CYTOGENETIC AND PHYTOCHEMICAL ASPECTS OF SOME SELECTED SPECIES OF *AMOMUM* ROXB. (ZINGIBERACEAE)

Thesis
submitted to the University of Calicut
for the award of the degree of

DOCTOR OF PHILOSOPHY IN BOTANY

By

SINITHA K.

CELL & MOLECULAR BIOLOGY DIVISION
DEPARTMENT OF BOTANY
UNIVERSITY OF CALICUT
KERALA – 673 635, INDIA
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DECLARATION

I, Sinitha K., hereby declare that the thesis entitled **"Cytogenetic and phytochemical aspects of some selected species of *Amomum* Roxb. (Zingiberaceae)"** submitted to the University of Calicut, for the award of the degree of DOCTOR OF PHILOSOPHY IN BOTANY is a record of original research work done by me under the supervision and guidance of Dr John E. Thoppil, Professor, Department of Botany, University of Calicut and that it has not formed the basis for the award of any degree/diploma to any candidate of any University.

Date: **Sinitha K**

UNIVERSITY OF CALICUT
DEPARTMENT OF BOTANY
CALICUT UNIVERSITY (P.O.) - 673635,
KERALA, INDIA

Dr JOHN E. THOPPIL
Professor
Cell & Molecular Biology Division

Date:.....

CERTIFICATE

This is to certify that the thesis entitled **"Cytogenetic and phytochemical aspects of some selected species of *Amomum* Roxb. (Zingiberaceae)"** submitted to the University of Calicut, for the award of the degree of DOCTOR OF PHILOSOPHY IN BOTANY is an authentic record of original research work done by **Sinitha K.** during the period of study (2012-2017) at the Cell and Molecular Biology Division, Department of Botany, University of Calicut under my supervision and guidance and that it has not formed the basis for award of any degree or diploma. Also certified that the contents in the thesis is subjected to plagiarism check using the software URKUND, and that no text or data is reproduced from others work.

Dr JOHN E. THOPPIL
Supervising Teacher

CONTENTS

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Sinitha K.

ABBREVIATIONS

BHA	:	Butylated Hydroxy Anisole
BHT	:	Butylated Hydroxy Toluene
BSA	:	Bovine Serum Albumin
CA	:	Chromosome Aberrations
CFP	:	Cyan Fluorescent Protein
COX-2	:	Cyclooxygenase-2
DLA	:	Dalton Lymphoma Ascites cell lines
DMEM	:	Dulbecco's Modified Eagle's Medium
DMSO	:	Dimethyl Sulphoxide
DPPH	:	1,1-Diphenyl-2-Picrylhydrazyl
DW	:	Dry Weight
EAC	:	Ehrlich Ascites Carcinoma cell line
EDTA	:	Ethylene Diamine Tetra Acetate
ELISA	:	Enzyme Linked Immunosorbent Assay
ESI	:	Electron Spray Ionization
FBS	:	Fetal Bovine Serum
FRAP	:	Ferric ion Reducing Antioxidant Power
FRET	:	Fluorescent Resonance Energy Transfer
GC/MS	:	Gas Chromatography/Mass Spectrometry
GSH	:	Glutathione
H_2O_2	:	Hydrogen Peroxide
HEPES	:	4-(2-hydroxyethyl)-1-piperazine ethane sulfonic acid
HPLC	:	High Performance Liquid Chromatography
HR-LC/MS	:	High Resolution-Liquid Chromatography/ Mass Spectrometry
HT 29	:	Human colorectal adenocarcinoma cell line

HTAB	:	Hexadecyl Trimethyl Ammonium Bromide
IC_{50}	:	Inhibition Concentration 50%
IL	:	Interleukins
iNOS	:	inducible Nitric Oxide Synthase
LOX	:	Lipoxygenase
LPS	:	Lipopolysaccharide
µg/mL	:	microgram/milliliter
µM	:	micro Molar
mg/kg	:	milligram/kilogram
MI	:	Mitotic Index
min	:	minute
mM	:	milli Molar
MPO	:	Myeloperoxidase
MTT	:	3-(4,5-Dimethylthiazol-2-yl)-2,5-Diphenyltetrazolium Bromide
NBT	:	Nitro Blue Tetrazolium
NCCS	:	National Centre for Cell Sciences
NF-κB cells	:	Nuclear Factor Kappa light chain enhancer of activated B
NIST	:	National Institute of Standards and Technology
NO	:	Nitric oxide
NOS	:	Nitric Oxide Synthase
NSAIDs	:	Non Steroidal Anti-inflammatory Drugs
OD	:	Optical Density
PBS	:	Phosphate Buffered Saline
PG	:	Prostaglandins
RAW 264.7	:	Mouse leukaemic monocyte macrophage cell line
ROS	:	Reactive Oxygen Species

RPMI	:	Rosewell Park Memorial Institute Medium
RT	:	Retention Time
SCAT	:	Sensor for activated caspases based on FRET
SDS	:	Sodium Dodecyl Sulphate
SE	:	Standard Error
SOD	:	Superoxide Dismutase
TAE	:	Tris Acetate EDTA
TBA	:	Thio Barbituric Acid
TBARS	:	Thio Barbituric Acid Reactive Substances
TNF	:	Tumor Necrosis Factor
TpTz	:	2,4,6,-tripyridyl-s-triazine
v/v	:	volume per volume
w/v	:	weight per volume
WHO	:	World Health Organization
YFP	:	Yellow Fluorescent Protein

CHAPTER I
GENERAL INTRODUCTION

The greatest share towards the economy of India was reliant on agriculture right from the prehistoric days itself. As per 2001 evaluation, more than 56.6% of fundamental labourers in India are engaged in farming and related works. Green revolution which started in 1960s ensured critical increment in the generation of food crops. Consequently, the significance of agriculture in Indian economy and society can never be denied.

1.1. Need for enhancing crop yield

The population in the world has been increasing every year in an uncontrolled manner and it is relied upon to achieve approximately 9.2 billion by the year 2050with an approximate increase of more than 40% (Balk *et al*., 2006). The yearly increase in human populace is (2.5-3%) in many developing countries that is more than that of the yearly rise in the food production. In this way, to address the issues of the increasing human population, it will be important to produce more food in the following years than it has since the onset of agricultural production roughly 10, 000years back.

In addition to the expansion in the population, there are numerous reasons for the loss in crop productivity; insect pest attack is one of the major problem to agricultural productivity in India. Which significantly undermines the attempt to expand production of food with the rising demand. Each year, around 20-25% of the yield is lost because of pest attack. This issue is more intensed in tropics and subtropics, where the atmosphere gives a totally favorable condition for the survival as well as for the multiplication and rearing of an extensive variety of insects.

During the most recent few decades, significant advance has been made in expanding crop productivity around the world. The increase in food production can be accomplished by expanding the cultivation area, crop yield as well as decreasing the product loss due to pests and pathogens. However, there is no expansion in the area of the agrarian land and infact, because of different human activities, it is getting reduced. Consequently, the enhancement in food production could be accomplished just in the accessible agricultural land by producing crop varieties with high yield and stability. In this manner, so as to counteract the perpetually increasing population, crop protection plays a key and essential part in the present day farming practices to limit losses and subsequently the farmers are trying to introduce the harvest variety with high yield and stability to improve the crop production.

1.2. Crop damage by the insect pests with special reference to *Spodoptera litura*

Arthropods are the most far reaching and diverse group of animals with an expected 4–6 million species around the world (Novotny *et al.,* 2002). While just a little part of arthropods are included in insect species, they cause great losses to crops in the world yearly harvest. The losses are much more huge in food crops, and in addition to direct losses brought by insects, there are extra expenses in the utilization for pest control. Besides from the harm brought about by feeding, insects cause extra yield losses by infecting crops with disease causing pathogens. Phytophagous (plant-eating) insects and mite pests are more dangerous to food production for human utilization and the larval types of lepidopteran insects are viewed as the most destructive living beings (Nicholson, 2007).

Spodoptera litura, the common cutworm, (otherwise called as tobacco cutworm, cotton leaf worm, tomato caterpillar, bean armyworm, cluster caterpillar, taro cutworm, *Prodenia* and so forth) is an economically important

and polyphagous agricultural pest dispersed all over Asia, Australia and Pacific Islands. It has an immense host range of more than 120plants (belonging to 44 families) around the world and 60plants in India, including crops like cotton, rice, maize, groundnut, soyabean, jowar and vegetables (tomato, potato and so forth), ornamental plants, aromatic and medicinal plants (Gothama *et al*., 1995). Generally it nourishes on tobacco, castor, cauliflower, groundnut, tomato, potato, cabbage and other cruciferous harvests (Qin *et al*., 2004). Formerly, it was perceived as a sporadic pest, later, it has been turned out to be a major pest of many harvest crops bringing about economic loss in many parts of India especially the state of Punjab All the larval stages incure extensive damage by decreasing the yield and nature of products. It may pass through 5-6 overlapping generations every year (Singh *et al*., 1999). If had it not been controlled conveniently, it would have caused a large crop losses in different parts of India. Its attack causes around 10-40% yield loss of harvests on which they feed. For this pest, the vital control measures used in the field include chemical insecticides and bio-control agents. Excessive and repeated use of synthetic insecticides against *S. litura* have led to the generation of imperviousness towards vast majority of the commercially available insecticides such as organophosphates, organochlorines, carbamates and synthetic pyrethroids (Kranthi *et al*., 2002).

1.3. Insect-plant interaction

Of the millions of insect species, approximately one half is plant sustaining forms. But, not any single plant species is susceptible to invasion by all phytophagous insect species and likewise, no single insect is fit for using all plant hosts. However an exceptionally wide range of host- plant specificities are evident. A host plant is here characterized as one on which the insect finishes its development and growth. The way that these two living systems (plant and insect) have evolved and existed together more than

several millions of years, they distinctly exhibit their elaborate association and interaction.

A comprehension of insect- plant interaction requires a fundamental learning of the development of insects and plants and the elements that promote the feeding of insects and also the defense systems of plants. This co-operation is a dynamic one, subjected to endless variations and changes. For instance, environmental conditions affect the interaction system prominently. Biological relationships between host plants and insects are studied previously by (Painter, 1936) and it has been reported that different species and assortments of host plants, different conditions and so on, may impose enormous impacts upon the rate of growth, development and life span of the insects sustaining upon them. So far, very less review has been made of the components in the plant that bring about these impacts i.e., regardless of whether they are of varieties in the general nutritive level of the plant, presence or absence of particular substances giving an appropriate or inadmissible taste, presence of toxic substances, lethal proteins, deficiency of vitamins or salts.

The foliage of the plants, roots, fruits, bark or twigs, seeds, some of these or all of these or any of the parts of the plant are damaged by the insects. A single plant might be attacked by just a single pest or by many insects influencing the yield or by injuring the plants. A very persuading case has been proposed recommending that the secondary plant compounds have really evolved with insects that would conceivably unequivocal them as a food source. These compounds might possibly be harmful to specific groups or just a single group of animals. If they are dangerous, they may influence the ordinary functioning of a cell and in this manner may change the biomolecular activities. These compounds, however synthesized and stored by the plant, might not affect much the pest in their natural conditions. In any

case, when isolated, extracted and standardized, these metabolites may change their chemical arrangement and turn in to a source of a specific insecticide, which might be successful for the control of the pest. These chemicals are generally considered as harmful and have favoured the development of behavioural and biochemical adaptations, and also have changed some physiological processes in the pest.

Almost all insects are the host specific and they select their most favoured food so as to extract the maximum benefit out of it. However most of them eat a considerable number of varieties (Bruce, 1946). For a better understanding of the insect-plant relationship, studies on quantitative aspect of nutrition of any insect are very much essential (Bhattacharya and Pant, 1976). The nutritional composition of plant tissues strongly affect the performance parameter like growth, development, survival and reproduction associated with healthy condition of the larvae of phytophagous insects (Mattson *et al*., 1987).

It is clear that the larvae accomplish maturity and lastly adulthood by devouring the fullest feeding requirements. They use the consumed food at a specific rate to accomplish development with the goal that the energy in the body later on helps in performing different metabolic activities during non-feeding phases of moulting and subsequent metamorphosis. More over, the nature of food directly or indirectly affect the quality and quantity of products obtained from insects. A balanced nutritious food is the important factor responsible for the healthy growth and development of any insect, as it gives a source of energy. In this manner, both quantitative and qualitative aspect of nutrition contribute to a significantly more conspicuous information and understanding of the insect - plant interaction that exists between them.

The growth and development of insects rely on the chemical components of the host plants (Kerkut and Gilbert, 1985). Environment

affects the physiology and chemical properties of plants. Seasonal change tends to alter the quality of host plant, which thus influences the growth, development and survival of the insect. Seasonal variations relate to changes in light intensity, temperature and water content which has an immediate impact in the metabolism of the plant. In this way, the seasonal assessment of the biochemical components of the leaves are important to recognize the changes that happen in leaves during various seasons. A number of insects are sensitive to changing ecological variables such as light and temperature which are thought to be of prime significance in the control of growth and development of insects. (Beck, 1977).

Insect body temperature has a tendency to be the same as the ambient temperature, that is they are poikilothermic. A diverse temperature influence different processes relating with the specific growth and development. The nutritional components of the food incorporates both the absolute and relative measures of proteins, amino acids, lipids, unsaturated fats, sugars, sterols, water, minerals, vitamins and so forth. With a specific goal to achieve its optimal growth, development, and reproductive performance, an insect must get sufficient measure of important nutrients in a suitably relative balance (Kerkut and Gilbert, 1985).

1.4. Secondary metabolites

Plants use metabolic pathways and biochemical co-factors for converting carbon dioxide and water to sugars, nitrogen to amino acids and to form nucleotides, lipid and simple organic acids. These pervasive substances formed in plants and animals are known as primary metabolites. The primary plant metabolites are used to synthesize the more complex compounds known as secondary metabolites. Plants produce different types of secondary metabolites for survival and resistance in the environment and the major part formed of 1-3 % of dry plant weight, are synthesized in particular cells at

specific developmental stages and have exceptionally complex structures. Secondary metabolites incorporate a large number of alkaloids, terpenoids, phenolic compounds and other minor secondary chemicals. Practically, every plant species has built up unique chemical compounds that protect it from pests.

Vacuoles and chloroplasts are the most critical storage site for some hydrophilic secondary metabolites. Plant secondary metabolites are not distributed equally all through the plant, either qualitatively or quantitatively in space and time. The amount of secondary metabolites in many plants differs diurnally. Besides, the amount of these compounds are probably changed by climatic and edaphic components, grazing herbivores and other natural elements. Besides the genetic difference, some factors such as temperature, water stress and intensity, age of plants, mineral insufficiencies, pathogens and predators and light quality also influence the synthesis of secondary metabolites. The versatility of secondary metabolite formation by stress may help in plant adaptation and survival.

The secondary metabolites may vary from antifeedants, growth inhibitors, development disrupters, moult inhibitors, factors influencing larval duration, pupal period, life span, fecundity, rate of egg hatchability and factors bringing about higher mortality and deformity of larvae, pupae and adults. They may influence the eclosion of eggs and result in abnormal larval and pupal development by arresting the typical hormonal discharge by the endocrine organs. They may influence the discharge of juvenile hormone and moulting hormone (ecdysone) and their life cycle might be altered.

1.5. Host plant preference of insects

Host plant specificities can be related to the plant part being used and furthermore by the distinctions in the feeding specificities of the larval and

adult phases of the insect. Consequently phenological measurement is required in the host plant specificity. Different insect species consume a given plant tissue at various circumstances of the year or at various developmental phases of the plant. For an insect, the host plant is not only something to feast upon, but also something to live on. Based on the specificity of the host plants, phytophagous insects are categorized into three; monophagous species are limited to just single type of plant or a few firmly related species; oligophagous insects prefer plants with in one family or closely related families and polyphagous insects use host plants of more than one botanical order.

Plant resistance to polyphagous insects ought to be the most reasonable, in view of this fact, the pressure for adaptation would be reliably lower than in both monophagous and oligophagous insects. In the polyphagous insects, tactile discrimination of specific plants are not generally specialised and the metabolic machinery for avoiding deleterious plant chemicals must be developed (Feeny, 1976). Since polyphagous insects enjoy a more prominent scope of feeding alternatives they may experience less pressure to adjust to any given plant species, and are furnished with a more extensive range of metabolic detoxification capacities.

From the pattern seen in host plant specificities among insects and the defense development and procedures among plants, an agricultural methodology for the development and utilization of insect-resistant crop plant should be possible. Beck (1950)reported about the high growth rate, greater body weight, better fecundity and life span of some lepidopteran species fed on artificial diet than on host plants. But the artificial dietary media lack some sensory factors (attractants,stimulants) that are crucial for the insects to survive under natural conditions.

The maintenance of stable resistance must be the most significant in the agricultural strategy. The evolutionary history of insect-plant interactions distinctly demonstrates that no plant defense system can be viewed as safe to counter adaptation by at least one type of phytophagous insects.

1.6. Biochemical constituents in experimental tissues

The midgut is a vital organ in insect not just in the view of the fact that it possesses an extensive space in their haemocoel, or it is an important part of the digestive tract; it additionally assumes critical role in other physiological regulations for example, digestion, immune response, circulation, osmotic pressure and homeostasis of electrolytes etc. It is the significant site for the assimilation and nutrient absorption.

The absorption of amino acids takes place in the gut. If higher concentration of amino acids occur in the gut lumen they diffuse across the epithelium down a concentration gradient, at lower concentration its absorption occurs at symports coupled with movement of a cation. Cells in different parts of midgut vary in their ability to take up different amino acids (Wolfersberger, 1996). Different amino acids enter the haemocoel at different rates and in different amounts relative to their presence in the gut lumen partly as a result of their differential uptake, but also they may be metabolized with in the gut epithelium.

Carbohydrates are absorbed mainly as monosaccharides. In some cases, it diffuses from a high concentration in the gut to a low one in the haemolymph. This is facilitated by the immediate conversion of glucose to the disaccharide trehalose in the fat body surrounding the gut so that the concentration of glucose in the blood never build up. Mannose and fructose are also absorbed in a similar manner but more slowly than glucose.

Absorption of water from the lumen of the gut tends to maintain relatively high concentration of sugars in the gut.

Lipids are mainly absorbed as fatty acids. In caterpillars the turnover of triglycerides is rapid and it appears that they are transported to the basal part of the cell where they are actively exported as triglycerides. The turnover of phospholipid is slower and substantial amounts remain in the midgut cell 24 hours after a meal (Turunen and Chippendale, 1989). Sterols appear to be absorbed unchanged, but in some caterpillars it is esterified in the gut cell.

Haemolymph is a watery liquid containing molecules, ions and cells. Haemolymph plays the roles of both blood and lymph. It is made out of water, inorganic salts (generally sodium, chlorine, potassium, magnesium and calcium) and organic compounds (generally carbohydrates, proteins and lipids). It helps to transport nutrients and remove waste materials. Protein and starch acquired from nourishment are circulated in haemolymph by dissolving in the aqueous portion of the fluid. Insect haemolymph is an important pool of free amino acids. In some insects the free amino acid content in the haemolymph is sixty times or more than that in the human blood (Chen, 1962). Protein synthesis, osmotic haemostasis and energy production for flight, mainly depend on the amino acids (Buck, 1953).

Insect haemolymph contain numerous proteins with various functions. The total concentration of these proteins vary during the developmental stages but the peak concentration occur in the late larval stage. Lepidoptera attain about 100mg /ml. These proteins function as storage protiens, lipid transport proteins, enzymes, vitellogenins, chromoprotiens etc.

Trehalose is the most important sugar found in the insect haemolymph. Usually its concentration is in the range 4-20mg/ml but sometime it is present in greater amounts. But it is not present in all insects. Glucose often presents

in much lower concentration but higher concentration is also present in some insects. Sugar level is maintained at constant level through the hormonal actions. Sometimes carbohydrates such as inositol, glycerol, mannitol are also present in the insect haemolymph. The concentration of lipid in the haemolymph generally varies between about 1 and 5 mg/ml but in some insects it reaches about 15mg/ml. Most of the lipid is in the form of diacylglycerols. They are normally carried by lipophorin.

The fat body plays significant functions in the life of insects. It is a dynamic tissue performing numerous metabolic functions. It stores and release energy with respect to insect's energy need. It is the prime area for metabolism and detoxification activity and also for storage and release of glycogen, lipids and proteins. The larval fat body cells, known as storage reserves contain storage proteins, lipids and glycogen. It is structurally well organized and is fully exposed to the haemolymph for absorption as well as release of metabolites.

Fat body is the major site of synthesis of haemolymph protein. In some insects about 90% of the total haemolymph protein is synthesized by the fat body (Palli and Locke, 1988). It also synthesizes diapause proteins and vitellogenins. Proteins that are synthesized in the fat body are released in to the haemolymph and are later stored in the fat body as granules. Uptake of protein is selective, different proteins are taken up to different extents.

Carbohydrates are stored as glycogen in insects. It is built up in the fatbody during the periods of active feeding. This store becomes depleted during the moult or sustained activity or when the insect is not feeding or under starvation. The level of sugars is maintained in the insect body through the conversion of glycogen to trehalose in the fat body.

Fat body is the major storage site of lipids in the insect. Most of the lipid is present as triacyl glycerol. More than 70% of the dry weight of fat body constitute lipids. The amount of lipid varied with the developmental stage and feeding state of the insect. Lipid store normally increases during the active feeding and it declines when feeding stops or when large quantities of lipids are utilized during oogenesis or prolonged flight. The quantity of lipid accumulating in the fat body may exceed the amount of lipid absorbed from the food. The additional lipid is synthesized from carbohydrate. Not only the total lipid content is increased by greater quantities of carbohydrate in the diet but also relative proportion of different fatty acid may also vary (Thompson, 1979).

1.7. Role of dietary components in insects

Generally insects have similar nutritional requirements because the chemical composition of their tissues and their metabolic processes are similar. Most of these requirements are usually attained by them through diet. Some chemicals are obtained directly from the diet and some others may be synthesized by the insects from the dietary components. In spite of the overall similarities, major differences in the nutritional requirement do occur. This may be due to the evolutionary changes related with the feeding on substrate with quantitatively and sometimes qualitatively different amounts of nutrient chemicals.

Amino acids are essential for the synthesis of proteins, which are used for the structural maintenance, as enzymes, as storage, transport and receptor molecules. Amino acids are usually present in the diet as proteins. The values of any ingested protein depends on its amino acid content and its digestive ability of the insect. There are ten essential amino acids in the diet and absence of any one of the essential amino acid prevents growth. The non essential amino acids synthesized by the insect are necessary for optimal

growth. Amino acid synthesis primarily occurs in the fat body although it also occurs in other tissues.

The survival and growth of the insects mainly depends on the environmental factors- biotic and abiotic. Abiotic factors include temperature, humidity, soil type and biotic factors such as quality of leaf, fecundity spacing etc., both have important roles during each stage of insect development. Several investigators studied and reported the relationship between environmental factors and changes in duration of different stages in the life cycle of insects (Qin *et al*., 2004; Zhu *et al*., 2005).

In insects, the growth and development is associated with protein metabolism. One fifth of the total body weight of the organism contain protein including both structural and soluble proteins (Swaminathan, 1983). Proteins are high molecular weight compounds made up of simple amino acid units. They have a vital role in the body building of the organism and in the metabolism of other biochemical compounds such as carbohydrates, fats etc. They also provide the energy required for insect body. Tissue proteins are utilized by the body at extreme condition like lack of availability of the other sources due to starvation. Proteins also work as metabolic regulators. They act both as enzymes and hormones and regulate most of the physiological functions. The total protein content of the tissue of an organism is used as a tool to evaluate the physiological condition of the organism.

Carbohydrates are the most abundant and nutritionally important components of many foods. In insects they are the focal point of cell metabolic pathways and are generally utilized as the key energy source needed for fuel biosynthetic pathways. They may be converted to fats and may contribute to the production of amino acids. They are therefore important component of the insect diet. Most insects so far examined require some amount of carbohydrate in the diet and grow better as the proportion is

increased. The utilization of different carbohydrates by the insect depends upon its capability to hydrolyze the polysaccharide, its absorption capacity and the presence of enzyme system capable to carry out the metabolic process. Some insects can use a wide range of carbohydrate because they are capable of digesting the more complex structures. Glycogen act as the storage of carbohydrate in insect tissues and trehalose delivered by the fat body,which constitutes the significant disaccharide in the insects blood.

The obvious accomplishment of insects on this planet has been their capacity to use lipids productively for metabolic needs and also as substrates for propagation, embryogenesis, transformation and flight. Besides these, lipids are utilized as methods for communication (pheromones), for control of variety of physiological activities (hormones), as a protection against a desiccating environmental situation (cuticular lipids), and as cell constituents (membranes) (Gilbert and Chino, 1974). Insects are able to synthesize many fatty acids and phospholipids.So usually they are not essential dietary components, but many insect do require a dietary source of polyunsaturated fatty acids and all insect require sterols. In insects the fatty acids are present as diacyl glycerides or triacyl glycerides. The major fatty acids in insect triglycerides and phospholipids are those with skeleton of 16 and 18 carbon atom. Poly unsaturated fatty acids with 20carbon atoms are present in many insect species. Poly unsaturated derivative eicosanoids are important in the reproduction, thermoregulation, and in lipid mobilization of all insects (Stanley Samuelson *et al*., 1991).

Some insects are able to synthesize poly unsaturated fatty acid from dietary acetate. But some require a small quantity of dietary unsaturated fatty acids. Lepidopteran insects generally require linolenic or linoleic acid in the diet. Some insects are able to synthesize C20from C18 fattyacids but others require a dietary source (Stanley Samuelson *et al*., 1991). Sterols are the

essential structural components of the insects. Insects are unable to synthesize sterols so they require sterols in the diet. Most of the plant feeding insect process the common plant sterol to synthesize cholesterol. Lepidoptera and some Coleoptera require a dietary source of inositol but some others synthesize it from glucose. Phosphatidyl choline is the major phospholipids in all insects. So its dietary source is very essential.

All foods differ in their protein and starch content, however variation of these two components in plants is substantially more prominent than in animals. For plants, changes in proteins and carbohydrates exists at various levels, including within species, between species, with in an individual plant depending upon the kind of tissue (*i. e*., leaves, stems, flowers and seeds) and furthermore its age (*i. e*., young versus old leaves). Moreover, a plant's protein and sugar content can change because of ecological factors, including the measure of light it gets, the soil composition and availability of water (Walter *et al*., 2012).

Chlorophyll content is one of the most important parameters in the relationship between plants and herbivores. Chlorophyll levels change during plant development. Increased phenolic concentrations in plants decrease herbivory despite the fact that species richness can be increased accordingly by co-evolutionary processes. Tannins are water soluble phenolic compounds and have the capacity to bind and precipitate the proteins and other micronutrients. Nutritional status of the larvae significantly affects the other stages such as pupae and adult.

1.8. Relevance of the study

There are various factors that decide the efficacy of a pest control methodology. The most vital of all is to have knowledge of the important environmental roles played by "pest" species in both agricultural and

unmanaged conditions and exhaustive information of the physiological, morphological and biochemical aspects of the pest. The biological information will unquestionably give the signal to build up an exclusive technology which depends on the exceptional characteristics of the insect species. In brief, lack of sufficient knowledge in the biology or technology will lead to the prevention of successful accomplishment of the control programmes. So this work would lay groundwork for future biological advantages and for the development of eco-friendly management of *S. litura.* The present study, deals with the influence of five host plants such as colocasia, papaya, banana, castor and sweet potato were tested on the developmental profile and the effect of these food materials on total protein, amino acid, carbohydrate and lipid content in the midgut tissue, fat body and haemolymph of the last instar larvae of *S. litura* were studied.

With this background, the present work is aimed at the following objectives:

- To study the detailed biology of *S. litura*.
- To study the effect of feeding of leaves of selected host plants on the total protein and free amino acid concentration in the midgut tissue, fat body and haemolymph of last instar larvae of *S. litura.*
- To study the effect of feeding the leaves of selected host plants on the total carbohydrate concentration in the midgut tissue, fat body and haemolymph of last instar larvae of *S. litura.*
- To study the effect of feeding the leaves of selected host plants on the total lipid concentration in the midgut tissue, fat body and haemolymph of last instar larvae of *S. litura.*
- To study the difference in the biochemical constituents and secondary metabolites in the leaves of selected host plants.

CHAPTER II
REVIEW OF LITERATURE

2.1. Introduction

Today the insect pests occur as a significant concern for the farmers over the world. About 1.4 million species of invertebrates have been reported so far, of which 75, 000species are insects (Applebaum, 1985). The insects are said to be in charge of decimating one fifth of the world's annual crop production. More than 10, 000species of insects damage the food crops throughout the world (Dhaliwal *et al.,* 2007). Sometimes the insect makes a higher percentage of damage and bring about 60-70% of yield loss. According to Dhaliwal *et al. (*2010) the Indian agriculture is presently suffering a yearly loss of about Rs.8, 63, 884 million because of the insect pests.

Even though different control measures are available against various pests, the farmers mainly depend on chemical control measures which cause consistent increment in crop loss (Dhaliwal and Koul, 2010). This is because of continuous and over usage of insecticides which cause resistance and increase in the survival rate of insect pests. Therefore, the farmers are forced to use the pesticides in large quantity that result in heavy crop loss (Aktar *et al.,* 2009). This lead to harmful impact on non-target living organism (Cork *et al*., 2003).

It is very important to know the biological characteristics and nutritional status of the insect for the development of innovating biological control. Nutritional ecology is the understanding of insect life within its most fundamental nutritional conditions. The changes in the suitability and availability of food, variations in temperature, humidity and other fluctuations in the regular habitat prevent the insects from accomplishing the physiological

potential life history achievement, that they would accomplish if they were placed in a perfect absolutely favourable environmental condition. (Kerkut and Gilbert, 1985).

Nutrition of any insect is of crucial importance for understanding the insect-plant interaction. The biochemical properties of host plant leaves of economic importance, on insect pest like *Spodoptera litura*, have a great significance in deciding their food value. This review include the information regarding the biological parameters of the insect, nutritional aspects of insects, biochemical properties of host plant leaves, and biochemical constituents of the insect tissues after fed with different host plant leaves in different seasons.

2.2. *Spodoptera litura*

Spodoptera litura, the tropical armyworm, is a common phytophagous insect and a member of an economically important polyphagous pest that cause serious damage to many crops in various agricultural fields. *S. litura* infested more than 290species of plants belonging to 40families (Wu *et al*., 2004). Since this pest is polyphagous in nature the larvae feed on different host plants including crops, weeds, vegetables, flowers, and even the leaves of citrus plants and rice (Pogue, 2003). Moussa *et al. (*1960) reported about its feeding on 112 species of plants and pest outbreak under rainfall season after a long dry spel. It is a folivor pest and its cosmopolitian behaviour was reported by Common Wealth Institute of Entomology (1967).

The first record of *Spodoptera litura* was from New Zealand as a pest of tobacco and it has appeared in higher numbers in home garden and on crops (Cottier and Gourlay, 1955). It is distributed throughout Asia and Oceania in temperate, tropical and subtropical zones (Venette *et al.,* 2003). Singh and Jalali (1997) reported about its wide spread distribution in India

and in other countries such as Fiji, China, Formosa, Japan, Bahrain, Thailand, Indonesia, Vietnam, Iran, Australia, Korea, Philippines and Egypt. About 25.8-100% of economic damage was caused by this insect (Higuchi *et al.,* 1994). It was reported as an important insect pest of tobacco in South China. It was causing serious defoliation so that its management became difficult (Jayanthi and Padmavathamma, 2001). Gao *et al*. (2004) reported about the management failures of *S. litura.* It occurs commonly in countries such as Southeast Asia, India and China, necessitating the development of novel control methods. Many biotic and abiotic factors affect the out breaks of *S. litura*. Its outbreak led to more than 90per cent yield loss of sunflower crop germplasm (Sujatha and Lakshminarayana, 2007).

The caterpillars, which make the damage measures 35 to 40mm in length during the development. They are smooth black with yellowish green stripes and lateral white bands. The adult moths are about 22 mm long and have 40mm wingspan. Forewings are golden and grayish brown in colour and are very beautiful (Atwal and Dhaliwal, 1997). Eggs are round (0.3 mm diameter) in shape and laid on the underside of leaves in bunches of 100-300, and secured with hair-scales. Every female lays some 1000-2000eggs and hatching period is 2-6 days (Hill, 1987). Avidov and Harpaz (1969) reported that the maximum number of eggs laid by *S. litura* was 3700.

2.3. Insect – Plant Interaction

Insect plant relationship has been extremely intrinsic one governed by various factors including the plants and insects concerned as well as environmental conditions. The establishment of insects on plants is represented by a number of factors involving responses of insects to plants as well as charactersistics of the plants eliciting these responses (Horie and Watanable, 1983)**.** Insects have the ability to discriminate the nature of the food, which is aided by the physical and chemical characteristics of the food.

The insects searching for a suitable host plant must locate first and identify an acceptable plant species (Dadd, 1985). Exact choice of host taxa and appropriate assessment of individual plant quality should also be accomplished with least time under most natural conditions.

Extensive host range is viewed as important for better opportunity to survive during evolutionary strategies (Lee *et al*., 2003; Raubenheimer and Simpson, 2003). Host plant range of common insect pests like *S. litura* may change because of their higher level of feeding on various plant species and all parts of these plants (Schoonhoven *et al.,* 1998). Host preference also depends on presence of plant metabolites which either attract or repel the pests (Ehrlich and Murphy, 1988). Host plant selection may be related with primary metabolites as well as the secondary metabolites present in these plants which help the insect to choose preferred hosts because of the nutritional variation (Ehrlich and Murphy, 1988; Lee *et al*., 2003). Presence of plant metabolites may slow down their development and also decrease deleterious impacts due to gregarious feeding (Lee *et al*., 2003).

Feeding is a basic process important to all animals and in spite of the fact herbivore insects have a suite of system available to overcome nutritionally imperfect food (either as a function of low concentration or imbalanced macronutrient content with respect to species-specific requirements) they show good performance when they approach the foods containing protein and digestible carbohydrate in the correct ratio, and at high concentrations. They can manage their protein and carbohydrate intake by mixing their diet, either by exchanging between plants or plant tissues (Villalba and Provenza, 2002; Felton *et al*., 2009). But, these mechanisms can be restricted sometimes under natural conditions. For instance balancing of the intake of protein and of carbohydrate by alternating host plants won't be possible due to the risk of predation (Hawlena and Schmitz, 2010). The

probability of attack by predators is higher when herbivores are forced for actively feeding for a longer period of time to compensate feeding on foods that have low concentrations of nutrient (Bernays and Minkenberg, 1997).

Plant-herbivore relationships have developed with respect to their feeding, survival and multiplication of generation. This mode of selection of different host plants cause them to maintain their numbers and to multiply and establish their diversity in nature (Raubenheimer and Simpson, 2003; Lee *et al.,* 2003). *S. litura* is generally an important leaf feeder, uses green matter and in severe shortage of food, feeds on almost all parts of the plants. This observation were clear when the leaves were either eaten up completely or when they were exchanged to other parts like flowers and fruits of host plants (Simpson *et al.,* 2002).

2.4. Morphological changes in the insect in relation to the different food materials under seasonal variation.

Feeding is an active and dynamic process with various feedback interactions with considerable impacts on growth, reproduction and dispersal (Hagen *et al*., 1984**).** Preference of host plant is based on many factors such as nutritional composition of the host plants (Thorsteinson, 1960). The quality, amount, and rate of food consumed by the adult insects influence their fecundity, movement and survival, while in larval insects, they influence the development duration, growth rate, final body weight and survival (Horie and Inokuchi, 1984**).** The biology and feeding habits of *S. litura* on different host plants were studied by many workers (Patel *et al.,* 1987). The significant difference in the growth and development of the larvae of *Prodenia litura* after fed with host plants were reported by *(*Pandey and Rangarajan, 1967).

Latheef and Harcourt (1972) compared the food consumption, assimilation and growth of *Leptinotarsa decemlineata* on two different host

plants and he observed that the larvae reared on tomato took longer time to feed and consumed more amount of the leaves and had a lower survival rate compared to the performance of the larvae of the same species on other host plant potato. But among the two host plants the food assimilation efficiency was more in the potato fed larvae. This was the reason for the increase in the body weight of the larvae reared on potato. Joshi and Mishra (1979) observed the changes in the weight of larvae, puapae and cocoon of eri silkworm reared on interchanged castor (C) and tapioca (T) leaves.

Studies of Koul *et al. (*1979) on the larval silk gland weight, protein consumption and silk production of silk worm *Bombyxmori,* on three different varieties of Mulberry revealed that the larvae fed on RRL race produced silk with high yield. The oviposition site distribution on various plants seemed to be independent of the plant species due to the fact that in all crops, a large portion of the egg clusters are laid within 10cm above the soil surface. The effect of larval nutrition on egg production of *Rhodnius prolans* was reported by Patterson (1979). The numbers of eggs laid by S. litura on different host plants were studied by Monobrallah (2003) and he found that the percentage of eggs on tomato, cabbage, cauliflower and soybean was 74.5, 91.7, 87.9 and 81.5% respectively on the lower side of the lowest leaves within 10cm from the soil surface but he observed that there was no eggs above 20cm from the soil except on the host plant tomato. An oviposition period of 6.80to 9.40days with a fecundity of 4586 -3163 eggs and 83- 89% egg hatching of *S. litura* on sunflower crops were recorded by Thomas and Bilapate (2007).

Response of phytophagous insects to different host plants affect the growth and larval weight of insect (Lazarevic and Peric-Mataruga, 2003). There is a positive correlation between the nutritional requirement and the mass of the insect (Schroeder, 1981). Body weight is the fitness indicator of insect population dynamics (Liu *et al.,* 2004). Variations in the larval weight

of of *Helicoverpa armigera* fed with different soybean varieties are reported by Naseri *et al. (*2010).

The effect of different host plants such as castor, Indian bean, lucerine, ivy gourd and cabbage on the growth and development of *S. litura* were studied by Patel *et al*. (1987). From the data it was observed that the larval survival is maximum on castor and cabbage followed by lucerine and ivygourd. The larval development was shorter in castor than lucerine, cabbage and ivy gourd respectively. *S. litura* larva shows maximum pupal weight, size, less duration of development and high growth index when it reared on castor. (Bhalani, 1989) reported that the order of the suitability of some host plants of *S.litura* were cotton> groundnut> cowpea> greengram>sorghum> maize

Shamacharye *et al. (*1980) reported that the weight of full grown larvae determine the ratios of cocoon weight, pupal weight and shell weight of the silk moth. Thangavelu and Phukan (1985) reported that the larval duration of *Bombyxmori* was longer on kesseru where as cocoon weight was higher in castor fed larvae. Chibber *et al. (*1985) observed food consumption and utilization of *S. litura* on nine host plants and he reported that even though the intake of food is significantly lower on *Ricinus communis,* approximate digestibility and conversion capability of ingested and digested food to body substance was higher in the case of *Ricinus communis* and *Helianthus annus.*

The effect of temperature in the developmental and physiological processes in determining the size and fecundity of six mayflies were studied and reported by Sweeney and Vannote (1981). Ratte (1985) studied about the effect of temperature on insect size and he reported that some insects have direct relationship between weight and temperature. Weight gain from feeding greatly influence the quality and performance of insects (Slansky and Scriber, 1985). The great influence of abiotic factors and food plants in the growth and

development of muga silkworm, shell weight, silk content and reliability were reported by Chandrasekhar and Thangavelu (1986).

Studies on the effect of three constant temperatures on larval critical weight, latent feeding period, larval maximal weight and fecundity of *Cnephasia jactatana* were carried out by Ochieng'-Odero (1992) and he reported that the adult and pupal weight were increased at 15°C. Female reared at 15°C were heavier and showed significantly higher fecundity. The effect of dietary moisture on the development and larval duration of *Bombyxmori* were observed by Paul *et al. (*1992) and they reported that outright utilization and growth rate/day/larvae increased with increasing level of leaf moisture. Larval days was prolonged under low water content however without a relating increment in the amount of leaf consumed. Sarkar and fijita. (1994) found out a positive correlation between crude protein content in the mulberry leaves and cocoon weight, shell weight and cocoon yield.

2.5. Biochemical composition of leaves and changes observed in insect development in relation to different seasons

Study of the effects of host plants on the biology of insects is important in understanding host suitability of plant infesting insect species. There have been a number of studies on the biological parameters of *S. litura* on different host plants under different environmental conditions, particularly in India (Patel *et al*., 1986, 1987**)**.

In plants, variation in proteins and carbohydrates exist at a number of levels, including between the species (Yeoh *et al.,* 1992), within the species (Sattelmacher *et al*., 1994), and within an individual plant (Mattson, 1980) depending on the type of tissue (i.e., leaves, flowers, seeds, and stems) and its age (i.e., young versus old leaves). Additionally, a plant's protein and carbohydrate content can vary in response to environmental factors like the

amount of light it receives and the chemical composition and water content in soil (Felton, 1996; Walter *et al*., 2012). Nutrition is one of the most important extrinsic factors which influence the growth and development of insects (Vedham and Muralirangan, 1999)

Hering and Taguchi (1951) reported that the chemical constituents of the leaf of a food plant may change under seasonal factors and as such the leaf eventually may become inadmissible for the insect feeding on it. Kozhanchiker (1950) portrayed the importance of the age related changes in the leaves of oak in the nutrition of *Antherae pernyi.* He revealed the significance of seasonal changes in the chemical composition of the food plants, which thereby influence the nutrition of oak silkworm and certain other lepidoptera.

The amount of crude protein present in the mulberry leaves was estimated by Tanaka (1964) and he reported that the values were diminishing with the increase in the age of the leaves. But the carbohydrate content was observed to be increased with the advancement of maturation of leaves. Further he noticed that vitamin 'A' present in the mulberry leaves caused increase in body weight, cocoon weight and cocoon layer ratio in the silkworm *Bombyx mori,* but high crude protein to carbohydrate ratio in mulberry leaves caused numerous diseases.

The growth of larva of *Prodenia litura* feeding on different wild host plants were observed by Pandey (1967) and he reported that there was marked differences in their growth and development on different host plants. A number of the tassar fauna stopped feeding on English oak after mid june because of the slow and consistent deposition of tannin content (Fenny, 1968). The nutritional requirements of the insect fluctuates throughout the developmental stages and such fluctuations results in changes in the feeding behaviour and food consumption (Barton Browne, 1995). Balancing of gut

amylase and proteinase levels based on the diet composition and larval developmental stages were reported in *H. armigera* (Kotkar *et al*., 2009).

Parpiev (1968) reported the positive impacts of high moisture content in the leaves on the palatability and assimilability of nutrients. Paul *et al.,* (1992) reported that leaves moisture content may be used as one of the criteria for assessing the leaf quality. The quality of leaves rely upon moisture content, nitrogen, protein, minerals, fibres, sugars and starch content and they play an important role in the proper development and growth of silkworm to deliver more healthier cocoon (Sinha and Jolly, 1971).

Jolly *et al*., (1974) completed their analytical works on leaves of various host plants of tassar silkworm *Antheraea mylitta* and noticed changes in constituents of the nutrients present in the leaves. The constituents under observation were total mineral, moisture, sugar, nitrogen, crude fibre, and starch contents which varied in every species and within the species too. Dutta *et al*. (1997) analysed the leaf constituents of various host plants of muga silkworm *Antheraea assama* and noticed the changes in the percentage values of moisture content, crude fibre, total nitrogen, protein, crude fat, soluble sugar, starch, phosphorus, potassium and calcium respectively. Horie (1978) did his work on qualitative requirements of nutrients important for the development of the silkworm *Bombyxmori* and evaluated the dry matter energy, carbon, carbohydrate, vitamins, lipids, proteins, amino acids and mineral required for growth and development.

Ruan and Wu (2001) reported about the influence of different nutritive values of host plants in the rate of development and the population dynamics of *Helicoverpa armigera* larvae. The population out breaks of polyphagous insects mainly depends on the availability of different host plants (Singh and Parihar, 1988). The growth, development and reproduction of insects are greatly affected by the quality and quantity of food they consumed (Scriber

and Slansky, 1981). Cohen and Patana (1984) reported the efficiency of food utilization by *Helicoverpa zea* raised on artificial diets or green beans. According to Samraj and David (1988) the variation in the survival and development of insects on different crops might be due to poor nutritional quality of the food, pericarp thickness, secondary plant biochemicals or antibiotic effects. A low dietary protein can cause an increase in the rate of feeding of the larvae (Slansky, 1993) similarly a high protein diet can reduce the rate of larval feeding (Mattson, 1980).

Study of nutritional indices give a proper understanding of the physiological and behavioural basis of insect response to different host plants. (Lazarevic and Peric-Mataruga, 2003). The test on feeding preference of the larvae on 8 different host plants were conducted by Vanish and Agarval (1978). The most preferred host plants of *S. litura* are sunflower followed by cowpea (*Vignaunguiculata* L.), radish (*Raphanussativus.*L.), green gram (*Vigna radiate* L.), black gram (*Vignamunga* L.), rose leaf and rose petal (*Rosa indica* L.), and tur (*Cajanuscajan* L.) in the order of preference. According to Chibber *et al. (*1985) the most suitable host plants of this pest were castor and sunflower. This identification was based on the food intake, growth rate, digestibility and ability to convert the ingested biomass of the body.

2.6. Variation in Protein, Free Amino acids, Carbohydrate and Lipids in the different tissues of insects:

Haemolymph is the main extracellular fluid in insects and it exists in an unbound, non-vascular state, in close contact with tissue and organs. Wyatt (1961) has done numerous work on biochemistry of insect haemolymph. He evaluated the general and physical properties of insect haemolymph and also the inorganic and organic components of it. The haemolymph proteins of insect have been examined from different focuses (Chen and Levenbook,

1966). These incorporate -1) the mapping of protein parameters in different species for taxonomic purposes, 2) determination of protein components at progressive developmental stages by both electrophoretic and immunological techniques and 3) detection of function of protein components on the basis of enzymological and histological tests. Physical properties and chemical structure of insect blood is broadly assessed by Florkin and Jeuniaux (1974). As indicated by these authors water which form the major part of haemolymph constitute around 84-92% of the total plasma.

Fat body of insects have the main function in the storage of reserve substances which require the rapid mobilization during the moulting and metamorphosis stage. Several studies have recently emphasized the role of the fat body as perhaps the most important centre of intermediary metabolism in insects (Kilby, 1963; Chefurka, 1965; Gilby, 1965). It has central importance in the intermediary metabolism of insects and is responsible for the synthesis and supply of haemolymph substances. Fat body cells, known as trophocytes, are clustered together by a thin basal lamina that expands into the haemocoel and forms amorphous lobes or ribbons that increase the organ surface area, which in turn enhance the exchange of substances between the organ and the haemolymph (Martins and Pimenta, 2008; Arrese and Soulages, 2010). It consists of a mass of cells located underneath the epidermis and in some insects, the fat body also surrounds the digestive and reproductive organs (Roma *et al.,* 2010). Wyatt (1957) emphasized that all proteins are synthesized in the fat body.

Caterpillars generally lack a foregut but have a large midgut (which is the primary site of absorption), and a relatively short hindgut. The midgut cells are actively involved in the production and secretion of enzymes as well as the digestion and absorption of nutrients. Regardless of the feeding habit most insect digest protein, carbohydrate and lipid in their diet because of their

similarity in the array of enzymes in the midgut. When the opportunity to mix their diet is limited, or constrained, herbivore insect can adjust feeding responses to balance the concentration of nutrients in their food (Fanson *et al*., 2012).

The metabolism of the nutritional components like protein, lipid and carbohydrate play role in many vital activities of insects. Numerous factors like sex, age, developmental stages, diapause, nutrient quality and quantity, seasonal conditions, temperature, host type in some species, sexual activity, use of insecticides also influence the levels of these substances (Shuxia and Adams, 2000; Nakasuji and Mizumoto, 2001; Barsagade and Tembhare, 2002; Giron and Casas, 2003). The major pathways such as glycolysis, TCA, fatty acid, β-oxidation, fatty acid synthesis, amino acid metabolism and pentose phosphate nucleotide metabolism have been already established for insect systems(Bursell, 1981).Proteins and carbohydrates are the major dietary nutrients (Simpson and Raubenheimer, 2012), even though they have similar caloric value, functionally they are entirely different.

Cook *et al*. (1972) established a connection that the increased protein and carbohydrate metabolism hindered the free amino acid concentration. The quantitative investigations on protein, carbohydrates and fatty acid contents in the haemolymph of normal cockroach showed a more elevated amount in the nymphs than in the adults (Reddy and Rao, 1982). The increase in the weight of larval body, silk gland and testis and ovary of fifth instar larvae of *Bombyx mori* was noticed by Reddy and Benchamin (1989). This increased weight of these tissues during their development period might be due to the accumulation of biochemical components like protein, carbohydrate and nucleic acid.

2.6.1. Protien and Amino acid

In insects, the growth and development is associated with protein metabolism (Singhman and Baquaya, 1971). Protein is considered as the most important limiting nutrient for herbivore insects (Schoonhoven *et al.,* 2005). Among the substances used as the fuel, proteins are the last option. Proteins are acting effectively on growth, metamorphosis and formation of cocoon and cuticule. Lepidopteran insects need a greater amount of protein and their nutritional requirements are varying during ultimate and penultimate instars (Simpson *et al*., 1988). Protein concentration changes especially before or during the metamorphosis in developmental stage (Meats and Leighton, 2004)**.**

Protein synthesis is essential for the maintenance of body growth and reproduction and a number of factors had been implicated in the control of protein synthesis (Carlisle *et al*., 1987). Dietary nitrogen strongly affects growth, consumption and food utilization of insects. (Jeyabalan and Murugan, 1996)**.** In all the viable cells the proteins play a vital role, as nucleoproteins, are required for the cell division and as enzymes and hormones, are essential to control many biochemical reactions in the cell metabolism (Hassan, 2002). Proteins also play roles in various reactions and they get incorporated in the cell as structural component along with the carbohydrate and the lipids (Cohen, 2010).

With a few exceptions, caterpillars typically live in protein-rich habitats and demonstrate protein-biased intakes (Behmer, 2009). In insects protein help to synthesize the microsomal detoxifying enzymes with respect to the foreign compounds (Wilkinson, 1976). ie., proteins can bind with these foreign compounds that lead to a decrease in proteins that may reflect the decrease in activity of these enzymes (Kyung and Kim, 1990). Studies on *Chironomus riparius* exposed to anoxia had shown a decrease in total protein

content due to degradation in to amino acids as they contribute to energy in insect (Forcella *et al*., 2007**)**.

Numerous accessory factors have been appeared to influence the protein content of insect including temperature, moulting status, developmental stages (Firling, 1979), diet (Riley, 1980) photo period or stress (Widdow *et al.,* 1972).Proteins are not a source of energy in colder environment but involve in lowering the super cooling and freezing points and protects the larvae from injury (Omana and Gopinathan, 1995). *Philosamia ricini* larvae under cold stress conditions have shown the decrease in protein content in the fat body and silk gland (Anithasingh *et al*., 2010). Etebari and Matindoos (2004) reported that different stresses on the silkworm *B. mori* can inhibit the total protein in haemolymph. This could be due to the breakdown of protein into amino acids, so with the entrance of these amino acids to TCA cycle as a keto acid, they will help to supply energy for the insect.

Several workers reported that the insect haemolymph is very rich in protein and free amino acids (FAA) (Ranjini and Mohamed, 2004). The fundamental role of free amino acids and protein in the maintenance of osmotic pressure in insect haemolymph was studied by many workers (Florkin and Jeuniaux, 1974). Laufer (1960) reported that the protein concentration increased rapidly from 3rd to 5thinstar in *Sarnia cynthia.* Nowosielski and Patton (1965) noticed that protein and amino acids in the young ones of *Acheta domestica* were initially high yet there was rapid fall during adult stage which is related with development process including protein synthesis. Protein synthesis in insect haemolymph has been found out in numerous species during the development and diapause (Laufer, 1960; Wyatt, 1961; Chen and Levenbook, 1966). A few important role of blood proteins have been reported e.g. they may work as catalysts, function as

amino acid stores for adult tissues or be utilized intact in developing adult structures ((Laufer, 1960)

Levenbook (1985) reported that the protein content in the haemolymph increased quickly during the later half of the larval development, fall at metamorphosis and decreased in early adult life. The insect haemolymph proteins are also considered as storage protein and in various insects it reached maximum during the last instar stage of the larvae. In *Drosophila* the increase in total protein content closely resemble that in both dry and wet weight during the initial 72 hr of development (Church and Robertson, 1966). The protein content in insect haemolymph is like that of the blood of man and other vertebrates and is higher than that of the internal fluid of the other invertebrates. The normal protein content is 5gm/100ml in Hymenoptera, 3-4gm/100ml in Coleoptera, 2gm/100ml in Lepidoptera and 1gm/100ml in Orthoptera (Florkin, 1936a).

Lue and Dixon (1967) found out that the number of various proteins is highly variable among the species. Kulkarni and Mehrotra (1970) had given the comparative outcomes regard to sex of an animal group where as Bodnaryk and Morison (1966) concerning diet and as for starvation and Florkin and Jeuniaux (1974) as for ontogenic stage. Proteins (catalysts) have a major role in all metabolic procedure and in the structure and function of muscles and different tissues. Insects feeding on protein rich host plants will be more successful than those that consume plant material that is less protein enriched (Vitthalrao**,** 2012).

Amino acids provided by the dietary proteins are utilized to build the new tissues, proteins and enzymes. Amino acids and proteins play important roles in various stages of insect life cycle and the overall metabolic pathways for the insects are largely the same. Amino acids and its derivatives have numerous functions in insects body. The most important of these is of the

synthesis of protein for which all 20common amino acids are used simultaneously. Lack of any one of these amino acids lead to the prevention of protein synthesis and to increased degradation of the other amino acids (Horie and Inokvehi,1978). The amino acids required for synthesis of proteins are derived from hydrolysis of dietary food proteins, from turnover of cell proteins and in some insects through the action of symbiotic microorganisms. Extensive review on insect biochemistry was also reported by Gilmour (I961).

Quantitative and qualitative analysis of the free amino acid variation patterns during larval growth and moulting of insects have been carried out by Levenbook, (1985). The presence of wide variety of amino acids in the insect haemolymph was reported by Wyatt (1961). Sharma *et al. (*1994) reported about sixteen free amino acids present in the haemolymph of *A. assama* during fourth and fifth instar larval stages in different seasons. The fall of free amino acid in the winter season is related with low carbohydrate metabolism (Cook *el al.,* 1972).

2.6.2. Carbohydrates

While studying the biochemical processes behind the growth and development of the insects it has been seen that glycogen and glucose, whose functions are firmly established among almost every other animals, play equally important roles in the organization and metabolic activity of this largest class of arthropods (Steele, 1981). Glycogen and trehalose are the largest stores of carbohydrate for energy metabolism with its glucose subunit playing minor role.

In general, glycogen is synthesized and stored in the fat body during the larval development of insects which is considered to be a “mobile reservoir”. The concentration is based on the stage of the insect life cycle, nutrition and on demands of different energy requiring processes (Kilby,

1963). The stored glycogen in the cell can act as substrate directly without the need for transport in to the cell. The process of glycolysis in insects is controlled on various points along the pathway. All foods vary in their protein and carbohydrate content, but generally variation of these two nutrients in plants is much greater than in animals (Schoonhoven *et al.,* 2005; Clissold *et al*., 2009; Behmer and Joern, 2012).

Non-reducing disaccharide trehalose is the major carbohydrate in insect haemolymph. It occurs in various tissues of different insects with prevalent activity in the salivary gland and digestive system. Wyatt and Kalf (1957) showed the occurance of trehalose in various insect orders and noticed its presence during larval, pupal and adult stages. The phosphorylated glucose can be either changed over to trehalose or glycogen or mobilized by means of glycolysis and the pentose phosphate pathways. The rate at which trehalose is utilized has been seen to be almost at a similar rate at which it is synthesized and secreted. Intensive investigations on disaccharides and their hydrolysis have been completed in a few insects including the silkworm, *Philosamia ricini* and *Bombyx mori* by Pant and Morris (1974). In some species, carbohydrate utilization occurs more during the early stages of metamorphosis (Ranjini and Mohamed, 2004).

The decrease in total carbohydrate promptly before pupation could be attributed to its use for the quick anabolic processes associated with the biosynthesis of glycogen (Crompton and Birt, 1967). Hayakaewa and Chino (1968) reported that in the diapausing pupae of *Philosamia cynthia* a large portion of the glycogen in the fat body at first is changed over to trehalose when the pupae are exposed to a low temperature of 2°C and afterward the reverse reaction, trehalose to glycogen occurs when the pupae are returned to a higher temperature of 20°-25°C. In this manner, the inter conversion between glycogen and trehalose is temperature dependent in the insect and

dietary glucose is changed over to trehalose at low temperature. The accumulation of these sugars (trehalose or glucose) was expected to function as cryoprotectant and fuel for basal metabolism during winter (Somne, 1982).

Numerous over wintering insects accumulate sugar alcohols (glycerol, sorbitol or inositol) and/or sugars (trehalose or glucose) through the breakdown of stored glycogen (Somne, 1982). In some insects low temperature caused the synthesis of sugar alcohols or sugar (Baust, 1982). The variations in total soluble carbohydrates in the haemolymph, fat body and silk gland during third, fourth and fifth instar were studied and compared with the pupal period. The fluctuation was more during larval and early days of pupal period and then started accumulating till the adult emergence (Unni, 1988). These variations in carbohydrate supported more or less the findings about the conversion of fats in to carbohydrates during the developmental stages of the insect (Wyatt, 1957).

2.6.3. Lipid

The primary role of lipids is in the formation and functioning of insect cuticle and nutritional requirements of insects. Lipids have a structural role in all membrane systems of the cell. Phospholipids and steroids are important for this function. In addition to that the biochemistry and physiology of lipoidal hormones and pheromones has been studied extensively. Lipids have a role in regulation and information transfer since some hormones (ecdysone and JH) are lipoidal in nature and pheromones are volatile lipid. Deposition of lipids has a significant physiological value, theoretical calculations suggest that the conversion of hexoses to storage fat can take up to 20–25% of the energy content of the supplied food (Westerterp, 1993).

Fat body is the main site for the synthesis and storage of lipids in insects (Sun and Brookes, 1968; Thomas, 1974). The most important lipid

class in the fat body of all species which have been reported by Chino and Gilbert (1965) was triglyceride, which comprises up to 98% of fat body lipid in pupal and adult stage of *Hyalophora cecropia*. Other classes of lipid that have been detected in the fat body in small amounts including diglyceride, monoglyceride, sterols, sterol esters, free fatty acids, phospholipids (Thomas, 1974), glyceryl ethers (Tan, 1973); quinnones and tocopherol (Sridhara and Bhat, 1965). Sexual dimorphism of fat body lipids has been reported in numerous species (Bhakthan and Gilbert, 1972). The changes in the lipid in insect tissue have also been associated with diet, metamorphosis, reproduction, aging and exercise (Dutkowski and Ziajka, 1970).

Florkin and Jeuniaux (1973) reported about the presence of 5.5% lipid in the haemolymph of few species. Significant changes in the lipid content of the insect haemolymph during metamorphosis, development, exercise and oogenesis have been reported by Bollade and Boucrot (1971). Changes in the lipid content of holometabolous insects through the larval development was reported by (Gilbert and Schneiderman, 1961) Developmental changes in the lipid also have been reported by various investigators (Yurkiewicz, 1970). The fatty acid profile of the insect during the developmental stages also reported by numerous researchers (Madariago *et al.,* 1974; Fernandez-Sousa *et al.,*1971b).

Larval development of *S. litura* varied greatly depending on host plants and temperature, and the development was prolonged under low or high temperatures (Chen *et al.,* 2002; Seema *et al*., 2004). In addition to feeding, the environmental factors also affect the variations in biochemical components of the insect tissues. The temperature plays a major role in the physiological behaviour of the insects. The insects will get adapted to the low temperatures by synthesizing various cryoprotectants like glycerol, trehalose, sorbitol etc. with a drastic change in the components like protein,

carbohydrates, pyruvate, total free amino acids, total lipids, phospholipids and triglycerols (Pant and Radha, 1984).

Metabolic implications of insect physiological activities such as ecdysis, metamorphosis, flight ,nutritional behaviour lead to major biochemical changes (Neville, 1975). The biochemical changes associated with metamorphosis in holometabolous insects are well documented (Agrell and Lindquist, 1973). The rate of lipid utilization is known to vary during metamorphosis (D'Costa and Birt, 1966). These studies suggest that carbohydrates and lipids are primary energy reserves in these events.

Reduced intake of amount of food at low temperature is one of the factors for increase in protein content (Anithasingh *et al*., 2010). Carbohydrate is the energy requirements in case of organisms under low temperature acclimatization (Lee *et al*., 1993). Studies on cold acclimation of insects have shown that carbohydrates undergo profound metabolic changes and sugars of low molecular mass get accumulated (Lee *et al*., 1991).

Dietary compensation in herbivore insect is well documented (Raubenheimer and Simpson, 1993; Chambers *et al*., 1995). In contrast, to caterpillars the size of pupae was similar regardless of the intake of protein and carbohydrate, whereas caterpillars on treatments in which both foods had high macronutrient content developed fastest (Lee *et al.,* 2002). Carbohydrates are required for optimal growth and are mainly used as the source of energy needed to fuel the biosynthetic processes. Orr (1964a, *b)*have reported that the changes in the biochemical substances during maturation may be due to the juvenile hormone that regulates their mobilization from the fat body. Duration of development and gain in mass are two important performance variables for herbivore insect (often associated with fitness) and results from the analysis demonstrated that duration of development was a function of an interaction between the protein content and

the carbohydrate content of food, whereas gain in mass was a function of carbohydrate content.

The importance of keeping lipid levels low has been demonstrated in caterpillars reared on carbohydrate-biased foods for multiple generations. In only eight generations they evolved the ability to eat excess carbohydrate without laying it down as fat (Warbrick-Smith *et al*., 2006). Therefore fat body is regarded as a storage depot for lipids, carbohydrates and proteins and also as an important site of intermediary and synthetic metabolism. In the locust, fat and to a lesser extent, glycogen form the chief energy reserves for locomotion (Weis-Fogh, 1952).

2.7. Phytoconstituents in host plant leaves

Phytochemicals are the compounds which are formed during the plant's normal metabolic processes. These chemicals are commonly referred to as *"Secondary metabolites"* of which there are numerous classes including alkaloids, flavonoids, polysaccharides, phenols, coumarins, glycosides, terpenes , terpenoids and tannins (Okwu, 2004). In addition to these compounds, plants also contain other chemical substances and these can act as agents to avert unconsiderable side effects of the fundamental dynamic compounds or to aid in the assimilation of the main compound. Plants have an almost limitless capacity to synthesize aromatic compounds, mainly secondary metabolites of which 12,000have been isolated and that is estimated to be less than that of 10% of the total (Mallikharjuna *et al.,* 2007).

Bahorun *et al. (*2005) reported the phytochemical components of *Cassia fistula.* Maheswara *et al. (*2006) reported about two new homo isoflavonoids isolated from *Caesalpinia pulcherrima*. Rahaman *et al*. (2008) observed 3, 5, 7, 4-tetrahydroxy flavone from the leaves of *Cassia alata.* Preliminary phytochemical screening of the crude leaf extracts of *Pteridium*

aquilinum were conducted by Kardong *et al*. (2013). Agnel Ruba *et al. (*2013) carried out preliminary phytochemical analysis of *Arthocnemum fruticosum* leaf using five different solvents. Lincy *et al*. (2013) conducted the preliminary phytochemical study of *Ventilagoma deraspatana* whole plant, using different solvents. Imaga *et al*. (2010) and Yogiraj *et al*. (2014) has reported phytochemical and antioxidant constituents of *Carica papaya* leaf extracts. Phytochemical screeing indicate the presence of, alkaloids, saponins, glycosides, tannins folic acid, vitamin B_{12} and anthraquinons.

Awoyinka *et al. (*2007) reported different phytochemical compounds from the water and ethanol extracts of dried leaf of *Cnidoscolus aconitifolius*. Mohanta *et al. (*2007) analyzed different extracts of *Semecarpus anacardium* for their phytochemical properties. Uma Devi *et al. (*2007) carried out the phytochemical analysis in *Achyranthes bidentata.* Vaghasiya and Chanda (2011) reported the presence of tannins, cardiac glycosides, steroids and saponins in the methanol and acetone extracts of 14 plants belonging to different families. Various researchers reported about the phytochemicals present in the different leaf extracts of *Ricinus communis* (Henriques *et al*., 2005).

Vaghasiya *et al. (*2011) carried out preliminary phytochemical screening and estimated total phenolics and flavonoid contents in 5 traditionally used medicinal plants from western region of India. Khare (2007) carried out the phytochemical screening of C*colocasia esculenta* and found the presence of flavones, apigenin, luteolin, and anthocyanins. Sathishkumar *et al. (*2008) screened the invitro antioxidant properties of ethanol extract of *Canthium parviflorum* leaves. Mature and immature leaves and stems of eight plant species belonging to 7 families were screened for alkaloids, saponins, tannins and total phenolics contents by Achakzai, *et al. (*2009). Qualitative analyses were carried out by Chitravadivu *et al. (*2009). for detecting the bioactive compounds present in *Acalypha indica, Cassia auriculata, Eclipta alba* and *Phyllanthus niruri*.

A comparative phytochemical study between six Malaysian medicinal plants, belonging to different families, was carried out by Krishnaiah *et al. (*2009). The leaf, stem and root of *Ichnocarpus frutescens* were analysed for their phytochemical properties by Mishra *et al. (*2011). The dried leaf aqueous and methanol extracts of *Carica papaya* were carried out by Asaolu *et al. (*2010) for their phytochemical constituents. Ayo (2010) analyzed the extract of *Cassia nigricans*for determining the phytochemical constituents. Igwe *et al. (*2010) evaluated the phytochemicals, minerals and vitamin A and vitamin C compositions present in the leaves of *Spondiasmombin*.

Thenmozhi *et al. (*2011) examined the phytochemicals present in methanol extracts of *Eclipta alba* and *Emilia sonchifolia* using HPTLC. Quantitative analysis of phytochemicals by HPTLC was done by Mishra *et al. (*2011) in *Eucalyptus hybrid* leaves. Yamunadevi *et al. (*2011) investigated alkaloids profile of *Aerval anata* using HPTLC. Karthishwaran *et al*. (2010) carried out preliminary phytochemical screening in *Pergulariadaemia.* They also separated and identified compounds from the crude leaf extract using TLC, HPLC and HPTLC.

Abirami and Murugan (2011) determined flavonoids in *Cassia occidentalis*by HPTLC. Priti *et al. (*2009) carried out qualitative and quantitative analysis of phytochemical components in *Leidium sativum* using HPTLC. HPTLC fingerprint was drawn for the phytochemicals derived from the methanol leaf extract of *Acacia nilotica* by Venkataswamy *et al*. (2010). Joshi *et al. (*2011) examined the entire plant extract of *Cyathocline lyrata* for phytochemical constituents by TLC and HPLC. Bhise and Salunkhe (2009), by using TLC and HPTLC techniques, screened the phytochemical components from Ashwagandha, Tulsi, Mulethi, Awala, Shatavari, Gokharu, Arjun, Giloy, Safedmusli, Kalimirchi, Haldi and Jaiphal. The aerial parts of *Hypericum perforatum* were analysed to find out the bioactive compounds (Gioti *et al.,* 2009). Nirupama *et al. (*2012) reported the presence of

phytochemical constituents in aqueous and methanol extract of different parts of *Aeglemar melos* using HPTLC.

Photosynthesis is the production of organic compounds by utilizing radiant energy in living creatures having chlorophyll. Green pigment called chlorophyll and sunlight are required for photosynthesis. Chlorophyll absorbs sun light and converts it to chemical energy (Yakar and Bilge, 1987). The chlorophyll content is a significant experimental parameter in agronomy and plant biology research (Lamb *et al.,* 2012). Amount of chlorophyll indicates variations depending on numerous edaphic and climatic factors such as salt stress (Yıldırım *et al*., 2008; Acar *et al.,* 2011), light (Dai *et al.,* 2009), water stress (Demirel *et al.,* 2010), air pollution (Elkoca, 2003), fertilizing (Tunalı *et al.,* 2012) and also it shows alteration depending on time in vegetation period (Zavoruev and Zavorueva, 2002).).

Determination of the amount of chlorophyll can be utilized in many fields. Foliage chlorophyll content indicate absorbance of the leaf , and thus the amount of light absorbed for any given incident light availability (Niinemets, 2010). The chlorophyll content is an indicator in determination cold tolerance of plants (Perks *et al*., 2004). Demirel *et al., (*2010) stated that chlorophyll measurements can be used in order to decide water stress at especially beginning of flowering period and ripening period.

Influence of various shadow conditions on chlorophyll in leaves have been presented by numerous researches (Dai *et al.,* 2009). The amount of chlorophyll in leaves shows an alteration by being influenced by numerous factors. In addition to this, it is known that plant species and position of leave influence the amount of chlorophyll in leave (Gond *et al.,* 2012).

CHAPTER III
MATERIALS AND METHODS

3.1. MATERIALS

3.1.1. Experimental insect: *Spodopteralitura* (Fabricius) (Lepidoptera: Noctuidae)

Tobacco caterpillar, was chosen as the experimental organism since it is one of the most imperative polyphagous pest making economic loses to a large number of crops like cotton, groudnut, cabbage, cauliflower and so forth. In addition to this, it has also developed resistance to a substantial number of insecticides belongs to various groups.

Life cycle of *Spodopteralitura*

The life cycle of the *S. litura* involves four stages, egg, larval, pupal and adult stage. Many intrinsic and extrinsic factors such as climatic conditions, population density, the plant leaf quality and larval stage etc. determine the feeding rate and development of the larva, adult fecundity, and life span. Based on these factors, the life cycle of this insect varies from 23-37 days.

Eggs

The eggs of *S. litura* are characteristically laid in an irregular manner on the lower surface of the host plant leaves. The egg masses strongly adhere to the leaf surface. The hair scales from the tip of the female abdomen covers the egg as a felt-like covering. The egg hatches within 3-4 days. The unhatched eggs are fed by the neonates in the initial stage.

Larva

After hatching, the first instars have 2-3mm length with black head and white body. As the caterpillars start feeding their colour changes to a translucent green with a dark thorax. During the day time they hide under the leaf or bottom of rearing bottles. There are six larval instars and each instar has a duration of 2-3 days but the sixth instar stage is longer, lasting for 4-6 days. Soon after hatching, the development of characteristic marking can be seen on their back. These marking patterns varies during the different larval period. Initially on the dorsal surface there appear white marking which gradually changes to a dark yellow stripe. The older larvae completely defoliate the plant by feeding stem, buds, flowers and fruits. The larval duration varies from 19-20days. When the larva reaches the pre-pupal stage it burrows in to the soil below the plant for few centimeters and there it pupate by making a covering with soil. It produces a fluid during the pre-pupal period.

Pupa

The pupal size varies from 15 to 20mm in length. They are red brown in colour. Generally the pupae of females are larger than the male. At the posterior end of the pupae, there is five circular marking in both the sexes, but the female pupa can be differentiated by the presence of an opening on 5^{th} circular mark where as in male it is on the 4^{th} circular mark. The duration of the pupal stage is about 7-9 days in winter, but is prolonged in summer. The female moths emerge in a day or two before the male moth.

Adult

The adult moth is brown in colour with a complex pattern of cream streaks crisscrossing the forewing and silvery white hind wings. Wing- span of adult is about 4cm. The proboscis helps the adult moth to feed on the nectar

from flowers. Pheromones secreted by the female moth help to attract the male. An adult female lays about 300-1200eggs. Egg laying starts with in 2-4 days after adult eclosion. Oviposition starts from third day and can extend up to seventh day and then subside. The female moth is having characteristic tufts of anal hairs. A blue-grey band running from the apex to the inner margin of each forewing is characteristic of male moths.

Nature of damage

S. litura is a ploy phagous pest and reported on more than 120host plants everywhere throughout the world (Moussa *et al.,* 1960) and known to make severe damage to many crops. It is a major defoliator. The polyphagous character demonstrates the capability of tobacco caterpillar to use effectively, an extensive variety of host plants. The damage is done by the larvae, which feed gregariously on leaves and fresh tender shoot. Neonate and second instar larvae nibble epithelium of the leaf, third and fourth instar larvae damage the leaves by making holes, later fifth and sixth instars defoliate the green foliage completely leaving only veins and midribs. The host plants play an important role in maintaining the continuity of the pest throughout the year.

3.1.2. Experimental hostplants

Five different host plants *viz*., castor (*Ricinuscommunis*Linn.), papaya (*Carica papaya* Linn.) , colocasia (*Colocasiaesculenta* (L.) Schott.),banana *(Musa acuminate* (L.) Colla*)* and sweetpotato (*Ipomoea batatas* (L.) Lam.) were selected for the study. Fresh host plant leaves were collected daily from the local areas near Malabar Christian College campus, Calicut for maintaining the culture.

***Colocasiaesculenta* (L.) Schott**

Family : Araceae

Common Name: Taro, Elephant Ears.

It is otherwise called *Arum esculentum L.* and, *Colocasiaantiquorum* Schott. Though originated in Tropical Asia, it occurs all through India and is cultivated around the world. It is a wild plant and cultivated all through the hotter parts of India and Ceylon. It flourishes in hot, humid conditions and is found growing in moist forests and wet territories in riparian habitats, riverbanks, along streams, marshes and canals or cultivated near farm houses, in water fields or as under-planting in coconut groves.

C. esculenta is an enduring herb 3-7 feet tall, tuberous or with a heavy short caudex, flowering and leafing together. Leaves all basal from a corm, sharp edges to 2 feet long, upper surface smooth green to somewhat bluish black between primary veins, petioles green to violet or reddish; spathe 15 inches long, basally green or red-purple, blade extended, reflexed and yellow. Flowers are yellow to orange, deciduous or wilting. Plant bears a massive, fleshy, starchy altered underground stem known as corm.

Mostly leaves contain calcium oxalate, fibers, minerals (calcium, phosphorus, and so on.), vitamin A, B, C, and so forth. Phytochemically, they contain flavones, apigenin, luteolin, anthocyanins, sterols, glycosides and different micronutrients. Tubers contain globulins representing 80% of the aggregate tuber proteins, amino acids, starch, nitrogen and lipids.

Generally it is utilized as a stimulant, astringent, expectorant, otalgia, and appetizer internal hemorrhage. It is additionally used to arrest arterial hemorrhage, ear ache, diarrhea and body ache. It is also used in cases of piles and congestion of the portal system, as an antidote to the stings of wasps and

other insects. Pharmacological reports revealed that the plant has different pharmacological activities like analgesic, anti-inflammatory, anti-cancer, anti-diarrheal, astringent, nervine tonic, hypolipidemic activity, hypoglycemic property and antifungal activity. It also have important roles in heart health, reduce blood pressure, immune system and digestive health, boost vision, enhance learning, against diabetes, circulation stimulation, against rheumatoid arthritis, dental and skin health etc.

***Ricinuscommunis* L.**

Family : Euphorbiaceae (Spurge).

Common name : Castor

It is one of the tropical flowering plant species and has been found to grow generally over the world. Castor bean is native to the tropics (Africa) however it is planted as a garden plant all through the U. S. for its expansive, striking appearance. It is currently commercially developed in the U. S. in Illinois, Missouri, Kansas, Oklahoma, Oregon and California. As a result, it is naturalized in the south where winters are gentle and is regularly found near streambeds, dumping grounds, barnyards or along roadsides. It is a woody herb, grown as an ornamental in gardens, sometimes as a houseplant and further more develops as a weed. It is annual in the south and perennial in the tropics and it might achieve 15 feet tall outdoors.

Castor bean is a tall herbacious annual, which can reach to nearly 2– 4 meters high when growing in open spaces in warm atmosphere. Large leaves are alternate and are about 15- 45 cm long, palmately lobed with 5-11 toothed lobes. Leaves are glossy and regularly red or bronze tinted when young. Flowers are formed in groups at the end of the primary stem in late summer.

The distinctive parts of the plant are utilized by nearby communities and backwood tenants in the treatment of different afflictions. Its ancient and

current remedial uses have been studied and detailed and different parts of the plant were utilized for therapeutic purposes. The extracts from plant parts possess different important impacts, like analgesic, diuretic, antidiarrhea, antiasthmatic, antihelminthic and numerous other therapeutic advantages. The leaves and roots extract and the seed oil have been utilized as a part of the customary drug as diuretic, and in the medicines of headache, backache, rheumatism, abscesses, dropsy, warts, ringworms, hypoglycemia, inflammation and liver disorder. External use of the leaves extract was appeared to increase milk flow in nursing mothers while the oil reduces pregnancy labour, resulting in quick delivery.

Three terpenoids and a tocopherol-related compound have been found in the aerial parts of *R. communis*. The lethality of raw castor beans is due to the presence of ricin. This poison gives the castor plant with some level of natural protection from insect pests such as aphids. Ricin has been explored for its potential use as an insecticide. The plant is likewise an exceptionally strong trigger for asthma and allergies to *Ricinus* are ordinary and severe. The sap of the plant causes skin rashes. The castor plant is also the source for undecylenic acid, a natural fungicide.

Castor oil has many uses in prescription and different applications. Methanolic extracts of the leaves of *R. communis* were indicated antimicrobial properties. The pericarp of *R. communis*showed central nervous system impacts in mice at low doses. At high measurements mice immediately died. Water extracts of the root and bark demonstrated analgesic action in rats. Antihistamine and anti inflammatory properties were found in ethanolic extracts of *R. communis* root bark. Castor oil is an effective engine oil and has been utilized in internal combustion engines.

***Musa acuminata (*L.)Colla**

Family : Musaceae.

Common Name :Banana

Edible bananas originated in the Indo-Malaysian region coming to northern Australia. Presently it is distributed in numerous nations such as Mediterranean region, Europe, South America, and Hawaii. Bananas normally grow in all humid tropical areas and constitute the fourth biggest fruit crop of the world. Banana is one of the most economically important crops in the world which is widely cultivated for its delicious fruit.

The banana plant is the largest perennial herb in the world and can grow up to15 m tall. It is a herb, with succulent, extremely juicy stem ("pseudostem") which is a cylinder of leaf-petiole sheaths, reaching a tallness of 20to 25 feet (6-7.5 m) and emerging from a fleshy rhizome or corm.

The banana is astaple crop, providing nourishment from fruit, bud and stem. As a food, banana is used in various ways. The fruits can be essentially peeled and eaten, or used to make distinctive drinks, cakes, syrups etc. Ripe bananas are fermented into beer and wine. Banana leaves are used instead of plates and for wrapping and packing away foods. The peel of dried banana has high tannin content and is utilized for darkening the leather. The ash remains from the dried peel of the banana is rich in potash and is used as a part of cleanser making.

Banana has numerous therapeutic uses, the flowers are utilized to treat bronchitis and diabetes, the astringent plant sap is presumed to be successful in treating epilepsy, hysteria, fever, diarrhea and can likewise ease haemorrhoids and insect bites and stings, the young leaves are utilized as poultices on burns. The roots are utilized for stomach related problems, the

peel and pulp of ripe bananas are found to have antibiotic and antifungal properties.

***Carica papaya* L**

Famil : Caricaceae.

Common name: Papaya

Inspite of the fact that the exact area of origin is unknown, the papaya is believed to be native to tropical America. But now it is distributed in numerous countries such as Panama, Dominican Republic , South and Central America, Southern Mexico, West Indies, Bahamas, Philippines , Malacca, India, Naples, Florida, Colombia, Puerto Rico Cuba, and New York. Today successful commercial production is primarily in Hawaii, tropical Africa, the Philippines, India, Ceylon, Malaya and Australia.

Papaya is a short-lived perennial, growing upto 30ft (9.14 m) high. Its hollow, herbaceous stem is normally unbranched. The profoundly lobed, palmate leaves are borne on long, succulent, green or more or less dark purple, hollow horizontal petioles 1 to 3.5 ft (30-105 cm) long, rising up out of the stem apex. The blade, deeply divided into 5 to 9 main segments, each sporadically subdivided, varies from 1 to 2 ft (30-60cm) in width and has prominent yellowish ribs and veins. The life of a leaf is 4 to 6 months. Both the stem and leaves contain copious white milky latex.

Ripe papaya is typically consumed fresh as a breakfast or dessert fruit; it can also be processed and utilized in a variety of products such as jams, fruit juices, and ice cream. Papaya is also consumed as a dried fruit. Unripe fruits and leaves are consumed as vegetables. The latex from papaya is either sun-dried or oven-dried and sold in powdered form to be utilized as a part of beer clarifiers, to treat wool and silk before dyeing, as an adjunct in rubber

manufacturing, meat tenderizers, digestion aids, wound debridement aids, tooth-cleaning powders, cosmetics and detergents, and in pharmaceutical preparations to help digestion. Papaya is a rich source of vitamin C and A. A root decoction is claimed to expel roundworms. Dried leaves have been smoked to relieve asthma or as a tobacco substitute.

***Ipomoea batatas* (L.) Lam.**

Family : Convolvulaceae

Common name : Sweet potato

Ipomoea batatas is native to the tropical regions in America (Central America or South America). Sweet potatoes are cultivated throughout the world wherever there is sufficient water to support their growth. Sweet potato is widely cultivated in Philippines, Indonesia, Vietnam, India, and some other Asian countries. The sweet potato is a dicotyledonous plant. Its large, starchy, sweet-tasting, tuberous roots are used as vegetable. The young leaves and shoot are sometimes eaten as raw. This is a herbaceous perennial plant, bearing alternate heart-shaped or palmately lobed leaves and medium-sized sympetalous flowers. Its root pulp ranges from beige through white, red, pink, violet, yellow, orange and purple. The plant does not tolerate frost. It grows best at an average temperature of 24^0C (75^0F) with abundant sunshine.

Besides simple starches, raw sweet potatoes are rich in complex carbohydrates, dietary fiber and beta-carotene (a provitamin Acarotenoid), while having moderate contents of other micronutrients, including vitamin B_5, vitamin B_6 and manganese. Sweet potato leaves and shoots are rich sources of vitamin A, C, and B_2 (riboflavin) and are a good source of lutein. The young leaves and vine tips of sweet potato leaves are widely consumed as a vegetable and also used in baby foods, salads and soups. Tubers are used mainly for breakfast. It is also used to make baked products such as cakes,

chapatis, bread, buns and cookies. Other uses are to make dye for cloth, as food colouring. All parts of the plant are used for animal fodder and for biofuel production.

Extracts from the starchy root and leaves may offer health benefits. It is used to treat combat diabetes, heart disease, as well as for anti-inflammatory activities.. Sweet potato contains properties that help to fight heart disease. It is effective at promoting healthy blood sugar, and significantly reduce cholesterol and fasting glucose. The high antioxidant activity of sweet potato appears to exert anti-cancer activity.

3.1.3. Chemicals and Equipments

Chemicals

1) Acetone
2) Acrylamide
3) Amino acid standard kit
4). Ammonium persulphate
5). Anthrone
6). Bovine serum albumin
7). Bromophenol blue
8). Cadmium acetate
9). Calcium chloride
10). Catechol
11). Chloroform
12). Citric acid
13). Coomassie brilliant blue
14). Copper sulphate
15). Diethyl ether

16). Disodium hydrogen phosphate

17). Disodium phenyl phosphate

18). 80% ethanol

19). Folin-ciocalteau reagent

20). Glacial acetic acid

21). Glucose

22). Glycerol

23). Glycine

24). Honey

25). Hydrochloric acid

26). Hepes

27). Methanol

28). Mono sodium hydrogen phosphate

29). Magnesium Sulphate

30). Ninhydrin

31). Olive oil

32). Orthophosphoric acid

33). Phosphomolybdic acid

34). Potassium chloride

35). Sodium bicarbonate

36). Sodium carbonate

37). Sodium chloride

38). Sodium dodecylsulphate

39). Sodium hydroxide

40). Sodium potassium tartarate

41). Sodium tungstate

42). Sucrose

43). Sulphuric acid

44). Tannic acid

45). TEMED

46). Toluene

47). Tris-base

48). Tris-HCl

49). Vanilline

50). Wax

Instruments

1). Capillary tube

2). Centrifuge tube

3). Cotton

4). Digital pH Meter

5). Dissection set

6). Eeppendorf tube

7). Electronic balance

8). Electrophoresis apparatus.

9). Gel documentation unit.

10). Glass slide

11). Glass wares

12). Gloves

13). Incubator

14). Magnetic stirrer

15). Micro pipette

16). Microscope

17). Microwave oven

18). Petri plates

19). Pipette

20). Plastic bottles

21). Plastic trough

22). Reagent bottles

23). Rearing cage

24). Spectrophotometer

25). Tissue homogenizer

26). Tissue paper

27). Ultracentrifuge

28). Vortex mixer

29). Watch Glass

30). Water bath

3.2. METHODS

3.2.1. Mass rearing of *S. litura*

Maintenance of Adult moth

The pupae obtained from NBAIR, Bangaluru were kept in rearing cages containing moistened cotton to avoid pupal death from dehydration during summer months. On emergence males and females were transferred in to small plastic containers for mating, having 20cm height and 15 cm diameter covered with cotton cloth. A sheet of paper is lined below the bottle as a substratum for the females to lay eggs. Cotton swab soaked with 20%honey were provided as the food source, which were hanged along the side of the containers. The adults were transferred to fresh bottles every day to avoid the feeding of eggs by the newly hatched larvae.

Maintenance of egg

The paper containing patches of eggs was kept in a tray in the cage with high humidity till it hatched. The eggs were observed daily for hatching. The newly emerged larvae were transferred to plastic bottles with fresh leaves.

Maintenance of larvae

Larvae were reared in plastic containers and the mouth of the containers was covered with cotton cloth. The larvae were fed with excess of leaves of five selected host plants (castor, papaya, banana, colocasia, sweet potato) in separate containers. Daily the old leaves were replaced with the fresh leaves. At the fifth instar stage before the prepupal stage they were transferred to the plasic trays for pupation. The tray contained moist soil at the bottom to avoid desiccation and is covered with muslin cloth.

Maintenance of pupae

The pupae from the sand were taken out by using forceps, taking care not to cause any injury. Then they were transferred to the cage for adult emergence.

3.2.2. Temperature and humidity.

The mass rearing of the culture was carried out in different seasons at different ranges of temperature and humidity under laboratory conditions.

1) Early summer (February to March middle): The average temperature maintained was 32± 2 ^{0}C and average relative humidity was 80%.

2). Monsoon (June to September) : The average temperature maintained was 27± 2^{0}C and average relative humidity was 88%.

3). Post monsoon (October to January): The average temperature maintained was 23± 2^0C and average relative humidity was 85%.

3.2.3. **Determination of Moisture Content:**

To determine the moisture content of the leaves, leaf samples of different host plants were collected.

They were washed thoroughly with distilled water, drained and the remaining water was blotted off with a blotting paper. Then weighed accurately about 10gm of fresh leaves of each host plant and placed it in a tared evaporating dish and kept it a hot air oven and then dried at 105°C for two hours and weighed. Continued the drying and weighing in each half an hour interval until to get a constant weight. The percentage of moisture was determined by the formula:

$$\text{Moisture content} = \frac{I-F}{I} x100$$

Here,

I = Initial weight of the leaf sample

F = Final weight of the leaf sample after drying

3.2.4. Determination of larval instars

Once the larvae moulted, the exuviae with head capsules were collected and kept in 70% ethanol. The width of the collected head capsules was measured using a Leica M 205A Stereozoom Microscope with imaging software [LAS V 4.7.1]. In addition, to distinguish different larval instars by length, the sizes of larvae were measured immediately after each ecdysis. Measurements were taken until the prepupal stage, when feeding was terminated.

3.2.5. Developmental studies

The developmental studies were carried out in the laboratory in three different seasons (early summer, monsoon and post monsoon seasons). The newly laid egg masses were placed inside plastic containers and were labeled with the date on which the eggs were laid. Eggs were incubated under laboratory conditions in different seasons at respective temperature and relative humidity. Upon hatching, the neonates were transferred individually to five separate plastic containers and each set of larvae in theses containers were fed separately with freshly excised leaves of five selected host plants. The larva inside each container was examined daily for ecdysis and upon moulting, the larval length and weight was measured. The instar duration and total larval duration were also noticed. When the larvae turned into the pre-pupal stage, the container was filled with soil. The prepupal and pupal period was recorded. After pupation the weight of male and female pupae were taken. Upon emergence of the adults, pairs of female and male moths were placed inside a container for mating. The preoviposition and oviposition duration, the number of eggs laid and moth longevity were recorded. A total of 150larvae from three subsequent generations (50larvae from each generation) in three different seasons were used. The survival of each individual larva was checked daily during the whole developmental period. Percentage stage-specific survival was calculated by dividing the number of the individuals still alive at the end of each life stage by the number of specimens at the beginning of each life stage. The overall survival rate percentage was calculated by dividing the number of emerged adults by the number of eggs tested. The life cycle duration was obtained from 50larvae which completed their development during the study.

3.2.6. Food consumption and utilization

Newly exuviated sixth instar larvae of *S. litura* that had been reared on each of the five selected host plant species were used in this study. Larvae of approximately the similar size were selected and the larvae were allowed to starve for 3 hrs and then each larva was individually coded and weighed. For each host plant, twenty larvae were used and they were divided into two sets of 10each-one maintained as control group and the other as test group. Likewise the leaves of each host plant was cut into large bits of similar size and they were also divided into two sets of 10bits- one set as control and the other test. In the control group, the 10larvae and 10fresh leaf bits were individually weighed and marked and then dried in an oven at 80° C and weighed again. The dry weights were used as the standard. In the test group , 10larvae and 10bits of leaves were weighed individually and marked. Then each larva was provided with one large bit of marked leaf for 24hrs. feeding. The larvae were then starved for 6 hrs to allow the larvae to defecate. The larvae, leaf tissues, and faeces in each container were weighed and then dried in an oven at 80° C. The dried leaf tissues , faeces and larvae were weighed again. Food utilization rates were then calculated based on the formula of Waldbauer (1968):

Relative growth rate = $\frac{D-C}{\frac{C+D}{2}}$ ----------[1]

Relative consumption rate = $\frac{A-B}{\frac{C+D}{2}}$----------[2]

% Efficiency of conversion of ingested food = $\frac{D-C}{A-B} X100$----------[3]

% Efficiency of conversion of digested food = $\frac{D-C}{A-B-E} X100$-----[4]

Approximate digestibility = $\frac{A-B-E}{AB} X100$---------[5]

where A is the weight of dried leaf tissues in the control, B is the weight of the dried leaf tissue in each test, C is the weight of dried larvae in the control, D is the weight of dried larvae in each test, and E is the weight of dried faeces in each test.

3.2.7. Biochemical Analysis

3.2.7.1. Quantitative estimation of total protein in different tissues of *S. litura*

The estimation of total protein in different tissues was carried out using the standard method of Lowry*et al. (*1951)

Reagents

1. 2% sodium carbonate in 0.1Nsodium hydroxide (Reagent A)
2. 0.5% copper sulphate ($CuSO_{4.5}H_2O$) in 1% potassium sodium tartarate (Reagent B)
3. Alkaline copper solution. Mixed 50ml of reagent A and 1 ml of reagent B prior to use. (Reagent C)
4. Folin-Ciocalteau Reagent;
5. Protein standard solution (Stock Standard): Weighed accurately 50mg of bovine serum albumin (Fraction v) and dissolved it in distilled water and made up to 50ml in a standard flask.

6. Working Standard: Diluted 10ml of the stock solution to 50ml with distilled water in a standard flask. One ml of this solution contains 200µg protein.

Sample preparation

Midgut

S. litura larvae were reared on five selected host plants. The midgut homogenates were prepared by using the last instar larvae of *S. litura*. Five sets of final instar larvae each fed with selected host plant leaves were taken and each set consisted of five larvae and for each plant 5 samples were prepared with tissue from one larva each. The larvae were anaesthetized on ice, midgut was dissected out, weighed after removing the adhered fat body and trachea by using forceps and after ringer was blotted off , it was homogenized in ringer (1 ml for each gut). To the homogenate added 80% ethanol to precipitate the protein from the sample and was centrifuged at 12, 000rpm for10min. The residue was dissolved in 0.1N sodium hydroxide by boiling in a water bath for 5 min. The clear supernatant was transferred to an eppendorfs tube. The protein present in the sample was estimated using the method of Lowry*et al. (*1951).

Fat body

Last instar larvae of *S. litura* reared on five different host plants were used for preparing fat body homogenate. The final instar larvae were anaesthetized on ice, fat body was dissected out, weighed after blotting off ringer adhered to it and homogenized in ringer (1 ml). Five samples were prepared for each plant and each sample consisted of tissue from single larva.80% ethanol was added to the homogenate to precipitate the protein from the sample and was centrifuged at 12, 000rpm for10min. The residue was dissolved in 5 ml of 0.1N sodium hydroxide by boiling in a water bath for

5 min. The clear supernatant was transferred to an eppendorfs tube and the protein present in the sample was estimated using the method of Lowry *et al. (*1951).

Procedure

For both the midgut and fat body the procedure for the quantitative estimation of protein is common. To 1 ml of the protein homogenate of midgut and fat body added 5 ml of alkaline copper reagent and was kept for incubation at room temperature for 10-15min. Then added 0.5ml of Folin-Ciocalteau reagent (1:1dilution) and allowed to stand for 30min for incubation at room temperature. The colour developed was read in a visible spectrophotometer at 650nm against the reagent blank. A standard set of BSA was also carried out in the same manner.

Haemolymph sample

Five sets of five last instar larvae fed with five selected host plants were separated. The haemolymph was collected from the larvae by amputing the thoracic legs and using a capillary tube it was taken in to a prechillled vial containing 0.5ml of 10% sodium tungstate to avoid melanization. The protein present in the sample was estimated using the method of Lowry*et al*. (1951). Five replicates were maintained for each estimation consisting of haemolymph from single larva.

Procedure

The collected haemolymph sample was centrifuged after adding 0.5 ml of 2/3N sulphuric acid for 5 minutes. Then the residue was dissolved in 1ml of 0.1N sodium hydroxide by boiling in a water bath for 5minutes. This sample was used to estimate the protein content as mentioned in 3.2.7.1.

3.2.7.2. Protein profiling by SDS-PAGE

Protein profiling of different tissues of *S. litura* by feeding different host plants were carried out by Sodium Dodecyl Sulphate-Poly Acrylamide Gel Electrophoresis (SDS-PAGE) at room temperature according to the Laemmli (1970) method.

Sample preparation

The midgut and fat body tissues were collected as described in section 3.2.7.1. From this 100mg of tissue was taken and homogenized in 1 ml of phosphate buffer and centrifuged and the clear supernatant was used for the analysis. The haemolymph was collected from the larvae in pre chilled phosphate buffer containing vials and kept under deep freeze until use. From the stored haemolymph 100µl of the haemolymph sample was taken and centrifuged after adding 1ml of phosphate buffer and the supernatant was taken for the analysis. The protein samples were mixed with equal volumes of sample buffer and the samples were heated in a water bath for 3 minutes at 95^0C. The denatured samples were kept in a refrigerator at -20^0C till use.

Procedure

A 12% linear resolving gel and 5% stacking gel were used to separate the proteins. A sandwich with two glass plates separated with a spacer strip were made (1.5mm thickness). The resolving gel mixture was poured in to the space between glass plates. To exclude air from inhibiting polymerization and to ensure a uniform flat gel surface a seam of distilled water was layered on to the gel. Formation of a sharp interface between the polymerized gel and the overlay was an indication of complete polymerization. After decanting the water layer from the surface 5% stacking gel was prepared and poured above the resolving gel and a teflon comb was inserted between the glass plates to form well. Care should be taken to avoid trapping of air bubbles during the

gel casting. The teflon comb was removed after polymerization. After removing the basal strip the glass plate with polymerized gel was fixed on to the electrophoretic apparatus. Protein samples as prepared earlier loaded in to each well and running buffer was added. Electrophoresis was carried out for 3 hrs at a constant voltage of 30V in the region of stacking gel and 90V in the region of resolving gel.

After electrophoresis, the gel was stained with staining solution Coomassie brilliant blue R-250for 6 hours. The gels were later destained with acetic acid and methanol. The photographs of the gel was taken and gel documentation and molecular weight determination were carried out.

3.2.7.3. Quantitative estimation of total free amino acids in different tissues of *S. litura*

The total free amino acid estimation in different tissues was carried out by standard method of Lee and Takahashi (1966).

Reagents

1. Amino acid Standard: Dissolved 5mg of glycine from a standard kit in 80% ethanol, so as to get 0.1% amino acid standard solution.

2. Ninhydrin - Cadmium acetate reagent: Dissolved 0.5g of ninhydrin in 12.5ml ethanol and added 0.5gm cadmium acetate. Covered the bottle with black paper and refrigerated

a) Midgut and fat body sample preparation

The supernatant collected after the precipitation of protein by homogenizing the midgut and fat body by adding 80% ethanol (as described in section 3.2.2.1) were used for the estimation of amino acid.

b) Collection and processing of haemolymph

The larvae were anesthetized, and the haemolymph was collected by amputing the legs using a calibrated capillary tube. The haemolymph was deproteinized using 80% ethanol and centrifuged at 10,000rpm for 10minutes. Supernatant was used for estimation.

Procedure

0.5ml of the supernatant left after the precipitation was mixed with 0.5mlof Ninhydrin-Cadmium acetate reagent and added 5 ml distilled water to form a total of 6ml. Boiled the mixture in a waterbath for 20minutes. Then cooled and allowed to stand for 15 minutes. The colour developed was measured at 540nm in a spectrophotometer. A set of glycine standards were also carried out in similar manner.

3.2.7.4. Estimation of total carbohydrate in different tissues of *S. litura*

The carbohydrate content estimation in the midgut, haemolymph and fatbodytissues were carried out by the standard procedure of Anthrone method (Mu, P. and Plummer,1988).

Reagents

1. Anthrone reagent; Dissolved 200mg of anthrone in 100ml of ice cold 95%H_2SO_4, prepared fresh before use.
2. Standard Glucose Stock: Dissolved 100mg in 100ml distilled water
3. Working standard-10ml of stock diluted to 100ml with distilled water and stored in refrigerator after adding a few drops of toluene.

Preparation of tissue samples

The midgut and fat body homogenates were prepared as described in section (3.2.2.1) similarly haemolymph sample was also collected as described in section (3.2.1.1). To the midgut, fat body and haemolymph samples 80% ethanol was added and then centrifuged at 12,000rpm for 10min. The supernatant obtained after the precipitation of protein is transferred in to a small dish and allowed to evaporate by keeping it overnight in an oven at 60^0C. The total carbohydrate content in the sample was precipitated at the bottom of the dish, it was scraped using a glass rod. This was then dissolved in 1ml of distilled water, centrifuged again and collected the supernatant.

Procedure

From the collected supernatant 0.5 ml sample was used for analysis. Prepared the standards by taking 0.2, 0.4, 0.6, 0.8 and 1 ml of the working standard. The volume was made to 1ml in all the tubes including the sample tubes by adding distilled water.1ml distilled water was used as blank. Then added 4 ml of anthrone reagent to all tubes and heated for eight minutes in a boiling water bath. It was cooled rapidly and the green to dark green colour developed was read at 630nm.

3.2.7.5. Estimation of total lipids in different tissues

Total lipids in animal tissues and leaf tissue was estimated according to the method of Frings and Dunn (1970).

Reagents

1. Hepesbuffer:Composition:NaCl (10mμ), KCl (12mμ), $MgSO_4$ (2mμ), Na_2HPO_4 (1mM), Hepes (30mM), Glucose (50mM), $CaCl_2$ (1mM), Sucrose (50mM) and BSA (2%). These ingredients were dissolved in

distilled water by keeping it in a magnetic stirrer. The pH was adjusted to 7.2 using a digital pH meter.

2. Conc. H_2SO_4

3. Phospho-vanillin reagent: Dissolved 0.6 g of vanillin in 100ml water in a 100ml volumetric flask and made up the volume with water (vanillin reagent). Mixed 35ml of vanillin reagent and 60ml of concentrated phosphoric acid with constant stirring and added 5.0ml of water and stored in a brown bottle at room temperature .

3. Standard-olive oil-stock is prepared by dissolving 1 g in 100ml chloroform.

4. Working standard - Prepared by diluting 1ml stock to10ml (50-500µg) chloroform.

Fat body and midgut sample preparation

The fat body and midgutwere removed from the larvae and chopped and then taken in pre-weighed incubation vials containing fixed volume of incubation buffer (200µl) and the weight of the fatbody and midgut were determined and kept for incubation.

Haemolymph sample preparation

The haemolymph was collected from the larvae as described in section (3.2.2.1) and the collected haemolymph was transferred in to a vial containing fixed volume of incubation buffer and kept for incubation

Extraction of lipid from the samples

After the *invitro* incubation of the samples of midgut, haemolymph and fatbody with the incubation buffer, the lipid released in to the medium was extracted by the standard methodof Frings and Dunn (1970).

A known volume of the incubation medium was drawn out from the sample incubation vials by using a micropipette. While drawing out the sample care must be taken to avoid the tissue pieces. The drawn out samples were allowed to stand for 15 minutes. Then 1ml of1M sodium chloride and 1ml of chloroform were added to the sample. The mixture was shaken well and kept for 15 minutes (or centrifuged for 3min at 10000rpm) to separate the aqueous and organic phase. The lower chloroform layer containing the extracted lipid was drawn out by removing the aqueous phase. The lipid sample was kept at room temperature to evaporate the chloroform from the sample. From this sample the amount of lipid in the tissues was estimated by using the method of phosphovanilline (Frings and Dunn, 1970).

Procedure

From the extracted samples 0.1ml was taken in each test tube and added 0.1 ml of concentrated $H_2SO_4$to each tube and they were heated in a boiling water bath for 10minutes. The test tubes were cooled and 5 ml phospho-vanillin reagent was added to all the tubes and incubated at 37^0c for 15 minutes. The optical densities were measured against the blank at 540nm in a spectrophotometer. A series of olive oil standards were also carried out in the same manner.

3.2.7.6. Quantitative estimation of total protein in leaf tissue

Estimation of protein in leaf tissue was carried out by using the standard method of Lowry *et al. (*1951)

Extraction of protein from sample

Weighed 500mg of the leaf sample and ground well with a pestle and mortar in 5-10ml of the phosphate buffer. It was centrifuged and the supernatant was used for protein estimation.

Procedure

Reagents and procedure as described in section 3.2.7.1.

3.2.7.7. Estimation of total free amino acid in leaf tissue by Ninhydrin Method

Procedure

Ninhydrin :0.8 gm of ml stannous chloride dissolved in 500ml of 0.2M citrate buffer (pH 5). Mix this solution with 20g of ninhydrin in 500ml of methyl cellosolve.

0.2M. Citrate buffer

Diluent solvent :Mix equal volume of water and n-propanol.

Preparation of standard: 50mg of leucine dissolved in 50ml of distilled water in a standard volumetric flask.10ml of this stock was diluted to 100ml in another volumetric flask for the working standard. A series of volume 0.1 ml to 1ml of this standard was used and proceeded as that of the sample**.**

Preparation of sample

500mg of the leaf sample weighed and ground it in a pestle and mortar with a small quantity of acid washed sand. Add 5-10ml of 80% ethanol to this homogenate and centrifuged. Saved the filtrate. Repeated the extraction twice with the residue and collected the supernatants and these extracts were used for the estimation of total free amino acids.

Procedure

To 0.1 ml of the extract added 1 ml of ninhydrin solution and made the volume to 2 ml in all the test tube with distilled water. Heated the tubes in a boiling water bath for 20min. Added 5 ml of the diluent and mixed well.

After 15 minutes read the intensity of the developed purple colour against the reagent blank in a spectrophotometer at 570nm.

3.2.7.8. Estimation of total carbohydrate in leaf tissue by Anthrone Method

The carbohydrate content estimation in the leaf tissue was carried out by the standard procedure of Anthrone method (Plummer, 1971).

Reagents

1 2.5 N-HCl

2. Anthrone reagent : 200mg of anthrone dissolved in 100ml of ice cold 95%H_2SO_4. prepared fresh before use.

3. Standard Glucose Stock: Dissolved 100mg in 100ml distilled water

4. Working standard-10ml of stock diluted to 100ml with distilled water and stored in refrigerator after adding a few drops of toluene.

Procedure

100mg of the leaf tissue was weighed in to a boiling tube and it was hydrolysed by keeping it in a boiling water bath for three hours with 5 ml of 2.5N HCl and cooled to room temperature. It was neutralized with solid sodium carbonate until the effervescence ceased. Then the volume was made up to 100ml and centrifuged. The supernatant was collected and taken 0.5 and 1ml aliquots for analysis. The standards were prepared by taking 0.2, 0.4, 0.6, 0.8 and 1 ml of the working standard.0served as blank. The volume was made up to 1ml in all the tubes including the sample tubes by adding distilled water. Then added 4 ml of anthrone reagent and heated for eight minutes in a boiling water bath. It was cooled rapidly and the green to dark green colour developed was read at 630nm.

3.2.8. Phytochemical analysis:

Plant Material: Leaves of selected host plants (Castor, Colocasia, Papaya, Banana, Sweet potato) were collected from the areas near by Malabar Christian college, Calicut.

Preparation of extracts from the leaves of selected host plants using the solvent methanol

Extraction: The dried leaves were ground into a coarse powder using a mixer grinder. Fine pulverized material was dissolved in solvent methanol as described below.

About 10gm of powdered leaves were extracted with 80ml methanol in a solvent extractor for about 3 hours. Then the extract was filtered through Whatmann no.40filter paper. Then it was made up to 100ml in standard flask using methanol. This extract was then evaporated to dryness and it was collected, weighed and stored in refrigerator at $4^{o}C$ for further phytochemical analysis.

3.2.8.1. Qualitative Detection of Phytochemical Constituents:

Detection of active phytochemical constituents was carried out for all the extracts using the standard procedures (Tang, 2005).

1. **Detection of alkaloids:** 50mg of leaf extracts of five plants were dissolved in 2 ml of dilute hydrochloric acid and filtered. The filtrates were tested carefully with various reagents for detection of alkaloids as follows.

a). **Mayer's Test:** Filtrate was treated with Mayer's reagent (Potassium Mercuric Iodide). Formation of a yellow coloured precipitate indicated the presence of alkaloids.

b). **Wagner's Test:** A small amount of filtrate was treated with Wagner's reagent (Iodine in Potassium Iodide). Formation of brown/reddish precipitate indicated the presence of alkaloids.

2. Detection of Flavonoids: Detection of flavones and flavonones

a). **Aqueous sodium hydroxide test:** A small amount of extract was added to 2ml of aqueous sodium hydroxide solution, blue to violet colour indicated the presence of anthocyanins, yellow to orange colour indicated flavones and orange to crimson colour indicated flavonones.

b). **Concentrated sulphuric acid test:** A small fraction of extract was treated with one ml of concentrated sulphuric acid, yellowish orange colour indicated the presence of anthocyanins, yellow to orange colour indicated flavones and orange to crimson colour indicated flavonones**.**

3. Detection of Steroids and Terpenoids:

A small fraction of the extract was treated with potassium hydroxide and filtered. One portion of the filtered extract was dissolved in chloroform and other portion is dissolved in equal volume of petroleum ether.

a). **Liebermann - Burchard test**: The extract portion dissolved in petroleum ether was treated with 2ml of chloroform and 2ml of acetic anhydride. Then 2ml of concentrated H_2SO_4 was added slowly and blue green colour was observed for terpenoids and reddish brown colour for steroids.

b). **Salkowski test** :To the portion of extract dissolved in chloroform add 2 drops of concentrated H_2SO_4 along the sides of the test tube. Red coloured ring at the junction and green coloured fluorescent layer at the bottom indicated the presence of steroids.

4. Detection of Saponins:

a). Foam test: 100mg of extract was mixed with 20ml of distilled water. The suspension was shaken vigorously in a graduated cylinder for 15 minutes for a stable persistent froth. Appearance of 2 cm layer froth indicated the presence of saponins.

5. Detection of phenolic compounds and tannins

a). Ferric chloride test: 50mg of extract was dissolved in distilled water in a test tube, and then filtered and to this 0.5 ml neutral 5% ferric chloride solution was added. Appearance of dark green colour indicated the presence of phenolic compounds.

b). Lead acetate test: 50mg of extract was dissolved in distilled water and to this, 3 ml of 10% lead acetate solution was added. Development of white turbidity indicated the presence of tannins**..**

6. Detection of carbohydrates and glycosides

100mg of all the extracts were dissolved in 5 ml of distilled water separately and filtered. The filtrate was subjected to following tests.

a). Molisch's test: 2 ml of filtrate was added to two drops of alcoholic solution of α-naphthol. The mixture was shaken well and one ml of concentrated sulphuric acid was added along the sides of the test tube and allowed to stand. A violet or purple ring indicated the presence of carbohydrates.

b). Fehling's test: One ml of filtrate was boiled in water bath with one ml each of Fehling's solution A and B. Formation of a brick red precipitate indicated the presence of carbohydrates.

c). **Benedict's test:** To one ml of filtrate, one ml of Benedict's reagent was added. The mixture was heated on a boiling water bath for 2 minutes. Yellow to red precipitate indicated the presence of carbohydrates.

7. Detection of proteins and amino acids

100mg of all the extracts were dissolved in 10ml of distilled water and filtered through filter paper and the filtrate was subjected to tests for proteins and amino acids.

a) **Ninhydrin test:** To 2ml of aqueous filtrate, 2 drops of ninhydrin solution was added. Appearance of characteristic purple colour indicated the presence of amino acids.

3.2.8.2. Estimation of total phenolic content in leaf tissue

Total phenol content estimation in leaf tissue was carried out with Folin-Ciocalteau reagent (Malik and Singh, 1980).

Reagents

1. 80% Ethanol: 80ml of ethanol mixed with distilled water to make up to 100ml.
2. Folin-Ciocalteau Reagent: Commercial grade (used in 1:1 dilution with distilled water).
3. 20% Na_2CO_3:20gm of sodium carbonate was dissolved in distilled water and the volume made up to 100ml
4. Standard: 100mg of catechol was dissolved in 100ml distilled water. Diluted 10times for a working standard.

Sample extraction

Weighed exactly 0.5 to 1.0g of the sample and ground it with a pestle and mortar in 10-times volume of 80% ethanol. The homogenate was centrifuged at 10,000rpm for 20minutes. The supernatant was collected, re-extracted the residue with 80% ethanol about five-times the volume of residue, centrifuged and pooled the supernatants. The supernatant was evaporated to dryness. The residue was dissolved in a known volume of distilled water (5ml).

Procedure

Pipetted out different aliquots (0.2 to 2ml) in to the test tubes. The volume was made up in each tube to 3ml with water.0.5ml of Folin-Ciocalteau reagent was added. After 3 minutes, added 2ml of 20% Na_2CO_3 solution to each tube. It was mixed thoroughly and placed the tubes in boiling water for exactly one minute, cooled and measured the absorbance at 650nm against a reagent blank.

3.2.8.3. Estimation of chlorophyll content in leaf tissue

Reagent

Analytical grade acetone was diluted to 80% acetone.

Procedure

1gm of leaf tissue was weighed out, finely cut and ground with 20ml of 80% acetone in a clean mortar to make a fine pulp. The pulp was centrifuged at 5000rpm for 5 minutes. The supernatant was transferred to a 100ml volumetric flask. The residue was again ground with 80% acetone and the supernatant was collected in the same flask. Repeated the procedure until the residue becomes colourless. The mortar and pestle was washed with 80%

acetone and collected the clear washings in to the volumetric flask. The volume was made up to 100ml with 80% acetone and the absorbance was read at 645, 663, and 652 nm against the reagent blank, 80% acetone. Chlorophyll content was calculated as described by Porra *et al*. (1989).

$$\text{Chl a} = \frac{(12.7 \text{ x } A663 - 2.69 \text{ x } A645) \text{ x } V}{1000 \text{x} W}$$

$$\text{Chl b} = \frac{(22.9 \text{ x } A\ 645 - 4.68 \text{ x } A663) \text{x} V}{1000 \text{x} W}$$

$$\text{Chl (a+b)} = \frac{(20.2 \text{ x } A\ 645 + 8.02 \text{ x } A\ 663) \text{ x } V}{1000 \text{x} W}$$

3.2.9. Statistical analysis:

One -way ANOVA test:

All the results are statistically analyzed with ANOVA and post hoc test (Scheffe test) with SPSS16 package. Results with $p<0.05$ were considered to be statistically significant.

CHAPTER IV

INFLUENCE OF DIET AND SEASONAL VARIATION ON THE BIOLOGICAL PARAMETERS OF *SPODOPTERA LITURA*

4.1. Introduction

4.1.1 *Spodoptera litura*

*Spodoptera litura (*Lepidoptera: Noctuidae) is a major polyphagous pest which infest more than 290plant species.

The systematic position of *Spodoptera litura* is as given below :

Kingdom - Animalia

Phylum - Arthropoda

Class - Insecta

Order - Lepidoptera

Family - Noctuidae

Genus - *Spodoptera*

Species - *litura*

Common name - tobacco cut worm

4.1.2. Biology

Female moths lay egg masses on the underside of young leaves. After the eggs hatch, caterpillars feed on leaves. They are first gregarious and later solitary. They may also feed on stems, buds, flowers and fruits. Pupation

occurs in soil few centimeters deep without a cocoon. The life cycle completes on an average range of 23-37 days.

The adult moth lives for 7-9 days and lays maximum of 300-1200eggs. The egg incubation period is 3-5 days and 6 larval instar stages completed within an average period of 19 days. The pupal period is completed within duration of 7-9 days and the total life cycle is completed within 23-37 days depending up on various biotic and abiotic factors. The neonate larvae initially attack the foliage of the plants and in the later stage feeds on the developing seeds in the pod. This is a serious pest of various economically important crops such as cotton, groundnut, chilly, tobacco, castor and pulses and also developed resistance for almost all commercially available chemical pesticides.

4.1.3 Economic significance

Spodoptera litura, the oriental leafworm, is regarded as an economically important polyphagous defoliator pest in India. This insect pest attacks a wide range of host plants belonging to different botanical origins (290cultivated food plants belonging to 44 families all over the world; in which 60plants are known from India) (Moussa *et al*., 1960; Chari and Patel, 1983). The higher reproductive ability and migration capacity over long distances make *S. litura* a serious pest of many economically important crops such as rice, cotton, groundnut, tomato, tobacco, citrus, cocoa, potato, rubber, castor, millets, sorghum, maize and many other vegetables and crops (Hill, 1993). Because of its wide host plant range, its introduction into a crop field, lead to a potential disruption of production and marketing of many agricultural and ornamental crops.

4.1.4 Potential Economic impact and Description of damage

This pest is considered to be of concern from a regulatory perspective. It is believed to have potentially high economic impact in terms of its direct pest damage and trade implications.

4.1.5. Effect of host plants on biology and food utilization of *Spodoptera litura*.

It is a well-known fact that the food materials have a significant role in the growth and development of insect pests, ultimately leading to a higher or lower rate of population increase. Nutritional indices serve as appropriate tools that could provide a profound understanding of the behavioural and physiological bases of insect-host plant interactions (Bryant *et al*., 1987). The food consumption rate, efficiency of growth and utilization are key indicators of herbivore's performance. The insect herbivores responses to changes in host plant quality differ in accordance with food sources (Awmack and Lether, 2002). From a nutritional point of view, utilization efficiency reflects the quality of food consumed (Baghery *et al*., 2013).

Study of the effects of host plants on the biology of insects is important in understanding host suitability of plant infesting insect species. There have been numerous studies reported on the biological parameters of *S. litura* on different host plants under different environmental conditions particularly in India (Patel, 1987), China (Zhu *et al*., 2000; Qin *et al*., 2004; Zhu *et al*., 2005), Pakistan (Ahmad *et al*., 2007), Korea (Bae *et al.,* 1997; Bae, 1999, a; Bae and Park, 1999), and other Asian countries (Etman and Hooper, 1979; Holloway, 1989) where *S. litura* has been an important pest on various crops.

The plant volatiles are important communication signals, as the presence and emission of plant secondary metabolites not only help in the

location of suitable hosts but also in the avoidance of unsuitable ones (Bruce and Pickett, 2011). With regard to insect-plant interactions, it is very essential to study the effect of different host plants/cultivars on the performance of herbivores (Azidah and Sofian-Azirun, 2006; Saeed *et al.,* 2009). The insects prefer high quality plant species to feed and oviposit inorder to ensure the availability of better food for their progeny (Prudic *et al*., 2005). According to McIntyre and Gooding (2000) host plant characteristics greatly influence the feeding, biology and overall life history of herbivore insects.

The analysis of the quality factor (s) of the host plants are of significant importance for further development of insect-resistant varieties through cross breeding and genetic modifications and also important to evolve other efficient pest management strategies. There have been a number of studies reported on the biological parameters of *S. litura* feeding on different host plants by various researchers (Patel *et al.,* 1986; 1987).

Oviposition by females varied greatly depending on different host plants under different environmental conditions (Patel *et al*., 1986). Bae and Park (1999) reported that about 935 and 3,467 eggs were laid by a single female on soyabean and cotton respectively. According to Patel *et al*. (1986) pupal development was not affected by the host plant on which their larvae fed. Cotton fed female adult lived 12.30days compared to 6.3 days for male adult. Larval and pupal survival varied on different host plants (Patel *et al.,* 1987) where as 100percentage larval and pupal survival were observed only in castor. On poor quality host plants, the presence of low nutritional composition or the secondary metabolites reduced the larval survival and development (Herms and Mattson, 1992).

4.1.6. Effect of temperature on insect biology

Temperature is considered to be the most important abiotic factor influencing the establishment and growth of pest populations (Ratte, 1985). Temperature has proven to be a vital component in an insect's development and survival with relatively small variations having disproportional effects on the growth rate of one or more of their life stages (Tanaka and Yabuki, 1978). As the temperature raised the developmental and metabolic rates apparently increased, however, as the temperature approaches the upper lethal limit, the metabolic rate and the developmental rate decreases (Amarendra and Tripathi, 2008). To manage insects efficiently, it is essential to determine the influence of temperature on its food consumption and utilization parameters. The present study was conducted to elucidate the effect of three seasons (summer, monsoon and post monsoon) upon growth and development parameters of final instar *S. litura* larvae on five selected host plants.

The indiscriminate and continued use of particular pesticides by the farmers over the years have led to the development of resistance in the pest populations as well as resurgence in the sucking pest populations (Kumar, 2007). The development of alternative methods of pest control are therefore more promising. So for the development of efficient control strategies for *S. litura*, it will require information regarding its biological interaction with different host plants. Among these, an essential part will be an understanding of host suitability.

Quantitative investigation of consumption and utilization of host plants by insect herbivores is a usually utilized tool in studies of plant insect interactions (Scriber and Slansky, 1981). Factors depicting the consumption of food by an insect, how well this food is changed over to insect biomass and the rate at which the insect develops can lead to an understanding of how specific insect species respond to variation in host plant suitability. Study of

the impact of food on the biology of insects is of specific significance in understanding host suitability of plant infesting species and assessing the magnitude of injury to the crops attacked by them. This may help accordingly, in designing more economic control strategies.

4.2. Materials and methods

The materials used and methods employed for carrying out the studies in biology of *S. litura* on different host plant leaves are given in the sections 3.2.1 to 3.2.6.

4.3. RESULTS

The present investigation was carried out to study the changes in the developmental biology of cutworm *S. litura* through different larval instars up to pupal formation upon feeding with five selected host plants- castor, colocasia, papaya, banana and sweet potato in different seasons viz. early summer, monsoon and post monsoon seasons. The detailed morphological investigation was conducted after each moult in every instar stages. The growth and developmental parameters such as the length and weight of the larvae; larval, prepupl and pupal duration; pupal weight; larval, prepupal and pupal survival; adult longevity and fecundity were taken into account. The experiments were done in three generations in a year feeding on five selected host plants to evaluate the nutritional effect and seasonal impact on the growth and development.

4.3.1. Determination of larval instars

The measurement of larval head capsule width and length indicated the occurrence of six instars in *S. litura* reared on selected host plants leaves. No overlapping was observed in measurement extremes between instars. The mean head capsule width and length was computed for different instars

(Table. IV.1, IV.2; Fig.IV.1,IV.2). It showed significant differences between head capsule width and length of different instars in *S. litura.*

In addition, comparison of the mean using Tukey's test revealed that the lowest head capsule size was that of the first instar and the highest head capsule size was for the sixth instar, which indicated that the head capsule width and length increased corresponding with the larval growth. The Dyar's growth ratios (Dyar, 1890) were computed for head-capsule width (TableIV.1). According to this ratio, head capsule width increased in a regular linear progression by a ratio of 1.5 (range 1.3 to 1.7) in successive instars. Dyar's ratios range for the first to the sixth larval instar interval in *S. litura* for selected host plants were 1.49-1.65 (sweet potato), 1.47-1.71 (castor), 1.48-1.69 (colocasia), 1.45-1.64 (papaya), 1.49-1.64 (banana) respectively. The obtained Dyar's ratios indicated that *S. litura* larval growth rate followed Dyar's rule, and confirmed that none of the larval instars were disregarded.

Table . IV.1. Variation in the head capsule width of *Spodoptera litura* reared on selected host plant leaves.

Host plants	Head capsule width in mm						
	First instar	Second instar	Third instar	Fourth instar	Fifth instar	Sixth instar	Dyar's ratio range
Sweet potato	0.265±0.005	0.395± 0.005	0.633±0.026	1.046±0.015	1.636±0.013	2.570±0.040	1.49- 1.65
Castor	0.271±0.014	0.410±0.020	0.625±0.007	1.015±0.019	1.737±0.016	2.65±0.024	1.47-1.71
Colocasia	0.261±0.001	0.427±0.014	0.713±0.008	1.212±0.012	1.832±0.027	2.713±0.018	1.48-1.69
Papaya	0.265±0.002	0.402±0.014	0.618±0.011	1.008±0.018	1.640±0.017	2.559±0.023	1.45-1.64
Banana	0.264±0.005	0.407±0.020	0.671±0.018	1.005±0.012	1.623±0.019	2.435±0.032	1.49-1.61
F value	0.557	1.419	26.62	11.75	19.56	38.99	
P value	0.322	0.264	0.000	0.000	0.000	0.000	

The values presented in the table are the mean value of ten replicates for each host plant with standard error (Mean ±Standard error). All the values were found to be significant at 1% level.

Table. IV.2. Variation in the head capsule length of *Spodoptera litura* reared on selected host plant leaves.

Head capsule length in mm						
Host plants	**First instar**	**Second instar**	**Third instar**	**Fourth instar**	**Fifth instar**	**Sixth instar**
Sweet potato	0.203±0.004	0.301±0.002	0.612±0.028	0.948±0.013	1.200±0.050	1.564±0.030
Castor	0.217±0.006	0.312±0.002	0.739±0.012	1.104±0.015	1.419±0.017	1.934±0.019
Colocasia	0.216±0.006	0.314±0.003	0.738±0.015	1.062±0.022	1.508±0.004	2.060±0.029
Papaya	0.212±0.005	0.306±0.003	0.640±0.056	1.007±0.011	1.374±0.020	1.937±0.016
Banana	0.206±0.005	0.286±0.022	0.468±0.010	0.817±0.007	1.207±0.029	1.547±0.038
F value	1.249	1.159	13.74	55.33	21.62	70.84
P value	0.322	0.358	0.000	0.000	0.000	0.000

The values presented in the table are the mean value of ten replicates for each host plant with standard error (Mean ±Standard error). All the values were found to be significant at 1% level.

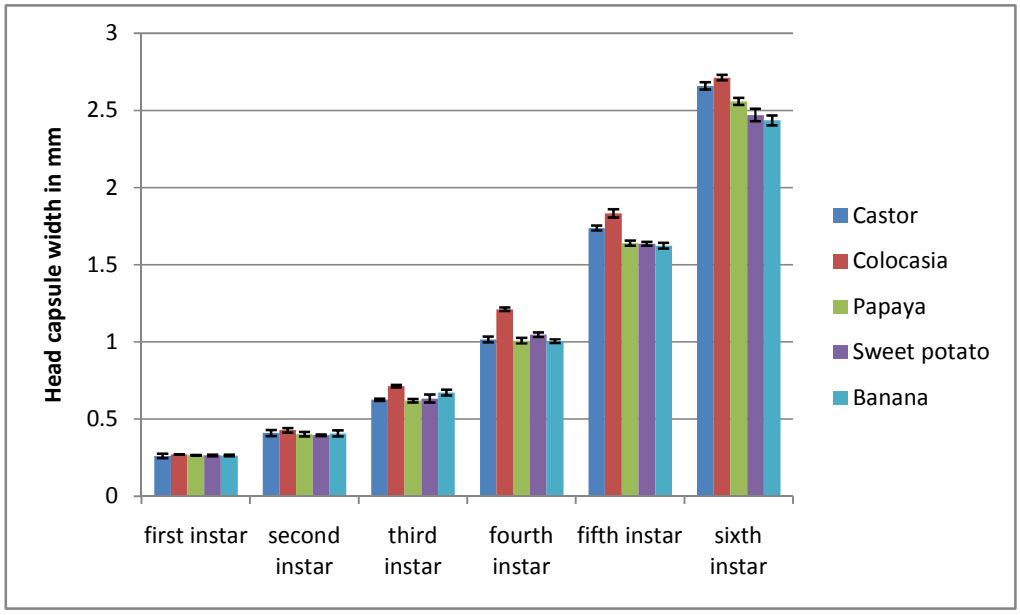

Fig.IV.1. Variation in the head capsule width of *Spodoptera litura* reared on selected host plant leaves.

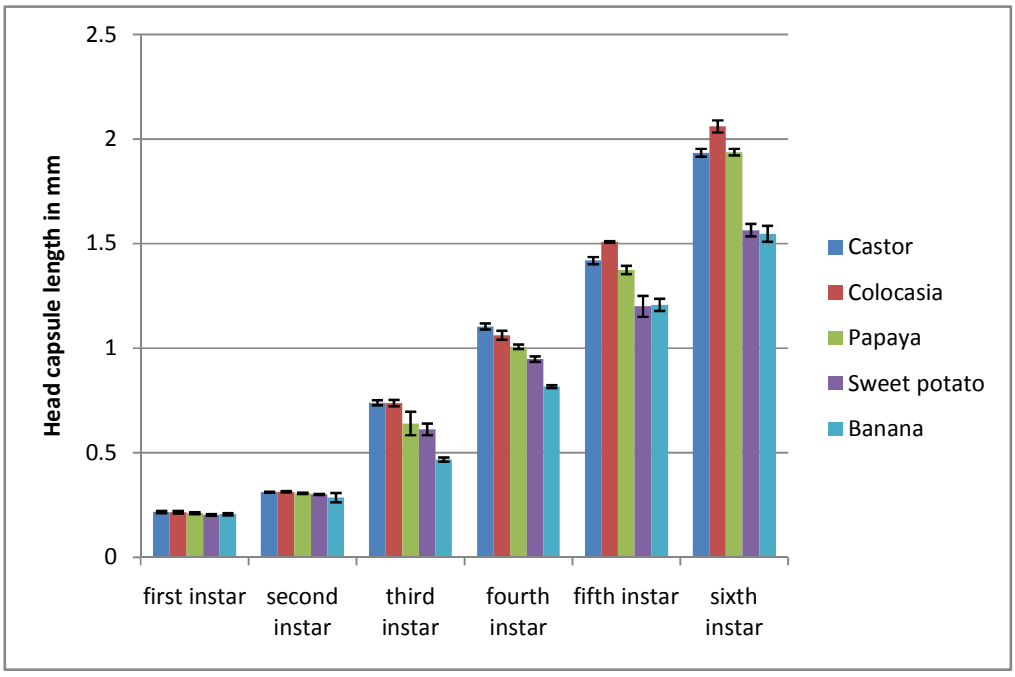

Fig.IV.2. Variation in the head capsule length of *Spodoptera litura* reared on selected host plant leaves.

4.3.2. Larval length and weight

1st instar

The newly hatched larva between the time of emergence and the first moulting or ecdysis is termed as the 1st instar. The length was measured in centimeter in three different seasons fed on five selected host plants. The length of larvae during summer, monsoon and post monsoon seasons were, for castor 0.37±0.016cm, 0.33±0.008cm, and 0.31±0.006cm; for papaya 0.33±0.013 cm, 0.32±0.008cm, 0.29±0.063cm; for colocasia 0.36±0.013cm, 0.28±0.013cm, 0.24±0.063cm; for sweet potato 0.33±0.015cm, 0.28±0.020cm, 0.25±0.016cm; for banana 0.26±0.008cm, 0.23±0.011cm and 0.22±0.007cm respectively (Table. IV.3 and fig. IV.3). The first instar length was highest for castor fed larvae and lowest in banana fed larvae and for other host plants it is intermediate between them. Seasonal variation in length of the larvae was also noticed.

Similarly the larval weight was measured in grams in three different seasons fed on five selected host plants. Larval weight for castor fed larvae was 0.090±0.044gm, 0.054±0.002 gm, 0.045±0.002gm; for colocasia fed ones 0.062±0.028gm, 0.041±0.002gm, 0.038±0.00gm; for papayafed larvae 0.062±0.028gm, 0.031±0.000gm, 0.028±0.002gm; for banana fed larvae 0.026±0.001gm, 0.022±0.001gm, 0.020±0.000gm; for sweet potato fed ones 0.058±0.001gm, 0.034±0.002gm and 0.032±0.001 gm respectively in the summer, monsoon and post monsoon seasons (Table IV.4 and Fig IV.4). The highest larval weight was observed for the larvae fed on castor and lowest weight was for banana fed larvae. Seasonal variations in the weight of the larvae was noticed.

2nd instar

After the first ecdysis the larva enters the 2nd instar. The length of the castor fed second instar larvae during summer season was 0.87±0.015cm. The larval length of, colocasia, papaya, banana and sweet potato fed larvae were 0.90±0.021cm, 0.87±0.013cm, 0.39±0.023cm and 0.87±0.021cm, respectively in summer season. Similar changes were noticed in monsoon and post monsoon seasons. The values were 0.84±0.022cm and 0.79±0.027 cm for castor fed larvae, 0.83±0.026cm and 0.78±0.046cm for colocasia fed ones, 0.79±0.017cm and 0.75±0.016cm for papaya fed larvae, 0.37±0.015cm and 0.35±0.016cm for banana fed ones and 0.84±0.030cm and 0.77±0.026cm for sweet potato fed larvae in monsoon and post monsoon seasons respectively (Table. IV.3and Fig. IV.3).Seasonal variation as well as difference in length of the larvae with respect to host plants were observed.

Similarly the weight of the second instar castor fed larvae were 0.116±0.004gm, 0.111±0.002 gm and 0.105±0.001 gm; of papaya fed larvae were 0.083±0.002gm, 0.077±0.003gm and 0.069±0.004gm; of colocasia fed larvae were 0.095±0.007gm, 0.087±0.004 gm and 0.078±0.004gm; of banana fed larvae were 0.035±0.000gm, 0.035±0.000gm and 0.032±0.00gm and for sweet potato fed larvae were 0.054±0.001gm, 0.048±0.002gm and 0.042±0.003gm respectively in the summer, monsoon and post monsoon seasons (Table IV.4 ; Fig.IV.4). The weight of second instar larvaewas higher in the castor fed case and lowest in the banana fed larvae in all the three seasons and the decline in weight was observed in the order castor >colocasia >papaya>sweet potato>banana. The weights of the larvae were greater during summer season than that in monsoon which was also slightly higher than that in post monsoon season.

3rd instar

The 3rd instar larvae attained a length of 1.80±0.014cm, 1.78±0.020cm, and 1.68±0.024cm when fed on castor; 1.86±0.033cm, 1.81±0.040cm and 1.78±0.038cm when colocasia fed; 1.64±0.037cm, 1.59±0.040cm and 1.52±0.048cm when papaya fed; 0.80±0.021cm, 0.76±0.016cm and 0.74±0.016cm when banana fed and 1.73±0.052cm, 1.69±0.023cm and 1.61±0.027cm when sweet potato fed during the summer, monsoon and post monsoon season respectively (Table IV.3; Fig. IV.3). Highest length was noticed for colocasia fed larvae and lowest was noticed for banana fed larvae in all the seasons. The length of larvae fed on other host plants showed the length in between that of colocasia and banana fed larvae. Seasonal variation was also noticed.

Similarly the weight of third instar larvae were 0.193±0.001gm, 0.186±0.003gm, 0.176±0.002gm for castor fed larvae, 0.127±0.010gm, 0.121±0.009gm, 0.110±0.004gm for papaya fed ones, 0.134±0.003gm, 0.133±0.002gm, 0.127±0.003gm for colocasia fed ones 0.082±0.002gm, 0.079±0.002gm, 0.071±0.002gm for banana fed ones, 0.104±0.006gm, 0.098±0.006gm, 0.090±0.002gm for sweet potato fed larvae during the summer, monsoon and post monsoon seasons respectively (Table. IV.4; Fig. IV.4). When the weight of the third instar larvae were compared the highest value was recorded for castor fed larvae during summer, monsoon and post monsoon season and the weight of the larvae showed decline in the order castor>colocasia>papaya>sweet potato>banana in all the three seasons. Seasonal variation of larval weight was also noticed.

4th instar

The data showed that the length of fourth instars varied with the feeding material and with the change in seasons. The length of the larvae was

in the order colocasia >castor> papaya >sweetpotato>banana and the values were 2.69±0.050cm for colocasia fed larvae, 2.56±0.022cm for castorfed larvae, 2.50±0.044 cm for papaya fed larvae, 1.98±0.020cm for sweetpotato fed larvae and 1.85±0.040cm for banana fed larvae in the summer season. Similar changes in the length were noticed in other seasons also. The values were 2.47±0.030cm and 2.38±0.041cmfor castor; 2.64±0.047cm and 2.55±0.037cm for colocasia; 2.45±0.022 cm and 2.26±0.121 cm for papaya; 1.79±0.034 cm and 1.69±0.027 cmfor banana and 1.91±0.023cm and 1.83±0.039 cm for sweet potato fed larvae respectively during monsoon and post monsoon seasons. Seasonal variation in length was also noticed (Table IV.3; Fig. IV.3).

Likewise the weight of the fourth instar larvae was observed as 0.247±0.006gm, 0.230±0.011gm and 0.222±0.005 gm for castor fed ones; 0.304±0.025 gm, 0.285±0.020gm, 0.240±0.116 gm for colocasia fed cases; 0.235±0.132gm, 0.237±0.008gm and 0.217±0.012gm for papaya fed larvae; 0.139 ±0.005gm, 0.134±0.005gm and 0.124±0.004gm for banana fed ones; 0.209±0.010gm, 0.197±0.001gm and 0.192±0.003gm for sweet potato fed larvae respectively in the summer, monsoon and post monsoon seasons (Table. IV.4; Fig. IV.4). Seasonal variation in instar weight was also recorded.

5th instar

When the length of the fifth instar larvae of *S. litura* was compared by feeding selected host plants it was noticed that the maximum length attained by the larvae that was fed on the castor followed by colocasia, papaya, sweet potato and banana. The length of the larvae was 3.45±0.022cm for castor fed; 3.18±0.082cm for colocasia fed; 3.16±0.037cm for papaya fed ; 2.75±0.026cm for sweetpotato fed and 2.66±0.037cm for banana fed larvae in the summer season and the values were 3.39±0.031cm and3.28±0.024cm for

castor fed; 3.14±0085cm and 3.01±0.060cm for colocasia fed ; 3.07±0.044cm and 2.96±0.045cm for papaya fed ; 2.69±0.027cm and 2.63±0.021cm for sweet potato fed and 2.57±0.039cm and 2.48±0.038cm for banana fed larvae respectively for monsoon and post monsoon seasons. Seasonal variation in length was also noticed (Table IV.3; Fig. IV.3).

Similarly the weight of fifth instar larvae were 0.559±0.042gm, 0.535±0.030gm and 0.491±0.024gm when fed with castor; 0.563±0.016gm, 0.542±0.013gm and 0.503±0.014gm when colocasia fed; 0.342±0.007gm, 0.336±0.006gm and 0.309±0.008gm when papaya fed; 0.738±0.049gm, 0.686±0.049gm and 0.644±0.036gm when sweet potato fed and 0.306±0.021gm, 0.274±0.009gm and 0.254±0.010gm when banana fed during summer, monsoon and post monsoon seasons respectively. Variation in weight with respect to the host plant and season was noticed (Table IV.4; Fig. IV.4).

6th instar

The length of the 6th instar larvae fed on selected host plant leaves was in the order colocasia> castor > banana> papaya> sweet potato in the summer season. The highest length was observed for colocasia fed larvae (4.07±0.053cm) and the lowest length was noticed in sweet potato fed larvae (3.66±0.063cm) during the summer season. But in the monsoon season the highest length was observed in colocasia fed larvae (3.96±0.045cm) and the lowest length was reported in the sweet potato fed larvae (3.36±0.087cm). In the post monsoon season the highest length was noticed in the banana fed larvae (3.82±0.032cm) of *S. litura* and the least value was reported in the sweet potato fed larvae (3.21±0.062cm)and the length of the larvae fed with other host plants were in between them. When comparing the larval length in different seasons, the length was highest during the summer season for all the

insects fed with selected host plant leaves and the lowest length was noticed in the post monsoon season (Table IV.3; Fig. IV.3).

The data obtained indicated a significant difference in the weight of the sixth instar larvae of *S. litura* reared on five selected host plants. The weight of the sixth instar larvae was in the order 0.847±0.031gm (colocasia fed)> 0.806±0.014gm (castor fed)> 0.750±0.043gm (papaya fed)> 0.738±0.049gm (sweet potato fed)>0.661±0.048gm (banana fed) in the summer season. Similarly the weight was 0.767±0.020gm and 0.681±0.017gm for castor fed larvae, 0.820±0.032gm and 0.787±0.028gm for colocasia fed larvae, 0.732±0.034gm and 0.686±0.032gm for papaya fed larvae, 0.570±0.029gm and 0.461±0.017gm for banana fed larvae and 0.686±0.049gm and 0.644±0.036gm for sweet potato fed larvae respectively in the monsoon and post monsoon seasons (Table. IV.4; Fig. IV.4).

Highly significant variations were observed in the larval length and weight in different seasons with respect to the feeding materials. Larval length and weight were highest in the summer season than in the monsoon and post monsoon seasons.

IV.3. Variation of the larval instar length of *Spodoptera litura* reared on selected host plants in different seasons.

Length in cm							
Seasons	**Host plants**	**First instar**	**Second instar**	**Third instar**	**Fourth instar**	**Fifth instar**	**Sixth instar**
Summer	Sweet potato	0.33±0.015	0.87±0.021	1.73±0.052	1.98±0.020	2.75±0.026	3.66±0.063
	Castor	0.37±0.016	0.87±0.015	1.80±0.014	2.56±0.022	3.45±0.022	3.86±0.022
	Colocasia	0.36±0.013	0.90±0.013	1.86±0.033	2.69±0.050	3.18±0.082	4.07±0.053
	Papaya	0.33±0.013	0.87±0.021	1.64±0.037	2.50±0.044	3.16±0.037	3.73±0.128
	Banana	0.26±0.008	0.39±0.023	0.80±0.021	1.85±0.040	2.66±0.037	3.98±0.077
	F value	12.21	123.8	276.8	99.95	49.7	4.8
	P value	0.000	0.000	0.000	0.000	0.000	0.002
Monsoon	Sweet potato	0.28±0.020	0.84±0.030	1.69±0.023	1.91±0.023	2.69±0.027	3.36±0.087
	Castor	0.33±0.008	0.84±0.022	1.78±0.020	2.47±0.030	3.39±0.031	3.81±0.017
	Colocasia	0.28±0.013	0.83±0.026	1.81±0.040	2.64±0.047	3.14±0.085	3.96±0.045
	Papaya	0.32±0.008	0.79±0.017	1.59±0.040	2.45±0.022	3.07±0.044	3.61±0.060
	Banana	0.23±0.011	0.37±0.015	0.76±0.016	1.79±0.034	2.57±0.039	3.84±0.058
	F value	10.14	78.75	210.8	130.8	44.6	16.3
	P value	0.000	0.000	0.000	0.000	0.000	0.000
Post monsoon	Sweet potato	0.25±0.016	0.77±0.026	1.61±0.027	1.83±0.039	2.63±0.021	3.21±0.062
	Castor	0.31±0.006	0.79±0.027	1.68±0.024	2.38±0.041	3.28±0.024	3.76±0.016
	Colocasia	0.24±0.063	0.78±0.046	1.78±0.038	2.55±0.037	3.01±0.060	3.74±0.054
	Papaya	0.29±0.063	0.75±0.016	1.52±0.048	2.26±0.121	2.96±0.045	3.48±0.062
	Banana	0.22±0.007	0.35±0.016	0.74±0.016	1.69±0.027	2.48±0.038	3.82±0.032
	F value	8.09	42.98	156.09	33.38	61.14	26.8
	P value	0.000	0.000	0.000	0.000	0.000	0.000

The values presented in the table are the mean value of ten replicates for each host plant with standard error (Mean ±Standard error). All the values were found to be significant at 1% level.

T able. IV.4. Variation of the larval instar weight of *Spodoptera litura* reared on selected host plants in different seasons.

Weight in gram							
Seasons	**Host plants**	**First instar**	**Second instar**	**Third instar**	**Fourth instar**	**Fifth instar**	**Sixth instar**
Summer	Sweet potato	0.058±0.001	0.054±0.001	0.104±0.006	0.209±0.010	0.345±0.018	0.738±0.049
	Castor	0.090±0.044	0.116±0.004	0.193±0.001	0.247±0.006	0.559±0.042	0.806±0.014
	Colocasia	0.062±0.028	0.095±0.007	0.134±0.003	0.304±0.025	0.563±0.016	0.847±0.031
	Papaya	0.062±0.028	0.083±0.002	0.127±0.010	0.235±0.132	0.342±0.007	0.750±0.043
	Banana	0.026±0.001	0.035±0.000	0.082±0.002	0.139±0.005	0.306±0.021	0.661±0.048
	F value	1.075	58.862	49.029	17.705	26.988	3.186
	P value	0.380	0.000	0.000	0.000	0.000	0.022
Monsoon	Sweet potato	0.034±0.002	0.0486±0.002	0.098±0.006	0.197±0.001	0.328±0.010	0.686±0.049
	Castor	0.054±0.002	0.111±0.002	0.186±0.003	0.230±0.011	0.535±0.030	0.767±0.020
	Colocasia	0.041±0.002	0.087±0.004	0.133±0.002	0.285±0.020	0.542±0.013	0.820±0.032
	Papaya	0.031±0.000	0.077±0.003	0.121±0.009	0.237±0.008	0.336±0.006	0.732±0.034
	Banana	0.022±0.001	0.035±0.000	0.079±0.002	0.134±0.005	0.274±0.009	0.570±0.029
	F value	40.595	110.112	53.152	23.474	59.413	7.489
	P value	0.000	0.000	0.000	0.000	0.000	0.000
Post monsoon	Sweet potato	0.032±0.001	0.042±0.003	0.090±0.002	0.192±0.003	0.299±0.014	0.644±0.036
	Castor	0.045±0.002	0.105±0.001	0.176±0.002	0.222±0.005	0.491±0.024	0.6819±0.017
	Colocasia	0.038±0.002	0.078±0.004	0.127±0.003	0.240±0.116	0.503±0.014	0.7873±0.028
	Papaya	0.028±0.002	0.069±0.004	0.110±0.004	0.217±0.012	0.309±0.008	0.6869±0.032
	Banana	0.020±0.000	0.032±0.001	0.071±0.002	0.124±0.004	0.254±0.010	0.4610±0.017
	F value	21.881	94.815	151.273	29.250	57.634	18.562
	P value	0.000	0.000	0.000	0.000	0.000	0.000

The values presented in the table are the mean value of ten replicates for each host plant with standard error (Mean ±Standard error). All the values were found to be significant at 1% level.

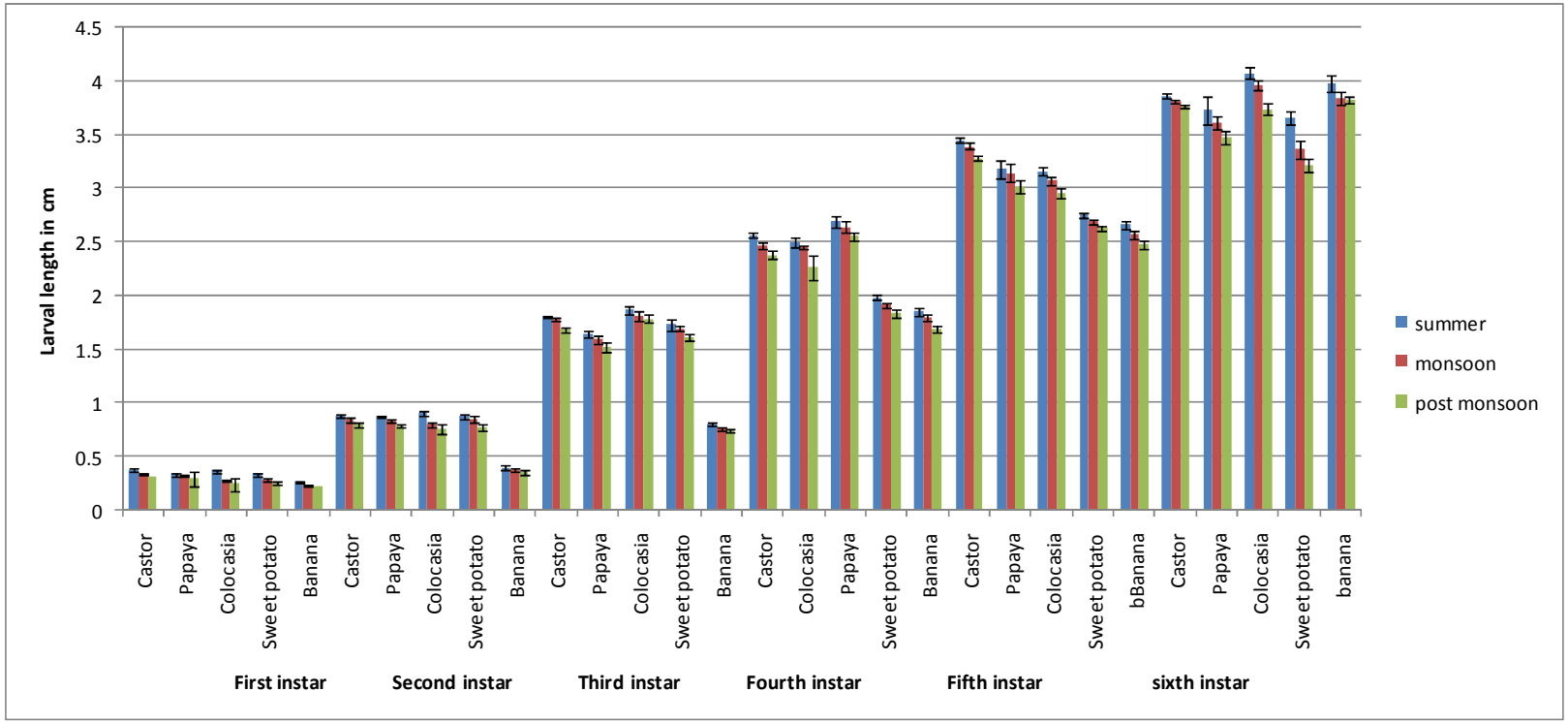

Fig. IV.3. Variation of the larval instar length of *Spodoptera litura* reared on selected host plants in different seasons.

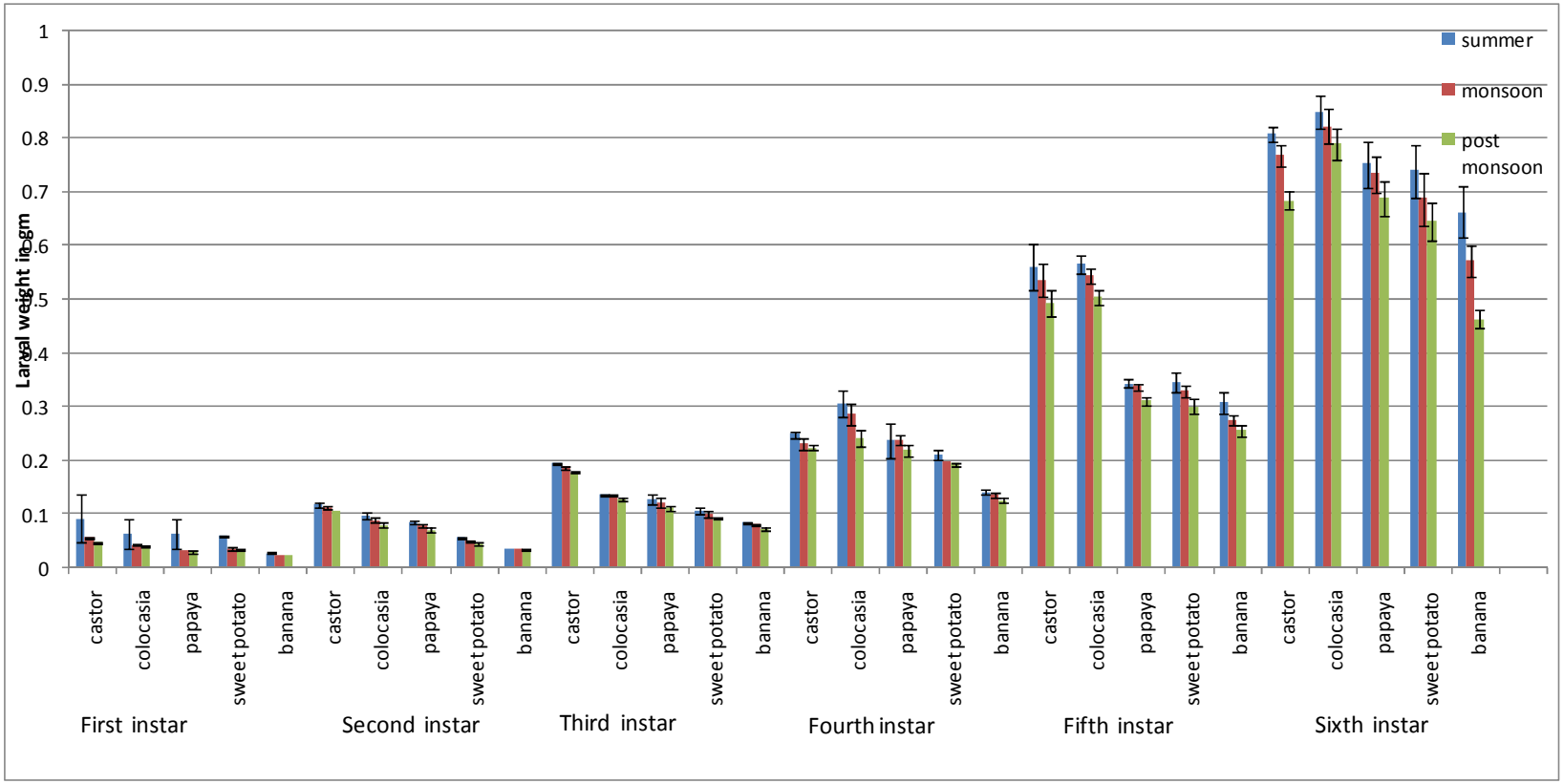

Fig IV.4. Variation of the larval instar weight of *Spodoptera litura* reared on selected host plants in different seasons.

4.3.3 Larval duration

The duration of each larval instars of *S. litura* feeding on the five host plants during three different seasons were recorded (Table. IV.5; Fig. IV.5). Significant variations were noticed in the total larval duration and instar duration when the *S. litura* were reared on selected host plants. The larval duration of *S. litura* reared on selected host plants during summer, monsoon and post monsoon seasons were 11.7±0.153 days, 13.6±0.163 days and 15.2±0.200days for castor; 12.6±0.163 days, 13.1±0.314 days and 16.4±0.163 days for colocasia; 13.0±0.149 days, 14.3±0.448 days and 16.4±0.221 days for papaya; 16.1±0.314 days, 17.6±0.400days and 20.3±0.335 days for banana and 15.4±0.221 days, 16.8±0.291 days and 18.5±0.268 days for sweet potato respectively. Both the total larval duration and the duration of each instar stage were maximum in the post monsoon season and shortest in the summer season. Larval duration and instar duration was found to be longest for banana fed larvae and shortest for the caster fed larvae and larval duration for the other host plant cases were in between them. The larval food had a great effect on different developmental stages of the insect. The mean larval duration was significantly affected by food.

Table IV.5 Variation of the instar and total larval duration of *Spodoptera litura* on selected host plants in different seasons

Larval instar duration in days								
Seasons	**Host plants**	**First instar**	**Second instar**	**Third instar**	**Fourth instar**	**Fifth instar**	**Sixth instar**	**Total larval period**
Summer	Sweet potato	3.3±0.153	2.3±0.153	2.9±0.100	1.9±0.100	2.0±0.000	3.0±0.000	15.4±0.221
	Castor	2.6±0.163	2.7±0.153	2.0±0.000	1.2±0.133	1.7±0.153	1.5±0.167	11.7±0.153
	Colocasia	2.8±0.133	2.7±0.153	2.1±0.100	1.8±0.200	1.7±0.153	1.6±0.163	12.6±0.163
	Papaya	2.8±0.133	3.0±0.000	2.2±0.133	1.2±0.133	1.8±0.133	2.0±0.000	13.0±0.149
	Banana	3.8±0.133	2.0±0.000	2.2±0.133	2.9±0.100	2.0±0.000	3.2±0.133	16.1±0.314
	F value	11.5	10.9	11.43	25.4	1.78	43.4	81.4
	P value	0.000	0.000	0.000	0.000	0.149	0.000	0.000
Monsoon	Sweet potato	3.5±0.167	2.4±0.163	3.2±0.133	2.2±0.133	2.3±0.153	3.2±0.133	16.8±0.291
	Castor	2.7±0.153	2.9±0.100	2.1±0.100	1.5±0.167	2.2±0.133	2.2±0.133	13.6±0.163
	Colocasia	2.9±0.100	2.9±0.100	2.2±0.133	1.6±0.163	2.0±0.000	2.2±0.133	13.1±0.314
	Papaya	3.4±0.163	4.1±0.100	2.5±0.167	1.5±0.167	2.3±0.153	3.2±0.133	14.3±0.448
	Banana	3.9±0.179	2.2±0.133	2.5±0.167	3.2±0.133	2.3±0.153	3.5±0.167	17.6±0.400
	F value	9.66	36.6	9.14	22.7	0.968	19.1	35.1
	P value	0.000	0.000	0.000	0.000	0.434	0.000	0.000
Post monsoon	Sweet potato	3.8±0.133	2.6±0.163	3.4±0.163	2.6±0.163	2.6±0.163	3.5±0.167	18.5±0.268
	Castor	3.7±0.153	3.3±0.153	2.9±0.100	2.2±0.133	2.5±0.167	2.5±0.167	15.2±0.200
	Colocasia	3.7±0.153	3.3±0.153	3.8±0.133	2.9±0.100	2.6±0.167	2.5±0.167	16.4±0.163
	Papaya	3.6±0.163	4.3±0.153	2.8±0.133	2.2±0.200	2.6±0.163	3.3±0.153	16.4±0.221
	Banana	4.2±0.200	2.4±0.163	3.9±0.100	3.6±0.163	2.8±0.133	3.8±0.133	20.3±0.335
	F value	2.09	22.5	15.3	14.0	0.478	14.1	68.5
	P value	0.097	0.000	0.000	0.000	0.752	0.000	0.000

The values presented in the table are the mean value of ten replicates for each host plant with standard error (Mean ±Standard error). All the values were found to be significant at 1% level.

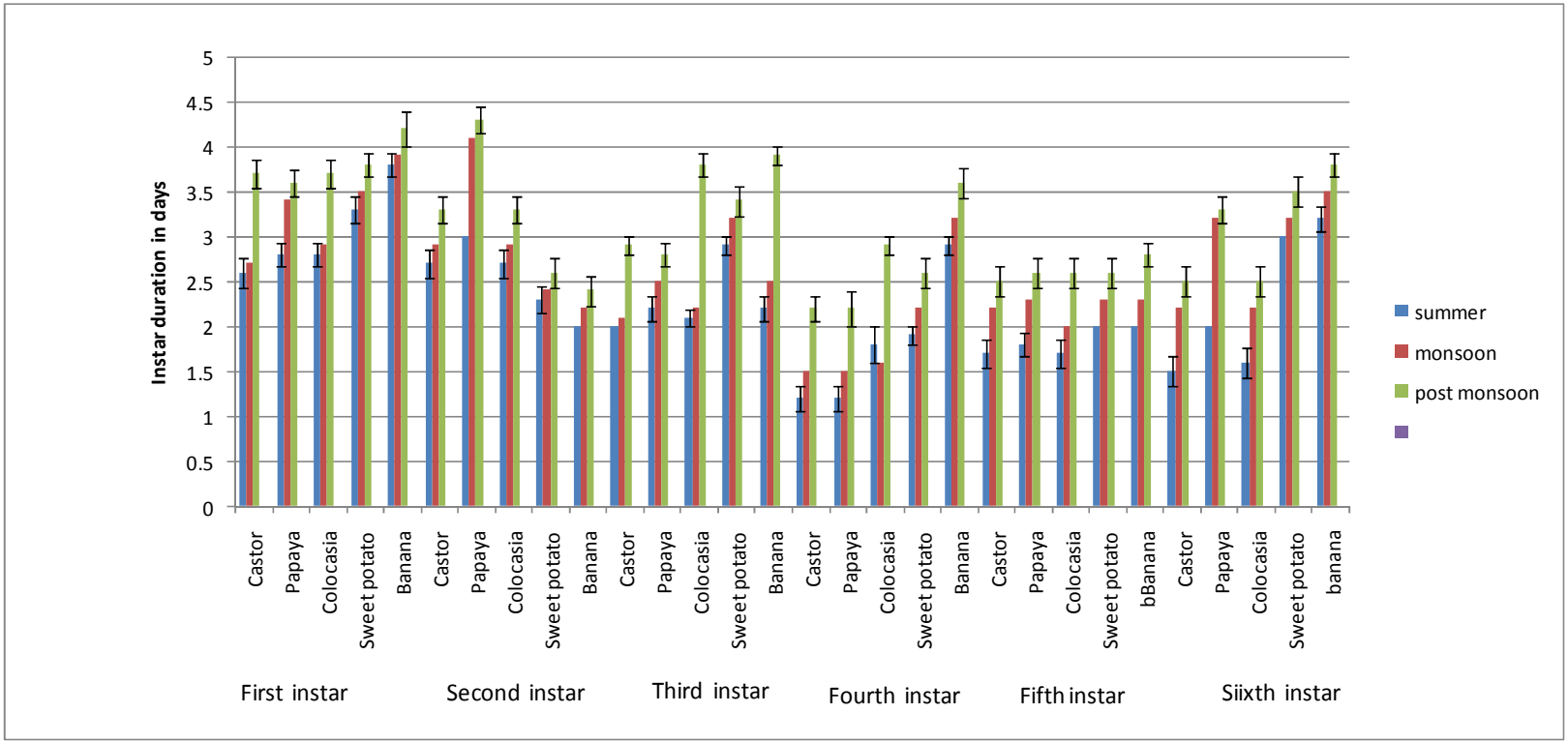

Fig. IV.5 Variation of the instar duration of *Spodoptera litura* on selected host plants in different seasons

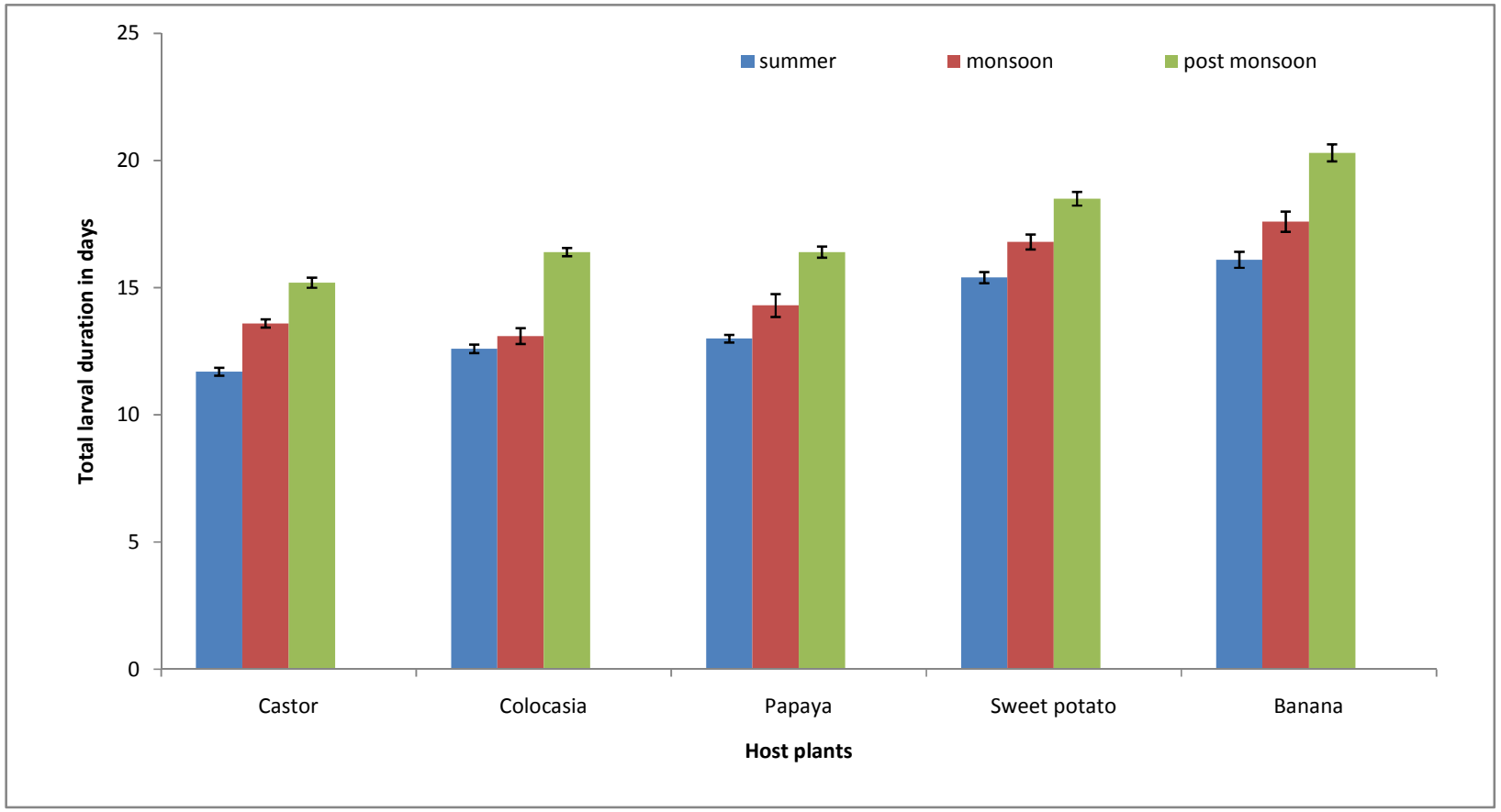

Fig IV.6 Variation of the total larval duration of *Spodoptera litura* on selected host plants in different seasons

4.3.4 Pupal characters

4.3.4.1. Prepupal and pupal duration

Highly significant variation was observed both in the prepupal and pupal duration of *S. litura* when fed with selected host plant leaves during different seasons. Prepupal duration for sweet potato fed larvae was 1.9±0.100days, 2.5±0.167 days and 2.7±0.153 days; for castor fed larvae it was 1.0±0.000days, 1.6±0.163 days and 1.9±0.100days; for papaya fed larvae 1.6±0.163 days, 2.3±0.153 days and 2.5±0.167 days; for colocasia fed larvae 1.0±0.000days, 2.0±0.211 days and 2.1±0.100days and for banana fed larvae 2.0±0.258 days, 2.8±0.133 days and 2.9±0.100days respectively in the summer, monsoon and post monsoon seasons (Table IV.6; Fig. IV.7).

Similarly the pupal duration was 8.2±0.200days, 8.9±0.276 days and 9.6±0.305 days for sweet potato fed larvae; 7.3±0.153 days, 7.8±0.200days and 8.5±0.167 days for castor fed larvae; 7.5±0.167 days, 8.2±0.249 days and 8.6±0.163 days for colocasia fed larvae; 8.0±0.258 days, 8.7±0.153 days and 9.4±0.305 days for papaya fed larvae and 8.6±0.221 days, 9.3±0.153 days and 11.2±0.326 days for banana fed larvae respectively in the summer, monsoon and postmonsoon seasons (Table. IV.6; Fig. IV.8).Both the pupal and prepupal days were maximum in the post monsoon season. Among the larvae fed with selected host plants banana fed larvae showed the longer and castor fed one showed the shortest period for the prepupal and pupal development.

Table. IV.6. Changes in the prepupal and pupal development of *Spodoptera litura* reared on selected host plant leaves.

Hostplants	Prepupal days			Pupal days		
	Seasons			Seasons		
	Summer	Monsoon	Post monsoon	Summer	Monsoon	Post monsoon
Sweet potato	1.9±0.100	2.5±0.167	2.7±0.153	8.2±0.200	8.9±0.276	9.6±0.305
Castor	1.0±0.000	1.6±0.163	1.9±0.100	7.3±0.153	7.8±0.200	8.5±0.167
Colocasia	1.0±0.000	2.0±0.211	2.1±0.100	7.5±0.167	8.2±0.249	8.6±0.163
Papaya	1.6±0.163	2.3±0.153	2.5±0.167	8.0±0.258	8.7±0.153	9.4±0.305
Banana	2.0±0.258	2.8±0.133	2.9±0.100	8.6±0.221	9.3±0.153	11.2±0.326
F value	11.13	7.6	10.6	6.702	7.692	16.936
P value	0.000	0.000	0.000	0.000	0.000	0.000

The values presented in the table are the mean value of ten replicates for each host plant with standard error (Mean ±Standard error). All the values were found to be significant at 1% level.

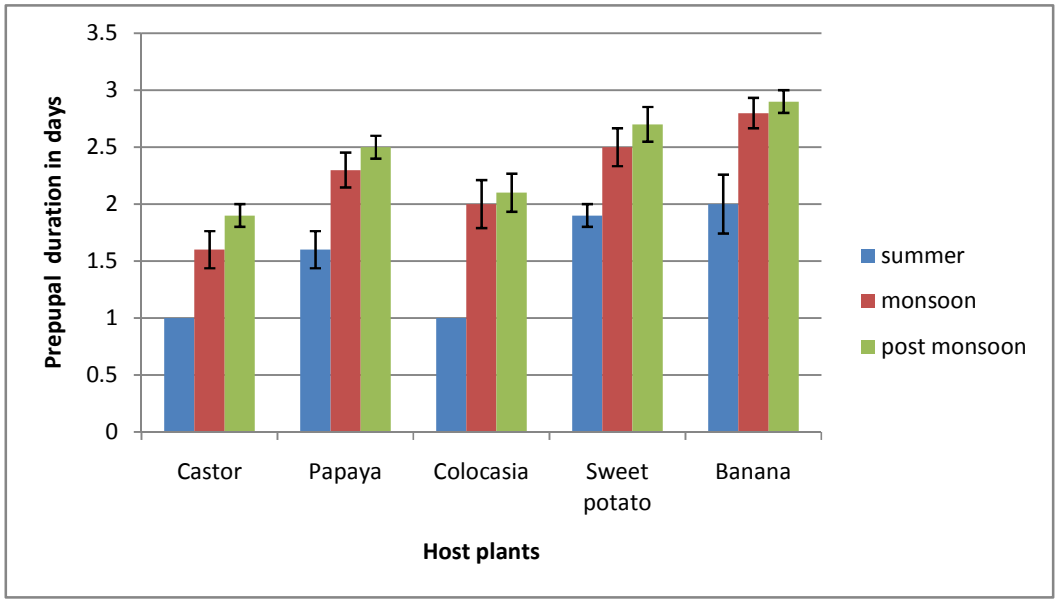

Table . IV.7. Changes in the prepupal development of *Spodoptera litura* reared on selected host plant leaves.

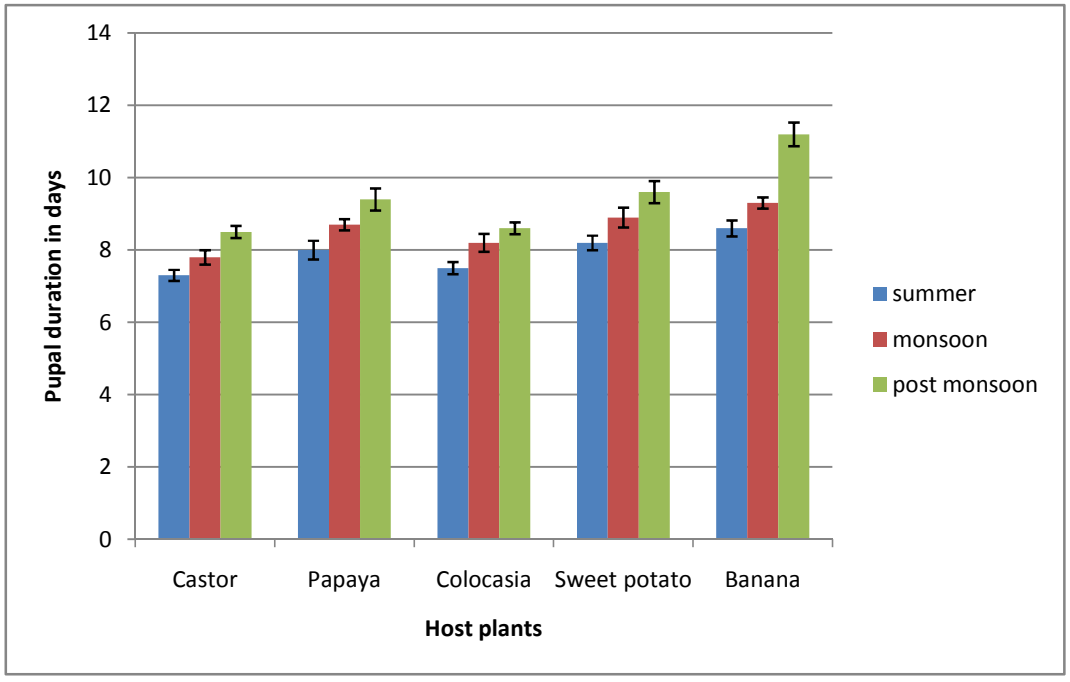

Fig. IV.8. Changes in the pupal development of *Spodoptera litura* reared on selected host plant leaves.

4.3.4.2. Pupal weight

The weight of the pupae of castor fed larvae during summer, monsoon and post monsoon seasons was recorded to be 0.313±0.015gm, 0.293±0.018gm and 0.279±0.008gm for male and 0.319±0.014gm, 0.305±0.012gm and 0.288±0.008gm for female respectively (Table IV.7 and Fig IV.9). The weight of other host plant fedcases was found to be lower than that of castor fed. For papaya it was 0.303±0.018gm, 0.284±0.010gm and0.274±0.007gm for male and 0.311±0.012gm, 0.304±0.012gm and 0.286±0.014 gm for female respectively during summer, monsoon and post monsoon seasons. In the case of colocasia it was 0.298±0.007gm, 0.283±0.005gm and 0.269±0.006gm for male and 0.309±0.012gm, 0.288±0.013gm and 0.274±0.011gm for females during summer, monsoon and post monsoon seasons respectively. The pupal weight of banana fed larvae was 0.267±0.014gm, 0.258±0.012gm and 0.241±0.010gm for male and 0.279±0.009gm, 0.269±0.005gm, and 0.250±0.010gm for females respectively in the summer, monsoon and post monsoon seasons. Similarly for sweet potatao it was 0.291±0.010gm, 0.262±0.008gm and 0.243±0.009gm for males and 0.308±0.010gm, 0.280±0.008gm and 0.262±0.008gm for females in the summer, monsoon and post monsoon seasons respectively. Castor fed larvae recorded highest pupal weight than that of other host plants during summer, monsoon and post monsoon seasons. Similarly the lowest pupal weight was recorded for the larvae fed on banana. The small changes were observed in the pupal weight of the *S. litura* reared on selected host plants in different seasons but the variation was not significant. Similarly comparing the male pupae with the female pupae, a slight increase in weight was noted in the latter case.

Table. IV.7. Variation of the pupal weight of *Spodoptera litura* reared on different host plants in selected seasons.

Host plants	Pupal weight gm/pupa±SE					
	Summer		Monsoon		Post monsoon	
	Male	Female	Male	Female	Male	Female
Sweet potato	0.291±0.010	0.308±0.010	0.262±0.008	0.280±0.008	0.243±0.009	0.262±0.008
Castor	0.313±0.015	0.319±0.014	0.293±0.018	0.305±0.012	0.279±0.008	0.288±0.008
Colocasia	0.298±0.007	0.309±0.012	0.283±0.005	0.288±0.013	0.269±0.006	0.274±0.011
Papaya	0.303±0.018	0.311±0.012	0.284±0.010	0.304±0.012	0.274±0.007	0.286±0.014
Banana	0.267±0.014	0.279±0.009	0.258±0.012	0.269±0.005	0.241±0.010	0.250±0.010
Fvalue	1.55	1.67	1.64	2.04	4.41	2.15.
P value	0.203	0.172	0.180	0.105	0.004	0.090

The values presented in the table are the mean value of five replicates for each host plant with standard error (Mean ±Standard error). All the values were found to be significant at 1% level.

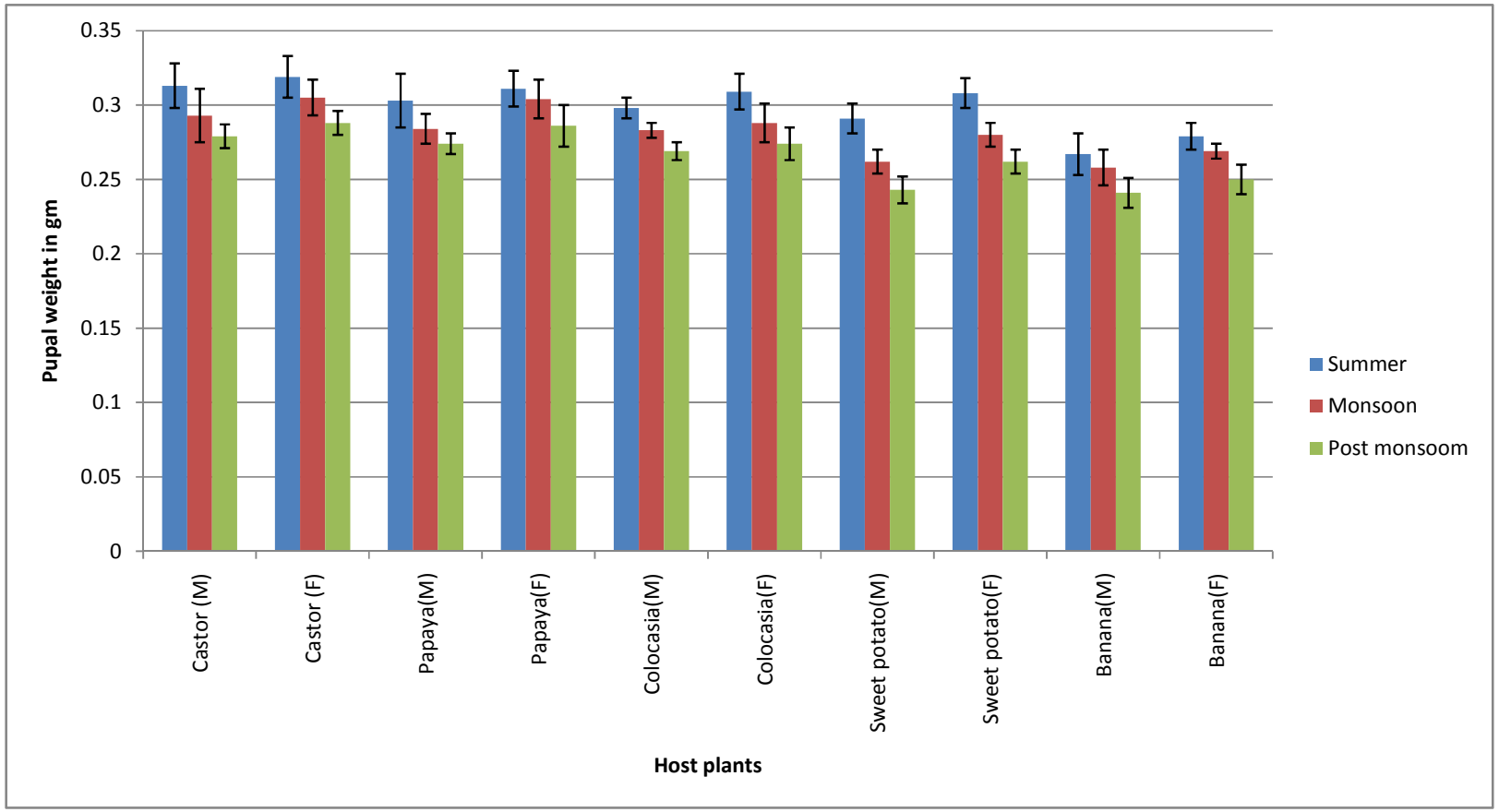

Fig. IV.9. Variation of the pupal weight of *Spodoptera litura* reared on selected host plants in different seasons

4.3.5. Oviposition and pre oviposition periods of S*podoptera litura* on selected host plants.

4.3.5.1 Pre-oviposition period

There was no significant variation in the pre-oviposition days of *S. litura* larvae fed with different host plants. The pre oviposition days of *S. litura* on selected host plants were noted as follows, 2.1±0.179 days , 2.5±0.167 days and 2.7±0.153 daysin sweet potato; 1.8±0.200days, 1.9±0.179 days and 2.1±0.17 daysin castor; 2.1±0.179 days, 2.5±0.167 days and 2.6±0.163 daysin colocasia; 2.0±0.149 days, 2.2±0.133 days and 2.6±0.163 daysin papayaand 2.4±0.163 days, 2.5±0.167 days and 2.8±0.133 days in banana respectively in the summer, monsoon and post monsoon seasons (Table.IV.8; Fig.IV.10).

Table. IV.8. Oviposition and pre-oviposition periods of S*podoptera litura* on selected host plants

Hostplants	Pre oviposition days			Oviposition days		
	Seasons			Seasons		
	Summer	Monsoon	Post monsoon	Summer	Monsoon	Post monsoon
Sweet potato	2.1±0.179	2.5±0.167	2.7±0.153	5.9±0.100	5.4±0.163	5.1±0.179
Castor	1.8±0.200	1.9±0.179	2.1±0.179	7.1±0.179	6.8±0.200	6.0±0.298
Colocasia	2.1±0.179	2.5±0.167	2.6±0.163	6.4±0.221	6.3±0.153	5.5±0.167
Papaya	2.0±0.149	2.2±0.133	2.6±0.163	6.0±0.211	5.7±0.153	5.5±0.167
Banana	2.4±0.163	2.5±0.167	2.8±0.133	5.7±0.213	5.3±0.153	4.8±0.133
F value	1.533	2.700	2.882	8.475	14.817	5.323
P value	0.209	0.042	0.033	0.000	0.000	0.001

The values presented in the table are the mean value of five replicates for each host plant with standard error (Mean ±Standard error). All the values were found to be significant at 1% level.

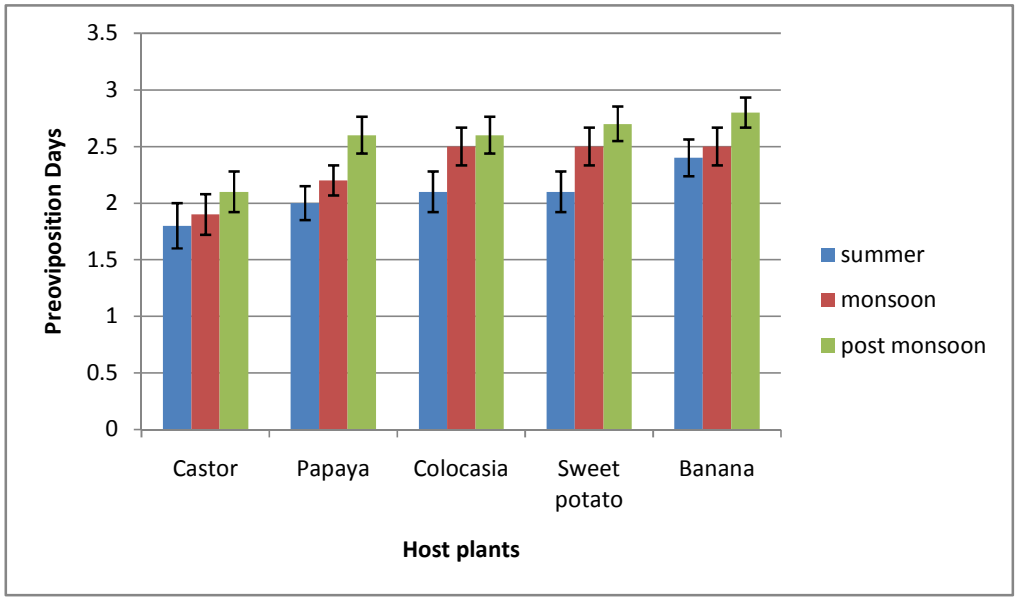

Fig. IV.10. Pre-oviposition periods of *Spodoptera litura* on selected host plants in different seasons

4.3.5.2. Oviposition period

Likewise the oviposition days of *S. litura* showed a significant difference among the larvae fed with selected host plant leaves. The values were 5.9±0.100days, 5.4±0.163 days and 5.1±0.179 days for sweet potatofed case; 7.1±0.179 days, 6.8±0.200days and 6.0±0.298 days for castor fed case; 6.4±0.221 days, 6.3±0.153 days and 5.5±0.167 days for colocasia fed case; , 6.0±0.211 days, 5.7±0.153 days and 5.5±0.167 days for papaya fed case and 5.7±0.213 days, 5.3±0.153 days and 4.8±0.133 days for banana fed case respectively in the summer, monsoon and post monsoon seasons (TableIV.8; Fig. IV.11). Oviposition dayswere less in the post monsoon and maximum was in the summer season. Maximum oviposition was shown by the adult of castor fed larvae and minimum number of oviposition days was for the adult of banana fed larvae. While observing the daily oviposition pattern it was noticed that the peak oviposition time occurred between the third andsixth day after emergence.

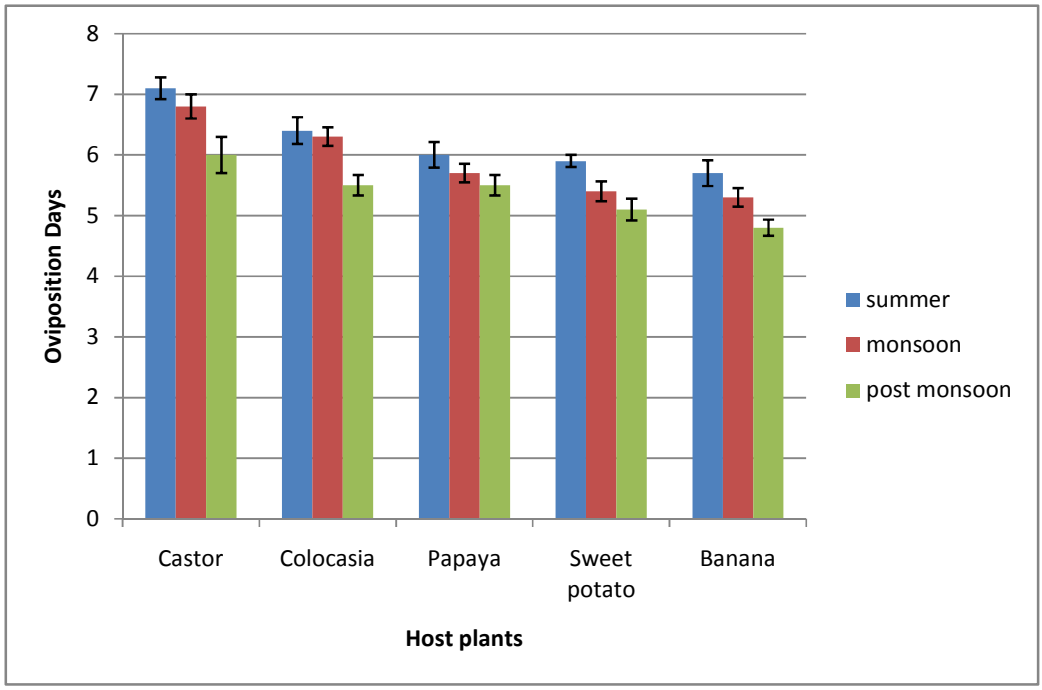

Fig.IV.11. Oviposition periods of *Spdoptera litura* on selected host plants in different seasons

4.3.6. Fecundity

The fecundity or the number of eggs laid by*S. Litura* reared on five selected host plants were recorded and given in Table IV.9 and Fig IV.12. The results showed a significant variation in the fecundity of adult moth reared from larvae fed on different host plants. The fecundity of *S. litura* larvae fed on castor leaves was found to be higher than the larvae fed on the other host plants. The total number of eggs laidby a female ranged from 695.80±84.65 to 1137.3±44.92 eggs. The lowest fecundity rate was noticed in the insect reared on banana. The fecundity rate was higher in the summer season followed by monsoon and post monsoon seasons.

Table IV.9. Fecundity of *Spodoptera litura* reared on selected host plants

Host plants	Fecundity		
	Summer	monsoon	Post monsoon
Sweet potato	783.90±58.24	749.80±41.258	496.60±25.12
Castor	1067.1±105.19	880.80±66.49	792.50±27.14
Colocasia	1137.3±44.93	822.80±36.36	691.00±55.80
Papaya	822.20± 56.43	763.10±33.53	387.10±35.60
Banana	695.80±84.65	594.80±90.19	251.60±30.79
F	6.77	3.43	36.14
P	0.000	0.016	0.000

The values presented in the table are the mean value of ten replicates for each host plant with standard error (Mean ±Standard error). All the values were found to be significant at 1% level

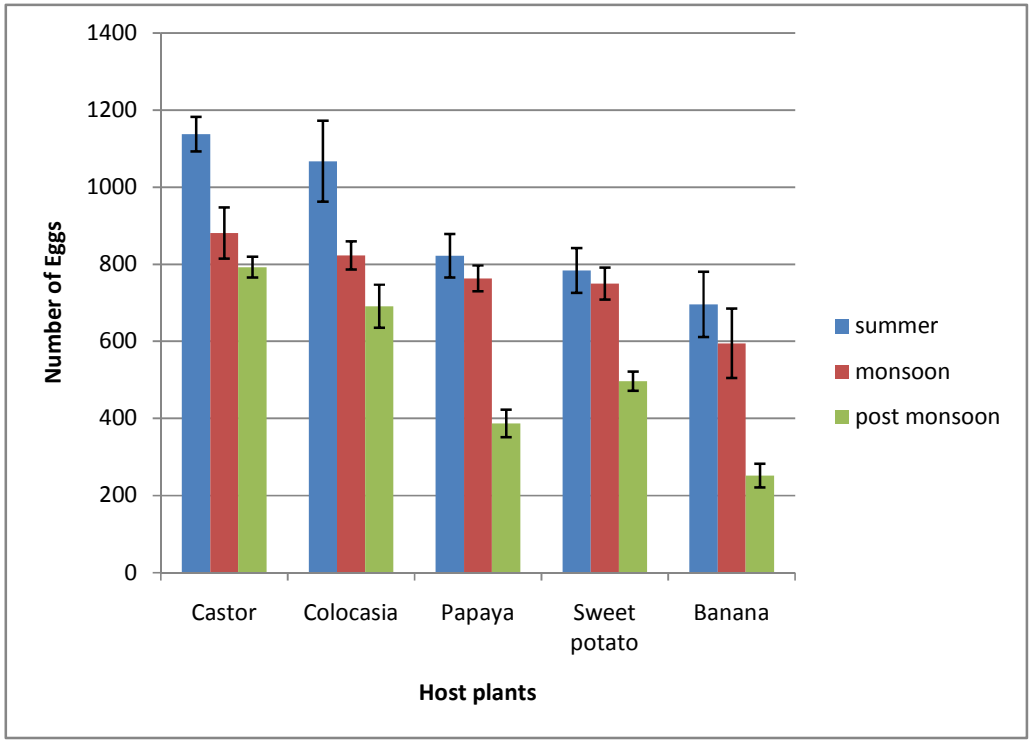

Fig. IV.12. Fecundity of *Spodoptera litura* reared on selected host plants

4.3.7. Variation in adult longevity of *S. litura* on selected host plants in different seasons.

The longevities of both female and male of *S. litura* adults were also significantly affected by the different seasons and also by the host plants on which their larvae fed. The highest female and male longevity were recorded for castor fed larvae 9.7±0.153days , 9.4±0.221days , 9.0±0.210days for male and 8.0±0.149days , 7.0±0.149days , 6.7±0.260days for female in summer, monsoon and post monsoon seasons respectively (Table IV.10; Fig. IV.13). In the case of colocasia it was 8.7±0.153days, 8.4±0.221days and 7.9±0.100days for male and 7.0±0.258 days, 6.9±0.233days, and 6.5±0.223days for females; in the case of papaya it was 8.6±0.163days, 8.2±0.249days and7.8±0.326days for males and 6.5±0.223days, 6.3±0.153days and 6.2±0.133days for females; in the case ofsweet potato it was 8.2±0.326days, 7.9±0.233days and 7.5±0.268days for male and 6.9±0.233days, 6.7±0.152days, 6.2±0.133days for females and in the case of banana it was 7.9±0.233days, 7.5±0.223days, and 7.3±0.153days for males and 6.4±0.266days, 6.0±0.298days and 5.6±0.266days for females respectively in the summer, monsoon and post monsoon seasons. The lowest male and female longevity were found in banana fed case. The adult longevity was maximum in the summer season. In the present study male adult lived longer (7.9±0.233 to 9.7±0.153days in summer, 7.5±0.223 to 9.4±0.221days in monsoon, and 7.3±0.153 to9.0±0.210days in post monsoon) than females (6.4±0.266 to 8.0±0.149daysin summer, 6.0±0.298 to 7.0±0.149 daysin monsoon and 5.6±0.266 to 6.7±0.260daysin post monsoon) which varied with different feeding material (selected host plant leaves).

Table. IV.10. Changes in the adult longevity of *Spodoptera litura* reared on selected host plant leaves

Hostplants	Adult longevity –Days ±SE					
	Summer		Monsoon		Post monsoon	
	Male	Female	Male	Female	Male	Female
Sweet potato	8.2±0.326	6.9±0.233	7.9±0.233	6.7±0.152	7.5±0.268	6.2±0.133
Castor	9.7±0.153	8.0±0.149	9.4±0.221	7.0±0.149	9.0±0.210	6.7±0.260
Colocasia	8.7±0.153	7.0±0.258	8.4±0.221	6.9±0.233	7.9±0.100	6.5±0.223
Papaya	8.6±0.163	6.5±0.223	8.2±0.249	6.3±0.153	7.8±0.326	6.2±0.133
Banana	7.9±0.233	6.4±0.266	7.5±0.223	6.0±0.298	7.3±0.153	5.6±0.266
F value	9.960	7.62	9.58	4.17	8.47	3.85
P value	0.000	0.000	0.000	0.006	0.000	0.009

The values presented in the table are the mean value of ten replicates for each host plant with standard error (Mean ±Standard error). All the values were found to be significant at 1% level

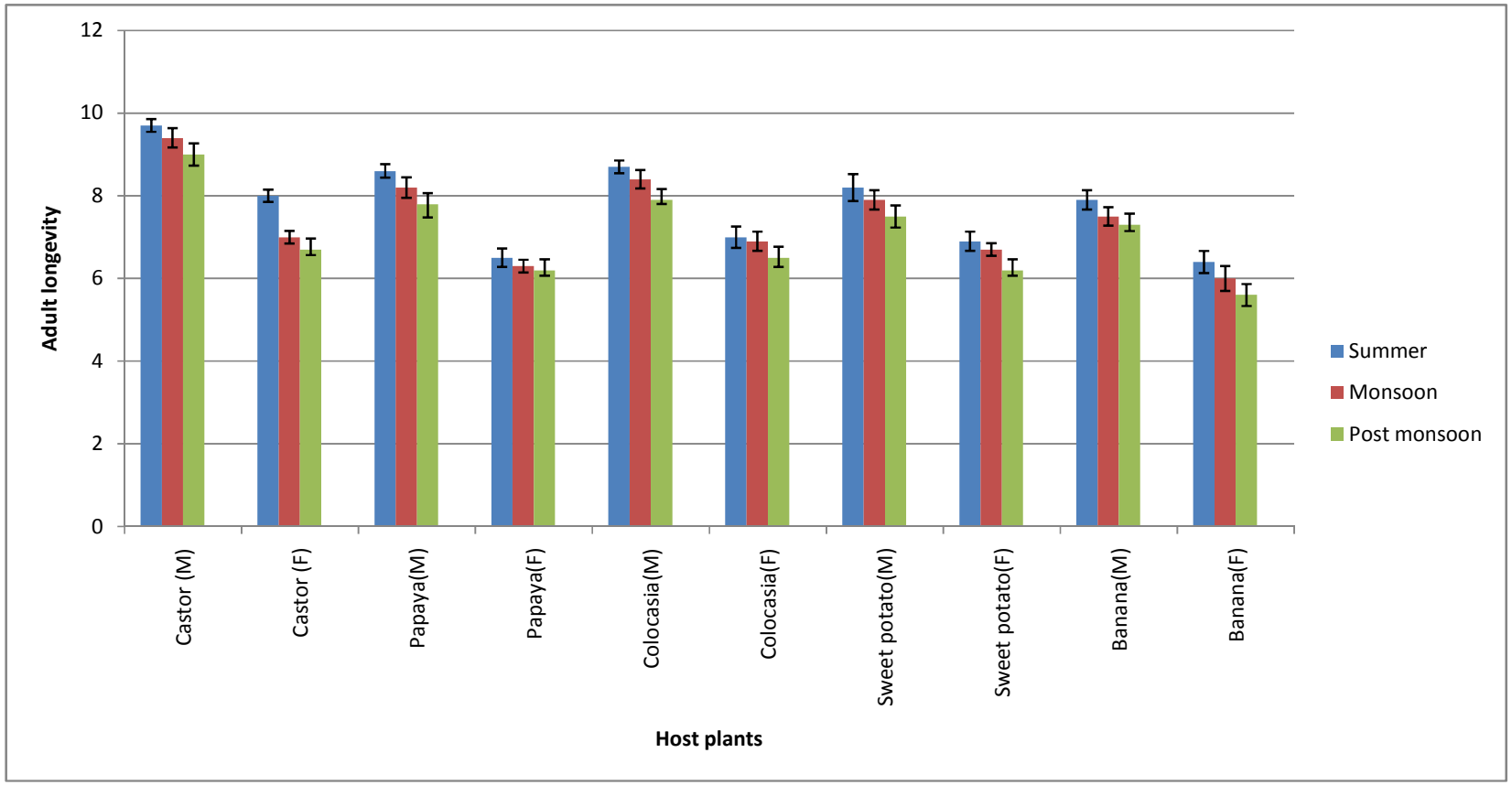

Fig. IV.13. Changes in the adult longevity of *Spodoptera litura* reared on selected host plant leaves

4.3.8. Variation in the instar, larval, pre pupal and pupal survival rate of *Spodoptera litura* reared on selected host plants

4.3.8.1. Instar survival rate

From the data presented in the table IV.11 it was observed that a significant difference exist in the survival rate of larvae, prepupae and pupae when reared on selected host plants under different temperature conditions. By comparing the instar survival rate from the maximum of 100instars, it was observed that in the case of first instar the maximum survival showed by the castor fed larvae was 98.50%, followed by colocasia fed larvae (97.00%), sweet potato fed larvae (93.20%), papaya fed larvae (80.70%) and least in the banana fed ones (67.20%) during the summer season. Similar changes were also noticed in the survival rate during the other two seasons also. The values were 95.30%and 88.90% (for castor), 94.00% and 88.00% (for colocasia), 90.40% and 85.00% (for sweet potato), 78.80%and 68.10% (for papaya), 59.00%and 54.20% (for banana) in the monsoon and post monsoon seasons respectively (Table. IV.11).

Similarly for the second instar the maximum survival rate was shown by the castor fed larvae in all the seasons 98.20% in summer, 95.30% in monsoon and 89.30% in post monsoon seasons. Similarly, 93.90%, 90.30%, 84.00% of survival rate was recorded for colocasiafed larvae; 79.20%, 75.40%, 68.20% for papaya fed larvae; 74.80%, 71.20%, 65.70% for sweet potato fed ones and 58.70%, 55.70%, 51.00% for banana fed case respectively in the summer, monsoon and post monsoon seasons. Least survival rate noticed in the banana fed case. For third instar larvae it was 97.30%, 93.20%, 86.20% for castor fed larvae ; 92.90%, 89.00%, 83.30% for colocasia fed larvae; 75.40%, 71.20%, 66.20% for papaya fed case; 73.60%, 69.40%, 62.10% for sweet potato fed ones; 52.20%, 50.10% and 47.00% for banana fed larvae in the summer, monsoon and post monsoon seasons respectively (Table. IV.11.). In this case also maximum survival rate was noticed in castor fed larvae and minimum survival rate was in banana fed larvae.

Table. IV.11. Changes in the instar survival rate of *S. litura* reared on selected host plants in different seasons

Survival rate in percentage							
Seasons	**Host plants**	**First instar**	**Second instar**	**Third instar**	**Fourth instar**	**Fifth instar**	**Sixth instar**
Summer	Sweet potato	93 2±0.153	74.8±0.163	73.6±0.153	69.3±0.233	86.0±0.163	88.4±0.133
	Castor	98.5±0.133	98.2±0.133	97.3±0.153	98.4±0.133	98.2±0.133	99.5±0.100
	Colocasia	97.0±0.153	93.9±0.153	92.9±0.200	94.8±0.163	91.4±0.179	99.2±0.100
	Papaya	80.7±0.258	79.2±0.233	75.4±0.167	56.2±0.305	87.1±0.153	58.2±0.290
	Banana	6.72±0.200	58.7±0.249	52.2±0.200	46.1±0.267	82.4±0.249	57.0±0.260
	F value	67.64	68.66	102.5	99.64	11.31	118.34
	P value	0.000	0.000	0.000	0.000	0.000	0.000
Monsoon	Sweet potato	90.4±0.149	71.2 ±0.233	69.4±0.179	65.0±0.268	80.0±0.258	82.6±0.290
	Castor	95.3±0.167	95.3±0.167	93.2±0.213	95.6±0.167	95.8±0.167	96.3±0.163
	Colocasia	94.0±0.163	90.3±0.149	89.0±0.179	89.3±0.179	87.5±0.153	96.0±0.163
	Papaya	78.8±0.249	75.4±0.268	71.2±0.179	53.0±0.300	82.4±0.290	57.0±0.260
	Banana	59.0±0.233	55.7±0.167	50.1±0.258	43.4±0.153	78.2±0.359	56.3±0.267
	F value	59.14	62.17	71.52	103.03	6.99	71.71
	P value	0.000	0.000	0.000	0.000	0.000	0.000
Post monsoon	Sweet potato	85.0±0.167	65.7±0.167	62.1±0.249	61.1±0.348	76.2±0.305	75.2±0.223
	Castor	88.9±0.133	89.3±0.277	86.2±0.267	88.9±0.133	89.0±0.100	88.2±0.133
	Colocasia	88.0±0.133	84.0±0.267	83.3±0.300	82.1±0.290	82.6±0.290	89.1±0.100
	Papaya	68.1±0.467	68.2±0.249	66.2±0.267	49.0±0.276	79.0±0.314	52.3±0.133
	Banana	54.2±0.163	51.0±0.233	47.0±0.213	40.3±0.211	69.2±0.433	54.4±0.163
	F value	37.17	40.09	37.75	62.05	5.74	130.62
	P value	0.000	0.000	0.000	0.000	0.001	0.000

The values presented in the table are the mean value of ten replicates for each host plant with standard error (Mean ±Standard error). All the values were found to be significant at 1% level

In the case of fourth instar larvae castor fed ones showed 98.40%, 95.60%, 88.9 0% ; for colocasia fed larvae it was 94.80%, 89.30% , 82.10%; for papaya fed larvae56.20%, 53.00%, 49.00% ; for sweet potato fed larvae 69.30%, 65.00%, 61.10% and for banana fed ones 46.10%, 43.40% and40.30%respectively in the summer, monsoon and post monsoon seasons. For the fifth instar larvae the survival rate was 98.20%, 95.80%, 89.00% for castor fed larvae; 91.40%, 87.50%, 82.60% for colocasia fed larvae ; 87.10%, 82.40%, 79.00% for papaya fed ones; 86.00%, 80.00%, 76.20% for sweet potato fed larvae and 82..40%, 78.20%, 69.20% for banana fed larvae in the summer, monsoon and post monsoon seasons respectively.

In the case of sixth instar the survival rate was maximum both in colocasia fed and castor fed larvae in the summer season (99.50%) and in monsoon season (96.30%) and in post monsoon season the colocasia fed larvae showed the maximum survival rate (89.10%). Like wise similarity were also noticed in the survival rate of banana fed and papaya fed larvae in the summer (57.00% and 58.20%respectively) in monsoon (56.30% and 57.00%respectively) and in post monsoon season (54.40% and 52.30% respectively) (Table. IV.11).

4.3.8.2. Total larval survival rate

The table IV.12. showed significant fluctuations in the larval survival rate of *S. litura* reared on selected host plants. In the summer season the survival rate of larvae reared on selected host plants was 92.80% for castor , 89.60% for colocasia , 76.00% for papaya, 62.20% for sweet potato, and 56.00% for banana. Similarly in monsoon and post monsoon season it was 89.20%, and 85.20% (for castor); 86.30% and 83.00% (for colocasia); 73.00% and 69.80% (for papaya); 58.90% and 55.50% (for sweet potato) and 54.00% and 51.00% (for banana) respectively. The larval survival rate was least on banana and maximumon castor.

4.3.8.3. Prepupal survival rate

From the analysis of data from the tableIV.12 significant variation was observed in the prepupal survival rate of *S. litura* reared on selected host plants. Prepupal survival rate was lowest in papaya 61.30%, 58.60% and 53.00% in summer, monsoon and post monsoon seasons. Prepupal survival rate was noticed highest in castor, 92.00%, 89.60%, and 86.00% in summer, monsoon and post monsoon respectively (Table. IV.12 and Fig. IV.15). The survival rate of other host plants were in between that for castor fed and that of papaya fed case. The prepupal survival rate was maximum in the summer season and minimum in the post monsoon season. The prepupal survival rate was in the order of castor>colocasia>banana>sweet potato>papaya.

4.3.8.4. Pupal survival rate

The pupal survival ratenoticed was approximately similar both in the castor and colocasia fed case – 92.10% and 91.20% respectively in the summer ; 88.10%in the monsoon and 84.50% and 82.00% respectively in the post monsoon (Table. IV.12). The lowest pupal survival rate was noticed in the papaya – 66.80%in summer, 64% in monsoon and 58%in post monsoon. The survival rate of other host plants were in between the papaya fed and castor fed cases. The pupal survival rate was maximum in the summer season and it was minimum in the post monsoon season in all the host plants selected.

The larval, prepupal and pupal survival rate was maximum for castor fed larvae in all the seasons. The larvae, prepupae and pupae reared on banana and papaya showed the lowest survival rates. Similarly the maximum survival rate was on the summer season and lowest in the post monsoon season.

Table. IV.12. Changes in the larval, pre pupal and pupal survival rate of *S. litura* reared on selected host plants in different seasons.

Seasons	Host plants	Survival rate		
		Lavalsurvival	Prepupal survival	Pupal survival
Summer	Sweet potato	62.2±0.133	74.5±0.400	74.3±0.163
	Castor	92.8±0.200	92±0.133	92.1±0.133
	Colocasia	89.6±0.179	89.4±0.179	91.2±0.100
	Papaya	76.0±0.221	61.3±0.277	66.8±0.305
	Banana	56.0±0.221	78.3±0.133	71.6±0.314
	F value	67.63	25.41	28.92
	P value	0.000	0.000	0.000
Monsoon	Sweet potato	58.9±0.200	71.6±0.314	71.0±0.233
	Castor	89.2±0.100	89.6±0.100	88.1±0.133
	Colocasia	86.3±0.163	85.0±0.268	88.1±0.133
	Papaya	73.0±0.260	58.6±0.249	64.0±0.266
	Banana	54.0±0.163	75.0±0.166	69.7±0.276
	Fvalue	73.49	27.63	26.6
	P value	0.000	0.000	0.000
Post monsoon	Sweet potato	5.5±0.167	6.7±0.300	6.7±0.260
	Castor	85.2±0.223	86.0±0.163	84.5±0.305
	Colocasia	83.0±0.153	79.2±0.314	82.0±0.290
	Papaya	69.8±0.233	53.0±0.153	58.6±0.290
	Banana	51.0±0.179	70.1±0.211	66.0±0.266
	Fvalue	64.65	27.79	15.5
	P value	0.000	0.000	0.000

The values presented in the table are the mean value of ten replicates for each host plant with standard error (Mean ±Standard error). All the values were found to be significant at 1% level

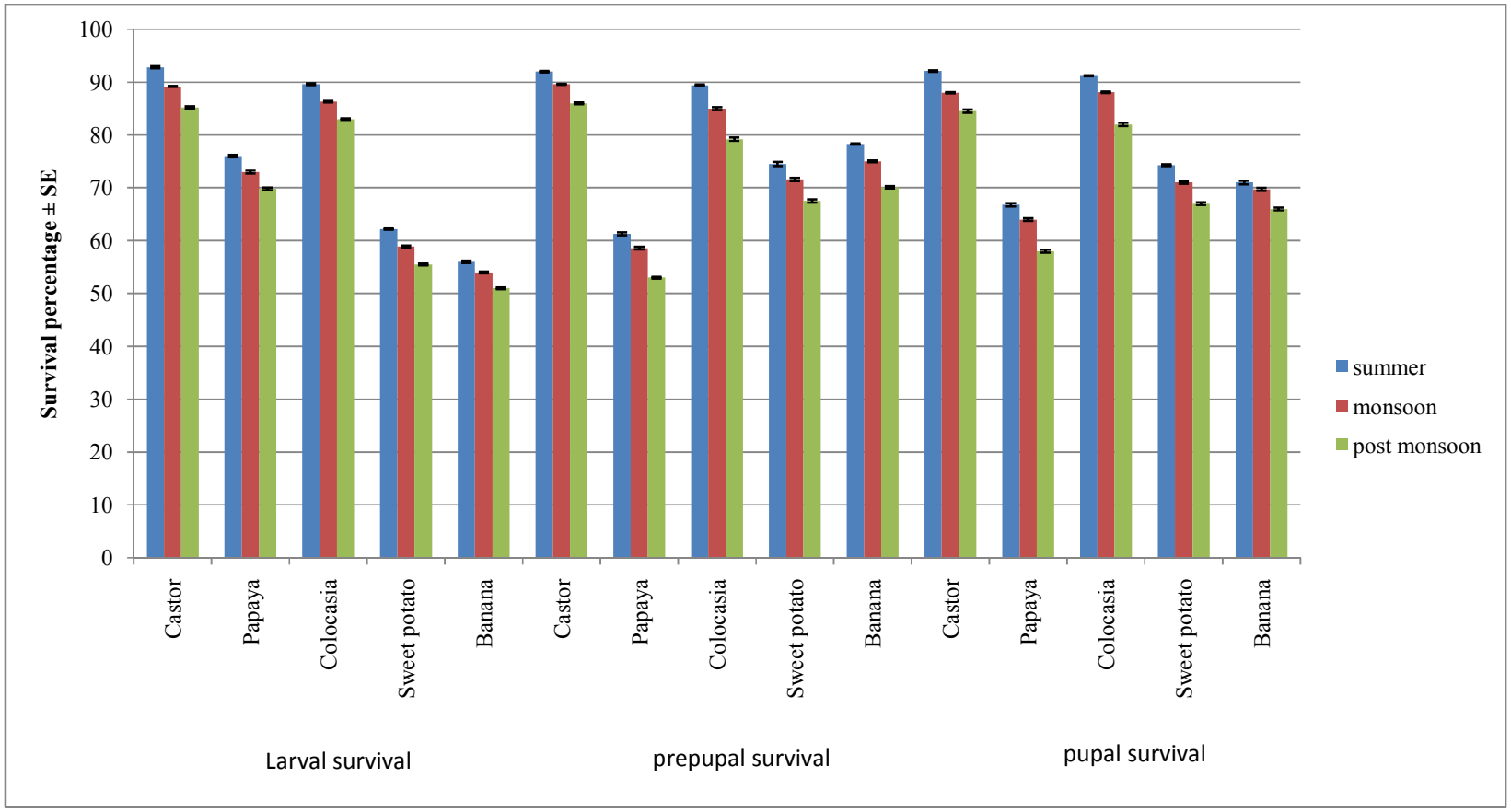

Fig. IV.14. Variation in the larval, prepupal and pupal survival rate of *Spodoptera litura* reared on selected host plants

4.3.9. Moisture content

The significant difference in the moisture content was noticed among the leaves of selected host plants. The highest moisture content was noticed in the colocasia leaves (86.32%) followed by papaya (83.86%), sweet potato (80.53%), banana (78.6%) and castor (75.2%).

Table. IV.13. Variation in the moisture content of selected plant leaves

Host plants	Moisture content in percentage
Castor	75.2
Colocasia	86.32
Papaya	83.86
Banana	78.6
Sweet potato	80.53

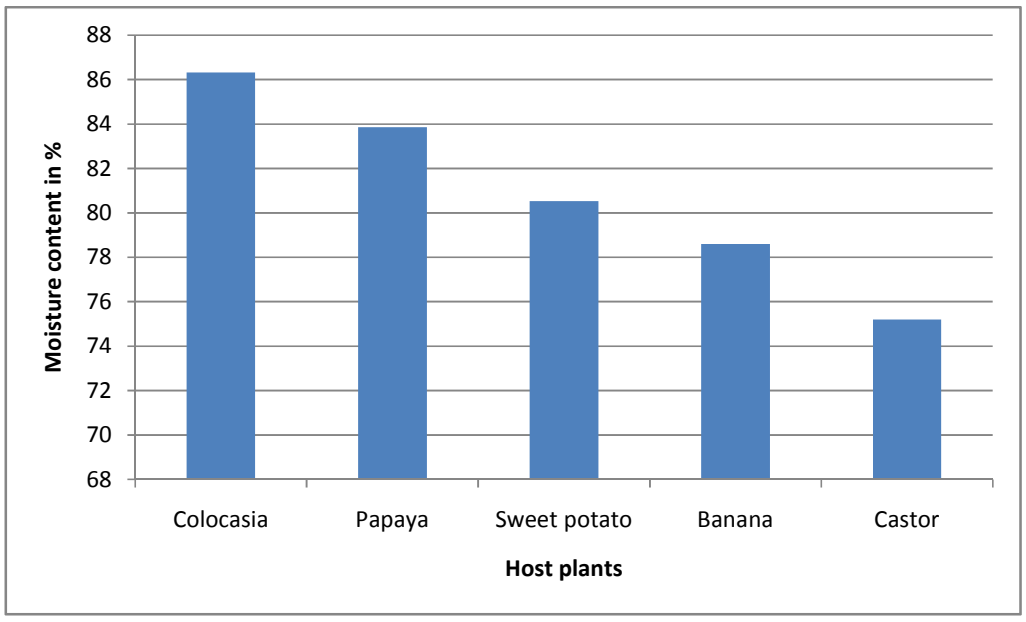

Fig. IV.15. Variation in the moisture content of selected plant leaves

4.3.10. Food consumption and utilization

Food consumption and conversion of ingested and digested food by *S. litura* larvae differ significantly among the five selected host plant leaves that the larvae consumed (Table IV.12). The relative growth rates of larvae reared on castor (0.335 ±0.01), colocasia (0.292 ±0.03) papaya (0.251 ±0.02) and banana (0.169 ±0.01) were higher than that on sweet potato (0.157 ±0.02). The relative consumption rates were highest when the larvae fed on sweet potato (2.53±0.17), followed by that on castor (1.97±0.07), then on papaya (1.44±0.12) and colocasia (1.37±0.10) and the lowest on banana (1.19± 0.03). The efficiency of conversion of ingested food was highest on banana (31.8 ±1.15), followed by that on castor (30.9±0.62) then that on colocasia (28.2±0.35), then on papaya (24.6±0.39) and lowest on sweet potato (8.91±0.26). The efficiency of conversion of digested food was higher when the larvae fed on banana (51.22 ±1.60) than when fed on the other four host plants. However, the larvae that fed on castor, colocasia and papaya were approximately similar and lower than those on sweet potato and banana. The approximate digestibility of *S. litura* larvae on the five host plants differed significantly and were higher on castor and colocasia than on papaya, sweet potato and banana.

Table. IV.14. Nutritional indices of *Spodoptera litura* larvae feeding on five selected host plants

CATEGORY	Rate ±SE						
	Sweet potato	**Castor**	**Colocasia**	**Papaya**	**Banana**	**F**	**P**
Relative growth rate	0.157 ±0.02	0.335 ±0.01	0.292 ±0.03	0.251 ±0.02	0.169 ±0.01	11.069	0.000
Relative consumption rate	2.53±0.17	1.97±0.07	1.37± 0.10	1.44±0.12	1.19±0.03	24.020	0.000
Efficiency of conversion of ingested food	8.91±0.26	30.9±0.62	28.2±0.35	24.6±0.39	31.8 ±1.15	174.498	0.000
Efficiency of conversion of digested food	36.1 ±0.80	37.52 ±2.11	49.46±1.22	24.50±1.37	51.22 ±1.60	166.255	0.000
Approximate digestibility	52.91 ±1.12	93.31 ±0.75	91.53 ±0.34	62.02 ±1.85	41.80± 2.05	279.901	0.000

4.4. DISCUSSION

The quality and quantity of food consumed affect growth, development and reproduction of insects (Scriber and Slansky, 1981). The present study was designed to evaluate the impact of five different species of food plants on the growth and developmental parameters of the polyphagous insect *S. litura* in different seasons (summer, monsoon and post monsoon seasons).

The data clearly showed that the larval and pupal development and survival, pupal weight and oviposition of emerged females of *S. litura* reared on selected host plants varied greatly. Many similar research works have been carried out but direct comparison of these data was difficult because different host plants and environmental conditions were used in these studies. Although the same insect was used, it differed in origin and it could be different strains or biotypes.

The consumption and utilization of food act as an essential component for growth, development and reproduction of an animal. The quality, amount and rate of food consumed by a larva influences its characteristic features viz. developmental time, final body weight, growth rate, probability of survival and dispersal ability (Kerkut and Gilbert, 1985). In phytophagous chewing insects, leaf shape may have a role since some of these insects prefer or require an edge and rolled leaves make feeding difficult (Chapman, 1974).

In the present work it was noticed that there were six larval instars for *S. litura* when reared on each selected host plant. The number of instars was identified by the head capsule measurement. The mean width of head capsule ranged from 0.2610±0.014mm in the first instar to 2.7134±0.018mm in the last instar. The largest width of the head capsule was reported for colocasia fed larvae. However, as recorded for several species, depending up on the diet, the size of the head capsules, especially at the end of developmental

stage can vary greatly (Parra *et al.,* 1977; Mattana and Foerster, 1988; Santos *et al*., 2003). The effects of food suitability on the number of instars is described by Parra (2009) and there are several examples demonstrating the effects of the quality of food on the number of instars in polyphagous insects (Santos *et al*., 1980). Esperk *et al. (*2007) reported that the occurrence of additional instars is a compensatory mechanism during adverse conditions where temperature, humidity, photoperiod, density of individuals and quality and quantity of food are the most common factors that affect the number of instars.

In the present study, it was observed that the size of *S. litura* larvae fed on selected host plants exhibit small differences during the early larval stages. However, the colocasia fed larvae showed a slight increase in length and castor fed larvae showed an increase in weight than the larvae fed on other host plants during the 1st and 2ndinstar stage. This difference may be due to high moisture content or nutritional content in the colocasia and the highest protein content in the castor leaves compared to the other host plants. In the 3rdand 4th instar stage the highest length was observed in the colocasia fed larvae but the weight is highest for castor fed larvae in 3rd instar and for colocasia fed ones in the 4th instar. In the 5th instar stage the maximum length was noticed for the castor fed larvae and in the 6th instar stage increased larval size was observed for the colocasia fed larvae. But highest larval weight was noticed for the colocasia fed larvae both in the 5th and 6th instar stage.

In the present study high larval weight of colocasia and castor fed larvae of *S. litura* during the last instars can be explained with reference to percentage of moisture content or the highest nutrients content in food plants. Parpiev (1968) reported that low water content influence the energy expenditure, nutritional efficiency and growth of herbivorous insects. The present work corroborate with the work of Paul *et al. (*1992) who suggested

that absolute consumption and growth rate/day/larva increased with increasing percentage of leaf moisture. Similarly Parpiev (1968) also reported that high water content influence both edibility and assimilability of leaves in the silkworm. Thus high moisture content in leaves of colocasia may cause an increase in length and weight of the colocasia fed larvae during the 6th instar.

Ratte (1985) reported that holo and hemi metabolous insects terminate their growth at the time of metamorphosis and when comparison of the growth curves of the initial development of these insects indicated a remarkable similarity, but it varied during the final instar. The duration of the instars of *S. litura* maintained in selected host plants produced different responses, since the early instars showed reduced periods while the later ones were longer. This is possibly related to the fact that, at the end of the larval stage, the larvae of Lepidoptera utilize more food to meet their nutritional demands acquiring the necessary reserves for the development of pupae and consequently adults (Bortoli *et al*., 2005).

A comparatively higher larval size and weight was recorded during the early summer season than monsoon and post monsoon season (Table IV.3, IV.4 and fig IV.3, IV.4). Ratte (1985) observed the influence of temperature on insect size and reported that some insects have direct relationship between weight and temperature. The interaction of temperature in developmental process and physiology, in determination of size and fecundity of sixco-existing mayflies were excellently described by Sweeney and Vannote (1981). Thus higher temperature during summer than post monsoon may be reflected in the higher weight and size of the *S. litura* larvae. The nutrient contents in the host plants were also recorded high during summer than in post monsoon which also may cause this variation.

The growth rate during the early instars were found to be quite low for all the larvae fed with selected host plants. It was supported by the work of

Poonia (1978) who suggested that most of the food consumed during early instars is spent in energy expenditure for maintenance and very little was used for growth. Panizzi and Parra (2009) suggested that the ingestion in the early life stages is of great significance for phytophagous insects because some dietary requirements of the host plant must properly fill their nutritional needs, so as to develop them normally into the adult stage.

It was observed that depending upon the feeding materials the larval period varied in *S. litura*. Shortest larval duration was noticed in the castor fed larvae (11.7 days) and longest was in the banana (16.1 days). This findings supported by Patel *et al*. (1973) who reported that the larval period varied from 10.98 days on castor to 30days on tobacco. Due to the lower amount of nutrients in the banana plant, larvae fed on this could not utilize sufficient amount of metabolites for its growth which led to prolonged larval duration. It was clear that the deficiency in any essential nutrient in insects usually lead to merely cessation of growth and prolonged survival of the insect (Gordon, 1984).

The shortest and the longest larval duration were found during the summer and post monsoon seasons respectively. The larval duration during monsoon was slightly longer than the summer season. Longer larval duration during post monsoon may be attributed to low protein and moisture content in the leaves and/or due to the minimal food intake by the larvae during post monsoon season.

Similarly high protein and moisture content in leaves and increased food intake by the larvae during summer and monsoon season gives shortest larval duration. These findings were supported by the works of Zhu *et al*. (2000), Chen *et al*. (2002) and Seema *et al. (*2004) who reported that the larval development of *S. litura* differ greatly depending on host plants and temperature, and the development was prolonged under low or high

temperatures. Rattanlal and Nayak (1963) observed the duration of 18 days of larval stage at 23^0C on castor. The larval development also greatly influenced by the kind of host plants (Moussa *et al*., 1960).

It has been reported that pupal development was not affected by host plants on which their larvae fed (Patel *et al.,* 1986). In the present study significant variation was observed in both the prepupal period and pupal period of *S. litura* when reared on selected host plants. Shortest prepupal and pupal period was found in castor fed insect and longest for banana in all the seasons. Seasonally the shortest prepupal and pupal period was observed in the summer season and longest in the post monsoon season. Nagoshi (2011) reported that the viable adult forming larvae reached pupation as much as three days earlier than those that have failed to successfully complete emergence.

Feeding material also influence the pupal weight. In the present observation the weight of pupae of castor fed larvae was highest than the other host plants in all the seasons. It was also noted a slight variation in the pupal weight of female and male. The female pupae showed a small increase in the weight than the male pupae. This findings were supported by the Garad *et al. (*1985) who reported the pupal weight within the range of 0.32 to 0.37 g for the *S. litura* reared on castor and okra. Various researchers also documented this sexual dimorphism among different species of *Spodoptera* (Mattana and Foerster 1988; Bavaresco *et al.,* 2004; Santos *et al.,* 2005; Xue *et al.,* 2010) and other lepidoptera. According to Gazzoni and Tutida (1996) high pupal mass in female lepidoptera may be related to a high level of reserves, which are essential for adequate activities in the reproductive phase including mating, oviposition and egg viability.

The influence of feeding material in the pre-oviposition and oviposition periods of *S. litura* were recorded in this study. It was observed

that there was no significant variation in the pre oviposition periods of the adults reared from larvae fed with selected host plant leaves. Even though the shortest pre oviposition days were observed in the adult formed from castor fed larvae and the longest period were noticed in the banana fed ones. The pre-oviposition period was shortest in the summer and longest in the post monsoon season. A significant variation was recorded in the oviposition periods, the oviposition days were maximum for the adult of the castor fed larvae and minimum for the adults of banana fed larvae. The longest oviposition period was noticed in the summer and shortest in the post monsoon season. This variation in oviposition by females on different hosts under different environmental conditions were also reported by Patel *et al. (*1986) and Bae and Park (1999).

The fecundity rate of castor fed larvae was found to be higher than the the larvae fed on other host plants. The larval diet quality influence the fecundity of the adult (Zucoloto Fernandes, 1997). So this higher fecundity rate may be due to the feeding nutritiously rich castor leaves. Seasonally, summer was found to be ideal for the highest fecundity rate and in post monsoon fecundity rate was the lowest. It was revealed that the reproductive dynamics and fecundity of female insects were dependent upon the climatic conditions as noticed in Colarado beetle *Leptinotarsa decemlineata* (Opyichalowa *et al.,* 1976). The average number of eggs laid per female has been reported to be 1,038 (Sankarperumal *et al.,* 1989), 2,088 (Seth and Sharma, 2001), and 3, 166.8 (Garad *et al.,* 1985) eggs per female when *S. litura* larvae was fed on castor.

Observations on longevity of *S. litura* adults on selected host plants showed significant variation. The highest longevity was reported for the adults of castor fed larvae in all the three seasons and least longevity was observed in the adults of banana fed larvae. By comparing the longevity of

male and female adults it was noticed that a slight increase in the male than in the female. Similarly adult longevity also changed depending on seasonal variation. In the present study the highest longevity was observed in the early summer season than in the monsoon and post monsoon seasons. Similar results were reported by Bae and Park (1999) and Xue *et al*. (2010) although the differences were generally less than one day. But Patel *et al. (*1986) found that on cotton, male adults lived 6.3 days as compared to 12.30days for female adults. It has been found that adult longevity became shorter as the temperature increases beyond the limit (Bae and Park, 1999)

In the present work variations in the survival rate of *S. litura* was noticed in each of the selected host plants during each instar stage. The maximum survival rate was shown by each instar of the castor fed larvae and minimum was shown by the banana fed larvae in all the three seasons. But when comparing the instars least survival percentage was reported during the third instar stage and maximum survival was shown in the first and sixth instar stages. Even though the papaya fed larvae showed the highest survival rate in the early instar stage, it showed a reduction in survival percentage during the sixth instar stage. Seasonal comparison showed that the maximum percentage of survival was during the early summer season and least in the post monsoon season.

Larval survival was observed maximum in the castor fed larvae and minimum in the banana fed ones and in other host plants it was intermediate between them. The nutritional content of the host plants also influence survival rate. The reduced protein content in the larval diet delay the larval duration and reduce the survival rate (Nestel and Nemny-Lavy , 2008). It was noticed that the survival rate changed with different seasons. Maximum survival rate was observed in the early summer season. The high rate of larval survival indicated that both the diet and the rearing conditions were

satisfactory for the development of the insect in the laboratory. This corroborate with the findings of Esperk *et al. (*2007) that the most common factors influencing instar number include temperature, photoperiod, food quantity and quality, humidity, injuries, inheritance and sex. According to Patel *et al. (*1987) the larval survival or pupation rate of *S. litura* differ greatly on different host plants, rangingfrom 100% on *Ricinus communis*.

Both the pupal and prepupal survival rates were highest in the castor fed larvae in the early summer season than the other host plants. Lowest survival rate for both the prepupal and pupal period was noticed in the papaya fed larvae. During the prepupal period a relatively high survival rate was observed, with a relatively short duration and without any significant difference between sexes. La Rosa *et al*. (2002) reported 100% survival for prepupal period, regardless of large larval mortality. Difference in pupal survival, longevity and fecundity may also be affected by temperature , diet and other environmental conditions (Zhu *et al*., 2000; Chen *et al.,* 2002; Seema *et al*., 2004).

The overall results showed that the development of *S. litura* was most affected by the host plant banana when compared to the other host plants since an increase in the duration of the larval, pupal and total life cycle occurred, as well as a reduction in pupal weight. The reason for this variations in these biological parameters may be probably related to the low nutritional content like carbohydrate, protein and amino acid of this leaves. This findings was supported by various authors who reported about the influence of larval diet in the variation of the duration of the life cycle of the different species of *Spodoptera* (Azidah and Sofian-Azirun, 2006; Barros *et al*., 2010; Saeed *et al.2*009; Farahani *et al.,* 2011) or may vary even among biotypes of the same species (Giolo *et al*., 2002; Busato *et al*., 2004).

Depending on the rearing conditions discrepancies in the number of instars were reported (Efron and Tibshirani, 1993). Since the immature stages are hypersensitive to abiotic and biotic elements in their environment, there is constant pressure on them to finish their immature stages as quickly as possible to achieve the adult stage and onset of maturity. However, the shorter developmental periods experienced at higher temperatures may sharply accelerate the immature stages, leading to an early onset of maturity which, in turn, would prompt an increase in progeny and larger populations. In most insect populations, there occurs discrepancy in developmental rates between individuals and among the sexes.

The results of the present investigation on nutritional parameters of last instar larvae of *S. litura* are in conformity with the findings of previous studies (Hegazi and Schop, 1984). The relative consumption rate (RCR) calculated from the dry weight of food consumed by *S. litura* is an indicator of its relative intake of nutrients. Relative growth rates (RGR) defines the physiological capacity of *S. litura* to convert food material into biomass. Both of relative consumption rates and relative growth rates were observed to be significantly higher on castor in comparison to other host plants. But in the case of sweet potato the relative consumption rate is higher but the relative growth rate is lower. Similarly both the consumption rate and growth rate were lesser in the banana. However, the values of larval approximate digestibility on the five host plants were significantly higher on castor and least on banana. It may be apprehended that the low approximate digestibility (AD) of *S. litura* larvae on the host plant banana was perhaps compensated by higher values of the efficiency of conversion of ingested food (ECI) and digested food (ECD). ECI is an index of an insect's ability to utilize the food consumed for growth and development, while ECD is a general index of the efficiency of conversion of digested food into biomass (Nathan *et al*., 2005) *S. litura* larvae reared on castor leaves exhibited considerably higher values of

growth rate, AD, ECI and ECD. However, the least efficiency of conversion of digested food for *S litura* was observed on sweet potato in comparison with castor , colocasia, papaya and banana although the relative consumption rate was high on sweet potato. Leaf texture, nutritional quality and secondary metabolic content may have influenced low feeding activity of *S. litura* larva on different host plants.(Bryant *et al*., 1987; Lindroth *et al*., 1988).

Cook (1927) and Matteson and Decke (1965) reported that the alternating or fluctuating temperature accelerated the development of some insects. But Guppy (1969)reported that there was no special effect of fluctuating temperature in the development of *Pseudo letia* unipuncta. They discussed this in relation to the metabolic activities. Since the insects living in the field are always affected by atmospheric temperature which fluctuates with a daily rhythm it is considered that their activities such as feeding, resting and so on must have a close connection to the daily rhythmic change of the atmospheric temperature. It is possible that this rhythmic change of temperature gives a favourable effect to the insect through a change in the metabolic rate. Thus it is very likely that the development of the insect is delicately affected by the presence or lack of such a rhythm..

In conclusion, based on oviposition preference, larval development and survival, prepupal and pupal weight and duration, and emergence fecundity and longevity of adults of *S. litura*, the preference and nutritional values of the selected host plants were ranked as castor > colocasia> papaya>sweet potato>banana. The summer season was most favourable for the growth and development of *S. litura*. Hence, the present study has shown the suitability of selected host plants for the development, longevity, and survival of *S. litura*. during different seasons. This may provide the useful information regarding the development of forecasting models and implementing a timely intervention for the control of *S. litura* on agricultural crops

CHAPTER V

SEASONAL VARIATION IN PROTEIN AND AMINO ACID CONTENT OF DIFFERENT TISSUES OF *SPODOPTERA LITURA* IN RESPECT TO FEEDING WITH THE SELECTED HOST PLANT LEAVES.

5.1. Introduction

Proteins are the complex nitrogenous compounds found in both plants and animals. Proteins play a vital role in different biological activities in the living organisms (Murthy *et al*., 2014). They act as enzymes or as biocatalyst, contractile elements, hormones, more over play important role in metabolic reactions in a cell. The amount of total protein at any time in a tissue depends on the storage and utilization of organic matters. The proteins are degraded by the hydrolyzing enzymes in to its corresponding amino acid units and these act as the precursor for the other protein synthesis and for the gluconeogenesis (Krishnamohan Reddy, 1986).They play an important role in meeting the energy needs and help in metabolic maintenance and further more in the cell volume regulation (Ferguson, 1982).

In insects, the growth and development is associated with protein metabolism (Singhman and Baquaya, 1971). In living organisms body protein constitute about 80-90% of all organic substances (Baker, 1996). Quality of protein is measured by the type of amino acids present in it. Eight amino acids are essential and they have to be obtained through food because they are not synthesized by the animal. These include Lysine, Leucine, Isoleucine, Methionine, Phenylalanine, Threonine, Tryptophan and Valine (Sen and Bhattachargya, 2001). Dietary protein which contain all the essential aminoacids in required quantity to the body is said to be of high quality and if

the protein is deficient in one or more of the essential amino acids, the protein is said to be of low quality (Tomoskozi *et al*., 2001).

Many insect biochemists, widely used proteins as a biochemical tool because of their crucial role in the development, morphogenesis and in almost all intermediary metabolic pathways. Proteins function as an indicator of the gene expression because all gene expressions lead to the synthesis of one or more proteins. Besides this the variation of protein in different genotypes provide the basis to understand the phylogeny, genetic distance, and establish taxonomic relationship between species (Lewontin and Hubby , 1966; Ayala *et al*.**,** 1974**).**

Like any other organism every plant also needs the basic component like protein and optimum climatic factors for growth. The plants synthesize the amino acid from the carbohydrate, which are formed from elements such as carbon, oxygen and hydrogen through the photosynthesis followed by the incorporation of nitrogen through the metabolic pathways. Plants synthesize only L-amino acids. These aminoacids are the basic units for the synthesis of protein. Plant's structure is mostly composed of a very high amount of carbohydrate (cellulose and others), but plants contain low level of protein compared with the animal cells. In recent years many works has been reported in the protein metabolism (Nelson *et al*., 2014).

Apart from their role as protein constituents, amino acids are also participating in a number of cellular reactions, hence they influence a number of important physiological activities such as plant growth and development, generation of metabolic energy or redox power, intracellular pH control and stress resistance (Pratelli and Pilot, 2014). The amino acids also have a role in the signaling pathways in plants (Timm *et al*., 2012; Ros *et al*., 2014).

Some amino acids act as precursors for the biosynthesis of other nitrogenous compounds like phytohormones, nucleotides, secondary metabolites (formation of glucosinolates from methionine, alanine, and branched-chain aromatic amino acids) (Halkier and Gershenzon, 2006). During the conditions such as scarcity of aminoacids in plants, protein contribute reservoirs of aminoacids by the catabolic programms like proteasome mediated degradation and autophagy (Araujo *et al*., 2010). Amino acids are subsequently reused and distributed for the synthesis of specific proteins required under nutrient limitation. Besides, during carbon starvation or the typical life cycle, proteins are degraded and the complete oxidation of their amino acids delivers the energy required to fuel the particular needs of specific organs (e. g. stressed leaves or roots). Hence, the exact detection of amino acid levels seems to be a key point for the effective regulation of protein and amino acid synthesis and catabolism and also for the control of energy production. In this way, regulation of the amino acid content and its transport through the plants are important for the adaptation of plant to carbon and nitrogen status, development and defense (Zeier, 2013; Pratelli and Pilot, 2014).

During the stress condition the pools of all amino acids are much induced. The concentration of proline significantly increase during the stress condition in several plants (Jacoby *et al*., 2011). Branched-chain amino acids are also much induced during different stress conditions (Joshi *et al*., 2010). These large variations reflect the different functional roles of the individual amino acids. In leaves, its concentration was seen high under several stress situations (Obata and Fernie, 2012)

Even though the animals get some of their nutrients through the endosymbionts (Karasov and Douglas, 2013), their requirement of multiple nutrients for the growth and development is attained mainly through eating.

The nutrient concentration is generally low in plant tissues compared to the animal tissue (Bernays and Chapman, 1994; Karasov and Martınez, 2007). Thus the challenge for herbivore is to search and find the food that contain the nutrients at higher concentration. Nutritionally optimal vegetative tissues are rare in plants, even though herbivore attain their nutrient requirement through pre-ingestive and post-ingestive mechanisms (Behmer, 2009).

The quality and quantity of host plants have a major role in the growth and reproduction of an insect. Each host plant contains numerous nutritional and anti-nutritional substances, for which regulation of their uptake by an insect shows integration of a highly complex set of interacting processes (Simpson and Raubenheimer, 1999). Insects can regulate their intake of protein by mixing their diet either by switching between plants or plants tissues (Villalba *et al.,* 2002; Felton *et al*., 2009). When the phytophagous insect have limited chance to mix their diet , they can adjust their feeding responses to balance the diet and the nutrient concentration in their food (Raubenheimer and Simpson, 1993; Fanson *et al*., 2012). This balancing mechanism of nutrients by alternating host plants may not be possible at all time under the natural environmental condition due to the threat of predators. (Schmitz and Suttle, 2001; Hawlena and Schmitz, 2010).

Due to the low concentration of required nutrients the herbivore need to actively feed on foods for a long time to compensate for the required nutrient concentration. This will lead to the increased risk of attack by predation (Bernays and Minkenberg, 1997). When the digestable dietary carbohydrate contents are low, the aminoacids synthesize glucose through the gluconeogenic pathway (Thompson *et al.,* 2002). Usually caterpillars live in protein rich habitats and exhibit a protein based food intake (Behmer, 2009).

Basically caterpillars lack a foregut but have a large midgut (which is the principal site of absorption) and a very short hindgut. This arrangement is

responsible for the rapid growth of the caterpillars (Yang and Joern, 1994a, b). The midgut is an important organ for insects because it occupies a major part of the digestive tract; it also play vital roles in other physiological activities like immune response, metabolism, homeostasis of electrolytes, osmotic pressure, circulation and more. The midgut is a complex organ that experience a gross change at metamorphosis to support diverse feeding habits at different developmental stages. In most insect species the midgut is the primary site for protein digestion (Ferreira *et al*., 1990). Thus any change in the microbial assemblage of the larval midgut results in the change in health condition and biology of the animal (Gilbert and Hazelwood, 1993).

The fat body is the primary source for the synthesis of haemolymph proteins. The fat body synthesizes and secretes numerous proteins of specific functions during the immature stage of life in numerous insect species. These proteins function as storage reserves for amino acids which are recovered and used later for the growth and development of new adult tissues during metamorphosis (Wyatt and Pan, 1978; Ranjini and Mohamed, 2002). The fat body produces numerous unique and physiologically important proteins like vitellogenins for oocyte development; stage - specific amino acid storage proteins such as calliphorin, drosophilin and manducin (Levenbook and Bauer, 1980; Ranjini , 2002).

Haemolymph is a fluid that fill and circulate in the body cavity of insects by bathing the tissues directly (Chapman, 1969). It consist of fluid plasma which bath all the tissues, comprising about 5-40% of the total body weight of the insects. Plasma contain haemocytes, inorganic constituents such as electrolytes or ions, phosphates and organic constituents like free amino acids, proteins, carbohydrates, lipids, uric acid etc. The proteins present in the haemolymph usually plays an important role in the transport function as well as the enzyme action. The synthesis and usage of haemolymph proteins are

controlled by genetic and hormonal factors. (Hurliman and Chen, 1974). The influence of the nutritional status in the synthesis of haemolymph or storage proteins during the larval development of silkworm is reported by Ramesh Babu *et al*. (2009) and by environmental conditions is reported by Rameshaet *al*. (2010).

Insect haemolymph is the major site of amino acid pool. Fluctuation of protein content in the insect haemolymph is more than that of free amino acids and other non-protein nitrogen. Previous studies reported that in lepidopteran insects, enhancement in protein concentration is gradual during the first larval stage then shows quick increase in the fourth and fifth instar stages, small changes occurs during the spinning and the early stage of pupal period and a sharp fall appeared during the adult development (Ranjini and Mohamed, 2004).

Amongst the organic constituents protein is one of the major biochemical component. Apart from biochemical analysis the protein patterns can be detected by electrophoretic technique, in which molecular sizes and molecular weights of a single protein molecule can also be determined. Key elements of classical proteomics are the separation of proteins in a sample using SDS-PAGE. Many investigators have carried out experiments to detect the protein pattern from vertebrate and invertebrates. The present study seeks to investigate the comparative estimation and electrophoretic profile of different tissues of *S. litura* fed with different host plants. This chapter include the quantitative and qualitative analysis of total protein and total free amino acids in the midgut tissue, fatbody and haemolymph of *S. litura* fed with different food materials in different seasons and the analysis of changes in the total protein and total free amino acids in the leaves of selected host plants in different seasons.

5.2. Materials and methods

The materials used and methods employed for carrying out the quantitative estimation of protein in different tissues of *S. litura* and its host plant leaves were given in the section 3.2.7.1. and 3.2.7.6 respectively. The methods for the quantitative estimation of amino acids in insect tissue and plant tissue were described in the section 3.2.7.3 and 3.2.7.7. respectively and the electrophoretic studies were discussed in the section 3.2.7.2.

5.3. Results

The results of the present work includes the quantitative and qualitative estimation of total protein and free aminoacids content in the midgut, fat body and haemolymph of last instar larvae of *S. litura* by giving different food materials in different seasons. The results showed the seasonal changes of the protein concentration and amino acid concentration in host plant leaves and the corresponding variation in the insect tissues. The changes in the protein concentration in plant tissue, midgut, fat body and haemolymph were given in the table V.1, table V.2, table V.3, and table V.4.

5.3.1. Seasonal variation in protein concentration in the leaves of selected host plants of *Spodoptera litura.*

The total protein content in five selected host plants in different seasons were presented in the Table V.1. and Figure. V.1. Variation in protein content in each host plant was observed in all the seasons. Comparison of the results obtained from the data indicated that among the five selected host plants the castor showed the highest protein content than the other four host plants and it contained 1.32±0.08mg/ml of protein in the summer season. The protein content in other host plants were 0.59±0.02mg/ml for papaya ; 0.36±0.01mg/ml for colocasia; 0.35±0.06 mg/ml for banana and 0.24±0.00mg/ml for sweet potato in summer season. Similar changes were

observed in monsoon and post monsoon seasons and the values were 1.05±0.03 and0.46±0.10mg/ml for castor, 0.29±0.02 and 0.18±0.01mg/ml for papaya, 0.19±0.00and 0.14±0.00mg/ml for colocasia, 0.13±0.01and 0.12±0.00mg/ml for banana and 0.12±00and 0.11±0.00mg/ml for sweet potato respectively. By comparing the seasonal variation it was observed that in the summer season the host plant leaves showed the highest protein content than in the other two seasons.

Table. V.1. Seasonal variation in protein content in the leaves of selected host plants of *Spodoptera litura*

Seasons		Protein concentration in	Host plants					F	P
			Castor	Papaya	Colocasia	Banana	Sweet potato		
	Summer	Mg/ml	1.32± 0.08	0.59±0.02	0.36±0.01	0.35±0.06	0.24±0.00	91.5	0.000
		Mg/gm	2.65±0.17	1.18±0.04	0.72±0.02	0.71±0.13	0.48±0.29	29.5	0.000
	Monsoon	Mg/ml	1.05±0.03	0.29±0.02	0.19±0.00	0.13±0.01	0.12±0.03	264.2	0.000
		Mg/gm	2.08±0.07	0.58±0.04	0.39±0.00	0.26±0.03	0.24±0.02	378.5	0.000
	Post monsoon	Mg/ml	0.46±0.10	0.18±0.01	0.14±0.00	0.12±0.00	0.11±0.00	12.06	0.000
		Mg/gm	0.92 ±0.03	0.36±0.02	0.28±0.00	0.24±0.01	0.22±0.01	578.2	0.000

The values presented in the table are the mean value of five replicates for each host plant with standard error (Mean ±Standard error). All the values were found to be significant at 1% level. The total protein content was expressed in the mg/ml and mg/gm tissue.

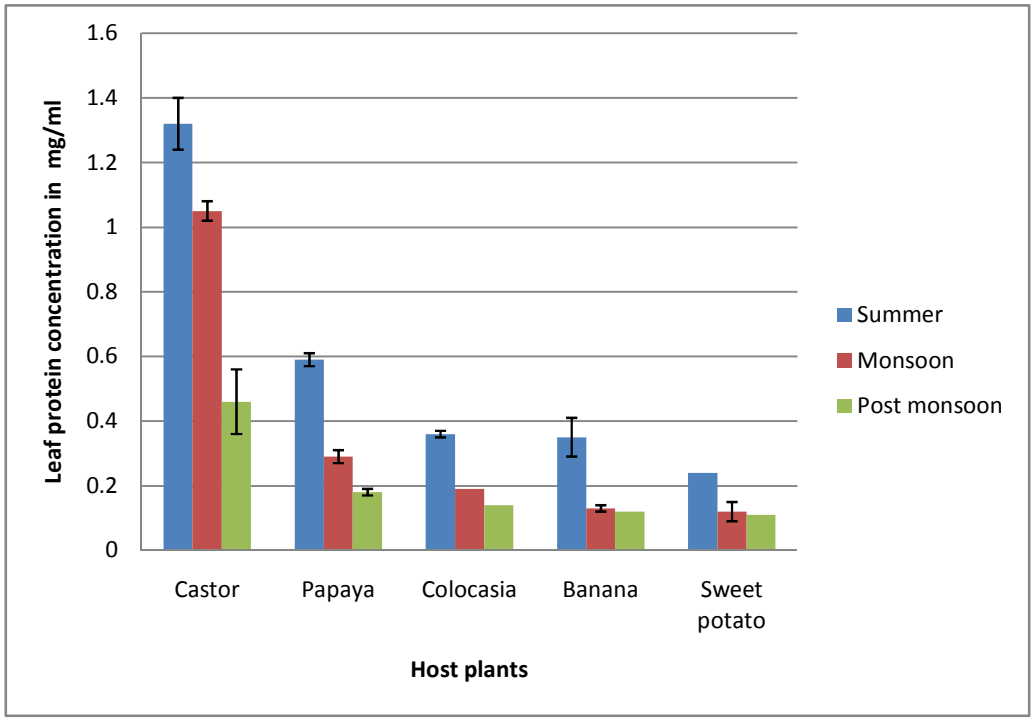

Fig. V.1. Seasonal variation in protein content in the leaves of selected host plants of *Spodoptera litura*

- **Seasonal variation in protein concentration of midgut tissues of last instar larvae of *Spodoptera litura* fed with selected host plant leaves**.

The variations in protein content of midgut tissue of the insect fed with five selected host plants (castor, colocasia, papaya, banana and sweet potato) in three different seasons were presented in Table. V.2. and Figure. V.2. The data indicated the variation in protein content of the midgut tissue in each season with the corresponding food materials. The midgut tissues of the castor fed larvae showed the maximum protein content in each season than the other host plants. The concentration of protein in the midgut tissue of insect fed with selected host plants were 4.15±0.04mg/ml (castor), 3.9±0.22mg/ml (papaya), 3.7±0.14mg/ml (colocasia), 3.2±0.16mg/ml (banana) and 3.09±0.05mg/ml (sweet potato) in the summer season. Similar changes were

observed in the monsoon and post monsoon seasons. They were 3.88±0.18 mg/ml and 3.25±0.18mg/ml for castor; 3.30±0.12 mg/ml and 2.87±0.45mg/ml for papaya; 3.08±0.19 mg/ml and 2.42±0.17mg/ml for colocasia; 2.93±0.09 mg/ml and2.79±0.04 mg/ml for banana and 2.8±0.08 and 2.09±0.27mg/ml for sweet potato in the monsoon and post monsoon seasons respectively.

Table. V.2. Seasonal variation in protein content of the midgut tissue of last instar larvae of *Spodoptera litura* after fed with the leaves of selected host plants.

	Protien concentration in	Host plants					F	P
		Castor	Papaya	Colocasia	Banana	Sweet potato		
Summer	Mg/ml	4.15±0.04	3.9±0.22	3.7±0.14	3.2±0.16	3.09±0.05	9.78	0.000
	Mg/gm	26.7±0.96	21.2±0.38	19.4±1.06	17.2±0.38	12.5±0.85	40.25	0.000
Monsoon	Mg/ml	3.88±0.18	3.30±0.12	3.08±0.19	2.93±0.09	2.8±0.08	8.22	0.000
	Mg/gm	23.05±1.2	16.8±0.33	15.7±0.35	13.3±0.37	11.3±1.3	37.51	0.000
Post monsoon	Mg/ml	3.25±0.18	2.87±0.45	2.42±0.17	2.79±0.04	2.09±0.27	2.83	0.052
	Mg/gm	19.3±0.97	11.9±0.99	10.4±0.74	8.97±0.46	7.31±0.35	38.03	0.000

The values presented in the table are the mean value of five replicates for each host plant with standard error (Mean ±Standard error). All the values were found to be significant at 1% level. The total protein content was expressed in the mg/ml and mg/gm tissue

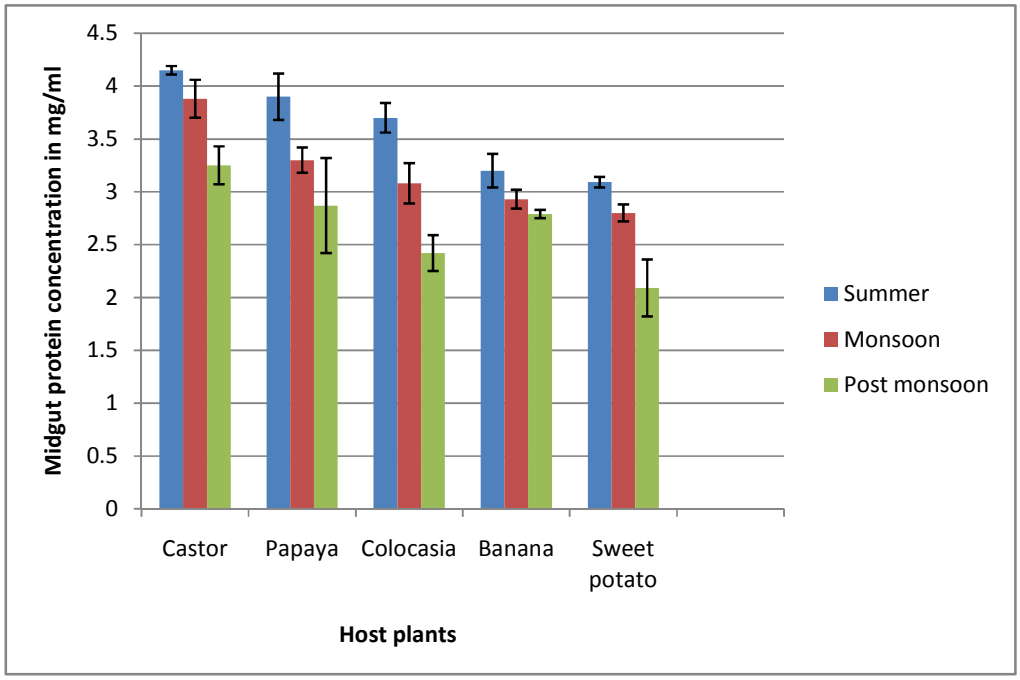

Fig. V.2. Seasonal variation in protein content of the midgut tissue of last instar larvae of *Spodoptera litura* after fed with the leaves of selected host plants.

- **Seasonal variation in protein concentration of fat body of last instar larvae of *Spodoptera litura* after fed with the leaves of selected host plants.**

The variation in protein content of the fat body of the larvae fed with five host plants (castor, colocasia, papaya, banana, and sweet potato) in three different seasons were presented in the Table. V.3. and Figure. V.3. The results showed that the protein content of the fat body varied with the feeding material and with the change in seasons. The protein content in the fat body was in the order castor> papaya> colocasia>banana>sweet potato and the values were 3.8±0.20mg/ml (castor), 3.5±0.04mg/ml (papaya), 2.9±0.20mg/ml (colocasia), 2.4±0.13mg/ml (banana), 2.0±0.15mg/ml

(sweetpotato) respectively. Similar changes were noticed in other seasons also. The values were 3.6±0.13mg/ml and 2.7±0.09mg/ml for castor, 3.2±0.18mg/ml and 2.06±0.31mg/ml for papaya, 2.4±0.22mg/ml and 2.04±0.26 mg/ml for colocasia, 2.0±0.26mg/ml and 1.77±0.15 mg/ml for banana and1.6±0.05mg/ml and 1.49±0.09 mg/ml for sweet potato respectively.

Table. V.3. Seasonal variation in protein content of the fat body of last instar larvae of *Spodoptera litura* after fed with the leaves of selected host plants.

Seasons		Protien concentration in	Host plants					F	P
			Castor	Papaya	Colocasia	Banana	Sweet potato		
	Summer	Mg/ml	3.8±0.20	3.5±0.04	2.9±0.20	2.4±0.13	2.0±0.15	26.9	0.000
		Mg/gm	19±1.6	17.5±0.48	16.5±1.6	12.5±1.1	12.16±1.5	5.1	0.005
	Monsoon	Mg/ml	3.6±0.13	3.2±0.18	2.4±0.22	2.0±0.26	1.6±0.05	19.17	0.000
		Mg/gm	13.9±0.75	12.6±0.56	11.9±0.65	10.1±0.97	9.1±0.19	8.13	0.000
	Post monsoon	Mg/ml	2.7±0.09	2.06±0.31	2.04±0.26	1.77±0.15	1.49±0.09	4.49	0.009
		Mg/gm	8.8±0.34	6.77±0.21	6.64±0.37	6.35±0.11	6.26±0.31	13.99	0.000

The values presented in the table are the mean value of five replicates for each host plant with standard error (Mean ±Standard error). All the values were found to be significant at 1% level. The total protein content was expressed in the mg/ml and mg/gm tissue

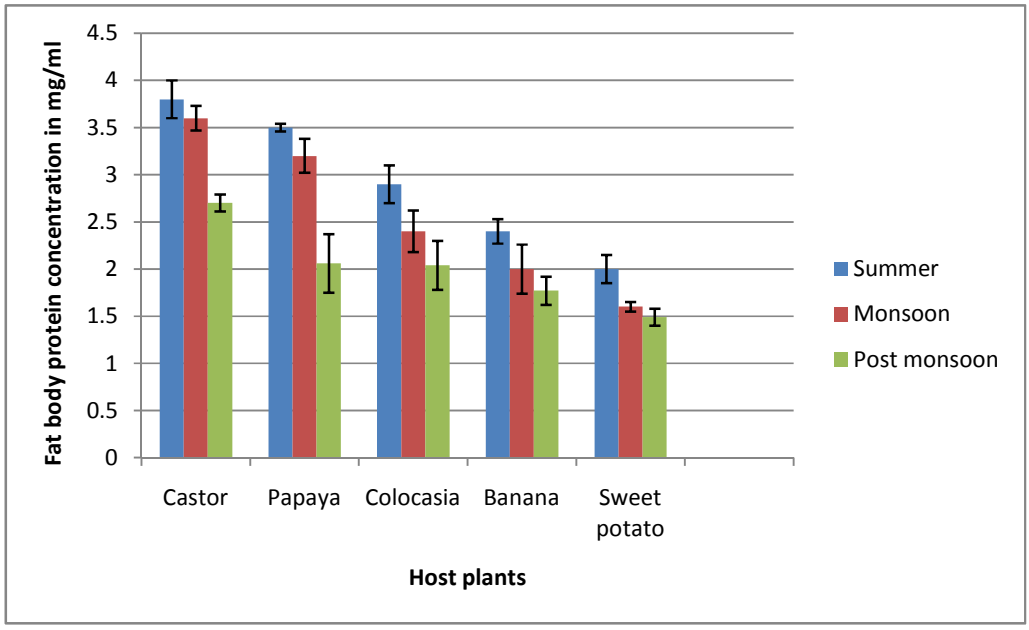

Fig. V.3. Seasonal variation in protein content of the fat body of last instar larvae of *Spodoptera litura* after fed with the leaves of selected host plants.

- **Seasonal variation in protein concentration of haemolymph of last instar larvae of *Spodoptera litura* after fed with the leaves of selected host plants**

The changes in the protein concentration of haemolymph with respect to the feeding material and with the changing seasons were given in Table. V.4. and Figure. V.4. From the data it was noticed that the significant variations in protein content in the haemolymph occurred with respect to the host plant feeding material in different seasons. The protein content was higher in the haemolymph of castor fed larvae than the other host plants in the summer season. The concentration of protein was in the order 53.0±1.3mg/ml (castor fed), 28.6±1.8 mg/ml (papaya fed), 24.5±0.50mg/ml (colocasia fed), 23.0±2.0mg/ml (banana fed) and 17.6±4.1 mg/ml (sweet potato fed)

respectively in summer season. Similar changes was observed in other seasons also. They were 43.5±0.24mg/ml and 28.7±0.97 mg/ml for castor fed larvae; 15.6±1.0mg/ml and9.75±0.52 mg/ml for papaya fed larvae; 14.3±1.03mg/ml and 11.4±0.50mg/ml for colocasia fed larvae; 12.7±0.32mg/ml and 9.4±0.28 mg/ml for banana fed larvae and 11.3±0.36mg/ml and 8.36±0.36 mg/ml for sweet potato fed larvae respectively.

From the above results a positive correlation was noticed between the protein content in the insect tissues and in the leaf tissues in different seasons.

Table. V.4. Seasonal variation in protein content of haemolymph of last instar larvae of *Spodoptera litura* after fed with the leaves of selected hostplants

Seasons		Protien concentration in	Host plants					F value	P value
			Castor	Papaya	Colocasia	Banana	Sweet potato		
	Summer	Mg/ml	53.0±1.3	28.6±1.8	24.5±0.50	23.0±2	17.6±4.1	31.8	0.000
		Mg/insect	10.6±1.5	5.7±1.05	4.9±0.35	4.6±1.4	4.6±0.41	5.7	0.003
	monsoon	Mg/ml	43.5±0.24	15.6±1.0	14.3±1.03	12.7±0.32	11.3±0.36	110.4	0.000
		Mg/insect	8.6±0.72	3.1±0.21	2.8±0.20	2.5±0.22	2.2±0.07	54.1	0.000
	Post monsoon	Mg/ml	28.7±0.97	9.75±0.52	11.4±0.50	9.4±0.28	8.36±0.36	216.8	0.000
		Mg/insect	5.7±0.19	1.9±0.19	2.2±0.21	1.8±0.05	1.6±0.07	111.3	0.000

The values presented in the table are the mean value of five replicates for each host plant with standard error (Mean ±Standard error). All the values were found to be significant at 1% level. The total protein content was expressed in the mg/ml and mg/insect tissues.

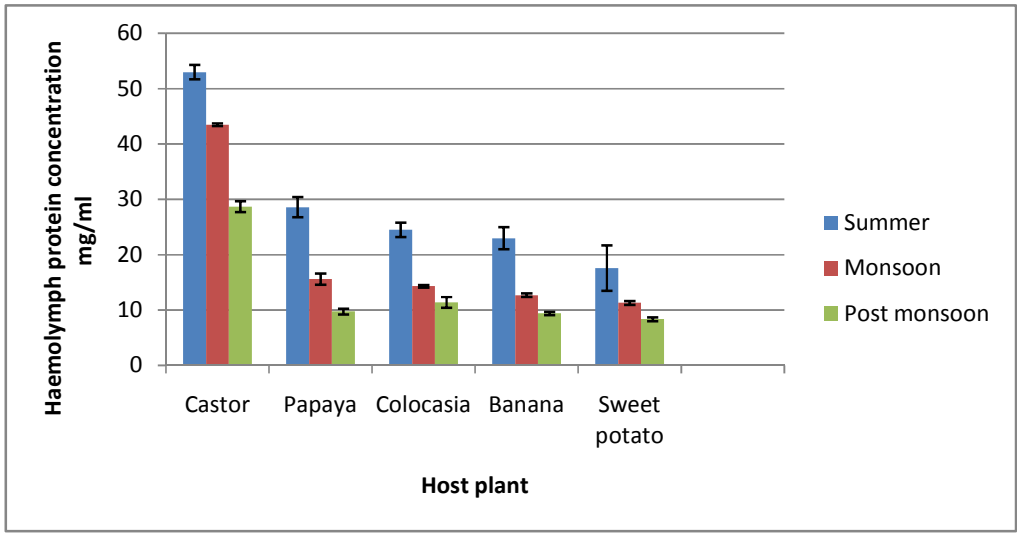

Fig. V.4. Seasonal variation in protein content of the haemolymph of last instar larvae of *Spodoptera litura* after fed with the leaves of selected host plants.

5.3.2. Electrophoretic studies

Electrophoretic analysis of protein in the midgut, fat body and haemolymph of last instar larvae of *S. litura* fed with different host plants were given in plates V.1, V.2 and V.3. The band analysis and pixel intensity revealed the presence of a varying number of bands in the same tissue of *S. litura* when fed with different host plants. Minor quantitative differences were observed in the protein in some bands among the tissues and the intensity of some of the bands differ significantly.

- **Protein profiling of midgut tissue of *Spodoptera litura* after fed with selected host plant leaves.**

The protein profiling of midgut tissue is presented in the plate V.1. With the molecular markers in the range of molecular weight 100KDa to 18KDa, proteins of this range present in the tissues were detected. The protein profile of castor fed midgut tissue showed the highest expression with

10protein bands with molecular weight ranging from 29 KDa to 104 KDa. Similarly there were 7 protein bands present in papaya fed midgut tissue with molecular weight 30KDa to 102 KDa. In the case of colocasia the number of protein bands was 2 and molecular weight ranging from 49 KDa to 71 KDa. For banana fed larval midgut tissue 5 protien bands were observed with a molecular weight in the range of 28 KDa to 98 KDa. The least expression of protein bands were observed in the case of sweet potato, it showed only one protein band with molecular weight 74 KDa. The highest molecular weight protein with molecular weight 104 KDa was present in the castor fed tissue and the lowest molecular weight 28 KDa protein was present in the case of banana. While comparing the protein bands of these different midgut tissues some similar proteins were observed in between them. Occurance of 71 KDa protein is common in the case of castor and colocasia and 66 KDa protein was present in both castor and banana fed cases. The common occurrence of 38 KDa protein was observed in the castor, papaya and banana fed midgut tissues.

- **Protien profiling of fat body tissue of *Spodoptera litura* after fed with selected host plant leaves.**

The protein profiling of fat body is presented in the plate V.2. With the molecular markers with molecular weight ranging from 100KDa to 30KDa, the proteins in this range present in the fat body of *S. litura* fed with selected host plant leaves can be detected. The maximum number of protein bands was 4, which exhibited in the protein profile of colocasia, banana and sweet potato fed larval fat body. Castor and papaya fed cases showed 3 protein bands. Fat body of castor fed larvae showed 3 protien bands with molecular weight ranging from 72 KDa to 91 KDa. Similarly the range of protein bands in papaya fed case was 36 KDa to 70KDa. In the case of colocasia fed larvae the molecular weight ranging from 41 KDa to 92 KDa and in banana fed case

protein bands of 48 KDa to 83 KDa were observed. In the case of sweet potato proteins detected were in the range between 39 KDa to 83 KDa. The highest molecular weight protein with molecular weight 92 KDa was present in the colocasia fed tissue and the lowest molecular weight 36 KDa protein was present in the case of banana. While comparing the protein bands of these different fat body tissues some proteins were commonly observed in between them. The 70KDa protein was common for papaya and colocasia fed tissue. Similarly 83 KDa and78 KDa proteins were present in both sweet potato and banana fed tissue.

- **Protien profiling of haemolymph of *Spodoptera litura* after fed with selected host plant leaves**.

The protein profiling of haemolymph is presented in plate V.3. With the molecular markers in the range of molecular weight from 100KDa to 30KDa, the proteins in this range can be detected. The expression was highest in the protein profile of papaya fed larval haemolymph which showed the presence of 7 bands with molecular weight ranging from 41KDa to 102 KDa. Similarly there were 6 protein bands in the case of banana fed tissue with molecular weight 41 KDa to102 KDa. In the case of colocasia fed larvae, the number of protein bands was 3 with molecular weight ranging from 72 KDa to 97 KDa. For castor fed larval haemolymph 3 protien bands were observed with a molecular weight 72 KDa to 105 KDa. The number of protein bands observed in the sweet potato fed case was 5, with molecular weight ranging from 34 KDa to 104 KDa. The highest molecular weight protein (105 KDa) was present in the castor fed case and the lowest molecular weight (34 KDa) protein was present in the sweet potato fed larval haemolymph. When comparing the protein bands of these different haemolymph common occurrence of some proteins were observed. The 102 KDa and 76KDa proteins were present both in the banana and papaya fed cases. Similarly 72

KDa protein was common in castor, papaya and colocasia fed tissue and 41 KDa protein was present in both papaya and banana fed larval haemolymph.

5.3.3. Seasonal variation in amino acid concentration of different tissues

The changes in the aminoacid concentration in different tissues of *S. litura* were given in Table. V.5, Table. V.6, Table. V.7 and Table. V.8. From the results obtained it was seen that there was a significant variation in the concentration of total free amino acid content of different tissues of both *S. litura* larvae and host plant leaves in different seasons.

- **Seasonal variation in amino acid concentration in leaves of selected host plants of *Spodoptera litura*.**

The total free aminoacid concentration in five selected host plants in three different seasons were tabulated in the table V.5. and Figure. V.5. Changes in the amino acid concentration of selected host plant leaves in different seasons were noticed. The statistical analysis of the data revealed the significant difference in the amino acid content in each season with respect to the feeding material.

Comparison of the data revealed that among the five selected host plants, the castor leaves showed the highest amino acid content of 26.7±0.17 mg/ml. The amino acid concentration in other host plant leaves were 25.8±0.47mg/ml (papaya), 24.4±0.19mg/ml (colocasia), 23.4±0.34mg/ml (banana) and 22.8±0.17mg/ml (sweet potato) respectively in summer season. Similar changes was noticed in the other seasons also and the values were 24.1±0.51mg/ml and 22.8±0.51mg/ml for castor; 23.2±0.23mg/ml and 20.3±0.04mg/ml for papaya; 22.7±0.19mg/ml and 19.4±0.07mg/ml for colocasia; 22.4±0.11mg/ml and 18.4±0.50mg/ml for banana and 21.8±0.15mg/ml and 17.1±0.47mg/ml for sweet potato in the monsoon and post monsoon seasons respectively. By comparing the seasonal variation it was observed that in the summer season the plant leaves showed the highest amino acid content than that in the other two seasons.

Table. V.5. Seasonal variation in amino acid content in the leaves of selected host plants.

Seasons		Amino acid concentration in	Host plants					F	P
			Castor	Papaya	Colocasia	Banana	Sweet potato		
	summer	Mg/ml	28.7±0.17	25.8±0.47	24.4±0.19	22.4±0.34	20.8±0.17	28.6	0.000
		Mg/gm	0.53±0.00	0.51±0.00	0.49±0.00	0.47±0.00	0.46±0.00	28.6	0.000
	monsoon	Mg/ml	24.1±0.51	23.2±0.23	22.7±0.19	19.4±0.11	18.8±0.15	9.5	0.000
		Mg/gm	0.48±0.01	0.45±0.00	0.40±0.00	0.36±0.00	0.29±0.00	9.5	0.000
	Post monsoon	Mg/ml	22.8±0.51	20.3±0.04	19.4±0.07	18.4±0.50	15.1±0.47	27.7	0.000
		Mg/gm	0.45±0.01	0.40±0.00	0.39±0.00	0.34±0.01	0.24±0.00	27.7	0.000

1The values presented in the table are the mean value of five replicates for each host plant with standard error (Mean ±Standard error). All the values were found to be significant at 1% level. The total amino acid content was expressed in the mg/ml and mg/gm tissue.

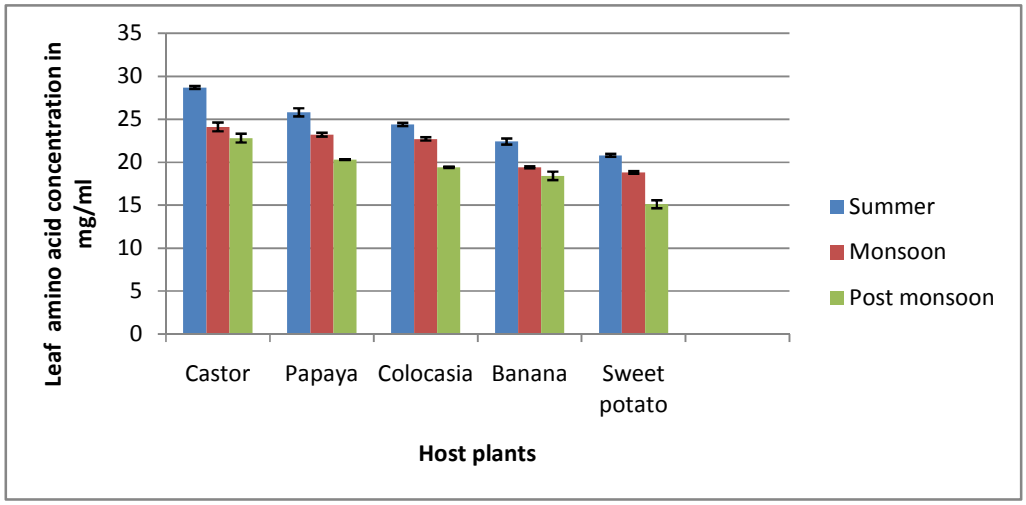

Fig. V.5. Seasonal variation in the amino acid content of the selected host plant leaves.

- **Seasonal variation in aminoacid content of the midgut tissue of last instar larvae of *Spodoptera litura* after fed with the leaves of selected host plants.**

In the case of midgut, the maximum concentration of amino acid was reported in the castor fed larval tissue (31.68±1.4mg/ml). The concentration of free amino acids in tissues fed with the other host plants were 30.8±1.1mg/ml (papaya), 25.3±2.1mg/ml (colocasia), 24.4±1.2 mg/ml (banana) and 23.5±1.0mg/ml (sweetpotato) in summer season (Table. V.6. and Figure. V.6.). Similar changes were observed in other two seasons also and the values were 22.3±1.2 mg/ml and15.8±2.6 mg/ml for castor; 21±1.6 mg/ml and 13.8±0.63 mg/ml for papaya; 19±0.46 mg/ml and 10.1±0.58 mg/ml for colocasia; 17.3±0.66 mg/ml and 9.6±0.54 mg/ml for banana and 15.3±0.92 mg/ml and 8.2±0.40mg/ml for sweet potato in the monsoon and post monsoon seasons respectively.

Table. V.6. Seasonal variation in aminoacid content of midgut tissue of last instar larvae of *Spodoptera litura* after fed with the leaves of selected host plants

Seasons	Amino acid concentration in	Host plants					F	P
		Castor	Papaya	Colocasia	Banana	Sweet potato		
Summer	Mg/ml	31.68±1.4	30.8±1.1	25.3±2.1	24.4±1.2	23.5±1.0	6.9	0.001
	Mg/gm	120.6±17	116±29	87.8±9	86.1±1.2	83.8±3	1.22	0.332
monsoon	Mg/ml	22.3±1.2	21±1.6	19±0.46	17.3±0.66	15.3±0.92	7.4	0.001
	Mg/gm	115±4.3	111±3.8	81.5±3.4	75.6±2.5	71.6±1.9	38.3	0.000
Post monsoon	Mg/ml	15.8±2.6	13.8±0.63	10.1±0.58	9.6±0.54	8.2±0.40	6.2	0.002
	Mg/gm	108.6±6	102.2±3.2	70.4±0.94	68.3±1.1	65.5±1.7	43.3	0.000

The values presented in the table are the mean value of five replicates for each host plant with standard error (Mean ±Standard error). All the values were found to be significant at 1%level. The total amino acid content was expressed in the mg/ml and mg/gm tissue**.**

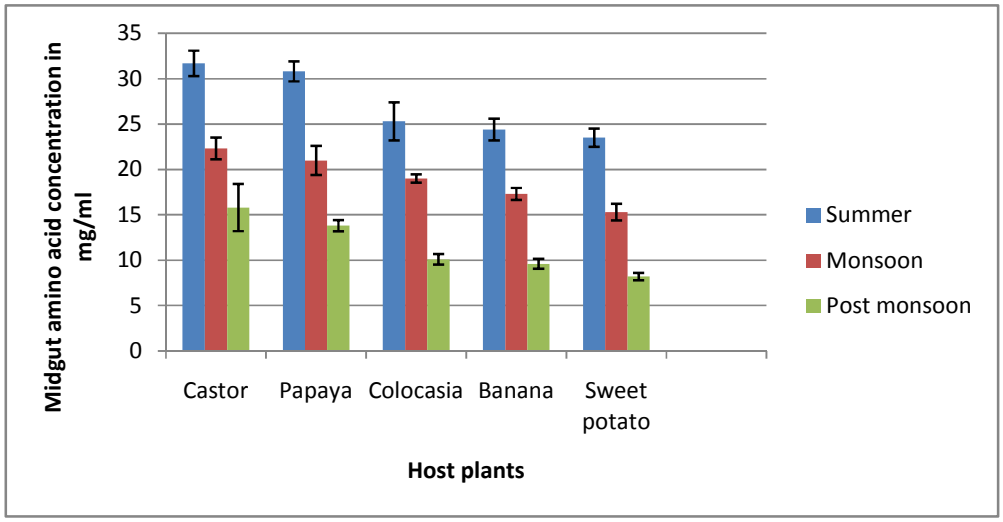

Fig. V.6. Seasonal variation in amino acid content of the midgut tissue of last instar of *Spodoptera litura* after fed with the leaves of selected host plants.

- **Seasonal variation in amino acid content of the fat body of last instar larvae of *Spodoptera litura* after fed with the leaves of selected host plants.**

In the fat body also similar changes was observed as in the case of midgut. The variation in the aminoacid content was noticed in fat body with respect to the feeding materials and different seasons (Table V.7. and Figure. V.7.). The highest concentration was seen in the case of castor fed tissue followed by papaya, colocasia, sweet potato and banana. The concentration of amino acid in the fat body was 20±2.4mg/ml for castor fed larvae, 18.7±1.6mg/ml for papaya fed larvae, 17.1±0.32mg/ml for colocasia fed larvae, 15.5±1.2mg/ml for banana fed larvae and 15.1±1.2mg/ml for sweetpotatofed larvae in the summer season. The values obtained in monsoon and postmonsoon seasons were 17.5±1.5mg/ml and 11.5±0.31mg/ml (for castor fed), 15.9±0.78mg/ml and 8.8±0.26mg/ml (for papaya fed), 14.4±1.5mg/ml and 7.7±0.79mg/ml (for colocasia fed), 12.3±1mg/ml and 7.0±0.36mg/ml (for banana fed) and11.9±1.2mg/ml and 5.1±0.31mg/ml (for sweet potato fed) respectively.

Table. V.7. Seasonal variation in aminoacid content of the fat body of last instar larvae of *Spodoptera litura* after fed with the leaves of selected host plants.

Seasons		Amino acid concentration in	Host plants					F	P
			castor	papaya	colocasia	banana	Sweet potato		
	summer	Mg/ml	20±2.4	18.7±1.6	17.1±0.32	15.5±1.2	15.1±1.2	1.4	0.263
		Mg/gm	125.9±14	118±13	114.7±2.6	112.7±4	109.7±5.2	0.464	0.761
	monsoon	Mg/ml	17.5±1.5	15.9±0.78	14.4±1.5	12.3±1	11.9±1.2	3.65	0.022
		Mg/gm	108.6±5.8	107.4±4.5	106.4±2.4	102±0.49	99.3±1.2	1.24	0.326
	Post monsoon	Mg/ml	11.5±0.31	8.8±0.26	7.7±0.79	7.0±0.36	5.1±0.31	26.3	0.000
		Mg/gm	100±0.76	98.2±1.9	97.3±1.2	94.1±0.92	88±0.61	16.4	0.000

The values presented in the table are the mean value of five replicates for each host plant with standard error (Mean ±Standard error). All the values were found to be significant at 1% level. The total amino acid content was expressed in the mg/ml and mg/gm tissue

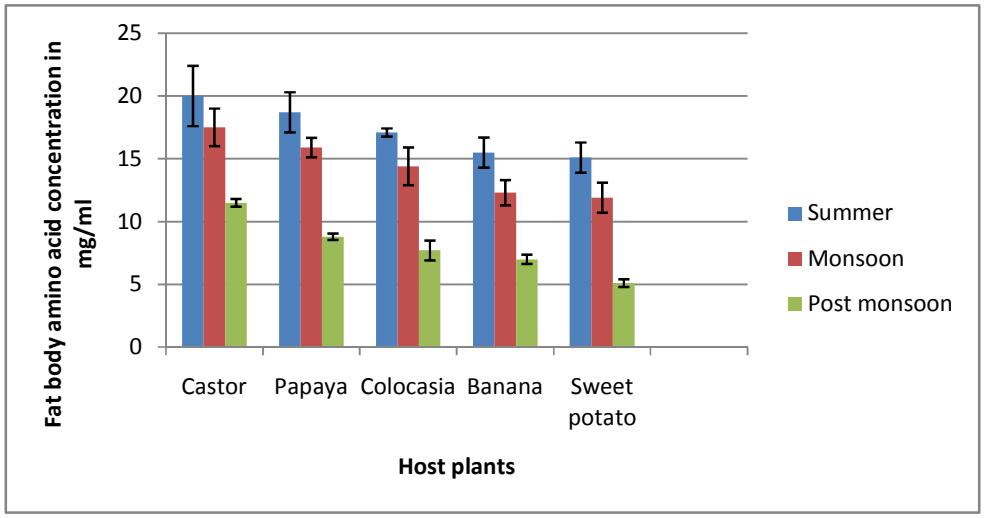

Fig. V.7. Seasonal variation in aminoacid content of the fat body of last instar larvae of *Spodoptera litura* after fed with the leaves of selected host plants.

- **Seasonal variation in amino acid content of the haemolymph of last instar larvae of *S. litura* after fed with the leaves of selected host plants.**

In haemolymph the amino acid concentration was very high. The data revealed significant difference in the aminoacid concentration of the haemolymph of *S. litura* larvae fed with selected host plant leaves (Table V.8. and Figure. V.8.). The concentration of amino acid in the haemolymph of the larvae fed with different host plants were in the order castor>papaya> colocasia > banana> sweetpotato. In all the three seasons the proportion of variation was in the same order as the above. The variation in free amino acid content in larval haemolymph fed with selected host plant leaves were 35.6±0.86mg/ml, 34.1±1.2mg/ml and 32.3±0.27mg/ml for castor; 34.5±0.89mg/ml, 33.1±0.39 mg/ml and 30.5±1.1 mg/ml for papaya; 33.1±2.46mg/ml, 30.2±2.5mg/ml and 28±2.7 mg/ml for colocasia; 29±1.8 mg/ml , 27.1±0.97 mg/ml and 26±1.2 mg/ml for banana and 20.9±0.75 mg/ml, 18.7±0.46 mg/ml and 17.4±0.66 mg/ml for sweet potato, in the summer, monsoon and post monsoon seasons respectively.

Table. V.8. Seasonal variation in aminoacid content of the haemolymph of last instar larvae of *Spodoptera litura* after fed with the leaves of selected host plants.

	aminoacid concentration in	Host plants					F	P
		castor	papaya	colocasia	banana	Sweet potato		
summer	Mg/ml	35.6±0.86	34.5±0.89	33.1±2.46	29±1.8	20.9±0.75	20.08	0.000
	Mg/insect	7.12±0.17	6.91±0.17	6.63±0.49	5.8±0.36	4.19±0.15	15.4	0.000
monsoon	Mg/ml	34.1±1.2	33.1±0.39	30.2±2.5	27.1±0.97	18.7±0.46	20.08	0.000
	Mg/insect	6.8±0.24	6.6±0.07	6.5±0.27	5.4±0.19	3.7±0.09	43.5	0.000
Post monsoon	Mg/ml	32.3±0.27	30.5±1.1	28±2.7	26±1.2	17.4±0.66	15.4	0.000
	Mg/gm	6.4±0.05	6.1±0.22	5.6±0.55	5.1±0.24	3.5±0.13	15.4	0.000

The values presented in the table are the mean value of five replicates for each host plant with standard error (Mean ±Standard error). All the values were found to be significant at 1% level. The total amino acid content was expressed in the mg/ml and mg/insect haemolymph.

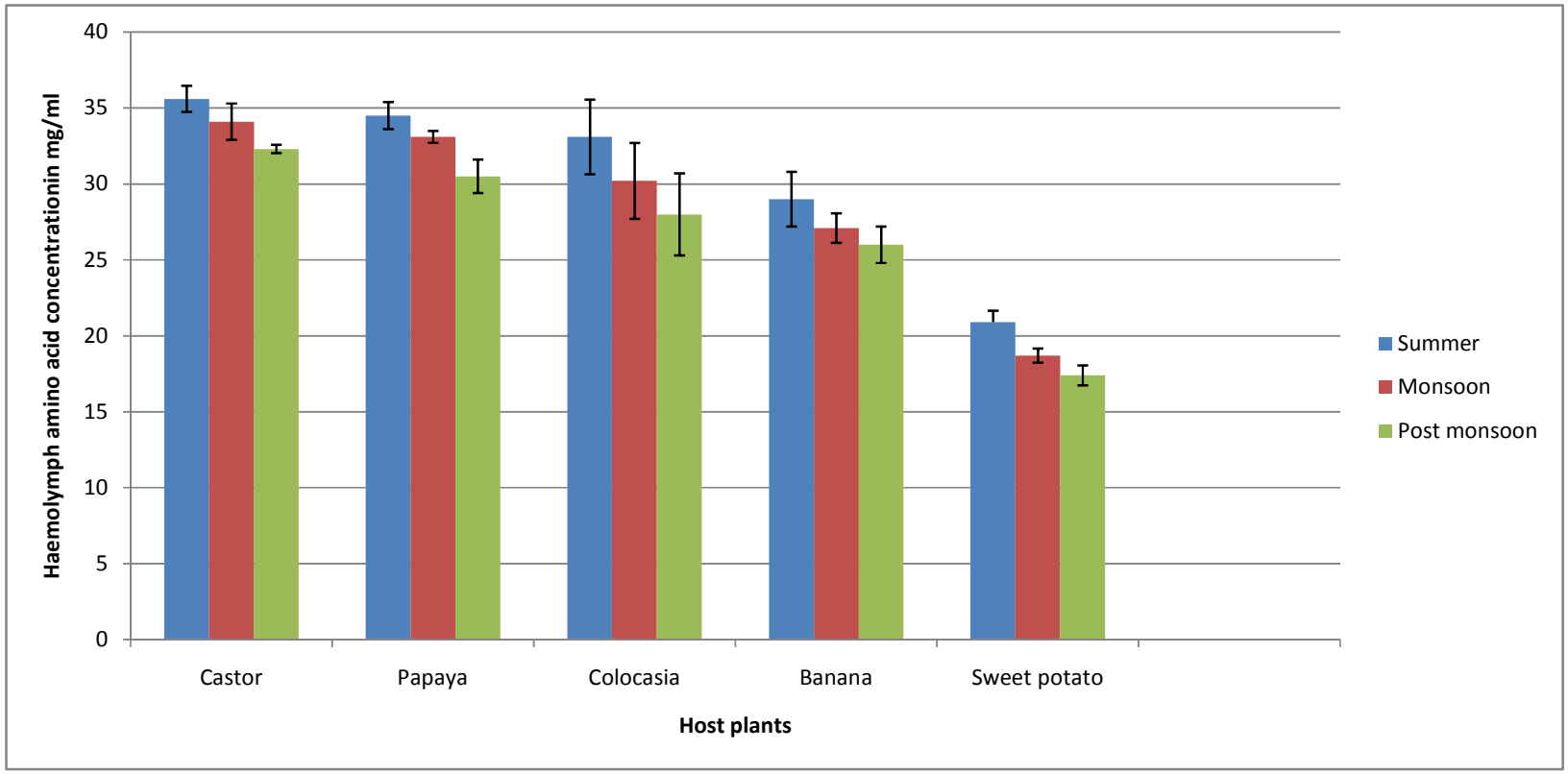

Fig. V.8. Seasonal variation in amino acid content of the haemolymph of last instar larvae of *Spodoptera litura* after fed with the leaves of selected host plants.

The findings revealed a positive correlation between the aminoacid content in the plant leaves and the tissues of the insect feeding on the specific plant materials. Among the test plant materials , castor leaves showed highest content of amino acid which was found to be correlated with a corresponding increase in content of amino acid concentration in the tissue of the larvae fed with castor leaves. Generally the concentration of amino acid was found to be reduced in the tissues of the larvae fed with sweet potato with the corresponding reduction in the amino acid content in host plant leaves. These findings reveals the influence of changes in biochemical components of host plant leaves on the biochemical components in insect tissues under the seasonal variation.

5.4. Discussion.

The present study revealed the effect of different dietary components in the larval tissues. Seasonal variation in biochemical components was seen in different host plant leaves. The quality of host plants determine the tissue components of the insects (*e. g*., the levels of protein, carbohydrate, trace elements and defensive compounds) that can affect the feeding and digestion positively or negatively in the herbivorous insects. Possible variations in secondary components or nutrient quality of host plants may affect the survival, growth, fecundity and development time of insects (Berynes and Chapman, 1994).

The primary metabolites proteins, carbohydrates and lipids are the major biochemical components in the living organism. Among the primary metabolites proteins are the main constituents. In chromosomes, each allele codes for the formation of amino acids that string together to form proteins. Thus, the difference in the nucleotide sequence of alleles lead to the production of slightly different strings of amino acids or variant forms of proteins. These proteins code for the development of anatomical,

morphological and physiological characteristics of the organism and are also responsible for determining the aspect of the behaviour of the organism (Bhat *et al*., 2010).

Among the five different host plants selected, the castor leaves showed the highest concentration of proteins. This corroborates with the findings of Martin *et al*. (1975), that the castor bean has high leaf-protein content in the mature leaf. The tissues (midgut, haemolymph and fat body) of castor fed larvae of *S. litura* showed highest protein and amino acid concentration. This may be due to the feeding of higher protein containing castor leaves. Aruga (1994) reported the presence of high protein concentration of haemolymph was correlated with high rate of feeding of mulberry leaves and afterward high rate of conversion and their accumulation in haemolymph of *Bombyx mori,* this correlated with the present work.

In the present study the protein concentration in the larval tissues fed with selected host plant leaves were highest during the summer seasons than the monsoon and post monsoon seasons. This variation may be due to the feeding of leaves with highest protein content in the summer season compared to low protein content in the leaves during the the monsoon and post monsoon seasons. This finding was supported by the work of Sheth (2011) who reported that the protein content in all the parts of *Calotropis procera* was highest during the summer seasons and lowest in the winter seasons. But the findings of Abd El-Rahman (1975) revealed that the effects of high temperature and high humidity induce salt and water stress to plants, which lead to a decrease in protein content.

The present work also reported the highest amino acid content in the leaves of different host plants during the summer season compared to the monsoon and post monsoon seasons. This finding was supported by the findings of Kramer (1983) who reported that water stress disrupted the

nitrogen metabolism leading to proteins solubility and accumulation of amino acids. But Ferrario *et al*. (1998) found that amino acid concentration decrease because of decrease in nitrate reductase enzyme responsible for transforming NO_3 to NO_2 in tobacco plants under drought stress.

In the present work the highest content of amino acids were observed in the castor leaves than the other selected host plants and the larval tissues fed with this host plants showed highest amino acid content. Similarly all the larval tissues showed the higher content of amino acid during the summer seasons than the monsoon and post monsoon seasons. This findings supported by the work of Mullins (1985), that various factors like temperature and diet influence the composition of the insect tissues.

Besides the feeding materials and seasonal variation, many other factors also influence the variation in tissue proteins and amino acids. Morphogenetic hormones regulate both quantitative and qualitative changes in proteins during development of insects. Results available from various biochemical works indicated the considerable importance of protein metabolism during different developmental stages of the insects (Chen, 1966). The present work showed the presence of high concentration of amino acid in the haemolymph of *S. litura* larvae. This is supported by the work done by Sutcliff (1963) who reported the presence of a high level of amino acids in the insect silkworm, *Bombyx mori* silk gland and haemolymph.

The factors like diet, temperature and diseases also affect the composition of the haemolymph (Mullins, 1985). Similarly the haemolymph composition is largely varied among species and during the different developmental stages of the same species (Ranjini and Mohamed, 2004). Many works reported the quantitative and qualitative analysis of haemolymph in various insects such as *Drosophila*, *Periplanata*, *Bombyx mori*, *Calliphora* and other lepidopteran insects (Mine *et al*., 1983).

During the larval feeding stage the rate of synthesis of protein is generally higher in the fat body and in the mature larvae the protein content is higher in both the haemolymph and fat body. The protein concentration decreases during the pupal stage, protein distribution changes and the specific haemolymph proteins are sequestered in the fat body (Ranjini , 2002; Ranjini and Mohamed, 2004).

The storage of protein in the haemolymph and fat body is a common process during growth and development. These storage proteins seems to be special adaptations during the moulting, metamorphosis, and cyclic reproduction of the insects and have no analogue in the vertebrates (Wyatt and Pan, 1978). Total proteins, triacylglyceride and glycogen are the three main storage macromolecules in insect fat bodies. Because of the difference in concentration of these molecules in host plants, utilization of different host plants by the insect might lead to a gain in various amount of these storage macromolecules in the insects. According to Florkin (1936) the protein concentration was increased seven folds during the larval stage of *Bombyx mori.* The importance of nutrition in the development of silkworm has been reported by Singh *et al. (*2011). Srivastava *et al*. (2002) reported that nutrition play a significant role in the development and metamorphosis especially in lepidopteran insects.

Proteomics is a large-scale study of the gene expression at the protein level, which ultimately provides direct measurement of protein expression levels and insight into the activity state of all relevant proteins. Besides the biochemical estimations protein profiles are also determined by the electrophoretic techniques, in which separations are based on the molecular size and molecular weight.

Many researchers worked out in electrophoretic technique to determine the protein profiles in vertebrates and invertebrate animals. In the present

investigation, an attempt was made to know the protein profile of different tissues of the larvae of *S. litura* fed with different host plant leaves in order to identify the variation in protein content in the tissues with respect to the feeding materials. The quantitative and qualitative analysis of protein among the tissues of *S. litura* fed with five different host plants revealed the variation in the protein content with respect to the feeding materials. The electrophoretic analysis has shown the presence of different bands of proteins with different molecular weight in the tissues of *S. litura* fed with different host plant leaves.

Similar studies in different insect tissues were reported by various researchers. Electrophoretic separation of haemolymph protein and lipoproteins of seven different lepidopteran species were described by Whitemore and Gilbert (1974). Singer and Nordlander (1973)described the protein profile of *Periplanata* from different body parts. Waehneldt *et al*. (1973) reported proteins from nervous tissues of several invertebrate and vertebrate animals.

CHAPTER VI

EFFECT OF FOOD MATERIALS AND SEASONAL VARIATION ON THE TOTAL CARBOHYDRATE CONCENTRATION IN DIFFERENT TISSUES OF *SPODOPTERA LITURA*

6.1. Introduction

Nutrition is one of the most essential environmental factors that decides both the growth and development of organisms. Dietary supplements are not just required for respiration and metabolism to provide energy requirements but in addition furnish fundamental chemical building blocks that are utilized for tissue development and overall growth. In this way, nutrition serve as prime factor for both growth and development and for phenotypic variation like body size (Chown and Nicolson, 2004; Simpson and Raubenheimer, 2012).

Carbohydrates are the most important nutritional components which provide energy source for all living beings whether plants or animals. It is composed of carbon, hydrogen and nitrogen. The simple sugar unit is monosaccharides which are linked together covalently to form different types of oligosaccharides and polysaccharides (starch, cellulose, and glycogen). The breakdown of these covalent bonds release energy.

In plants the carbohydrates occur in the form of starches, fructosans and sugars. The sugars are most significant and are universal phagostimulants. The polysaccharide cellulose is the supporting material in the plant tissues. Starch is the storage carbohydrate in plants. It is present in granules associated with the chloroplast or with the photosynthetic tissues. About 5-8% of the leaf dry weight is starch. The major disaccharide in plants is sucrose, its concentration varies considerably. The hexose sugar concentration is very low

in plants. Both hexoses and sucrose are present in the cytoplasm and mostly in photosynthetic tissues of plants. Sucrose is the carbohydrate that is transported through the tissues of the plants from the production site to the other tissues. Hence its level is higher in the phloem. In some plants mannitol and sorbitol are the translocated carbohydrates.

Carbohydrates are the main nutrients for phytophagous insects. Carbohydrates, such as simple sugars, starch and different polysaccharides are the principal components present in most of the insect diet. They are the common respiratory fuel. They provide the carbon skeleton for the synthesis of numerous amino acids. Carbohydrates can be converted to lipids. Besides this there are many other functions for this biomolecule. By utilizing the metabolic intermediates obtained from the breakdown of lipids and amino acids, all insects synthesize glucose by the path way of gluconeogenesis. Carbohydrates are the basic elements in the structure and function of all insect tissues and can be found in the cytoplasm, nuclei, membranes of cells, in the extra cellular haemolymph and supporting tissues.

Insect carbohydrates are present either in free form or in combination with other molecules such as purines, pyrimidines, proteins and lipids. Carbohydrates have substantial role in all level of cellular organization. The major structural component of an insect's outer covering is made up of a polysaccharide, chitin, found abundantly in nature (Bernys and Chapman, 1994). Glycogen is the storage carbohydrate in animals. Usually most of the insects require some amount of carbohydrate in their diet and they can show a better performance at a particular proportion of the dietary carbohydrate relative to other nutrients that varies from species to species.

The differences in the utilization of carbohydrates by the insects are based on their ability to hydrolyse the polysaccharides, the efficiency to absorb the different compounds and the ability of their enzyme system to

bring about these compounds in to metabolic processes. The insects having very broad digestive capacity utilize very wide range of carbohydrates. There is also difference in the utilization of carbohydrates between the larvae and the adult.

The selection of higher sugar containing host plants by the insect will decrease the corresponding intake of proteins. Besides, the protein concentration is higher in the younger leaves but the carbohydrate concentration is higher in the older leaves. Specialist insects maintain a balance of carbohydrates and proteins by feeding the different aged leaves from the host plants (Bernys and Chapman, 1994).

Wyatt and Kalf (1956) first reported the presence of trehalose in the haemolymph of insects. The disaccharide trehalose occurs in all insects, but it is not necessarily at all stages of insect and it is the most abundant sugar in the haemolymph of the insects. It has also been reported as the major haemolymph sugar in *Schistocerca gregaria* (Howden and Kilby, 1956), blowfly *Phormia regina* and honeybees (Evans and Dithier, 1957). Irrespective of the nutrition of the insect, the concentration of trehalose in the haemolymph is relatively constant at any one stage of the development. The two glycolytic intermediates, glucose-1-phosphate and glucose-6-phosphate are utilized by the fat body to synthesize the trehalose. The glucose source for the trehalose synthesis includes dietary sucrose, glycogen, and by gluconeogenesis (Friedman, 2002).

Trehalose play multiple roles, such as storage carbohydrate that provide energy for flight and act as a cryoprotectant protecting insects from damage during overwintering in cold climates. The level of trehalose in the haemolymph plays a significant role in regulating carbohydrate intake and maintaining nutritional homeostasis. Levels of trehalose in the haemolymph are maintained by a complex interaction of nutrient intake and metabolism.

Only a few portion of the glucose absorbed from the food is instantly oxidized through the metabolism or flight and the remaining portion of sugars are converted to glycogen and trehalose by the fatbody, this process normally takingplace during the resting period.

Deposition of carbohydrate as glycogen in the fat body and the formation of protein polysaccharide complexes in the haemolymph are possible pathways which clarify the reduction of the levels of haemolymph glucose and trehalose during development of the fifth instar larvae of *Manduca sexta* (Dahlman, 1973).

The cells of most insect tissues utilize glucose, the liberation of which is carried out inside the cells by the trehalase enzyme that is present in the haemolymph, fat body, midgut epithelium, lumen and theinsect muscles. Glucose does not leave the fat body apparently, where it is stored as glycogen rather it is mobilized in to the haemolymph as trehalose (Evans and Dithier, 1975; Clegg and Evans, 1961). The concentration of glucose in the haemolympyh is typically low. For instance, in the silkworm larvae it averages only 0.22%. In the larvae of *Prodenia* the haemolymph glucose level increases within fifteen minutes of feeding and during flight the blood sugar level is markedly decreased or depleted and replenished from the gut and fat body. In non-feeding insects the significant part of glucose is converted to trehalose (Clements, 1959).

During the moulting stage the glucose in the haemolymph is utilized for the synthesis of chitin when the new cuticle is being laid down (Bade and Wyatt, 1962). Carbohdrates are used for the synthesis of protein and in numerous energy metabolism. The carbohydrate content of the haemolymph and fatbody increased during the time of intensive feeding in the locust *Schistocerca gregaria* (Walker *etal*., 1970). Most of the intermediary metabolism, including storage and synthesis of carbohydrate and fat, take

placein the fat body. Under fed condition the dietary carbohydrates are the sole source of glucose.

To date, nutritional investigations have concentrated fundamentally on biochemical compounds such as carbohydrate which are important to support optimum growth, development, regenerative action and survival of individual species. For most species, glucose, fructose and sucrose are healthfully sufficient sugars. The quantitative prerequisites of carbohydrate differ as per age, sex and metamorphic stages. These biochemical parameters are valuable in assessing and predicting the life cycle of the insects. The present investigation deals with the variation in dietary carbohydrate and its effect on biochemical constituents in insect tissues and the resulting changes in the biological parameters of *S. litura.*

6.2. Materials and methods

The materials utilized and methods employed for carrying out the quantitative estimation of carbohydrate in the insect tissues and leaf tissues are given in the section 3.2.7.4 and 3.2.7.8 respectively.

6.3. Result

6.3.1. Seasonal variation in carbohydrate concentration

The results of the present work include the quantitative estimation of total carbohydrate content in the midgut, fat body and haemolymph of last instar larvae of *S. litura* by giving different food materials in different seasons. The results showed the seasonal changes of carbohydrate concentration in plant tissues and the corresponding variation in the insect tissues. The changes in the carbohydrate concentration in host plant leaves, midgut, haemolymph, and fat body are given in tables VI.1,VI.2 ,VI.3 and VI.4 and figures VI.1,VI.2 ,VI.3 and VI.

- **Seasonal variation in carbohydrate concentration in leaf tissues of selected host plants.**

The total carbohydrate content in the leaves of five selected host plants in different seasons were presented in the Table. VI.1. Variation of carbohydrate content in each host plant leaf was noticed in each season. Comparison of the results obtained from the data indicated that from the five different host plants selected the castor shows the higher carbohydrate content than the other four host plants and it contain 0.037±0.00mg/ml of carbohydrate. The carbohydrate content in other host plants papaya, colocasia, sweet potato and banana are in the order 0.036±0.00mg/ml, 0.034±0.00mg/ml, 0.033±0.00mg/ml and 0.028±0.0mg/ml respectively in summer season. Similar changes were noticed in monsoon season and post monsoon season. The values were 0.32±0.00mg/ml and 0.029±0.00mg/ml for castor; 0.030±0.00mg/ml and 0.021±0.00mg/ml for papaya; 0.027±0.00mg/ml and 0.020±0.00mg/ml for colocasia; 0.027±0.00mg/ml and 0.013±0.01mg/ml for sweet potato and 0.025±0.00mg/ml and 0.007±0.00mg/ml for sweet potato in monsoon and post monsoon seasons respectively. By comparing the seasonal variation it was observed that in the summer season all the selected plant leaves showed the highest carbohydrate content than in the monsoon and post monsoon seasons.

Table. VI.1 Seasonal variation in carbohydrate content of the leaves of selected host plants.

Seasons		Carbohydrate concentration in	Host plants					F	P
			Castor	Papaya	Colocasia	Sweet potato	Banana		
	Summer	Mg/ml	0.04±0.00	0.04±0.00	0.03±0.00	0.03±0.00	0.028±0.0	0.936	0.463
		Mg/gm	0.37±0.00	0.36±0.03	0.34±0.01	0.33±0.01	0.28±0.00	0.657	0.629
	Monsoon	Mg/ml	0.03±0.00	0.03±0.00	0.03±0.00	0.03±0.00	0.025±0.0	7.80	0.001
		Mg/gm	0.32±0.00	0.30±0.00	0.27±0.01	0.27±0.01	0.25±0.00	7.77	0.001
	Post monsoon	Mg/ml	0.03±0.00	0.02±0.00	0.02±0.00	0.01±0.00	0.01±0.0	3.59	0.023
		Mg/gm	0.29±0.08	0.21±0.01	0.20±0.00	0.13±0.01	0.07±0.03	3.59	0.023

The values presented in the table are the mean value of five replicates for each host plant leaves with standard error (Mean ±Standard error). All the values were found to be significant at 1% level. The total carbohydrate content was expressed in the mg/ml and mg/gm tissue.

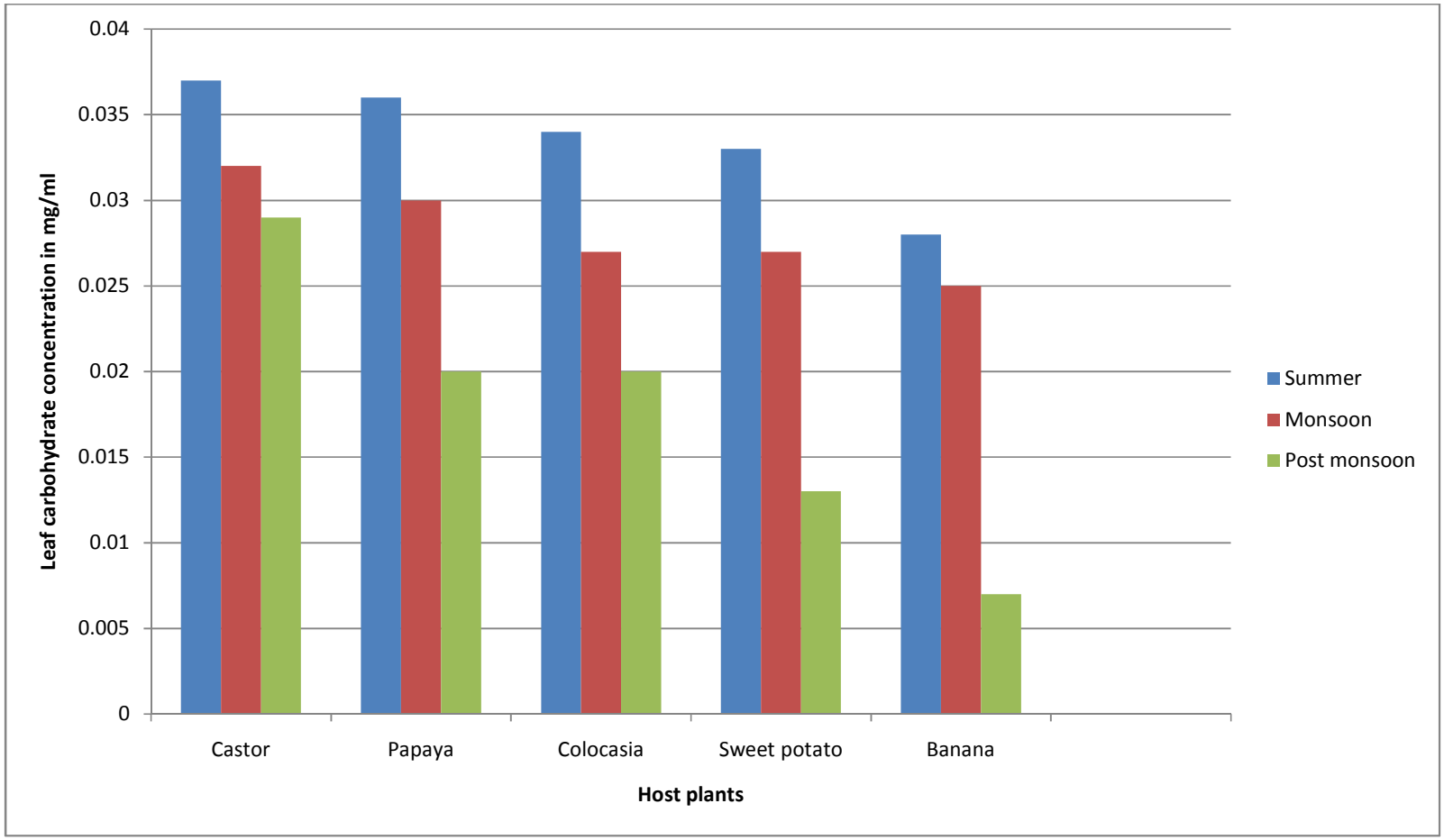

Fig. VI.1 Seasonal variation in carbohydrate content of selected host plant leaves.

- **Seasonal variation in carbohydrate concentration of midgut tissues of *Spodoptera litura* fed with selected host plant leaves**.

The variation in carbohydrate content of midgut tissue of the insect by feeding with five different host plant leaves (castor, colocasia, papaya, banana, sweet potato) in three different seasons were presented inTable. VI.2and Fig. VI.2. The data indicated that the variation in protein content was observed in the midgut tissue in each season with the corresponding food materials. The midgut tissue of castor fed larvae showed the maximum protein content than the other host plants.

The concentration of carbohydrate in the midgut tissue of larvae fed with castor, papaya, colocasia, sweet potato and banana, were 0.36±0.01 mg/ml, 0.34±0.01 mg/ml , 0.32±0.00mg/ml, 0.26±0.00mg/ml and 0.21±0.00mg/ml respectively in the summer season. Similar changes in the carbohydrate content was noticed in the monsoon and post monsoon seasons. The values were 0.33±0.00mg/ml and 0.09±0.02 mg/ml for castor, 0.31±0.00mg/ml and 0.07±0.01 mg/ml for papaya, 0.22±0.01 mg/ml and 0.06±0.00mg/ml for colocasia, 0.25±0.01 mg/ml and 0.06±0.00mg/ml for sweet potato and 0.14±0.01 mg/ml and 0.04±0.00mg/ml for banana in the monsoon and post monsoon seasons respectively.

Table. VI.2. Seasonal variation in carbohydrate content of the midgut tissue of last instar larvae of *Spodoptera litura* after fed with the leaves of selected host plants.

Seasons		Carbohydrate concentration in	Host plants					F	P
			Castor	Papaya	Colocasia	Sweet potato	Banana		
	Summer	Mg/ml	0.36±0.01	0.34±0.01	0.32±0.00	0.26±0.00	0.21±0.00	37.6	0.000
		Mg/gm	1.17±0.13	1.06±0.01	0.97±0.04	0.51±0.02	0.33±0.00	33.2	0.000
	Monsoon	Mg/ml	0.33±0.00	0.31±0.00	0.22±0.01	0.25±0.01	0.14±0.01	23.1	0.000
		Mg/gm	1.05±0.04	0.95±0.03	0.33±0.05	0.84±0.07	0.24±0.02	54.9	0.000
	Post Monsoon	Mg/ml	0.09±0.02	0.07±0.01	0.06±0.00	0.06±0.00	0.04±0.00	2.43	0.081
		Mg/gm	0.48±0.08	0.44±0.06	0.31±0.03	0.24±0.01	0.16±0.02	7.11	0.001

The values presented in the table are the mean value of five replicates for each host plant leaves with standard error (Mean ±Standard error). All the values were found to be significant at 1% level. The total carbohydrate content was expressed in the mg/ml and mg/gm tissue

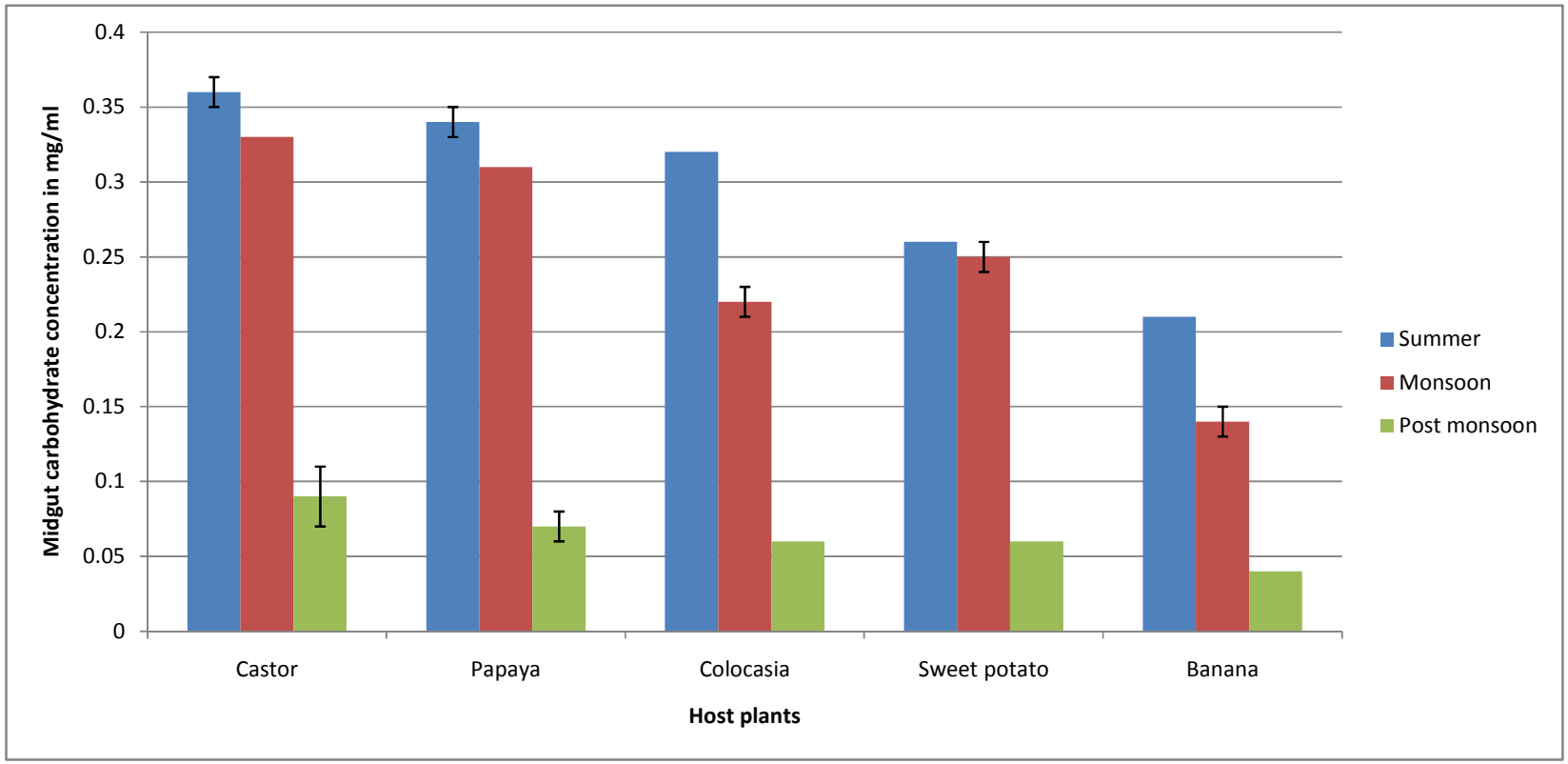

Fig. VI.2. Seasonal variation in carbohydrate content of the midgut tissue of last instar larvae of *Spodotera litura* after fed with the leaves of selected host plants.

- **Seasonal variation in carbohydrate concentration of fat body of *Spodoptera litura* fed with selected host plant leaves**.

The variation in carbohydrate content of the fat body of the insect fed with five selected host plant leaves (castor, colocasia, papaya, banana, sweet potato) in three different seasons were presented in the Table. VI.3 and Fig. VI.3. The results showed that the carbohydrate content varies with the feeding material and also with the change in seasons. The carbohydrate content in the fat body of the larvae fed with selected host plant leaves was in the order castor>papaya>colocasia> sweet potato>banana and the values were 0.31±0.02mg/ml, 0.23±0.02mg/ml, 0.21±0.01mg/ml, 0.16±0.00mg/ml, and 0.15±0.00mg/ml for castor, papaya, colocasia, sweet potato and banana respectively in the summer season. Similar changes were noticed in the monsoon and post monsoon seasons and the values were 0.29±0.03mg/ml and 0.25±0.02mg/ml for castor; 0.21±0.00mg/ml and 0.14±0.00mg/ml for papaya; 0.11±0.00mg/ml and 0.09±0.00mg/ml for colocasia; 0.17±0.00mg/ml and 0.05±0.00mg/ml for sweet potato and 0.09±0.00mg/ml and 0.05±0.00mg/ml for banana in the monsoon and post monsoon seasons respectively.

Table. VI.3. Seasonal variation in carbohydrate content of the fat body of last instar larvae *Spodoptera litura* after fed with the leaves of selected host plants.

Season		Carbohydrate concentration in	Host plants					F	P
			Castor	Papaya	Colocasia	Sweet potato	Banana		
	Summer	Mg/ml	0.31±0.02	0.23±0.02	0.21±0.01	0.16±0.00	0.15±0.00	14.48	0.000
		Mg/gm	2.2±0.17	1.5±0.11	1.1.2±0.05	0.91±0.02	0.79±0.01	33.07	0.000
	Monsoon	Mg/ml	0.29±0.03	0.21±0.00	0.11±0.00	0.17±0.00	0.09±0.00	34.6	0.000
		Mg/gm	1.8±0.14	1.17±0.06	0.66±0.02	0.71±0.15	0.54±0.02	29.2	0.000
	Post Monsoon	Mg/ml	0.25±0.02	0.14±0.00	0.09±0.00	0.05±0.00	0.05±0.00	35.2	0.000
		Mg/gm	1.6±0.04	0.73±0.02	0.55±0.05	0.44±0.07	0.38±0.02	98.9	0.000

The values presented in the table are the mean value of five replicates for each host plant leaves with standard error (Mean ±Standard error). All the values were found to be significant at 1% level. The total carbohydrate content was expressed in the mg/ml and mg/gm tissue.

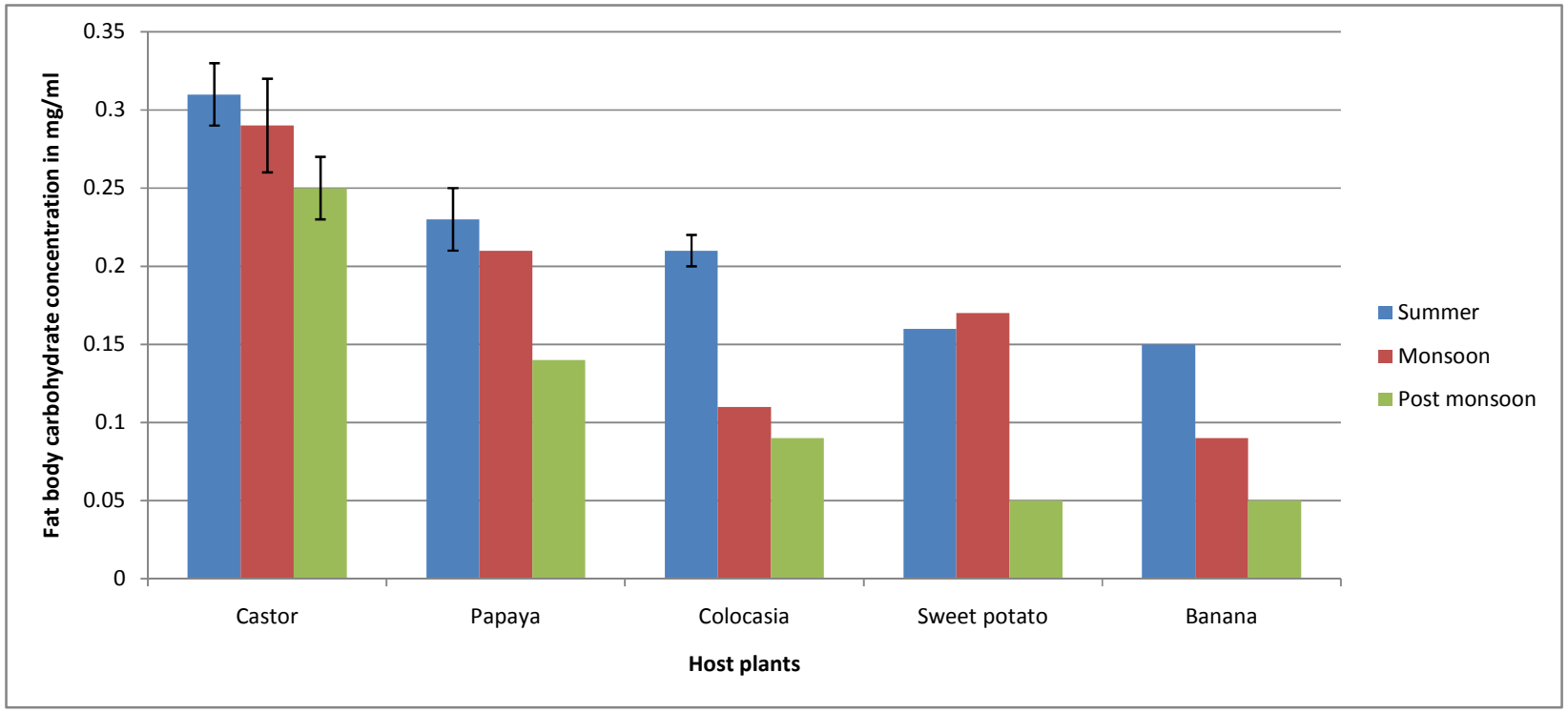

Fig. VI.3. Seasonal variation in carbohydratecontent of the fat body of last instar larvae *Spodoptera litura* after fed with the leaves of selected host plants.

- **Seasonal variation in carbohydrate concentration of Haemolymph of *Spodoptera litura* fed with selected host plant leaves**.

The changes in the carbohydrate concentration of haemolymph with respect to the feeding material with the changing seasons are given in the Table. VI.4 and Fig. VI.4. From the data it was observed that there occurred variation in carbohydrate content with respect to the host plant leaves and with the seasons. The carbohydrate content was higher in the haemolymph of castor fed larvae in the summer season than in the larvae fed with the other host plants. The concentration of carbohydrate in the haemolymph was in the order 1.8±0.01mg/ml, 1.7±0.04 mg/ml, 1.4±0.08mg/ml, 1.2±0.02mg/ml and 0.78±0.03mg/ml in castor, papaya, colocasia , sweet potato and banana respectively in the summer season. Similar changes were observed in other two seasons also. The values were 1.5±.07mg/ml and 1.3±0.06mg/mlfor castor; 1.1±0.06mg/mland 0.70±0.05mg/ml for papaya; 0.89±0.03mg/ml and 0.47±0.02mg/ml for colocasia; 0.84±0.03mg/ml and 0.53±.00mg/ml for sweet potato and 0.67±0.02mg/ml and 0.24±0.00mg/ml for banana respectively in the monsoon and post monsoon seasons. From the above results a positive correlation was seen in the insect tissues with respect to the carbohydrate content in the leaf tissue with respect to the seasons.

Table. VI.4. Seasonal variation in carbohydrate content of the haemolymph oflast instar larvae of *Spodoptera litura* after fed with the leaves of selected host plants

Seasons		Carbohydrate concentration in	Host plants					F	P
			Castor	Papaya	Colocasia	Sweet potato	Banana		
	Summer	Mg/ml	1.8±0.01	1.7±0.04	1.4±0.08	1.2±0.02	0.78±0.03	71.5	0.000
		Mg/i	0.36±0.00	0.34±0.00	0.27±0.01	0.25±0.00	0.17±0.01	50	0.000
	Monsoon	Mg/ml	1.5±.07	1.1±0.06	0.89±0.03	0.84±0.03	0.67±0.02	43.8	0.000
		Mg/i	0.25±0.06	0.24±0.01	0.22±0.01	0.19±0.00	0.16±0.00	1.5	0.245
	Post Monsoon	Mg/ml	1.3±0.06	0.70±0.05	0.53±.00	0.47±0.02	0.24±0.00	100	0.000
		Mg/i	0.27±0.00	0.14±0.01	0.09±0.00	0.07±0.00	0.05±0.00	220	0.000

The values presented in the table are the mean value of five replicates for each host plant leaves with standard error (Mean ±Standard error). All the values were found to be significant at 1% level. The total carbohydrate content was expressed in the mg/ml and mg/insect tissue

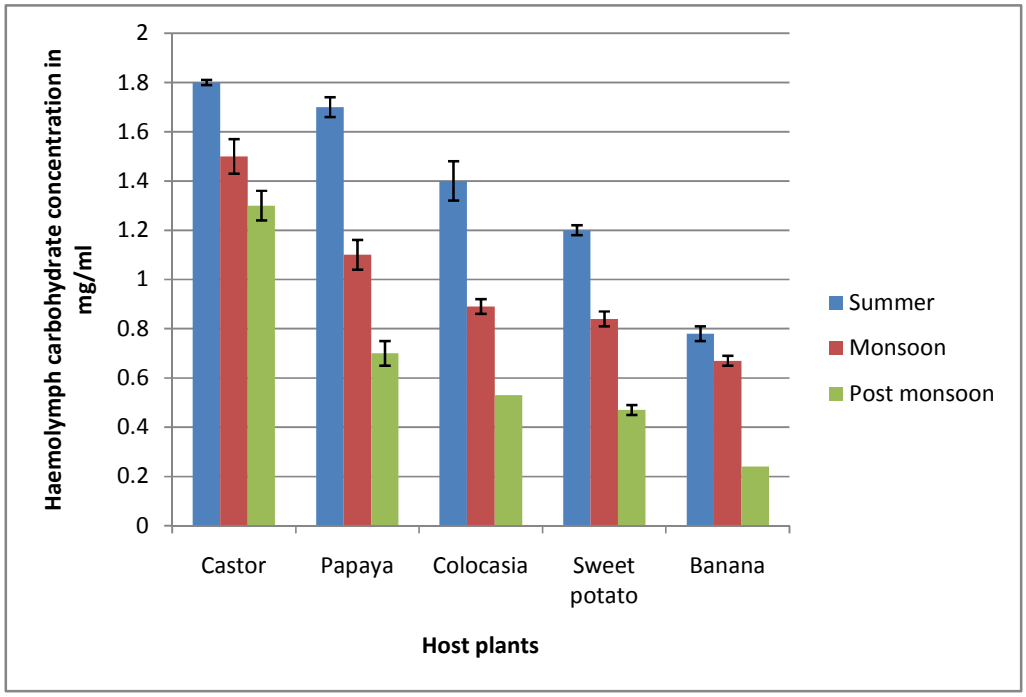

Fig. VI.4 Seasonal variation in carbohydrate content of the haemolymph of last instar larvae of *S. litura* after fed with the leaves of selected host plants

6.4. Discussion

The food quality is very important for the growth, development and reproductive potential which depends mainly on nutritional composition, including both the absolute and relative amount of water, proteins, amino acids, carbohydrates, lipids, minerals etc Slanky and Scriber (1985). Carbohydrate plays a significant role in determining the leaf quality which in turn influence the growth and development of the insects. The amount of dietary prerequisite of carbohydrate in insect larvae differ variably. The larvae of few species don't require carbohydrate since they are capable of substituting dietary protein or lipid for carbohydrate and meet their energy requirements for growth and development from amino acid and fatty acid oxidation. Other species require only moderate amounts of carbohydrate

during their larval period. The adult insects usually consume large amount of carbohydrates. These ingested carbohydrates together with nutrient reserve carried over from the larval stage are important to meet the energy demand of the insects (Wyatt, 1967).

The results of the present study revealed that there was considerable variation in the carbohydrate content in midgut tissue, haemolymph and fat body of the last instar larvae of *S. litura* based on its feeding on different host plants during different seasons. This may be due to the significant difference in the nutritional value of the host plant leaves with changing seasons. The quality of an herbivore insect diet changes both within and between its host plants and these fluctuations can be predictable, such as seasonal changes in plant quality (Table VI.1 and Fig VI.1) or unpredictable, such as the changes caused by environmental stress.

In this work among the selected host plants it was noticed that the amount of carbohydrate content varied in all the host plant leaves. The castor leaves showed highest amount of carbohydrate followed by papaya, colocasia, sweet potato and banana. The higher amount of carbohydrate content in castor leaves were reported by many workers (Sannappa and Jayaramaiah , 2002 ; Chandrappa *et al.,* 2005) The least carbohydrate content was noticed in banana leaves. The highest amount of carbohydrate content in the leaves was noticed during the summer season than in the monsoon and post monsoon season. The present observations were in agreement with the findings of previous works of many researchers. According to them for plants, changes in carbohydrates existed at a number of levels, including between the species (Yeoh *et al.,* 1992) within the species (Sattelmacher *et al*., 1994) and within an individual plant (Mattson , 1980) depending on the type of tissue (i. e., leaves, flowers, seeds, and stems) and its age (i. e., young versus old leaves). Besides, a plant's protein and carbohydrate content can vary in response to

environmental factor including the amount of light it receives, the chemical composition of the soil, and inputs of water (Felton 1996; Walter *et al*., 2012).

Similarly the ecological factors either work through each other or react together, as the change in physiographic conditions at a place may bring about a change in local climate that, in turn, may influence the soil and soil nutrient based inter-plant competition resulting in the individual plant variations. These alterations are felt right from the biochemical constituents, through the structure (anatomy) and functions (physiology) to the genetic makeup (Miller, 2003).

In this study, while comparing the preference of the host plant, voracious feeding of *S. litura* larvae was observed on the castor leaves than the other host plant leaves. Among the five different host plants selected the higher carbohydrate content was observed in the tissues (midgut, haemolymph and fat body) of *S. litura* larvae fed with castor leaves. This may be due to the increased rate of feeding of castor leaves which contain higher amount of carbohydrate. This indicated the influence of dietary components on the biochemical constituents of the insect tissues. This findings is supported by Friedman *et al*. (1991). Who reported that the carbohydrate in diet of *Heliothis zea* markedly influenced the trehalose level in the haemolymph.

Carbohydrate content in insect tissues (midgut, haemolymph and fat body) was observed to be low in post monsoon in contrast with the summer. This may be due to the feeding of host plant leaves with low carbohydrate content during the post monsoon season. It has been generally accepted that numerous over wintering insects accumulate sugar alcohols such as sorbitol as well as glycerol through the breakdown of glycogen storage (Grubor*et al.,* 1992).

The diet decided the body size and energy of the insects. Among the diet components insect utilized carbohydrate as building materials and fuels (Cohen, 2010). Carbohydrates play a vital role in insect development like metabolism, reproduction and embryonic development, metamorphosis, development of flight muscles, insect behaviour and as reserve food during diapauses (Chapman, 1998). A few carbohydrates can't be used by the vast majority of the insects, they might be valuable as pillers that assist in intestinal mobility. A few insects particularly phytophagous insects are unsuccessful to survive on artificial diet that are low in carbohydrates (House, 1974).

It has been reported that the carbohydrates fed by adults might be an extra food supply that aids the vitellogenin synthesis and egg development, in this way increasing the fecundity (Tisdale and Sappington, 2001). Pratissoli *et al. (*1995) reported that sugars aid to maintain egg development during adult ageing. The energy stores in the fat body can be affected significantly by the carbohydrate quality (Nestel , 2004 ; Nestel and Nemny-Lavy , 2008). It was fascinating to consider the more extensive impacts of this on phenotype and on reproductive potential (Aguila *et al*., 2013)

A carbohydrate rich diet modulates insect metabolic rate by diminishing the energy use on reproduction (Naya *et al*., 2007). The energy obtained from a carbohydrate-rich diet is mainly utilized for the maintenanace of the insect's life, therefore prolonging the insect life span (Lardies *et al.,* 2004). In other words, sugar plays only a small or no role in life history trade-offs in arthropods (Zera and Larsen, 2001). According to Eggert *et al. (*2003) stored glucose is utilized by the insect for the reproductive processes.

A number of studies reported about the variation of carbohydrate contents in the insect tissues during different developmental stages of the insects. Lohr and Gade (1983) noticed that the higher concentrartion of

carbohydrate in the haemolym ph of last instar larvae of *Carausius morosus* decreased towards ecdysis. This low level of sugar before ecdysis might be because of the effect of refusal to take food by the insect during that period, for which tissue, haemolymph, and fat reserve carbohydrates, protein and lipids are utilized for its maintenance.

Bade and Wyatt (1962) reported that in *Cecropia* silkworm the glycogen concentration decreased during the larval-pupal transformation. The same was also reported by Crompton and Birt (1967) for *Lucilia cuprina*. During larval-pupal transformation stage histolysis of organs happen, which may cause the fluctuation of carbohydrate concentration. During the final instar stage due to the rapid development, the larvae have to face an increasing demand for glucose; this requirement is achieved by the larvae through the rapid and voracious feeding. Glucose utilization in the haemolymph drastically decreased during the moulting time due to the increased synthesis of chitin. (Bade and Wyatt, 1962).

Carbohydrates is stored as glycogen in the fat body and as protein-polysaccharide complex in the haemolymph (Green and Dhalman, 1973). The energy stores in the fat body can be influenced by the quality of dietary carbohydrate (Nestel *et al*., 2004). The trehalose diffuses in to the midgut where it is hydrolysed by the enzyme trehalase to provide the glucose in the haemolymph. It is well known that the food reserves are stored in the fat body and released in to the haemolymph for utilization and again sequestered in to the fat body depending on the physiological status of the animal (Kilby, 1963).

Since the insects have trehalose as the circulating saccharide unit, the enzyme trehalase in the haemolymph serves the role of releasing glucose by hydrolyzing trehalose. Trehalase in the haemolymph is most active during the moulting time and make glucose available for chitin synthesis and for other tissues which lack trehalase. During the intermoults, trehalose is stable because the enzyme trehalase is inhibited (Friedman , 1961). Haemolymph

glucose is used for metabolic purposes as well as for providing carbohydrate material during the chitin synthesis by the epidermis at each moult, because the epidermal cells appear to lack trehalase (Duchateau *and florkin*., 1959).

Trehalase is present in the midgut epithelium and to a lesser extent in its lumen. Since trehalase is not found in the higher plants or in vertebrates it is unlikely that the major function of this enzyme in the midgut is digestion. Possibly the enzyme hydrolyses the trehalose, which diffuse out of the haemolymph in to the midgut, the glucose so formed then tending to diffuse back to the haemolymph (Chapman, 1998). Sacker (2015)while discussing the control of oxidative metabolism in insect flight muscle has suggested that trehalase in the haemolymph controls the entrance of carbohydrate in to the catabolic pathway.

The level of haemolymph trehalose sharply decreases at each moult and also during fasting period corresponding to spinning and this fall in trehalose level corresponds to the increase in glucose at the same period. (Florkin and Jeuniaux, 1969). Haemolymph trehalase inhibition is suppressed during the beginning of each molting stage which lead to a fall in the trehalose concentration of the blood. (Duchateau Bosso *et al*., 1963). Glucose released from the trehalose hydrolysis is rapidly removed from the blood by the cells and is utilized for the metabolic activities and for providing carbohydrate for the chitin synthesis by the epidermis (Candy and Kilby, 1961). In the fat body glycogen disappearing almost completely at each moult, while the amount of trehalose tends to remain at nearly constant level. On the other hand the bulk of the fat body is consumed to a large extent during the period of chitin synthesis. These observations suggested that the trehalose level of haemolymph is supplied at the expense of the glycogen of the fat body.

CHAPTER VII

CHANGES IN TOTAL LIPID CONCENTRATION OF DIFFERENT TISSUES OF *SPODOPTERA LITURA* FED WITH SELECTED HOST PLANT LEAVES DURING DIFFERENT SEASONS

7.1. Introduction

The amount of energy reserves in an animal provide essential implications of its ability to survive, grow and reproduce. Lipids play a variety of important roles in both plants and animals (Hadley, 1985). To a great extent, the apparent success of insects on this planet has been their capacity to utilize lipids efficiently as substrates for reproduction, embryogenesis, metamorphosis and flight. Besides, lipids are utilized as a means of communication (pheromones), for regulation of different physiological processes (hormones), as protection against a desiccating environment (cuticular lipids) and as cell constituents (membranes).

Lipids comprise a fundamental and integral part of cell membranes, in addition to that they provide an important source of metabolic energy for cell maintenance. The complex pattern of insect development and the environmental changes which are frequently associated with this process result in dramatic physiological and biochemical changes within an insect during the course of its life history. The physiology and biochemistry of insect development have been reviewed by many workers (Chen, 1971; Agrell and Lundquist, 1973). The present investigation was aimed to determine the variation in total lipid concentration of various tissues (midgut , fat body and haemolymph) of *S. litura* in relation to seasonal changes and difference in the host plants.

Lipids are very suitable energy source for insect tissue because an iso caloric amount of triglycerides occupies substantially less storage space than the equivalent amount of glycogen. This is extremely significant in insects which have to fly for long periods of time. Lipid reserves are also utilized as energy sources for various processes other than flight. Many insects characteristically accumulate lipid in high concentrations at physiological stages of development for the succeeding periods of non-feeding state, such as pupation or diapause and also in maturing females for deposition of eggs. Insect lipids are derived from the diet and /or synthesized from the non lipid precursors such as carbohydrate and proteins which are present in the diet or stored in the tissues.

Insect fat body is comprised of a diffuse aggregate of cells enclosed by delicate connective tissue membranes. In numerous insects it extends from the terminal abdominal segment into the head capsule and represents a major constituent of the haemocoel. Functionally it may be viewed as closely resembling to vertebrate liver and adipose tissue, serving not only for the storage of carbohydrate, lipid and protein, but also as an organ of intermediary metabolism (Kilby, 1963). Whole body nutrient reserves are not only stored, but also detected by the fat body; the fat body utilizes this information to organize the storage and utilization of insect energy reserves for the coordination of insect growth, metamorphosis and reproduction (Arrese and Soulages, 2010).

In insects the fat body is the main site for the synthesis and storage of lipids (Sun and Brookes, 1968; Thomas, 1974). The predominant lipid class in the fat body of all species which have been previously reported by Chino and Gilbert (1965) was triglyceride, which comprises up to 98% of fat body lipid in pupal and adult stage of *Hyalophora cecropia*. Other classes of lipid that have been detected in the fat body in small amounts including diglyceride,

monoglyceride, sterols, sterol esters, free fatty acids, phospholipids (Chang, 1974; Thomas, 1974), glyceryl ethers (Tan, 1973); quinnones (Sridhara and Bhat, 1965) and tocopherol (Sridhara and Bhat, 1965). Sexual dimorphism of fat body lipids has been reported in numerous species (Bhakthan and Gilbert, 1972) and changes have also been associated with diet, metamorphosis, reproduction, aging and exercise (Walker and Bailey, 1970; Dutkowski and Ziajka, 1970). These changes serve to emphasize the role of the fat body in responding to altered metabolic and physiological demands.

Insects have an open circulatory system in which the haemolymph circulates through body cavity and directly bathes the various organs. Haemolymph is the primary means by which materials might be transported from sites of absorption or synthesis to storage organs and afterward to sites of utilization; consequently the haemolymph lipid profile differs with the physiological condition of the animal. Significant changes have been accounted during metamorphosis, development, exercise and oogenesis (Bollade and Boucrot, 1971.

The principal contributor of total lipid content in insects is triacylglycerol. The total lipid content of an insect can range from 1% to more than 50% of wet mass in which 90% of wet mass is triacyl glycerol (Gilbert and Chino, 1974). Lipid concentrations of up to 5.5% have been reported in the haemolymph of a few species (Florkin and Jeuniaux, 1974) and this tissue must be viewed as a promptly accessible store of lipid. However, the precise contribution of lipid in this regard cannot be defined until data are available concerning the rate of lipid turnover in haemolymph under a variety of physiological conditions.

In many insects the major lipid component of haemolymph is diglyceride, with triglyceride and free fatty acids also present (Chang, 1974; Thomas, 1974). The hemipteran *Pyrrhocoris apterus* contains extensive

quantities of triglyceride-containing cells, adipoleucocytes in the haemolymph and these account for the high haemolymph triglyceride levels which have been accounted in this insect (Martin, 1969). Other lipid classes which have been recognized in insect haemolymph include monoglycerides, phospholipids, hydrocarbons, carotenoid and ketone bodies (Chang, 1974; Thomas, 1974; Diehl, 1975). Also the insect hormones released from the corpora allata and ecdysial glands are of a lipoidal nature and are present in the haemolymph at different concentrations during the course of insect development (Gilbert and King, 1973).

The lipid content of holometabolous insects increases consistently during larval development although the rate of increase is not constant all through every single larval stage (Gilbert and Schneiderman, 1961; Wimer and Lumb, 1967). Developmental changes have been reported in the relative amounts of the major lipid classes (Yurkiewicz, 1970; Castillon *etal.,* 1971;) and the fatty acid profile (Madariago *et al.,* 1974; Fernandez-Sousa *et al.,* 1971 b). These changes reflect the metabolic requirements of the larva, as well as indicate the accumulation of reserves required for maintenance during metamorphosis.

Significant amount of neutral lipids is deposited in the developing oocyte during oogenesis and in many species the predominant portion is triglyceride (Svoboda *et al.,* 1966; Gilbert, 1967b). This fraction declines during embryogenesis and the reduction in glyceride is often accompanied by an increase in phospholipid (Yurkiewicz and Oelsner, 1969; Lipsitz and McFarlane, 1970, 1971). These discoveries propose that some glyceride is being used for the synthesis of structural phospholipid while the rest serves as an energy source for the developing embryo.

During the metamorphosis of holometabolous insects, the animal is reliant upon reserves accumulated during larval development for energy and

for provision of anabolic precursors. Lipid and/or carbohydrate may contribute to the energy consumptionof pupae with the relative importance of each substrate dependent upon the species and sex of the animal under investigation (Chen, 1971; Agrell and Lundquist, 1973). The pattern of lipid utilization varies during the course of metamorphosis (; D'Costa and Birt, 1966) with some interconversion of carbohydrate to lipid is reported at mid-metamorphosis for two species of blowfly (D'Costa and Birt, 1966; Tate and Wimer, 1974).

Lipid is likewise an important substrate for the migratory flights of locusts (Weis-Fogh, 1952). Diglyceride pool is utilized during flight (Spencer and Candy, 1974). Within few days after adult emergence the insects enter a period of rapid lipogenesis during which dietary carbohydrate might be changed over to lipid and stored in the fat body as triglyceride (Walker *etal.,* 1970). In late winter the insects accumulate large stores of lipid in preparation for anortherly spring migration and Cenedella (1971) has assessed that these reserveswhich occur principally as triglycerideare sufficient to provide metabolic fuel for the northward migration. Brown and Chippendale (1974) extended this observationto demonstrate that the adult females canrapidly incorporate glucose into abdominal glycerides and suggested that dietary sugars may supplement the available triglyceride reserves.

Similar accumulation of lipid by diapausing insects has been reported for *Coccinella septempunctata* (Hodek and Cerkasov, 1961); *Pectinophora gossypiella (*Adkisson *et al.,* 1963); *Anthonomus grandis (*Lambremont *etal.,* 1964); *Hypera postica (*Tombes, 1964) and *Trogoderma granarium (*Karnavar and Nair, 1969). Accumulation of lipids prior to overwintering in some species were reported by Schaefer andMiura (1972). Lipids facilitate the process of embryogenesis by giving an efficient source of metabolic energy and an essential source of precursors for the synthesis of cellular and

subcellular membranes (Gilbert and Schneiderman, 1961). During vitellogeneis appreciable amount of lipid are stored in the eggs (Martin, 1969a; Dutkowski and Ziajka, 1970) and in at least some species the source of this yolk lipid is the fat body (Martin, 1969a; Dutkowski and Ziajka, 1972).

7.2. Materials and methods

The materials used and methods employed for carrying out the quantitative estimation of total lipid in different tissues of *S. litura* fed with selected host plant leavesindifferent seasons are given in the section **3.2.7.5**.

7.3. Results

7.3.1. Seasonal variation in total lipid concentration of different tissues of *Spodoptera litura* with respect to feeding of selected host plant leaves.

The results of the quantitative estimation of total lipid content in the midgut, fat body and haemolymph of last instar larvae of *S. litura* by giving different food materials in different seasons showed the seasonal changes of lipid concentration in the insect tissues with respect to feeding different host plant leaves. The changes in the lipid concentration in midgut, haemolymph, and fat body are given in tablesVII.1, VII.2 and VII.3 and figures VII.1, VII.2 and VII.3)

- **Seasonal variation in total lipid concentration in midgut tissues of *Spodoptera litura* fed with selected host plant leaves.**

The variation of lipid content in midgut tissue of the insect by feeding with five different host plants (castor, colocasia, papaya, banana and sweet potato) in three different seasons were represented in tableVII.1 and Fig. VII.1. The data indicated significant variation in lipid content (at $P<0.05$) in the midgut tissue during each season with the corresponding food materials.

In the summer season the migut tissue of castor fed larvae showed the maximum lipid content than the other host plants (1.05±0.04mg/ml). The lowest concentration of lipid was noticed in the banana fed larvae (0.74±0.02 mg/ml) and the intermediate values were noticed in other three host plant fed larvae and the values were 0.93±0.02mg/ml for sweet potato, 0.83±0.01 mg/ml for papaya and 0.81±0.03 mg/ml for colocasia fed larvae. Similar results were noticed in the monsoon season with highest concentration in castor fed larvae (0.90±0.05mg/ml) and lowest in banana fed ones (0.65±0.02mg/ml). In the post monsoon season banana fed larvae showed the least lipid content (0.43±0.00mg/ml) and castor fed larvae showed the highestconcentration (0.82±0.02mg/ml). The lipid content in the midguttissues showed the higherconcentration during the summer season than in the monsoon and post monsoon seasons.

Table. VII.1. Seasonal variation in total lipid concentration in the midgut tissue of last instar larvae of *Spodoptera litura* after fed with the leaves of selected host plants.

Seasons	Lipid concentration in	Host plants					F	P
		Castor	Sweet potato	Papaya	Colocasia	Banana		
Summer	Mg/ml	1.05±0.04	0.93±0.02	0.83±0.01	0.81±0.03	0.74±0.02	29.2	0.000
	Mg/gm	4.4±0.26	3.8±0.09	3.6±0.17	2.9±0.07	2.8±0.09	17.3	0.000
Monsoon	Mg/ml	0.90±0.05	0.74±0.01	0.73±0.04	0.69±0.02	0.65±0.02	13.6	0.000
	Mg/gm	3.4±0.32	2.9±0.18	2.5±0.13	2.1±0.07	2.2±0.05	8.4	0.000
Post monsoon	Mg/ml	0.82±0.02	0.68±0.03	0.62±0.00	0.53±0.03	0.43±0.07	6.7	0.001
	Mg/gm	2.9±0.23	2.3±0.13	2.0±0.14	2.6±0.22	2.2±0.04	4.3	0.011

The values presented in the table are the mean value of five replicates for each host plant with standard error (Mean ±Standard error). All the values were found to be significant at 1% level. The total lipid content was expressed in the mg/ml and mg/gm tissue

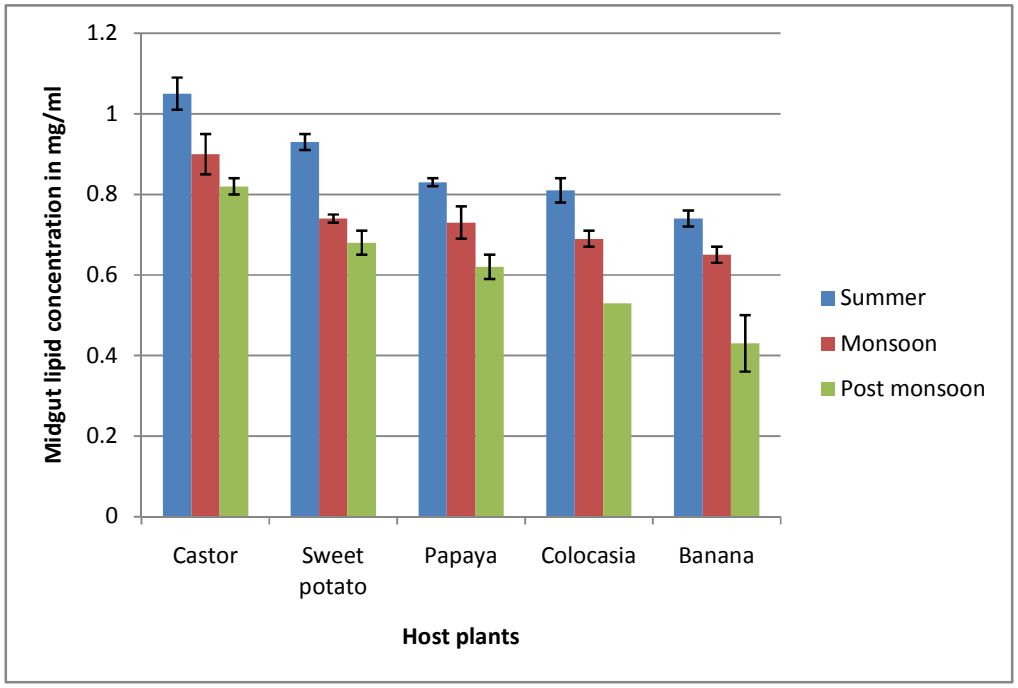

Fig. VII.1 Seasonal variation intotal lipid concentration in the midgut of last instar larvae of *Spodoptera litura* after fed with the leaves of selected host plants.

- **Seasonal variation in total lipid concentration offat body of last instar larvae of *S. litura* fed with selected host plants**

The variation intotal lipid content of the fat body of *S. litura* fed with five selected host plant leaves (castor, colocasia, papaya, banana, sweet potato) in three different seasons were presented in the tableVII..2 and Fig. VII..2. The results revealed significant variation at $P<0.05$ in the lipid content of fat body of *S. litura* with the feeding material and with the changing seasons. The lipid content in the fat body of the larvae fed with different host plant leaves was in the order castor>papaya> sweet potato > colocasia> banana in the summer season. The highest value was observed in the fat body of castor fed larvae (1.1±0.07mg/ml) and the lowest value noticed in banana fedlarvae (0.69±0.01mg/ml) during the summer season. But in the monsoon

season the highest value was observed in the fat body of castor fed insect (0.91±0.01mg/ml) and the lowest value was noticed in the fat body of banan fed larvae (0.60±0.01mg/ml). In the post monsoon season the highest value of lipid content was noticed in the fat body of castor fed larvae (0.69±0.01mg/ml) of *S. litura* and the least value was found in the banana fed larvae (0.27±0.01mg/ml)and the values of the other host plants were in between them. When comparing the seasonal variation , the lipid content was highest in the summer season in all the fat body of larvae fed with different host plant leaves and the lowest value was noticed in the post monsoon season. The lipid content in monsoon season was in between the summer and post monsoon seasons.

Table VII.2. Seasonal variation in total lipid concentration of the fat body of *Spodoptera litura* fed with selected host plant leaves.

Seasons	Lipid concentration in	Host plants					F	P
		Castor	Sweet potato	Papaya	Colocasia	Banana		
Summer	Mg/ml	1.1±0.07	0.92±0.02	0.83±0.01	0.77±0.03	0.69±0.01	41.45	0.000
	Mg/gm	8.0±0.49	7.1±0.33	4.2±0.06	3.8±0.04	3.4±0.05	51.7	0.000
Monsoon	Mg/ml	0.91±0.02	0.88±0.01	0.75±0.01	0.65±0.01	0.60±0.01	23	0.000
	Mg/gm	6.8±1.6	5.3±0.16	4.5±0.24	3.0±0.12	3.0±0.12	40.7	0.000
Post monsoon	Mg/ml	0.69±0.01	0.68±0.01	0.39±0.02	0.31±0.02	0.27±0.01	19.3	0.000
	Mg/gm	5.9±0.22	4.7±0.17	2.7±0.10	2.2±0.18	1.6±0.08	12.4	0.000

The values presented in the table are the mean value of five replicates for each host plant with standard error (Mean ±Standard error). All the values were found to be significant at 1% level. The total lipid content was expressed in the mg/ml and mg/gm tissue

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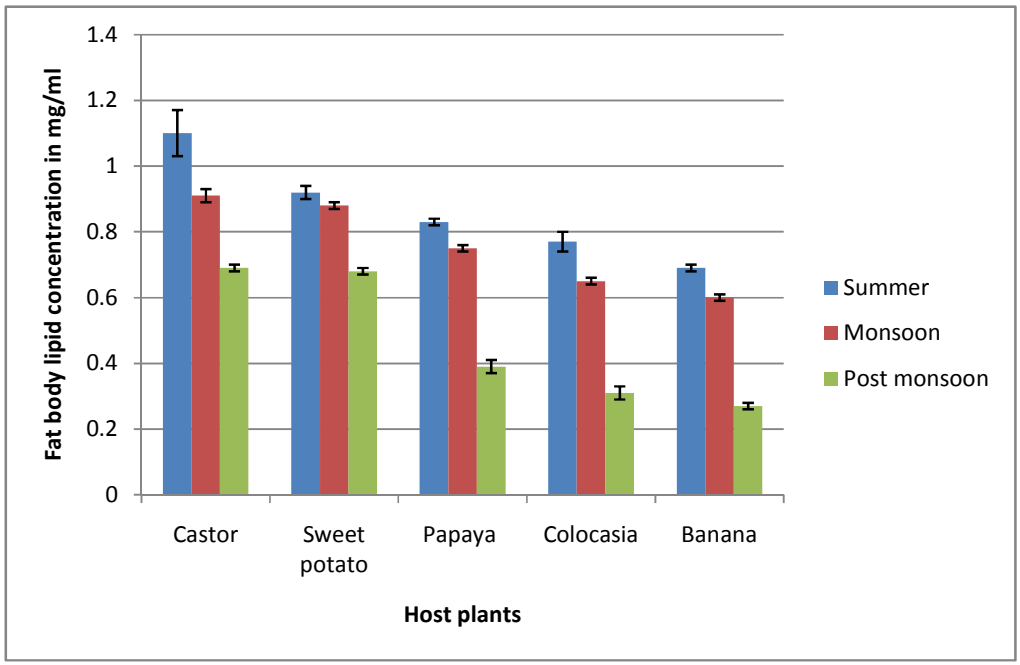

Fig. VII.2. Seasonal variation in lipid concentration of the fat body of *Spodoptera litura* fed with selected host plant leaves

- **Seasonal variation in total lipid concentration of haemolymph of *S. litura* fed with selected host plant leaves.**

The variation in total lipid content of the haemolymph of the *S. litura* fed with five different host plants (castor, colocasia, papaya, banana and sweet potato) in three different seasons were presented in the tableVII.3. and Fig.VII.3.The results showed significant variation at $P<0.05$ in the lipid content in haemolymph of *S. litura* with the feeding of different host plant leaves and with the changing seasons. The lipid content of the haemolymph of larvae fed with different host plant leaves was in the order castor> sweet potato > papaya> colocasia>banana in the summer season. The highest value was observed in the haemolymph of castorfed larvae (0.88±0.05mg/ml) and the lowest value was noticed in banana fed larvae (0.33±0.03mg/ml) during the summer season. In the monsoon season also the highest value was

observed in the haemolymph of castor fed larvae (0.56±0.05mg/ml) and the lowest value was reported in the haemolymph of banana fed larvae (0.20±0.03mg/ml). In the post monsoon season the highest value in lipid content was noticed in the haemolymph of castor fed larvae (0.46±0.02mg/ml) of *S. litura* and the least value was found in the banana fed larvae (0.21±0.00mg/ml) and the values of the other host plants were in between them. When comparing the seasonal variation, the lipid content was highest in the summer season for haemolymphof larvae fed with selected host plant leaves and the lowest value was noticed in the post monsoon season. The lipid content of monsoon season was in between the summer and post monsoon season.

Table. VII.3. Seasonal variation in total lipid concentration of haemolymph of *Spodoptera litura* fed with selected host plant leaves.

Seasons	Lipid concentration in	Host plants					F	P
		Castor	Sweet potato	Papaya	Colocasia	Banana		
Summer	Mg/ml	0.88±0.05	0.68±0.01	0.66±0.01	0.65±0.01	0.33±0.03	5.7	0.003
	Mg/insect	4.4±0.27	3.9±0.07	3.6±0.14	2.6±0.25	1.7±0.18	5.5	0.004
Monsoon	Mg/ml	0.56±0.05	0.37±0.02	0.31±0.00	0.23±0.01	0.20±0.03	22.1	0.000
	Mg/insect	3.1±0.56	2.8±0.28	1.9±0.03	2.2±0.15	1.5±0.04	53.6	0.000
Post monsoon	Mg/ml	0.46±0.02	0.36±0.01	0.25±0.01	0.23±0.01	0.21±0.00	108.3	0.000
	Mg/insect	2.1±0.04	1.7±0.11	1.1±0.04	1.2±0.06	0.90±0.08	26.2	0.000

The values presented in the table are the mean value of five replicates for each host plant with standarderror (Mean ±Standard error). All the values were found to be significant at 1% level. The total lipid content was expressed in the mg/ml and mg/insect tissue

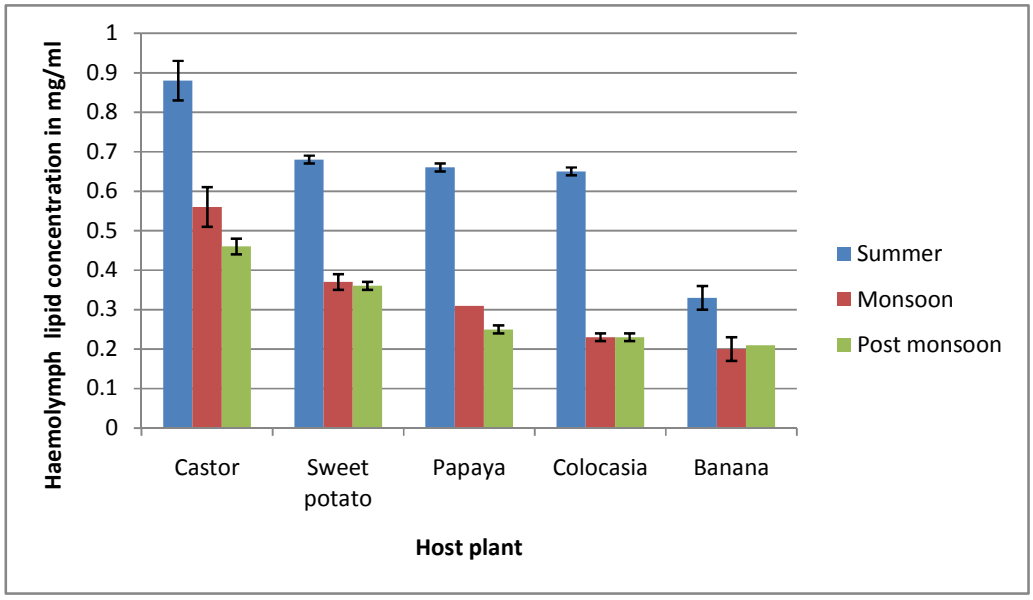

Fig. VII.3. Seasonal variation in total lipid concentration of haemolymph of *S. litura* fed with selected host plant leaves.

When comparing the lipid concentration in different tissues, the fat body showed the maximum lipid content than the midgut tissue and haemolymph. The concentration of lipid in each tissue showed maximum concentration during the summer season. The above results indicated significant variation in lipid content in the insect tissues with changing seasons and different food materials.

7.4. Discussion

Lipids have an important role in serving as an energy reservoir in insects (Hadley, 1985) and lipid content indicate an insect's available resources. On account of its high-energy content, lipid is the primary stored nutrient in insects and most other animals (Downer and Matthews, 1976). Insects require finite resources to grow and reproduce. As a result, life-history strategies require lipid resources to storage, maintenance, growth and reproduction. Allocation patterns are influenced by many environmental

factors including water and food availability, photoperiod, humidity, as well as temperature (Chown and Nicolson, 2004). The results of this study demonstrated the variations of total lipid content in different tissues of *S. litura* in relation to the feeding materials and also with seasonal variation.

Scoggin and Tauber (1950) reported that numerous factors influence the lipid content of the insects such as nutrition, developmental stage, sex, starvation, environmental temperature, diapause, cold hardiness etc. Similarly in this study, variation in total lipid content was noticed in tissues of *S. litura* with respect to the seasons and feeding materials. The larvae of *S. litura* fed with different host plants showed corresponding variation in lipid content in their tissues. The tissues of castor fed larvae showed the maximum lipid content than the larvae fed with other host plants. This finding was supported by the work of Mullins (1985) who reported that the dietary components influence the lipid components in the insect tissues. So this higher concentration of the lipid content in the tissues of castor fed larvae may be due to the extensive feeding of the nutritiously rich castor leaves. Beenakkers and Scheres (1971) studied and reported the impact of the fatty acid composition of dietary lipid on the fatty acid composition of neutral lipid in the fat body of adult male *Locusta migratoria*.

Behavioural features like selective foraging or feeding and physiological processes like digestion, assimilation and allocation are responsible for insect nutrition (Raubenheimer and Simpson, 1998). Insects tune themselves to maximize benefits and limit costs by making appropriate nutrition choices, which inturn reflect on growth and reproduction (Babic *et al*., 2008). The quality of the dietary lipid source may be of great influence in the maturation of insects. Cahu and Quazuguel (1989) reported that successful maturation is dependent on diet. These factors imply that the lipids are nutrients important for the maturation of insects. The total lipid content in

various insects at different developmental stages has been reported by Gilbert (1967a) who found that the difference in lipid content of insect tissue of different orders and often with in a single family. Triglycerides play the major role of the lipid during the developmental stages of the insect

The gut is the major site for digestion of food and absorption of nutrients and secretion of digestive enzymes (Pauchet *et al*., 2008). Gut will contain extensive amount of lipid after the intake of large quantity of lipid-containig meal. In the present work variation in the lipid content was observed in the midgut tissue when the larvae were fed with selected host plant leaves. The concentration of lipid content in the gut of castor fed larvae was higher. This may be due to the presence of the higher lipid content in the castor leaves. The macromolecular components present in the foods are catalyzed by three major digestive enzymes in the gut *viz.* amylases , proteases and lipases. Lipases secreted in to the midgut lumen of insects break down a variety of dietary lipids such as triacylglycerol and phospholipids, in to fattyacids (Weintraub and Tietz, 1973).

Among insects most studies have concentrated on the role of lipases in the fat body as compared to the gut digestive enzymes. Grillo *et al*. (2007) depicted the role of triacylglycerol (TAG) lipase in lipid digestion in *Rhodnius prolixus* midgut. The products of digestion were absorbed by the midgut epithelium and after that it was used to synthesize complex lipids, such as TAGs, diacylglycerols, and phospholipids. Horne and Haritos (2008) have reported a neutral lipase gene cluster in *Drosophila* and proposed that the lipase cluster has undergone dynamic evolutionary changes to maximize absorption of lipid nutrients from the diet.

The fat body of an insect is a tissue with multiple metabolic functions, performing important biosynthetic activity during the whole life of the insect and serves as the store of nutrients (Keeley, 1985). Fat body also synthesize

lipid from the non-lipid precursors. The fat body oxidizes the lipid so as to obtain the energy to carry out the functions such as gluconeogenesis. A higher concentration of lipid was observed in the fat body of *S. litura* larvae when fed with different host plant leaves. The fat body of castor fed larvae of *S. litura* showed higher concentration than the fat body of larvae fed with other host plant leaves. Torstensen *et al*. (2000) reported that the fatty acid composition of tissue lipids of Atlantic salmon were readily influenced by the fatty acid composition of dietary lipid. The present work showed similar results and it may be due to the presence of higher lipid content in the insect diet when fed with castor leaves. Considerable variation was noticed in the lipid content in the fat body as had previously been found for total body lipid analysis by Gilbert (1967).

Variations in fatty acid composition occur from insect to insect and also in the same insect at different developmental stages. From the work of Beenakkers and Scheres (1971) it was clear that the much of the variation may be because of the distinction in dietary lipid fatty acid composition. Tietz (1967) reported that release of free fatty acids occurred from the fat body of flown locusts, but not from rested insects and suggested that free fatty acid release may subsequently rely upon the physiological state of the insect.

The lipid content in the haemolymph is less compared to the fat body even though it constitutes an important energy reserve. Many researchers worked in the quantitation of haemolymph lipid in various insects such as *Formia regina* (Hopf, 1940), *Papillia japonica* (Lud wig and Wugmeister, 1954), *Bombyx mori* (Sreedhara and Bhat, 1965) and many others. The work of *Baiely et al*. (1975) indicated that in *Locusta migratoria* the lipid content and composition of haemolymph was highly dependent on the age, sex, dietary and hormonal status. In the present study the variation in lipid content in the haemolymph of *S. litura* was noticed when it was provided with

different feeding materials. Higher concentration of lipid was noticed when it was fed with castor leaves. This variation in the haemolymph lipid content with respect to the diet corroborate with the suggestion of Mullins (1985)that several factors such as diet, temperature and disease influence the insect hemolymph composition.

In general either diglyceride or triglycerides are the principal components in the insect haemolymph but a large variation exist in relatively small number of species so far investigated. Haemolymph diglyceride is derived from fat body triglyceride with some metabolic rearrangement of the glyceride fatty acid moieties occurring prior to release. Inspite of the fact that diglyceride is the major lipid component of haemolymph in almost all species of insects, triglyceride and free fatty acid release from fat body have been reported in certain developmental stages of few species (Chang, 1974).

Studies on several insects have demonstrated that when fat body is incubated in haemolymph, the diglyceride content of the haemolymph rises (Tietz, 1967; Chang, 1974; Thomas, 1974). Lipolytic activity has been reported in the haemolymph of several species (Price, 1975) and a capacity for fatty acid esterification has also been depicted in this tissue (Chang, 1974).

Chippendale (1973) suggested that the total lipid content in the haemolymph is normally not as much as that in the fat body. Similar results were observed in the present work also. *S. litura* larvae fed with different host plant leaves showed variations in the lipid content in different tissues. When comparing the tissues, the fat body showed higher amount of lipid than midgut and haemolymph. Chippendale (1973)suggested similar results in *Sitotroga cerealella*larvae in which fat body contained 0.7mg/ml lipid and lipid content in the haemolymph was 0.07mg/ml. Walker (1970) demonstrated that haemolymph and fat body lipid content differ during the

developmental stages of *Schistocerca gregaria* and in most stages there is far less lipid in the haemolymph than in the fatbody.

Larval nutrition can influence the adult lipid storage (Hahn, 2005). The grasshoppers *S. gregaria and L. migratoria* both accumulate significant lipid reserves in adulthood for use in dispersal and in both some of these lipid reserves have been appeared to accumulate during larval feeding (Raubenheimer and Simpson, 1997; Simpson *et al*., 2002). Since the nutritional quality of host plants in the field can vary widely, a polyphagous grasshopper like *Schistocerca americana* may experience huge variety in diet quality during its life time (Mattson, 1980; Bernays and Bright, 2001).

Seasonal variation influence the biochemical and physiological processes of the insect. Food consumption and utilization is also affected by the existing atmospheric temperature and humidity at the rearing time (Benchamin and Jolly, 1986). Depending on the species, organisms can tolerate temperature differences within certain limits through altering the metabolic activities Sharma 2013) reported that lipid metabolism relied up on oxygen utilization which frequently increased with temperature increasing upto a critical point. Anoxic condition causes the inability to metabolize lipids and carbohydrate. In this study the seasonal variation was noticed in the lipid content of different tissues of *S. litura* fed with selected host plant leaves. The concentration of lipid content in midgut, haemolymph and fat body was reported higher during the summer season than in the monsoon and post monsoon seasons. This variation may be either due to the fluctuations in temperature or humidity or by changes in any other environmental factors. Various studies done on numerous insect species revealed that temperature rise lead to increased metabolic rate (Mankin *et al.,* 1999; Taveras *et al.,* 2004).

In this context, it has been possible to recognize the complexity of insect lipid patterns and to show the changes in lipid content and form which occur during the life of an insect. Significant variations in dietary lipid may be found among insects and indeed the life history strategies of these insects may be influenced accordingly.

CHAPTER VIII
PHYTOCHEMICAL ANALYSIS

8.1 PRELIMINARY PHYTOCHEMICAL SCREENING AND DETECTION OF CHLOROPHYLL CONTENT IN LEAVES OF DIFFERENT HOST PLANTS.

8.1.1. Introduction:

Plants create primary and secondary metabolites with varying functions (Middleton, 1998). The primary metabolites constituting amino acids, simple sugars (glucids), proteins and lipids are associated with cellular processes. Secondary metabolites are chemically active compounds (flavonoids, alkaloids, terpenoids, steroids, saponins, etc.), which are produced in response to stress with complexity in structure and more restricted in distribution and function than the primary metabolites (Amalesh *et al.,* 2011). Plants can produce various kinds of secondary metabolites, which elicit their effects on other organisms (Lattanzio *et al*., 2006).

Phenolics are broadly distributed, structurally different natural compounds in plants and more than 2000different compounds are reported occurring both in the free state or in the form of glycosides (Mukerjee, 2002). They act as flower pigments and important natural animal toxicants, protect the plant from the infecting organisms, serve as signal molecules, function as allelopathic compounds, affect cell and plant growth and some may work as pesticides (Ndakidemi and Dakora, 2003; Sadasivam and Thayumanavan, 2003). Approximately 8000naturally occurring plant phenolics are known and about half of them are flavonoids.

Flavonoids are low molecular weight secondary polyphenolic metabolites present in plants characterized by their flavannucleus (Thilakarathna and Rupasinghe , 2013). There are over 700characterized flavonoids which have been shown to be responsible for the flavour and colour, pigment intensities in flowers, fruits and leaves (Samappito and Butkhup , 2010; Thilakarathna and Rupasinghe , 2013). Their presence in plants assist in protection against UV radiation, pathogens and herbivores (Butkhup *et al*., 2010).

Tannins are groups of plant polyphenolics, high molecular weight secondary metabolites that have been used by human for decades. The word tannins originated from the French word "*tan"* meaning the bark of the Holm oak tree which was used for tanning (ability to darken colour) and have been found to be present not only in the tree bark but also in leaves, stems, roots, buds and seeds (Frutos*et al*., 2004). They create dry, astringent and bitter taste in mouth when consumed in unripe fruit, strong tea or red wine (Ashok and Upadhyaya, 2012).

Terpenoidsare the largest group of secondary metabolites which contain more than 40, 000different molecules and are significant in the defensive function of plants (Garcia andCarril, 2009). They are commonly insoluble in water and their main role in plants is to protect or attract useful organisms. They also play a role in defense against herbivory with their analogous structure to that of the moulting hormone of insects which causes interference in the moulting process (Taiz and Zeiger, 2002).

High-performance thin layer chromatography (HPTLC) based methods could be regarded as a good alternative, as they are being explored as an important technique in phytochemical analysis. The main advantage of HPTLC is its ability to analyze more number of samples at the same time using a very small amount of mobile phase. This reduces time and cost of

analysis. Moreover, it minimizes exposure risks and significantly reduces disposal problems of toxic organic effluents, thereby reducing possibilities of environment pollution. HPTLC also facilitates repeated detection of chromatogram with same or diverse parameters (Devi *et al*., 2013). The present study deals with the development of HPTLC fingerprints of methanol extracts of the leaves of selected host plants of *S. litura* which can be used for identification, authentication and characterization.

Chlorophyll is an active biological component which is essential for photosynthesis. It helps the plants to absorb energy from the sun light (Patane and Vibhute, 1998). Here carbon dioxide and water are powered by the presence of sunlight and chlorophyll to generate glucose and oxygen. Chlorophyll is a pigment which act as the strong absorber of light energy in blue region of electromagnetic spectrum followed by the red region. It is the penniless absorber of light in green and near-green portion of electromagnetic spectra. This is the reason which makes the green colour of chlorophyll-containing tissues. Chlorophyll, the photoreceptor play the central role in the photosynthetic oxidation-reduction reaction spotted into different kinds such as: Chlorophyll 'a' which is found in all the plants and algae; Chlorophyll 'b' which is seen only in green plants and it absorbs only the blue light; Chlorophyll 'c' found only in the photosynthetic members of the Chromista as well as the dinoflagellates; Chlorophyll 'd 'absorbs far red light at 710nm, just outside the optic range and occursin marine red algae; Chlorophyll 'e'which is rare type of chlorophyll that is present in certain bacteria like *Vaucheria hamate* and *Tribonema bombycinum*; Chlorophyll ' f 'which is recently discovered, absorbs light at 706 nm which is shorter than that of chlorophyll d.

Chlorophyll is one of the most important parameters of the crop on which crop growth and health depends. The chlorophyll is a photosynthetic

pigment and its relative concentration dictates the photosynthetic potential of plant. The changes in concentration of this pigment relate strongly to the status of plant and its growth. Chlorophyll also supplies energy for the maintenance of the plant. In some vegetal species, leaf chlorophyll concentrations in relation to pest presence (Baldy *et al*., 1996 a, b). Leaf colour is directly proportional to its chlorophyll content and the health of the plant. The healthy crop shows delight appearance compared to the diseased crop.

The present study helps to assess the status of phytochemical compounds in leaves of five selected host plants of *S. litura*, which could account for its varied functions in the insect and also to determine the chlorophyll content of the selected host plant leaves.

8.1.2. MATERIALS AND METHODS

The materials and methods employed for carrying out phytochemical analysis were described in section 3.2.8.1 and 3.2.8.3

8.1.3. RESULTS

8.1.3.1. Phytochemical screening

Preliminary phytochemical screening of the crude methanolic extracts of leaves of selected host plants revealed the presence of different kinds of chemical groups such as alkaloid, carbohydrate, glycoside, steroid, protein, tannin, terpenoid, flavonoid and phenolic compounds (Tables VIII.1 to VIII.3.). The presence of these bioactive compounds indicated the effect of these compounds in *S. litura* larvae when the larvae were fed with these host plant leaves.

- **Qualitative analysis of carbohydrates and proteins**

Table VIII.1 illustrated the qualitative analysis of carbohydrates and proteins in the leaves of different host plants selected. It was evident from table that among the leaves of different host plants all the leaves were found to have the presence of carbohydrates and protein. The carbohydrate was found to be strongly present in the castor leaf and all the other plant leaves contain moderate amount of carbohydrate. Similarly the protein content was maximum in castor, moderate was present in colocasia , papaya and minimum amount of protein was showed in the banana and sweet potato.

Table VIII.1. The qualitative analysis of carbohydrate and protein in the methanolic extract of selectedhost plant leaves.

Phyto constituents	Reagents /chemicals	Castor	Colocasia	Papaya	Banana	Sweet Potato
Carbohydrates	Molisch's Test	+++	++	++	++	+
	Fehling's test	+++	++	++	++	++
	Benedict's test	+++	++	++	++	+++
Proteins and Aminoacids	Biuret's test	+++	++	++	+	+
	Ninhydrin Test	+++	+++	++	+	+

+++ = maximum; ++ = moderate; + = minimum.

- **The qualitative analysis of alkaloids, flavonoids, phenolics, and terpenoids in the methanolic extract of selected host plant leaves.**

The qualitative analysis of phenolics, terpenoids, flavonoids and alkaloids in the leaves of the selected plants were shown in Table. VIII..2 which revealed that the selected host plant leaves showed variation in their content of phenolics, terpenoids, flavonoids and alkaloids. Among these phytochemicals flavonoids and phenolics occur in highest level. Alkaloids were absent in papaya, where as the alkaloids occur in moderate level in castor, colocasia and sweet potato. Banana showed minimum amount of alkaloids. Maximum amount of flavonoids were present in castor, colocasia and banana. Papaya contain moderate amount and sweet potato contain minimum amounts of flavonoids. Higher concentration of phenolics were present in castor. Similarly moderate amount of phenolics were present in colocasia, papaya, banana and sweet potato. Castor, papaya , sweet potato and neem contain minimum amount of terpenoids and it was absent incolocasia and banana.

Table VIII.2. The qualitative analysis of alkaloids, flavanoids, phenolicsand terpenoids in the methanolic extract of selected host plant leaves.

Phyto constituents	Reagents /chemicals	Observations				
		Castor	Colocasia	Papaya	Banana	Sweet Potato
Alkaloids	Mayer's test	+	++	_	+	++
	Dragendroff's Test	++	++	–	+	++
	Hagers test	++	+	_	+	+
	Wagners test	++	++	+	+	++
Flavonoids	Aq. NaOH test	+++	+++	++	+++	+
	Conc. H_2SO_4	+++	+++	++	+++	+
	Shinoda's test	+++	+++	+	+++	+
Phenolics	Ferric chloride test	+++	++	++	++	++
	Lead acetate test	+++	++	++	++	++
Terpenoids	Salkowski's test	+	-	+	-	+

+++ = maximum; ++ = moderate; + = minimum; - = absent.

- **The qualitative analysis of saponins, glycosides, phytosterols, fixed oils and fats, gums and mucilage in the methanolic extract of selected host plant leaves.**

The results of the qualitative analysis of glycosides, phyto sterols, saponins and fixed oils and fats in the leaves of the selected plants were presented in Table VIII.3. It was evident that different plant leaves were found to have varying content of glycosides, phytosterols, saponins and fixed oils. Glycosides and phytosterols were present in castor, colocasia and sweet potato in moderate amounts. Similarly papaya, banana and sweet potato contain minimum amount of glycosides. Moderate amount of fixed oils and fats were present in castor and colocasia and minimum amount were present in the papaya, banana and sweet potato. Gums and mucilage were present moderately in all plant leaves. Castor contain moderate amount of saponins and minimum amount of saponins were showed in th ecolocasia, papaya, banana and sweet potato.

Table VIII.3. The qualitative analysis of saponins, glycosides, phytosterols, fixed oils and fats, gums and mucilage in the methanolic extract of selected host plant leaves.

Phyto constituents	Reagents /chemicals	Observations				
		Castor	Colocasia	Papaya	Banana	Sweet Potato
Glycosides	Borntrangers's test	+++	++	+	+	++
Phytosterols	Legal's test	++	++	+	+	++
	Salkowski's test	+	+	+	+	+
Fixed oils and fats	LibermannBuchard's test	++	+++	+	+	+
	Spot test	+	++	+	+	+
Gums and mucilage	Saponification test	+	+	+	+	+
	Precipitation test	+	+	+	+	+
Saponins	Foam test	++	+	+	+	+

+++ = maximum; ++ = moderate; + = minimum; - = absent.

8.1.3.2. High Performance Thin Layer Chromatography

High performance thin layer chromatographic studies were conducted for theleaf extracts of selected host plants in order to identify the presence of phytochemical components. Qualitative analysis for the identification of the components of different bands was performed with spraying of specific reagents for visualization. The results from HPTLC finger print scanned at wavelength 254nm, 366nm UV light and 554 nm visible light (after derivatization with Anizaldehyde reagent)for methanolic leaf extracts of selected host plants and the corresponding chromatogram were shown in plate. VIII..1.1 The HPTLC densitometric profiling of methanolic extract of selected hostp lant leaves were presented in plate. VIII.1.2. A number of polyvalent phytoconstituents were present.

Table. VIII.4. Showing the number of spots of selected host plant leaves with their R_f values and area in percentage under 254 nm.

R_f values of phytoconstituents under 254 nm										
peaks	Castor		Colocasia		Papaya		Banana		Sweet potato	
	R_f	Area in %	R_f	Area in %	R_f	Area in %	R_f	Area in %	R_f	Area in %
1	0.09	12.70	0.08	4.54	0.08	4.81	0.07	6.99	0.08	8.79
2	0.14	7.45	0.14	1.49	0.13	1.61	0.17	4.00	0.14	2.85
3	0.19	12.84	0.19	3.34	0.21	4.58	0.21	6.01	0.19	3.00
4	0.22	5.72	0.21	2.61	0.34	3.95	0.27	2.54	0.21	3.61
5	0.24	4.13	0.35	0.98	0.39	5.59	0.33	2.49	0.25	2.03
6	0.31	4.11	0.40	4.57	0.43	1.93	0.40	5.62	0.35	2.25
7	0.40	1.61	0.45	1.12	0.46	5.45	0.46	2.90	0.40	6.23
8	0.45	1.07	0.51	0.57	0.51	4.53	0.82	2.22	0.46	4.12
9	0.53	0.43	0.56	1.42	0.56	13.68	0.87	3.43	0.51	2.66
10	0.68	0.41	0.60	3.62	0.63	4.91			0.56	9.41
11	0.82	1.53	0.66	0.35	0.70	0.62			0.63	4.81
12	0.88	0.56	0.77	2.92	0.74	2.86			0.74	1.16
13	0.92	0.35	0.82	1.14	0.78	1.68			0.78	0.61
14	0.96	0.91	0.86	12.84	0.82	2.57			0.82	1.28
15					0.87	10.65			0.87	6.82
16					0.96	0.74			0.96	5.13

Table. VIII..5. Showing the number of spots of selected host plant leaves with their$R_f$values and area in percentage under 366nm.

	R_f values of phytoconstituents under 366 nm									
	Castor		Colocasia		Papaya		Banana		Sweet potato	
	R_f	Area in %	R_f	Area in %	R_f	Area in %	R_f	Area in %	R_f	Area in %
1	0.09	14.91	0.08	8.62	0.08	6.14	0.08	11.11	0.08	8.47
2	0.14	11.35	0.14	2.42	0.13	2.89	0.13	3.10	0.13	3.67
3	0.19	19.80	0.19	7.74	0.19	2.23	0.19	8.50	0.19	5.48
4	0.22	8.23	0.21	3.30	0.21	4.74	0.21	6.40	0.21	2.33
5	0.28	1.53	0.35	2.97	0.34	6.25	0.35	5.80	0.26	3.19
6	0.35	1.96	0.40	8.25	0.39	6.93	0.40	12.29	0.34	4.25
7	0.40	2.81	0.56	5.05	0.43	2.42	0.56	5.64	0.40	8.30
8	0.46	0.87	0.60	5.25	0.46	5.56	0.63	1.29	0.46	3.50
9	0.61	0.67	0.66	1.36	0.51	6.68	0.88	1.15	0.51	5.42
10	0.68	2.04	0.71	0.32	0.56	14.97	0.96	1.81	0.56	13.26
11	0.82	0.46	0.77	1.08	0.63	5.59			0.63	5.01
12	0.88	1.56	0.87	5.01	0.66	1.96			0.67	1.48
13	0.95	1.70	0.95	19.19	0.70	2.12			0.69	1.21
14					0.74	4.83			0.74	2.97
15					0.78	2.82			0.78	0.75
16					0.87	8.40			0.88	6.00
17					0.96	3.11			0.96	10.03

Table. VIII.6. Showing the number of spots of selectedhost plant leaves with their $R_f$values and area in percentage under 550nm.

	R_f Values of phytoconstituents under 550nm									
	castor		colocasia		papaya		banana		Sweet potato	
	R_f	Area in %	R_f	Area in %	R_f	Area in %	R_f	Area in %	R_f	Area in %
1	0.01	2.52	0.01	18.17	0.01	15.82	0.02	31.99	0.02	31.31
2	0.06	3.36	0.06	5.90	0.06	6.14	0.09	3.04	0.06	8.98
3	0.10	1.20	0.09	1.97	0.09	3.70	0.13	1.72	0.10	4.91
4	0.15	7.22	0.20	1.96	0.15	1.02	0.18	2.34	0.23	4.59
5	0.20	2.31	0.22	1.01	0.18	1.33	0.23	4.05	0.26	2.51
6	0.23	3.25	0.25	0.67	0.22	4.03	0.32	2.14	0.36	2.13
7	0.26	4.48	0.35	0.81	0.36	3.34	0.36	1.13	0.41	6.04
8	0.32	0.54	0.41	4.14	0.41	6.30	0.41	3.04	0.47	1.27
9	0.35	0.79	0.47	0.91	0.47	2.72	0.47	1.06	0.58	9.90
10	0.41	30.40	0.57	7.17	0.52	3.99	0.58	6.25	0.65	6.99
11	0.60	4.27	0.64	8.01	0.57	11.47	0.66	12.05	0.76	11.91
12	0.65	7.36	0.74	15.83	0.63	6.54	0.75	10.45	0.81	3.65
13	0.74	5.01	0.77	9.36	0.75	4.82	0.80	2.02	0.90	3.45
14	0.80	1.27	0.91	23.12	0.84	7.54	0.91	18.72	0.93	2.14
15	0.91	0.41	0.97	0.98	0.93	21.24			0.98	0.20
16	0.96									
17										

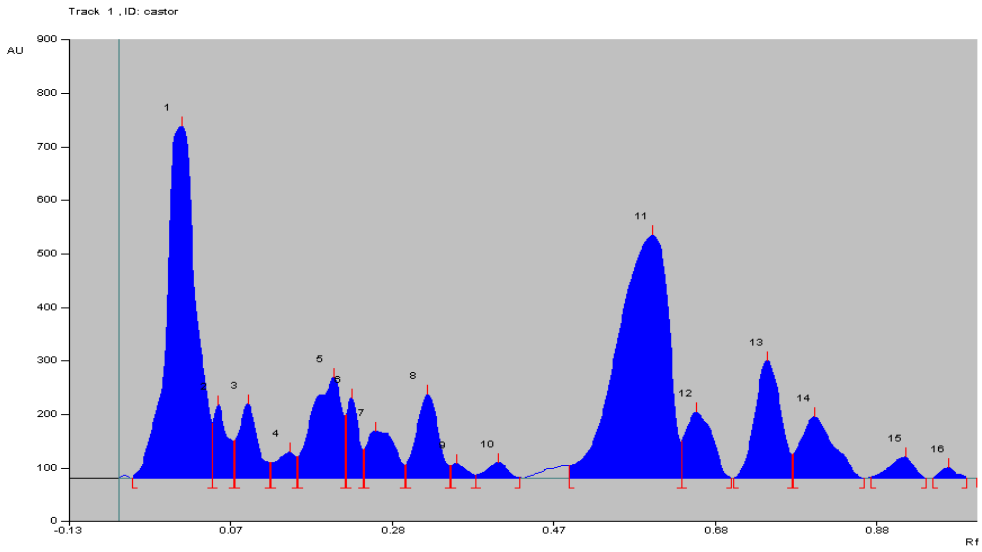

Figure. VIII.1. HPTLC Finger print of methanolic extract of castor leaves showing different peaks of phytochemicals at 550nm.

It was clear from FigVIII.1. that there were 16 spots that were visualized from the developed chromatogram of methanolic leaf extracts of castor plant scanned at 550nm. Table. VIII.6. indicated the occurrence of at least 16 different components in the methanol extract of castor leaves with R_f values 0.01, 0.06, 0.10, 0.15, 0.20, 0.23, 0.26, 0.32, 0.35, 0.41, 0.60, 0.65, 0.74, 0.80, 0.91and 0.96 and the corresponding percentage area was 2.52%, 3.36%, 1.20, 7.22%, 2.31%, 3.25%, 4.48%, 0.54%, 0.79%, 30.40%, 4.27%, 7.36%, 5.01%, 1.27% and 0.41% respectively.

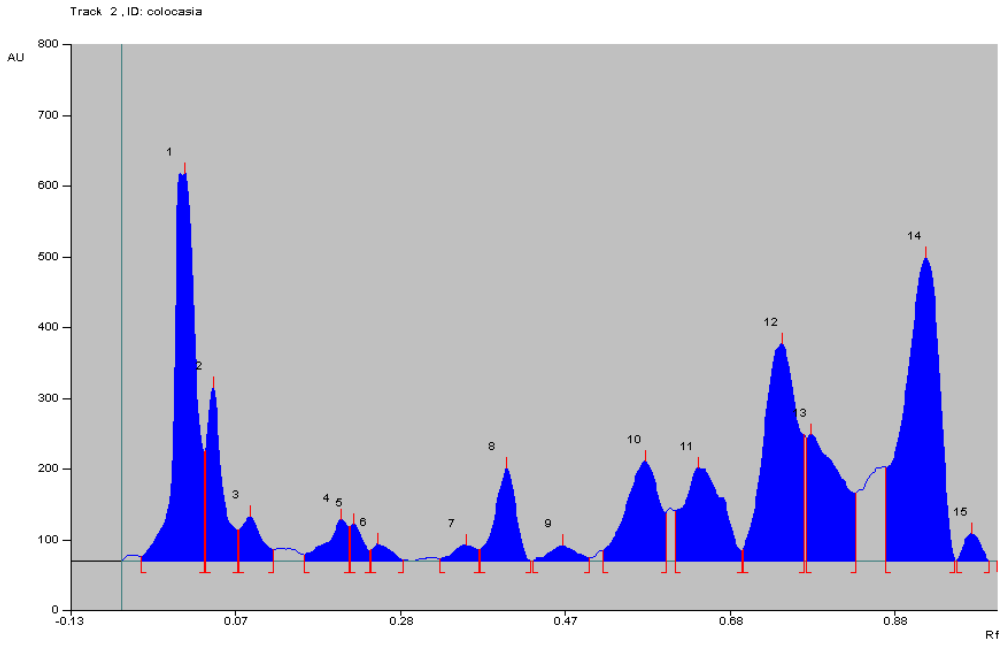

Figure. VIII.2. HPTLC Finger print of methanolic extract of colocasia leaves showing different peaks of phytochemicals at 550nm.

It was apparent from Fig. VIII.2 that there were 15 spots that were visualized from the developed chromatogram of leaf extracts of colocasia plant scanned at 550nm. Table. VIII.6.indicated the occurrence of at least 15 different components in the methanolic leaf extract of colocasia with their R_f values 0.01, 0.06, 0.09, 0.20, 0.22, 0.25, 0.35, 0.41, 0.47, 0.57, 0.64, 0.74, 0.77, 0.91 and 0.97 and the corresponding percentage area was 18.17%, 5.90%, 1.97%, 1.96%, 1.01%, 0.67%, 0.81%, 4.14%, 0.91%, 7.17%, 8.01%, 15.83% , 9.36%, 23.12% and 0.98% respectively.

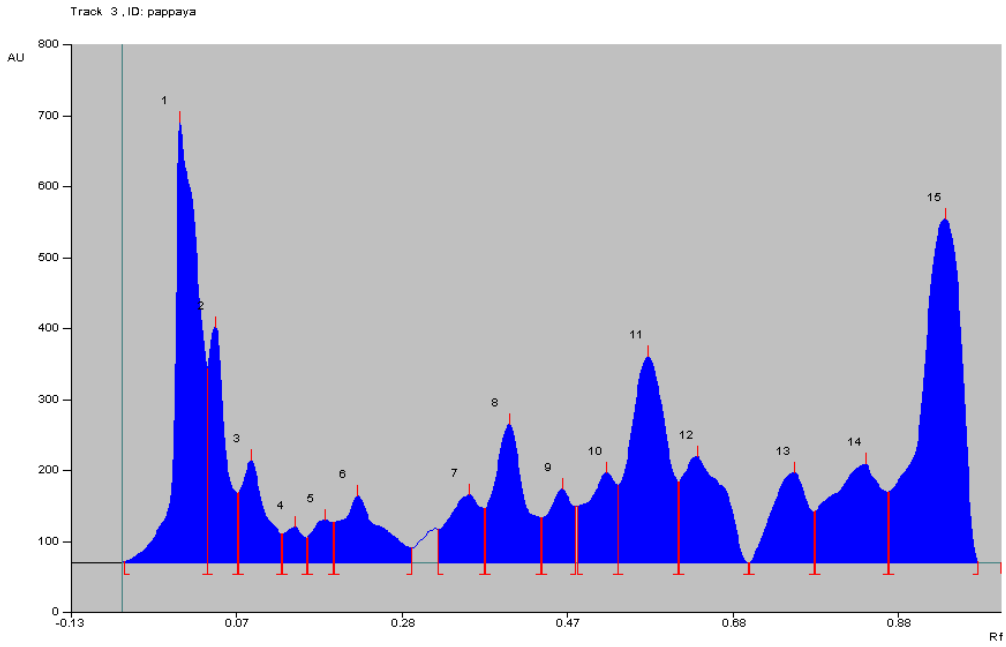

Figure. VIII.3. HPTLC Finger print of methanolic extract of papaya leaves showing different peaks of phytochemicals at 550nm.

It was evident from Fig. VIII.3 that there were 15 spots that were visualized from the developed chromatogram of leaf extracts of papaya scanned at 550nm. Table. VIII.6. indicated the occurrence of at least 15 different components in the methanolic leaf extract of papaya with their R_f values 0.01, 0.06, 0.09, 0.15, 0.18 , 0.22, 0.36, 0.41, 0.47, 0.52, 0.57, 0.63, 0.75, 0.84 and 0.93 and the corresponding percentage area was15.82%, 6.14%, 3.70%, 1.02%, 1.33%, 4.03%, 3.34%, 6.30%, 2.72%, 3.99%, 11.47%, 6.54% , 4.82%, 7.54% and 21.24% respectively.

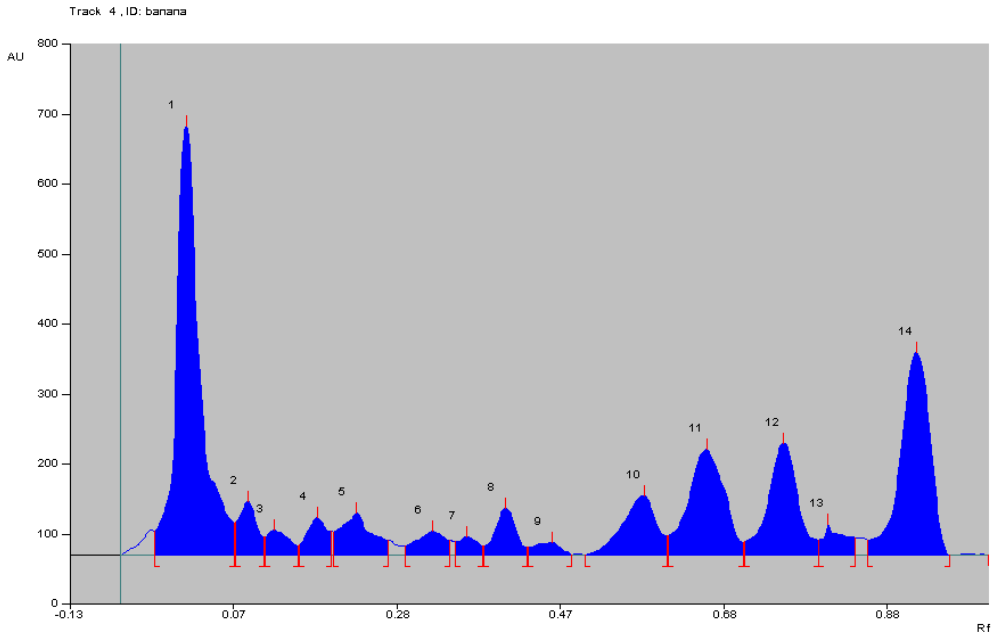

Figure. VIII.4. HPTLC Finger print of methanolic extract of banana leaves showing different peaks of phytochemicals at 550nm.

It was clear from Fig. VIII.4 that there were 14 spots that were visualized from the developed chromatogram of leaf extracts of banana scanned at 550nm. Table. VIII.6. indicated the presence of at least 14 different components in the methanol extract of banana with R_f values 0.02, 0.09, 0.13, 0.18, 0.23, 0.32, 0.36, 0.41, 0.47, 0.58, 0.66, 0.75, 0.80and 0.91 and the corresponding percentage area was 31.99% , 3.04%, 1.72%, 2.34%, 4.05%, 2.14%, 1.13%, 3.04%, 1.06%, 6.25%, 12.05%, 10.45%, 2.02% and 18.72% respectively.

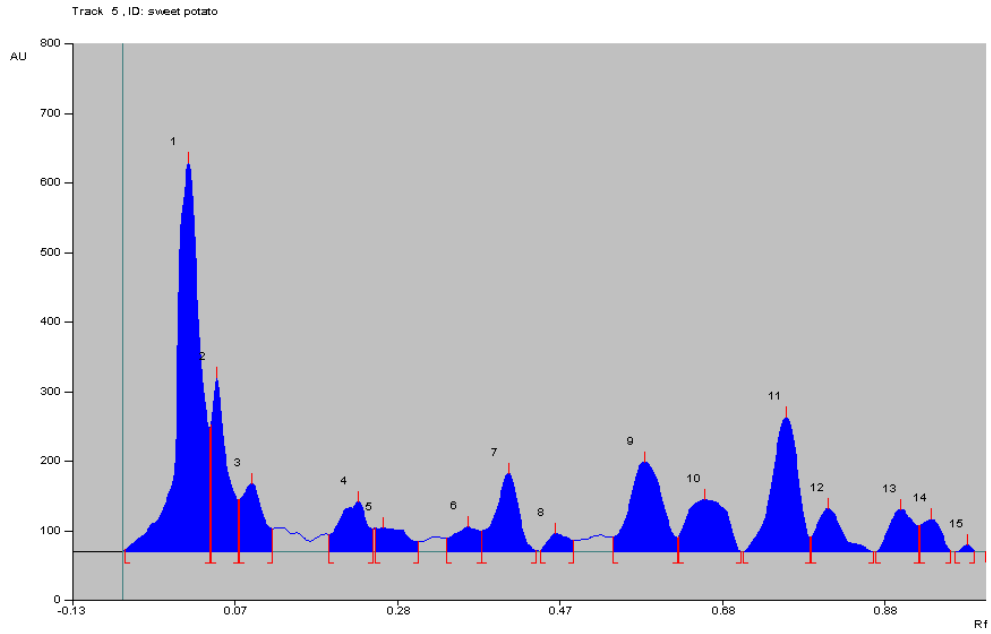

Figure. VIII.5. HPTLC Finger print of methanolic extract of sweet potato leaves showing different peaks of phytochemicals at 550nm.

It was apparent from the Fig. VIII.5 that there were 15 spots that were visualized from the developed chromatogram of leaf extracts of sweet potato scanned at 550nm. Table. VIII.6 indicated the occurrence of at least 15 different components in the methanol extract of sweet potato with R_f values 0.02, 0.06, 0.10, 0.23, 0.26 , 0.36, 0.41, 0.47, 0.58, 0.65, 0.76, 0.81, 0.90, 0.93 and 0.98 and the corresponding percentage area was 31.31%, 8.98%, 4.91%, 4.59%, 2.51%, 2.13%, 6.04%, 1.27%, 9.90%, 6.99%, 11.91%, 3.65%, 3.45%, 2.14% and 0.20% respectively.

The HPTLC chromatogram of methanolic extract of leaves of castor showed maximum number of peaks of 16 at 550nm while the number of peak was 13 at 366nm and 14 at 254nm. The number of peaks for colocasia were 14, 13, 15; for papaya 16, 17, 15; for banana 9, 10, 14 and for sweet potato16, 17, 15 at 254nm, 366nm and 550nm respectively. Among the methanolic

extracts of leaves of five selected plants banana showed the minimum number of peaks in all the three wavelength.

8.1.3.3. Estimation of chlorophyll content

In the present study the chlorophyll pigment levels of selected host plant leaves were estimated using UV spectrophotometer. Marked differences existed among the five selected plant leaves in respect of chlorophyll contents *viz*., 'a', 'b' and total chlorophyll (TableVIII.7 and Fig. VIII.6. Significantly higher chlorophyll contents were recorded in castor (1.116±0.076 mg/gm, 0.876±0.144mg/gm and 2.0878±0.7515mg/gm) and least in sweet potato (0.573±0.238mg/gm, 0.483±0.083mg/gm and 1.055±0.3068 mg/gm). However, in the remaining plants the total chlorophyll contents varied from1.991±0.1812mg/gm to 1.331±0.6574mg/gm in the order of castor>papaya>colocasia>banana>sweet potato. Chlorophyll'a' was higher in all plant leaves than chlorophyll 'b'.

Table. VIII.7. Shows the chlorophyll contents in five different host plants.

Plant	Chlorophyll a	Chlorophyll b	Chlorophyll (Total)
Castor	1.116±0.076	0.876±0.144	2.0878± 0.7515
Colocasia	1.009±0.117	0.959±0.402	1.9685± 0.4330
Papaya	1.0835±0.328	1.005± 0.442	1.991±0.1812
Banana	0.6744±0.333	0.657±0.360	1.331±0.6574
Sweet potato	0.573±0.238	0.483±0.083	1.055±0.3068

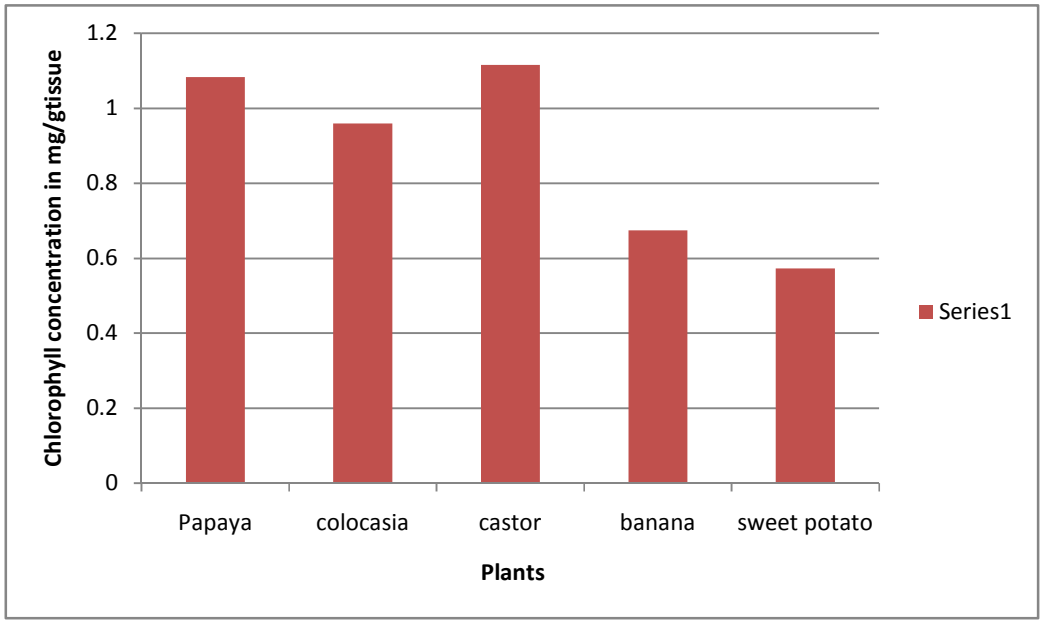

Fig. VIII.6. Showing the chlorophyll content in the leaves of five selected host plants.

8.1.4. Discussion

Plants are rich in secondary metabolites. The qualitative analysis of crude extract of different plant leaves exemplified differential occurrence of phytochemicals including alkaloids, glycosides, steroids, terpenoids, saponins, tannins and reducing sugars. In the present study preliminary phytochemical screening of methanolic extract of five different plant leaves showed the presence of alkaloid, carbohydrate, glycoside, steroid, protein, tannin, terpenoid, flavonoid and phenolic compounds.

Alkaloids, which are chemically heterogenous group of natural substances and pharmacologically active compounds, are composed of more than 6000basic nitrogen containing organic compounds which present in more than 150different plant families. About 15% of all vascular plants contain alkaloids. In the present study, the presence of moderate amount of alkaloids

were found in the castor and colocasia and low level of alkaloids were found in papaya, banana and sweet potato.

The flavonoids are a large group of naturally occurring phenolic compounds found in fruits, vegetables, grains, roots, stems, bark and flowers (Samappito and Butkhup, 2010). Tannins are members of polyphenol chemical family. Tannins are produced to a greater or lesser degree by all plants. They draw the tissues together and improve their resistance to infection. In this work the higher amount of flavonoids were found in the castor, colocasia and banana and the minimum level was found in the sweet potato and papaya leaves.

Steroids are organic compounds with four cyclohexane rings. Terpenoids are small molecular products synthesized by plants and are probably the most widespread group of natural products. Saponins are heterogeneous group of natural products found in many plant-derived foods and medicinal plants. There are two types of saponins: triterpenoids and steroidal saponins. Many plants containing steroidal saponins have a marked hormonal activity while triterpenoids, and saponins are often strong expectorant and aid in the absorption of nutrients. In the present work the presence of saponins, terpenoids and steroids were noticed. Castor and sweet potato showed the presence of moderate amount of saponins , while the papaya, banana and colocasia showed the minimum amount of saponins. Minimum level of steroids and terpenoids were reported in all the selected plant leaves.

Phenolic compounds are plant secondary metabolites that constitute one of the most common and wide spread groups of substances in plants. Above all, phenols have a high affinity to chelate metals and scavenge the free radicals in cells. In the present work castor showed maximum phenolic content but the papaya, colocasia, banana and sweet potato showed moderate

amount of phenolics. Glycosides are sugar molecules which is bound to a non-carbohydrate moiety, usually a small organic molecule. In this work castor showed the maximum concentration of glycosides , colocasia and sweet potato showed moderate amount and papaya and banana showed minimum amount of glycosides.

All the above findings about the presence of phytochemical compounds in the extract of selected plant leaves were supported by the works of many researchers. Kang *et al*. (1985) conducted the preliminary phytochemical study of *Riccinus communis* and revealed the presence of steroids, alkaloids, flavonoids, saponins and glycosides. He reported that the dried leaves of *R. communis* showed the presence of alkaloids, ricinine (0.55%) and N-demethylricinine (0.016%), and six flavones, glycosides quercetin3-O-β-D- xylopyranoside, quercetin-3-O-β-D-glucopyranoside, kaempferol-3 O-β-rutinoside, kaempferol-3-O-β-D-xylopyranoside, kaempferol-3-O-β-D-glucopyranoside, and quercetin-3-O-β- rutinoside. Darmanin *et al*. (2009) reported the presence of monoterpenoids (1, 8-cineole, α P inene and camphor) and sesquiterpenoid (β-caryophyllene), quercetin, epicatechin and ellagic acid, gentisic acid, gallic acid, rutin as the major phenolic compounds isolated from leaves of castor plant. Cherish and Ibraheem (2014) also reported the presence of various classes of flavonoids and tannins present in the different parts of castor plant. Obumselu (2011) also reported the presence of saponins in the leaf extracts of *R. communis*.

Similarly Willson *et al*. (2007) and Ikeyi *et al*. (2013) reported the presence of alkaloid, saponin, tannin, glycoside and flavonoids in the papaya leaves. In the present work protein and amino acids were noticed in moderate amount in the leaves of papaya. Yogiraj *et al*. (2014)reported the presence of phenolic compounds, flavonoids, fats, triterpenoids, xanthones, glycosides,

carbohydrate and alkaloids but in contrast to this they reported the absence of proteins and amino acids in the *Carica papaya* leaves.

The qualitative phytochemical screening of *Ipomoea batatas* leaves were carried out by Ahmed Awol (2014) and revealed the presence of tannins, saponins, flavonoids, terpenoids, quinones, phenol, amino acid and protein. Márcia Thais (2011) also reported that the sweet potato leaves showed the presence of triterpenes and/or steroids, alkaloids, anthraquinones, coumarins, flavonoids, saponins, tannins, and phenolic acids as secondary metabolites with potential biological activities.

Repon Kumer *et al*. (2013)reported that the extracts of *Musa sapientum. var.* showed the presence of various types of phytochemical active compounds including alkaloids, flavonoids, steroids, glycosides and saponins. Similarly Pothavorn *et al. (*2010) has reported the presence of bioactive compounds like apigenin glycosides, myricetin glycoside, myricetin-3-*O*-rutinoside, naringenin glycosides, kaempferol-3-*O*-rutinoside, dopamine, *N*-acetyl serotonin and rutin in different species of *Musa*.

Vaibhavi and Chanda, (2016) reported the presence of saponins, alkaloids, phenols and coumarin in *Colocasia esculenta* and they observed that tannins, flavonoids, terpenoids, anthraquinones, quinones, cardiac glycosides and anthocyanins were completely absent in *Colocasia esculenta.* They conducted the quantitative analysis of phytochemicals in *C. esculenta* and reported the presence of high amount phenols and saponins as compared to other phytochemicals. Phenol and saponin concentration was more when compared to the alkaloid content.

The phytochemical screening of plants shows that they are rich in alkaloids and flavonoids. It has been noted that the presence of phytochemical compounds in the plants are responsible for the observed biological activities

including antibacterial, antiviral and anti-diabetic properties of these plants (Nicholson and Hammerschmidt, 1992.).

Plants are composed of many constituents and are therefore very variable in nature. Hence it is very important to obtain reliable chromatographic fingerprints that represent chemically characteristic components of the plants. HPTLC fingerprinting profile is very important parameter for the standardization and for the proper identification of plants. HPTLC chromatogram of methanolic extract showed that there are many compounds in plant leaves.

In this study the HPTLC finger printing characteristics of methanolic extracts revealed difference in number of peaks and R_f values. It was apparent that the R_f values were comparatively different irrespective of peaks and solvents selected for HPTLC analysis. The similarity in few R_f values showed the evidence of the presence of specific compounds among methanolic extracts of these plants. The difference in R_f values in most of the appeared peaks reflected qualitative variation in the phytocompounds.

The HPTLC fingerprinting profile of the selected plant can also be utilized as an important source of information to ascertain the identity and to determine the quality and purity of the plant material in future studies. This study could serve as a suitable tool for phytochemical authentication of species, isolation of new bioactive compounds, preparation of natural and or semi synthetic herbal products and exploration of its clinical aspects in care of livestock.

Secondary metabolites in plants are responsible for several biological activities in man and animals. There is a growing awareness in correlating the phytochemical constituents of a medicinal plant with its pharmacological activity. The environmental conditions sometimes have a direct effect on

plant physiology and behavior. Stress responses in plants are dynamic and engage complex cross stalk at different regulatory levels. Plants might overcome these stresses through avoidance or tolerance which include metabolic adjustment through alteration of compatible solutes or secondary metabolites (Ramakrishna and Ravishankar, 2011; Krasensky and Jonak, 2012).

Phytochemicals may also have potential uses as larvicides, repellents, ovicides and oviposition deterrents and growth and reproduction inhibitors (Govindarajan *et al*., 2008 a). The bioactive compounds in plants that induced larvicidal or adulticidal response might be from various compounds including phenolics, terpenoids, flavonoids and alkaloids as single compound or as joint compounds (Elumalai *et al*., 2012). Anuskha and Dhivya (2017) reported the ovicidal activity of chloroform and petroleum ether extract of papaya leaves due to the presence of secondary metabolites such as tannins, phenols, flavonoids, sterols, terpenoids, saponins, anthroquinones and proteins.

From the findings it is very evident that among the five host plants used the castor showed the highest chlorophyll content and sweet potato showed the lowest chlorophyll content. Similar studies were reported by Chandrasekhar *et al. (*2000) who reported the variation in chlorophyll 'a', 'b' and chlorophyll a/b ratio in leaves of host plants of eri silkworm. These results were in conformity with the observations of Sannappa and Jayaramaiah (2002) who recorded chlorophyll 'a', 'b' and total chlorophyll contents in local castor genotype as1.226, 1.353 and 2.579 mg/gm. Similarly, Chandrappa *et al*. (2005) recorded higher chlorophyll 'a', 'b' and total chlorophyll contents in 48-1 castor genotype (2.948, 1.251 and 4.234 mg/g) and these contents were lower with genotype JI-226.

The larvae of Lymantriidae, Tortricidae, Noctuidae (Lepidoptera) and Curculionidae or Chrysomelidae (Coleoptera) which are chewing insects, eat

leaves, flowers, buds and twigs. They can seriously damage foliage, reducing plant fitness (Hochwender *et al.,* 2003) and regenerative capacity and also affect photosynthesis and plant growth (Doyle *etal*., 2002). Plants develop several compensatory mechanisms to these damages by increasing their relative growth rate, activating the growth of meristems or enhancing photosynthetic activity (Mizumachi *et al*., 2006). Other factors such as leaf age, nutritional status and a range of environmental and phenological conditions may also influence the relationship between photosynthetic rate and chlorophyll content (Nagaraj *et al*., 2002; Barry *et al*., 2009). The pattern of leaf senescence may also affect the chlorophyll content (Gratani and Bombelli, 2001). Demarez *et al*. (1999) reported that chlorophyll concentration in leaves strongly increased at the beginning of the growing season.

8.2 VARIATION OF TOTAL PHENOLIC CONTENT IN THE LEAVES OF SELECTED HOST PLANTS AND ITS EFFECT ON S. LITURA LARVAE.

8.2.1 Introduction

Plants produce a large variety of allelochemicals which are not directly participated in the primary metabolic processes. These allelochemicals influence the insects in diverse ways such as through acute toxicity, growth inhibition, hormonal disruption or imbalance and alteration of the consumption and/or utilization of food (Rosenthal and Berenbaum, 1992). Secondary metabolites in plants comprise an inbuilt chemical barrier to herbivorous insects and may protect plants from the attack of herbivores and pathogens. Dietary intake of allelochemicals may produce reactive oxygen species in insects which can cause extensive damage to metabolically active tissues. Insects possess a well equipped endogenous resistance mechanism comprising of antioxidant and detoxifying enzymes to protect them from the toxic impact of the allelochemicals (Zheng and Wang, (2001). The present study was envisaged to investigate the influence of total phenolic compounds on *S. litura* larvae at developmental as well as biochemical level.

The secondary metabolites mainly include phenolics, alkaloids, terpenoids and glucosinolates (Freeman and Beattie, 2008). Among the secondary metabolites phenolic compounds are pervasive in plants which are characterized by the presence of an aromatic ring bearing one or more hydroxyl groups (Parr and Bolwell, 2000). Phenolics are structurally different, broadly distributed natural compounds in plants and more than 2000different compounds are reported occurring both in the free state or in the form of glycosides (Mukerjee, 2002). They act as flower pigments, protect the plant from the infecting organisms, serve as signal molecules, function as allelopathic compounds, affect cell and plant growth, important natural

animal toxicants and some may work as pesticides (Ndakidemi and Dakora, 2003; Sadasivam and Thayumanavan, 2003). Based on the number of carbon atoms phenolic compounds are classified into various groups. The important general categories are flavones, isoflavones, flavonones, anthocyanidins and flavanols. Out of these, simple phenols, phenolic acids, flavonoids, tannins and hydroxycinnamic acid derivatives are widely distributed in plants (Sadasivam and Thayumanavan, 2003). Phenolic compounds are derived from the amino acid phenyl alanine. Plants contain a large variety of phenolic derivatives such as cinnamic acid and benzoic acid derivatives, isoflavonoids, flavonoids, tannins and lignans (Shahidi, 2000). Approximately 8000naturally occurring plant phenolics are known and about half of this are flavonoids.

Flavonoids are the biggest and most widely distributed group of phenolic compounds which have more than 4000structures (Ateyyat *et al*., 2012). The flavonoids have closely related structures, they contain C_{15} heterocyclic nucleus of flavones and they differ mainly in the number of phenols such as phenyl propanoids, phenolic quinines and phenolic acids (Harborne and Boxter, 1995). They (C6-C3-C6 group) are low molecular weight compounds and have an extensive range of biological activities. They have been recognized to a great extent for their antioxidant, anti-cardiovascular and anticarcinogenic disease capacity (Parr and Bolwell, 2000; Hoffman, 2001). Quercetin is one of the most studied member of this group which has been reported to be present in higher amounts in apple, onions and broccoli (Saric *et al*., 2007). Glycosidic form of quercetin-rutin has also been reported to be available in plant tissues. The negative impact of quercetin and rutin has been shown in some insect pests like woolly apple aphid *Eriosoma lanigerum* and fruit fly *Drosophila melanogaster* (Saric *et al.,* 2007; Ateyyat *et al*., 2012).

Tannins are polyphenolic compounds having molecular weight more than 500units (Mehansho *et al*., 1987). They are present in the bark and fruits of many plants (Haslam, 1989). The tannins can be divided into two-they are hydrolysable tannins and the condensed tannins. The hydrolysable tannins incorporate polyesters of gallic and hexahydroxydiphenic acid (gallotannins and ellagitannins respectively), while the condensed tannins are made up of oligomers and polymers consisting of flavan-3-ol nuclei (Bruyne *et al*., 1999). Tannic acid also act as antimutagenic and anticarcinogenic agents (Kuo *et al*., 1992). Tannins can also serves as protein precipitating agents and can also penetrate the peritrophic membrane and enter into body cavity of insects thus lead to toxic effects on herbivore insect (Karowe, 1989). Adverse impact of tannins and gallic acid have been reported on some lepidopteran and other insect pests (Nomura and Itioka, 2002).

The prevention afforded by phenols against the plant pathogen and herbivore was the important factor in their selection during the plant evolution (Harborne, 1998). These compounds mainly act in defense against the predators and pathogens and giving the reproductive advantages as attractants of self-pollinators and seed dispersers. Phenolic compounds are commonly white solids except flavonoids (yellow) and anthocyanins (red)). Some phenolic compounds isolated from the plants produce alterations in the insect behaviour and physiology. The characterization of these compounds in response to herbivory is important to understand the host plant-herbivore interaction.

Significance of phenolic compounds

Phenols play many vital roles in plants and animals. They show antioxidant property and function as free radical scavengers. They act as the structural polymers. Lignin is the important phenolic compound which function as the structural unit in plants. Cutin and suberin are the other two

phenolic compounds which provide mechanical strength and development of internal water system of plants. Phenolic compound tannins act as a feeding deterrent. The interaction and precipitation of tannin with protein make bitter tasteto plants which make them unfit for feeding by the insect (Appel, 1993). Phenolic compounds act as the signaling compounds in many metabolic pathways. Methyl salicylate and dehydrodiconiferyl alcoholglucoside (DCG) are reported as the signaling phenolic compounds. Phenols such as anthocyanins and flavonoids (carotenoids) are the low molecular weight compounds which are responsible for the aroma and attractive colouration of the flowers to attract the pollinating agents.

8.2.2. Materials and methods

The materials used and the methods employed in the estimation of total phenolic content was given in the section 3.2.8.2.

8.2.3. Results

The variation of the total phenolic content in the selected host plant leaf extracts were presented in the table VIII.8. and Fig. VIII.7

The amount of total phenol was determined with the Folin-Ciocalteu reagent. The calibration curve showed linearity for gallic acid in the range of 3.37-30.87mg/ml, with a correlation coefficient (R^2) of 0.988 (Figure.8.8). While comparing the total phenolic content in different host plant leaves, the results showed that the richest source of the phenolic content was the leaves of *Ricinus communis (*30.83±1.53 mg GaE/g), *Colocasia esculenta* (8.13±0.06 mg GaE/g), *Ipomoea batatas* (7.53±0.06 mg GaE/g), *Carica papaya* (6.73±0.06 mg GaE/g) and the lowest phenolic content was in *Musa acuminata colla* (3.37 ±0.12 mg GaE/g).

Table. VIII.8. Variation in total phenolic content in the leaves of selected host plants

Plant	Mean±SD	R^2
Carica papaya L (papaya)	6.73±0.06	
Ricinus communis L (Castor)	30.83±1.53	
*Ipomoea batatas (*sweet potato*)*	7.53±0.06	0.988
Musa acuminata colla (banana)	3.37±0.12	
Colocasia esculenta (L.)	8.13±0.06	

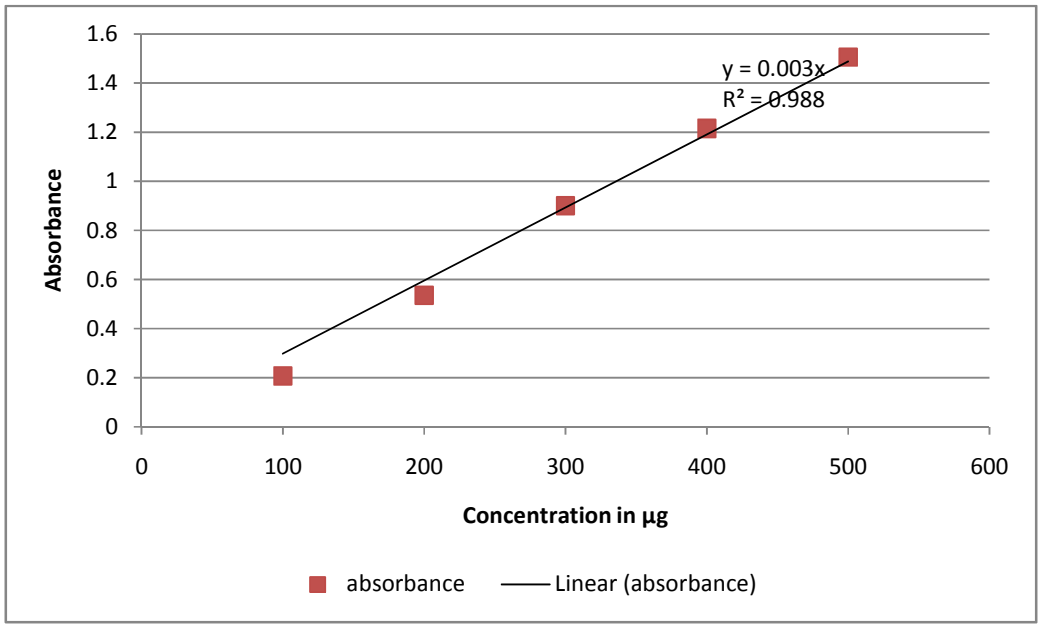

Fig. VIII.7: Standard calibration curve of gallic acid at concentrations of 100, 200, 300, 400, 500µg/ml. Spectrophotometric detection was at 765 nm

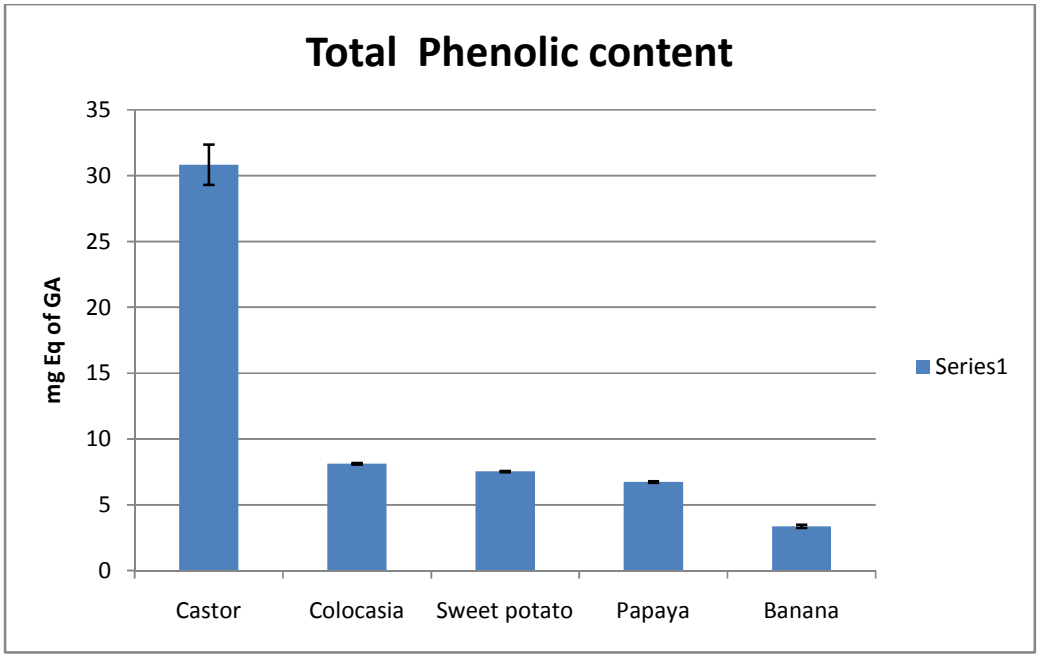

Fig. VIII.8. Variation of total phenolic content in the leaves of selected host plants

8.2.4. Discussion

Phenolic compounds are the most commonly found secondary metabolites in plants of significant medicinal value. Phenolic compounds are known to be responsible for antioxidant potential of plants by neutralization of free radicals that are produced during stress, metabolism, diseases and other numerous factors (Orcic *et al.,* 2011). High phenolic content in plant extracts is believed to be responsible for high antioxidant potential in plants.

In the present study, significant difference in total phenolic contents was recorded among methanolic extract of host plant leaves. Highest phenolic contents was observed in the methanolic extract of castor leaf (20.22±0.57 mg of GAE/g) where as lowest was found in methanolic leaf extract of banana. The environmental factors such as climatic conditions, soil type, biotic and abiotic stress and nutrition were found to influence the presence as well as the

amount of important phytochemicals in plants. In case of *in vitro* phytochemical and antioxidant potential studies in plants, factors such as type of solvents, method of extraction, type of plant parts and geographical location of plant material can alter the results significantly (Jain *et al*., 2011**).** Also it has been reported that different plant parts vary in the amount as well as quality of phytoconstituents (Tiwari *et al*., 2011**).**

All plants differ in their phenolic contents. Darmanin *et al. (* 2009) reported that phenoloic compounds such as the monoterpenoids (1, 8-cineole, α P inene and camphor) and sesquiterpenoid (β-caryophyllene), quercetin, epicatechin and ellagic acid, gentisic acid, gallic acid and rutin are present in the leaves of *Ricinus communis*. Islam *et al. (*2002) and Komaki and Yamakawa (2007) reported that *Ipomoea batatas* leaves were an excellent source of antioxidative polyphenolics such as caffeoylquinic acid, anthocyanins, as well as beta-carotene. Islam *et al. (*2006) reported that leaves contained about fifteen different anthocyanin compounds and about six different polyphenolic compounds and were good sources of bioactive anthocyanin and polyphenolic constituents.

Effect of plant phenolic compounds on *Spodoptera litura* biology

Plant phenolic compounds are getting more attention in the research field due to an increased interest in their interaction with other organisms. Most of these compounds inhibit the growth rate at different stages of the insects. All plants differ in their phenolic contents and even different parts of the same plant differ in their phenolic content. So based on the difference in the amount of total phenolic content and on the difference in the individual phenolic compound of the host plant leaves, variations were observed in the biological parameters of the insect (Chapter IV)

Effects of phenolic compounds on egg hatching of *Spodoptera litura*

Previous studies (Sohal and Sharma, 2011) reported that some phenolic content reduced the egg hatchability of the insects. But in the present study among the five host plants selected the higher concentration of total phenolic content was observed in the castor leaf. While comparing the egg hatchability of *S. litura* larvae fed with different host plants, the castor fed insect showed maximum hatchability of eggs. So there was a positive correlation with the total phenolic content of the host plant and egg hatchability of the insect. The reason for this difference may be due to the variation in the individual phenolic compounds of the host plant leaves. Sohal and Sharma (2011)reported the decreasing ovicidal effect of phenolic compound, pyrogallol on eggs of *Bactrocera. cucurbitae.* Similar results were reported by Manoukas (1996) in olive fruitfly, *Bactrocera. oleae* after treatment with phenolic compounds viz. pyrogallol, resorcinol, hydroquinone and phloroglucinol. The effect of flavonoids on ovicidal activity of bruchids and *S. littoralis* were also been reported(Salunke *et al.,* 2005).

Effect of phenolic compounds on larval and total developmental duration of *Spodoptera litura*

The observations recorded for larval duration in *S. litura* revealed that the larvae fed with the leaves of castor which contain high phenolic content, the larval period was shortened (TableIV.5 , fig. IV.5 and fig. IV.6). But larvae fed with banana which have low phenolic content showed extended larval period. This finding was supported by Saric *et al. (*2007)who suggested that a shortened larval period on *Drosophila melanogaster* flies when reared on 1.75% quercetin diet. Taman (2005) also reported the antifeedant and growth inhibitory effects of plant quercetin at higher concentrations on *S. littoralis*. Inhibition and development retardation have also been reported in *S. littoralis* at higher concentrations of quercetin whereas low concentrations

(less than 2000ppm) had no effect on biological measures of the insect (Mesbah *et al*., 2007).

Tannic acid also play an important role in the larval duration. In this work the highest larval duration was showed by the banana fed larvae of *S. litura*. So this may be due to the presence of highest tannin content in the banana leaf. The previous works reported that the larval period of tobacco cutworm, *S. litura* and cotton bollworm, *H. armigera* also increased by the tannic acid (Kathuria and Kaushik, 2005). On the contrary, Panzuto *etal. (*2002) had reported a reduction in the larval duration of oblique banded leaf roller, *Choristoneura rosaceana* with tannic acid.

Rutin also prolonged the non-feeding duration and insect moulting in *H. armigera* and *S. litura* at higher concentrations (Jadhav *et al.,* 2012). Rutin had no significant effect on the larval period of three larval instars of melon fly whereas gallic acid significantly prolonged the larval period of 88-96hrs old larvae. Gallic acid has been noticed to play a concomitant role in anti-herbivoredefense in plants (Ananthakrishnan, 1997). Mansour (1982) had also suggested a prolongation in the larval duration of a lepidopteran, *S. littoralis* after treatment with gallic acid. In the mosquito, *A. aegypti* and in mealworm beetle *Tenebrio molitor* larvae, growth inhibitory and toxic effects of quercetin has been reported by Sosa *et al. (*2000). Chrzanowski and Leszczyński (2008) reported that gallic acid isolated from blackcurrant and sour cherry leaves have increased the pre reproductive duration in grain aphid, *Sitobion avenae*. The present findings indicate that variation in the concentration of certain secondary metabolite in plants may be of potential value in biology of*S. litura*.

Effects of phenolic compounds on larval growth and total growth of *spodoptera litura.*

The instar growth rate and total growth rate of *S. litura* were observed in chapter IV. The maximum growth was observed in the castor fed larvae and slow growth rate was observed in the banana fed larvae. The phenolic compounds gallic acid and tannic acid have growth inhibitory effect on insects. Study of Santos *et al*. (2010) revealed the presence of varying amount of total tannin content in the green banana fresh leaf depending on the genotypes and the growing conditions of the plants. Kubo *et al. (*2003) reported the growth inhibitory activities of both gallic acid and tannic acid seen in artificial diet bioassays against *Pectinophora gossypiella* larvae. High mortality were recorded at higher concentrations of quercetin in *S. littoralis* which got reduced with the decrease in concentration of the phenolic compound (Mesbah *et al.,* 2007). The changes in the phenolic activity against different larval instars of melon fruit fly could be a result of variation in their structure or differences in their phenolic oxidation state. Several other factors may also be added to tolerance or susceptibility of the larvae, among them are antioxidant enzymes (Krishnan and Kodrick, 2006), low molecular weight antioxidants (Barbehenn *et al.,* 2003), the peritrophic envelope (Barbehenn, 2001) and surfactants (De Veau and Schultz, 1992).

Effects of phenolic compounds on nutritional indices of *Spodoptera litura.*

The decline in larval weight and pupal weight of *S. litura* showed an antinutritional effect of phenolic compounds which results either from inhibition of digestion or from post absorption inhibition of metabolism. In order to pupate successfully, the larvae have to attain critical weight (Trumble and Millar, 1996). Quercetin inhibited the larval weight of tobacco cutworm, *S. litura (*Beninger and Abou-Zaid, 1997). Reduction in larval and pupal weight has also been showed in *S. littoralis, S. litura*, and *H. armigera* fed on

gallic acid and tannic acid incorporated diet (Kathuria and Kaushik, 2005; Mrdakovic *et al.,* 2011). Quercetin have no effect on larval weight of *cucurbitaceae* pest, *Epilachna paenulata* (Napal *et al.,* 2010). But decrease in larval weight was common in western spruce budworm, *Celtis occidentalis,* cabbage looper*, Trichopulsia ni* and crucifer pest, *Mamestra configurata* treated with flavonoids (Hoffman-Campo *et al.,* 2001; Onyilagha *et al.,* 2004).

All the phenolic compounds had detrimental effect on food assimilation and mean comparative growth rate of the insect. Reese and Beck (1976a, b) have reported reduction in food assimilation in black cutworm, *Agrotis ipsilon* fed on catechol and gallic acid. Similar findings have also been reported in forest tent caterpillar, *Malacosoma. disstria* and cabbage looper, *T. ni* fed on rutin and tannic acid (Hoffman-Campo *et al.,* 2001). Decreased weight gain and depressed growth rate have been the most often observed antinutritional effects of dietary tannins (Salunke *et al.,* 1990). The protective effects of phenolics is the outcome from their several modes of action including binding with molecules such as proteins, lipids, metals and carbohydrates via both covalent and non-covalent interactions (Pierpoint, 1983) and oxygen radical formation (Felton and Duffey, 1991. Thus, they may stand as a nutritional protein precipitating agents resulting in impaired enzyme functions and reducing availability of amino acids (Felton *et al.,* 1992). Toxic effects of tannins have also been portrayed in the form of lesions in the gut epithelium of insects or tannin products may act on tissues elsewhere in the body (Steinly and Berenbaum, 1985).

Effects of phenolic compounds on oviposition behaviour of *Spodoptera litura*

The variation in the oviposition behaviour of *S. litura* when fed with different host plant leaves were recorded in chapter IV (TableIV.8 and fig. IV.11). Maximum oviposition was shown by the adult of castor fed larvae and

minimum number of oviposition days was for banana fed insect. Chemical inhibitors have a vital role in the inhibition of oviposition on the host plant and inturn, resulting in larval number decline and survival of progeny (Chapman, 1974). Kaur *et al. (*2010) had also reported deterrent aspects of polyphenolic rich extracts from the bark of *Acacia auriculiformis* on the oviposition of the melon fruit fly, *B. cucurbitae*. Quercetin extracted from *Ricinus communis* had also shown a significant deterrent effect on ovipositional behaviour of pulse beetle, *Callosobruchus chinensis (*Upasani *et al.,* 2003). Salunke *et al. (*2005) had also reported oviposition deterrent activity of quercetin and rutin against chinese bean weevil, *C. chinensis*. Lactones and flavonoids in ethanolic extract of *Andrographis paniculata* also showed oviposition deterrent activity against malarial vector, *A. stephensi (*Chenniappan andKadarkarai, 2008). The addition of rutin at lower concentration decreased egg laying by zebra swallow tail, *Eurytides marcellus* while at higher concentrations the decrease was not significant (Haribal and Feeny, 2003). Tannin also decreased oviposition by western flower thrips, *Frankliniella occidentalis (*Whittaker and Kirk, 2004). Under choice-test all the phenolics except rutin treatment effectively inhibited oviposition by adult flies. Four other flavonoid compounds, poncirin, rhoifolin, naringin and marmesin from *Poncrius trifoliata*were also reported to have an oviposition deterrent activity against the yellow fever mosquito, *A. aegypti (*Rajkumar andJebanesan, 2008).

On the other hand, gallic acid in contrast to the presesnt findings showed stimulatory effect on oviposition behaviour of spruce budworm, *Choristoneura.fumiferana (*Grant and Langevin, 2002). Results also revealed lesser number of punctures on experimented pumpkin pieces under no-choice and multiple choice tests. The role of flavonoids in modulating oviposition behaviour of insects has also been noted by Simmonds (2001). An insect before ovipositing delves the surface of the leaf or stem with its antenna or by walking which brings the contact sensilla located on the tarsi and antenna into close association with compounds on the plant's surface. Nair and Thomas

(2001) had reported that before oviposition melon fruit flies thoroughly probed the substrate treated with extracts of *Acorus calamus* with the help of chemoreceptors seen in their mouth parts and ovipositors. During such probing, they intercepted the extracts and avoided oviposition on the treated surface. The present findings clearly revealed that phenolic compounds were effective in the preference of oviposition of *S. litura* when fed with different host plant leaves.

So the findings from this work revealed that the phenolic content play an important role in influencing some of the biological parameters of *S. litura* larvae.

CHAPTER IX
GENERAL DISCUSSION

The phytophagous insects use plant nutrients for their growth and development. The quality and quantity of host plants have a great role in the growth and reproduction of an insect. Each host plant contains various nutritional and anti-nutritional substances for which regulation of their uptake by an insect shows integration of a highly complex set of interacting processes (Simpson and Raubenheimer, 1999). Any change in the nutritional quality of the host plant may result in adverse physiological adaptation. The environmental factors have a direct influence on the host plants in different seasons (Hering and Taguchi, 1951).

The diet of phytophagous insects has got a significant influence on the biochemical constituents of the insect tissues. Continuous feeding generally depends on continuous phagostimulation and an insufficient concentration of phagostimulants causes early cessation of feeding (Bdmays *et al*., 1975). The present study revealed the effect of selected host plants on biochemical components of the insect tissues. The quality of host plants describes the tissue components of the insects (*e. g*., the levels of protein, carbohydrate, trace elements and defensive compounds) that can affect the feeding and digestion positively or negatively in herbivorous insects.

The protein content in different tissues (midgut, fat body and haemolymph) of last instar castor fed larvae of *S. litura* was found to be comparatively very high than in the tissues of the larvae fed with other host plants. Among the selected host plants castor showed the highest protein concentration than the other host plants in the summer, monsoon and post monsoon seasons. This variation in plant proteins may be due to the

environmental factors such as the amount of light it receives, the chemical composition of the soil and inputs of water (Felton, 1996; Walter *et al*., 2012). So the feeding of castor leaves may have caused an increase in the concentration of protein in the midgut, fat body and haemolymph of *S. litura* larvae during all the seasons. During the larval feeding stage the rate of synthesis of protein is generally higher in the fat body and in the mature larvae the protein content is higher in both the haemolymph and fat body (Thompson, 1975).

In all the selected host plants protein content was highest during summer than in monsoon and post monsoon seasons. Similar work was reported by Sheth (2011) who suggested the presence of highest protein content in all the parts of *Calotropis procera* during the summer season and lowest in the winter season. However, Chaluvachari and Bongale (1995) have reported lower value of nitrogen, protein and sugar content in leaf during summer. But Obata and Fernie (2012) reported higher concentration of protein in leaves under stress conditions. The temperature and humidity variations prevailing during summer must have caused the variation in protein level in the food plants. This seasonal variation in the protein content of the host plants also influenced the protein content in the midgut, fat body and haemolymph of the *S. litura* when they were fed with these host plants. So the midgut, fat body and haemolymph of *S. litura* showed higher concentration of protein during summer than in the monsoon and post monsoon seasons when fed with all the selected host plants.

Similarly the low intake of food or the intake of low quality food by *S. litura* during post monsoon season may reduce the protein content in its tissues. The fluctuation in the protein content in the whole body tissue may be attributed to a variety factors such as diet, temperature, photoperiod, developmental stages and soil condition of host plants. The influence of all

these factors will implicit seasonal changes in the protein and free amino acid pool (Graney and Giesy, 1986). Therefore it may be assumed that the variable nutritional value caused by seasonal variation may be responsible for difference in protein level in different tissues of *S. litura* during different seasons. Variations in protein content of insect tissues due to the effect of feeding and physiological activities are reported by various researchers (Banno *et al*., 1993; Aruga, 1994).

The present work recorded the highest amino acid content in the leaves of selected host plants during the summer season comparing to the monsoon and post monsoon seasons. This results corroborate with the findings of Kramer (1983) who reported that during summer the water stress disrupted the nitrogen metabolism leading to solubility of protein and accumulation of amino acids. But Ferrario *et al*. (1998) reported that under drought stress amino acids concentration in tobacco plants decrease due to the low activity of nitrate reductase enzymes which is responsible for transforming NO_3 to NO_2. This seasonal variation in the aminoacid content of the selected host plants has also influenced the amino acid content in the tissues of *S. litura* when they were fed with these host plants.

The highest concentration of amino acids was recorded in the midgut tissue, fat body and haemolymph of larvae of *S. litura* during the summer season. This may be due to the highest concentration of amino acid in the host plant leaves during the summer season. Among the selected host plants the castor leaves showed highest amino acid content and the banana leaves showed least amino acid content than the other selected plant leaves. This variation in the aminoacid content of the host plants also influenced the concentration of amino acid in the midgut tissue, fat body and haemolymph of the larvae of *S. litura* fed with these host plants. Due to the presence of higher concentration of aminoacid in the castor leaves all the tissues of the larvae fed

with castor leaves exhibited a higher concentration of amino acid than the tissues of larvae fed with other host plants. In the present work, it was noticed that a high concentration of amino acid was present in the haemolymph of the larvae than in the midgut tissue and fat body. Various researchers made detailed investigation on haemolymph free amino acid level in different insects (Wyatt , 1961; Sutcliff, 1963; Florkin and Jeuniaux, 1974).

Cook *et al. (*1972) reported that the decline in free amino acid content in insects during winter season is associated with the carbohydrate metabolism. In banana fed larvae, the low level of free aminoacid during the post monsoon season may be due to their utilization for generating energy via gluconeogenesis. Influence of dietary components in the concentration of amino acids in insect larvae was reported by Burnet and Sang (1968) and Strong (1964).

Other growth related functions, like metamorphosis (Ducbateau and Florkin, 1959), detoxification (Cauda, 1955) organ formation (Goldberg and Demeillon, 1948), energy production, histogenesis (Jolly *et al*., 1972) and during different metabolic, metamorphic and stressful situations like osmoregulation (Bishop *et al*., 1925) and protein synthesis (Buck, 1953) also affect the variations in amino acid content of the insect tissues.

Carbohydrates are probably the most widely distributed and widely occurring compounds in plants in which their quantity and quality vary exceedingly. In the present study, among the selected host plants the amount of total carbohydrate content was recorded highest in castor in comparison to other selected host plants. This variation may be due to the following factors as described by various researchers that is in plants fluctuation in carbohydrates exists at different levels, including between the species (Yeoh *et al.,* 1992), within the species (Sattelmacher *et al*., 1994) and within an individual plant (Mattson , 1980) depending on the type of tissue (i.e., leaves,

flowers, seeds and stems) and its age (i.e., young versus old leaves). The feeding of these host plants by the larvae of *S. litura* may lead to the corresponding variations in carbohydrate content of their midgut tissue, fat body and haemolymph. The castor fed larval tissue showed highest carbohydrate concentration. This indicated the influence of dietary components on the biochemical constituents of the insect tissues. This finding was supported by Friedman *et al. (*1991) who reported that the quality of a herbivore insect diet changes both within and between its host plants, and these fluctuations can be predictable, such as seasonal changes in plant quality or unpredictable, such as the changes caused by environmental stress.

Plant's protein and carbohydrate content can differ in response to environmental factors, such as the amount of light it receives, the chemical composition of the soil and inputs of water (Felton, 1996; Walter *et al*., 2012). In the present work, concentration of carbohydrate in leaves during post monsoon was lower than that of summer for all the selected host plants. This finding is supported by Pandey (1995) who reported that the carbohydrate concentration in plants fluctuate according to season. Miller (2003) reported that the ecological factors may bring about a change in local climate that, in turn may influence the soil and soil nutrient based inter-plant competition resulting in the individual plant variations. These variations are felt right from the biochemical constituents, through the structure (anatomy) and functions (physiology) to the genetic makeup. This alterations in the carbohydrate content of the host plants caused corresponding variation of these compounds in the larval tissues when the larvae feed on them. This may be the reason for the low carbohydrate content in the midgut, fat body and haemolymph of *S. litura* larvae during the post monsoon season when fed with these host plants.

It has been generally accepted that numerous over wintering insects accumulate sugar alcohols such as sorbitol as well as glycerol through the

breakdown of glycogen storage (Grubor *et al.,* 1992). The variations of carbohydrate in different organs, supported the speculation about the conversion of fat into carbohydrate during developmental stages. (Nestel, 2004; Nestel and Nemny-Lavy, 2008). In insects the variation of carbohydrate also occurred during the larval pupal transformation (Wyatt, 1962) and ecdysis (Lohr and Gade, 1983).

Lipids have an important role in serving as an energy reservoir in insects (Hadley, 1985). Allocation patterns of lipids are influenced by many environmental factors including water and food availability, photoperiod, humidity as well as temperature (Chown and Nicolson, 2004).

Scoggin and Tauber (1968) reported that numerous factors influenced the lipid content of the insects such as nutrition, developmental stage, sex, starvation, environmental temperature, diapause, cold hardiness etc. Food consumption and utilization is also affected by the existing atmospheric temperature and humidity at the rearing time (Benchamin and Jolly, 1986).

The tissues (midgut, fat body and haemolymph) of castor fed larvae showed the maximum lipid content than the larvae fed with other host plants. It may be due to the presence of higher lipid content in the insect diet when fed with castor leaves. This finding was supported by Mullins (1985) who reported that several factors such as diet, temperature and disease influence the insect haemolymph composition of a species. Beenakkers and Scheres (1971) reported the dietary lipid and its effect on the insect tissues.

In this study the concentration of lipid content in midgut , fat body and haemolymph was reported higher during the early summer than that of monsoon and post monsoon seasons. reported that lipid metabolism relies up on oxygen utilization which frequently increases with temperature increase upto a critical point. Various studies done on numerous insect species

revealed that temperature rise leads to increased metabolic rate (Mankin*et al.,* 1999; Taveras*et al.,* 2004).

Many researchers worked in the quantitation of haemolymph lipid in various insects such as *Formia regina* (Hopf, 1940), *Papillia japonica* (Ludwig and Wugmeister, 1954), , *Bombyx mori* (Sreedhara and Bhat , 1965) and they suggested that the lipid content and composition of haemolymph was highly dependent on the age , sex, dietary and hormonal status. In the present work, when comparing the tissues, the fat body showed higher amount of lipid than midgut and haemolymph. Chippendale (1973) suggested similar results in *Sitotroga cerealella* larvae in which fat body contained 0.7mg /gm lipid and lipid content in the haemolymph was 0.07mg/ml.

Total proteins, triacylglyceride, and glycogen are the three main storage macromolecules in insects. Because of the difference in concentration of these molecules in host plants, utilization of different host plants by the insect might lead to a gain in various amount of these storage macromolecules in the insects.

Plants are rich in secondary metabolites. The preliminary phytochemical screening of methanolic extract of five selected plant leaves revealed the differential occurrence of phytochemicals including, alkaloids, flavonoids, glycosides, steroids, terpenoids, saponins, tannins, proteins, amino acids, phenolic compounds and reducing sugars. The variations in the presence of phytochemical compounds in the different plant leaves extract were reported by many researchers in castor (Obumselu, 2011; Cherish and Ibraheem, 2014) in papaya (Willson *et al*., 2007 ; Ikeyi *et al*., 2013), in banana *(*Pothavorn *et al.,* 2010: Repon Kumer *et al*., 2013;), in colocasia (Vaibhavi and Chanda, 2016)*,* and in sweet potato (Márcia Thais, 2011; Ahmed Awol , 2014). This variations may be due to the environmental stress related factors in the plants. Plants might overcome the stresses through

avoidance or tolerance which includes metabolic adjustment through alteration of compatible solutes or secondary metabolites (Ramakrishna and Ravishankar, 2011; Krasensky and Jonak, 2012).

The quantitation of chlorophyll content in different host plant leaves revealed the variation in the chlorophyll content between the host plants and it was in the order castor>papaya>colocasia>banana>sweet potato. Highest chlorophyll content was noticed in the castor leaves. Sannappa and Jayaramaiah (2002) and Chandrappa *et al*. (2005) also reported higher chlorophyll content in castor. This variation in the chlorophyll content may be due to the factors such as leaf age, nutritional status and a range of environmental and phenological conditions, which influence the relationship between photosynthetic rate and chlorophyll content (Nagaraj *et al*., 2002; Barry *et al*., 2009). The leaf senescence pattern may also affect the chlorophyll content (Gratani and Moriconi, 1989; Gratani and Bombelli, 2001). Demarez *et al*. (1999) reported that chlorophyll concentration in leaves strongly increases at the beginning of the growing season.

Polyphenols are the most commonly found secondary metabolites in plants. All plants differ in their phenolic contents. In this work, significant difference in total phenolic content was recorded among methanolic extract of different host plant leaves. Highest phenolic content was recorded in castor leaf whereas lowest was found in banana. This difference may be due to the environmental factors such as climatic conditions, soil type, biotic and abiotic stress and nutrition which were found to influence the presence as well as the amount of important phytochemicals in plants.

Food quality is very important for the growth, development and reproductive potential which depends mainly on nutritional composition including both the absolute and relative amount of water, proteins, amino acids, carbohydrate , lipids, mineralsetc. (Slanky and Scriber, 1985). Possible

differences in secondary components or nutrient quality of host plants may have impact on the survival, growth, fecundity and developmental time of insects (Berynes and Chapman, 1994). The quantity, rate and quality of food utilized by a larva influences its performances such as growth rate, developmental time, final body weight, dispersal ability and probability of survival (Kerkut and Gilbert, 1985).

All plants differ in their phenolic contents and even different parts of the same plant may differ in their phenolic content. So based on the difference in the amount of total phenolic content and on the difference in the individual phenolic compound of the host plant leaves, variations were observed in the developmental activities of the larvae.

The present work indicated that all nutritional indices varied when *S. litura* fed on the five selected host plants. Efficiency of conversion of food on different host plants was found to differ considerably in *S. litura* larvae (Balasubramanian *et al.,* 1985) and in general by insects (Slansky and Scriber, 1985; Scriber and Slansky, 1991). The present result showed that *S. litura* had similar relative growth rates on castor , colocasia , papaya and sweet potato, but had the lowest relative consumption rate when feeding on banana compared with those for the other four host plants. *S. litura* larvae showed least preference for feeding on banana leaves and had lower relative growth rate, relative consumption rate, and approximate digestibility, but it had an extremely higher efficiency of conversion of ingested food and considerably higher rate of efficiency of conversion of digested food, indicating that the larvae are capable of compensating by more efficiently utilizing their limited banana leaf tissues than other host plants. This finding was supported by Zhu *et al*. (2005). One of the reason for such variation may include the homeostatic adjustment of consumption rates and efficiency parameters such that an insect can approach its "ideal" growth rate even with foods of varying

quality. The digestion rate in insects is affected by the enzyme activities on various feeding materials, including trehalase, invertase, and others. In practice however, it can be quite difficult to ascertain "cause" and "effect" responses with efficiency parameters. Efficiency parameters are very closely related to the physiological characters of the insects. Understanding of these basic principles of nutritional ecology can enhance the information of insect's adaptation to new food resources.

Depending up on the diet, the size of the head capsules, especially at the end of developmental stage, can differ greatly (Mattana and Foerster , 1988; Santos *et al*., 2003). The highest width of the head capsule was recorded for last instar of colocasia fed larvae of *S. litura*. This may be due to the increased moisture content or nutrient contents in the colocasia leaf. The influence of food suitability on the number of instars is reported by Parra (2009). *S. litura* passes through six instar stages on different host plants and there are several examples showing the effects of the quality of food on the number of instars in polyphagous insects (Santos *et al*., 1980).

Variations in the length and weight of the *S. litura* larvae was noticed when fed with selected host plants. The length and weight of the larvae influenced by the diet quality and the environmental factors (Davidowitz *et al*., 2003). Colocasia and castor fed larvae showed highest length and weight during the last instar stage. This difference may be due to high moisture content or the higest nutrient contents in the leaves of colocasia and castor compared to other host plants. With the increasing percentage of moisture content in leaf the absolute consumption and growth rate of larvae also increased (Paul *et al*., 1992). Parpiev (1968) also reported similar work in silkworm. The banana fed larvae showed lesser weight and length compared to the other host plants. This may be due to the lowest feeding rate, less water content and nutritional content of the banana leaves which may affect energy

expenditure, nutritional efficiency and growth of herbivorous insects. This finding was supported by Martin and Van't Hof (1988). The increase of larval weight with consumption of food indicated higher level of protein content in the later stages of development.

Ratte (1985) reported that some insects have direct relationship between weight and temperature. In this study larval size and weight was reported highest during the early summer than monsoon and post monsoon seasons. Higher temperature during the early summer reflected increase in weight of the *S. litura* larvae. Sweeney and Vannote (1981) reported the effect of temperature in the developmental and physiological processes and size and fecundity determination of mayflies. The nutrient contents such as carbohydrate, protein, amino acid and lipids in the selected host plants were also recorded high during the summer seasons. The feeding of nutrient rich host plants during the summer may be one of the reason for the increased length and weight of the *S. litura* larvae at the summer season.

The decline in larval weight and pupal weight of insects by the anti nutritional effect of phenolic compounds such as quercetin (Stevenson *et al.,* 1993; Beninger and Abou-Zaid, 1997) gallicacid , tannic acid (Nomura and Itioka, 2002; Kathuria and Kaushik, 2005 and Mrdakovic*et al.,* 2011) and flavonoids (Hoffman-Campo *et al.,* 2001; Onyilagha*et al.,* 2004) are also reported.

Carbohydrate and amino acids were higher in the castor leaf and lower in the banana leaf. The longer larval duration was observed in banana fed larvae compared to the other selected host plant fed ones. It can be suggested that due to lower amount of nutrients in the banana leaves than the other selected host plant leaves, the banana fed larvae cannot invest sufficient amount of metabolites for its growth which lead to prolonged larval duration (Gogoi and Yadav, 1995).

The *S. litura* larvae showed the shortest larval duration in the summer. This may be due to the high protein and moisture content in leaves and increased food intake by the larvae during summer season. Similarly longer larval duration during post monsoon may be due to low protein and moisture content in the leaves and/or due to the minimal food intake by the larvae during post monsoon. The larval development of *S. litura* varied greatly depending on the host plants and temperature and the development was prolonged under low or high temperatures as reported by Zhu *et al*. (2000); Chen *et al*. (2002) and Seema *et al. (*2004)

The total phenolic content also affect the larval duration. They acts negatively or positively on the larval duration of insects depends upon the individual phenolic compounds. The observations recorded for larval duration in *S. litura* revealed that the larvae fed with the leaves of castor which contain high phenolic content, showed shortened larval period. But larvae fed on banana leaves which have low phenolic content showed extended larval period. Similar variation in the larval duration due to the phenolic compounds such as tannic acid (Kathuria and Kaushik, 2005), gallicacid (Ananthakrishnan, 1997), rutin (Jadhav *et al.,* 2012). and quercetin (Saric *et al.,* 2007) was reported in various insects like *S. litura* , *Helicoverpa armigera,. Choristoneura rosaceana and Culex pipiens pallens.*

Patel *et al*. (1986) reported that pupal development was not affected by host plants on which their larvae fed. However the present results showed that pupae developed faster when the larvae fed with leaves of castor and colocasia than with leaves of papaya, sweet potato and banana ,lthough the difference was less than one day duration on papaya and sweet potato and more than one day duration on banana. Pupal development was faster in the summer season and slower in the post monsoon season. The Bae and Park

(1999) suggested that temperature also plays a vital role on pupal development.

The pupal weight differed significantly depending on the host plant on which the larvae were fed and differed significantly between males and females when they were fed on the same host plant and also when larvae fed on different host plants. These results showed that larval food directly affects pupal size and weight and the female were generally heavier than their male counterparts. This sexual dimorphism of *Spodoptera* species and other lepidopterans was reported by Mattana and Foerster (1988), Bavaresco *et al. (*2004), Santos *et al. (* 2005) and Xue *et al. (*2010). Low pupal weight was reported during post monsoon season. This may be due to the feeding of selected host plant leaves with low quantity of proteins and carbohydrates during the post monsoon season. Similar results were reported by Pandey (1995).

There was no significant difference in the preoviposition and oviposition periods of *S. litura* when fed with selected host plants. But slight variations were observed among the selected host plant fed insects. The adult of castor fed larvae showed shortest pre oviposition and and longest oviposition period but the longest pre-oviposition and shortest oviposition period were noticed in the adult of banana fed larvae. The temperature also affect the preoviposition and oviposition period. In this study the pre-oviposition days of *S. litura* was shortest in the summer and longest in the post monsoon season but oviposition period was longest in summer and shortest in post monsoon. Bae and Park (1999)also reported such changes in oviposition period by females reared on different host plants under different environmental conditions.

Chemical inhibitors present in the host plants also have an important role in the inhibition of oviposition (Chapman, 1974; Stotz *et al.,* 1999). The

variation in the oviposition of insects due to the action of phenolic compounds such as quercetin (Upasani*et al.,* 2003), lactones and flavonoids (Chenniappan and Kadarkarai, 2008), rutin (Haribal and Feeny, 2003), Tannin (Whittaker and Kirk, 2004), flavonoids (Rajkumar and Jebanesan, 2008) and gallic acid (Grant and Langevin, 2002) in different insects were also reported. In the present work the castor showed the highest total phenolic content than the other selected host plants. But the adult of the castor fed larvae showed longest oviposition period than the adults fed with other host plants. This may be due to the variation in concentration of the individual phenolic content in the selected host plants. These findings clearly revealed that the phenolic compounds in different plants have a great influence in the oviposition of *S. litura* when it was fed with different host plant leaves.

The number of eggs oviposited by the *S. litura* females reared on the five selected host plants varied significantly. The nutritional quality of the larval diet influence the egg production (Zucoloto and Fernandes, 1997). Maximum number of eggs were laid by the adults of castor fed larvae and least number of eggs laid by adult of banana fed larvae. This difference may be due to the variation in the nutrient contents of these host plants. The number of eggs laid by the *S. litura* from the larvae fed with selected host plants were generally within the range as reported on various host plants earlier. Bae and Park (1999) reported that the *S. litura* adults oviposited an average 803 eggs per female on artificial diet, 935 on soyabean and 3,467 on cotton (Patel *et al.,* 1986) and 5, 995 eggs per female on different artificial diet (Chu and Yang, 1991). Seasonally, summer was found to be ideal as the fecundity rate of adults of all the larvae fed with selected host plants was found to be high during this season. Opyichalowa *et al. (*1976) reported the influence of climatic condition on the reproductive dynamics and fecundity of females of colarado potato beetle *Leptinotarsa decemlineata*. Bae and Park (1999) also reported that the oviposition by females varied greatly on

different host plants under different seasonal conditions. Thus the present findings are more or less in agreement with the earlier findings and it showed that the diet influenced the oviposition of *S. litura*.

The longevities of both female and male *S. litura* adults were also significantly affected by the host plants on which their larvae fed and also by the seasonal variation. The highest female and male longevities were recorded on castor and the lowest male and female longevities were found on banana. Bae and Park (1999) and Xue *et al*. (2010) also reported similar differences in longevity of *S. litura*. Male adults lived longer than female adults. Similar findings in *S. litura* reared on cotton were reported by Patel *et al*. (1986). The adult longevity was maximum in the early summer season. But in contrast to the present result, Bae and Park (1999) reported that adult longevity became shorter as the temperature increased.

The survival rate of *S. litura* larvae varied on different developmental stages on the five selected host plants. The highest larval survival percentage was observed on castor in all the instar stages, but it was moderate on colocasia, papaya and sweet potato and lowest on banana. In the present work it was found that among the selected host plants castor is the most nutrient rich host plant compared to the other host plants. So the survival rate of the larvae fed on this host plant may be maximum. These observations were corroborated by the findings of Herms and Mattson (1992) and Slansky (1992) who observed that larval survival and development can be reduced on poor quality host plants due to nutritional composition and /or secondary plant metabolites. This findings also supports the result of Patel *et al*. (1987) who found out that larval or pupal survival rate of *S. litura* varied greatly on different host plants, 100percent survival was observed on castor. The survival rate for all the life stages of *S. litura* was maximum in the summer season. Bae and Park (1999) found that pupation rates were positively

correlated with high temperature. Similar results were noticed in the present study that pupal survival ranged from 92.1 to 71 percent on castor, colocasia, papaya, sweetpotato and banana during summer season. But it was reduced during the monsoon and post monsoon seasons.

Some phenolic content reduced the egg hatchability of the insects (Manoukas, 1996; Salunke *et al.,* 2005; Sohal and Sharma, 2011). But in the present study among the five selected host plants the higher concentration of total phenolic content was observed in the castor leaf. But comparing the egg hatchability of *S. litura* larvae fed with different host plants, in castor fed insect the maximum hatchability of eggs was noticed. So here a positive correlation with the total phenolic content of the host plant and egg hatchability was noted. The reason for this difference may be the variation of the individual phenolic compounds of each host plant leaves or the variation in the nutritional content of the different host plant leaves.

The results revealed that the developmental period for all the instars differed significantly in all host plants. The growth rate during the early instars were found to be quite low for all the host plants. It is similar to the work of Poonia (1978) who reported that most of the food consumed during early instars is spent in energy for maintenance. Over all larval development was significantly affected by host plants and was longest on banana followed by sweet potato, papaya, colocasia and shortest on castor. The longest pupal development was observed in banana and shortest pupal development was noticed in castor. Overall adult development was significantly affected by host plants and was longest on banana followed by sweet potato, papaya, colocasia and shortest on castor. Seema *et al*. (2004) reported that difference in pupal survival , longevity and fecundity may also be affected by temperature and other environmental conditions.

In the present studythe detailed information regarding the influence of nutrient quality and secondary metabolites of selected host plants in the biochemical and biological parameters of *S. litura* larvae during different seasons was recorded which may be useful for the selection of more eco friendly control measures such as biofensing and trap crop methods in future.

SUMMARY

Spodoptera litura is an economically important polyphagous pest. It attack numerous crops in wide areas of the world. The knowledge about the biochemical and morphological characters of the *S. litura* fed with selected host plants may be helpful in future in order to find out the eco-friendly control measures of this insect.

The diet of the phytophagous insect like *S. litura* has got a significant influence on its growth and developmental activities. It is the one and only source of proteins, carbohydrates, free amino acids and lipids of *S. litura*. The sixth instar larvae of *S. litura* fed with five selected host plants castor, colocasia, papaya, banana and sweet potato. Detailed study was conducted on food utilization and the variation in morphological characteristics such as, larval length and weight; pupal weight; larval, prepupal and pupal duration; larval, pupal and prepupal survival and fecundity of *S. litura* fed on five selected host plants during summer, monsoon and post monsoon seasons.

(i). *S. litura* passes through six instars when fed with five selected host plant leaves. The head capsule measurement recorded highest for colocasia fed larvae.

(ii). The variation in length and weight of different larval instars occurred with respect to different diet, which showed seasonal variation also. Castor and colocasia fed larvae during the last instar stage exhibited slightly greater length and weight than the larvae fed on other host plants. Summer recorded the highest larval length and weight in all instars than other two seasons.

(iii). The larval duration was found to be shorter in castor fed larvae than that of the other host plant fed ones. Shortest larval developmental

period was recorded during summer than in monsoon and post monsoon seasons.

(iv). Variation was observed in pupal weight of all the larvae fed with selected host plants in the three seasons. The weight of the pupae of colocasia fed larvae was higher than larvae fed on other selected host plants. During summer highest pupal weight was noted than that of monsoon and post monsoon seasons.

(v). Fecundity of castor fed *S. litura* was recorded to be highest than that of larvae fed on other host plants. Higher fecundity was recorded during early summer for all the larvae fed on selected host plants.

(vi). The survival rates also varied depending on the host plants. The survival rate (larval, prepupal and pupal) was significantly higher on castor fed cases than for other host plants fed case. Highest survival rate was noticed during summer for all the larvae fed on selected host plants.

(vii). Total larval, pupal and adult developmental period was recorded, which showed difference among the larvae depending on diet and season. Shortest developmental period for larvae, pupae and adult were recorded in castor fed case followed by colocasia, papaya, sweet potato and banana respectively. The longest developmental period was observed in banana in different seasons.

(viii). The variation in adult longevity was also observed depending on the feeding material. The longevity was higher for males than females.

(ix). The biochemical components of host plant leaves were analyzed during the summer, monsoon and post monsoon seasons. Variation in the biochemical components in selected host plant leaves were recorded

during different seasons. Castor leaves contained high protein content in all the seasons than the other selected host plants. In summer the highest protein content was recorded in all the plant leaves than monsoon and post monsoon season.

(x). Highest amino acid content was noticed in the castor leaf than other host plants. Summer recorded the highest amino acid content than monsoon and post monsoon seasons.

(xi). Carbohydrate content was found to be higher in castor leaves in all the seasons than the other selected host plants. During summer the higher carbohydrate content was recorded in leaves than monsoon and post monsoon seasons.

(xii). Phytochemical analysis of the selected host plant leaves revealed the presence of varied amount of alkaloids, flavonoids, phenolics, terpenoids , glycosides, etc.

(xiii). Phenolic content estimation reported the presence of highest phenolic content in the castor leaves.

(xiv). Chlorophyll content was recorded to be higher in the castor leaves than the other host plants.

Variation in the protein, carbohydrate, amino acid and lipid content in the mid gut, fat body and haemolymph of last instar larvae of *S. litura* during different seasons –summer, monsoon and post monsoon were studied and compared.

(i). Highest concentration of protein was recorded in the midgut tissue, fat body and haemolymph of last instars of castor fed larvae. The highest content of protein was recorded during the summer season in all the selected host plants.

(ii). The midgut, fat body and haemolymph of last instar castor fed larvae demonstrated higher carbohydrate than the larvae fed with other selected host plants. Fluctuation of carbohydrate content was observed during different seasons. During summer highest carbohydrate content was recorded in larvae fed with all the selected host plants.

(iii). The amino acid content of the body tissue showed significant difference between the larvae fed with selected host plants. The amino acid content was found to be high in the castor fed larvae compared to the larvae fed with other host plants. Amino acid content was recorded to be low during post monsoon season.

(iv). Variation was observed in the lipid content of different tissues of *S. litura* fed on five selected host plants. Castor fed larvae exhibited high lipid content in all the seasons than the larvae fed with other host plants. In summer season high lipid content was noticed in the larvae fed with all the selected host plants.

The variation in the food utilization of *S. litura* was also noticed when reared on selected host plants. Food consumption and utilization of castor, colocasia, papaya, banana and sweet potato by final instar larvae of *S. litura* as evidenced by higher values of RCR, RGR, ECI and ECD, on castor largely due to its relatively higher feeding and highest efficiency of conversion of digested food into biomass. But the RCR, RGR and AD values were least in the banana but it shows higher ECI and ECD.

In conclusion, based on oviposition preference, larval growth , development and survival, pupal weight, duration and emergence and fecundity of adults of *S. litura*, the preference and nutritional values of the five host plants were ranked as castor> colocasia > papaya > sweet potato >

banana. Hence, the present study has shown the suitability of selected host plants for the development, longevity and survival of *S. litura.*

An extensive study of various developmental aspects of *S. litura* and its food plants are very essential to improve the existing control measures in an eco-friendly manner. The biochemical constituents of the host plants, present in different concentration play a decisive role in food selection by *S. litura*. The present study which deals with morphological and developmental characters of *S. litura* providing selected food material indifferent season and the variation in biochemical parameters influencing the growth, development and food utilization of *S. litura* may provide important information for development of innovative control measures in future.

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