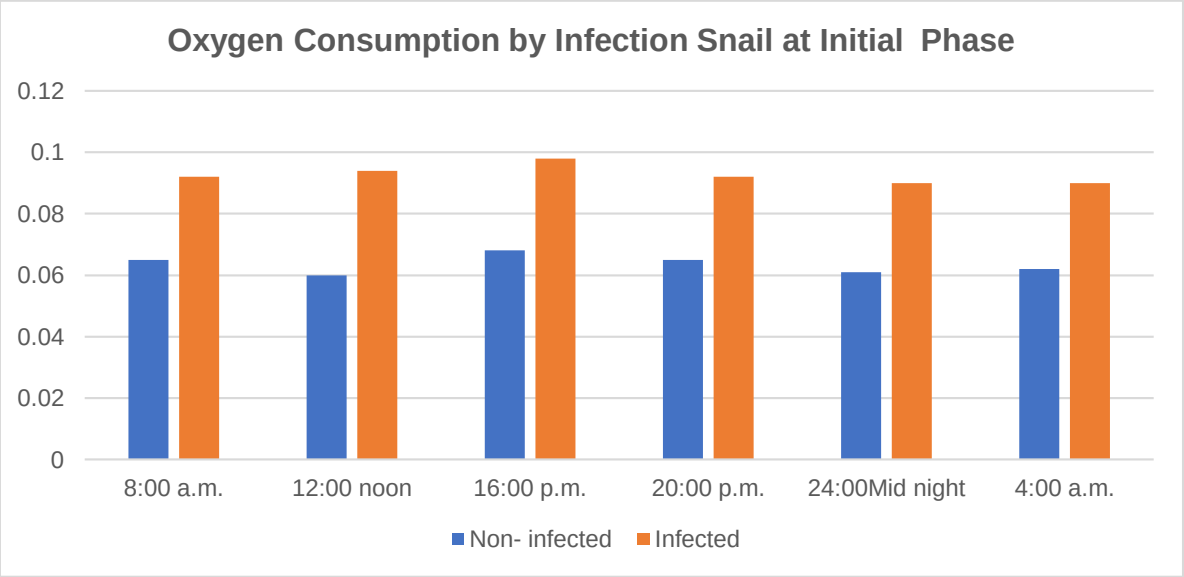



**Table 1.7 Amount of oxygen consumption during Peak phase of infection of snail. Melania tuberculate**

| Snail Category | Amount of oxygen consumed in mg $O_2$/liter/hr./gm. body weight at different hours during peak phase of the day | | | | | |
|---|---|---|---|---|---|---|
| **Time** | 8:00 a.m. | 12:00 noon | 16:00 p.m. | 20:00 p.m. | 24:00 mid night | 4:00 a.m. |
| **Non-infected** | 0.0942 ± 0.0063 | 0.0944 ± 0.0032 | 0.0948 ± 0.0049 | 0.0972 ±0.0009 | 0.0970 ±0.0067 | 0.0910 ±0.0078 |
| **Infected** | 0.0655 ±0.0051 | 0.0650 ±0.0090 | 0.0648 ±0.0068 | 0.0635 ±0.0012 | 0.0631 ±0.0023 | 0.0632 ±0.0036 |

*Source: Primary Data*

**Graph 1.6 oxygen consumption during Peak phase of infection of snail. Melania tuberculate**

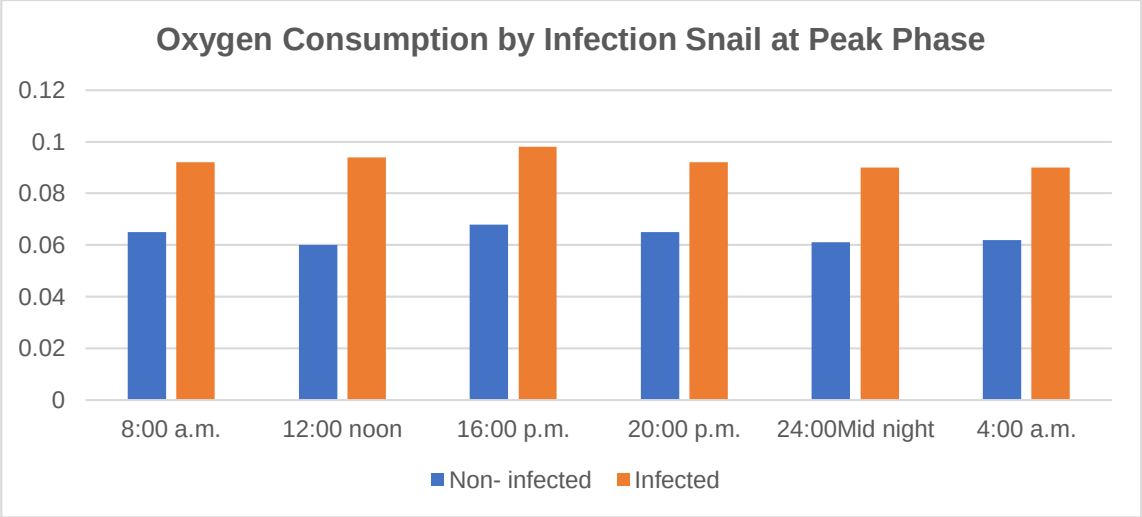



**Table 1.8 Amount of oxygen consumption during Peak phase of infected period of snail (*Melania scabra)***

| Snail Category | Amount of oxygen consumed in mg $O_2$/liter/hr./gm. Body weight at different hours during peak phase of the day | | | | | |
|---|---|---|---|---|---|---|
| **Time** | 8:00 a.m. | 12:00 noon | 16:00 p.m. | 20:00 p.m. | 24:00 mid night | 4:00 a.m. |
| **Non-infected** | 0.065 ± 0.0051 | 0.060 ± 0.0090 | 0.068 ± 0.0068 | 0.065 ± 0.0012 | 0.061 ± 0.0023 | 0.062 ± 0.0036 |
| **Infected** | 0.092 ± 0.0063 | 0.094 ± 0.0032 | 0.098 ± 0.0049 | 0.092 ± 0.0009 | 0.090 ± 0.0067 | 0.090 ± 0.0078 |

*Source: Primary Data*

**Graph 1.7 oxygen consumption during Peak phase of infected period of snail (*Melania scabra)***

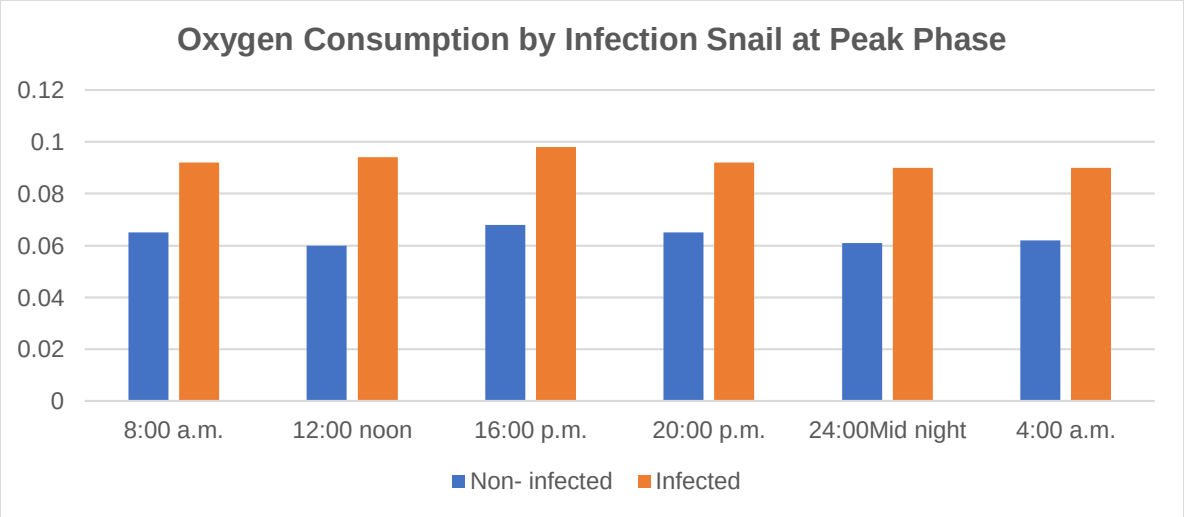



**Table 1.9 Amount of oxygen consumption during Initial phase of infected period of snail (*Melania scabra)***

| Snail Category | Amount of oxygen consumed in mg $O_2$/liter/hr./gm. body weight at different hours during Initial phase of the day | | | | | |
|---|---|---|---|---|---|---|
| **Time** | 8:00 a.m. | 12:00 noon | 16:00 p.m. | 20:00 p.m. | 24:00 mid night | 4:00 a.m. |

| | | | | | | |
|---|---|---|---|---|---|---|
| **Non-infected** | 0.0642 ± 0.0011 | 0.0638 ± 0.0020 | 0.0630 ± 0.0016 | 0.0632 ± 0.0017 | 0.0635 ± 0.0014 | 0.0640 ± 0.0013 |
| **Infected** | 0.0781 ±0.0036 | 0.0778 ±0.0021 | 0.0780 ±0.0022 | 0.0784 ± 0.0034 | 0.0779 ± 0.0010 | 0.0755 ± 0.0042 |

*Source: Primary Data*

**Graph 1.8 oxygen consumption during Initial phase of infected period of snail (*Melania scabra*)**

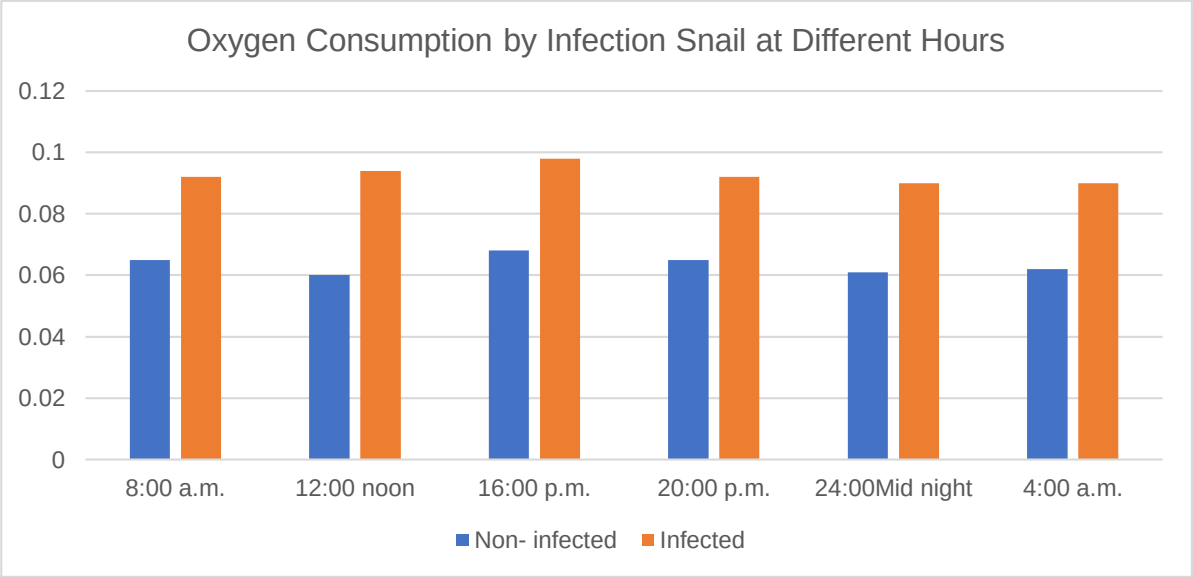



**Table 1.10 Amount of oxygen consumption during Peak phase of infected period of snail (*Melania scabra*)**

| **Snail Category** | **Amount of oxygen consumed in mg $O_2$/liter/hr./gm. Body weight at different hours during peak phase of the day** | | | | | |
|---|---|---|---|---|---|---|
| **Time** | 8:00 a.m. | 12:00 noon | 16:00 p.m. | 20:00 p.m. | 24:00 mid night | 4:00 a.m. |
| **Non-infected** | 0.065 ± 0.0051 | 0.060 ± 0.0090 | 0.068 ± 0.0068 | 0.065 ± 0.0012 | 0.061 ± 0.0023 | 0.062 ± 0.0036 |
| **Infected** | 0.092 ± 0.0063 | 0.094 ± 0.0032 | 0.098 ± 0.0049 | 0.092 ± 0.0009 | 0.090 ± 0.0067 | 0.090 ± 0.0078 |

*Source: Primary Data*

**Graph 1.9 oxygen consumption during Peak phase of infected period of snail (*Melania scabra*)**

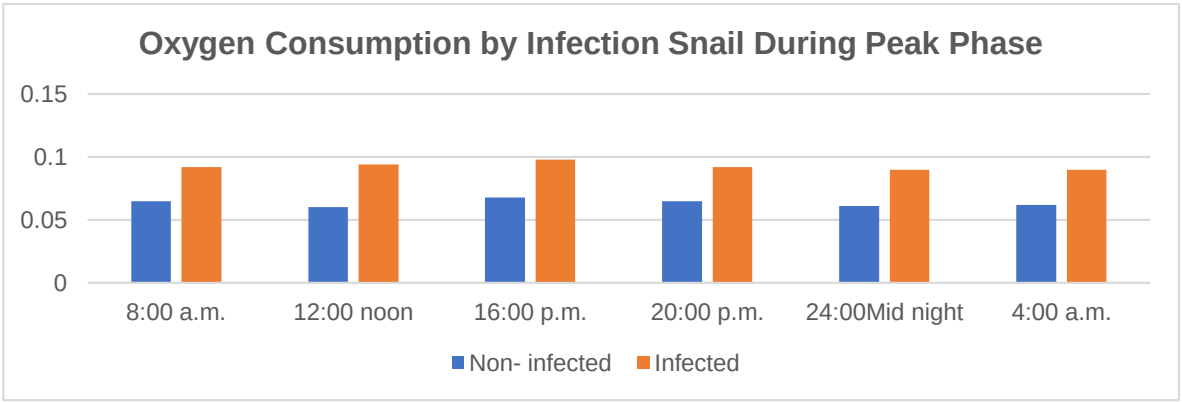


**Figure 1.1 Pharengeal Infected and Non-Infected (Melenia Tuberculated)**

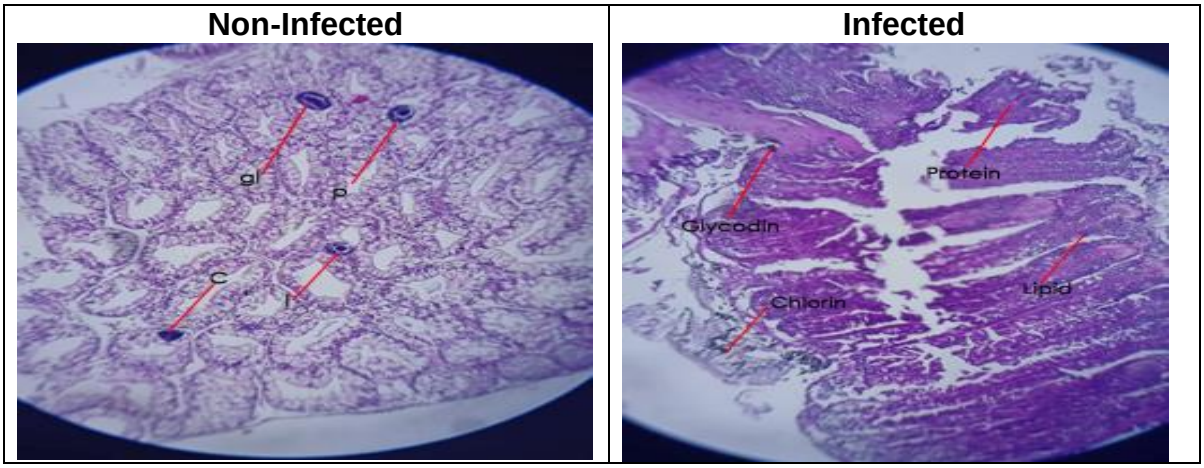
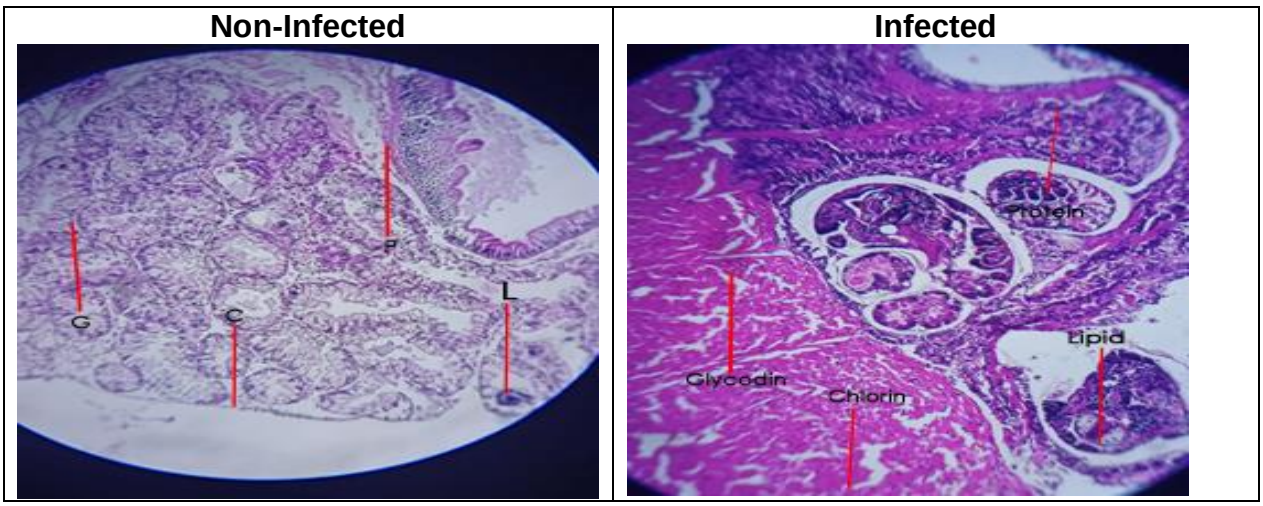



**Figure 1.2 Pharengeal Infected and Non-Infected (Melenia Scabra)**




**The discussion about Biochemical content of Melania tuberculata and Melania scabra.**

In aquatic animal's water oxygen is very important factor i.e. maintenance of BOD. *Melania tuberculata* and *Melania scabra* collected from various localities of study area. According to the localities there is also variation of B.O.D depends on environment and water contamination with infections of trematodes larval.In aquatic life the snails are compete the environment for metabolic and physiological interactions. The parasite affects the snail's behavior process referred to by Von Brand (1975) as food robbery. The host nutrition affected by parasitic mode of nutrition with gross physiological expression of the host snails. The present freshwater intermediate host *Melania tuberculata* and *Melania scabra* found invade various parasite during patency period. The pathogen shows alternation of generation due to their life cycle stages so that intermediate host snails are affected its metabolic rate in all the tissues of internal organisation with effects on the morphological texture. Reader (1971) Bithamiatentaculata infected by sporocyst of cercariae *helcitica* or rediae of *cercariae cytogenata*.

In the present study work in laboratory *Melania tuberculata and Melania scabra* during the 7 days infected period has been observed like hepatopancreas and gonadial complex found infections of various cercarial trematode. The *cercariae Diplostmum* trematode species mostly found in the

intermediate host. In present study various species of snails were found infected by the different types of the larval trematodes. The glycogen level in hepatopancreas was found depletion of glycogen level. It is half of the level of non-infected snails observed. According to this is the glycogen is energy requirement of the metabolism of snail. The infected snails have trematode larval forms like rediae and *metacercariae* which absorbs the glycogen and depleted the percentage and metabolism of snail tissue. Portel (1970) and Robson and Williams (1971) in the digestive gland as well as foot of snail Littorinalittorea. Isshauk et.al (1975) also observed decreased in the glycogen level of the infected snails. *Biomphalarialexandrina* and *Balinustruncatus*. The infected snail decreases the rate of glycogenesis and phosphate pyruvic acid cycle in the tissue by the process of energy breakdown.

**Conclusion:**

The present study material *Melania tuberculata and Melania scabra* reported that amino acids and protein level changes due to the trematode larval infection. Gilberston et.al (1967) reported progressively decreased *haemolymph* amino acid and protein concentration in *A. glabratus* during infection period by *schistosomanosoni* and protein concentration were also reported by Lee and Cheng (1972) infected *B.glabrata*. In the present studies total protein and amino acid similar progressive decreased levels in both infected study snail *Melania tuberculata and Melania scabra* due to continuous releasing of trematode larval cercariae. Over all decreased amino acid concentration was reported in digestive gland of *M.tuberculata and M.scabra* intermediate host snails. In the laboratory observation *M. tuberculata* and *M.scabra* having infections of larval trematode cercariae and development in hepatopancreas and gonads with ovatestis. It is believed that amino acid is the building block in development of cercariae and their simultaneous releasing.

During the observation period non infected snail *M.tuberculata* and *M.scabra* maintain the total protein and amino acid level double of the concentration in the various tissue and hepatopancreas and gonads. It can be concluded that parasitic infection may be cause an enhanced rate of tissue protein catabolism the be resulting depletion in total protein and amino acids in parasitized intermediate host snail and other other aquatic animals. Beaker (1980) observed a similar type of significant reduction in the total protein content in the *hymolyph* of *B. glabrata* parasitized by *S.mansoni*.

Lipid is the important bioenergtic biochemical present in all the living organisms. The snail actively synthesized lipid and stored in the form of sterols, fatty acids and triglycerides. Mostly lipids are utilized at the time of starvation period of animal same in aquatic larval system i.e. snails. The nutritional requirement of lipid less but it is also depleted due to the parasitize larval trematode infection to hepatopancreas and gonads properly Thomson and Lee (1987) demonstrated that the free phosphatide pool of the digestive glands in *B.glabratais* significantly reduced infected snail.

The present study *M.tuberculata* and *M.scabra* infection shows the depletion of lipids in the hepatopancreas and *ovatestis* due to responsible infection of cercariae schistosome trematode

(Thomson and et.al 1986). In this study some time *M.tuberculata* shows the stable levels of lipid content. In all living organisms including molluscan nitrogenous containing material may not be retain within animal body. Aquatic animal mainly retains the ammonia is major products of the nitrogenous waste material amino acid interacts the catabolism and eliminated nitrogenous wastes. In infected snail the catabolism of nitrogenous material is more because of parasite also shows the continuous process of catabolism of protein and amino acids. (Horne and Boonkoom 1970) some species pathway of ammonia detoxification and nitrogen excretion pH Osmo regulation *Limaxflavus* snail.

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