5. RESULTS AND DISCUSSION

5.1 SHORT DESCRIPTION OF GEOMETRID SPECIES WITH IDENTIFICATION KEYS OF THEIR IMMATURE (LOOPER) AND ADULT STAGES

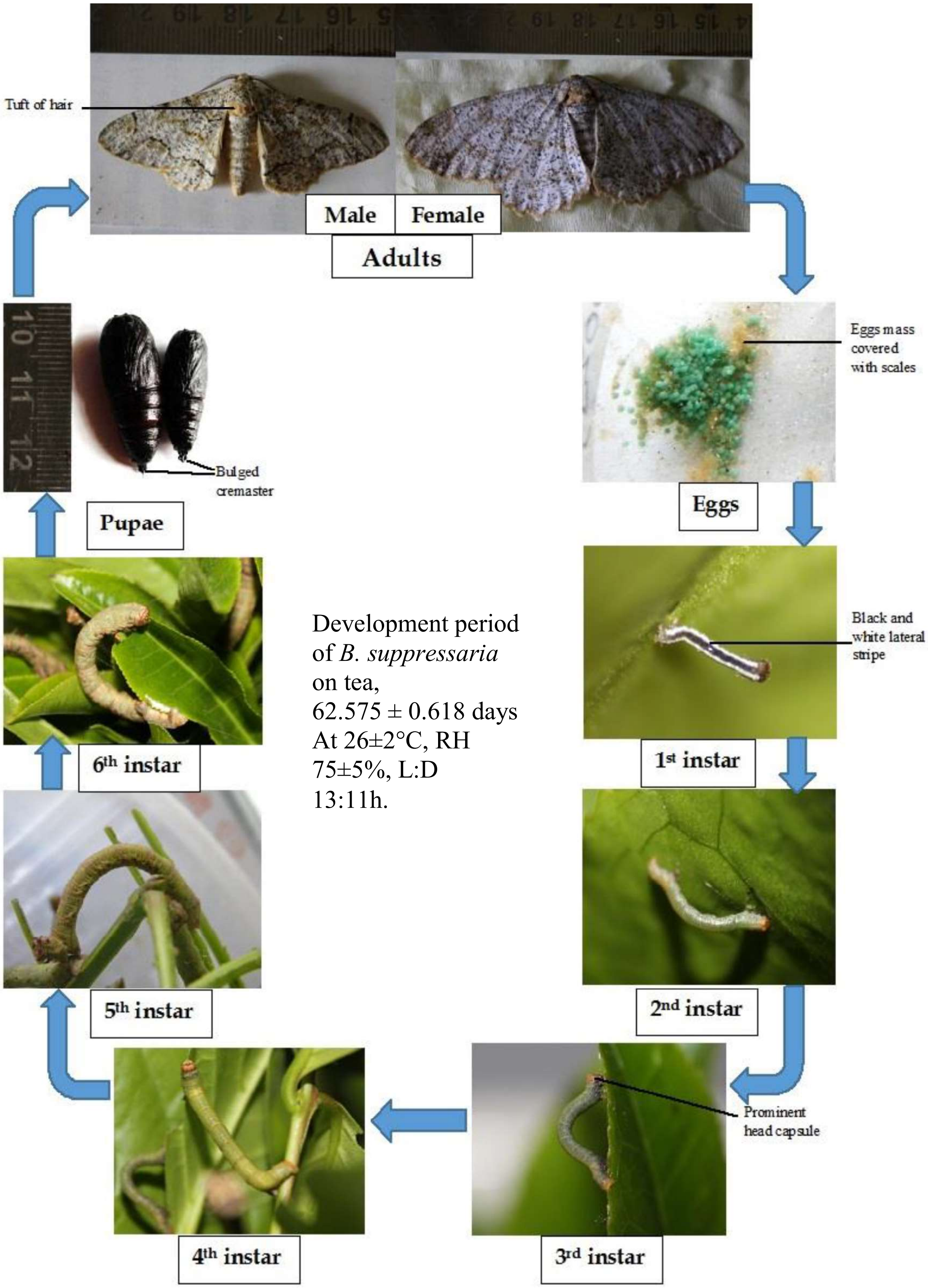
5.1.1 ***Biston* (=*Buzura*) *suppressaria*:** Life-cycle of *B. suppressaria* consisted of egg, six larval instars, pupa and adult (Fig. 5.1.1). Developmental period on tea, ranged between 60-65 days at ambient temperature of 26±2°C with RH 75±5% and L:D 13:11h.

Egg: Eggs laid in cluster and covered with scales; light green in colour which turn blackish grey one day before hatching.

Larva: 1st instar larva had black with white lateral stripe running through entire length of the body. 2nd instar onwards, body turned into light green with a prominent head capsule.

Pupa: Freshly formed pupa was green, which turned reddish brown within few hours. Two spines like crochets present on the bulged cremaster. Female pupa (≈2.6cm) was always larger than the male pupa (≈2cm).

Adults: Male moth smaller than female. Brownish white, with forewing span of around 7cm in female and 5cm in male. A tuft of hair was present on the thoracic segments. Bi-pectinate antenna in male moth, whereas female with filiform antenna.



Development period of *B. suppressaria* on tea, 62.575 ± 0.618 days At 26±2°C, RH 75±5%, L:D 13:11h.

Figure 5.1.3 Life-cycle of *B. suppressaria*

5.1.2 *Hyposidra talaca*: *H. talaca* (Fig. 5.1.2) on tea completed life-cycle in 30-32 days at an ambient temperature of 26±2°C with RH 75±5% and L:D 13:11h.

Egg: Eggs were laid in cluster, green in colour and generally not covered with scales. Eggs gradually turned from green to yellowish brown then to blackish grey, till neonates hatched.

Larva: Early larval instars of *H. talaca* were black with white dotted transverse lines on their body. In late instars, these dots disappeared with whole body turning brown.

Pupa: A pair of crochets and a pair of spine like processes were present laterally at pupal cremaster. Female pupa (≈1.7cm) was always larger than male pupa (≈1.4cm).

Adults: Adults had colour shades between grey and brown. Male smaller than female. The forewing span of male about 4cm and female 4.5cm. There was a notch present at distal inner margin of forewing. The antenna was bi-pectinate in male whereas filiform in female moth.

5.1.3 *Hyposidra infixaria*: *H. infixaria* (Fig. 5.1.3) on tea completed life-cycle in 35-38 days at a temperature of 26±2°C, RH 75% and L:D 13:11h.

Egg: Eggs were laid in cluster. Green when freshly laid and turned blackish grey with maturity.

Larva: Early instars (up to 3rd instar) were with similar features to that of *H. talaca*. In 4th and 5th instar, the 3rd pair of thoracic leg turned darker than other two pairs of thoracic legs and a black stripe extended from the legs to lateral side of thorax.

Pupa: A pair of crochets was present at cremaster; but cremaster was not bulged like that in pupa of *B. suppressaria*. Female pupa (≈1.7cm) was always larger than male pupa (≈1.4cm).

Adults: Adults were darker than that of *H. talaca*. Posterior margin of the wings fringed; typical notch was absent in the inner margin of forewing. The wing size was similar to that of *H. talaca*.

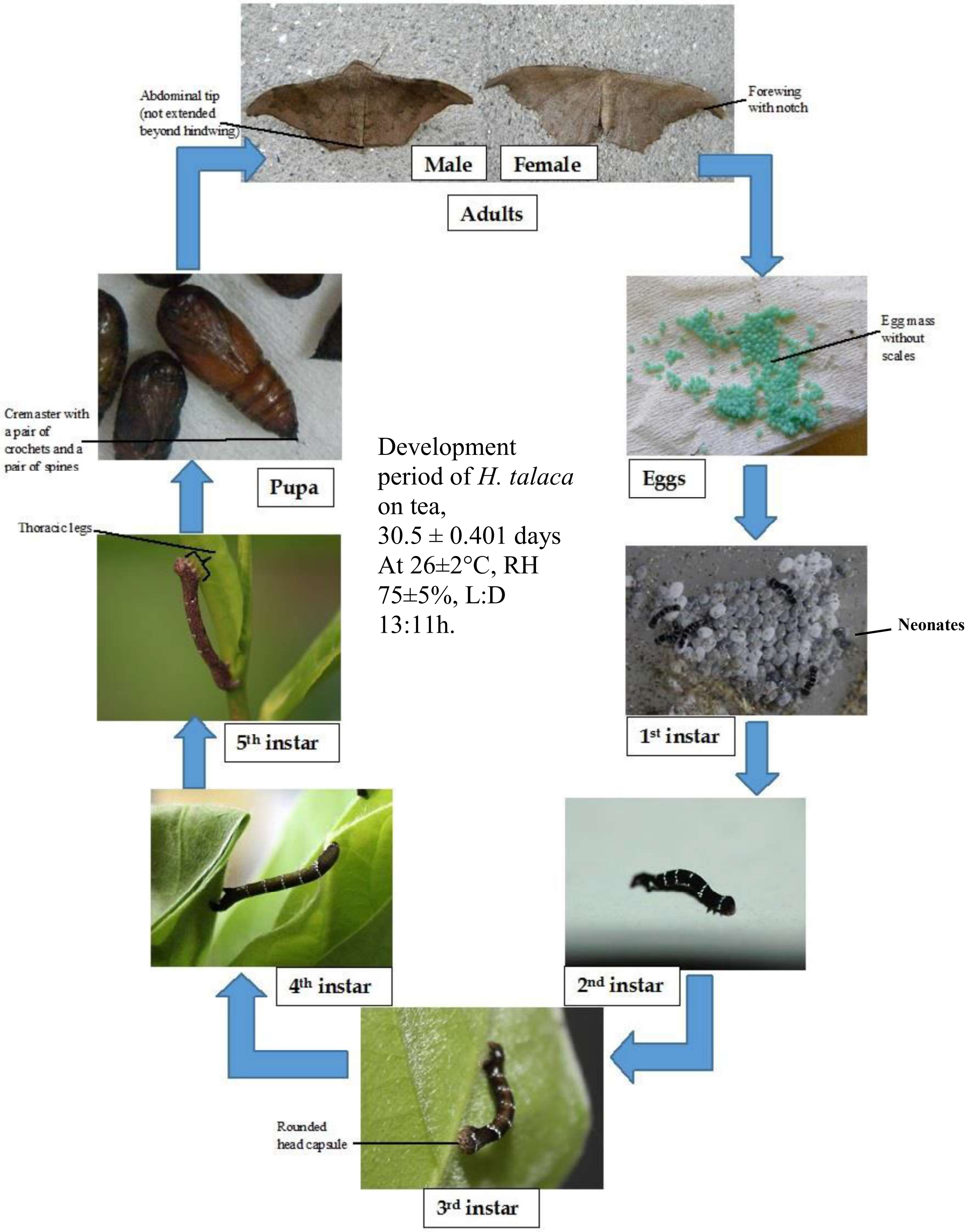
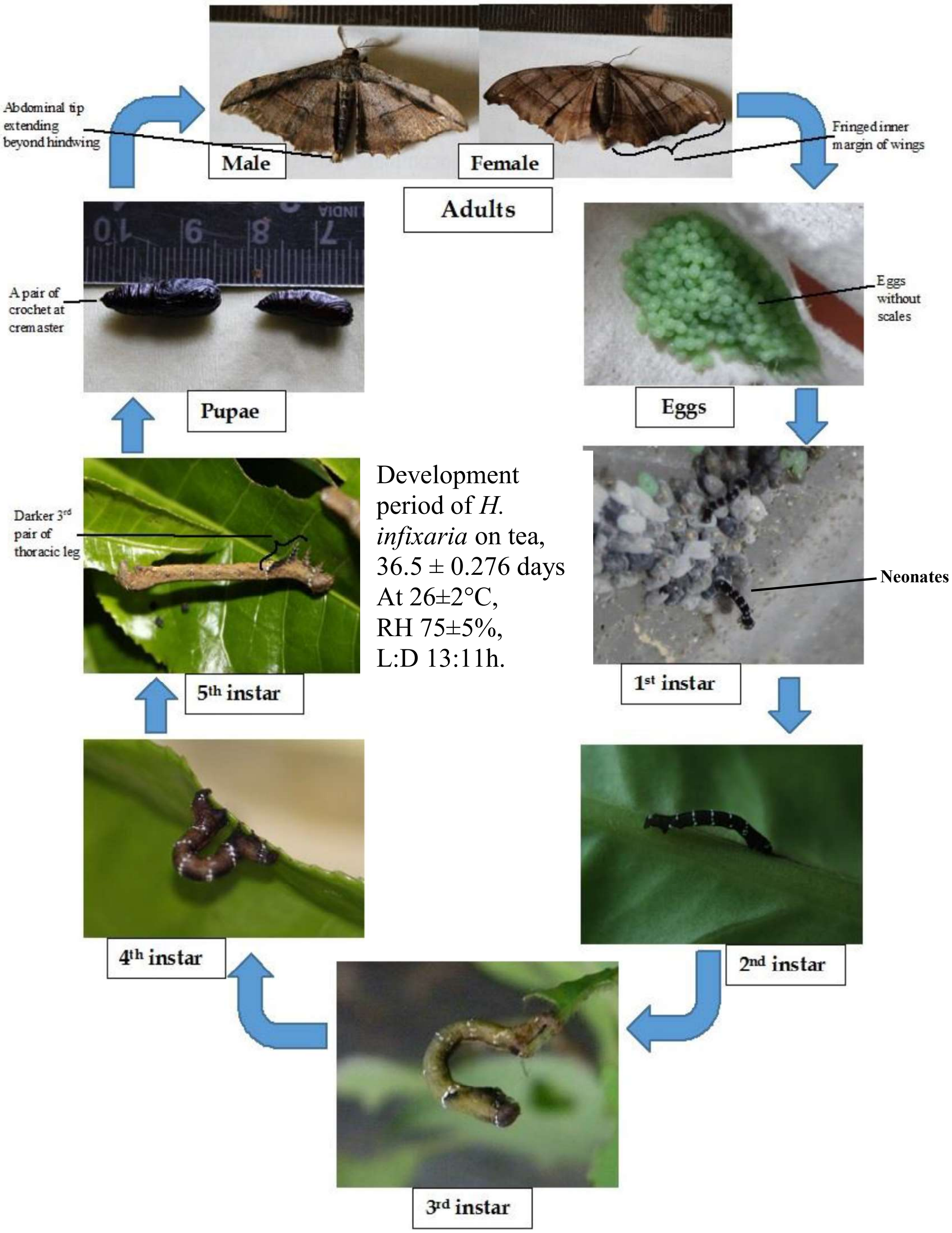


Figure 5.1.4 Life-cycle of *H. talaca*



Development period of *H. infixaria* on tea, 36.5 ± 0.276 days At 26±2°C, RH 75±5%, L:D 13:11h.

Figure 5.1.5 Life-cycle of *H. infixaria*

5.1.4 Field identification key to immature stages and adults of geometrid pests of tea

Eggs:

1. Egg mass covered with scales………………. ***Biston* (=*Buzura*) *suppressaria***

\- Egg mass not covered with scales…………... ***Hyposidra* spp**

Larva:

1\. Body green (early instar) to greenish brown (late instar) with prominent reddish spiracles at the lateral margin of the body and triangular head capsules……………………………………………… ***B. suppressaria***

\- Body black (early instar) to brown (late instar) with white transverse dotted stripes; small, rounded head capsules……. ……………………….. 2

2\. In 4th and 5th instars, a dark (blackish) coloured stripe develops through dorsal half of the body to tip of the meta-thoracic leg…………………. ***H. infixaria***

\- In 4th and 5th instars, all three pair of thoracic legs are uniformly light coloured.…………………... ***H. talaca***

Pupa:

1\. Posterior tip of pupa with two hooks like crochets and a bulged cremaster; pupae approximately 2cm (male) to 2.6cm (female) long………………………………………………….. ***B. suppressaria***

- Posterior tip of pupa with two hook like crochets; cremaster not bulged, pupae approximately 1cm (male) to 1.7cm (female) and long……..…….. **2**

2. Two spines like structure present at cremaster….. ***H. infixaria***

- No spine like structure present at cremaster… ***H. talaca***

Adult:

1. Body and wings golden brown to whitish, with a tuft of hair on thoracic segment; wing span approximately 7cm in female and 5cm in male…….. ***B. suppressaria***

- Body and wings brown to grey; wing span approximately 4.5cm in female and 3.2cm in male…. **2**

2. Forewing of female with notch in inner margin; abdominal tip of male not extending beyond the hindwing….…………………………………... ***H. talaca***

- Forewing of female with fringed inner margin (no notoch); abdominal tip of male extending beyond the hindwing………………………………... ***H. infixaria***

5.1.5 Discussion

Identification key prepared for immature and adult stages of commonly occurring geometrid moths would be helpful to the tea planters. They would be able to recognize the specific stage of the pest on field and relate the same with the damage symptoms if present. In the integrated pest management (IPM), economic injury level need to be determined before applying any pest control measures that would involve an extra cost. Correct identification of pest and their stages and population density is basic for making decision in any IPM. Quinn and Arnold (2009) emphasized on the routine inspection and accurate identification of pests as vital steps in IPM and effective control of those pests. The correct identification of the pest is also required to retrieve previous reports and information about the specific pest for better management (Rugman-Jones et al. 2010). *B. suppressaria* of the same plantation can be distinguished from *Hyposidra* spp. easily as all its stages are morphologically different. However, early instars (1st to 3rd) of the two *Hyposidra* spp. appear similar and cannot be morphologically distinguished. An apparent difference was noticeable in 4th instar looper caterpillar of *H. infixaria*, where a dark (blackish) coloured stripe develops through dorsal half of the body to tip of the meta-thoracic leg whereas there was no such colour difference in the legs of *H. talaca*. Pupae of all the three species could be differentiated by the structures present at the cremaster. Adults of the congeners could also be distinguished by the forewing pattern and their margin. Using such morphological features identification and distinction of several lepidopteran species are reported. Mosher (1915) classified a group of lepidopteran on the basis of pupal characters. Pljushch and Dolinskaya (2003) had used pupal cremaster as an identifying character for distinguishing different tiger moths. Pupae have important taxonomic characters which helped to distinguish various geometrid species (McGuffin 1987; Bolte 1990). Subfamily Notodontinae (Lepidoptera; Noctuidae) was classified on the basis of their pupal morphology (Miller 1992). For a better approach in pest management, Timm et al. (2008) prepared a key for four pest species of Noctuidae of deciduous fruit tree in South Africa. The key was based on the morphological characters of immature stages (first instar to pupae). Moths of genus *Spodoptera* could be differentiated by Brambila (2013) on the basis of their wing pattern. So utility of morphological characters in identifying of immature stages as well as adults of three common geometrid species of tea has been made evident in the present study. Such keys may further be expanded to include in future other less common geometrid species that are also found associated with tea plants but might not have assumed pest species.

5.2 DESIGNING AND DEVELOPMENT OF SYNTHETIC (ARTIFICIAL) DIET FOR REARING THE GEOMETRID (LOOPER) SPECIES

Basic ingredients used for preparing diets were: (i) Wheat germ, (ii) Cellulose powder, (iii) Casein, (iv) Potassium sorbate, (v) Dextrose, (vi) Sodium alginate, (vii) Sucrose, (viii) Wesson's salt mixture, (ix) Choline chloride, (x) Vitamins mixture, (xi) Cholesterol and (xii) Wheat germ oil. These chemical ingredients were mixed with autoclaved solution of agar dissolved in distilled water as discussed in the materials and methods chapter. A pictorial flow chart of the steps of diet preparation along with the rearing process undertaken for the geometrids has been diagrammatically presented in Fig. 5.2.1.

5.2.1 *Biston* (*=Buzura*) *suppressaria*

The aforesaid diet composition was initially used for rearing of *B. suppressaria*, but while rearing, different kinds of developmental defects were observed such as no pupation, incomplete pupation, deformed adult with un-stretched squeezed wings, etc. Addition of the folic acid and linoleic acid along with inositol to the basic diet was done to overcome these problems. Despite the said modifications of diet, the females of *B. suppressaria* emerged with deformed wings, although some of the males emerged with normal wings (Fig. 5.2.2). So the diet was further supplemented with linolenic acid. Since, linolenic acid is a component in the linseed oil, to make the diet cost effective, linseed oil was added to the diet (Table 5.2.1). Additional advantage of using linseed oil was that it also acted as an additional source of other fatty acids such as linoleic acid, palmitic acid, stearic acid and oleic acid. Therefore, addition of linseed oil in the diet could provide the essential linolenic acid besides linoleic acid. Incorporation of adequate amount of these oil ingredients (fatty acids) in diet appeared to be obligatory for successful rearing of *B. suppressaria*. Vitamin E was also added as antioxidant.

5.2.2 *Hyposidra talaca*

Diet recommended by Lyon and Brown (1970) if supplemented with folic acid, linoleic acid and vitamin E was adequate for rearing and sustaining the life-cycle of *H. talaca* with fair survival rate. For normal development of *H. talaca*, addition of only linoleic acid was sufficient to support the complete life-cycle (Fig. 5.2.3). It was noted that unlike the diet *B. suppressaria* addition of linolenic acid was not obligatory in the diet for normal development of *H. talaca*.

5.2.3 *Hyposidra infixaria*

H. infixaria when reared on the diet found ideal for rearing *H. talaca*, showed deformity in adults (Fig. 5.2.4). The diet formulated for rearing *H. infixaria* stood somewhat in between diets of both *B. suppressaria* and *H. talaca*. Only half of the volume of linseed oil used in diet of *B. suppressaria* was needed for normal development of *H. infixaria*. Whereas the early larval instar showed less mortality on the diet composition recommended for rearing *H. talaca*. Therefore, different diets were used for rearing early and late instar of *H. infixaria*.

The final compositions of the synthetic diet with proportion of the ingredients used for the three species of geometrids are listed in table 5.2.2. The formulated diets have all the basic nutrients such as carbohydrate, protein, lipids and essential elements such as vitamins and minerals. Each component has specific role to play which are listed in table 5.2.3.

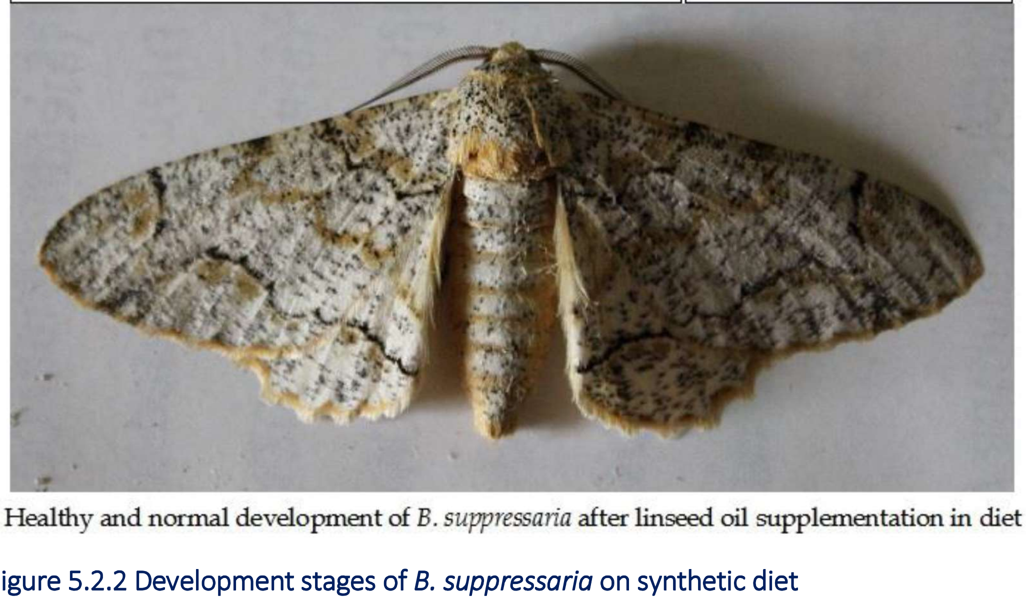
Figure 5.2.1 Generalized procedure of synthetic diet preparation and rearing of geometrid loopers

1. Pouring hot autoclaved agar media into blending container
2. Mixing other ingredients in agar media
3. Blending of the slurry
4. Pouring slurry into a plastic container
5. Cooling followed by refrigeration of diet
6. Solidified diet cut into cubes along with removal of moisture (soaked) before serving
7. Rearing containers with toothpick holding cubes of diet
8. Transferring neonates to containers with help of brush
9. Rearing container covered as shown in the figure
10. 40-50 neonates in a container maintained in BOD incubator
11. 3rd instar of looper, *H. talaca*
12. A 5th instar of looper, *H. talaca*
13. Compartmentalized container (cubicals) for culturing advanced instar individually followed by their pupation
14. Adult emerging from pupa
15. Front view of rearing setup inside the BOD incubator

Developmental defects in *B. suppressaria* when reared on basic un-modified diet (designed for *H. talaca*)

Final stage *B. suppressaria* larva (looper)

Fully formed pupa



Healthy and normal development of *B. suppressaria* after linseed oil supplementation in diet

Figure 5.2.2 Development stages of *B. suppressaria* on synthetic diet

Table 5.2.1: A comparison of the ingredients of synthetic diets showing modifications done for complete rearing of *B. suppressaria*

Diet formulated by Lyon and Brown (1970) for geometrid rearing	Diet ingredients initially used for rearing geometrid, *B. suppressaria*	Supplemented economical diet for *B. suppressaria* rearing
Vitamin-free casein	Vitamin-free casein	Vitamin-free casein
Dextrose	Dextrose	Dextrose
Wesson's salt mixture	Wesson's salt mixture	Wesson's salt mixture
Cholesterol	Cholesterol	Cholesterol
Potassium sorbate	Potassium sorbate	Potassium sorbate
Choline chloride	Choline chloride	Choline chloride
Wheat germ	Wheat germ	Wheat germ
Alphacel (Cellulose powder)	Alphacel (Cellulose powder)	Alphacel (Cellulose powder)
Wheat germ oil	Wheat germ oil	Wheat germ oil
Agar	Agar	Agar
Sodium alginate	Sodium alginate	Sodium alginate
Potassium sorbate	Potassium sorbate	Potassium sorbate
Vanderzant's fortification mixture	**Multivitamins+ Sucrose**	**Multivitamins+ Sucrose**
Distilled water	Distilled water	Distilled water
--------------------------------	**Linoleic Acid**	**Linoleic Acid+ Linseed oil (containing linolenic acid)**
Pupa formation & Adult Emergence on respective diets		
Incomplete pupation and if pupated adults with deformed squeezed wings	Pupal mortality was comparatively reduced but most of the adults found with scale less and/or partially deformed wings	Pupa with normal well-expanded wings with scales; mortality negligible

Developmental defects of *H. talaca* when reared on the diet formulated by Lyon and Brown (1970)

Final stage *H. talaca* larva (looper)

Fully formed pupa

Healthy and normal development of *H. talaca* adult when diet supplemented with Linoleic acid

Figure 5.2.3 Development stages of *H. talaca* on synthetic diet

Developmental defects in *H. infixaria* when reared on the diet designed for *H. talaca*

Final stage *H. infixaria* larva (looper)

Fully formed pupa

Healthy and normal development of *H. infixaria* after linseed oil supplementation of diet

Figure 5.2.4 Development stages of *H. infixaria* on synthetic diet

Table 5.2.2: Composition of synthetic (artificial) diets for *B. suppressaria*, *H. talaca* and *H. infixaria*

DIET COMPONENTS	*B. suppressaria*	*H. talaca*	*H. infixaria* (4th instar onwards)
Agar	9g	9g	9g
Distilled water	300ml	300ml	300ml
Casein	11.675g	11.675g	11.675g
Dextrose	11.675g	11.675g	11.675g
Wesson's salt mixture	3.35g	3.35g	3.35g
Cholesterol	1g	1g	1g
Potassium sorbate	2.075g	2.075g	2.075g
Choline chloride	0.35g	0.35g	0.35g
Wheat germ	16.675g	16.675g	16.675g
Alphacel/ cellulose powder	10g	10g	10g
Wheat germ oil	0.9ml	0.9ml	0.9ml
Linoleic acid	1g	1g	1g
Linseed oil	3 ml	-	1.5 ml
Sodium Alginate	1.675g	1.675g	1.675g
Ascorbic acid	1.2825g	1.2825g	1.2825g
Sucrose	0.9g	0.9g	0.9g
Folic acid	0.0025g	0.0025g	0.0025g
Inositol	0.114g	0.114g	0.114g
CoBaDex forte*	3 tablets	3 tablets	3 tablets
Evion Vit E**	3 tablets	1 tablets	2 tablets

*Composition of CoBaDex Forte (marketed by GlaxoSmithKline)

Thiamine Mononitrate IP	10mg
Vitamin B_2 IP	10mg
Vitamin B_6 IP	3mg
Nicotinamide IP	100mg
Calcium Pantothenate IP	50mg
Folic acid IP	1500mcg
Vitamin B_{12} IP	15mcg
Vitamin C IP	150mg
Biotin USP	100mcg

**Composition of Evion Vit E (marketed by MERCK)

Tocopheryl Acetate IP 400mg

Table 5.2.3: Components of synthetic diet and their function

Component	Function
Agar media in distilled water	Gelling agent
Casein	Protein source
Dextrose	Carbohydrate source
Wesson's salt mixture	Mineral source
Cholesterol	Sterol
Potassium sorbate	Antimicrobial agent
Choline chloride	Precursor of acetylcholine esterase
Wheat germ	Source of protein, minerals, lipid, rich in PUFA and phytosterols, vitamins except ascorbic acid, amino acids
Alphacel/ cellulose powder	Bulking and binding agent
Wheat germ oil	Lipid source
Linoleic acid	Lipid source
Linseed oil	Lipid source (including linolenic acid)
Sodium Alginate	Bulking and binding agent
Ascorbic acid	To maintain the normal growth and development of some insects, particularly phytophagous insects (Nation 2001)
Sucrose	Phago-stimulants for many leaf-eating insects (Chapman 1998).
Folic acid	Necessary for nucleic acid biosynthesis
Inositol	Component of Inositol triphosphate (IP_3), a transducer of neuropeptides, a secondary messenger
Vitamin B Complex	Cofactors of enzymes (catalysing metabolic pathways)
Vitamin E	Helping reproduction in some insects and improving fecundity (McFarlen 1992); antioxidant

5.2.4 Discussion

Synthetic diets are simplified and optimized version of natural diets. Numerous compounds and their polymers of natural diet get eliminated while simplifying it into synthetic diet. Presence of all these compounds of natural diet when absorbed may be beneficial or harmful to the insect. Optimization yields a diet that aims to achieve maximal growth rate and attainment of the final size of insect (Shapiro 1992). Moreover, success of insect rearing depends not only on its diet composition but also on the rearing setup (Schoonhoven 1972). Optimum moisture content has a big role in enhanced pupal survivability and adult emergence. Andrewartha and Birch (1984) emphasized on the importance of temperature and relative humidity on the vital activities of insect like metabolism, behaviour, reproduction, embryonic development and mortality.

While formulating a synthetic diet, trial and error approach may be undertaken where foods of various nutritional quality are offered to the concerned insect to see whether it is acceptable or not (Cohen 1992). Waldbauer (1968) described the development of synthetic diet with addition and deletion technique. Growth and development was assessed to determine which nutrient was essentially required, and same was incorporated in the diet. First diet for a geometrid moth, the fall cankerworm, *Alsophila pometaria* was formulated by Lyon and Brown (1970). Taking clues from Cohen (2004), the said geometrid diet was chosen for rearing the geometrid species of tea that are presently under study. As the fall cankerworm diet was found deficient in supporting the tea infesting geometrids, new formulated synthetic diets were tried, wherein modifications were contemplated with replacement of Vanderzant's vitamin fortification mixture by capsules of Vitamin B complex and Vitamin E along with Sucrose, Inositol and Folic acid. In order to overcome the developmental deformities further modifications were done for each species as per requirement.

Problems with adult emergence, adult deformity and crippled wings were mostly encountered among all the species of geometrid studied. Linoleic acid and vitamin E were added to overcome the wing deformity of adults of *H. talaca*. Linoleic acid is polyunsaturated fatty acid (PUFA). In insects, fatty acids are present in form of triacylglycerol (TAG) or diacylglycerol (DAG) which act as substrate for lipase (Fig. 5.2.5). The major fatty acids in insect associated with TAG are those with long chain (16-18 carbon atoms) that include Palmitic (C16:0), Palmitoleic (C16:1), Stearic (C18:0), Oleic (C18:1), Linoleic (C18:2) and Linolenic (C18:2) acids.

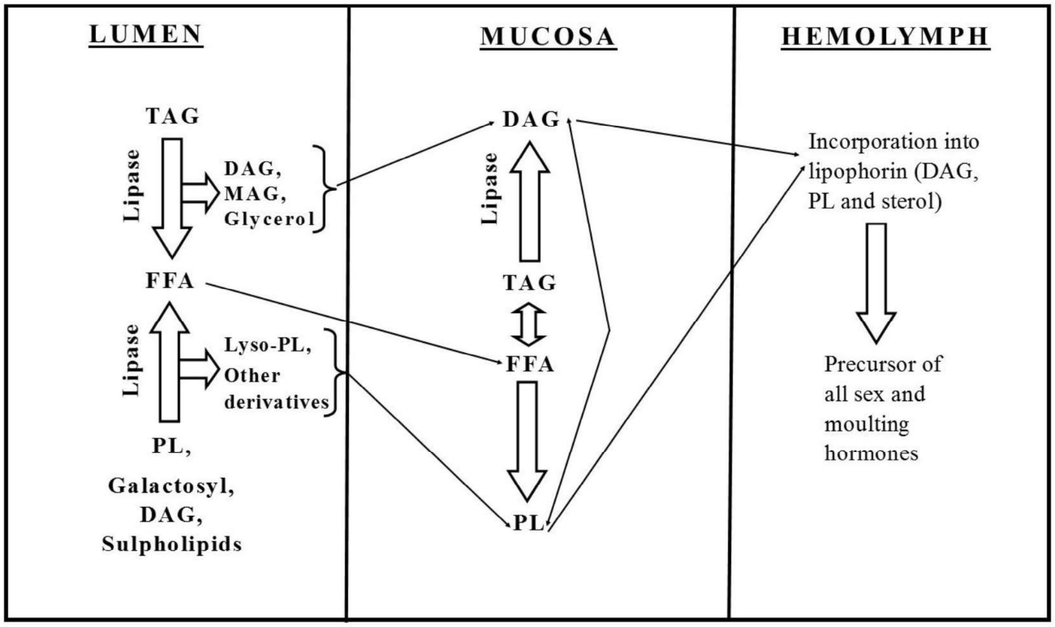


Figure 5.2.5 Lipid digestion and biosynthesis in insects [Adopted from Turunen (1985); TAG: Triacylglycerol; DAG: Diacylglycerol; FFA: Free Fatty acids; PL: Phospholipids; MAG: Monoacylglycerol]

Frankel and Blewett (1946) found that linoleic acid was essential in the Mediterranean flour moth, *Anagasta* (=*Ephestia*) *kuniella*, to produce intact wing scales, expansion of wings and emergence of the moths. Without linoleic acid these features were affected adversely. The extent of the abnormalities was found proportional to severity of the deficiency. The wing scales were actually formed but remained stuck to the cast off exuvium. *Pectinophora gossypiella* when reared on fat-deficient diets resulted in formation of faulty moth emergence and malformed scale-less wing (Beckman et al. 1953). Further linolenic acid was marked as more active than linoleic acid in promoting successful emergence of adults in *P. gossypiella* (Venderzant et al. 1957) which was also subsequently confirmed by Dadd (1985). Tan-Kristanto (2006) observed that a diet with low concentration of linoleic acid and linolenic acid did not result in larval mortality or prevention of adult emergence. However, they somewhat affected growth and enhanced frequency of adult abnormalities which mostly included crippled wings.

In the present study addition of linolenic acid was found effective in solving the problem related to the pupal and adult deformities in *B. suppressaria* and *H. infixaria*. Linolenic acid is a costly ingredient, therefore, to make the diet cost-effective, cheaper linseed oil was used. Linseed oil is a mixture of long-chain fatty acids, viz., α-Linolenic acid (51.9-55.2%), Linoleic acid (14.2-17%), Oleic acid (18.5-22.6%), Palmitic acid (7%) and Stearic acid (3.4-4.6%). As, α-linolenic acid is susceptible to autoxidation leading to the formation of harmful substances (Miyashita, 1990), vitamin E was added as an antioxidant. Holloway et al. (1991) also added linseed oil to the diet for butterflies to overcome the problem of extensive crippling of wings. Similarly Astuti (1992) stated that presence of linseed oil in the diet reduces the incidence of crippling in wings of *Papilio demoleus* adults.

As present study showed that larval development of *H. infixaria* took place satisfactorily on the diet without linseed oil supplementation, the early instars of *H. infixaria* could be reared on linseed oil-free diet up to 3rd instar. However, 4th instar onwards the larvae had to be reared on the diet supplemented with linseed oil for successful pupation and higher emergence. While studying the dietary regimes and preference of gypsy moth larvae from early to advanced instars, Stockhoff (1993) observed that there was a shift in food choice from 'high protein-low lipid' to 'high lipid-low protein' diet as the larval instars advanced. Stockhoff (1993) also reported that energy storage in terms of fat reserve was key factor for successful pupation and adult emergence in Gypsy moths. The importance of fatty acids in late instars of insect had been demonstrated by Rock et al. (1965) and Kato (1978). Unlike *H. infixaria*, its congener, *H. talaca* had no special requirement for linseed oil in the diet. Wheat germ oil and linoleic acid as source of fatty acid was found adequate for supporting development in *H. talaca* from egg to adult. In contrast to *Hyposidra* spp., *B. suppressaria* required both linoleic and linolenic acid throughout its larval stages for normal development. Being the primary consumer at the same ecological niche of tea ecosystem by and large with same food habit, such difference in nutritional requirement is somewhat intriguing or may be a species specific characteristics with some evolutionary significance.

Linoleic acid and linolenic acid are also the precursors of prostaglandins which is known to be involved in the regulation of ovarian development and ovipositional behaviour (Blomquist et al. 1991). As both these fatty acids are predominant components of ovarian triglycerides, linoleic acid and linolenic acid seem to be conveyed from the abdomen, possibly from the fat body, to the ovaries to carry out the functions related to reproduction (Murata 2001). In the pathway for the biosynthesis of arachidonic acid, Δ6 desaturase converts linoleic acid into linolenic acid. It is already mentioned that many lepidopterans cannot biosynthesize these two fatty acids. Therefore, both of them must be essentially supplemented through the diets. Moreover it is also known that all lepidopteran may not require both or may require them in less concentration.

There is one more aspect of the success story where these fatty acids play a very important role. The abundance of *H. talaca* in tea plantation areas of Darjeeling Terai and the Dooars is more than *H. infixaria* and *B. suppressaria* (Sinu et al. 2012). Sinu et al. (2011) also revealed that lack of any specialized natural enemies has given *H. talaca* the opportunity to build up its population in this region. Numerous studies have shown that plant defence system is triggered by substances in the regurgitants of the herbivores. The best known of these plant volatile elicitors are the fatty acid-amino acid conjugates (FACs) (Yoshinaga et al. 2010). FACs that gives out cues to attract natural enemies of the herbivore show maximum activity with glutamine-linolenic acid conjugates whereas, FACs show negligible activity when glutamine conjugates with linoleic, oleic and other minor fatty acids (Aboshi et al. 2007). It was also observed that some of the specialist herbivores (pests) have the ability to manipulate their FACs composition to avoid the natural enemies. Therefore, there is a possibility that *H. talaca* prefers fatty acids other than linolenic acid for bio-synthesising FACs which attracts less natural enemies thus having better chances of survival and success in evolving as a severe pest.

5.3 DEVELOPMENTAL TRAITS AND FOOD UTILIZATION EFFICIENCIES ON NATURAL HOST PLANTS (TEA AND NEEDLEWOOD)

5.3.1 *Biston* (=*Buzura*) *suppressaria*

The life-cycle traits of the geometrid species, *Biston* (=*Buzura*) *suppressaria* were studied on two natural hosts, tea (*C. sinensis*) and needlewood (*S. wallichi*) (Fig. 5.3.1).

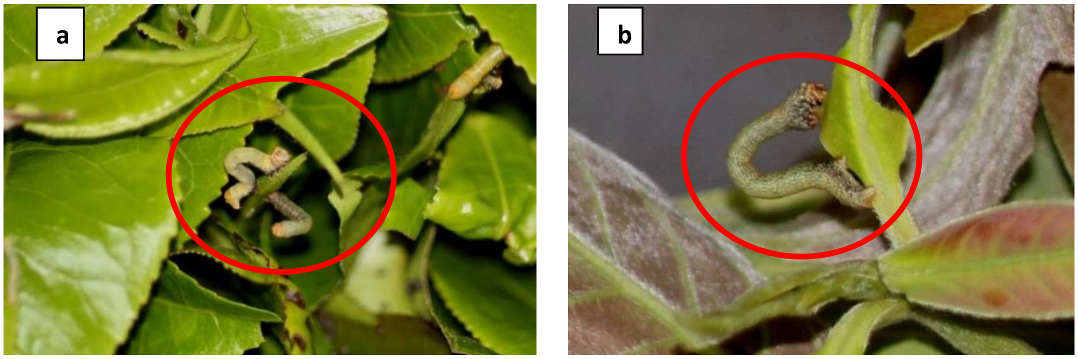


Figure 5.3.1 Rearing of *B. suppressaria* on (a) Tea and (b) Needlewood

Developmental traits: Larval period (p<0.001, Welch's approximate t= 7.544, df=27) and total developmental period (p<0.001, t= 4.783, df=38) along with fecundity (p<0.001, t= 4.776, df=18) were significantly different in *B. suppressaria* when reared on fresh tea and needlewood leaves, however no significant difference was observed in its pupal period (p=0.103, t= 1.669, df=38). Further there were no significant difference in weight of pupa (female: p=0.742, t= 0.331, df= 38; male: p=0.825, Welch's approximate t= 0.233, df=27) and adult (female: p<=0.204, t= 1.293, df=38; male: p=0.166, Welch's approximate t= 1.42, df=31) (Table 5.3.1).

Table 5.3.1: Comparison of parameters of life-cycle stages of *B. suppressaria* on tea and needlewood leaves (mean±SE)* (n=20) (*n=10, for fecundity)

	Larval Period (in days)	Pupal Period (in days)	Total Development Period (in days)	Fecundity *	Weight of Pupa		Weight of Adult	
					Female (in g)	Male (in g)	Female (in g)	Male (in g)
Tea	$31.425^a \pm 0.558$	$21.15^a \pm 0.302$	$62.575^a \pm 0.618$	$799^a \pm 15.27$	0.956 ± 0.025^a	0.74 ± 0.009^a	0.582 ± 0.015^a	0.268 ± 0.005^a
Needle-wood	$26.8^b \pm 0.255$	$22^a \pm 0.411$	$58.8^b \pm 0.491$	$698^b \pm 13.03$	0.945 ± 0.021^a	0.735 ± 0.02^a	0.609 ± 0.014^a	0.282 ± 0.008^a

*Means followed by different letter in each column are significantly different (Student *t*-test at $p \leq 0.05$, Welch's correction applied wherever required).

Table 5.3.2: Nutritional indices of fourth, fifth and sixth instars of *B. suppressaria*, reared on tea and needlewood leaves (mean± SE)* (n=20)

	RCR/h	RGR/h	ECI	ECD	AD	MC	PI
Fourth instar							
Tea	$0.301^a \pm 0.012$	$0.062^a \pm 0.002$	$20.656^a \pm 0.396$	$57.417^a \pm 2.818$	$37.207^a \pm 1.234$	$0.833^a \pm 0.089$	$0.574^a \pm 0.028$
Needle-wood	$0.427^b \pm 0.025$	$0.072^b \pm 0.004$	$17.227^b \pm 0.639$	$50.305^a \pm 2.155$	$34.855^a \pm 1.188$	$1.095^a \pm 0.141$	$0.503^a \pm 0.022$
Fifth instar							
Tea	$0.136^a \pm 0.002$	$0.02^a \pm 0.001$	$14.898^a \pm 0.586$	$47.212^a \pm 2.997$	$32.723^a \pm 1.154$	$1.315^a \pm 0.182$	$0.472^a \pm 0.030$
Needle-wood	$0.243^b \pm 0.012$	$0.031^b \pm 0.002$	$12.619^b \pm 0.425$	$35.606^b \pm 2.565$	$39.299^b \pm 2.405$	$2.197^b \pm 0.235$	$0.356^b \pm 0.026$
Sixth instar							
Tea	$0.165^a \pm 0.011$	$0.023^a \pm 0.002$	$14.013^a \pm 0.616$	$36.089^a \pm 1.919$	$39.850^a \pm 1.678$	$2.020^a \pm 0.179$	$0.361^a \pm 0.019$
Needle-wood	$0.289^b \pm 0.021$	$0.022^a \pm 0.002$	$7.670^b \pm 0.271$	$15.661^b \pm 1.017$	$52.217^b \pm 2.263$	$6.257^b \pm 0.588$	$0.157^b \pm 0.010$

*Means followed by different letter in each column in each instar are significantly different (Student *t*-test at $p \leq 0.05$, Welch's correction applied wherever required).

Food utilization efficiencies: In *B. suppressaria*, 4th instar larvae showed significantly higher RCR/h ($p<0.001$, $t=4.615$, $df=38$) and RGR/h ($p=0.026$, Welch's approximate $t=2.347$, $df=29$) on needlewood than on tea. Whereas, ECI ($p<0.001$, $t=4.566$, $df=38$) was significantly higher on tea along with marginally higher ECD ($p=0.052$, $t=2.006$, $df=38$), AD ($p=0.178$, $t=1.374$, $df=38$) and PI ($p=0.052$, $t=2.006$, $df=38$). The MC ($p=0.123$, $t=1.578$, $df=38$) was marginally lesser on tea. In 5th instar almost similar trends of food utilization efficiencies as in 4th instar were observed. RCR/h ($p<0.001$, Welch's approximate $t=8.983$, $df=38$) and RGR/h ($p<0.001$, $t=4.726$, $df=23$) were significantly higher on needlewood than on tea. ECI ($p<0.01$, t=3.15, $df=38$), ECD ($p=0.006$, $t=2.944$, $df=38$), AD ($p=0.002$, Welch's approximate $t=2.466$, $df=27.306$) and PI ($p=0.006$, Welch's approximate $t=2.944$, $df=37$) were significantly higher on tea than on needlewood along with lesser MC ($p=0.005$, $t=2.97$, $df=38$). In final larval instar (6th instar), RCR/h ($p<0.001$, Welch's approximate $t=5.209$, $df=29$) was significantly higher on needlewood than on tea, but with no significant difference in RGR/h ($p=0.518$, $t=0.653$, $df=38$). However, ECI ($p<0.001$, Welch's approximate $t=9.43$, $df=26$), ECD ($p<0.001$, Welch's approximate t=9.411, $df=29$), AD ($p<0.001$, $t=4.392$, $df=38$) and PI ($p<0.001$, Welch's approximate $t=9.411$, $df=29$) were significantly higher on tea than that on needlewood along with significantly lesser MC ($p<0.001$, Welch's approximate t=6.892, $df=23$) (Table 5.3.2).

5.3.2 *Hyposidra talaca*

The geometrid pest of tea, *Hyposidra talaca* was reared on its natural hosts, tea and needlewood (Fig. 5.3.2).

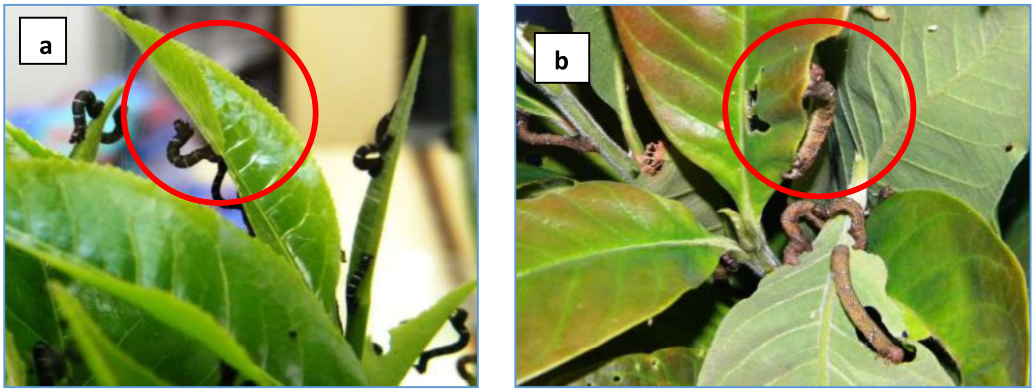


Figure 5.3.2 Rearing of *H. talaca* on (a) Tea and (b) Needlewood

Developmental traits: Larval development period ($p<0.001$, $t= 7.611$, $df=38$) of *H. talaca* on the natural diet tea and needlewood was significantly different whereas, there was no significant difference when pupal period ($p=0.123$, $t= 1.579$, $df=38$) and total developmental period (egg to adult) ($p=0.113$, $t=1.625$, $df=38$) were compared. Fecundity was significantly higher on tea than on needlewood ($p=0.001$, $t=3.476$, $df=38$). Both pupal (female: $p<0.001$, $t=6.589$, $df=38$; male: $p=0.002$, $t=3.342$, $df=38$) and adult (female: $p<0.001$, Welch's approximate $t=11.793$, $df=20$; male: $p<0.001$, $t=17.232$ $df=38$) weights were found to be significantly higher on tea than on needlewood (Table 5.3.3).

Food utilization efficiencies: In 4th instar of *H. talaca*, RCR/h ($p<0.001$, $t=4.615$, $df=38$) was found to be higher on needlewood than tea whereas, RGR/h ($p=0.026$, Welch's approximate $t= 2.347$, $df=29$), ECI ($p<0.001$, $t=4.566$, $df=38$) was higher on tea along with no significant difference in ECD ($p=0.052$, $t= 2.006$, $df=38$), AD ($p=0.178$, $t=1.374$, $df=38$), MC ($p=0.123$, $t=1.578$, $df=38$) and PI ($p=0.052$, $t=2.006$, $df=38$). However, MC was found to be marginally higher on needlewood along with slightly lesser value of PI. While comparing the food utilization indices of 5th instar, more or less same trend was observed. RCR/h ($p<0.001$, $t=3.971$, $df=38$) and MC ($p<0.001$, Welch's approximate $t=4.51$, $df=38$) were found to be higher on needlewood than that on tea. ECI ($p<0.001$, $t=6.270$, $df=38$), ECD ($p<0.001$, Welch's approximate $t=5.623$, $df=30$) and PI ($p<0.001$, $t=5.623$, $df=30$) values were higher on tea than that on needlewood along with no significant difference in RGR/h values ($p=0.47$, Welch's approximate $t=0.733$, $df=25$) and AD ($p=0.971$, $t=0.037$, $df=38$) (Table 5.3.4). Higher RGR of 4th and 5th instars on tea compared with needlewood indicated better utilization of the former plant as food. The ECI and ECD values in general were found to decline with advancement of instar.

Table 5.3.3: Comparison of parameters of life-cycle stages of *H. talaca* on tea and needlewood leaves (mean±SE)* (n=20)

	Larval Period (in days)	Pupal Period (in days)	Total Development Period (in days)	Fecundity	Weight of Pupa		Weight of Adult	
					Female (in g)	Male (in g)	Female (in g)	Male (in g)
Tea	16.5±0.185^{a}	7.3±0.179^{a}	30.5 ± 0.401^{a}	168.3±11.72^{a}	0.238± 0.012^{a}	0.174± 0.003^{a}	0.156±0.011^{a}	0.09± 0.004^{a}
Needlewood	19±0.271^{b}	7.7±0.179^{a}	31.35 ± 0.52^{a}	112.8±10.84^{b}	0.188±0.008^{b}	0.126±0.007^{b}	0.101±0.003^{b}	0.05± 0.002^{b}

*Means followed by different letter in each column are significantly different (Student *t*-test at $p \leq 0.05$, Welch's correction applied wherever required).

Table 5.3.4: Nutritional indices of fourth and fifth instars of *H. talaca*, reared on tea and needlewood leaves (mean± SE)* (n=20)

	RCR/h	RGR/h	ECI	ECD	AD	MC	PI
Fourth instar							
Tea	0.206±0.008^{a}	0.041±0.001^{a}	20.036±0.717^{a}	48.528±0.988^{a}	41.226±0.917^{a}	1.068±0.041^{a}	0.485±0.009^{a}
Needlewood	0.311±0.031^{b}	0.037±0.001^{b}	10.473±0.413^{b}	20.279±0.966^{b}	51.845±0.516^{b}	4.036±0.246^{b}	0.203±0.009^{b}
Fifth instar							
Tea	0.186±0.006^{a}	0.034±0.002^{a}	18.091±0.798^{a}	43.246±0.953^{a}	41.727±1.294^{a}	1.323±0.053^{a}	0.433±0.0095^{a}
Needlewood	0.163±0.011^{a}	0.014±0.001^{b}	9.006±0.419^{b}	15.957±0.812^{b}	56.598±0.576^{b}	5.426±0.352^{b}	0.159±0.0081^{b}

*Means followed by different letter in each column in each instar are significantly different (Student *t*-test at $p \leq 0.05$, Welch's correction applied wherever required).

5.3.3 *Hyposidra infixaria*

Study on life-cycle traits of another geometrid pest, *Hyposidra infixaria* were also conducted on two of its natural hosts (tea and needlewood) (Fig. 5.3.3).

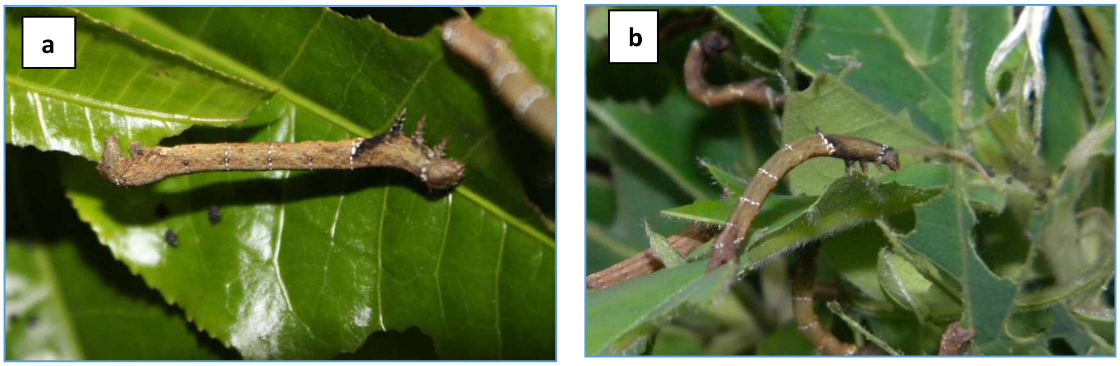


Figure 5.3.3 Rearing of *H. infixaria* on (a) Tea and (b) Needlewood

Developmental traits: Larval ($p<0.001$, Welch's approximate $t= 5.438$, $df=32$), pupal ($p=0.001$, $t= 3.525$, $df=38$) and total developmental period ($p<0.001$, $t=8.057$, $df=38$) of *H. infixaria* showed significant difference on tea and needlewood. Fecundity was not found to be significantly different on tea and needlewood ($p=0.221$ $t= 1.247$, $df=38$). Both female pupae ($p=0.885$, $t= 0.145$, $df=38$) and adults ($p=0.414$, $t= 0.826$, $df=38$) showed no significant difference whereas male pupae ($p<0.001$, $t= 5.471$, $df=38$) and adults ($p=0.001$, $t= 3.737$, $df=38$) were heavier in case of tea-reared ones (Table 5.3.5).

Food utilization efficiencies: In 4th instar of *H. infixaria*, significantly higher RCR/h ($p=0.006$, Welch's approximate $t= 2.94$, $df=34$), RGR/h ($p<0.001$, $t= 11.541$, $df=38$), ECI ($p<0.001$, Welch's approximate $t= 10.783$, $df=25$), ECD ($p<0.001$, $t= 5.017$, $df=30$), AD ($p<0.001$, $t= 5.412$, $df=38$) and PI ($p<0.001$, Welch's approximate $t= 5.017$, $df=30$) along with significantly lesser MC ($p<0.001$, Welch's approximate $t= 4.569$, $df=23$) were recorded on tea-reared ones than needlewood-reared ones. Whereas in 5th instar, RCR/h ($p<0.001$, $t=3.971$, $df=38$) was significantly higher in needlewood along with no significant difference in RGR/h (p=0.47, Welch's approximate t=0.733, df=25) on both the host plants. ECI (p<0.001, t=6.27, df=38), ECD (p<0.001, Welch's approximate t=5.623, df=30) and PI (p<0.001, Welch's approximate t=5.623, df=30) were significantly higher on tea with significantly reduced MC (p<0.001, Welch's approximate t=4.510, df=21) than that on needlewood. AD (p=0.971, t= 0.037, df=38) showed no significant difference (Table 5.3.6).

Table 5.3.5: Comparison of parameters of life-cycle stages of *H. infixaria* on tea and needlewood leaves (mean±SE)* (n=20)

	Larval Period (in days)	Pupal Period (in days)	Total Development Period (in days)	Fecundity	Weight of Pupa		Weight of Adult	
					Female (in g)	Male (in g)	Female (in g)	Male (in g)
Tea	20.6 ± 0.169^{a}	9.9 ± 0.24^{a}	36.5 ± 0.276^{a}	559 ± 29.49^{a}	0.274 ± 0.013^{a}	0.159 ± 0.004^{a}	0.199 ± 0.005^{a}	0.058 ± 0.003^{a}
Needlewood	18.85 ± 0.274^{b}	8.8 ± 0.200^{b}	33.65 ± 0.221^{b}	530 ± 28.65^{a}	0.273 ± 0.007^{a}	0.139 ± 0.004^{b}	0.195 ± 0.004^{a}	0.047 ± 0.001^{b}

*Means followed by different letter in each column are significantly different (Student *t*-test at $p\leq 0.05$, Welch's correction applied wherever required).

Table 5.3.6: Nutritional status and growth rate of fourth and fifth instars of *H. infixaria*, reared on tea and needlewood leaves (mean± SE)* (n=20)

	RCR/h	RGR/h	ECI	ECD	AD	MC	PI
Fourth instar							
Tea	0.293 ± 0.0104^{a}	0.0623 ± 0.002^{a}	21.314 ± 0.381^{a}	54.501 ± 1.775^{a}	39.385 ± 1.114^{a}	0.854 ± 0.065^{a}	0.545 ± 0.018^{a}
Needlewood	0.257 ± 0.15^{b}	0.035 ± 0.003^{b}	13.863 ± 0.915^{b}	$42.294\pm2.915^{\mathbf{b}}$	33.122 ± 1.151^{b}	1.487 ± 0.182^{b}	0.423 ± 0.029^{b}
Fifth instar							
Tea	0.227 ± 0.011^{a}	0.041 ± 0.002^{a}	23.127 ± 1.112^{a}	63.829 ± 2.209^{a}	36.579 ± 2.134^{a}	0.582 ± 0.053^{a}	0.638 ± 0.022^{a}
Needlewood	0.227 ± 0.014^{a}	0.038 ± 0.004^{a}	16.483 ± 1.087^{b}	46.329 ± 3.929^{b}	36.673 ± 2.221^{a}	1.327 ± 0.233^{b}	0.463 ± 0.039^{b}

*Means followed by different letter in each column in each instar are significantly different (Student *t*-test at $p\leq 0.05$, Welch's correction applied wherever required)

5.3.4 Discussion

It is evident from the studies on different geometrid pests that food intake and its utilization are largely responsible for their growth, development and reproduction. Quality and quantity of food consumed during post-embryonic developmental stages affect growth rate, developmental time, body weight, survival, fecundity, longevity and their capacity to convert into body mass (Parra et al. 2012). The majority of herbivorous insects live in heterogeneous environment that consist of variety of host plants, climate and biological conditions. As a result, these insects often show variation in morphological, biological and ecological attributes of their populations (Singh and Cunningham 1981; Helden et al. 1984; Powell et al. 2006). A number of studies on insect herbivores have shown significant intra-specific variation in characters associated with host plant utilization (Futuyama and Philippi 1987; Via 1990).

The forest tree needlewood (*S. wallichi*) and the tea bush (*C. sinensis*) provide different host environment and quality of food to the folivorous geometrid pests, subsisting on them. So, certain common phytochemicals characters of these host plants, belonging to the same family (Theaceae), were expected to be the basis for their selection as food by these looper species as previous exposure to host odours has been found to leave trail effect on their olfactory sensitivity (van Loon and Frentz 1991). However, the variable utilization of the two hosts by the loopers might be dependent on their physical characters and some biochemical parameters of their leaves used as food.

Both *Hyposidra* species are well-adapted to tea in having multiple broods as compared to only few broods of *B. suppressaria* a year. More number of broods of *Hyposidra* spp. on tea have possibly enabled *Hyposidra* spp. to utilize the host in a more efficient way than the alternate host (needlewood). Their better performance on tea has likely resulted from a directional selection mechanism through better adaptation to tea. The *B. suppressaria* however has failed to show a similar adaptive trend on tea. As a generalist, within few generations *Hyposidra* spp. showed development of the capability to efficiently utilize both forest (needlewood) and plantation host (tea). No significant difference in pupal and adult weight could be recorded in *B. suppressaria* when reared on two of these host plants, tea and needlewood (Das 2015). Despite the difference in developmental period on needlewood and tea, life-cycle of *B. suppressaria* could be successfully completed on both the hosts, resulting in same sized pupae and adults. A change in relative growth trend (RGR) of *B. suppressaria* was noticeable from 4th to 5th and 6th larval instars. While RGR was marginally more on needlewood leaf in 4th instar than that on tea, the trend changed to better relative growth in 5th and 6th instars on tea than on needlewood. Such a shift may be related to the instar specific host utilization strategy. Because of higher ECI, ECD and PI on tea, it may be assumed that availability of nutrients was more in tea leaves than in needlewood leaves which consequently resulted in higher fecundity of females. Optimum protein is required for secretion of juvenile hormones which is essential for development of ovaries and eggs (Genc 2006). The reproductive ability of adults largely depends on the larval diet (Awmack and Leather 2002; Hanks 1999). Similar observation was recorded by Golizaddeh et al. (2009) while rearing *Plutella xylostella* (Lepidoptera: Plutellidae) on different host plants, where they found that the shorter developmental period of the pest was on kohlari but cauliflower and cabbage were most preferred host as the species performed well in terms of reproductive potential and intrinsic growth rate.

In general, shorter developmental period with heavier pupae and adults were considered as the criteria to judge the suitability of any food regime. However, in contrast to the general idea, an insect may either shorten its development at the cost of its size, or may grow large at the cost of long development (Jarošik and Honek 2007) as has been observed in case of *H. infixaria*. Another concept of life-history theory which states that longer development time may result in bigger adult, supports similar observation made on *H. infixaria* (Poykoo and Hyvarinen 2011).

In both the congeners of *Hyposidra*, pupae and adults were found to be heavier on tea, whereas larvae reared on needlewood resulted into comparatively lighter pupae and adults. Many of the morphological features are considered as life-history traits because of their contribution to the reproductive success of a species (Agnew et al. 2000, 2002). The body weight is an important fitness indicator of insect population dynamics (Liu et al. 2004). Pupal weight can be an indirect, but easily measured indicator of lepidopteran fitness (Leuck and Perkins 1972). Appreciable reduction in weight of pupae and adults of *Hyposidra* spp. on 'needlewood' is possibly due to availability of suboptimal nutrients and moisture from its leaves which are essential for proper growth and development. Similar effect was observed by Mensah and Kudom (2011) while rearing *Hypolimnas missipus* (Lepidoptera: Nymphalidae) on less nutritious *Asystasia gangetica* (Acanthaceae) compared to other more nutritious host plants.

While comparing the nutritional indices of three geometrid species, it was found that, in general, the RCR was either higher on needlewood or marginally differed on the two hosts. This may be due to leaf quality. Leaves of different plants differ in their suitability as insect food because of variations in their nutrient and moisture content, type and concentration of secondary plant compounds and degree of sclerophyll (toughness/fibre) (Gullan and Cranston 1994). For instance, an increase in leaf toughness (Hunter and Lechowicz 1992) and a reduction in moisture content of the foliage (Martin and van't Hof 1988) decreases the leaf nutritional quality of a host variety, resulting into higher feeding rate and enhanced RCR in *Lymantria dispar* (Lindroth et al. 1997). Herbivores, especially tree foliage feeders often encounter with low levels of plant nutrients, and so their success of growth and reproduction depends upon their ability to convert ingested and digested food efficiently and to metabolize plant nitrogen with optimal level of leaf water (Scriber and Slansky 1981).

Higher ECD values suggest higher food efficiency and lower cost of maintenance (Sabhat et al. 2011). Significant differences in ECI and ECD values indicate varying nutritive value of host plants (Kouhi et al. 2014). Instar wise indices may have differences as the nutritional requirement of insect changes through development and such differences typically result in change of food consumption and utilization (Barton Browne 1995). In all three species of geometrid studied, ECI and ECD values got reduced as the instars advanced. The physiological changes in penultimate and ultimate larval instars probably were responsible for such reductions in ECD values of the last two larval instars as was also observed by Nation (2001)

Soo Hoo and Frankel (1966) noted that the negative correlations between RCR and ECI could have either of two explanations. First, when larvae consume less, the food tends to pass through their digestive system more slowly, and so it can be converted more completely and used by the insects. Second, it may be that insects consume less of a special food simply because they are capable of converting it more efficiently and therefore do not need to eat large quantities of that food to reach appropriate levels of growth.

The RGR value is known to decline with advancement of instars. This changed may be due to internal metamorphosis, partitioning and extra expenditure of energy prior to pupation as has been explained by Soo Hoo and Fraenkel (1966) for last instar larva of tree feeding southern armyworm *Prodenia eridania*. Usually little or no change in internal morphology occurs between larval moults, but major changes occur during transformation into pupa or adult (Nation 2001). Physiological changes in the nervous system of 5th instar cause cessation of feeding, on set of wandering behaviour, and induction of metabolic changes in the fat body (Naseri et al. 2010).

The maintenance cost of all three species on needlewood was higher than on tea. Increase in food consumption rate that enhanced the maintenance cost on needlewood may be due to its less nutritious food quality. Presumably, most of the ingested food was used up in basal metabolism, resulting in low utilization for growth of the insect. A similar phenomenon was observed by Mukherjii and Guppy (1970), Pradhan et al. (2006), Srivastava and Mukhopadhyay (2006) in the larvae of *Pseudaletia unipuncta*, *Euproctis latisfascia* and *Chrysomela chlorina* respectively. The production index was found to be higher on tea than on needlewood and this might be due to the better suitability of tea leaf as food in supporting the advanced life stages of the species. Plant defense chemicals also alter performance of digestibility in herbivores (Muthukrishnan and Pandian 1987).

5.4 DEVELOPMENTAL TRAITS AND FOOD UTILIZATION EFFICIENCIES ON SYNTHETIC DIET AND NATURAL HOST (TEA)

5.4.1 *Biston* (=*Buzura*) *suppressaria*

This was the first successful attempt to rear the *B. suppressaria* through generations on synthetic diet as an alternative to tea leaf diet (Fig. 5.4.1). A comparative study on performance of the pest on its natural host (tea leaf) and on newly developed synthetic diet revealed adequacy and superiority of the latter diet.

Figure 5.4.1 Rearing of *B. suppressaria* on synthetic diet

Developmental traits: The total development period was significantly shorter in synthetic diet-reared *B. suppressaria* than tea-reared ones (p<0.001, Welch′s approximate t=18.535, df=33). The pupa (female: p<0.001, t= 6.312, df=38; male: p<0.001, t= 3.894, df=38) and female moths (p<0.001, t= 4.919 df=38) were found to be significantly heavier on synthetic diet than on tea. Weight of male moths

(p=0.703, t= 0.384, df=38) however, did not differ significantly on the said diets (Table 5.4.1). Fecundity was not significantly different on synthetic diet and tea (p=0.794, t= 0.264, df=18). Adult emergence from pupae was better on synthetic diet (99%) than natural diet (94.44%) (Table 5.4.1). On both natural and synthetic diets, complete development of *B. suppressaria* from egg to adult stage took place for three continuous generations. In general, the survivorship curves on both the diets showed similar trends with constant rate of mortality at early stages (Fig. 5.4.2).

Table 5.4.1 Comparative account of some life-cycle parameters of *B. suppressaria* reared on natural diet (tea) and synthetic diet (diet) (mean± SE)* (n=20) (*n=10 for fecundity, **n=100 for survivorship study)

	Total Development Period (in days)	Fecundity * (eggs/female)	Survival %**	Adult Emergence %	Pupal weight (in g)		Adult weight (in g)	
					Male	Female	Male	Female
Tea	62± 0.74^{a}	799± 15.27^{a}	56.33	94.44	0.731± 0.019^{a}	0.936± 0.021^{a}	0.269± 0.005^{a}	0.585± 0.015^{a}
Diet	48± 0.468^{b}	792± 19.95^{a}	64.11	99	0.827± 0.015^{b}	1.185± 0.033^{b}	0.271± 0.005^{a}	0.722± 0.024^{b}

*Means followed by different letter in each column are significantly different (Student t-test at $p \leq 0.05$, Welch's correction applied wherever required).

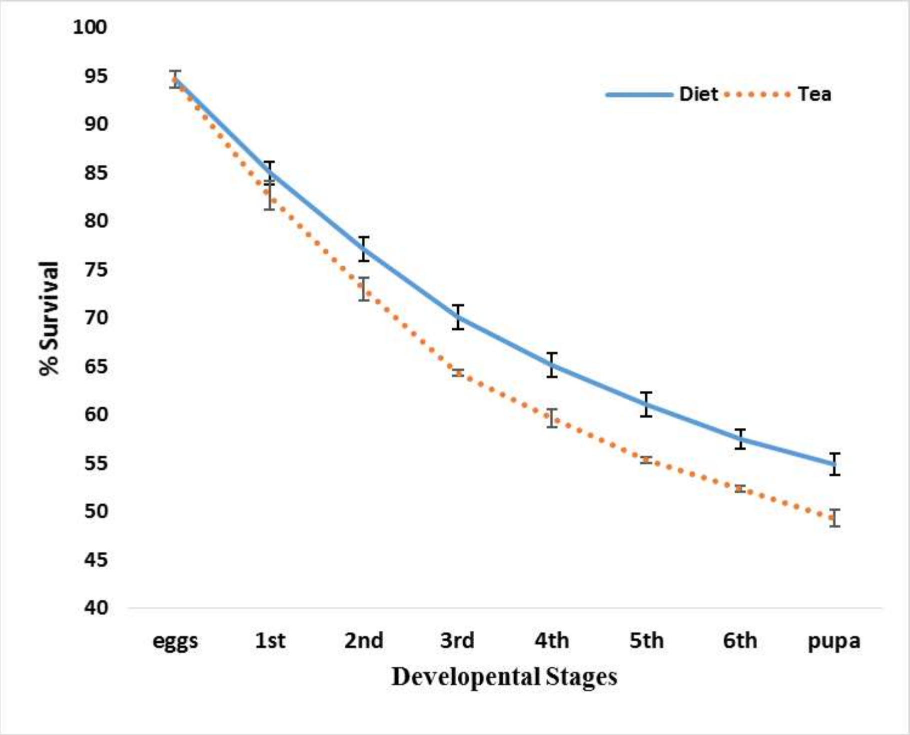


Figure 5.4.2 Survivorship curve of *B. suppressaria* reared on synthetic diet (Diet) and natural diet (Tea) (error bars indicate standard error of the mean)

Food utilization efficiencies: Low MC, higher PI, higher ECI and ECD, higher AD and higher RGR along with marginally higher RCR values (Table 5.4.2) recorded on the synthetic diet implied better sustaining quality of the newly formulated diet than the natural diet (tea) for rearing *B. suppressaria*. Comparison of the nutritional indices of 5th instar of *B. suppressaria* on tea and synthetic diet showed, significantly higher RGR/h value (p<0.001, Welch′s approximate t=14.125, df=23) on synthetic diet than on tea. There was no significant difference in RCR/h (p=0.863, Welch′s approximate t=0.175, df=20) on both the food regimes. Values of ECI (p<0.001, Welch′s approximate t=15.057, df=26), ECD (p<0.001, t=8.577, df=38), AD (p<0.001, t=8.7, df=38) and PI (p<0.001, t=8.577, df=38) were significantly higher on synthetic diet than on tea whereas MC value (p<0.001, Welch's approximate

$t=4.92$, $df=21$) was significantly less on synthetic diet. Again in 6th instar, RGR/h ($p<0.001$, Welch's approximate $t=6.933$, $df=28$), ECI ($p<0.001$, $t=12.093$, $df=38$), ECD ($p<0.001$, Welch's approximate $t=4.586$, $df=32$), AD ($p<0.001$, Welch's approximate $t=9.002$, $df=28$) and PI ($p<0.001$, Welch's approximate $t=4.586$, $df=32$) were significantly higher on synthetic diet than that on tea along with significantly lesser values of RCR/h ($p=0.001$, $t=3.784$, $df=38$) and MC ($p<0.001$, Welch's approximate $t=4.507$, $df=22$).

Table 5.4.2: Nutritional indices of fifth and sixth instars of *B. suppressaria* on natural diet (tea) and synthetic diet (diet) (n=20, mean*±SE)

	RCR/h	RGR/h	ECI	ECD	AD	MC	PI
Fifth instar							
Tea	0.135± 0.002^{a}	0.020± 0.000^{a}	14.898± 0.571^{a}	47.212± 2.921^{a}	32.723± 1.125^{a}	1.315± 0.177^{a}	0.472± 0.029^{a}
Diet	0.137± 0.008^{a}	0.041± 0.001^{b}	31.218± 1.014^{b}	67.492± 1.576^{b}	46.31± 1.073^{b}	0.499± 0.039^{b}	0.675± 0.016^{b}
Sixth instar							
Tea	0.165± 0.011^{a}	0.023± 0.002^{a}	14.013± 0.616^{a}	36.089± 1.919^{a}	39.85± 1.678^{a}	2.020± 0.179^{a}	0.361± 0.019^{a}
Diet	0.114± 0.0073^{b}	0.052± 0.004^{b}	26.357± 0.815^{b}	46.508± 1.218^{b}	56.659± 0.82^{b}	1.17± 0.054^{b}	0.465± 0.012^{b}

*Means followed by different letter in each column are significantly different (Student t-test at $p\leq 0.05$, Welch's correction applied wherever required).

5.4.2 *Hyposidra talaca*

Rearing of looper stage of *H. talaca* (Fig. 5.4.3) was done on synthetic diet for three consecutive generations with better survival (Fig. 5.4.4) and higher nutritional indices.

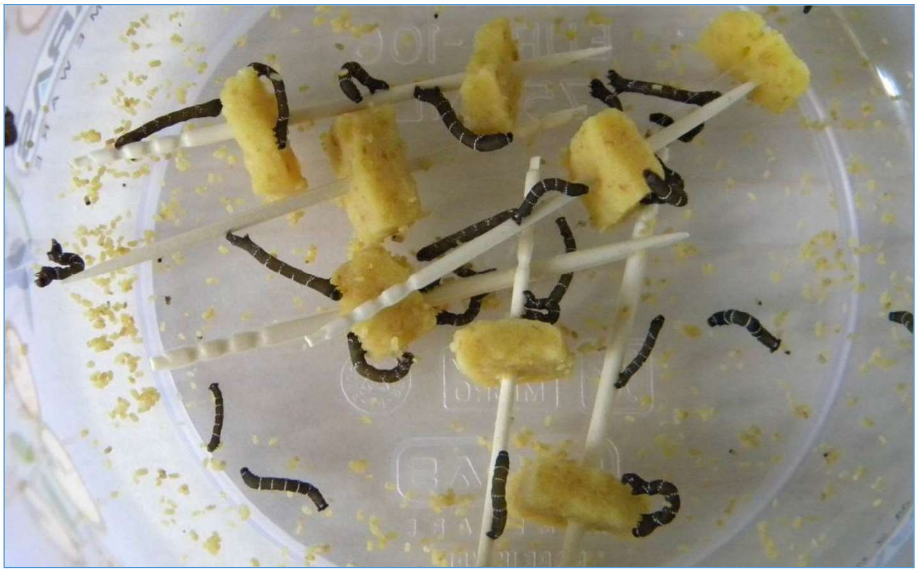


Figure 5.4.3 Rearing of *H. talaca* on synthetic diet

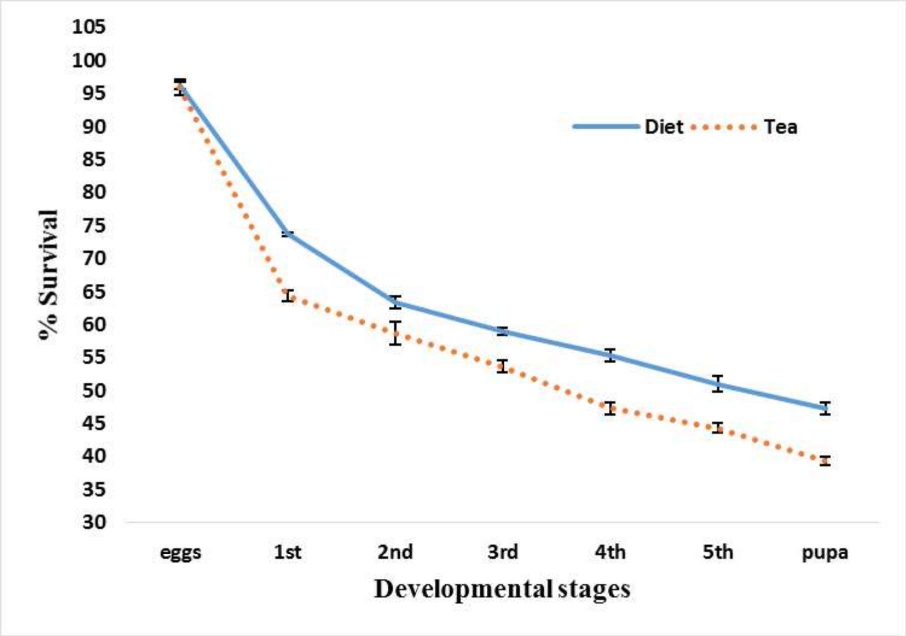


Figure 5.4.4 Survivorship curve of *H. talaca* on synthetic diet (Diet) and natural diet (Tea) (error bars indicate standard error of the mean)

Developmental traits: The total larval development period of *H. talaca* was significantly shorter on synthetic diet than on natural diet ($p<0.0001$, Welch's approximate $t= 7.857$, $df = 30$). Fecundity was more in *H. talaca* reared on synthetic diet than that on natural diet ($p=0.0267$, significant. $t=2.413$, $df=18$). Pupal weight (male: $p=0.083$, $t=1.781$, $df=38$, n=20 and female: $p=0.9779$, $t=0.02785$, $df=38$) was not significantly different on tea and synthetic diet. Similarly adult weight (male: $p=0.9971$, $t=0.0037$, $df=38$ and female: $p=0.1072$, $t=0.0037$, $df=38$) was also not significantly different on both food regimes (Table 5.4.3).

Table 5.4.3: Comparative account of life-cycle parameters of *H. talaca* reared on natural diet (tea) and synthetic diet (diet) (mean± SE)* (n=20) (*n=10 for fecundity, **n=100 for survivorship study)

Life-cycle parameters	Total Development Period (days)	Fecundity* (eggs/ female)	Survival (%)**	Adult emergence (%)	Pupal Weight (in g)		Adult weight (in g)	
					Male	Female	Male	Female
Tea	$30.3±0.38^{a}$	$168± 16.12^{a}$	39.00	78.38	$0.171± 0.004^{a}$	$0.23± 0.009^{a}$	$0.09± 0.0031^{a}$	$0.155± 0.0077^{a}$
Diet	$26.8±0.22^{b}$	$249± 27.25^{b}$	47.00	86.31	$0.172± 0.003^{a}$	$0.252± 0.008^{a}$	$0.094± 0.0023^{a}$	$0.186± 0.0051^{a}$

*Means followed by different letter in each column are significantly different (Student *t*-test at $p\leq 0.05$, Welch's correction applied wherever required).

Food utilization efficiencies: ECI ($p<0.0001$, $t= 7.406$, $df = 38$), ECD ($p<0.003$, Welch's approximate $t= 3.316$, $df = 23$), AD ($p=0.0004$, Welch's approximate $t= 4.011$, $df = 29$) and PI ($p=0.003$, Welch's approximate $t= 3.316$, $df = 23$) were significantly higher along with a lower MC ($p=0.0029$, Welch's approximate $t= 3.256$, $df = 29$) on synthetic diet as compared to natural diet. However, no significant difference in RGR ($p=0.7425$, $t= 0.3309$, $df =38$) was detected on the two diets, in spite of significantly lower RCR value (p=0.1123, t= 7.857, df = 30) on synthetic diet than natural diet (Table 5.4.4).

Table 5.4.4: Nutritional indices of fifth instar of *H. talaca*, reared on natural diet (tea) and synthetic diet (diet) (n=20, *mean± SE)

	RCR/h	RGR/h	ECI	ECD	AD	MC	PI
Tea	0.19± 0.01^{a}	0.03± 0.00^{a}	18.09± 0.79^{a}	43.25± 0.95^{a}	41.73± 1.29^{a}	1.32± 0.05^{a}	0.43± 0.01^{a}
Diet	0.14± 0.01^{b}	0.03± 0.03^{a}	24.63± 0.90^{b}	51.97± 2.39^{b}	47.84± 1.62^{b}	0.96± 0.08^{b}	0.52± 0.02^{b}

*Means followed by different letter in each column are significantly different (Student t-test at $p \leq 0.05$, Welch's correction applied wherever required).

5.4.3 *Hyposidra infixaria*

H. infixaria was reared (Fig. 5.4.5) on a slightly modified diet of *H. talaca* for three consecutive generation with better survivability than that on its natural host, tea (Fig. 5.4.6).

Developmental traits: Developmental period of *H. infixaria* was significantly longer on tea than on synthetic diet (p<0.0001, t=9.798, df=38). Fecundity was not significantly different on the two diets (p=0.2566, Welch's approximate t=0.166, df=21). Pupal weight (male: p=0.9484, Welch's approximate t=0.065, df=26 and female: p=0.6365, t=0.4764, df=38) as well as adult weight (male: p= 0.1239, t=1.573, df=38 and female: p=0.3682, t=0.9105, df=38) was also not significantly different on the two food

Figure 5.4.5 Rearing *H. infixaria* on synthetic diet

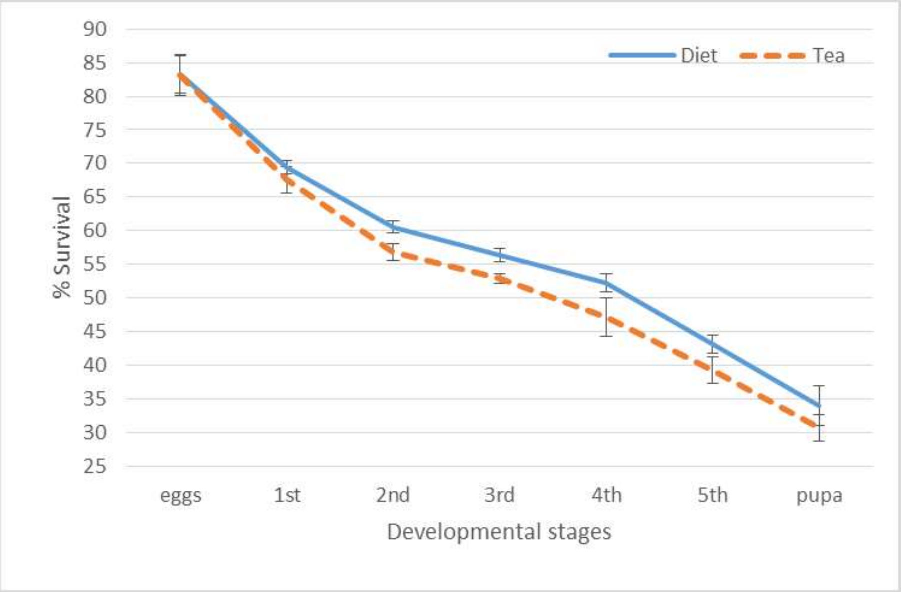


Figure 5.4.6 Survivorship curve of *H. infixaria* on synthetic diet (Diet) and natural diet (Tea) (error bars indicate standard error of the mean)

Table 5.4.5: Comparative account of some life-cycle parameters of *H. infixaria*, reared on natural diet (tea) and synthetic diet (diet) (mean± SE)* (n=20) (*n=10 for fecundity, **n=100 for survivorship study)

Life-cycle parameters	Total Development Period (days)	Fecundity* (eggs/ female)	Survival (%)**	Adult emergence (%)**	Pupal Weight (in g)		Adult weight (in g)	
					Male	Female	Male	Female
Tea	36.5± 0.27^{a}	559± 29.49^{a}	23.00	87.62	0.159± 0.003^{a}	0.27± 0.009^{a}	0.058± 0.003^{a}	0.2± 0.005^{a}
Diet	31.7± 0.4^{b}	523.5± 7.59^{a}	39.00	90.27	0.16± 0.006^{a}	0.28± 0.008^{a}	0.065± 0.003^{a}	0.193± 0.006^{a}

*Means followed by different letter in each column are significantly different (Student *t*-test at $p \leq 0.05$, Welch's correction applied wherever required).

Food utilization efficiencies: MC of 5th instar looper of *H. infixaria* was significantly higher on tea ($p<0.0001$, Welch's approximate $t=6.097$, $df=28$). PI ($p<0.0001$, $t=7.253$, $df=38$). AD ($p<0.0001$, $t=4.483$, $df=38$), ECI ($p<0.0001$, Welch's approximate $t=7.484$, $df=30$) and ECD ($p<0.0001$, $t=4.539$, $df=38$) were significantly higher on synthetic diet than tea. RCR/h was significantly higher on tea ($p<0.0001$, $t=5.488$, $df=38$) than on synthetic diet but RGR/h was not significantly different in two food regimes ($p=0.7692$, Welch's approximate $t=0.2967$, $df=24$) (Table 5.4.6).

Table 5.4.6: Nutritional indices of fifth instar of *H. infixaria*, reared on natural diet (tea) and synthetic diet (diet) (mean± SE)* (n=20)

	RCR/h	RGR/h	ECI	ECD	AD	Main. Cost	Prod. Index
Tea	0.177± 0.011^{a}	0.04± 0.001^{a}	23.19± 1.08^{a}	63.76± 2.15^{a}	36.75± 2.077^{a}	0.585± 0.051^{a}	0.638± 0.022^{a}
Diet	0.119± 0.01^{b}	0.041± 0.004^{a}	34.61± 1.87^{b}	75.66± 3.02^{b}	45.98± 2.04^{b}	0.335± 0.019^{b}	0.787± 0.02^{b}

*Means followed by different letter in each column are significantly different (Student *t*-test at $p \leq 0.05$, Welch's correction applied wherever required).

5.4.4 Discussion

Suitability of a synthetic diet can be gauged by comparing development time and survival rate of an insect on it when compared with their natural diet (Abdullah et al. 2000). In present study, shorter development period and better survival rate in all the three consecutive generations of the three geometrids implied that newly formulated synthetic diets prepared are of optimum quality to support a long term sustainable culture of the concerned geometrid species. Generally insects and especially lepidopterans take longer period to develop when fed with sub-optimal food plants and materials (Waters and Barfield 1989), but larvae reared on optimum synthetic diet often survive better and develop faster (Slansky and Scriber 1985; Chen et al. 2004).

In this maiden attempt to rear three geometrid species attacking tea plant, present survival rate of 64% in *B. suppressaria*, 47% in *H. talaca* and 39% in *H. infixaria* on synthetic diet could further be improved. In future, trials can be conducted with modified rearing system where less mortality of early instars may be expected from physical rearing problems. In the first rearing trial for Lesser Appleworm (Lepidoptera: Tortricidae) on lima bean-based semisynthetic diet, the mean survival rate at best was recorded 46.4% (Mantey et al. 2000). It was further evident that when reared for the first time on two synthetic diet formulations, Common Birdwing *Troides helena* (Lepidoptera: Papilionidae) showed a survival rate of 57.05% and 51.66% (Ngatimin et al. 2014). In another attempt, 62% survival of *Spodoptera exigua* was recorded when reared on synthetic diet (Abdullah et al. 2000). It was observed that mortality at early instar was higher in all the three geometrid species studied. Third instar onwards, survivorship of these species were more and in the tune of 80.23%, 60.36% and 78.29% for *H. talaca*, *H. infixaria* and

B. suppressaria respectively possibly indicating a better adaptation to the diet and the laboratory environment (rearing containers). Zalucki et al. (2002) pointed out in their review that mortality of early larval stages is high in many species of lepidoptera for unidentified reason. As observed during the rearing of three species of geometrids, the web formed by the secretion of *Hyposidra* spp. is more than *B. suppressaria*. This may be one of the causes behind their mortality. As size of neonates are smaller in case of *Hyposidra* spp. than those of *B. suppressaria*, they get entangled easily and die off. The synthetic diet seemed well-balanced as it supported emergence of mostly healthy moths (except few deformed ones), thus establishing the superiority of synthetic diet over natural diet for mass rearing of this pest. In a similar finding Felland and Hull (1992) and Slansky and Wheeler (1992) also observed that the larvae reared on optimal synthetic diet developed faster and survived better.

Studies on nutritional ecology and its indices have considerable significance in understanding insect development and assessing the quality diets on which they are reared (Cohen 2004; Waldbauer 1968). Broadly, these indices define or describe the efficiencies of digestion or utilization of diets or diet components, that is, how easily the insect can convert food to their own biomass.

In general, higher ECI, ECD, AD, PI and lower RCR which resulted into lower MC on synthetic diet in all three species of geometrids studied. Significantly higher ECI and ECD on synthetic diet indicated a better ability of these species to utilize the food ingested as well as digested, for growth and development. ECI may vary with digestibility of food and the proportional amount of digestible food which is converted to body mass and metabolized for energy needed for vital activity (Abdel-Rahman and Al-Mozini 2007). Lower AD in tea-reared caterpillars may be due to the presence of tannins in the leaves which can block the availability of protein by forming less digestible complexes (Feeny 1968). The young leaf of tea plants contains high levels of plant allelochemicals like polyphenolic compounds, caffeine, etc. (Banerjee 1993). Higher energy consumption in detoxification of allelochemicals might be resulting in significantly reduction in ECD (Appel and Martin 1992) as well as significantly higher MC and a reduced PI in loopers when reared on tea than those reared on synthetic diet. Reduction in RGR, ECI, ECD values led to delay in larval growth (Senthil-Nathan et al. 2007) and formation of smaller pupae which had a direct bearing on fecundity and longevity of the adult insect, besides, making them susceptible to diseases and natural enemies. The ability of an organism to convert nutrients, especially protein, positively influence its growth and development (Sogbesan and Ugwumba 2008). Decrease in AD, ECI and ECD values on tea leaf (natural diet) probably resulted from an increased allocation of assimilated energy from growth to MC required for detoxification of ingested plant allelochemicals (de Oliveira et al. 2011). The RCR can be used for measuring an insect's capability to exploit food source. This index shows the rate of feeding in an insect which much depends on water content as well as the physicochemical properties of food (Srinivasan and Uthamasamy 2005).

5.5 PRINCIPAL HYDROLASES AND DEFENSE ENZYMES OF LOOPERS REARED ON TEA, NEEDLEWOOD AND SYNTHETIC DIET

Enzymes activities in the midgut of the three geometrid species reared on three kinds of food, two natural host plants and one synthetic diet were estimated.

5.5.1 *Biston (=Buzura) suppressaria*

Amylase activity showed significant difference between the loopers reared on Tea and Synthetic Diet (henceforth called 'Diet') (*p*=0.006) and Needlewood and Diet (*p*=0.005), whereas no significant difference was observed between Tea and Needlewood reared specimens (*p*=0.92) (F=6.173, *p*=0.006, *df* =2, 27) (Fig. 5.5.1).

Invertase activity showed no significant difference between the cohorts reared on different foods (F=0.262, *p*=0.771, *df*=2, 27) (Fig. 5.5.2).

Protease activity showed significant difference between Tea and Needlewood (*p*=0.018) and Tea and Diet (*p*<0.001) reared cohorts but no significant difference between Needlewood and Diet (0.09) reared ones (F=9.218, *p*=0.001, *df*=2, 27) (Fig. 5.5.3).

Lipase activity showed no significance difference between *B. suppressaria* loopers reared on Tea and Needlewood (*p*=0.08), Needlewood and Diet (*p*=0.453) and Tea and Diet (*p*=0.3) (F=1.666, *p*=0.208, *df*=2, 27) (Fig. 5.5.4).

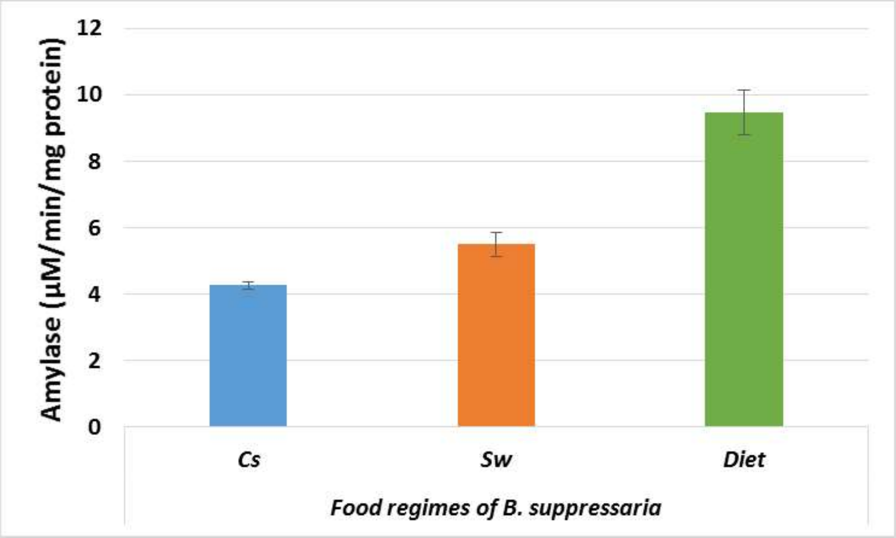


Figure 5.5.1 Amylase activity of *B. suppressaria* on three food regimes [Tea (*Cs*), Needlewood (*Sw*) and Synthetic diet (*Diet*)] (Error bars indicate standard error of mean)

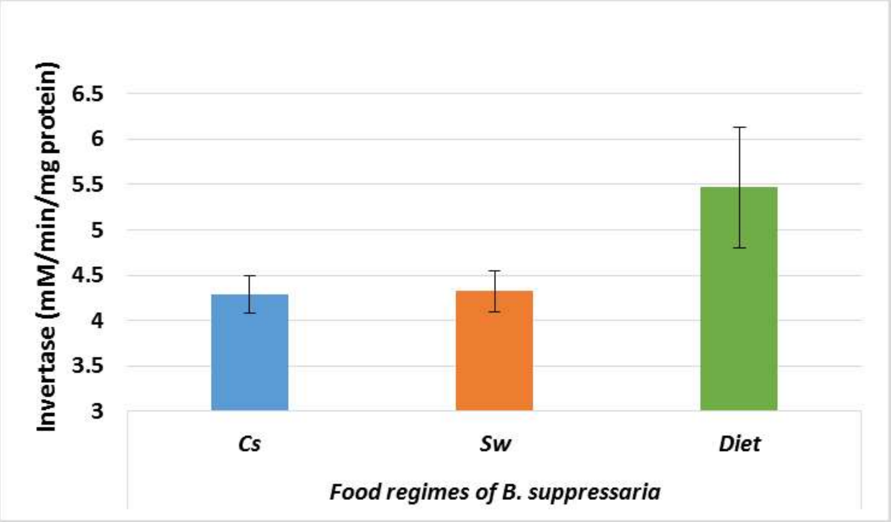


Figure 5.5.2 Invertase activity of *B. suppressaria* on three food regimes [Tea (*Cs*), Needlewood (*Sw*) and Synthetic diet (*Diet*)] (Error bars indicate standard error of mean)

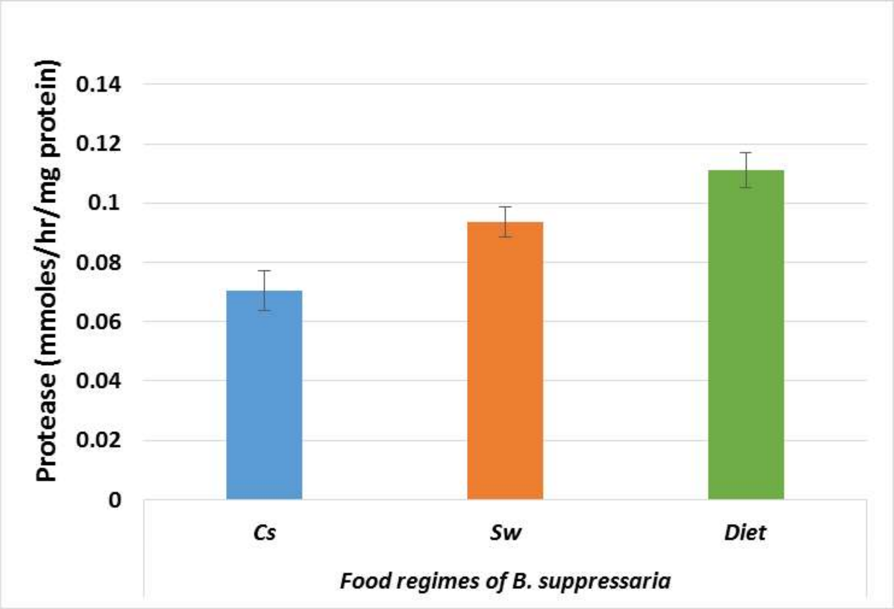


Figure 5.5.3 Protease activity of *B. suppressaria* on three food regimes [Tea (*Cs*), Needlewood (*Sw*) and Synthetic diet (*Diet*)] (Error bars indicate standard error of mean)

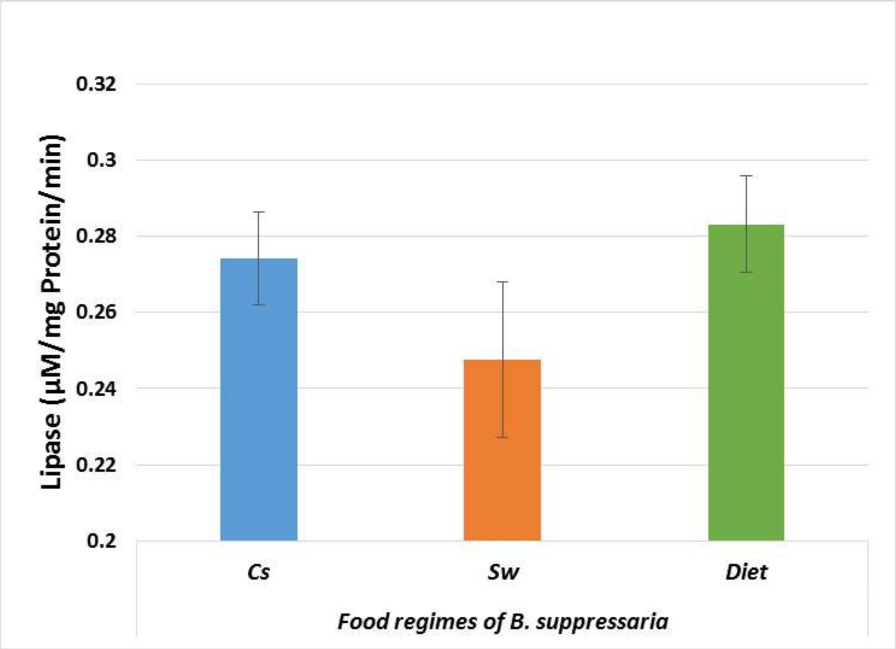


Figure 5.5.4 Lipase activity of *B. suppressaria* on three food regimes [Tea (*Cs*), Needlewood (*Sw*) and Synthetic diet (*Diet*)] (Error bars indicate standard error of mean)

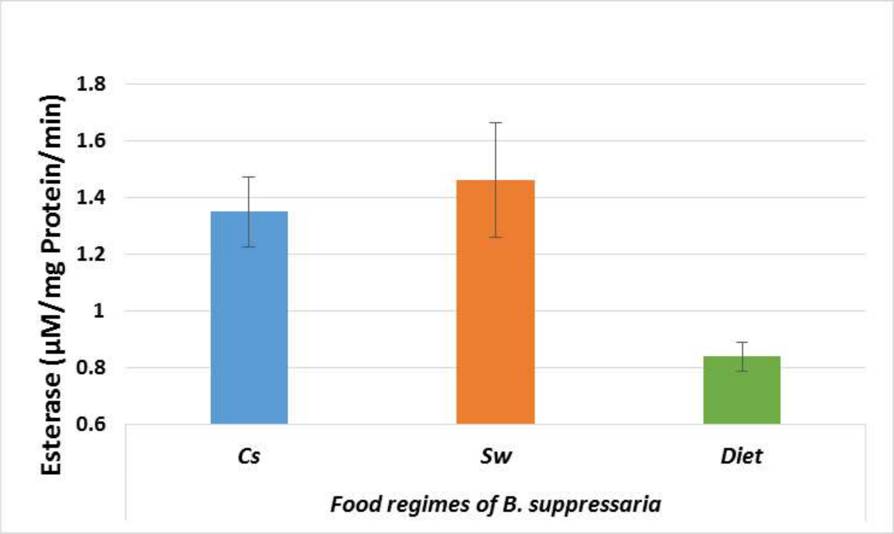


Figure 5.5.5 Esterase activity of *B. suppressaria* on three food regimes [Tea (*Cs*), Needlewood (*Sw*) and Synthetic diet (*Diet*)] (Error bars indicate standard error of mean)

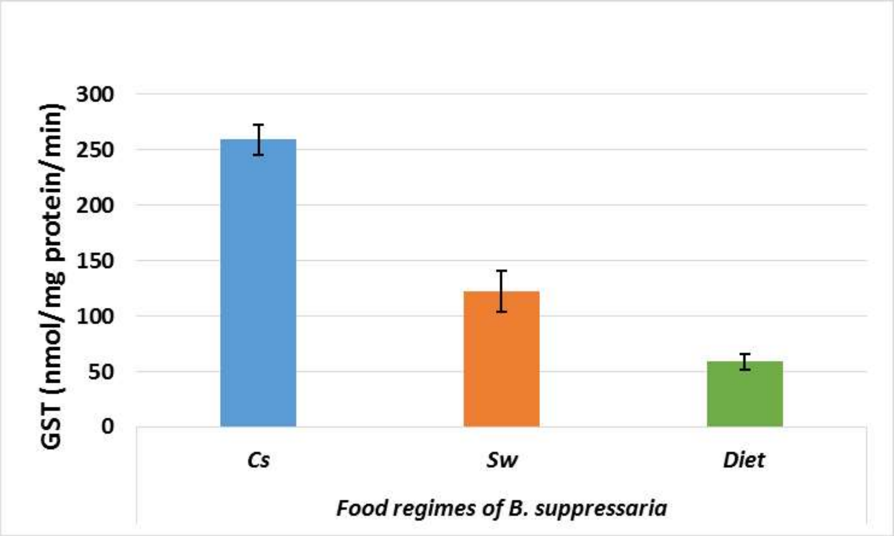


Figure 5.5.6 GST activity of *B. suppressaria* on three food regimes [Tea (*Cs*), Needlewood (*Sw*) and Synthetic diet (*Diet*)] (Error bars indicate standard error of mean)

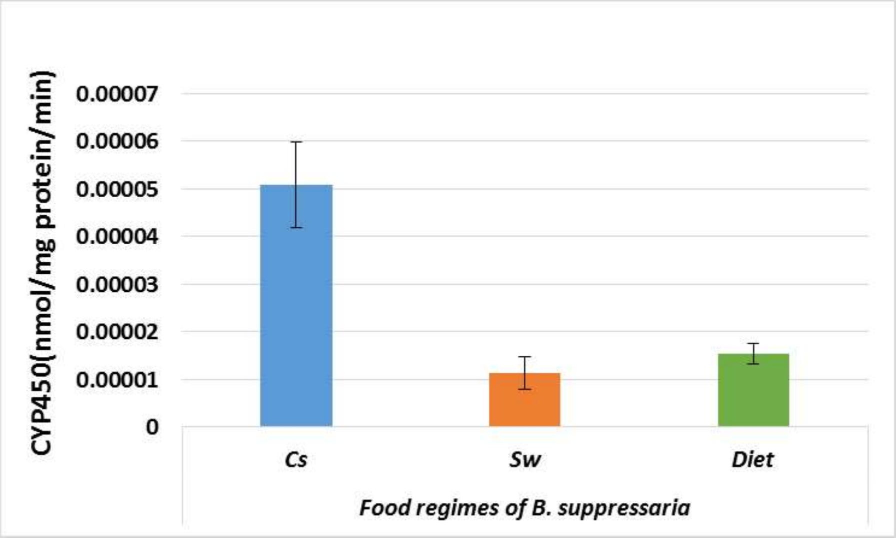


Figure 5.5.7 CYP450 activity of *B. suppressaria* on three food regimes [Tea (*Cs*), Needlewood (*Sw*) and Synthetic diet (*Diet*)] (Error bars indicate standard error of mean)

Esterase activity level in *B. suppressaria* showed no significant difference between Tea and Needlewood reared ones (*p*=0.175), whereas significant difference was recorded between Tea and Diet (*p* <0.001) and Needlewood and Diet reared ones (*p*=0.01) (F=8.957, *p*=0.001, *df*=2, 27) (Fig. 5.5.5).

GST activity showed significant difference between the loopers reared on all three food regimes (Tea and Needlewood, *p*<0.001; Tea and Diet, *p*<0.001; Diet and Needlewood, *p*<0.001) (F=35.749, *p*<0.001, *df*=2, 27) (Fig. 5.5.6).

Cytochrome P450 activity showed significant difference between loopers reared on Tea and Needlewood (*p*=0.001) and Tea and Diet (*p*=0.001), but no significant difference between Needlewood and Diet reared ones (*p*=0.848) (F=9.47, *p*=0.001, *df*=2, 27) (Fig. 5.5.7).

5.5.2 *Hyposidra talaca*

Amylase activity showed no significant difference between three cohorts of loopers reared on different food regimes, Tea, Needlewood and Synthetic diet (Diet) (F=0.211, *p*=0.811, *df*=2, 27) (Fig. 5.5.8).

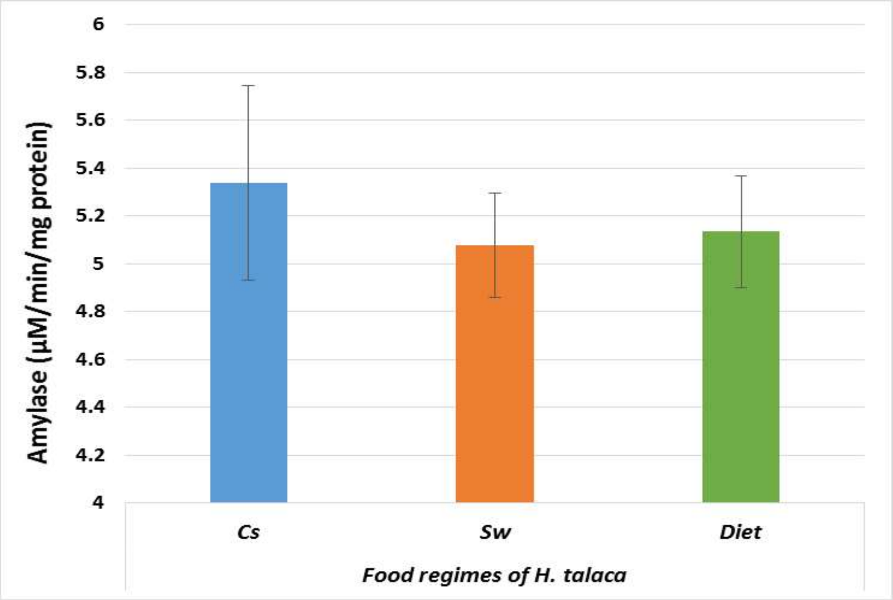


Figure 5.5.8 Amylase activity of *H. talaca* on three food regimes [Tea (*Cs*), Needlewood (*Sw*) and Synthetic diet (*Diet*)] (Error bars indicate standard error of mean)

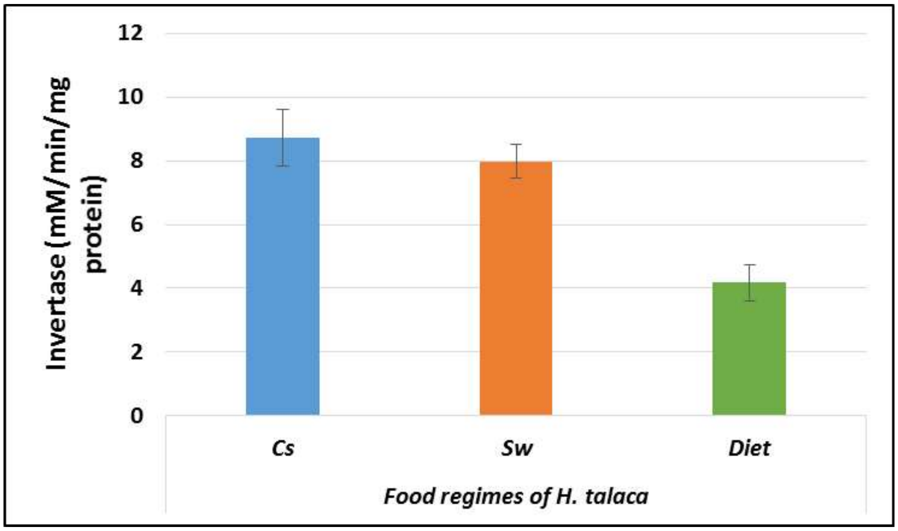


Figure 5.5.9 Invertase activity of *H. talaca* on three food regimes [Tea (*Cs*), Needlewood (*Sw*) and Synthetic diet (*Diet*)] (Error bars indicate standard error of mean)

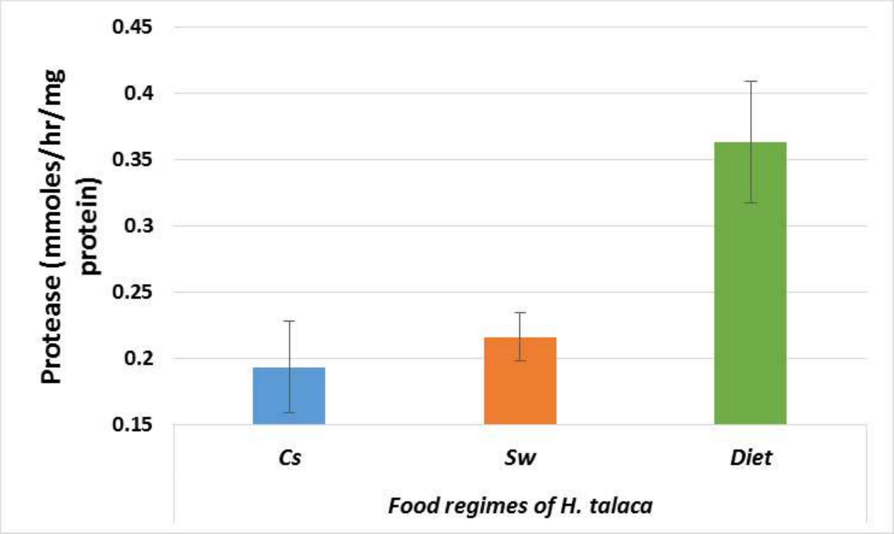


Figure 5.5.10 Protease activity of *H. talaca* on three food regimes [Tea (*Cs*), Needlewood (*Sw*) and Synthetic diet (*Diet*)] (Error bars indicate standard error of mean)

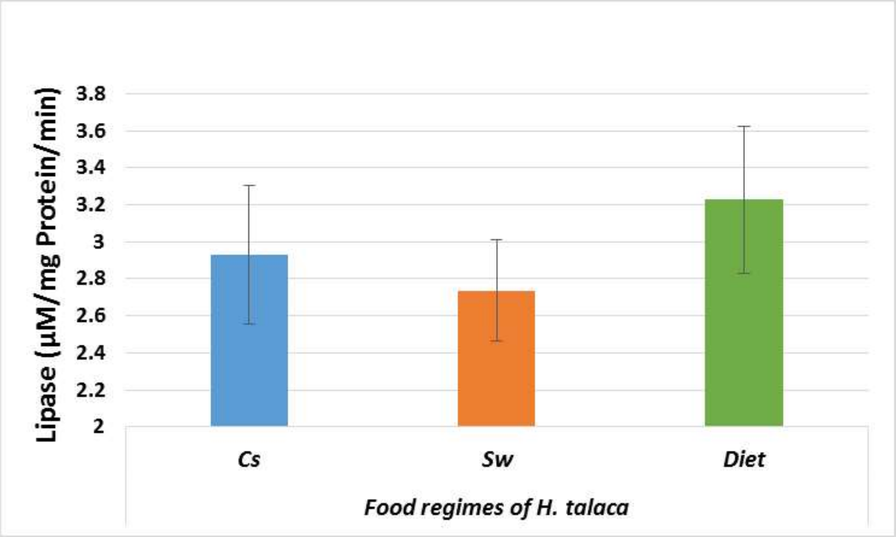


Figure 5.5.11 Lipase activity of *H. talaca* on three food regimes [Tea (*Cs*), Needlewood (*Sw*) and Synthetic diet (*Diet*)] (Error bars indicate standard error of mean)

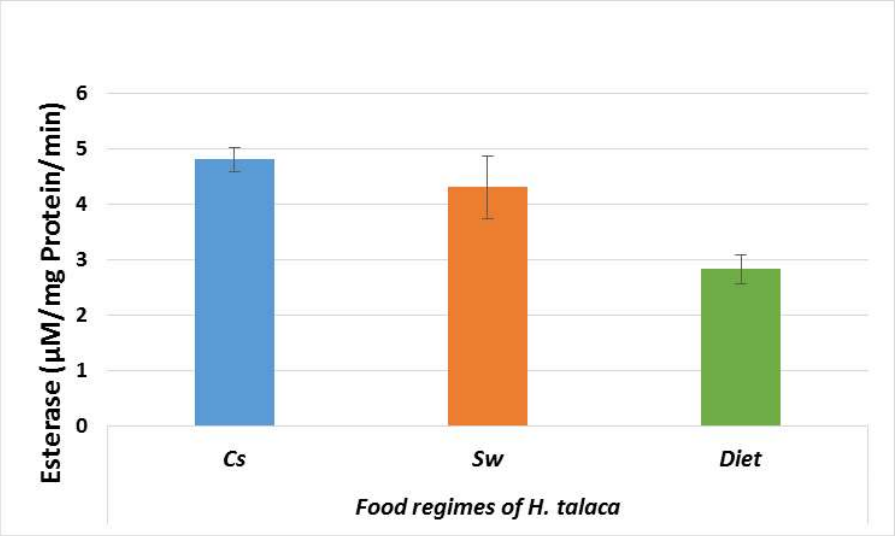


Figure 5.5.12 Esterase activity of *H. talaca* on three food regimes [Tea (*Cs*), Needlewood (*Sw*) and Synthetic diet (*Diet*)] (Error bars indicate standard error of mean)

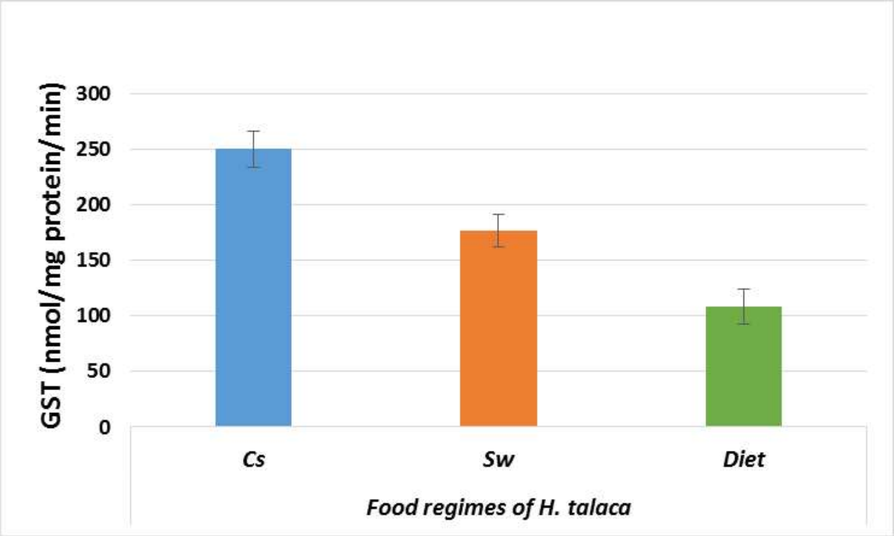


Figure 5.5.13 GST activity of *H. talaca* on three food regimes [Tea (*Cs*), Needlewood (*Sw*) and Synthetic diet (*Diet*)] (Error bars indicate standard error of mean)

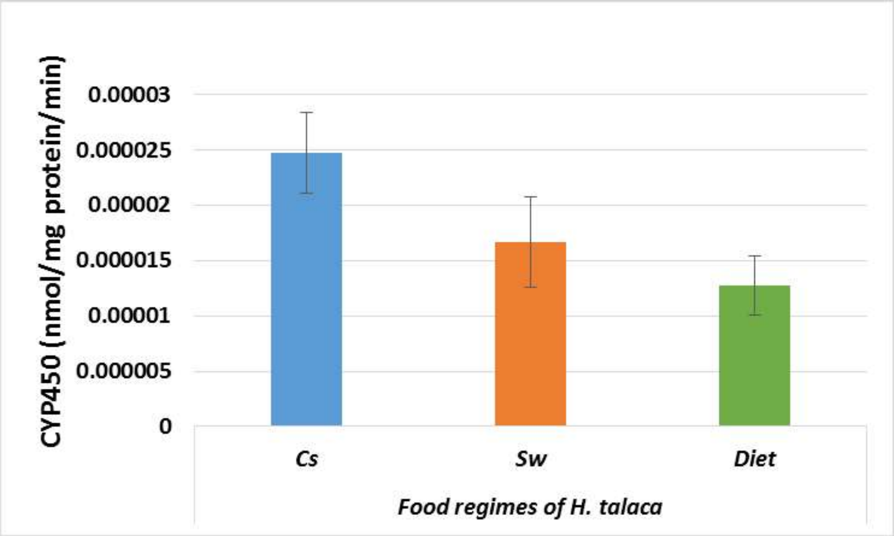


Figure 5.5.14 CYP450 activity of *H. talaca* on three food regimes [Tea (*Cs*), Needlewood (*Sw*) and Synthetic diet (*Diet*)] (Error bars indicate standard error of mean)

Invertase activity showed significant difference between loopers reared on Tea and Diet (*p* <0.001) and Needlewood and Diet (*p* <0.001), whereas no significant was observed between Tea and Needlewood reared groups (*p*=0.437) (F=13.121, *p* <0.001, *df*=2, 27) (Fig. 5.5.9).

Protease activity showed significant difference between loopers reared on Tea and Diet (*p*=0.002) and Needlewood and Diet (*p*=0.006), but no significant difference was observed between Tea and Needlewood-reared cohorts (*p*=0.648) (F=7.074, *p*=0.003, *df*=2, 27) (Fig. 5.5.10).

Lipase activity showed no significant difference between three cohorts reared on the three different food regimes (F=0.487, *p*=0.62, *df*=2, 27) (Fig. 5.5.11).

Esterase activity showed significant difference between Tea and Diet (*p*=0.001) and Needlewood and Diet (*p*=0.011), whereas no significant difference between loopers reared on Tea and Needlewood could be recorded (*p*=0.359) (F=7.274, *p*=0.003, *df*=2, 27) (Fig. 5.5.12).

GST activity showed significant difference between all three cohorts of loopers reared on Tea, Needlewood and Diet (Tea and Needlewood, p=0.003; Tea and Diet, p <0.001; Diet and Needlewood, p=0.004) (F=20.648, p <0.001, *df*=2, 27) (Fig. 5.5.13).

Cytochrome P450 activity showed significant difference between looper cohorts reared on Tea and Needlewood (p <0.001) and Tea and Diet (p <0.001), but no significant difference between Diet and Needlewood (p=0.884) (F=38.562, p <0.001, *df*=2, 27) (Fig. 5.5.14).

5.5.3 *Hyposidra infixaria*

Amylase activity showed significant difference only between Tea and Needlewood (p=0.018) reared loopers, whereas no significant difference between loopers reared on Tea and Diet (p=0.22) and Needlewood and Diet (p=0.219) were observed (F=3.162, p=0.058, *df*=2, 27) (Fig. 5.5.15).

Invertase activity showed significant difference between loopers feeding on all three food regimes (Tea and Needlewood, p<0.001; Tea and Diet, p<0.001; Needlewood and Diet, p=0.024) (F=22.417, p<0.001, *df*=2, 27) (Fig. 5.5.16).

Protease activity showed no significant difference between Tea and Needlewood (p=0.093) reared loopers, whereas there were significant difference between Tea and Diet (p=0.033) and Needlewood and Diet (p=0.00) reared ones (F=7.967, p=0.002, *df*=2, 27) (Fig. 5.5.17).

Lipase activity showed significant difference between all three cohorts of loopers reared on three food regimes: (Tea and Needlewood, p=0.020; Tea and Diet, p=0.001; Needlewood and Diet, p<0.001) (F=20.811, p<0.001, *df*=2, 27) (Fig. 5.5.18).

Esterase activity showed no significant difference between Tea and Needlewood reared loopers (p=238), whereas significant difference was observed between Tea and Diet (p=0.002) and Needlewood and Diet reared ones (p=0.033) (F=6.128, p=0.006, *df*=2, 27) (Fig. 5.5.19).

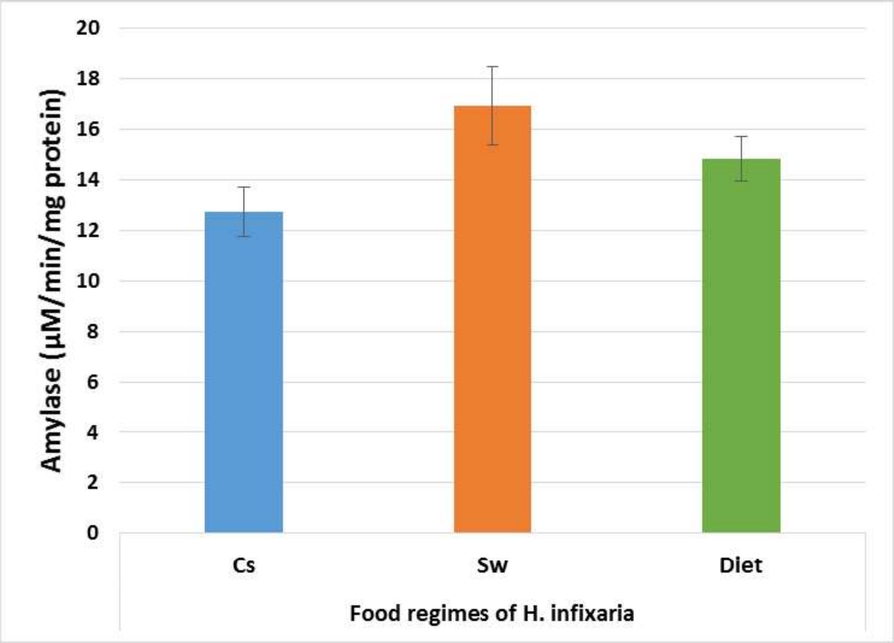


Figure 5.5.15 Amylase activity of *H. infixaria* on three food regimes [Tea (*Cs*), Needlewood (*Sw*) and Synthetic diet (*Diet*)] (Error bars indicate standard error of mean)

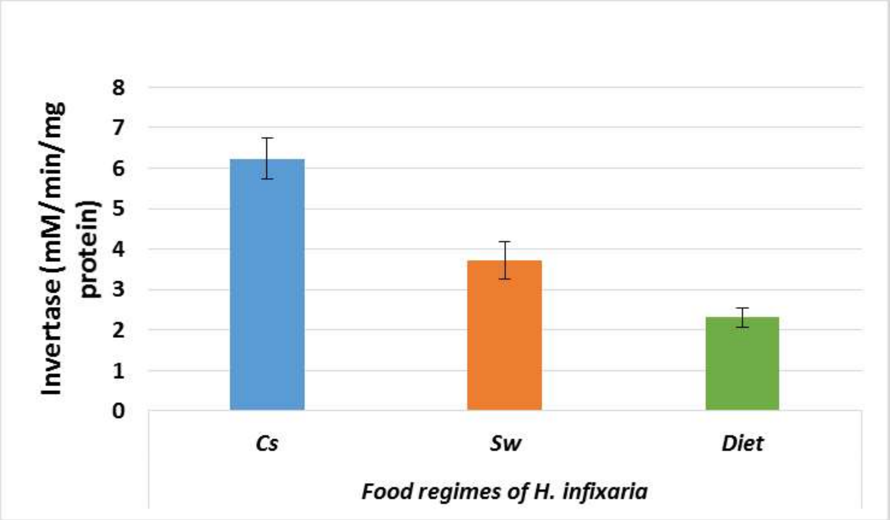


Figure 5.5.16 Invertase activity of *H. infixaria* on three food regimes [Tea (*Cs*), Needlewood (*Sw*) and Synthetic diet (*Diet*)] (Error bars indicate standard error of mean)

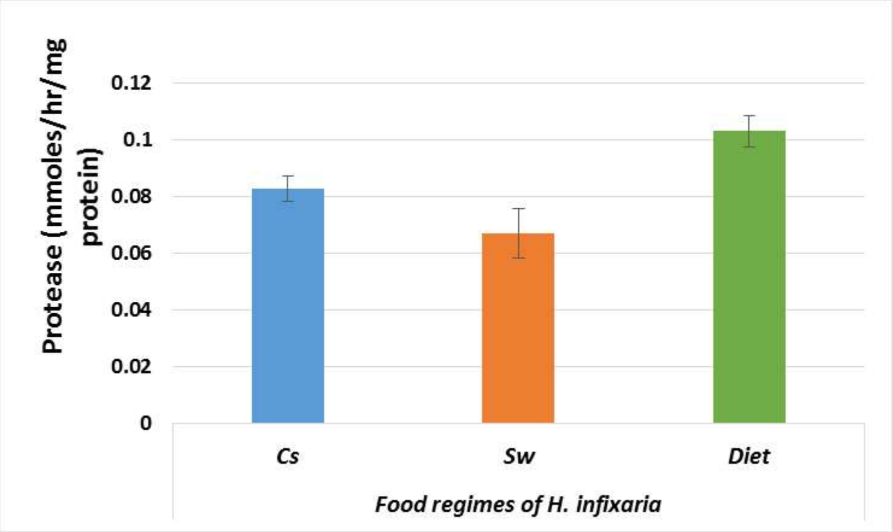


Figure 5.5.17 Protease activity of *H. infixaria* on three food regimes [Tea (*Cs*), Needlewood (*Sw*) and Synthetic diet (*Diet*)] (Error bars indicate standard error of mean)

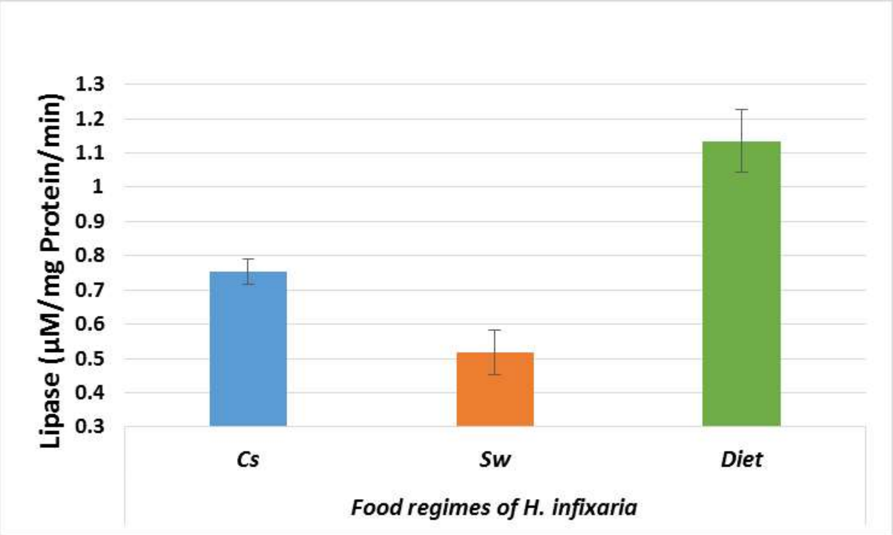


Figure 5.5.18 Lipase activity of *H. infixaria* on three food regimes [Tea (*Cs*), Needlewood (*Sw*) and Synthetic diet (*Diet*)] (Error bars indicate standard error of mean)

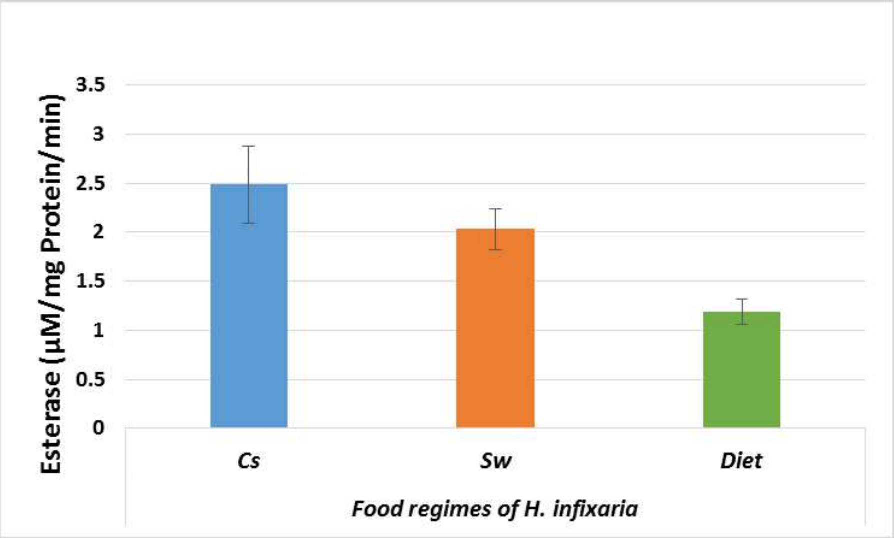


Figure 5.5.19 Esterase activity of *H. infixaria* on three food regimes [Tea (*Cs*), Needlewood (*Sw*) and Synthetic diet (*Diet*)] (Error bars indicate standard error of mean)

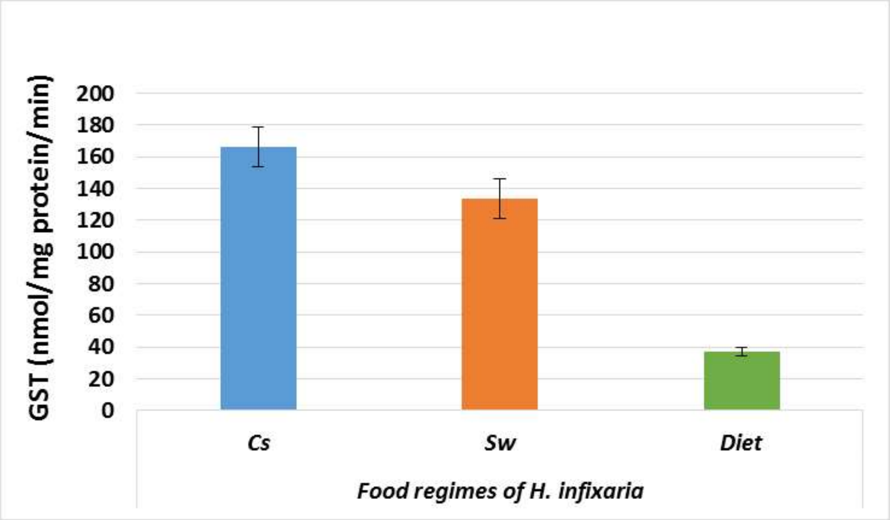


Figure 5.5.20 GST activity of *H. infixaria* on three food regimes [Tea (*Cs*), Needlewood (*Sw*) and Synthetic diet (*Diet*)] (Error bars indicate standard error of mean)

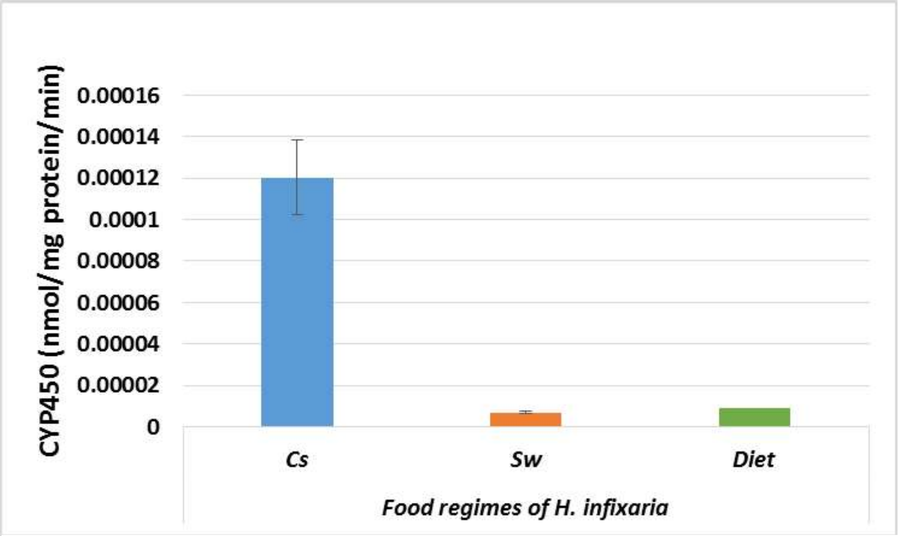


Figure 5.5.21 CYP450 activity of *H. infixaria* on three food regimes [Tea (*Cs*), Needlewood (*Sw*) and Synthetic diet (*Diet*)] (Error bars indicate standard error of mean)

GST activity showed significant difference between all three cohorts reared on three different food regimes (Tea and Needlewood, *p*=0.036; Tea and Diet, *p*<0.001; Needlewood and Diet, *p*<0.001) (F=41.476, *p*<0.001, *df*=2, 27) (Fig. 5.5.20).

Cytochrome P450 activity showed significant difference between Tea and Needlewood (*p*<0.001) and Tea and Diet (*p*<0.001) reared ones, but no significant difference between Diet and Needlewood (*p*=0.884) reared loopers (F=38.562, *p*=0.00, *df*=2, 27) (Fig. 5.5.21).

5.5.4 Discussion

Presence of a particular digestive enzyme in an insect, is an indication that the insect is capable of hydrolysing substrate of the enzyme concerned and it also means that the substrate is needed in that insect's diet (Fathipour and Naseri 2011). Analysis of digestive enzymes has proved to be important clue for development or improvement of synthetic diets for several species of insects (Gingrich 1972; Cohen 1992, 2001). The present investigations centres round the fitness performance of three geometrid species on natural as well as synthetic diets. An insight into the quantitative change of their principal hydrolysing enzymes along with the detoxifying enzymes that metabolize plant allelochemicals consumed with natural food (Schoonhoven et al. 2005), help to understand the interrelationship between the physiological processes.

B. suppressaria showed significantly higher amylase activity on synthetic diet than on natural diets. Similar observation was recorded by Merkx-Jacques and Bede (2005) in *Spodoptera exigua* when its larvae were fed on synthetic diets in comparison with the larvae fed on legume, *Medicago truncatula*. The *Hyposidra* spp. showed no significant difference in amylase quantity between natural and synthetic diets. In a similar finding, Teimouri et al. (2014) when performed amylase assay of *Ectomyelois ceratoniae* on synthetic diet and two pistachio cultivars, observed that there was no significant difference in amylase activity between one of the cultivars (Kalequechi) and the synthetic diet. High invertase activity found in alimentary canal of *Chrysoperla carnea* larvae facilitated the use of sucrose as a source of carbohydrate instead of fructose (Yazlovetsky 1992). In presence of dextrose in synthetic diet, *Hyposidra* spp. might be utilizing dextrose more than sucrose as a carbohydrate source whereas *B. suppressaria* may also require sucrose as another carbohydrate source.

Teimouri et al. (2014) also observed significantly higher value of protease and lipase activities on synthetic diet than two pistachio cultivars used as natural food. A similar type of finding in the present study uphold Teimouri's observation. The optimum nutritional quality available from the newly formulated synthetic diet may be the main cause of shorter developmental period and growth of robust larval instars. As "critical weight gain" is obligatory for the last larval stage to enter into pupation (Nijhout and Williams 1974), a better availability and utilization of nutrients from synthetic diet has possibly endowed the looper stages to attain critical weight in a shorter period than that on natural diets i.e. tea and needlewood.

Feeding activity on natural host plants elicits a high secretion and function of detoxifying enzymes, such as general esterase (GE), glutathione *S*-transferases (GST) and cytochrome P450 (CYP450) possibly directed to metabolize (detoxify) the allelochemicals that are available along with the basic nutrients in tea leaves. GE and GST activities of all three species were significantly lesser on synthetic diet than on natural host plants (Table 5.5.1), whereas CYP450 activity was significantly lesser both on needlewood and synthetic diet than that on tea, signifying possible absence of the allelochemicals in the former foods that require detoxification by cytochrome system.

Further, activity levels of digestive enzymes of insect midgut appear to have a correlation with its nutritional indices. The biosynthetic and metabolic activities of detoxifying enzymes involve significant energy expenditure. More energy allocated to detoxify would reduce the amount of energy available for other biological functions, generating energy trade-offs between detoxification capacity and growth rate and/or reproduction (Cresswell et al. 1992; Karban and Agarwal 2002). The percentage of reduction in ECI and ECD results from a foodstuff conversion deficiencies, which hinder the growth of insect, perhaps through a diversion of energy from biomass production to detoxification (Wheeler et al. 2001). Therefore, as compared to the newly designed synthetic diet, the natural diets stand as sub-optimal. Presence of some host plant derived toxic moieties, such as alkaloids, tannins, phenolics and even protease inhibitors, may impair the availability of nutrients to an insect, resulting in its poor performance in terms of growth, development and reproduction (Scriber et al. 1982; Morsy et al. 2001).

5.6 STANDARDIZATION OF LABORATORY CULTURE TECHNIQUE FOR THE GEOMETRID (LOOPER) SPECIES ON SYNTHETIC DIETS

5.6.1 Sterilization of rearing equipment and facility: A separate room (12ft x 14ft) for insect rearing that was allotted by Department of Zoology, was used. The room was equipped with BOD incubators and other rearing instruments (Fig. 5.6.1). Routine sterilization of culture room, BOD incubator, culture equipment, plastic containers etc. was done before using them for rearing purpose as mentioned in materials and methods section 4.2.12.

Figure 5.6.1 Laboratory set up for insect-rearing at Department of Zoology [insets: (a) Front elevation of Zoology Department, (b) Ante chamber for the rearing room, (c) Inner view of rearing cubicle within glass partition, (d) Rearing trays inside BOD incubator, (e) Set up inside BOD incubator for group and individual rearing]

5.6.2 Maintenance of rearing system: Sterilization was done as mentioned in materials and methods, section 4.2.12. Mortality during rearing was prevented by handling with care but in event of large scale death, whole container was discarded to avoid spread of any infection. Monitoring of any casualty was done as a daily routine to avoid contamination of culture.

5.6.3 Sexing and mating: After emergence, adults were sexed and release in a mating cages. Chances of successful mating in *Hyposidra* spp. was more when male and female were kept in the ratio of 2:3 in the containers (6cm height x 4cm diameter). Whereas in *B. suppressaria* successful mating took place when male and female were kept in the ratio of 3:4 in containers (10cm height x 8cm diameter). After mating males generally die within a short period, whereas females die after laying eggs. Dead moths were removed from the mating cages. Wild males were at times introduced in the running culture to maintain biological vigour and to reduce the inbreeding stress. In wild these geometrid adults were found during March to October in good number, so introducing wild male during early March and then after six month was found to be good option for the purpose.

5.6.4 Collection and sterilization of eggs: Eggs were collected from the mating jar with the help of a brush. Scales associated with the eggs were removed by gentle fanning. The surface sterilization of egg was done within 24-36h by dipping the cluster of eggs for 5 seconds in 1% formalin solution, followed by thoroughly washing with distilled water and complete drying with the help of autoclaved blotting paper. The sterilized eggs were then kept in BOD incubator at 27°C till hatching.

5.6.5 Hatching of Neonates: Fertilized eggs turned blackish grey before hatching (Fig. 5.6.2). These were collected and kept in a fresh container, which was then carefully covered with cotton cloth. The rearing containers were wrapped in paper to avoid the phototactic movement of neonates after hatching (Fig. 5.6.3). In case neonates moved in one direction (in absence of food) they were normally found to assemble at one side of the container and secrete webs around themselves and finally die. After hatching neonates were transferred as early as possible in containers with fresh food with optimum moisture content.

Figure 5.6.2 Hatching of loopers, *Hyposidra* sp.

Figure 5.6.3 Containers for rearing the first instar properly covered to avoid excess moisture and phototactic movement of neonates

5.6.6 Improvisation of rearing technique: Earlier, the diet slurry was poured on the floor (bottom) of rearing containers and refrigerated. The food laden containers released excess moisture that accumulated on the wall in the form of water droplets. Water therefore, had to be removed by blotting paper before the neonates were transferred to these containers. In spite of the removal of water droplets, the rate of mortality of early instars was very high due to subsequent accumulation of water droplets inside the containers. In order to get over this problem the semi-solid diet was used. Diet was cut into small cubes and suspended free with the help of toothpicks (Fig. 5.6.4 and Fig 5.6.5). These toothpicks also provided caterpillars a chance to cling and move freely as they do in nature on tea twigs and branches.

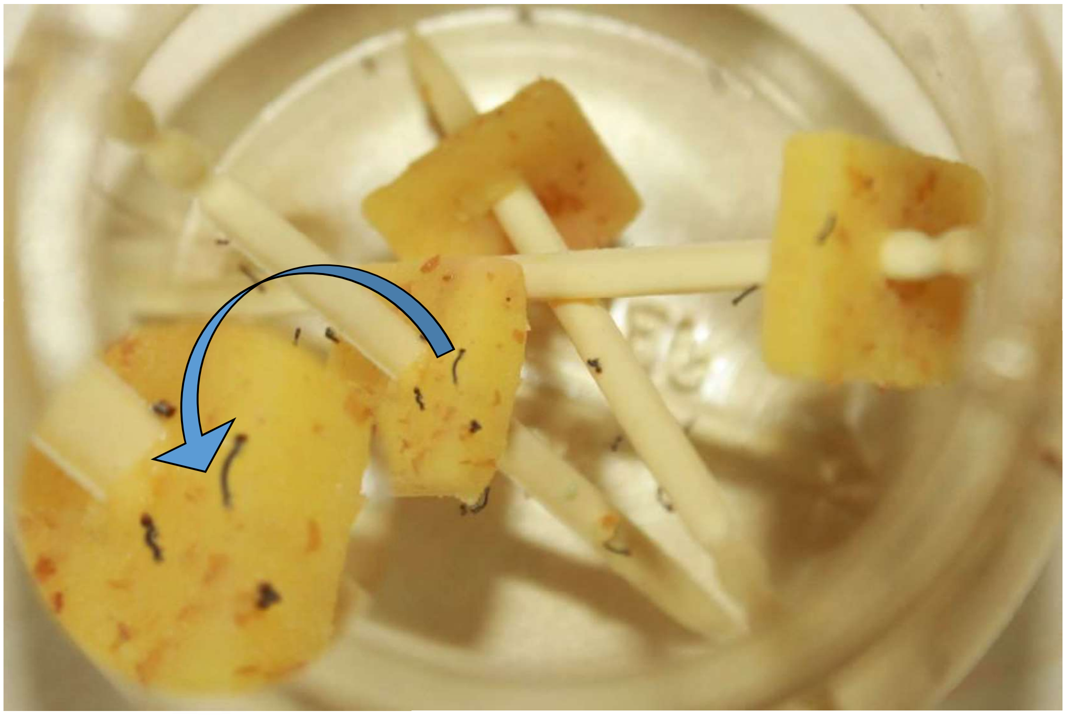
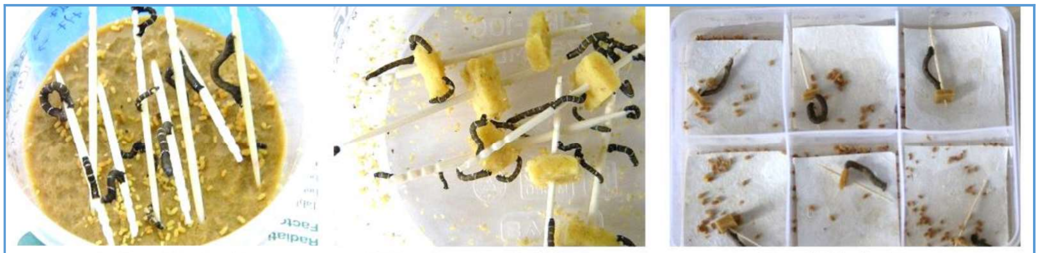


Figure 5.6.4 Neonates released on the diet cubes pegged with toothpicks

The containers were covered with lid in such a way that proper aeration as well as adequate moisture could be maintained. The manipulation of lids for proper ventilation was necessary as excessive aeration could lead to quick drying of diet without being consumed by caterpillars whereas an excess moisture would lead to drowning of early instar resulting in higher mortality (Fig 5.6.3). Care was taken not to keep any gap or hole between container and its lid as because the 1st instar could escape even through holes of 2mm width. Number of caterpillars had to be reduced per container as they grew bigger. Preferably, caterpillars of same age were kept together to have synchronized ecdysis. In spite of starting a culture on the same day different instars occurred in the same brood due to staggered development. So, it is recommended to remove caterpillars which take longer time to moult as they can be a source of infection to the rest of the culture.



A. Use of toothpick

B. Use of semisolid diet cubes

C. Individual culture

D. Individual culture tray stacked together

E. Proper aeration for normal pupation: covering individual container with lid (not airtight), instead of stacking

F. Stack culture tray after pupa formation kept for adult emergence

Figure 5.6.5 Modifications done in the rearing system to reduce exposure of neonates to excess moisture (accumulated water droplets) with consequent of their better survival; A: Use of toothpicks as wedge for clinging and movement; B: Use of semisolid cubes of diet suspended with toothpick to avoid drowning of neonates in water droplet formed on the surface of diet while pouring slurry in the floor of the container; C & D: Individual rearing gives better survival than mass culture in containers; E: Proper aeration of containers led to reduced moisture resulting in higher rate of successful pupation; F: Stacked culture tray for better space utilization in the rearing incubator

5.6.7 Modifications done for normal pupation: Particularly in synthetic diet-based rearing, humidity inside the container was regulated by manipulation of lid of the plastic container. For individual rearing, trays (9inch length x 6inch breadth) with partitioned cubicles were used which could be stacked or kept separately according to the requirement of the optimum moisture (Fig 5.6.5). Therefore, as soon as larvae stopped feeding and started 'wandering' before entering in to pupation, profuse aeration was maintained to keep RH under 75%. If needed a piece of autoclaved blotting paper was provided to larvae to avoid accumulation of excess moisture. That piece of blotting paper not only absorbed the moisture but also acted as a substrate for pupation.

5.6.8 Cost-benefit analysis of Synthetic diet and Natural diet (Tea): The present material cost for preparing, a 300ml of synthetic diet was approximately ₹1800/-. The diets consumed were best before 30 days if kept at 4°C. A 300ml of diet was sufficient to rear around 100 caterpillars of *Hyposidra* spp. and about 450ml of diet was sufficient to rear 100 caterpillars of *B. suppressaria* (Table 5.6.1).

As per the current government policy and directives of tea associations the minimum selling price of one kilogram of fresh young leaves is ₹12/- for conventionally produced (as per the local daily, Uttarbanga Sambad, Dated 1st September, 2015) and ₹30/- for organically produced tea (Personal Communication) in Darjeeling Terai-Dooars region. For lab-based rearing of the geometrid pests (looper), organically produced tea leaves are recommended in order to avoid pesticide contamination. Tea leaves always have to be provided in excess than that larvae could consume. On an average, to rear a cohort of 100 looper of *Hyposidra* spp and 100 looper of *B. suppressaria*, 16 kg and 30 kg of fresh leaf of Tocklai cultivar TV 26 was needed, respectively. Procuring of tea leaves from organic plantations would involve cost of transport from and to the rearing site and careful handling. Failing which a tea plantation has to be maintained in the vicinity of the rearing facility/laboratory for regular collection of tea leaves. Natural diet-based rearing require voluminous space for maintaining culture and large scale production. Synthetic diet-based rearing would require less space for dispensing diet cubes in containers with reasonable dimension.

Table 5.6.1: Cost-benefit and risk analysis of natural and synthetic diets for complete rearing of the geometrid species

Food regime		**Natural diet (tea)**	**Synthetic diet**
Cost of diet for rearing 100 loopers (in ₹)	*Hyposidra* spp	480/-	1800/-
	Buzura suppressaria	750/-	2700/-
Labor cost for maintaining culture in ₹250/- per day per person	*Hyposidra* spp	3750/- (for 15 man days)	2500/- (for 10 man days)
	Buzura suppressaria	7000/- (for 30 man days)	5000/- (for 15 man days)
Space and containers/cages		Large laboratory/ insectary	Relatively compact and smaller space
Risk of infestation/ contamination	**Parasites infestation**	High	Low
	Microbial infection	High	Low
	Fungal infection	High	Negligible due to antifungal agent
	Allelochemical interference	High	Negligible
Seasonal Availability		Variable	Consistent

5.6.9 Discussion

The rearing of insects in the laboratory is essential for resolving various problems associated with pure or applied entomology (Kogan 1980). In modern entomology, the advanced research depends on the availability of insect specimen and its continuous supply as and when needed (Parra 2012). The maintenance of the laboratory-based insect culture is indispensable part to modern strategies of pest management since a continuous supply of insect is always needed for both basic and applied research (Parra 2012).

Parra (2012) further emphasized on the development of facility for rearing large number of an insect pest on synthetic diet to study different aspects related to its IPM. However, maintaining synthetic diet-based culture is itself a sophisticated art which requires routine observation and hygienic conditions throughout the rearing process and at the working place. The sterilization of equipment, rearing containers and rearing room are the most important component of mass-rearing of concerned species to get a continuous supply of the same (Singh 1977; Cohen 2004; Dyck 2005). Proper sterilization helps to avoid any microbial or fungal contamination. The microbial contamination may collapse the whole culture. Not only sterilization of equipment and rearing system but sterilization of eggs are also essential to avoid viral infections (Cohen 2004; Dyck 2005). Cohen (2004) emphasized on the improvisation which can add some natural essence to the synthetic rearing system. Loopers prefer to move on tea twigs and rest on them. The diet cubes held with the help of toothpick provided almost similar kind of situation of clinging to twigs to the caterpillars. Avoiding phototactic movement of early instar after hatching could also be done by wrapping the container by black paper after Bolaji and Bosque-Perez (1998), who reared *Mussidia nigrivenella* (Lepidoptera: Pyralidae) on synthetic diets in the laboratory. Wild males were periodically introduced into the geometrid culture (colony) as has been prescribed for many insect cultures (Shorey and Hale 1965; Singh 1977; Guennelon et al. 1981; Proverbs 1982; Wajnberg 1991; Rogers and Winks 1993; Bloem et al. 1999, 2004) for maintaining the biological vigour of the species.

Acceptability of synthetic diet for insect rearing depends upon their cost-benefit ratio. While considering only the expenditure, it was observed that more or less same amount of money will be spent on both natural and synthetic diet-based rearing system. However, the major disadvantage of using natural diet is about inconsistent availability of quality of tea flush to be used as food. This might cause difficulty in maintenance of the insect culture especially in non-productive winter season of tea plantations. In synthetic diet-based rearing food availability is not a limiting factor. Other disadvantages of using natural host plant have already been discussed in 'Synthetic diet versus Natural diet' section 1.8. Although, the rearing cost of geometrids on both natural and synthetic diet is comparable, yet the risk of contamination of insect culture is much less on synthetic diet with a more consistent and quality production of disease-free insects and their stages.

Han et al. (2012) compared fitness parameters such as larval development, immature survival, pupal weight, pupation, adult emergence, egg hatchability and oviposition to evaluate the suitability of the synthetic diet. Wang et al. (2013) formulated a synthetic diet for Bean Pod Borer, *Maruca vitrata* (Lepidoptera: Crambidae). They compared various life-cycle parameters of the pest both on synthetic diet and natural diet. This pantropical pest of grain legumes was further studied by Wang et al. (2013) to develop a suitable synthetic diet for producing uniform insects for research as well as for commercial purposes. Kim et al. (2014)

developed a synthetic diet and studied its effect on developmental rate and life-cycle traits of swallowtail butterfly *Papilio xuthus* (Lepidoptera: Papilionidae) which is a common pest in South Korea. Sree and Varma (2015) mentioned several advantages of *in vivo* production of virus which can be successfully done on the synthetic diet-reared caterpillars. As the insect develops fast with high biomass on synthetic diet than field-collected insect, the yield of virus in synthetic diet-reared ones are more encouraging (Hedlund and Yendol 1974; Shapiro et al. 1981).

Optimization of NPV production for two pests *Spodoptera litura* (Lepidoptera: Noctuidae) (Kumar et al. 2005) and *Helicoverpa armigera* (Lepidoptera: Noctuidae) (Kalia et al. 2001; Gupta et al. 2007) has already been done in India based on synthetic diet rearing. The same kind of optimization of synthetic diet-based rearing has led to production of disease-free quality specimen of tea loopers for advanced experimentations (Prasad and Mukhopadhyay 2015a, b; Prasad and Mukhopadhyay 2016).

This rearing technology based on synthetic diet will certainly provide a better platform to come up with better control measures for these defoliators of tea, *B. suppressaria* and *Hyposidra* spp. Further amendments in infrastructure and diet composition may further improve the performance, survival and quality of these geometrid insects.