INFLUENCE OF NUTRITION AND OVIPOSITION STIMULANT OF HOST PLANT ON COMMON GRASS YELLOW (*Eurema hecabe*)

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By

MISS. TANAYA SARVANAND GOSAVI

Seat Number: 22P0440014
ABC ID:625972092497
PRN: 201812430

Under the Mentorship of

DR. NITIN SAWANT

School of Biological Sciences and Biotechnology

Discipline Zoology

GOA UNIVERSITY

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SBSB, M.Sc., Zoology ★ Taleigao Plateau, Goa 403206 ★ GOA UNIVERSITY

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1.INTRODUCTION

1.1 Background

Eurema hecabe commonly known as Common Grass yellow is widespread across Asia, Africa and Australia. In India *E.hecabe* is a very common butterfly found throughout the country. In India it uses several plants of Fabaceae as larval host plants (Nitin *et al*.,2018). The occurrence of butterflies depends on the climatic dicta and the presence of Suitable caterpillar foods. (Opler and Krizek 1984; Kunte 2007).

Adult butterflies have been considered to be Opportunistic foragers due to their foraging activity on a wide variety of plant species (Courtney 1986). In line with this, Kato *et al*. (2008) stated that forests with different flora and vegetation type harbour different assemblages of butterfly visitors, and thus the properties of plant-butterfly interactions are greatly affected by the composition of regional biota.

The Government of India has brought the Wildlife Protection Act 1972 into force long ago which has enough provisions for the conservation of natural resources including butterflies and it again revised the Act in 2022 for protection of IUCN red listed species and CITES listed species.

It is well established that nutrition affects the expression of life-history traits and trade-offs within species (Van Noordwijk A. 1986), and it has been hypothesized to shape life-history evolution across species (Arnold SJ.,1992). Indeed, variation in diet quality across species has been suggested to influence the evolution of development and body size in insects (Mattson WJ. ,1980). There are more than 1100 species in 83 genera in the family Pieridae, and they inhabit a wide range of environments (Braby, 2005) The Fabales (legumes and related plants), Brassicales (crucifers and related plants containing glucosinolates) and Santalales (mistletoes) are three major plant groups (orders) that the Pierids exploit. Species in the Fabales, especially the family Fabaceae, are considered the ancestral hosts of Pieridae (Ferrer-Paris *et al*. 2013).

Many butterfly groups, including the Pieridae, Riodinidae, and Lycaenidae, frequent the Fabaceae, suggesting that this plant family is the most likely ancestral host for butterflies (Janz and Nylin 1998). Nutrient availability across species over their evolutionary histories makes it difficult to investigate the evolutionary importance of nutrition. Specialist herbivores such as phytophagous insects are a useful system because nutrient content has been shown to vary systematically across plant families (Watanabe T. *et al.* 2007). Butterflies are a powerful system because most of an individual's essential nutrients come during larval feeding and larval host plants are known for most species. Furthermore, by mapping these host records onto lepidopteran phylogenies, we can estimate the relative timing of diet shifts (Braby MF and Trueman JWH, 2006).

Amphibians, holometabolous insects and many marine invertebrates can be particularly difficult to develop under nutritional constraints. Such creatures are characterized by distinct larval and adult stages that often live in distinct habitats with different nutritional conditions. Thus, individuals are restricted in their ability to acquire the essential materials for maturing into adulthood and reproducing. Rowe and Ludwig, 1991; Moran, 1994; Awmack, Leather, 2002; Roff, 2002). Insects that eat plants are a great way to study the connections between nourishment and health. Nitrogen is a key nutrient for all animal species, because it is required to build proteins, nucleic acids and many essential body structures (Mattson 1980; Bernays & Chapman 1994).

Various sensory cues are essential for insects to locate and reach the host plants properly and then perform the appropriate behaviour on those plants (Bernays &Chapman, 1994). When insects locate host plants from far away and approach them, visual and olfactory cues play essential roles (Prokopy & Owens, 1983). After landing, insects recognise the species and the quality of a plant to decide what behaviour they should perform (Renwick & Chew, 1994). In the coevolution of herbivores and food plants, on the other hand, plants are selected to prevent herbivores from feeding or ovipositing (Futuyma, 1986). It follows that most plants produce secondary metabolites that hinder the normal development of herbivores. Since many herbivores evolved a metabolism that detoxifies some

secondary metabolites, they feed on the plants of specific taxa. The contact chemical sense is, therefore, crucial for phytophagous insects to recognise whether the plant is an appropriate host or not (Renwick & Chew, 1994). Visual and olfactory cues are also important for phytophagous insects to land on appropriate food plants effectively. Gravid females alight non-randomly on plants with leaves of similar shape to those of food plants (Papaj, 1996; Mackay & Jones, 1989).

Some experimental studies indicate that the size, direction and arrangement of leaves also have roles in the recognition of an appropriate food plant (Rojas & Wyatt,1999). Plants produce numerous secondary metabolites for chemical defense against herbivores (Mithöfer and Boland 2012; Wink 2013). Phytophagous insects capable of overcoming these chemical obstacles are capable of exploiting certain plant species, often employing plant-specific secondary metabolites to locate and recognize their hosts (Nishida, 2014). A limited number of plant species are the focus of most butterflies' diets at the larval stage. Gravid females select and oviposit on preferred hosts using various plant cues (Thompson and Pellmyr 1991).

Despite the rich butterfly diversity in India, there are only a few studies from India that provide information on plant-butterfly interactions.

1.2 Aim

To study the influence of nutrition and oviposition stimulant of host plants on Common Grass yellow (*Eurema hecabe.)*

1.3 Objectives

- To study efficiency of utilization of larval host plants by carrying out life cycle of Common Grass Yellow on different host plants and monitoring growth indices.

- To analyse nutritional status of host plants with respect to macronutrients.

- To monitor presence of Common Grass yellow and Host plants availability affecting host plant preference.

- To validate presence and level of oviposition stimulant in host plants.

1.4 Hypothesis

Common Grass yellow (*Eurema hecabe*) butterfly number may vary with availability of host plants and may also affect host plant preference. Growth of the caterpillars may vary with different host plant species and oviposition response of butterflies towards the host plant may depend on the nutritional status and presence and level of oviposition stimulant.

2.LITERATURE REVIEW

Atsushi N.*et al.,* (1985) reported that arrestant for yellow butterfly larvae on host plant is mixture of chemical constiuents such as D-pinitol.

Mukai S.*et al*., (2016) stated that *Albizia julibrissin* and *Lespedeza cuneata* are host plants of common grass yellow (*Eurema hecabe mandarina*) belonging to family fabaceae. Chemical analysis revealed that host plant contained D-pinitol as the major component. Female butterfly response towards D-pinitol and found that it induced oviposition responses at concenteration greater than 0.1%.

Yoshiomi K. and Tatsuo N. (1989) studied male approach to pupae in yellow butterfly*, Eurema hecabe* and found that mate seeking males' approach both males and females. As per Kim, S. *et al.,* (2015) the optimal growth of *Eurema hecabe* occurred at 30ºC and at higher temperature development was completed at a faster rate. Hirota T. and Kato Y.(2001) studied influence of visual stimuli on host location in *Eurema hecabe* and reported females landed and deposited eggs predominantly on yellow-green model.

Yan k. *et al*., (2012) reported artificial diet of nutrient agar containing Caesalpinia pulcherimma leaves fed to *Eurema hecabe* larvae resulting in 70 % successful growth from larvae to adults.

Arju M. *et al*., (2015) studied developmental stages of a Common grass yellow butterfly, *Eurema hecabe* and positive correlation among the larval instars, amount of food consumption and excretion of faeces was stated.

Nitin R, *et al.,* (2018) reported presence of more than 18 host plants of Common Grass Yellow (*Eurema hecabe*) in the Western ghats.

Shah HA *et al*., (2021) studied and reported morphometrics and length-length relationships of the Common grass yellow butterfly (*Eurema hecabe).*

Narender S, *et al.,* (2006) gave description on eggs of common grass yellow is spindle shaped, upright base, micropylar end, weakly sculptured with ridges, slightly compressed in middle. shinning with white color and later turns pale yellow.

V.Ramana, *et al*., (2003) stated that consumption index, growth indices, approximate digestibility decreases as larva ages. Efficiency of conversion of ingested food and efficiency of conversion of digested food increases as larva ages.

Nevertheless, it remains unclear level of plant chemicals mediate host selection and oviposition in Fabaceae-feeding butterflies in India. By further investigating these relationships, we can gain a deeper understanding of butterfly ecology and potentially improve management practises to support their populations in the wild.

3.METHODOLOGY

3.1 Study Area

Current research was carried out at Goa University campus - located on Taleigao plateau with geographical location between 15º27'30" N and 73º50'04" E. The study area is spread across 1.2 sq.km Vegetation mainly consists of moist deciduous type mixed with evergreen species. Goa University Campus is home to variety of host and nectar plants for butterfly species.

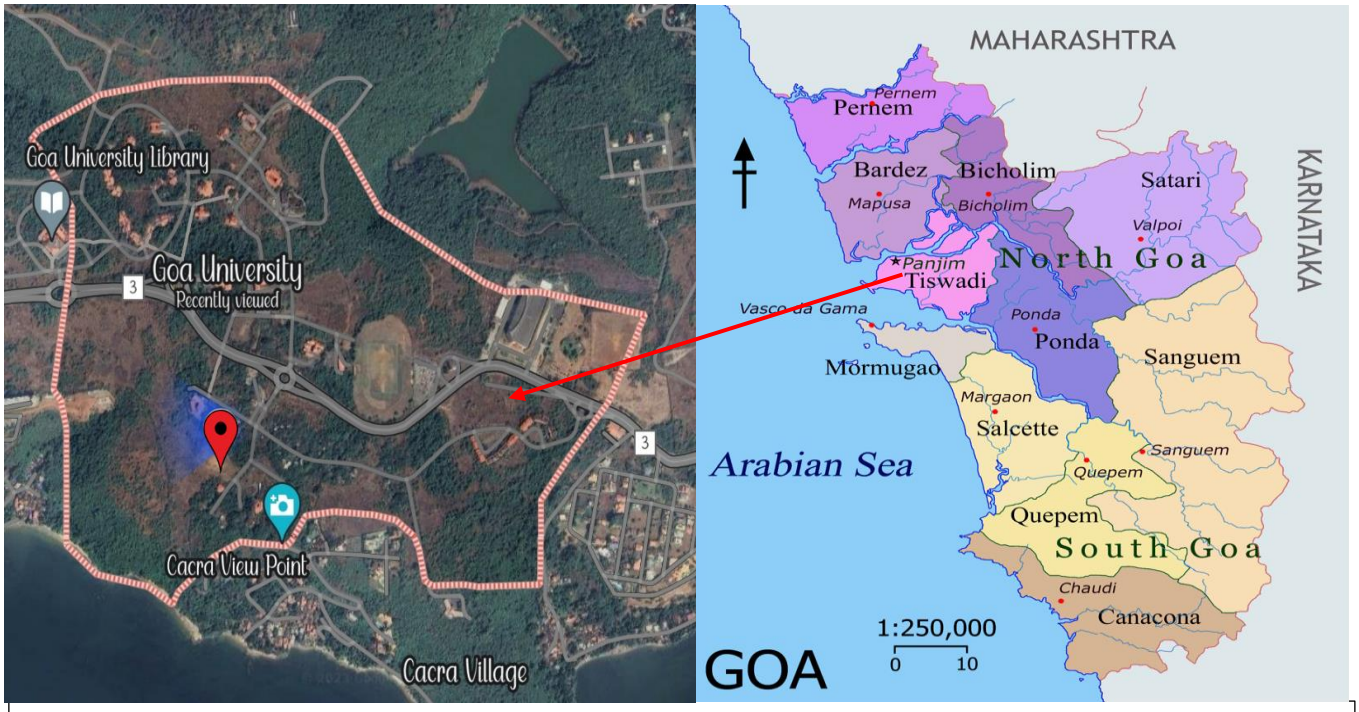


Fig 3.1.1: Map depicting Study area

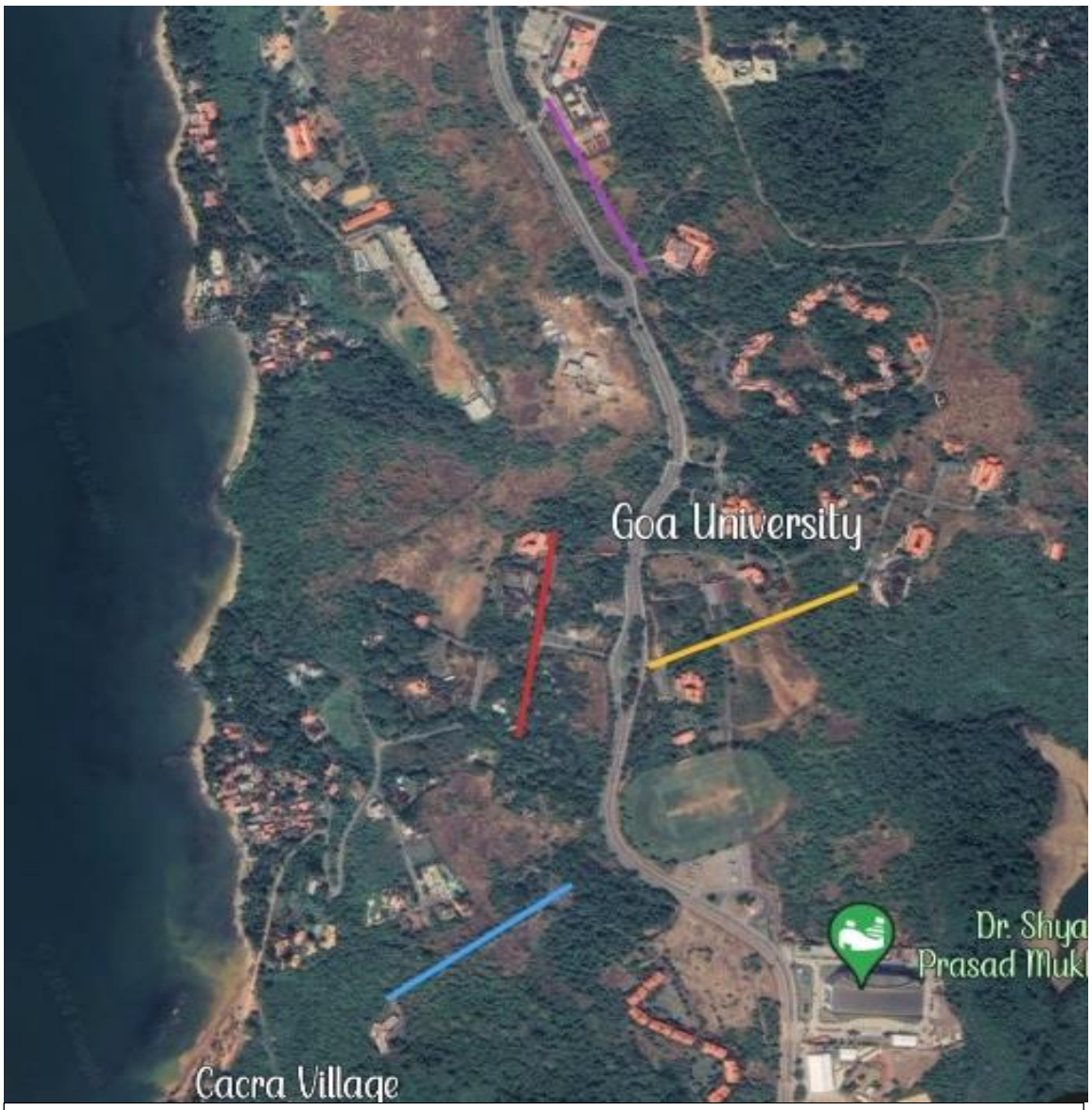


Fig 3.1.2: Transects marked in Study area.

Transect 1: ●, Transect 2: ●,Transect 3:●,Transect 4:●

3.2 Study of Presence of Common grass yellow and host plant availability and oviposition preference.

A preliminary survey was carried out to check presence of Common grass yellow (*E.hecabe*) and host plants availability in Goa University Campus. Transects were marked randomly based on availability of host plants following random transect method (Thomas *et al.*2010). Transect 1: Transect 2: Transect 3: transect 4. Observations were made from 4th June 2023 to 25th February 2024 on fixed four transects of 300m each. Each transect was surveyed twice in a month at an interval of 15 days approximately during good weather periods (no heavy rains or strong winds) from 8:00 am –10:30 am and 4:00 pm -6:00 pm and presence of Common grass yellow butterfly and host plant availability and oviposition preference was monitored.

3.3 Model organism

Fig 3.1.3: Common grass yellow (*Eurema hecabe*)

CLASSIFICATION

Kingdom - Animalia

Phylum - Arthropoda

Class - Insecta

Order - Lepidoptera

Family - Pieridae

Genus - *Eurema*

Species - *E.hecabe*

3.4 Indoor Butterfly Rearing Setup

Eggs of Common grass yellow (*Eurema hecabe*) were identified in field using "A Guide to Butterflies of Western Ghats" (Hemant O. and Milind B.,2018) and collected along with the host plants leaf by random sampling method (Bhardwaj,2018) and kept one egg in each container (7cm×4.5cm×6cm) and (12cm×9cm×5.5cm). But for non-egg laying host plants eggs were collected from egg laying host plant and after hatching an attempt was made to feed 1st instar caterpillar with non-egg laying host plant leaves. To maintain moisture inside the container for egg hatching was managed by keeping small ball of cotton soaked in water. After hatching of the eggs, 1st instar caterpillars were fed with fresh leaves of host plants. After providing fresh leaves larvae were replaced and the old foliage was removed and their growth indices and utilization efficiency of host plants was studied (Palem H. *et al*.,2015). Larvae was observed regularly for supplying food, measurements, and molting process. Also, larvae were handled with soft bristle brush to prevent any injury to the caterpillar. The larval instars were recorded between the time of first larval appearance to pupation with changes in morphological characteristics such as measurement of body size, change in body colouration, feeding quantity and excreta. Larvae were reared following the method of (Arju MH. *et al*, 2015). The amount of food consumption and faeces (gm) were recorded by using Precision Electronic weighing balances.

Formulas to calculate Food utilisation efficiency indices

$$\text{Consumption index(CI)} = \frac{\text{Weight of food consumed}}{\text{Weight of instar} \times \text{Number of feeding days}}$$

$$\text{Growth rate(GR)} = \frac{\text{Weight gain of instar}}{\text{Mean weight of instar} \times \text{Number of feeding days}}$$

$$\text{Approximate Digestibility} = \frac{\text{Weight of food consumed} - \text{Weight of faeces}}{\text{Weight of food consumed}} \times 100$$

$$\text{ECD} = \frac{\text{Weight gain of instar}}{\text{Weight of food consumed} - \text{weight of faeces}} \times 100$$

$$\text{ECI} = \frac{\text{Weight gain of instar}}{\text{Weight of food consumed}} \times 100$$

3.5 Statistical analysis

The data collected from the field study was subjected to statistical analysis using Prism 10.2.1. All the samples were analysed and were expressed as Mean SD (Standard Deviation) Wherever necessary. The data was subjected to a test of normality using Anderson -Darling test and Shapiro-Wilk test followed by one way ANOVA or Kruskal walis test based on Parametric and non-parametric data , When ANOVA test were significant, the mean were compared by post-hoc Tukey's test for multiple comparison and when Kruskal walis test was significant ,the mean were compared by Dunn's multiple comparison test was used.

3.6 Laboratary protocols

8 different host plant leaves samples such as *C.tora*, *P. dulce, Sesbania bispinosa, A. americana, C. fistula, M. pudica, P. pterocarpum and C. pulcherrima* were analysed to estimate amount of Carbohydrate, Proteins and lipids content and presence and level of D-pinitol in M.S.c laboratory ,Zoology department, SBSB, Goa -University.

Apparatus and instruments

Laboratory wares like measuring petri dish, cylinders, beakers, test tubes, glass rods, dropper, tripod stand, funnel, mortar and pestle, eppendorf tubes, micropipettes, reagent bottles, mortar and pestle, conical flasks, Whatmann No. I filter papers were used. Instruments like Hot plate, weighing balance (PGB, 200), Centrifuge (R-24), Hot air oven (MIC-165), UV-Visible spectrophotometer (BL 1073) and pH meter (TMP 3) were used.

3.6.1 Carbohydrate Estimation (Ludwig & Goldberg,1956)

Apparatus required: Beakers, test tubes, test tube stand, Measuring cylinder, Spatula, glass rod, micropipette, Centrifuge tubes.

Chemicals used: Anthrone, Distilled water, Glucose D, Concentrated H_2So_4.

Instrument used: UV visible spectrophotometer.

Chemical preparation:

<u>stock solution for standard</u>

0.005g Glucose D dissolved in 50 ml of distilled water

<u>Anthrone Reagent</u>

0.1g Anthrone dissolved in 50 ml concentrated H_2SO_4

Phosphate buffer

0.807099g Di-potassium hydrogen phosphate + 0.68045g Potassium dihydrogen phosphate→500ml Distilled water. Maintain pH 7 using pH meter.

Sample preparation and estimation.

0.1g of each plant tissue (leaves) was weighed and ground with mortar and pestle using phosphate buffer and poured in centrifuge tubes making the volume upto 10 ml. Then centrifuged in centrifugation machine at 5000 RPM for 10 mins. Then supernatant was collected from each tube and was used as unknown sample. Each plant sample was analysed in triplets to obtain precise readings.1m of each plant sample was taken in a test tube and to it 4ml of anthrone reagent was added and kept in waterbath for 10 minutes. After the time duration all test tubes were removed, cooled at room temperature and absorbance was taken at 620 nm. Quantification of carbohydrates content was done with standard curve of Glucose.

3.6.2 Protein estimation (Lowry et al.,1951)

Apparatus required-Test tubes, test tube stand, beakers, glass rod, Measuring cylinder, spatula, Micropipettes, Centrifuge tubes.

Chemicals used-Bovine Serum Albumin, Folin ciocalteau, Sodium hydroxide (NaOH), Copper sulphate ($CuSo_4$), Potassium sodium tartrate($C_4H_4O_6KNa.4H_2O$), Sodium carbonate (Na_2CO_3) and Distilled water.

Instrument used -UV visible spectrophotometer, Centrifugation machine.

Chemical Preparation:

Standard stock solution

5mg BSA (Bovine serum albumin) dissolved in 20 ml NaOH

4% Sodium hydroxide (NaOH)

0.2g sodium hydroxide dissolved in 50 ml distilled water

Phosphate buffer

0.87099g Dipotassium hydrogen phosphate + 0.68045 Potassium dihydrogen phosphate dissolved in 500ml Distilled water and pH 7 was maintained using pH meter.

Lowry's Reagent

A. 6% Na_2CO_3: 6g Sodium Carbonate dissolved in 150ml Distilled water.

B. 2% $CuSO_4$: 0.1 g Copper Sulphate dissolved in 5ml Distilled water.

C. 4% $C_4H_4O_6KNa.4H_2O$: 0.2g Sodium potassium tartrate dissolved in 5ml Distilled water.

150 ml Reagent was prepared by pipetting out 144ml Solution A+3ml Solution B + 3ml ml Solution C to make a volume of 150 ml.so as to maintain the proportion (100:1:1)

Folins Reagent (1:1)

20ml Folin Ciocalteau dissolved in 20ml Distilled water. The reagent was freshly prepared as it degrades faster due to exposure to light.

Sample preparation.

0.1 g of each plant tissue(leaves) was weighed and ground with mortar and pestle using phosphate buffer and poured in centrifuge tubes making the volume upto 10 ml. Then centrifuged in centrifugation machine at 5000 RPM for 10 mins. Then supernatant was collected from each tube and was used as unknown sample.

Estimation of Protein

Each plant sample was analysed in triplets to obtain precise readings. 1m of each plant sample was taken in a test tube and to each 5ml of Lowry's reagent was added and kept for incubation at room temperature for 15 mins and then 0.5 ml folins reagent was added and again test tubes were kept for incubation for 10 minutes and after time duration absorbance checked at 660 nm using UV visible

spectrophotometer. The intensity of the complex exhibiting a blue colour was compared to that of a suitable blank. Quantification of protein content was done with standard curve of bovine serum albumin (BSA).

3.6.3 Estimation of total lipids (Lee,1995)

Tissue:Plant tissue (leaves)

Apparatus required - Beakers, Seperating funnel, Glass rod, Measuring cylinder, Whatmann filter paper 1, funnel, tripod stand, Petriplates and mortar and pestle.

Chemicals required: Chloroform, Methanol,0.5% Nacl. Distilled water.

Chemical preparation:

Chloroform: Methanol mixture (2:1)
20ml chloroform +10ml methanol

0.5% NaCl
0.5g NaCl → 100 ml Distilled water

Estimation protocol
3g of plant tissue(leaves) weighed and homogenized using mortar and pestle with 30 ml Chloroform: Methanol mixture (2:1). Mixture poured in seperating funnel by filtering using whatmann filter paper 1. After filtration 10ml 0.5 % Nacl added. Lid was closed and tilted 5 times shaking and kept stagnant for layer formation. Lower layer formed was taken and measued.3ml each was poured in initially weighed three petriplates, kept in oven at 100ºC and final weight was taken.

Total lipids extracted was calculated using:

Lipid content (%)

$$= \frac{Lipid\ extracted(g)}{\text{Sample weight}} \times \frac{(\text{Chloroform layer} + \text{amount lost})\text{ml}}{3\text{ml}} \times 100$$

3.6.4 TLC analysis of Pinitol (Indumati *et al*.,2013)

Apparatus used: Beakers, TLC plates, Capillary tubes, measuring cylinder, Glass rod, dropper, Reagent spray bottle, glass rod, funnel.

Chemicals required: Methanol, Chloroform, ethanol, silver nitrate ($AgNO_3$), Sodium hydroxide (NaOH), Ammonia solution (NH_4OH), Distilled water.

Tissue: Plant tissue(leaves)

Chemical Preparation:
Chloroform: Methanol mixture (6:3) :60ml chloroform + 30ml methanol

Tollens Reagent
A.1g silver nitrate dissolved in 10ml Distilled water

B. 1g Sodium hydroxide dissolved in 10 ml Distilled water

solution B added to A stirring continuoulsy until Brown precipitate is formed and the precipitate was dissolved by addition of Ammonia solution dropwise.

Sample Preparation
Air dried plant material(1g) was weighed in 75ml of ethanol containing beaker and kept in water bath (100º C) for 1 hour. Filtered and concentrated and used for spot development.

Spot Development
1 ml of sample was dissolved in 1 ml of ethanol and with help of capillary sample was loaded on Silica gel coated TLC plates and kept to dry. Then TLC plates were kept in Beaker containing Chloroform: Methanol mixture (6:3) for solvent to travel for 30 mins. TLC plates were examined under UV lamp. Further Silver nitrate solution was sprayed. Development of an orange, brown spot for pinitol was noted and its R_1 and R_2 value was recorded. Same was followed and compared with pinitol standard.

3.6.6 Quantification of D-Pinitol

Apparatus required-Beakers, Test tubes, filter paper, funnel, Conical flask, test tube stand, glass rod, dropper, micropippetes.

Chemicals required- Methanol, TritonX-100, Disodium hydrogen phosphate, Sodium dihydrogen phosphate, Sodium chloride (Nacl).

Chemical Preparation:

50ml Nacl

0.45g Nacl dissolved in 50ml Distilled water

Phosphate buffer saline

A.1.06g Disodium hydrogen phosphate dissolved in 50 ml Distilled water

B.1.71g Sodium dihydrogen phosphate dissolved in 50 ml Distilled water

41ml solution A +9ml solution B → total volume 50ml

50ml Of Solution A+B mixture was added to 50ml Nacl(normal saline) to prepare 100ml phosphate buffer saline.

1% TritonX-100 (detergent water)

9.9ml Phosphate buffer saline + 0.1ml triton x-100

Sample preparation.

Oven dried leaves powder was soaked in methanol for 24 hours and filtered. The process was repeated thrice for residue. All three filtered portions were combined and evaporated in water bath to give greenish-brown leaf extract at 45-50ºC.The crude extract residue was kept in refrigerator.25mg crude methanolic extract was mixed in 25 ml detergent water and used as a stock solution and filtered.0.1ml of stock solution+5 ml detergent water was mixed in test tube and OD was taken at 229nm.D-pinitol standard optimization was done using D-pinitol as standard.

4.ANALYSIS AND CONCLUSION

4.1 Observations

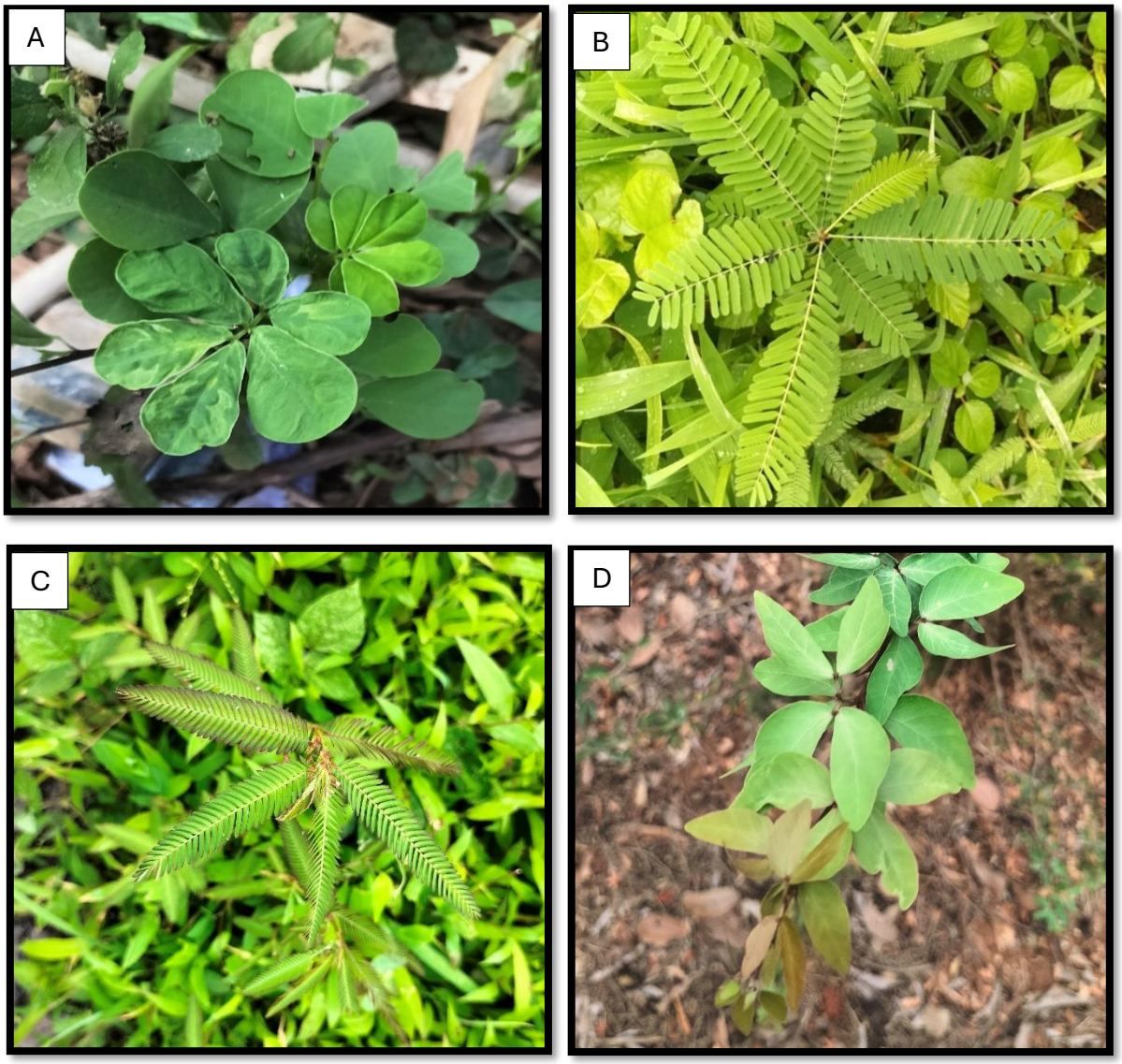


Fig 4.1.1: Egg laid larval Host plants of *E.hecabe*

A. *Cassia tora*, B. *Sesbania bispinosa*, C. *Aeschynomene americana,* D. *Pithecellobium dulce*

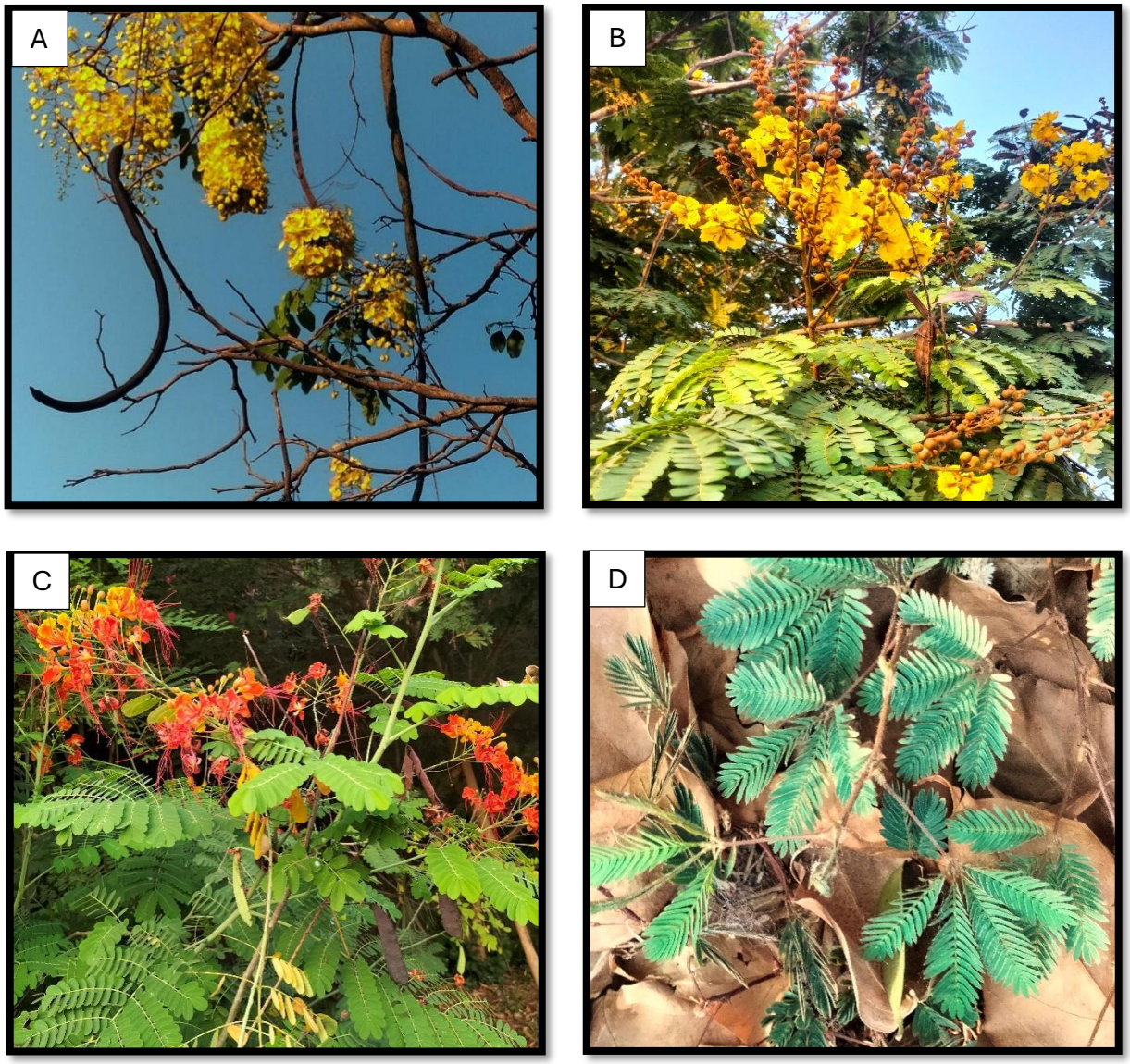


Fig 4.1.2: Non egg laid larval Host plants.

A. *Cassia fistula*, B. *Peltophorum pterocarpum*. C. *Caesalpinia pulcherimma*,

D. *Mimosa pudica*

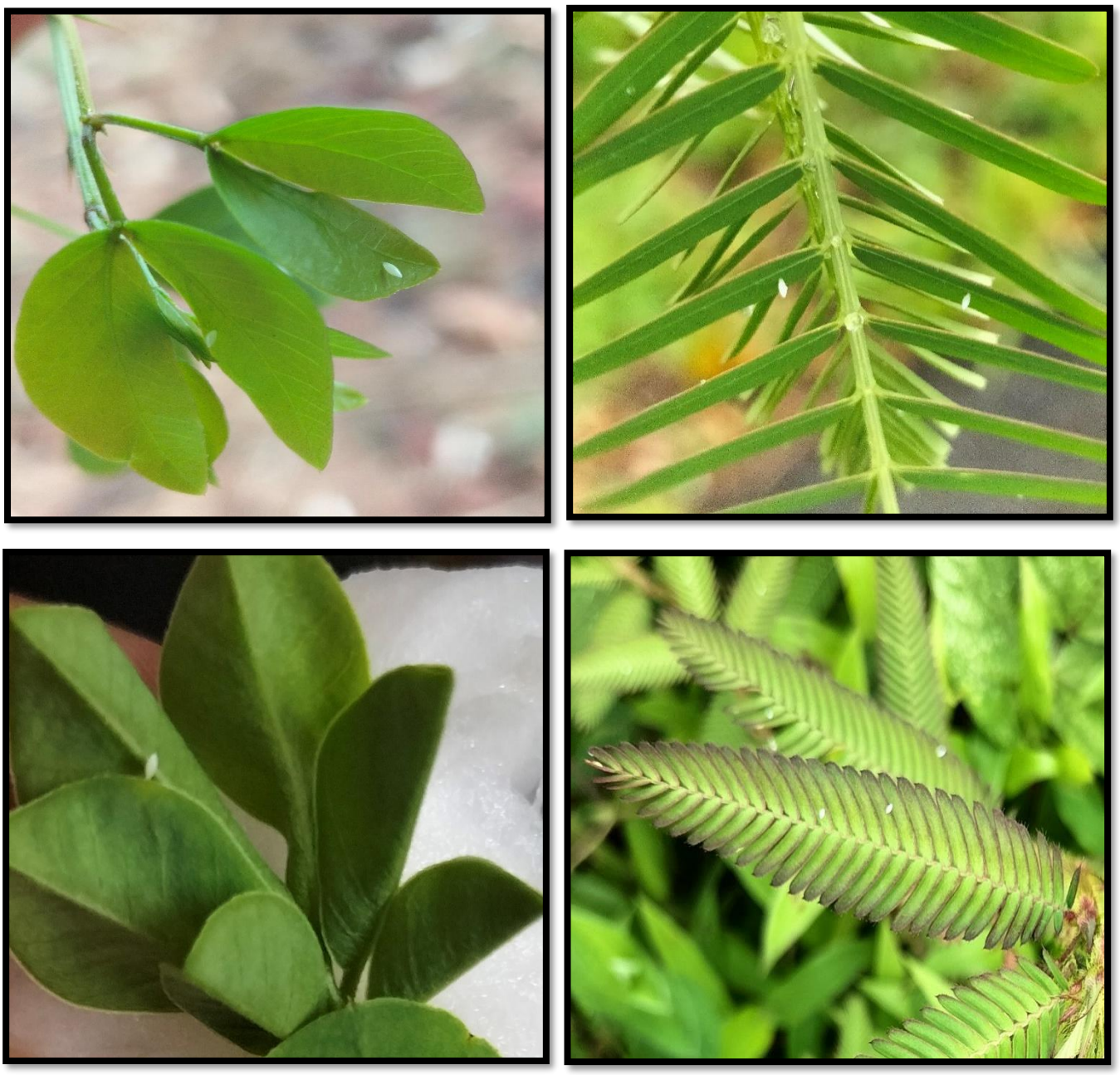


Fig 4.1.3: Eggs of *E.hecabe* observed on LHP .

A. *Pithecellobium dulce,* B. *Sesbania bispinosa*, C. *Cassia tora*, D. *Aeschynomene americana*

Table 4.1.1: Common grass yellow Butterflies presence during study period

No. Of observations	Month	Transect 1	Transect 2	Transect 3	Transect 4
1	June	+	+	+	+
2		+	+	+	+
3	July	+	+	+	+
4		+	+	+	+
5	August	+	+	+	+
6		+	+	+	+
7	September	+	+	+	+
8		+	+	+	+
9	October	+	+	+	+
10		+	+	+	+
11	November	+	+	+	+
12		+	-	+	+
13	December	-	-	-	+
14		-	+	-	-
15	January	-	-	-	-
16		+	+	-	-
17	February	+	-	-	+
18		+	+	-	-

Table 4.1.2: Host plants availability and oviposition preference of Common grass yellow (*Eurema hecabe*) during study period (□-Host plant, ● Ovipsition preference)

No. Of obs.	Month	*C.tora*				*S.bispinosa*				*P.dulce*				*A.americana*			
		T1	T2	T3	T4	T1	T2	T3	T4	T1	T2	T3	T4	T1	T2	T3	T4
1	June	□	□	□	□	□	□	□	□	□	□	□	□	□	□	□	□
2		□	□	□	□	□	□	□	□	□	□	□	□	□	□	□	□
3	July	•□	□	•□	□	□	□	□	□	□	□	□	□	•□	•□	•□	•□
4		•□	•□	•□	•□	□	□	□	□	□	□	□	□	•□	•□	•□	•□
5	August	•□	•□	•□	•□	•□	□	•□	•□	•□	□	•□	•□	•□	•□	•□	•□
6		•□	•□	•□	•□	•□	•□	•□	•□	□	•□	•□	□	•□	•□	•□	•□
7	September	•□	•□	•□	•□	•□	□	•□	•□	•□	□	•□	•□	•□	•□	•□	•□
8		•□	•□	•□	•□	•□	•□	•□	□	□	□	□	□	•□	•□	•□	•□
9	October	•□	•□	•□	•□	•□	•□	•□	•□	•□	•□	□	•□	-	-	-	-
10		•□	-	•□	•□	□	•□	•□	•□	□	□	•□	•□	-	-	-	-
11	November	-	-	-	-	•□	•□	•□	□	□	•□	□	□	-	-	-	-
12		-	-	-	-	•□	•□	•□	□	□	•□	□	□	-	-	-	-
13	December	-	-	-	-	-	-	-	-	□	□	•□	□	-	-	-	-
14		-	-	-	-	-	-	-	-	□	•□	□	□	-	-	-	-
15	January	-	-	-	-	-	-	-	-	•□	□	□	□	-	-	-	-
16		-	-	-	-	-	-	-	-	•□	□	□	□	-	-	-	-
17	February	-	-	-	-	-	-	-	-	•□	□	□	•□	-	-	-	-
18		-	-	-	-	-	-	-	-	□	•□	□	□	-	-	-	-

No. Of obs.	Month	*P.pterocarpum*				*C.fistula*				*C.pulcherimma*				*M.pudica*			
		T1	T2	T3	T4	T1	T2	T3	T4	T1	T2	T3	T4	T1	T2	T3	T4
1	June	□	□	□	□	□	□	□	□	□	□	□	□	□	□	□	□
2		□	□	□	□	□	□	□	□	□	□	□	□	□	□	□	□
3	July	□	□	□	□	□	□	□	□	□	□	□	□	□	□	□	□
4		□	□	□	□	□	□	□	□	□	□	□	□	□	□	□	□
5	August	□	□	□	□	□	□	□	□	□	□	□	□	□	□	□	□
6		□	□	□	□	□	□	□	□	□	□	□	□	□	□	□	□
7	September	□	□	□	□	□	□	□	□	□	□	□	□	□	□	□	□
8		□	□	□	□	□	□	□	□	□	□	□	□	□	□	□	□
9	October	□	□	□	□	□	□	□	□	□	□	□	□	□	□	□	□
10		□	□	□	□	□	□	□	□	□	□	□	□	□	□	□	□
11	November	□	□	□	□	□	□	□	□	□	□	□	□	□	□	□	□
12		□	□	□	□	□	□	□	□	□	□	□	□	□	□	□	□
13	December	□	□	□	□	□	□	□	□	□	□	□	□	□	□	□	□
14		□	□	□	□	□	□	□	□	□	□	□	□	□	□	□	□
15	January	□	□	□	□	□	□	□	□	□	□	□	□	□	□	□	□
16		□	□	□	□	□	□	□	□	□	□	□	□	□	□	□	□
17	February	□	□	□	□	□	□	□	□	□	□	□	□	□	□	□	□
18		□	□	□	□	□	□	□	□	□	□	□	□	□	□	□	□

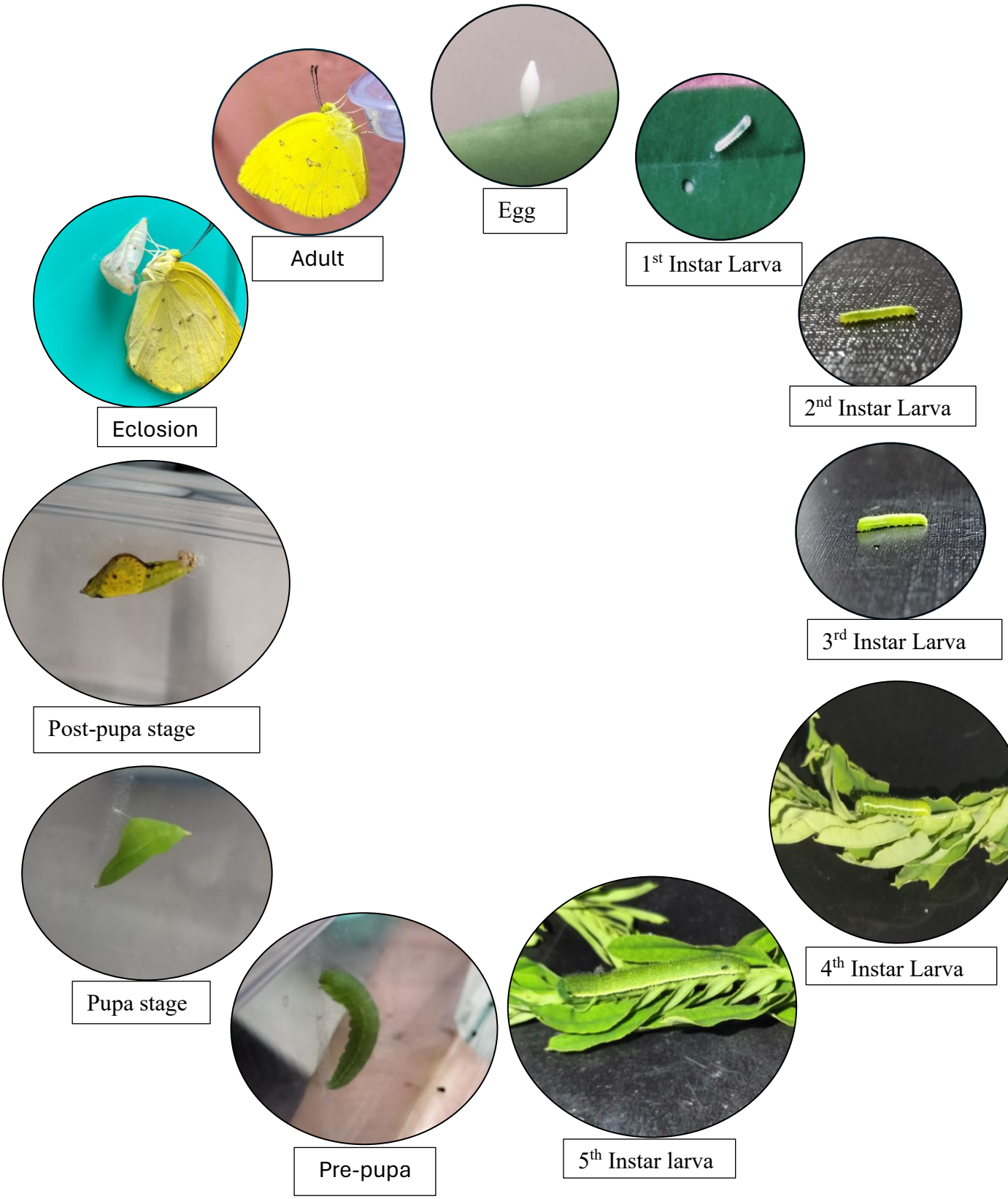


Fig:4.1.4: Life Cycle of Common grass yellow (*Eurema hecabe*)

Table 4.1.3: In-vitro studies of *E. hecabe* reared on *A. americana* from 16/07/2023 to 27/07/2023 (Ave ± S.D.)

Days	Instar	Weight (gms)	Length (cms)	Weight of feces Excreted (gms)	Amount of LHP Consumed (gms)	Growth Indices (G.I.)
		(Ave ± SD)	(Ave ± SD)	(Ave± SD)	(Ave ± SD)	
1	1	0.0002 ± 0	0.18±0.0447	0.0002 ± 0	0 ± 0	0.00027
2		0.0003 ± 0	0.3±0	0.0002 ± 0	0.0166 ± 0	0.0004
3	2	0.0005 ± 0	0.42±0.0447	0.0005 ± 0	0.0366 ± 0.0044	0.00067
4		0.002 ± 0	0.48±0.0447	0.001 ± 0	0.166 ± 0.0044	0.00271
5	3	0.0054 ± 0.0001	0.62±0.0447	0.003 ± 0.0003	0.177 ± 0.0044	0.00731
6		0.0102 ± 0.0002	1±0	0.0078± 0.00005	0.566 ± 0.0044	0.01385
7		0.0216 ± 5.4772	2.26±0.1341	0.0044 ± 0	0.772 ± 0.0001	0.02935
8	4	0.0494 ± 0.0001	2.58±0.0447	0.0216 ± 0	0.896 ± 0.0008	0.06704
9		0.16 ± 0.0547	3.14±0.1140	0.05 ± 0	0.9138 ± 0.0001	0.21687
10	5	0.118 ± 0.0044	3.44±0.0894	0.04 ± 0	1.01 ± 0.0089	0.15994
11		0.166 ± 0.0089	4.02±0.0447	0.04 ± 0	0.1692 ± 0.0008	0.22501
12		0.204 ± 0.0054	4.36±0.1516	0.03 ± 0	0.3409 ± 0.3946	0.27652

Table 4.1.4: In-vitro studies of *E. hecabe* reared on *C. tora* from 1/08/2023 to 12/08/2023(Ave ± S.D.)

Days	Instar	Weight (gms)	Length (cms)	Weight of feces excreted (gms)	Amount of LHP consumed (gms)	Growth Indices (G.I)
		(Ave ± SD)	(Ave ± SD)	(Ave± SD)	(Ave ± SD)	
1	1	0.0002 ± 0	0.48 ± 0.0447	0.0001 ± 0	0 ± 0	0.00047
2		0.0005 ± 0	0.58 ± 0.0447	0.0008 ± 0	0.012 ± 0.0044	0.00117
3	2	0.0012 ± 0.0004	1.12 ± 0.1303	0.0011 ± 0.0002	0.048 ± 0.0044	0.00282
4		0.0052 ± 0.0004	1.34 ± 0.1140	0.0025 ± 0	0.11 ± 1.5515	0.01223
5	3	0.0212 ± 0.0004	1.68 ± 0.0836	0.01 ± 0	0.3148 ± 0.0052	0.04989
6		0.0222 ± 0.0004	2.14 ± 0.1140	0.0125 ± 0.0004	0.5186 ± 0.0136	0.05224
7		0.0238 ± 0.0004	2.54 ± 0.1673	0.0234 ± 0.0002	0.635 ± 0.0008	0.05601
8	4	0.0292 ± 0.0004	2.84 ± 0.0547	0.0233± 0.0005	1.51094 ± 0.0030	0.06872
9		0.0548 ± 0.0004	3.02 ± 0.0447	0.0546 ± 0.0019	1.71352 ± 0.0460	0.12897
10	5	0.0778 ± 0.0004	3.2 ± 0	0.1308 ± 0.0049	1.54286 ± 0.0009	0.1831
11		0.0958 ± 0.0004	3.7 ± 0	0.1954 ± 0.0008	1.23184 ± 0.0014	0.22546
12		0.093 ± 0	4.02 ± 0.0447	0.0377 ± 0	0.0604 ± 0.0002	0.21887

Table 4.1.5: In-vitro studies of *E. hecabe* reared on *S. bispiniosa* from15/08/2023 to 26/08/2023 (Ave ± S.D.)

Days	Instar	Weight (gms)	Length (cms)	Weight of feces excreted (gms)	Amount of LHP consmed (gms)	Growth Indices (G.I)
		(Ave ± SD)	(Ave ± SD)	(Ave± SD)	(Ave ± SD)	
1	1	0.0001 ± 0	0.22 ± 0.0447	0.0002 ± 0	0 ± 0	0.00021
2		0.0005 ± 0	0.48 ± 0.0447	0.0006 ± 0	0.012 ± 0.0044	0.00109
3	2	0.0018 ± 0.0017	1.12 ± 0.0836	0.002 ± 0.0017	0.05 ± 0	0.00395
4		0.0182 ± 0.0004	1.82 ± 0.0447	0.0052 ± 0.0004	0.118 ± 0.0044	0.04
5	3	0.0214 ± 0.0008	2.18 ± 0.0447	0.012 ± 0.0044	1.302 ± 0.0044	0.04703
6		0.0222 ± 0.0004	2.46 ± 0.0894	0.11028 ± 0.0044	1.344 ± 0.0070	0.04879
7		0.0234 ± 0.0013	2.78 ± 0.0447	0.22198 ± 0.0004	1.43144 ± 0.0001	0.05142
8	4	0.0288 ± 0.0004	3.08 ± 0.0447	0.02354 ± 0.0012	1.44704 ± 0.0032	0.06329
9		0.0548 ± 0.0004	3.32 ± 0.0447	0.05516 ± 0.0003	1.51198 ± 0.0004	0.12043
10	5	0.0958 ± 0.0004	3.5 ± 0	0.1436 ± 0.0005	1.11908 ± 0.0045	0.21054
11		0.0928 ± 0.0004	3.6 ± 0	0.1968 ± 0.0004	1.1 ± 0.0707	0.20395
12		0.0952 ± 0.0004	3.82 ± 0.0447	0.0339 ± 0.0017	0.06048 ± 0.0001	0.20923

Table 4.1.6: In-vitro studies of *E. hecabe* reared on *P. dulce* from 01/09/2023 to 12/09/2023(Ave ± S.D).

Days	Instar	Weight (gms)	Length (cms)	Weight of feces excreted(gms)	Amount of LHP consmed (gms)	Growth Indices (G.I)
		(Ave ± SD)	(Ave ± SD)	(Ave± SD)	(Ave ± SD)	
1	1	0.0002 ± 0	0.1 ± 0	0.012 ± 0.0044	0 ± 0	0.00024
2		0.0003 ± 0	0.28 ± 0.0447	0.022 ± 0.0044	0.0211 ± 0	0.00037
3	2	0.0004 ± 0	0.58 ± 0.0447	0.04 ± 0	0.031 ± 0.0001	0.00049
4		0.0018 ± 0.0004	0.7 ± 0	0.054 ± 0.0054	0.56 ± 0.0001	0.00224
5	3	0.0520 ± 0.0007	0.88 ± 0.0447	0.09022 ± 0.0004	0.667 ± 0.0001	0.00476
6		0.0105 ± 0.0001	1.04 ± 0.0547	0.0912 ± 0	0.7307 ± 0.0001	0.0131
7		0.0323 ± 0.0009	1.14 ± 0.0547	0.219 ± 0.0004	0.8112± 0.0004	0.0403
8	4	0.0513 ± 0.0002	1.4 ± 0	0.111± 0.0044	1.263 ± 0.0008	0.06389
9		0.1200 ± 0.0447	1.58 ± 0.0447	0.111 ± 0.0134	1.156 ± 0.0008	0.14946
10	5	0.1420 ± 0.0044	1.76 ± 0.0547	0.12 ± 0	1.16 ± 0.0017	0.17686
11		0.1920 ± 0.0044	2.48 ± 0.0836	0.21 ± 0.0089	1.266 ± 0.0004	0.23913
12		0.2000 ± 0	3.24 ± 0.2073	0.314 ± 0.0447	0.333 ± 0.0004	0.2491

C.I.=Food consumption index; A.D.=Approximate digestibility; E.C.D.=Efficiency of conversion of digested food; E.C.I.=Efficiency of conversion of ingested food.

Table 4.1.7: Food Utility index for caterpillars grown on *A. americana* LHP

Instars	C.I.	A.D.	E.C. D	E.C. I
1	21.1	71.09005	3.333333	2.369668
2	134.3182	98.52792	0.377812	0.37225
3	7.760329	87.9397	4.884427	4.295351
4	7.060712	90.82265	7.796996	7.081439
5	1.722222	76.65821	25.24823	19.35484

Table 4.1.8: Food Utility index for caterpillars grown on *C. tora* LHP.

Instar	C.I.	A.D.	E.C. D	E.C. I
1	16.6	97.59036	3.08642	3.012048
2	40.52	99.25962	0.012432	1.233959
3	13.54614	98.99274	2.485764	2.460726
4	4.320157	96.04376	4.413655	11.57365
5	0.45955	92.76363	34.60747	32.10315

Table 4.1.9: Food Utility index for caterpillars grown on *S. bispinosa* LHP.

Insta	C.I.	A.D.	E.C. D	E.C. I
1	8.571429	92.5	6.306306	5.833333
2	12.34375	97.72152	4.145078	4.050633
3	7.28373	96.86979	4.72429	4.57641
4	19.19321	97.58149	2.669653	2.605087
5	3.544761	87.16447	10.78828	9.403548

Table 4.1.10: Food Utility index for caterpillars grown on *P. dulce* LHP.

Instar	C.I.	A.D.	E.C. D	E.C. I
1	10	93.33333	5.357143	5
2	4.2	95.71429	12.43781	11.90476
3	20.28577	91.55696	1.794717	1.643188
4	18.79936	97.34034	2.732335	2.659664
5	3.293686	83.58016	12.10858	10.12037

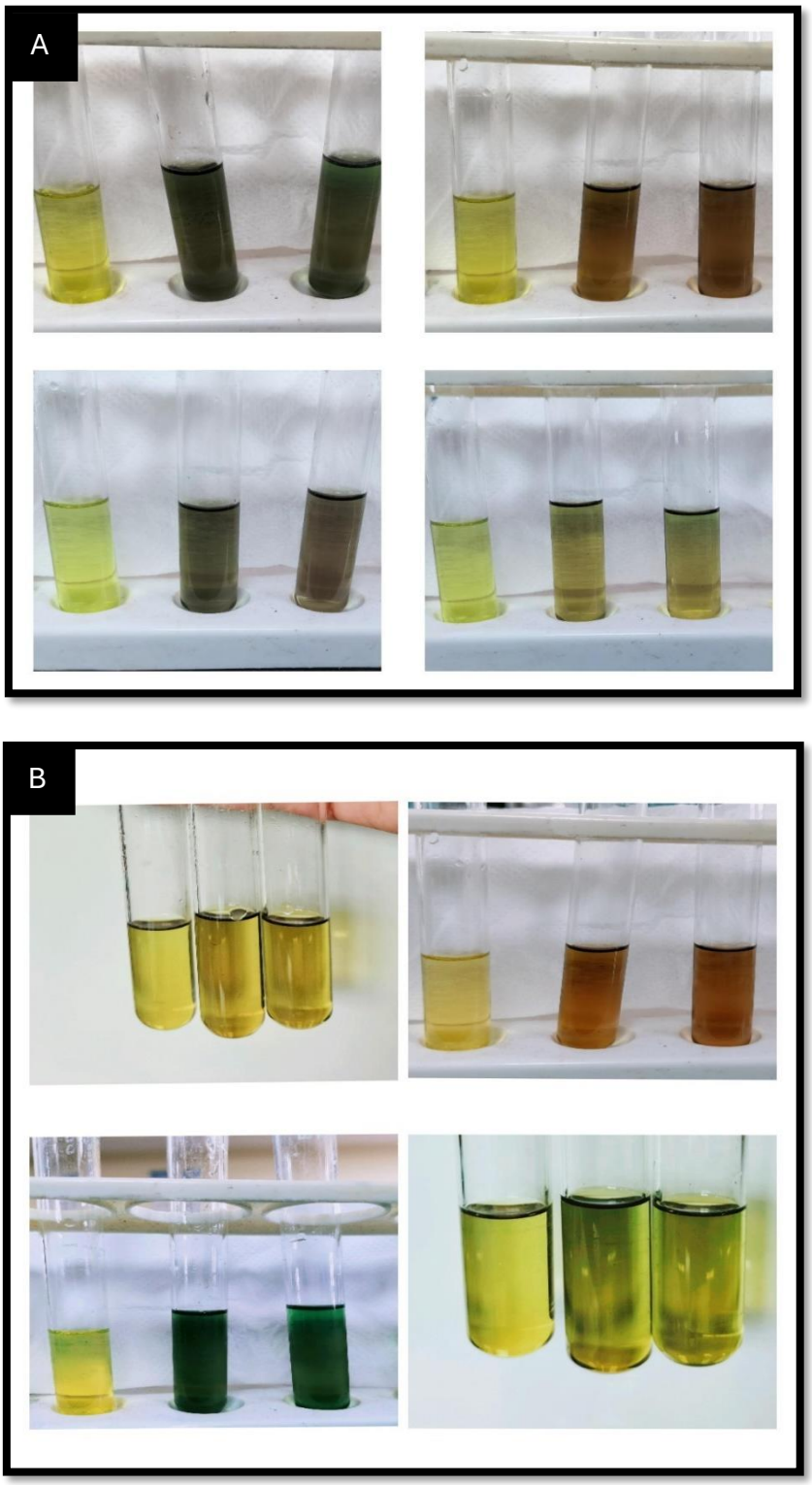


Fig 4.1.5: Carbohydrate estimation.

A. Carbohydrate estimation of Egg laid LHP, B. Carbohydrate estimation of Non egg laid LHP

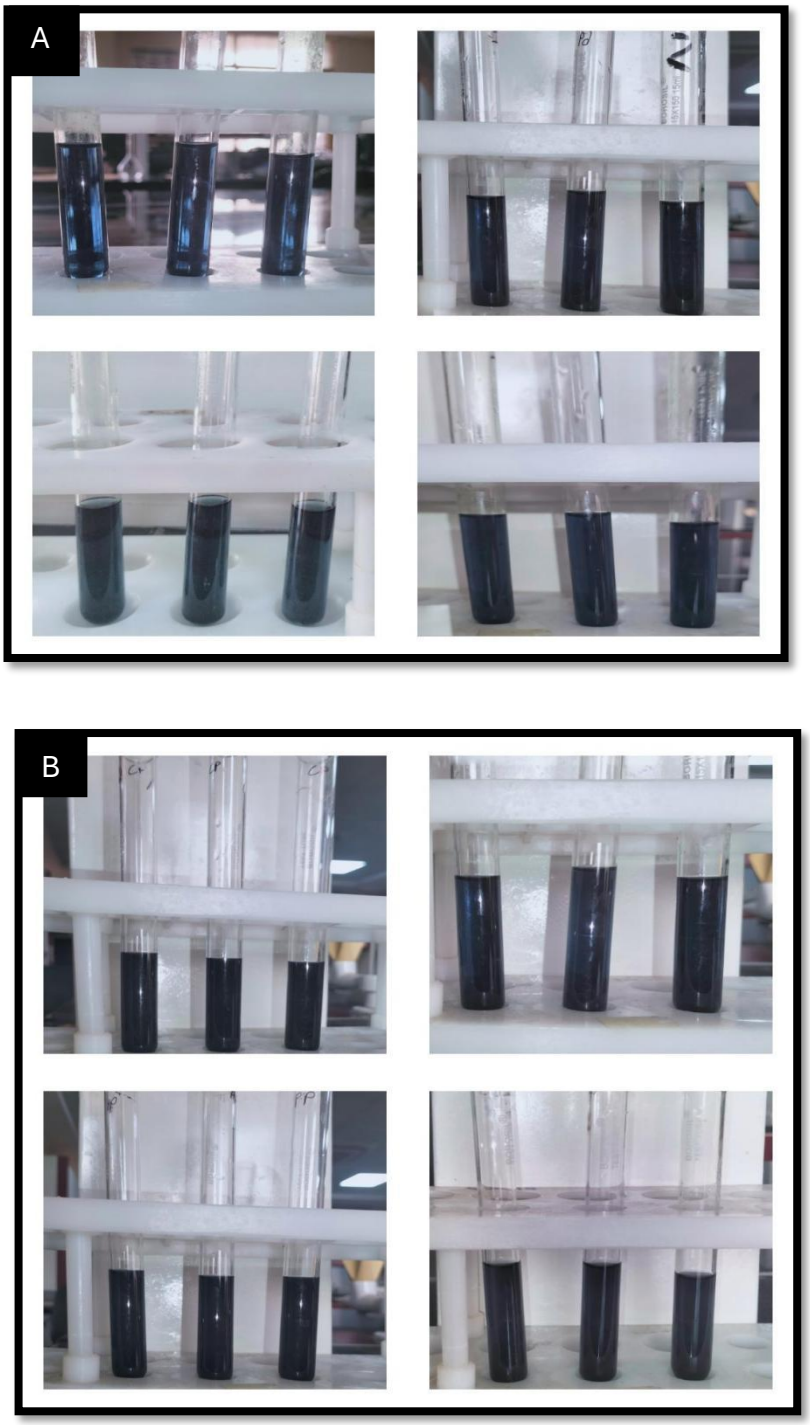


Fig 4.1.6: Protein estimation

A. Protein estimation of Egg laid LHP, B. Protein estimation of Non-egg laid LHP

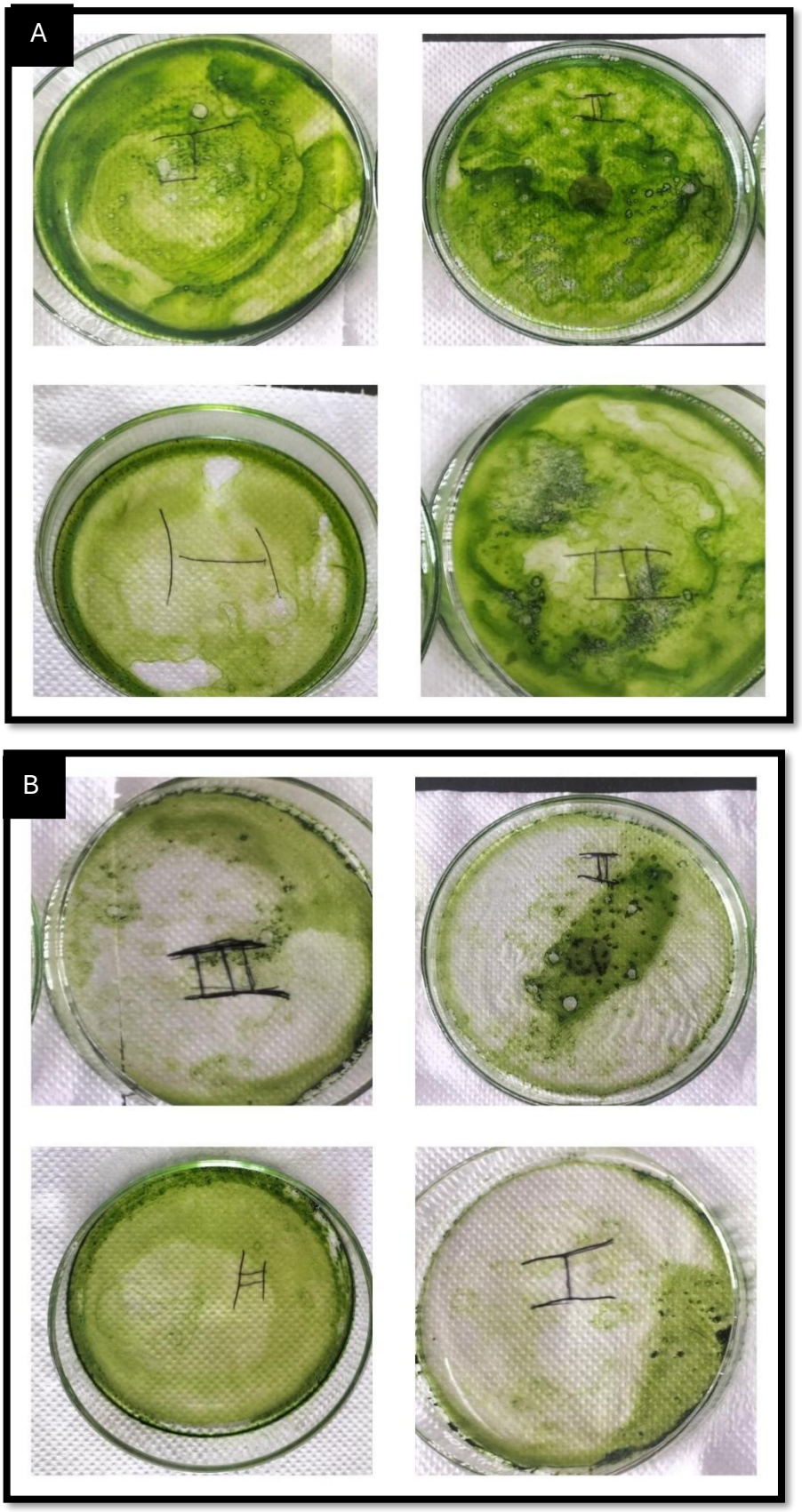
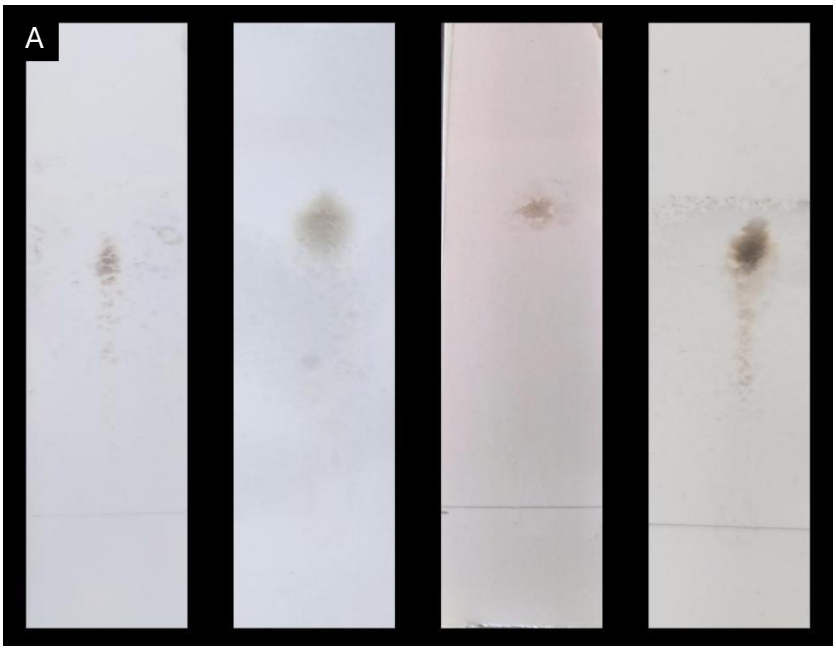


Fig 4.1.7: Lipid estimation

A. Lipid estimation of egg laid LHP, B. Lipid estimation of non-egg laid LHP



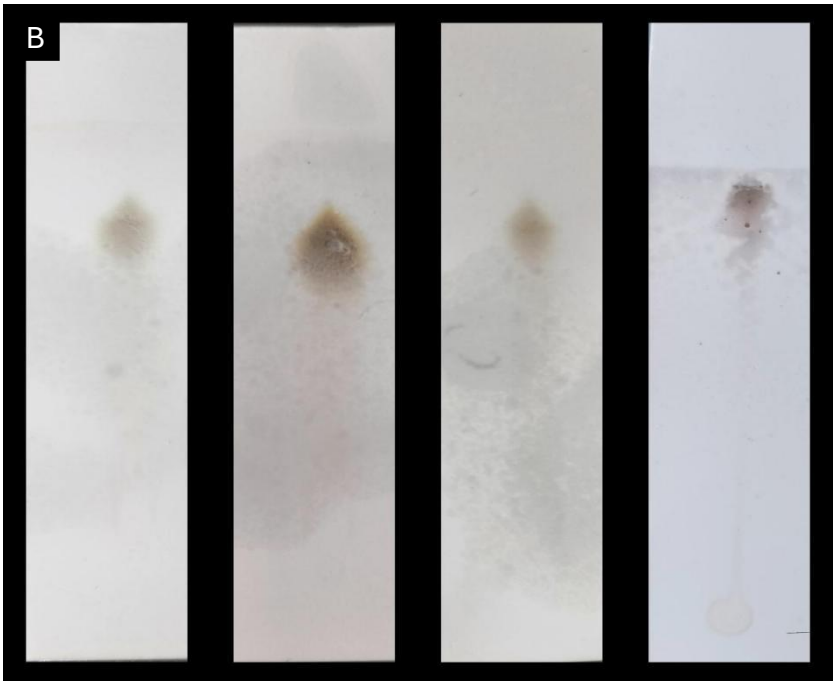


Fig 4.1.8: Pinitol spot development through TLC

A. Pinitol spot development of Egg laid LHP, B. Pinitol spot development of Non - egg laid LHP

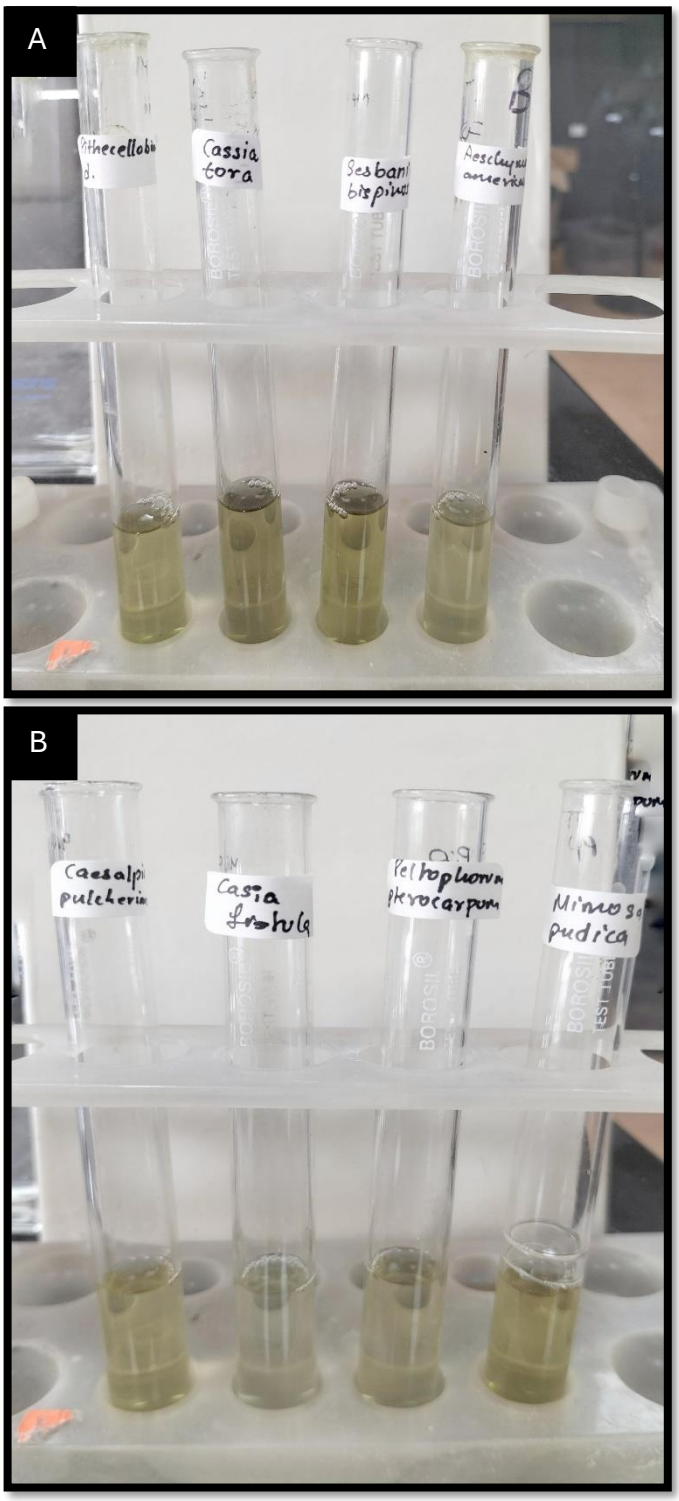


Fig 4.1.9: Estimation of D-pinitol

A. D-pinitol estimation of Egg laid LHP, B. D-pinitol estimation of Non-egg laid LHP

Table 4.1.11: Nutritional status of Egg laid LHP w.r.t Macronutrients.

Plant Name	Carbohydrate mg/g	Protein mg/g	Total Lipid content (%)
	Ave ± Stdev	Ave ± Stdev	
C. tora	1.53±0.0616	86.79±0.3230	42.08
S. bispinosa	9.23±0.1925	93.54±0.3437	1.9
P.dulce	6.08±0.5093	92.39±0.1552	4.8
A.americana	5.05±0.1111	89.57±0.9440	1.52

Table 4.1.12: Nutritional status of Non egg laid LHP w.r.t Macronutrients.

Plant Name	Carbohydrate mg/g	Protein mg/g	Total Lipid content (%)
	Ave ± Stdev	Ave ± Stdev	
C. fistula	17.61±0.3247	90.51±0.9972	1.5
P.pterocarpum	15.39±0.2021	160.10±0.3230	0.13
C.pulcherimma	23.40±0.0308	181.9±0.0338	0.75
M.pudica	7.24±0.4603	71.37±0.2893	0.61

Table 4.1.13: Rf values of for spot development of Pinitol using TLC.

Test Samples	Rf values
Standard	0.71
S1- *C. tora*	0.78
S2- *S. bispinosa*	0.76
S3- *P. dulce*	0.72
S4 - *A. americana*	0.74
S5-*C. fistula*	0.7
S6- *P. pterocarpum*	0.69
S7- *C. pulcherimma*	0.71
S8- *M. pudica*	0.71

Table 4.1.14: D-pinitol content of Egg laid LHP.

Plant Name	Pinitol (mg/g)	% Pinitol content
C. tora	16.23±0.6002	1.62
S. bispinosa	17.39±0.0944	1.69
P.dulce	11.26±0.4898	1.73
A.americana	6.97±0.2442	1.12

Table 4.1.15: D-pinitol content of Non egg laid LHP.

Plant Name	Pinitol (mg/g)	% Pinitol content
C. fistula	6.01±0.0315	0.6
P.pterocarpum	1.57±0.2746	0.15
C.pulcherimma	0.3104±0.2021	0.38
M.pudica	3.82±0.6018	0.03

4.2 Results

4.2.1 Identification and localization of larval host plants

In the present study, out of 18 larval host plants reported by Indian foundation for butterflies only 8 showed their existence in the choosen study area through preliminary survey carried out and based on their localization four transects were marked to carry out the study.

4.2.2 Presence of *E.hecabe* , host plants availability and oviposition preference

Variations were observed in sighting of common grass yellow with availability of host plant and oviposition response as given in table 4.1.1 and 4.1.2. During the 9 months of study period the sighting of common grass yellow (*E.hecabe*) in all four transects was more in the months from June to October and eventually from November 2023 the number of grass yellow started decreasing upto February 2024.On the contrary the availability of host plants and oviposition preference by common grass yellow was studied in which LHP *C. tora* was available in the month from June to October but preferred as oviposition LHP from July-October, LHP *S. bispinosa* was available in the month from June-November but prefered as a oviposition site from August- November. Also, LHP *A. americana* showed its presence in the month of June-September and preferred for oviposition in July-September. *C. tora*, *S. bispinosa* and *Aeschynomene americana* are seasonal plants and *P. dulce* is annual. Also, this led to shift oviposition on *P. dulce* which is annual plant and showed its presence during whole study period. Also, other host plants such as *P. pterocarpum*, *C. pulcherimma*, *C. fistula* and *M. pudica* were not preferred for oviposition during study period.

4.2.3 In-vitro studies of caterpillar growth

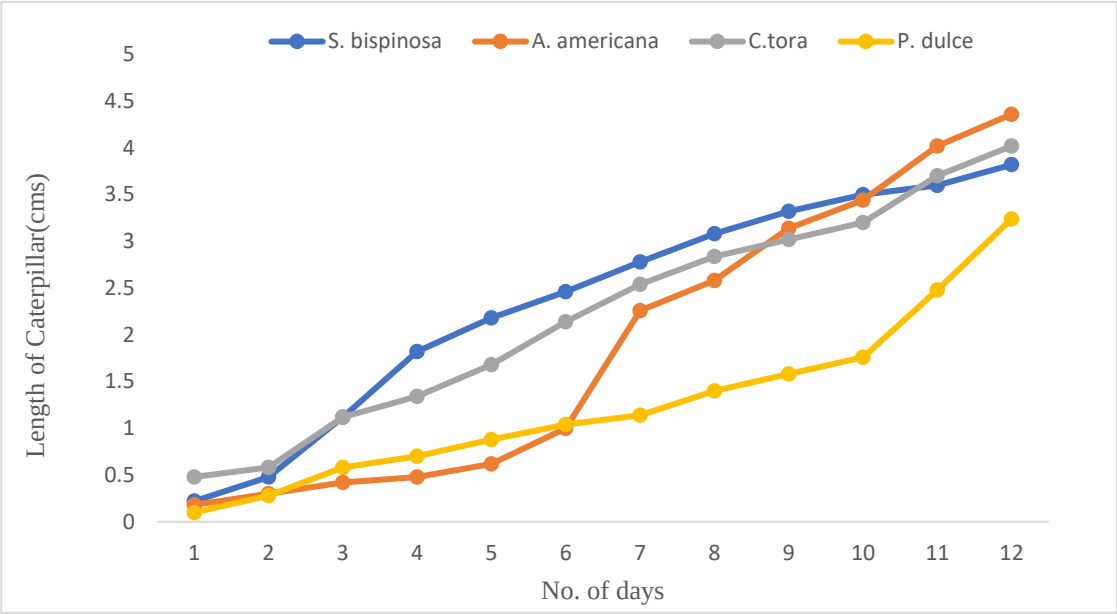


Fig :4.2.1-Invitro studies of caterpillar. Comparison of change in length of caterpilars reared on *S.bispinosa, A.americana, C.tora and P.dulce* .lengths do not differ significantly as per One way Annova test as P>.005.

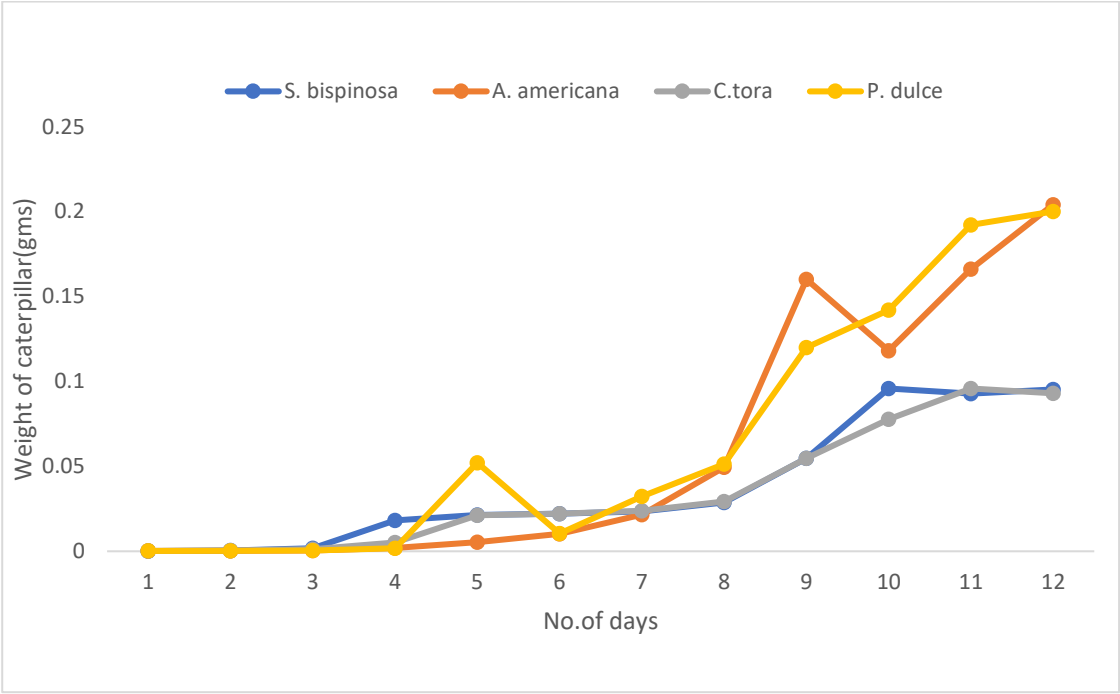


Fig:4.2.2- Invitro studies of caterpillar. Comparison of change in weight of caterpilars reared on *S.bispinosa, A.americana, C.tora and P.dulce* .Comparison of weights is non-significant as per Kruskal wallis test as P>.005.

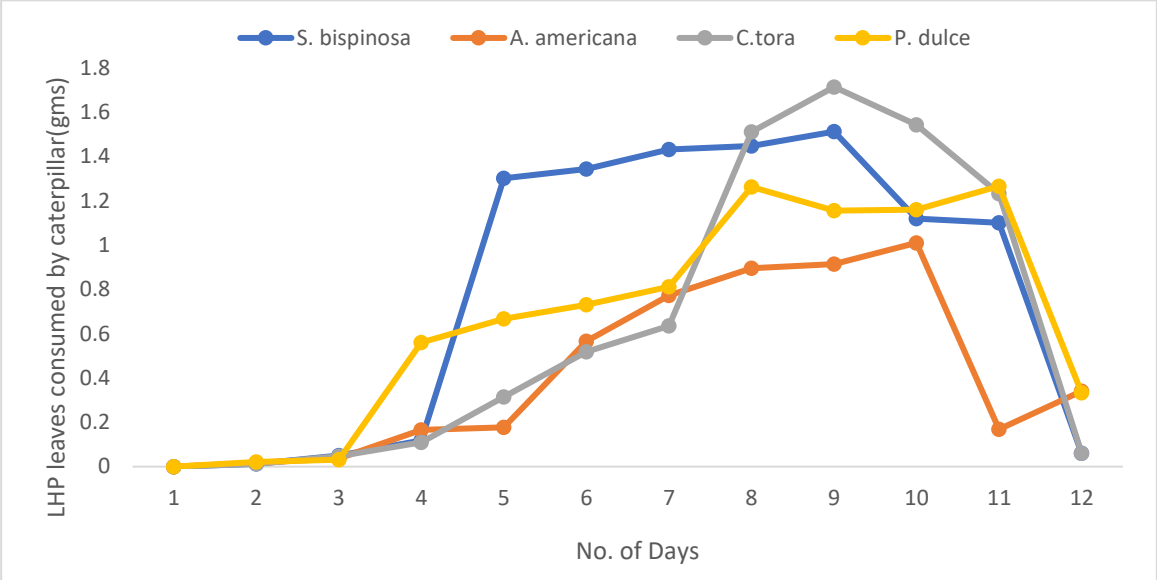


Fig:4.2.3- Invitro studies of caterpillar. Comparison of change in LHP consumed by caterpillars reared on *S.bispinosa, A.americana, C.tora and P.dulce* .Comparison of LHP consumption was not significant as per Kruskal wallis test as P>0.05.

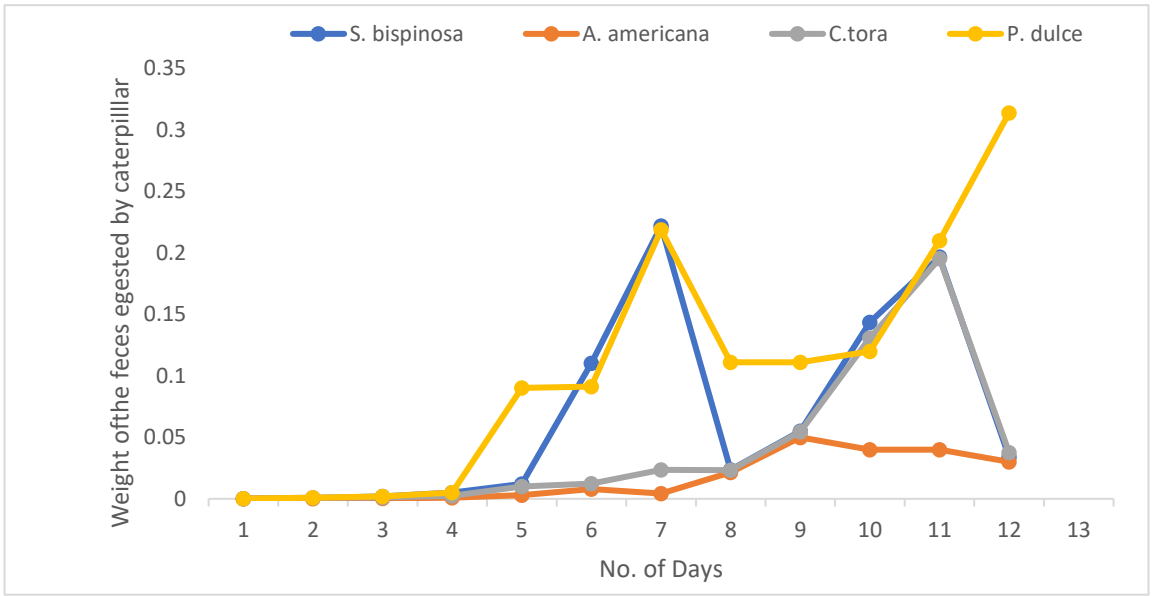


Fig:4.2.4- Invitro studies of caterpillar. Comparison of change in feces egested by caterpillars reared on *S.bispinosa, A.americana, C.tora and P.dulce* .Amount of feces do not differ significantly as per Kruskal wallis test as P>.005.

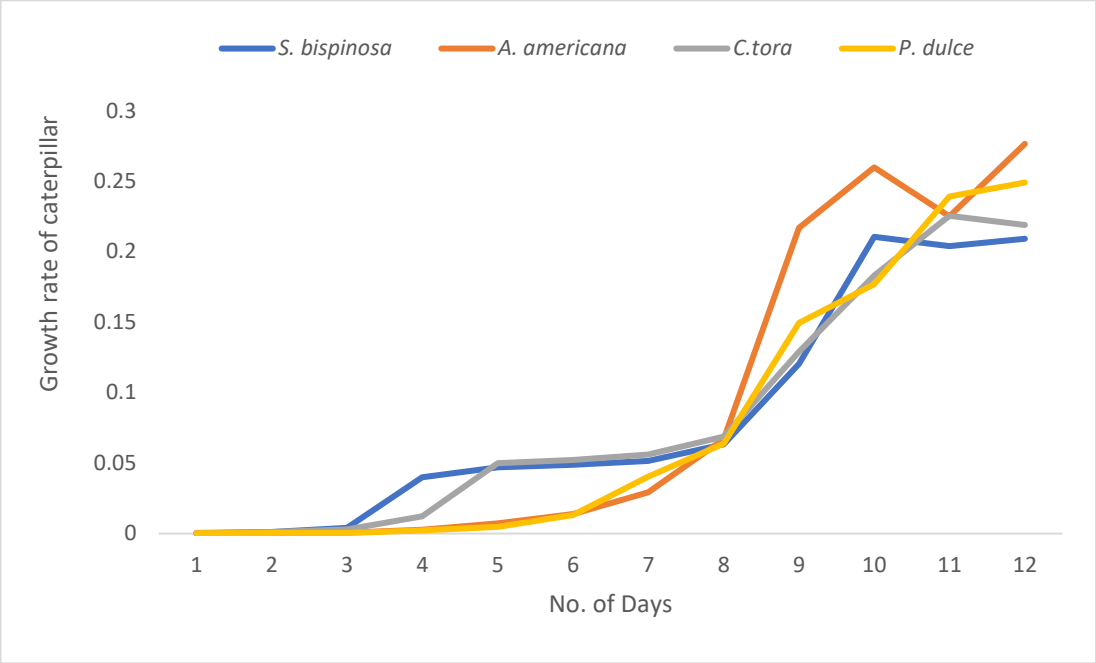


Fig: 4.2.5- In-vitro studies of caterpillar. Comparison of change in growth rate of caterpillars reared on *S.bispinosa, A.americana, C.tora and P.dulce* .Growth rate do not differ significantly in as per Kruskal wallis test as P>0.05.

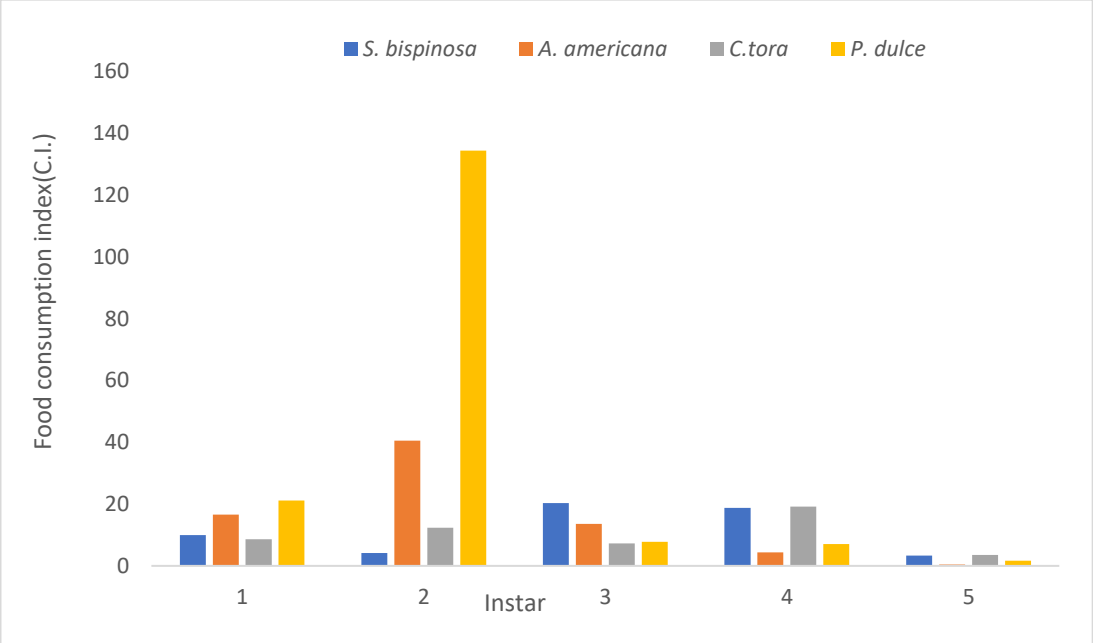


Fig 4.2.6-Food consumption index (C.I.) of invitro studies carried on caterpillar of *E. hecabe* reared on leaves of *S.bispinosa*, *A.americana*, *C.tora* and *P.dulce* .C.I. is non-significant as per Kruskal wallis test .

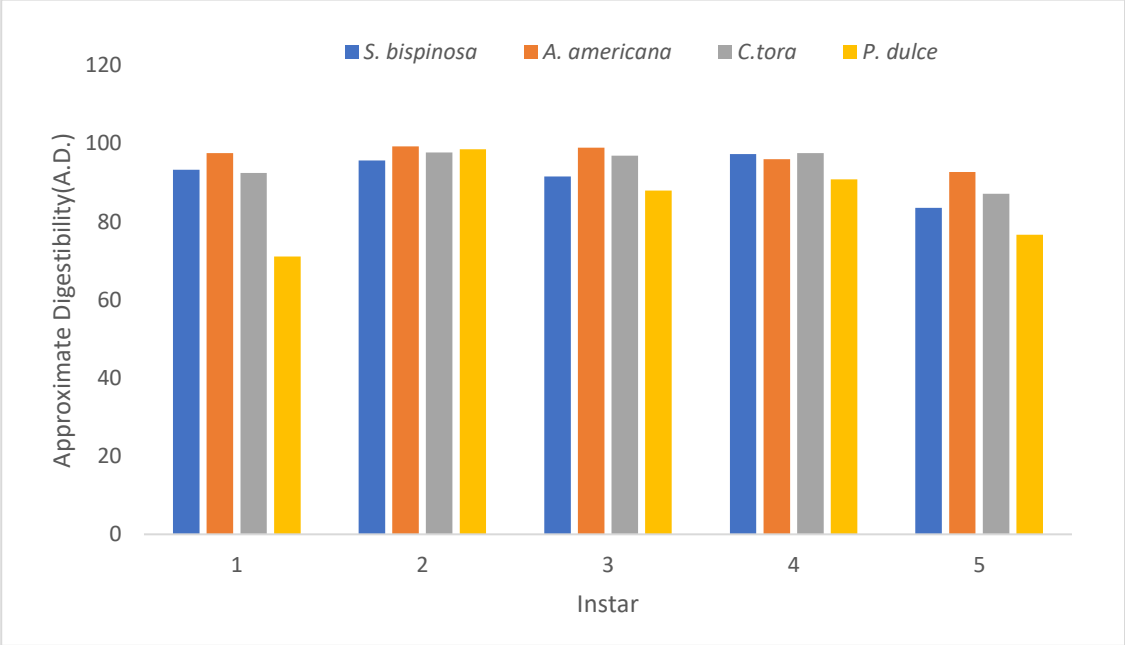


Fig 4.2.7- Approximate digestibility (A.D.) of invitro studies carried on caterpillar of *E. hecabe* reared on leaves of *S.bispinosa*, *A.americana*, *C.tora* and *P.dulce* . Compared A.D. of larval host plants is non-significant as per One-way Annova as P>0.05.

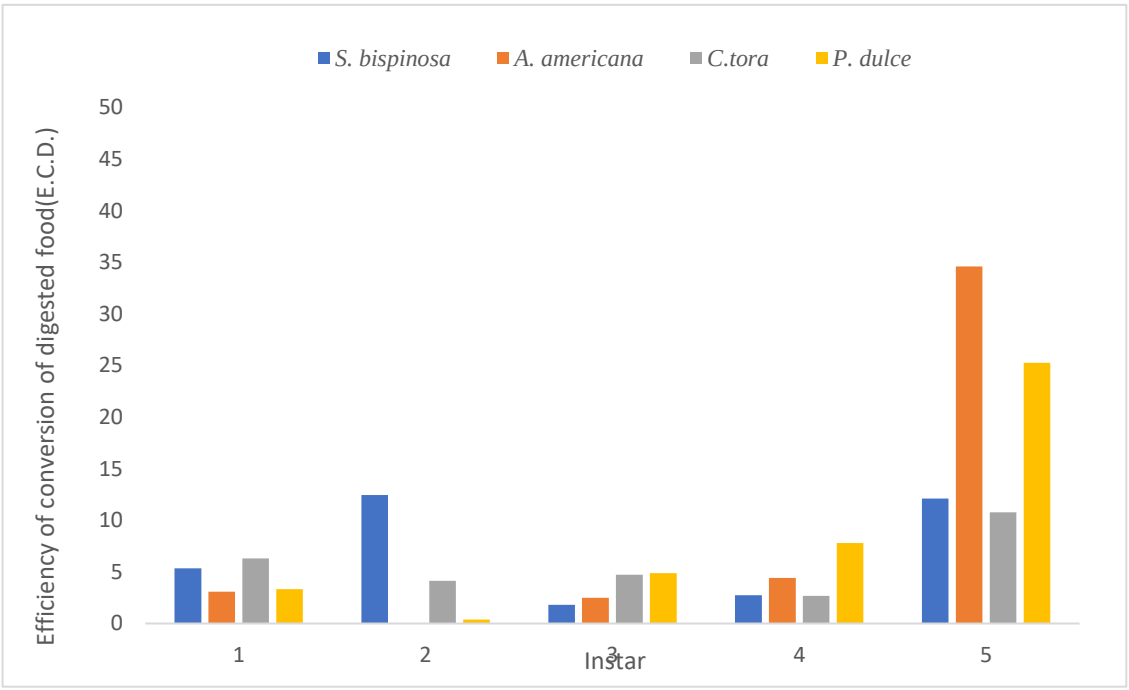


Fig 4.2.8- Efficiency of conversion of digested food (E.C. D) of invitro studies carried on caterpillar of *E. hecabe* reared on leaves of *S. bispinosa*, *A. americana*, *C. tora* and *P. dulce.* E.C.D. is non-significant as per Kruskal wallis test as P>0.05.

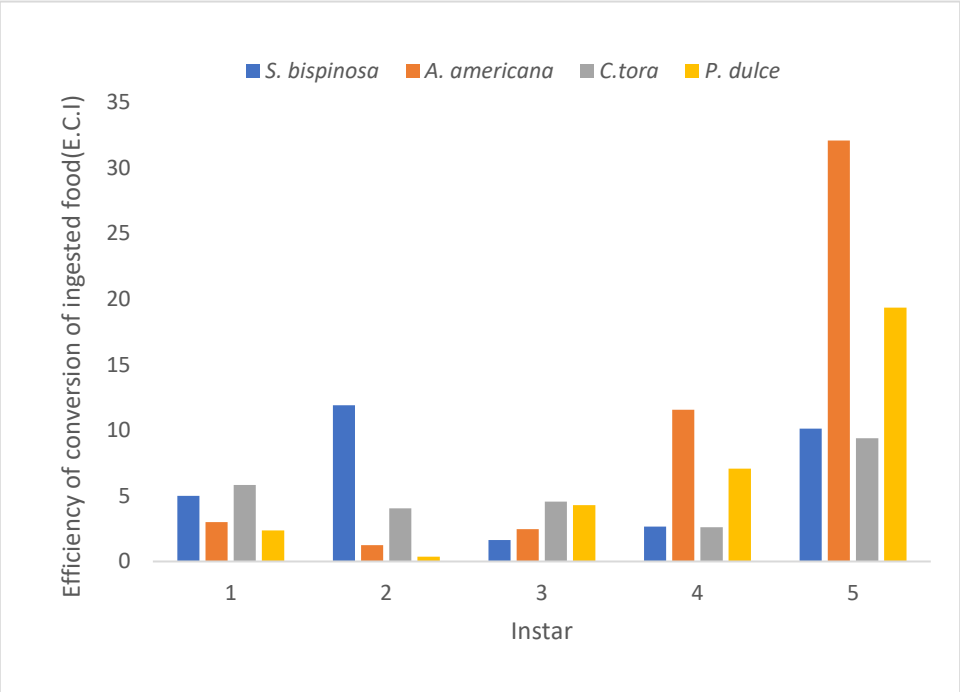


Fig 4.2.9- Efficiency of conversion of ingested food (E.C. I) of invitro studies carried on caterpillar of *Eurema hecabe* reared on leaves of *S. bispinosa*, *A. americana*, *C. tora* and *P. dulce.* E.C.I. is non-significant as peer Kruskal wallis test as P>0.05.

To understand effectiveness of feeding of caterpillars on Larval host plants, the caterpillars of *E. hecabe* were raised in campus premises provided suitable conditions and their morphological characters were observed such as length, weight, amount of leaves consumed and feces egested and larval performances in terms of food utilization indices were calculated on 4 different host plants such as *C. tora*, *S. bispinosa*, *A. americana* and *P. dulce* from 1st instar till it pupates. Whereas for other host plants such as *C. fistula*, *P. pterocarpum*, *C. pulcherimma* and *M. pudica,* caterpillars refused to feed.

The data of parameters measured such as length, weight of caterpillar, number of leaves consumed and excreted, growth rate are tabulated in tables 4.1.3- 4.1.6 and each of these parameters were compared with respect to all four LHP graphically in fig 4.1- 4.5.The data for efficiency of caterpillars to feed on its larval host plants is tabulated in table-4.1.7 to table-4.1.10 and each of these parameters were compared with respect to all four larval host plants and is graphically represented in figs 4.2.6 to 4.2.9.

4.2.4 Comparison of length, weight, LHP consumption and feces excreted

The length of caterpillar was steadily increasing on all 4 LHP with number of days in fig 4.2.1. Fluctuations in weight of caterpillars were observed in fig 4.2.2. Initially caterpillars reared on *S.bispinosa* and *A.americana* have similar weights but later weight increased more rapidly after day 6.Caterpillar weight on *C.tora* is stable until day 6,after which it experiences a noticeable increase .Weight of caterpillars reared on *P.dulce* exhibits a fluctuating pattern throughout the 12 days ,with a significant weight increase after day 10.

LHP consumption by caterpillars showed fluctuations in fig 4.2.3. For instance, caterpillars reared on *S.bispinosa* and *A.americana* initially have low LHP consumption rates but show significant increase in consumption after day 3 .Caterpillars exhibits a relatively steady consumption rate on *C.tora* throughout the observation period. Whereas on other hand consumption rates on *P.dulce* showed fluctuating consumption patterns ,with a notable decrease in consumption after day 8.

Graphically weight of feces excreted by each caterpillar species differed as shown in fig 4.2.4. Feces egested by caterpillars reared on *S.bispinosa* and *A.americana* initially showed stable feces weights, while on *S.bispinosa* feces weight showed slight increase after day 3.Feces weight after consumption of *C.tora* demonstrates a fluctuating pattern in feces weight throughout the observation period. However on *P.dulce* feces weight showed noticeable increase starting from day 8.

Variations observed in growth rates of caterpillar on different host plants are given in fig 4.2.5. Initially growth rates of caterpillars on *S.bispinosa and A.americana* were low . with a slight increase on *S.bispinosa* after day 3 and a more significant increase after day 6.Growth rates on *C.tora* exhibits a fluctuating pattern throughout the observation period, however demonstrates a steady increase in growth rate over the 12 days.

Graphically the length, weight, leaves consumption, feces egested and growth rate showed variations across no.of days but statistically all these parameters are non-significant as per One way annova and Kruskal wallis test.

4.2.5 Comparison of food utilization efficiency indices

The comparison of approximate food consumption index (C.I.) of caterpillar reared on 4 different host plants are shown in fig 4.2.6. Instar 1 shows lowest food consumption index on *C.tora* followed by *P.dulce* and *S.bispinosa* but shows high consumption index on *A.americana*. Instar 2 caterpillars on *A.americana* exhibits a significantly higher food consumption index compared to the others at this stage. But on *S.bispinosa* and *C.tora* caterpillars show moderate consumption , while on *P.dulce* consumption remains relatively low. Instar 3 caterpillars on *A. americana* maintains a high food consumption index, continuing to outpace the other species. On *S.bispinosa* and *C. tora* it showed moderate consumption, while on *S. bispinosa* slightly higher consumption index. Consumption index on *P. dulce* remains the lowest. Instar 4 caterpillars on *A. americanana* continues to lead in food consumption, followed by *S. bispinosa* and *C. tora* with relatively similar consumption levels. It remains at the bottom with the lowest consumption index on *P.dulce.* The trend continues with fifth instar caterpillars feeding on *A. americanana* having the highest food consumption index followed by *S. bispinosa* and *C. tora*, with S. bispinosa showing slightly higher consumption and *P. dulce* remains the least consuming species of LHP.

The comparison of approximate digestibility (A.D.) of caterpillar reared on 4 different host plants are shown in fig 4.2.7. Instar 1 caterpillars at this initial stage of development, exhibit higher digestibility on *S.bispinosa* and *C.tora* compared to *A. americana and P. dulce.* Instar 2 caterpillars on *A. americana* shows a significant rise in digestibility, surpassing other species. Instar 3 caterpillars digestibility continues to increase on all LHP species, with caterpillars on *A. americana* maintaining its lead in digestibility. *S. bispinosa* and *C. tora* also showed relatively high digestibility on *S. bispinosa* and *C. tora.* Instar 4 caterpillars digestibility remains high for *A. americana*, with *S. bispinosa* and *C. tora* following closely behind. On *P. dulce*, caterpillars exhibit lower digestibility compared to the other plant species. Instar 5 caterpillars maintains its high digestibility on

A. americana, while on *S. bispinosa* and *C. tora* exhibit a slight decrease. On *P. dulce,* caterpillars still show the lowest digestibility among the species.

Overall, caterpillars on *A. americana* consistently demonstrates higher digestibility across the instars, while *S. bispinosa* and *C. tora* also show relatively good digestibility, especially in the earlier stages of development. *P. dulce* consistently displays lower digestibility compared to the other species across all instars.

The comparison of Efficiency of Conversion of Digested food (E.C.D) of caterpillar reared on 4 different host plants are shown in fig 4.2.8. Instar 1 on *A.americana* exhibits the highest efficiency of conversion of digested food (E.C.D.), followed by *C. tora*, *S. bispinosa* and on *P. dulce* showed lower efficiency compared to the other two species. Instar 2 caterpillars maintained its lead on *A. americana* in E.C.D., while on *S. bispinosa* shows a notable improvement, surpassing *C. tora* and *P. dulce.* In case of Instar 3 efficiency of conversion of digested food continues to increase for all species. *A. americana* maintains its high E.C.D., while on *S bispinosa* it showed a slight decrease compared to Instar 2 but remains relatively high. It also exhibits increased efficiency compared to previous instars *C. tora* and *P. dulce.* Instar 4 caterpillars on *A. americana* still maintains the highest E.C.D., with *S.bispinosa* following closely behind. On *C. tora* and *P. dulce* showed comparable efficiency, although slightly lower than the other two species. Instar 5 caterpillars on *A. americana* demonstrates a substantial increase in E.C.D., reaching its peak efficiency among all instars and species. On *S. bispinosa* it maintains a relatively high E.C.D., while on *C.tora* and *P.dulce* show lower efficiency compared to the other species.

Overall, A. americana consistently exhibits the highest efficiency of conversion of digested food across all instars, followed by *S. bispinosa*. *C. tora* and *P. dulce* generally show lower efficiency compared to the other two species, with some variations across the instars.

The comparison of Efficiency of Conversion of Ingested food (E.C.I.) of caterpillar reared on 4 different host plants are shown in fig 4.2.9. Instar 1 caterpillars on *A. americana* displays the highest efficiency of conversion of ingested food (E.C.I.), followed by *S. bispinosa*. *C. tora* and *P. dulce* show lower efficiency compared to the other two species. Instar 2 on *A. americana* maintains its lead in E.C.I., while on *S. bispinosa* it showed a significant improvement, surpassing *C. tora* and *P. dulce.* Instar 3 caterpillars' efficiency of conversion of ingested food continues to increase for all species and maintains its high E.C.I *on A. americana*., while *S. bispinosa* shows a slight decrease compared to Instar 2 but remains relatively high. On *C. tora* and *P. dulce* also exhibit increased efficiency compared to previous instars. Instar 4 caterpillars on *A. americana* still maintains the highest E.C.I., with *S. bispinosa* following closely behind. *C. tora* and *P. dulce* showed comparable efficiency, although slightly lower than the other two species. Instar 5 caterpillars on *A. americana* demonstrates a substantial increase in E.C.I., reaching its peak efficiency among all instars and species. On *S. bispinosa* it maintains a relatively high E.C.I., while *C. tora* and *P. dulce* show lower efficiency compared to the other species.

Overall, caterpillars on *A.americana* consistently exhibits the highest efficiency of conversion of ingested food across all instars, while *S.bispinosa* generally follows closely behind. *C. tora* and *P. dulce* show lower efficiency compared to the other two species, with some fluctuations across the instars.

Graphically C.I., A.D., E.C.D, E.C.I showed variations but statistically these are non-significant as per Kruskal wallis test and One-way Annova.

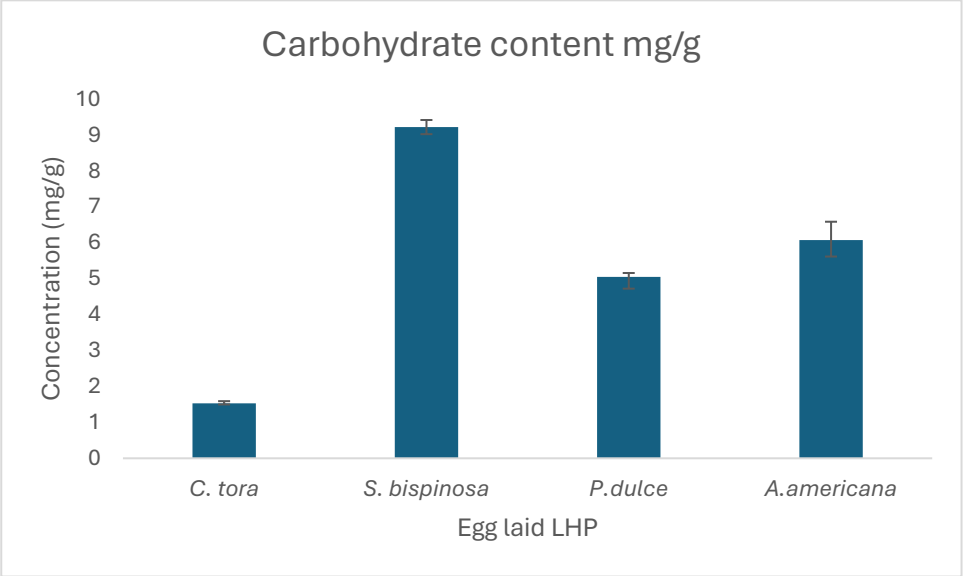


Fig 4.2.10- Carbohydrate content of egg laid larval host plants.

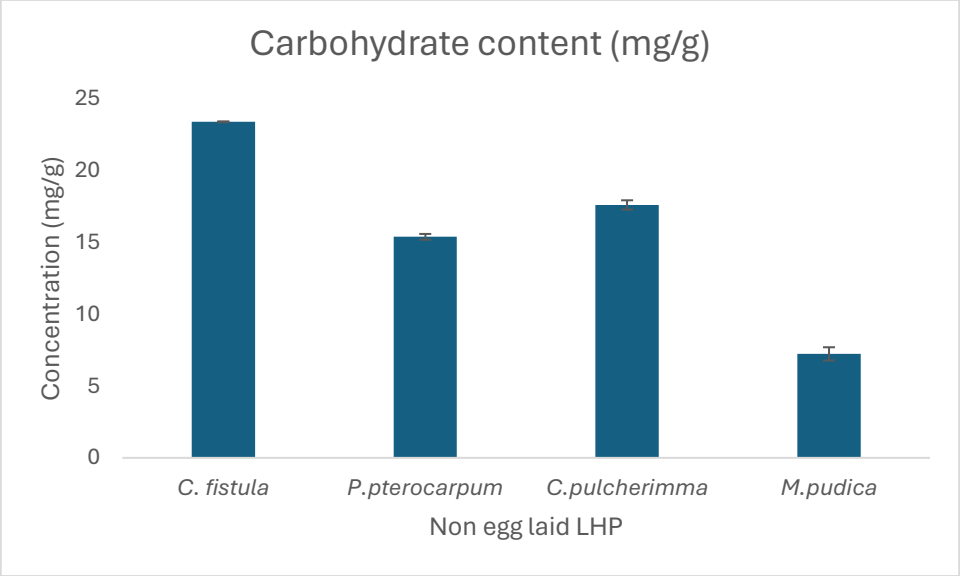


Fig 4.2.11-Carbohydrate content of non- egg laid larval host plants.

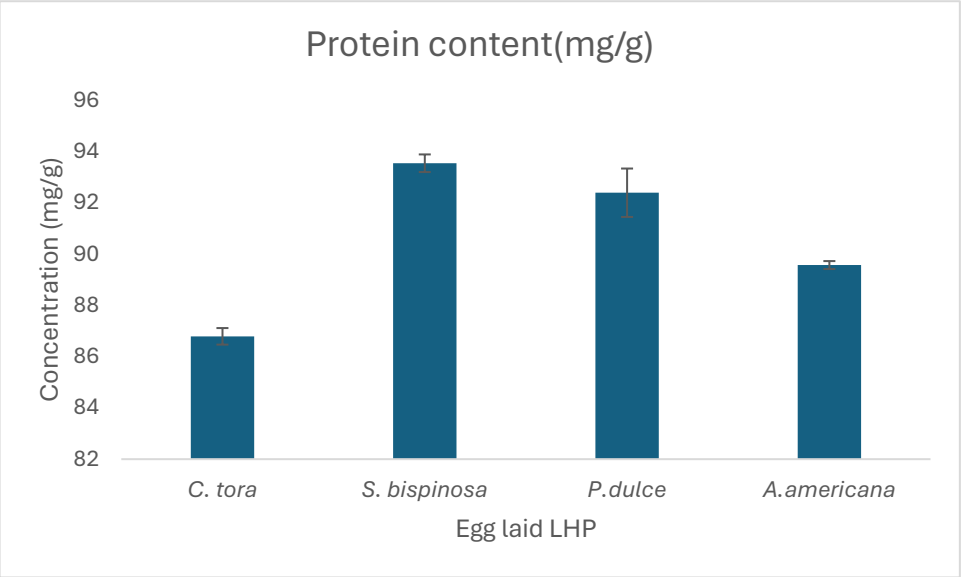


Fig 4.2.12-Protein content of egg-laid LHP.

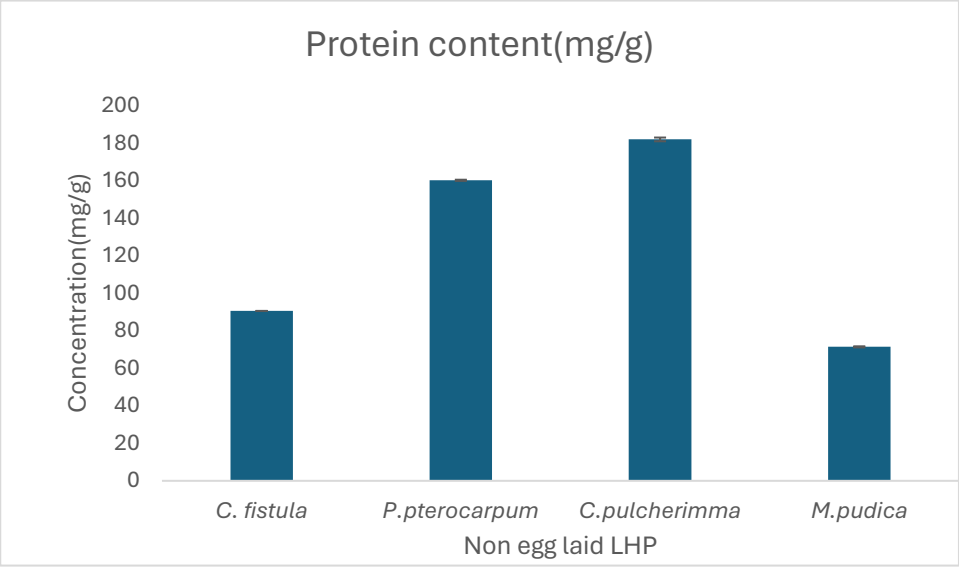


Fig 4.2.13-Protein content of non-egg laid LHP.

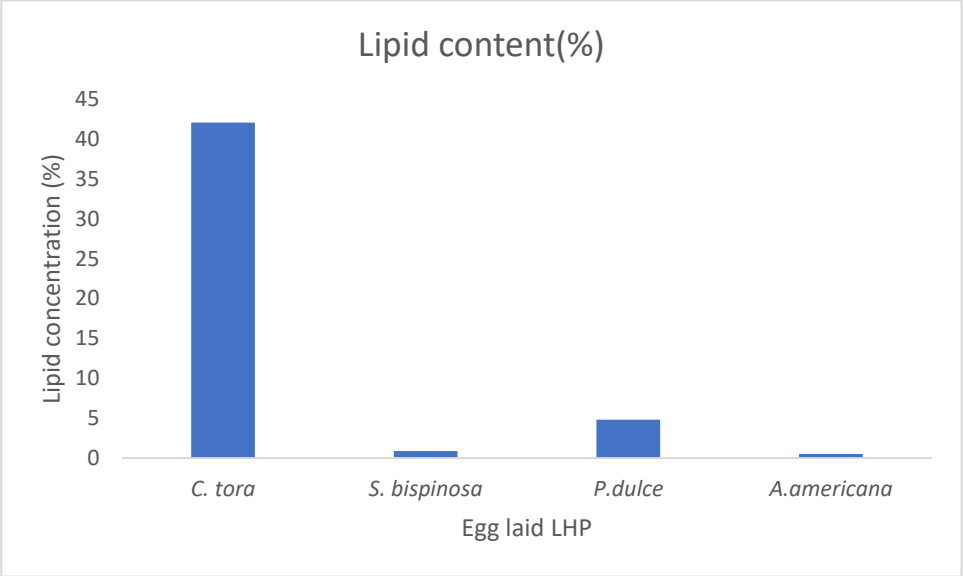


Fig 4.2.14-Lipid content of egg laid larval host plants.

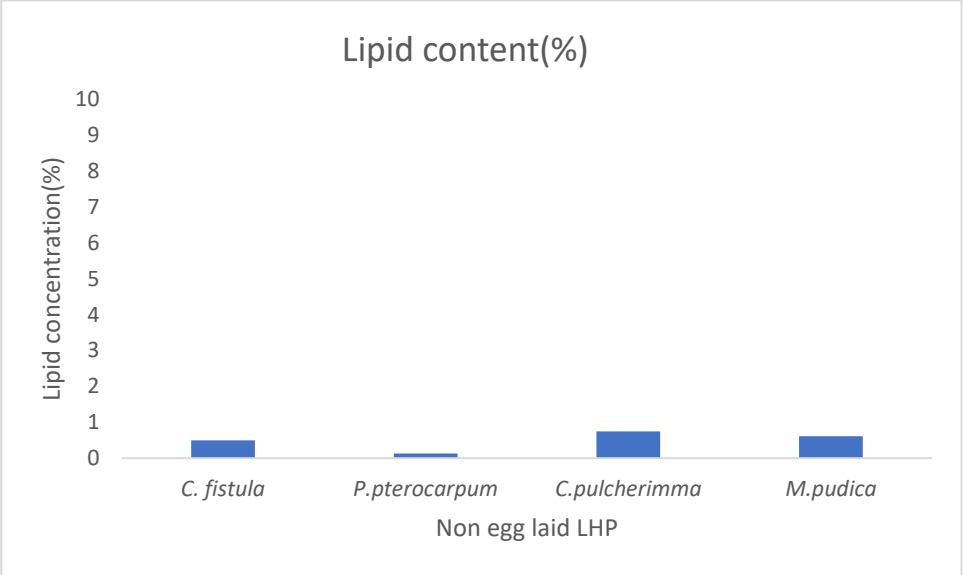


Fig 4.2.15-Lipid content of non-egg laid larval host plants.

4.2.6. Comparison of nutritional content of host plants

Nutritional content w.r.t macronutrients of egg laid LHP and non-egg laid LHP are given in table 4.1.11- 4.1.12. Comparing both the graphs fig 4.2.10 and fig 4.2.11 carbohydrate content is highest in 4 of Non egg laid LHP such as *C.pulcherimaa*, *P.pterocarpum*, *C.fisula* and *M.pudica* followed by egg laid LHP such as *S.bispinosa*, *P.dulce*, *A.americana* and *C.tora*. Larval host plant containing less carbohydrate content is prefered by *Eurema hecabe* for oviposition.

Graphically protein content is highest in *C.pulcherimma* followed by *P.pterocarpum, S.bispinosa, P.dulce, C .fistula, A.americana, Cassia tora* and *Mimosa pudica* as shown in fig.4.2.12 and fig 4.2.13. So protein doesnot affect ovposition response towards host plant as there is no uniform distribution across Egg laid LHP.

Comparison of lipid content across two graphs fig 4.2.14 -fig 4.2.15. Comparing the two graphs it seems that lipid concentration in the second graph is generally lower compared to those in first graph. It seems that larval host plants containing more lipid content is preferred by *Eurema hecabe* for oviposition.

4.2.7 Spot development using TLC to check presence of D-pinitol.

Brownish colour spot development was observed indicating presence of pinitol in all LHP. Also, the observed Rf value from table for standard was compared with that of pinitol standard from literature. Also, the values obtained of samples-1,2,3,4,5,6,7,8 are close in range with obtained standard value as shown in table 4.1.13.

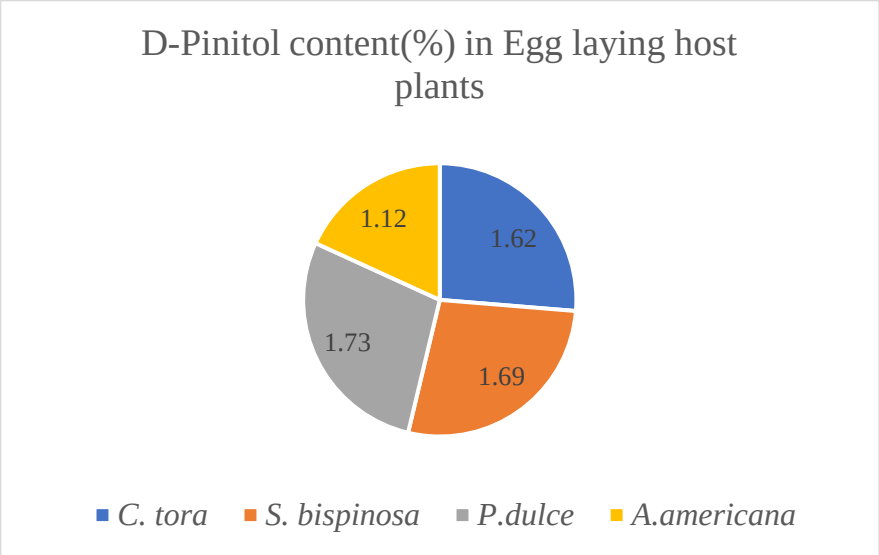


Fig 4.2.16-D-pinitol content in egg laid larval host plants.

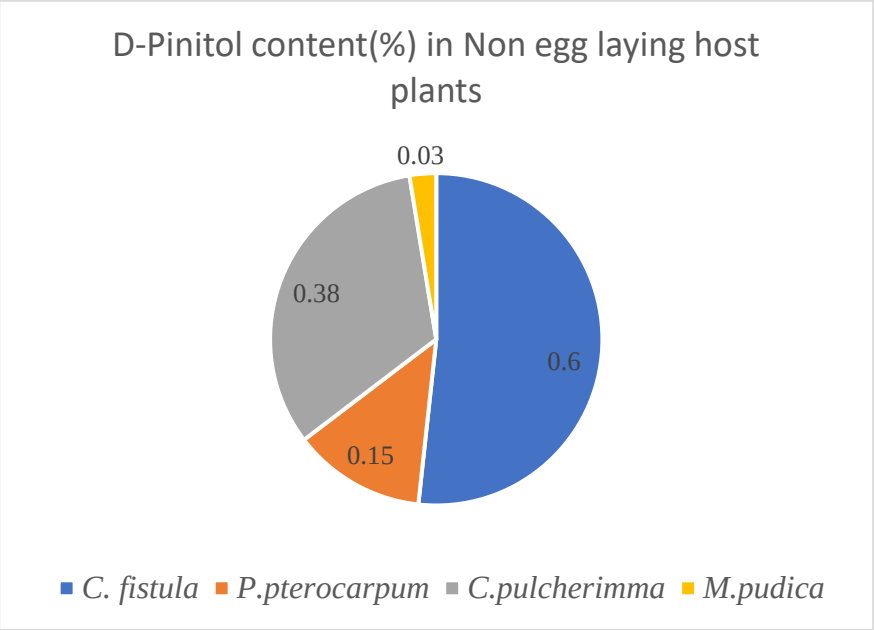


Fig 4.2.17- D-pinitol content in non-egg laid larval host plants.

4.2.8 Comparison of Pinitol Concentration across LHP

Pinitol concentration of Egg laid LHP and non-egg laid LHP are given in table 4.1.14 and 4.1.15. Comparison of pinitol content of egg laid LHP and non-egg laid LHP showed in fig 4.2.16-fig 4.2.17. D-Pinitol content is more in Egg laid LHP compared to Non egg laid LHP. It indicates that larval host plants containing D-pinitol content > 1% is preferred by *E. hecabe* for egg deposition as D-pinitol is refered to as oviposition stimulant for Common grass yellow (*E.hecabe*).

4.3 Discussion

It is a difficult effort for female butterflies to find and select a favorable spot for ovipositing, and the success of their progeny depends on that preference. A female butterfly's oviposition is indicated by its downwardly curled abdomen touching a leaf. One of the last stages of insect reproduction is called oviposition behavior; it entails the developed egg being deposited outside the female's body and involves several behavioral and physiological processes. (Janz, 2002). Similar behavior is observed in Common grass yellow (*Eurema hecabe*)

Out of the eighteen host plants that have been recorded for Common Grass yellow (*E hecabe*), only eight plants were found on the campus of Goa University, according to the study. The location of these host plants was aided by an initial survey. In nature, *E. hecabe* is polyphagous, consuming host plants that belong to the Fabaceae family. While most butterfly caterpillars have particular feeding preferences, the main objective when selecting an oviposition site is to optimize progeny survival with the least amount of energy expended during ovipositioning. Monarchs are the only insects that lay eggs on milkweed. Eggs are laid by black swallowtails on any plant belonging to the carrot family, including dill, parsley, and fennel, but not on other plants. A caterpillar cannot thrive on any other plant after it has consumed its first meal (Bartelett and Peterson, 1998). There may be many opportunities to expand the regional lists of food plants as a result of the research of geographic diversity in host plant use by polyphagous and oligophagous species (Tolman & Lewington, 1997).

The optimal oviposition theory, often known as the "oviposition preference-offspring performance hypothesis," is the subject of much of the study on oviposition site selection, either directly or indirectly. According to this theory, an adult female will choose hosts that enhance larval performance because doing so will increase her own fitness (Mayhew, 1997). Similar behavior is displayed by the *E. hecabe*, which limits its host plant preference to leguminous plants even though its caterpillars are polyphagous by nature. It is necessary to balance the preferences of females and the performance of larvae based on the specialization of each host plant.

C.tora, *S.bispinosa, P.dulce, A.americana, P.pterocarpum, C.fistula, M.pudica, C.pulcherimma* are reported as larval host plants of *E. hecabe* (Nitin *et al*.,2018).But larvae of *E.hecabe* refused consumption of *P.pterocarpum*, *C.fistula*, *M. pudica* and *C.pulcherimma* as well as no egg deposition was recorded.

It's possible that female choice differs from plant characteristics that promote the best larval success on hosts, or that there are additional factors influencing female choice. Not all plants provide a perfect match between the preferences of the females and those that guarantee the best possible larval performance (Forister, 2004). The choice of oviposition site should be associated with an individual's success because of its relationship to the "trade-off" between the fitness of three lifecycle stages: adult, ovum, and larva. It would be beneficial for females to conduct thorough research and select a host that has the best qualities for the development of larvae. In the current study, the Eurema hecabe is thought to be selecting *Cassia tora, Sesbania bispinosa*, *Pithecellobium dulce*, and *Aeschynomene americana* as its substrate. The utilization of this substrate for ovipositioning by adult females led to shift in host plant preference based on LHP availability.

The female must track signals at ever smaller physical scales, including environment, microhabitat, plant, and plant component, in order to perform oviposition. According to Janz (2002), the usual order would be search, encounter and orientation, assessment, and acceptance or rejection. The geographical scale at which the butterfly acts, the behavior of the species, the physical characteristics of the habitat, and the plants utilized all influence the ovipositioning sequence. The choices are primarily binary, requiring consideration of at least chemical or visual signals at each stage of the procedure (Dennis *et al*., 2006). When choosing a host plant after alighting, butterflies typically evaluate the form, nutrient content, and oviposition stimulant of the leaves. Getting closer or making physical contact is frequently necessary to gain more host approval. When they get close to a possible host, they change from a swift and focused flying style to a practically immobile hovering-like flight.

looking for volatile substances in the air before landing. Rejection or acceptance is based on one's attitude toward surface allelochemicals. Host selection may be better explained by loyalty to secondary plant substances than by plant taxonomy (Haribal & Feeny, 2003). Since *E. hecabe* may respond to many allochemicals, the key chemical signals, such as chemosensors and allelochemicals, that control the ovipositioning sequences are not well understood. According to a recent study conducted in Japan on *Eurema hecabe mandarina*, D-pinitol in the fabaceae family may stimulate the oviposition of common grass yellow (Mukae *et al*., 2016). To relate this to the current investigation, a detailed calculation of D-pinitol in each host plant used by *Eurema hecabe* larvae was analysed.

Species that lay their eggs on very nutrient-rich substrates, typically have eggs that develop quickly and larvae that begin feeding as soon as they hatch. When non-edible substrates are used for egg laying, the larvae either grow more slowly or the substrate acts as a support for longer egg development (Wiklund, 1984; Atluri *et al*., 2010). The factors determining growth over a given period of development are the amount and type of food consumed and the efficiency with which it is utilised (Browne and Raubenheimer,2003). The E.C.I and E.C.D. are important parameters of nutritional responses of an insects (Parra *et al.*2012).When ECI increase CI decrease or vice versa(Slansky and Scriber,1985).The observed values of the Approximate digestibility (A.D.), the efficiency of conversion of ingested food (E.C.I.), and the efficiency of conversion of digested food (E.C.D.) into the body substance on *S. bispinosa*, *C. tora*, *P. dulce and A. americana,* indicate that the larval food also appears to be highly nutritious. There was no uniform increase or decrease observed in those parameters. Comparison of parameters including growth rate, weight, leaves consumed, excreted feces, and efficiency of food utilization, such as the consumption index (C.I.), approximate digestibility (A.D.), efficiency of digested food (E.C.D.), and efficiency of ingested food (E.C.I.), are not significant in the current study because $P>0.005$ as per One-way Annova and Kruskal wallis test.

Large hosts offer excellent passive protection for juveniles and can act as a buffer against larval completeness. Host size and food availability are frequently associated (Wikilund, 1984). The absorption efficiency is influenced by the chemistry of the leaf, namely its water content (Pandian TJ & Marian P., 1986). The aforementioned research highlights the significance of the physical and chemical properties of the leaves of the larval host plants in serving as prospective caterpillar food. In the current study, it was discovered that the *E. hecabe* prefers larval host plants with high lipid and low carbohydrate contents for ovipositioning. This might be to enable them to fuel their rapid growth and metamorphosis. This finding highlights the knowledge that female butterflies require the nutrients found in leaves to support their caterpillars growth.

Butterfly phenology varies, indicating that various species have distinct life histories and react differently to the seasonality of their surroundings. As noted by Jones and Rienks, even distinct species within a genus might exhibit dissimilar behaviors (1987). Temperature and humidity are examples of microclimatic factors that may affect the survival and development of eggs. Thus, cotton soaked in water was kept within the rearing container for egg hatching and caterpillar growth in the current study in order to preserve moisture in in-vitro circumstances.

Wynter & Blyth (1957) determined that for butterfly reproduction activity, spring was the most suitable season for the majority of India, with post-monsoon and Southwest monsoon following closely behind. However, the current study indicates that oviposition on larval host plants is not influenced by the season. *Eurema hecabe* oviposition has also been documented throughout the dry season, monsoon, and early summer. According to the study, oviposition depends on the host plant's availability.

Changes in land use have expanded the "essential" niche condition required for oviposition. Human-driven landscape alteration can affect species distribution ranges or abundances via spatial or temporal (e.g., phonological) effects on habitat features involved in oviposition behavior in at least some instances (Fartmann & Poniatowski, 2003). There hasn't been much discussion of the connection between oviposition behavior and habitat fragmentation. Not surprisingly, in the highly dispersive ecosystem, connectivity was connected with egg densities (Rabasa et al., 2005). Similar plateau degradation was seen throughout the research period when plant patches were cleared for infrastructure development on the Goa university campus. This resulted in a decrease in the availability of host plants, which in turn caused Common grass yellow to shift to other host plants like *P.dulce* for egg deposition.

The female must track signals at ever smaller physical scales, including environment, microhabitat, plant, and plant component, in order to perform oviposition. According to Janz (2002), the usual order would be search, encounter and orientation, assessment, and acceptance or rejection. The geographical scale at which the butterfly acts, the behavior of the species, the physical characteristics of the habitat, and the plants utilized all influence the ovipositioning sequence. The choices are primarily binary, requiring consideration of at least chemical or visual signals at each stage of the procedure (Dennis *et al*., 2006).

According to a study conducted in Japan on *Eurema hecabe mandarina*, D-pinitol in the fabaceae family may stimulate the oviposition of common grass yellow on the leaves of *A. julibrissin* and *L. cuneata*, *E. Mandarina* females respond to D-pinitol as a contact chemical cue for oviposition. D-pinitol alone induced female oviposition at concentrations >0.1 %. D-Pinitol is widely distributed in fabaceous foliages, including *A. julibrissin* and *L. cuneata* (Mukae *et al*., 2016). Although particular fabaceous plants produce small concentrations of inositol together with D-pinitol (Negishi *et al*. 2015; Phillips et al.1984), *E. mandarina* females specifically show response towards D-pinitol. Therefore, D-pinitol likely plays a role in triggering Oviposition by *E. mandarina* on various fabaceous host

plants. An observation was that female responses to authentic D-pinitol was reduced at concentrations >0.5 %. These observations suggest that fabaceous plants with high concentrations of D-pinitol may not be oviposited on by Female *E. mandarina*.

Interestingly, in *E. mandarina*, female butterfly oviposition preference for D-pinitol tested in this Study is similar to that of larval preferences for feeding (Numata *et al*., 1985), suggesting that larvae and adults have a common sensory track to detect D-pinitol in host plants. Investigating the physiological and ecological affinity to cyclitols would be helpful in understanding the diversification and host plant switching within these lineages. Despite the omnipresence of D-pinitol in Fabaceae, *E. mandarina* and *C. erate* have considerably different host ranges; (Honda *et al*. 1997, 2012). Accordingly, several plant compounds, including D-pinitol, probably regulate host range and oviposition preferences of *E. hecabe* females. Further studies need to identify other plant components that serve as oviposition stimulants or deterrents for this butterfly. So in the present study oviposition of *E.hecabe* was seen on host plant containing D-pinitol concentration >1% and LHP containing below this amount *E.hecabe* refused to deposit eggs on the LHP.

4.4 Conclusion

The exploration of insect ecology, especially butterflies, has always intrigued humans because of their dynamic beauty and behavior. While study of individual species and their response to the microenvironment specially in terms of oviposition is one of the less explored fields in the ecology. Through this project "Influence of nutrition and oviposition stimulant of Host plant on Common grass yellow (*Eurema hecabe)* ". An attempt was made to study the availability of Common grass yellow, larval host plants and its preference for oviposition, growth indices of caterpillars and factors which influence the larval host plant selection in *Eurema hecabe* in a selected microhabitat (study site – Goa University Campus).

The study shows that out of 18 larval host plants reported by Indian foundation for butterflies only eight are prominently been growing in study area of which only four were been extensively used for oviposition by *E. hecabe* namely *C. tora*, *S. bispinosa, P. dulce and M. pudica*. The butterfly population was found to be highest from June to October and was found positively showing egg deposition on all four LHP whereas *E. hecabe* uses *P. dulce* evenly throughout the study period. In-vitro studies of caterpillars indicates that there is no variation in size, weight, LHP consumed, feces egested, growth rate, consumption index, approximate digestibility, Efficiency of conversion of digested food and efficiency of conversion of digested food a $P>0.05$ which indicates non-significant as per One-way Annova and Kruskal Wallis test.

The unavailability of all larval host plants of caterpillars with polyphagus nature in a micro habitat providing ultimate natural environment for in-vitro studies, lack of some chemicals and the instruments in the laboratory were found to be few of the limitations to the present studies.

From the present study we conclude that the process of ovipositioning by *E. hecabe* is influenced by multiple factors such as availability of larval host plant, quality of larval host plant based on its

nutritional value and oviposition stimulant (D-pinitol) available in micro habitat. The larval host plant selection by adult female *E. hecabe* butterfly follows trade of hypothesis and null hypothesis. The present research concludes that the population of Common grass yellow (*Eurema hecabe*) varies with host plant availability affecting ovipositioning. Nutritional status of host plant and oviposition stimulant influence oviposition, Alternate hypothesis accepted and also the statement claiming growth indices varies with different host plant, null hypothesis accepted.

The above conclusion Is based on the observation made for nine months (June 2023 to March 2024) in a small micro habitat and efforts are just a glimpse considering vastness and presence of multiple layers to the topic and furthermore research is essential for understanding and concluding better interpretation. Few of the future scope of the topic are working in different microhabitat with different environment and vegetation can be done. The larval host plants selection by both adult female butterflies as well as by its caterpillar in in-vitro condition and natural environment also needs a special attention. Also, caterpillar shift from one LHP to another needs to study. Other Chemoreceptors and Allelochemical importance and their roll in larval host plant selection need to be also investigated.

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