The Asian Journal of Animal Science (June, 2011), Vol. 6 Issue 1 : 58-59 | *RESEARCH ARTICLE*

On a new species of the genus *Calycobothrium* (Southwell, 1911) from *Chiloscyllium plagiosum* at Malvan, Sindhdurg district West Coast of Maharashtra, India

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ABSTRACT

The present investigation deals with a new cestode *Calycobothrium maharashtrii* from *Chiloscyllium plagiosum* at Malvan, Sindhurg District, West Coast of Maharashtra.The present parasites having scolex quadrangular, with four circular suckers, tentacles finger like, 18 in number, neck short, the mature proglottids broader than long , testes four to five in number, cirrus pouch rounded, the vas deference short, genital pore submarginal, ovary bilobed and granular vitellaria.

Key words : *Calycobothrium maharashtrii, Chiloscyllium plagiosum,* West Coast of Maharashtra

The genus *Calycobothrium* was erected by Southwell in 1911 with its type species *C. typicum* from *Aetobatis narinari* (Euphrasen, 1790) at Ceylon. Later on no species is added to this genus. The present communication deals with *Calycobothrium maharashtrii* n.sp. from the intestine of *Chiloscyllium plagiosum* (Anonymous, Bennett, 1830) at Malvan, Sindhdurg District, (West Coast of Maharashtra) India, during the period of December 2003 to November 2005.

MATERIALS AND METHODS

Twenty six specimens were collected from the intestine of *Chiloscyllium plagiosum* (Anonymous, (Bennett,) 1830) at Malvan, Sindhdurg District, (West Coast of Maharashtra) India, during the period of December 2003 to November 2005. Ten worms were taken for taxonomical studies.

RESULTS AND DISCUSSION

Scolex is quadrangular in shape. It measures 0.75 (0.4611-1.0387) in length and 0.854 (0.4854-1.2183) in width. Anterior region bears with four circular suckers. The suckers measures 0.1310 (0.1165-0.1456) in length and 0.1480 (0.1359-0.1601) in width. Posterior portion bearing 18 finger like tentacles spreading from central cavity. The tentacles measures 0.2645 (0.2330-0.2961) in length and 0.03883 (0.02427-0.05339) in width.Neck is short and measures 0.1796 (0.1553-0.2038) in length and 0.580 (0.5728-0.5873) in width.

The mature proglottids are broader than long, measures 0.5606 (0.5485-0.5727) in length and 1.02 (0.9660-1.0776) in width. Testes are four to five in numbers, oval in shape, overlapped, pre-ovarian, measures 0.1650 (0.1456-0.1844) in length and 0.4223 (0.4077-0.4368) in width. Cirrus pouch is rounded in shape, elongated, placed above the middle of the proglottids, measures 0.2572 (0.2378-0.2766) in length and 0.1140 (0.1067-0.1213) in width. Cirrus is long, protrusible in some proglottids, slightly curved, measures 0.3276 (0.3252-0.33) in length and 0.01456 (0.09708-0.01941) in width. The vas deferens is short, slightly curved, transversely directed and measures 0.2087 (0.2038-0.2135) in length and 0.0072 (0.004854-0.09708) in width. The genital pores are sub-marginal, large, oval, irregularly alternate and measures 0.02184 (0.01941-0.6247) in length and 0.01213 (0.09708-0.01456) in width.

The vagina is anterior to cirrus pouch, measures 0.6237 (0.5970-0.6504) in length and 0.1092 (0.07281-0.1456) in width. Receptaculum seminis is short, tube like, reaches to ootype and measures 0.0461 (0.04368-0.04854) in length and 0.03883 (0.02427-0.05339) in width.

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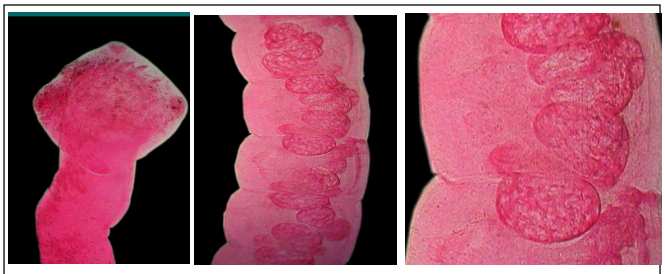
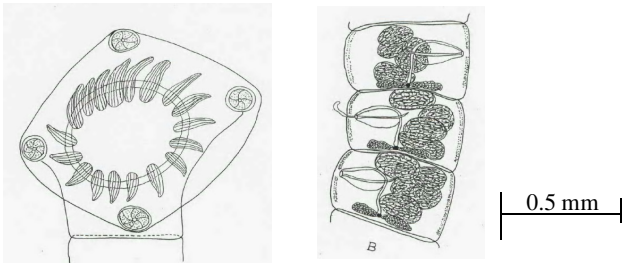


Scolex Mature Proglottids Mature Proglottids

Fig. 1: Micro-photograph of *C. maharashtrii*, n.sp.



Scolex Mature Proglottids

Fig. 2: Camera Lucida diagram of *C. maharashtrii*, n.sp.

Ootype is small, rounded, medially placed at the posterior region of the body, measures 0.03883 in diameter. Ovary is bilobed (unequal lobes), posterior side of the segment and measures 0.8737 (0.8252-0.9223) in length and 0.03398 (0.01213-0.06310) in width. Vitellaria granular and corticular in position.

The genus *Calycobothrium* was established by Southwell in 1911 as a type species *C. typicum* from *Aetobatis narinari* (Euphrasen, 1790) at Ceylon.

The present parasite *Calycobothrium maharashtrii* n.sp. is differ from *C. typicum* in having Scolex quadrangular Vs. circular, tentacles 18 Vs. 14 in numbers, mature proglottids broader than long Vs. longer than broad, testes 4 to 5 Vs. numerous, cirrus pouch rounded, elongated above the middle Vs. half way across proglottids, cirrus protrusible Vs. not Protrusible, Vas deferens present Vs absent and Ovary bilobed Vs. compact.

The above noted characters of these worms are valid enough to erect a new species *Calycobothrium maharashtrii* n.sp. is named after the state Maharashtra.

A key to the species of the genus *Calycobothrium*, Southwell, 1911

Scolex circular - *C. typicum*, Southwell, 1911.
Scolex quadrangular - *C. maharashtrii*, n.sp.

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The Asian Journal of Animal Science (June, 2011), Vol. 6 Issue 1 : 60-65 | *RESEARCH ARTICLE*

Effects of temperature on oxygen consumption and biochemical contents in fresh water crab, *Barytelphusa guerini*

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ABSTRACT

The influence of temperature on oxygen consumption in whole animal, glycogen and protein content in tissues (leg muscle and hepatopancreas) of *Barytelphusa guerini* was determined at cold (18 ± 0.5^0C), warm (35 ± 0.5^0C) and normal temperatures. Oxygen consumption increased significantly ($p < 0.05$) under warm condition and decreased insignificantly under cold condition. Leg muscle and hepatopancreas glycogen were found to be decreased in both the conditioned temperatures (cold and warm) except for hepatopancreas in warm condition where the value was increased significantly ($p < 0.001$). Whereas the protein content in both the tissues were found to be increased on cold and warm acclimation. It was concluded that the high rate of intermediary metabolism during thermal stress was supported by utilizing oxygen and involvement of various biochemical and physiological adjustments.

Key words : *Barytelphusa guerini*, Temperature, Oxygen consumption, Glycogen, Protein

Temperature is an environmental parameter that plays a key role in determining animal distribution in any environment. Relationships between animal occurrence and survival have been clearly recognized for a long time as being related to maximum and minimum temperatures. Precht *et al*. (1973) stated that the temperature influences on the unchanging or steady systems (normal physiological conditions) which are more important than those on the changing systems (during growth, reproduction, development etc.) for better assessment of the thermal stress. Temperature is known to affect the chemical composition of aquatic organisms (Landau, 1992 and Brown *et al.*, 1994).

Rate of respiration and metabolic rate is normally assessed in terms of oxygen consumption which is a highly complex physiological process and is influenced by various extrinsic and intrinsic factors such as – temperature (Pörtner *et al*., 2004; Valeria *et al.*, 2008 and Mandic *et al*., 2009); salinity (Hagerman, 1970 and Jones, 1974); oxygen tension (McMahon *et al*., 1974); body size (Kapoor, 1974) and starvation (Kotaiah and Rajabai, 1975). With increasing concern about global warming, habitat fragmentation and introductions of exotic species, it is imperative to understand how changes in species composition scale up to affect large-scale ecosystem processes (Vitousek 1990; Lawton and Jones, 1995; Hector *et al*., 2001).

Diwan and Nagabhushanam (1976) described that the variations in chemical constitution of tissues have been associated with differences in environmental temperatures. The effects of temperature acclimation on some aspects of carbohydrate metabolism in decapod Crustacea have also been reported (Dean and Vernberg, 1965). A much higher concentration of glycogen and significantly lower lipid reserves in the tissues of fish acclimated to 5^0 C than fish acclimated to either 15 or 25^0 C have also been reported (Johnston and Maitland, 1980; Stone and Sidell, 1981 and Johnston and Dunn, 1987).

The effect of temperature on protein synthesis and degradation rate under cold condition in fresh water male crab, *Barytelphusa guerini* have been reported (Ambore, 1974 and Kadam, 1980). Berger and Emlet (2007) have also shown that acclimation of *B. glandula* to relatively higher temperatures resulted in higher levels of protein synthesis. A mechanism facilitating the release of oxygen at cell level linked with O_2 demand at the cost of increased in protein synthesis have also been reported.

A comprehensive study of the aquatic poikilotherm in relation to thermal acclimation has been proposed to investigate the effect of cold and warm temperatures on the oxygen consumption in order to observe the changes in the metabolic profiles which enable us to arrive at a clear understanding of the total oxygen consumption of fresh water male crab, *Barytelphusa guerini*.

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Simultaneously changes in total protein content and glycogen content were also studied after acclimation to different temperatures.

MATERIALS AND METHODS

Suggesting a high degree of temperature adaptability to laboratory conditions and its wide range of natural habitat (Kadam, 1980), the fresh water male crab, *Barytelphusa guerini* has been chosen as the experimental animal. The male crabs with body weight (35 – 45 g) were collected from the paddy fields of the adjoining districts of Nanded (Maharashtra) and Nizamabad (Andhra Pradesh) for the present investigation. The crabs were brought to the laboratory and kept submerged in glass troughs containing sufficient quantity of tap water (pH: 7 – 7.5; Total hardness: 100 – 112 mg/L and Dissolved oxygen: 4.5 – 5.7 mg/L). During their sojourn in the laboratory, the animals were given *ad libitum* quantities of minced meat daily in the evening so that it coincides with the time of feeding in their natural habitat. The water in the troughs was replaced daily with fresh dechlorinated tap water.

The water temperature was around 24 ± 1^0C during winter months and 33 ± 1^0C during summer months. After one week of their adaptive sojourn in the laboratory in order to obviate the effect of environmental changes, the animals were divided into three groups and maintained at their respective temperatures consecutively for 20 days. The first set of the animals was maintained at the laboratory temperature only to serve as control or laboratory adapted normal animals. The second set of animals was subjected to cold temperature (18 ± 0.5^0C); while the third set of animals was subjected to warm temperature (35 ± 0.5^0C) and served as experimental animals. Uninjured intermoult stage (C_4) crabs were selected for the present investigation and the animals were not fed 24 hours prior to the commencement of the experiments to eliminate the influence of differential diet (Gilbert, 1959).

The total oxygen consumption of the crab was measured by using the apparatus as described by Saroja (1959). The amount of dissolved oxygen content of the water samples was estimated by the standard Winkler's iodometric method (Welsh and Smith, 1960). All duplications were done at the same period of the day to maintain constant temperature. The oxygen consumed by the animal in one hour was calculated and represented as ml of O_2/hr of the animal. The glycogen content of the tissue in normal and experimental animals was estimated by Anthrone Method (Seifter *et al*., 1950); since, the anthrone reaction forms the basis of a rapid and conventional method for the determination of glycogen. The values were read from the standard graph of glucose and the amount was expressed in mg of glycogen/gm wet weight of tissue. For the estimation of total protein content, the method recommended by Lowry *et al*. (1951) was employed and the amount of total protein content was expressed in mg of protein/gm wet weight of tissue.

Each experiment was repeated at least 8 times and the mean of the eight experiments with standard deviations were used to make comparisons with other experiments. Data were statistically analyzed using means with standard deviations and Student 'T' Test. The values of p = 0.05 were taken as significant.

RESULTS AND DISCUSSION

The influence of the acclimation to different temperatures on total oxygen consumption of whole animal and on the tissues (leg muscle and hepatopancreas) of fresh water male crab, *Barytelphusa guerini* was determined and the results are presented in Table 1 and in Fig. 1.

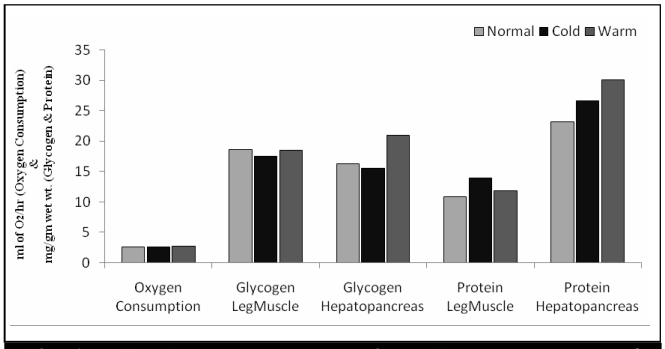

Fig. 1: Total oxygen consumption, glycogen content of fresh water made carab

From the data given in Table 1, it is clear that total oxygen consumption decreased in cold acclimation temperature and increased in warm acclimation temperature. The glycogen content in leg muscle decreased in both cold and warm conditions whereas in hepatopancreas, the values were found to be decreasing in cold temperature and increasing in warm temperature. The values of total protein content in leg muscle and hepatopancreas were found to be increased in both the conditions.

The physiological mechanisms limiting and adjusting cold and heat tolerance have regained interest in the light of global warming and associated shifts in the geographical distribution of ectothermic animals. In

accordance with Shelford's law of tolerance decreasing whole animal aerobic scope characterizes the onset of thermal limitation at low and high pejus thresholds (Shelford, 1931). This led to the hypothesis of a unifying concept, proposing that a mismatch between the O_2 – demand and O_2 – transport from gills to cells is the primary mechanism restricting animal tolerance to thermal extremes (Pörtner and Knust, 2007). And the aerobic scope of an animal is indicated by falling oxygen levels in the body fluids and the progressively limited capacity of circulatory and ventilatory mechanisms during thermal stress (Frederich and Pörtner, 2000; Satoris *et al*., 2003 and Metzger *et al*., 2007).

A decrease in oxygen consumption rate in a wide range of experimental temperatures which happen to be statistically insignificant was reported in White and North Sea snails (Sokolova and Pörtner, 2003). Whereas a significant increased in oxygen consumption in *H. Brachysoma* with increasing acclimation temperature between 15 to 31^0 C and 33 to 36^0 C have been reported by Dalvi (2009) indicating better capability for adapting to higher temperatures. The increased in the respiration rate of *Barytelphusa guerini* with the increased in temperature observed in the present study corresponds to results obtained in previous studies (Achituv and Cook, 1984 and Emmerson, 1985); which was expected as both studies used a closed bottle system. As temperature directly affects the rate of all biological processes the increase in respiration rate with an increase in temperature is not surprising (Schmidt – Nielsen, 1983).

It is conceivable that visceral blood oxygenation levels increased during warming to meet rising metabolic oxygen demands. This is possibly being a trade-off at the expense of reduced blood supply to the less active muscular tissue (Johnston and Dunn, 1987). The oxygenation levels can be increased or remain unchanged in more vital organs such as liver when oxygen supply to muscle is reduced (Mark *et al*., 2002). While Pörtner *et al*. (2005) and Wittmann *et al*. (2008) described that the increase in oxygen consumption rates with rising temperature is typically exponential within the passive thermal tolerance window set by upper and lower critical temperatures. And in contrast, non-exponential temperature-dependent oxygen consumption has been previously detected in larvae of *Cancer irroratus* (Sastry, 1979).

Finally it can be inferred that thermal acclimation caused a negligible significant variation in total oxygen consumption of fresh water male crab, *Barytelphusa guerini*. The adjustment to increase and decrease in total oxygen consumption was facilitated by activities involving biochemical and physiological adjustments where glucose and fatty acids are broken down to provide energy by producing more ATPs and of ATPases to support the increased rate of metabolism, locomotory, respiratory, and circulatory activities. Andrejew (1948) reported that glycogen serves as a reserve of carbon and energy. However, the demonstration of glycogen as a cell constituent suggests that the reserves may not be exclusively lipid (German *et al*., 1961). Antoine and Tepper (1969) also signify that, in *Mycobacterium phlei*, glycogen is a more labile storage material than lipid and is utilized before lipid.

A significant depletion in the glycogen content in the tissues (leg muscle and hepatopancreas) was observed in freshwater crab, *Barytelphusa guerini* exposed to cold and warm temperatures in the present investigation (Table 1) and it is supported by the findings of Kulkarni and Nagabhushanam (1978) and Krishnamoorthy (1979) who had briefed that the alteration in biochemical parameters to be eco-physiologically significance in counteracting the ambient thermal fluxes. Glucose being the predominant substrate in the energy yielding process, carbohydrate metabolism has been shown to play an important role in the energetic of thermal stress or adaptation (Bonthu, 1995). However, there has been an increase in the glycogen content in hepatopancreas upon exposure to warm temperatures (Table 1). Generally in poikilotherms maintenance of energy requirements are considerably lowered upon acclimation to cold temperature (Hochachka and Somero, 1973).

Considering the biochemical mechanisms in

Table 1 : Total oxygen consumption, glycogen content and protein content of fresh water male crab

	Mean and standard deviation			% change over normal		Student 'T' Test		Level of significance	
	Normal	Cold	Warm	Cold	Warm	Cold	Warm	Cold	Warm
Oxygen consumption	2.613± 0.15	2.578± 0.08	2.747±0.07	- 1.35	+ 5.09	0.59	2.31	p > 0.05	p < 0.05
Glycogen leg muscle	18.601±0.61	17.478±0.45	18.431±0.32	- 6.04	- 0.92	4.17	0.70	p < 0.001	p > 0.05
Glycogen hepatopancreas	16.323±2.46	15.544±2.41	20.932±1.69	- 4.77	+ 28.23	0.64	4.37	p < 0.05	p < 0.001
Protein leg muscle	10.903± 2.5	13.891±0.69	11.786±0.88	+ 27.41	+ 8.1	3.26	0.94	p < 0.01	p > 0.05
Protein hepatopancreas	23.124±0.68	26.579±0.69	30.034±3.65	+ 14.94	+ 29.88	10.11	5.27	p < 0.0001	p < 0.0005

acclimation to be similar in different organisms, three possible modes of triggering and control are suggested by Rao (1966) namely, direct effects of temperature, effects of the nervous system and, most important of all, regulation through the release of hormones or hormone-like substances. From the large number of studies carried out to date, it seems that animal cells utilise a multitude of volume regulatory mechanisms, including transport of inorganic and organic osmolytes across the cell membrane and alterations in metabolism to modify levels of organic metabolites (Lang *et al.,* 1998). Several metabolic pathways are sensitive to cell volume changes, including glycogen synthesis and glycolysis, leading to changes in the amount of carbohydrate metabolites that contribute to cellular osmolarity (Al-Habori *et al.,* 1992).

It is known that the protein metabolism, just like carbohydrate or lipid metabolism, shows some adaptive changes in poikilotherms when exposed to thermal stress. Enhanced rate of general protein synthesis measured by the incorporation of labelled amino acids into proteins has been reported after cold acclimation in the liver of gold fish (Das and Prosser, 1967). A significant increase in protein content in *Poecilobdella viridis* during warm and cold acclimation have been also reported by Kulkarni and Nagabhushanam (1978), and the same pattern was noticed in leg muscle and hepatopancreas of fresh water male crab, *Barytelphusa guerini* in the present investigation (Table 1).

Acclimation relatively to higher temperatures resulting in higher levels of protein synthesis has been reported in *B. glandula* (Berger and Emlet, 2007). Also of interest is the possibility that disassembly of the protein synthetic system at high temperatures is associated with a shift to production of heat shock proteins, as in other organisms (Lemaux *et al.,* 1978 and Moran *et al.,* 1978). Protein synthesis rates may also be influenced by membrane transitions in different temperature range and another factor that may play a role is the requirement for adjustment of blood pH with temperature change (Rahn and Baumgardner, 1972).

A remarkable increase of the total protein content in liver and gill and less in muscle of the goldfish during cold adaptation have been reported Das and Prosser (1967). Robertson *et al.* (2001) also reported an increase in whole body protein synthesis rates accompanied by increased in both RNA activity and RNA/protein ratio in the baltic isopod crustacean, *Saduria entomon*. Significant recovery from drop in serum protein concentrations following metabolic compensation has also been reported (Ennis, 1973) and depletion of available organic reserves by starvation resulting in a loss of the ability to respond to cold exposure by a compensatory increase in metabolic rate was also reported (Vernberg, 1959). These observations suggest that there exists an integrative mechanism capable of preventing temperature acclimation when available organic reserves are required for what appear to be more vital metabolic needs and a prolonged encounter to low temperatures necessitate a compensatory metabolic reorganization (Passano, 1960).

In conclusion, the present study suggests that the capacity of oxygen delivery is set to a level just sufficient to meet maximum oxygen demand between the average highs and lows of laboratory temperatures. The increase in activity during thermal stress and the need for transporting and utilizing oxygen to support high level of activity involves various biochemical and physiological adjustments, indicating high rate of intermediary metabolism, the substrates for which may come from the stored glycogen and fat in addition to ingested food and the variation in tissues constituents at different temperatures which reflect the differences in their energy requirements for maintenance of body physiology during thermal stress. Therefore, the recorded data indicate that temperature has a role in maintaining normal physiological functions of the fresh water male crab, *Barytelphusa guerini* which are of vital statistics for sustaining life. Further investigation emphasizing the importance of taking into account of other relevant factors (e.g. seasonal amplitude of temperatures, food availability and activity levels of animals) are required when studying metabolic adaptations to temperature.

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The Asian Journal of Animal Science (June, 2011), Vol. 6 Issue 1 : 66-70 | *RESEARCH ARTICLE*

Effect of Chitin biosynthesis inhibitor-diflubenzuron on the fifth instar larvae of *P. ricini* Fabr

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ABSTRACT

Fifth instar larvae of *Pericallia ricini* Fabr. (Lepidoptera, Arctiidae) were fed on the leaves of castor plant (*Ricinus communis* L.) treated with different concentrations of diflubenzuron. Larval mortality of 86.95% was recorded at 100 ppm level. Abnormalities on the body of adult were reported due to the toxicity of chemical. Abnormal adults who could not stretch their wings properly with swollen thoracic region, appeared at 50 ppm level of diflubenzuron. Maximum deformity was recorded at 10 ppm level. The larval and pupal life span was increased by 12 and 34%, respectively at 10 ppm level. The chemical has also affected the food intake capacity and growth of larvae and pupae. Maximum food intake, growth and approximate digestibility were manifested at the lowest concentration of the chemical.

Key words : *Pericallia ricini, Ricinus communis*, Diflubenzuron-Chitin biosynthesis inhibitor, ppm (parts per million)

Pericallia ricini is found all over India and is commonly known as castor hairy caterpillar. It is a serious pest of oil seed plant, castor and cucurbitaceous crops. The female moths lay eggs in large number on the lower surface of leaf. The larvae feed on young and full grown plant leaves and fruits. In heavy infestation only stem and branches are left behind.

In past many research workers attempted to study the lethal influence of chitin biosynthesis inhibitors on agricultural insect pests and reported almost complete lethal action by these chemicals (Gupta and Verma, 1992 and Baringbing and Karmawati, 1992) but the adequate literature is still lacking on the loss of crop by these pests which survive after exposure with these chemicals. Hence, the objective of the present research was to find out, the extent of crop damage done by the insect pest which avoid lethal dose of chitin biosynthesis inhibitor. Besides food consumption, growth of treated larvae was also studied as the reproduction of malformed individual is drastically affected and their population is checked to reach economic injury level.

MATERIALS AND METHODS

The eggs of *P. ricini* Fabr. were collected from castor leaves and reared in the laboratory on castor leaves. Eggs were kept between two leaves in the wooden cages, (60×60×45cm) and fresh clean fleshy leaves were provided daily. After hatching, larvae started feeding on fresh leaves, the excreta and other waste were removed daily from cages. For the protection of larvae from ants, the rearing cages were placed on water filled pots (Earthen cups) Larvae were reared till the pupa formation. Freshly emerged adults were transferred to separate jar, for ovipositor. Honey mixed sugar solution (10%) soaked in a cotton ball, was provided in the plastic cavity (2×2×1cm) for feeding the adults. Fresh castor leaves were placed in the glass chimney for egg laying. The females laid eggs on leaf surface; such leaves along with eggs were transferred into another glass jar. The eggs were kept between fresh succulent castor leaves to provide food for hatching larvae easily and also to prevent leaves from rapid evaporation. Fifth instar larvae of *P.ricini* were separated from the cages and were starved at least for six hours as the least variation in results is exhibited due to suitable starvation before treatments. Starvation also assures that all experimental insects feed on treated food. The larvae fed on castor leaves which were dipped in volatile solution of chemical at different concentrations.

To find out the toxicity of chemical, mortality, deformity, longevity, food consumption, growth and approximate digestibility was recorded.

Due to interaction of chemical in the biosynthesis of chitin in experimental insects, the mortality in different treatment was observed during the period of investigation. Moribund test insects were also considered as dead. Net mortality was calculated after necessary correction by using Abbott's formula (1925).

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Deformity due to the irregular deposition of chitin was observed in developmental stages of experimental insect pests. Different types of deformity were observed and deformity percentage was also calculated.

The longevity of larvae and pupae after treatment was recorded to see the effect of chitin biosynthesis inhibitors on development. Any increase or decrease in life period of larvae and pupae was compared with the control.

The chitin biosynthesis inhibitor weaken the mouth parts and foregut of experimental larvae due to the irregular deposition of chitin, so treated larvae consumed lesser amount of food than control. Hence the food intake capacity of test insects was also determined. For this purpose known amount of leaves was given to the experimental larvae for feeding. The consumed leaves were determined daily to record the food consumption of experimental insects. The difference between leaves supplied and unconsumed gave an index to food consumption. The weight loss of leaves due to evaporation of water during experimental period was adjusted with control by the formula of Evans (1939a, b).

To find out the effect of the chemical on growth of treated larvae, the larvae were weighed daily and weight of treated larvae was compared with the control experiment. The maximum weight per larva after treatment and average weight per larva before treatment was recorded. The weight gain per larva during feeding was calculated by subtracting the average weight per larva before treatment from the average maximum weight per larva after treatment.

For the calculation of approximate digestibility, food ingested and faecal matter produced by experimental insects was recorded daily and the approximate digestibility was calculated by the formula of Waldbauer (1964).

RESULTS AND DISCUSSION

The influence of diflubenzuron on *Pericallia ricini* (on survival, development, longevity, food consumption and growth) is described in the Table 1-4.

Mortality (Table 1):

The mortality rate, in larval feeding treatment was increased with increase of chemical concentration. At 100 ppm a maximum of 86.95% mortality was exhibited and the adults emerged were unable to stretch their wings properly due to poor blood supply in veins.Net mortality observed was 19.56, 34.78, 63.04, 80.43 and 86.95% at 0.1, 1.0, 10.50 and 100ppm concentration, respectively. Ahmad (1992) also studied the effect of dimilin on *Dysdercus cingulatus* and noted that this compound caused mortality in nymphs and a significant reduction in fecundity, fertility and progeny development of females emerging from treated nymphs.The reduction was more pronounced in fifth instar treated nymphs than in fourth instar nymphs. Beninger and Arnason (1993) observed the mortality, deformity, longevity and poor growth in European corn borer due to the toxic effect of chemical.

Deformity (Table 2):

The compound caused deformities in the larvae, pupae and adult insects. Deformity includes mostly larva-pupa intermediates. Pawar *et al.* (1989) reported the toxicity of IGRs due to the action of chemical,larva – pupa intermediates were formed, pupal and larval mortality was also seen in mosquitoes. The deformity observed in pupae was 16,28,33,13 and 11% at 0.1, 1.0, 10,50 and 100 ppm, respectively. The deformity percentage was highest at 10ppm level.

Swelling in thoracic region of adult was also seen which was due to the irregular deposition of chitin. At 50 and 100ppm deformity percentage was reduced due to high rate of mortality. Due to chitin inhibiting action, the deformity at lower doses was manifested. The adults were not capable of flight due to the folded wings. Cuticle softness and swelling in the body of larvae and adults were also reported. Gupta and Dogra (1990) also reported the toxic action of diflubenzuron and penfluron on potato

Table 1 : Mortality effect of diflubenzuron in fifth instar larval feeding treatment of *Pericallia ricini*

Concentration	Larvae treated	Larvae prepupated	Larvae pupated	Adults emerged	Total Mortality		Mortality	Net mortality
ppm	(no)	(no)	(no)	(no)	(no)	±SE	(%)	(%)
Control	10	9.6	9.6	9.6	0.8	±0.1	8.0	-
0.1	10	8.2	7.8	7.2	2.6	±0.2	26.0	19.56
1.0	10	7.6	6.2	5.4	4.0	±0.3	40.0	34.78
10	10	4.8	4.2	3.6	6.6	±0.4	66.0	63.04
50	10	2.2	1.8	1.2	8.2	±0.4	82.0	80.43
100	10	1.8	1.6	0.8	8.8	±0.5	88.0	86.95

Table 2 : Deformity effect of diflubenzuron in fifth instar larval feeding treatment of *Pericallia ricini*

Concentration	Larvae treated	Pupae deformed	Pupal deformity
ppm	(no)	(no)	(%)
Control	10	-	-
0.1	10	1.6	16.0
1.0	10	2.8	28.0
10	10	3.3	33.0
50	10	1.3	13.0
100	10	1.1	11.0

beetle. They observed the cuticle softness on insects due to the action of chitin biosynthesis inhibitors. The post ecdysal cuticle from diflubenzuron and penfluron treated adults was soft. Haynes and Smith (1994) also reported the cuticle softness of newly emerged adults of *A. Grandis* boll weevil by the action of diflubenzuron. Srivastava and Srivastava (1990) had seen the influence of chitin biosynthesis inhibitor on larval food consumption and growth of *P. ricini*. Effect of chitin biosynthesis inhibitor penfluron, also seen by Pugazhvendan and Soundararajan (2009) and Prakash *et al.* (2006).

Longevity (Table 3):

The larval and papal period increased significantly. The larval survival period was increased by 4, 8, and 12% and papal by 21, 32, and 34% at 0.1, 1.0, and 10ppm level. The decease in larval life span was by 2 and 7% ,papal by 1 and 21% at 50 and 100 ppm level. In the present study the larval life span was increased in the instars in which the larvae were treated while the life span of remaining instars was almost equal to control treatment. It shows that slow down of biochemical development process remains maximum in the treated larval instars but in the later stages, the effect of chemical is detoxified by the experimental larvae and subsequent larval duration becomes similar as in control. Senapati and Patnaik (1991) reported that the *E. Sparsa* males and females survival periods were decreased by diflubenzuron but in present study the prolongation of immature life stages was observed which was due to decrease in the rate of biochemical developmental process by the influence of chemical.Radwan *et al.* (1986) also reported that adult's life span decreased as the concentration increases in *S. littoralis* reduction in food consumption and growth was reported.

Food consumption and growth (Table 4):

The food consumption of experimental larvae decreased on increase of chemical concentration. The maximum food consumption was manifested at the lowest concentration and minimum food consumption at the highest concentration. The chemical weakens the mouth parts of test insects thus the food intake was reduced. Maximum food consumption per larva was 0.285g at 0.1 ppm and minimum 0.180g at 100ppm level in comparison to control (0.490g). The growth of larvae in comparison to control was reduced which is showing the toxicity of chemical. The maximum weight gain was exhibited at lowest concentration (0.160 g at 0.1 ppm) due to the good intake of food. The approximate digestibility was also reduced due to the action of chemical. At highest concentration (100ppm) it was 66.666 % and at lowest concentration (0.1ppm) 70.175 %. Singh *et al.*(2008) also seen the effect of chronic exposure of biorational insecticides on the growth and development of silk worm, *Bombyx mori*.

The reduction in food consumption may be due to the disruption of chitin-biosynthesis during moulting. Due to the cuticle softness by the chemicals the mouth parts became weak and larvae could not chew its food properly by weak jaws thus the food intake capacity was ultimately reduced. Though the larval life span is considerably prolonged (Khan and Srivastava 1990), the total consumption of food of experimental larvae was clearly reduced in comparison to control this is an additional

Table 3 : Longevity effect of diflubenzuron in fifth instar larval feeding treatment of *Pericallia ricini*

Concentration	Larvae treated	Average longevity of fifth instar larvae	Pupal longevity	Increase in larval longevity	Increase in pupal longevity	Increase in larval longevity	Increase in pupal longevity
ppm	(no)	(day)	(day)	(day)	(day)	(%)	(%)
Control	10	5.5	11.1	-	-	-	-
0.1	10	5.9	13.2	0.4	2.1	4.0	21.0
1.0	10	6.3	14.3	0.8	3.2	8.0	32.0
10	10	6.7	14.5	3.4	1.2	12.0	34.0
50	10	5.3	11.0	-0.2	-0.1	-2.0	-1.0
100	10	4.8	9.0	-0.7	-2.1	-7.0	-21.0