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Studies on the Feeding and Nutritional Influence on the Growth and Reproduction of Monarch Butterfly, *Danaus Chryssipus* (Insecta: Lepidoptera)

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Abstract

An investigation is carried out to assess the feeding biology, dietary utilization and reproduction of Lepidoptera in general, interaction of host plant biochemical and nutrients with the defensive mechanism of the Lepidoptera and host plant selection of Monarch butterfly, Danaus chryssipus. Danaid butterflies usually feeds on the plant juice from Calotropis gigantea. The attraction of danaid butterflies on the Calotropis gigantea is mainly due to the presence of PA s on the host plants. PA s of Danaid host plants is involved in male reproduction as well as pheromone production of insects. Danaus chrysippus reared on young leaves and inflorescence had shorter larval duration, improved longevity and higher fecundity. This may be due to the higher water content and glycoside concentration.

Keywords: Butterfly, *Danaus chryssipus, Calotropis gigantea.,* Pyrrolizidine alkaloids (PA).

Introduction

Invertebrates, which form an overwhelming majority of life on earth in terms of individuals, species and biomass, are important components in the functioning of natural ecosystems. Approximately 1.4 million species of invertebrates have been described so far, of which 75,000 species are insects[1]. Butterflies are perhaps the most conspicuous and colourful of insects. Due to their attractiveness and omnipresence, they have acquired a niche in the prose and poetry of various cultures. Butterflies are an important group of ecological indicators[2], which are often considered as flagship taxa in many ecosystems. They could be an important component in both species-based and system-based indicator groups. Butterfly diversity in the forest areas is reported to have significant relations with the forest area and isolation status[3].

India has a rich butterfly fauna comprising of 1,501 species out of 16,823 species recorded from all over the world. Of the various butterfly habitats found in India, the Western Ghats is one of the most diversified areas containing a wide variety of species (330 Species) due to the typical eco-climate and geographical features.

The butterflies of the family Nymphalidae are popularly known as the Brushfooted butterflies"[4]. It is a diverse family and now includes many subfamilies such as Acraeinae, Amanthusiinae, Danainae and Satyrinae, which were earlier treated as distinct families. The subfamily Danainae comprises of six species of tigers in peninsular India, three crows and one tree nymph and it is amongst the most studied groups of butterflies.

These butterflies have been and are being studied for their habits of long distance migrations and association with plants that are rich in unpalatable alkaloids. They usually feed on plants of the families Apocynaceae, Asclepiadaceae and Moraceae. A common characteristic feature of major host plant families is that they accumulate polyphenolics as quantitative defense compounds[5]. On the other hand, most lycaenids avoid plant families characterized by the accumulation of toxic, quantitative defense system. Such toxic or deterrent plant families eg. Aristolochiaceae, Brassicaceae, Apocynaceae, Asclepiadaceae are widely used by Papilionidae, Pieridae or Nymphalidae butterflies[6].

In plants from which butterflies and moths often sequester many of these substances, there are three principal building blocks for these compounds: acetate, which via the mevalonate pathway leads to mono-,sesqui-,and diterpenes, iridoid glycosides and cardenolides; aminoacids leading to cyanogenic glycosides, glucosinolates; pyrrolizidine alkaloids and glycosidase inhibitors and shikimic acid, the precursor of many aromatic compounds such as furanocumarine, aristolochic acid and β-carboline alkaloids (via aromatic aminoacids)[7]. These substances take part in the chemical defenses in Lepidoptera.

The aspects discussed in this investigation are studies on the feeding biology, larval and nymph duration,pupal duration, fecundity and adult longevity of *D. chrysippus* on different parts of *Calotrophis gigantea* (young, mature, senescent leaves and inflorescence), influence of different aged leaves of *C.gigantea* on food utilization measures of *D. chrysippus* with special reference to host plant water, nitrogen, protein and amino acids.

Material and Methods

Laboratory Culture of *Danaus chryssipus:* Freshly hatched larvae of the butterfly, *Daniaus chryssipus* were collected from the host plant, *Calotropis gigantea.* They were reared in plastic containers measuring 35 x 40 cm, covered with muslin cloth. They were offered adlibitum of their host leaves. The larvae were maintained in the containers until they undergo pupation. The adults emerged were released in separate cages and 10% sucrose solution was provided as food.

Rate of development on different leaves: The first instar larvae of *Danaus chryssipus* were segregated in groups and each group was fed with young, mature and senescent leaves of *Calotropis gigantea.* The comparative rate of development of *Daniaus chryssipus* on different ages of host leaves were studied based on the total body weight and the duration of post-embryonic development. For assessing the duration of larval development, the eggs were separated and observations were made on their incubation period and duration of each larval stage when fed on their different ages of host leaves.

Fecundity Studies: Freshly emerged adult male and female pairs, which were formerly reared on the respective host plant parts, were confined in the mating cages and host plant was provided for oviposition. Fecundity was assessed on the basis of the oviposition period and the number of eggs laid during the lifetime was noted. The mean longevity of male and female, which were reared on different host plants, were also noted. Reproduction per day was calculated by dividing the fecundity by the reproductive period.

$$\text{Reproduction per day} = \frac{\text{Fecundity}}{\text{Reproductive period}}$$

Feeding preferences: To assess the host preferences of the larvae of *Daniaus chryssipus*, the fourth instar larvae were allowed to select among three age classes of *Calotropis gigantea* ; young leaves (6 day old), fully expanded mature leaves (15 day old) and senescent leaves (28 day old). Three leaves of different ages were placed alternatively in a petridish (10 cm diameter), which was lined with moist filter paper. Five fourth instar caterpillars were placed in the centre. This experiment was repeated 10 times. The petridishes were kept for 3-6 h until half the leaf pieces in the dish had been fed upon. The leaf age class that was eaten most was visually determined and recorded.

Life history parameters: Life tables were constructed for Danaid butterflies on the host plant and on suspected alternative hosts. Host plant was systematically searched every day. Freshly hatched larvae of Danaid butterflies were collected and placed on the same host plant species and on suspected alternative hosts in the laboratory. Duration of each moulting was recorded and changes occurring at the time of larvae, pupal and adult period was noted frequently.

Quantitative Food Utilization: The gravimetric method was used for assessing the quantity of food utilized. All weights were taken using a monopan balance accurate to 0.1 mg. After the initial weights of the larvae were determined they were introduced into individual containers and allowed to feed on weighed quantities of their respective host plants for a period of 24 hrs. At the end of the experiment, the remaining food, excreta and the insects were weighed. The sample larvae were weighed oven dried (48 h at 60^0C) and reweighed to estimate the dry weight of the experimental larvae. The host plant remaining at the end of each day was oven dried and weighed. Quality of food ingested was estimated by substracting the diet (dry weight) remaining at the end of each experiment from the toal dry weight of diet provided. Faecal pellets were collected daily and weighed, than oven dried and reweighed to estimate the dry weight of excreta.

Various food utilization indices (all based on dry weight) were calculated to assess the growth and feeding efficiencies of *Daniaus chryssipus* in the traditional manner[8].

Absolute growth rate (AGR) = P / T, Absolute consumption rate (CR) = E / T, Relative growth rate (RGR) = P / TA, Consumption index (CI) = E / TA, Appropriate digestibility (AD%) = 100 (E-F) / E, Efficiency of conversion of ingested food (ECI) % = 100 P / E, Efficiency of conversion of digested food (ECD) % = 100 P / (E - F).

where, A = Mean dry weight of animal during T, E = Dry weight of food eaten, F = Dry weight of faeces produced, P = Dry weight gain of insect, T = Duration of experimental period

Efficiency of faecal pellet egestion of larvae fed on three different leaf stages of *Calotropis gigantean:* A group of four larvae were reared in two separate containers each of two fed with *Calotropis gigantea*. The time taken by the larvae to egest out the first fecal pellet was noted in respect to the *Calotropis gigantea*. The total amount of fecal pellets egested by each groups of larvae reared on *Calotropis gigantea* was noted.

Biochemical analysis of host leaves: The suitability of different host plant parts of the *Calotropis gigantea* from its nutritional point of view were assessed by subjecting the *Calotropis gigantea* leaves to various biochemical analysis, such as total protein, total lipids, total carbohydrate, total amino acids, nitrogen and water content.

Estimation of total soluble protein: A set of standard solutions of bovine serum albumin (BSA) containing 25 µg, 50 µg and 75 µg of standard solution were taken in a series of test tubes. The volume in each tube was made up to 1 ml with distilled water. 5 ml of alkaline copper reagent was added,mixed and allowed to stand for 10 min at room temperature. 0.5 ml folin – ciocalteau phenol reagent was then added to each tube and shaken well. The blue colour developed was read at 720nm after 20minutes, against a reagent blank in spectrophotometer[9]. The standard

graph was drawn by plotting the concentration of the standard solution on the ordinate and the optical density on the abaciss. For the estimation of tissue proteins, 0.01ml of extract were taken and it was made upto a first volume of 1ml with distilled water. The same procedure was followed as described for the standard solution on the X-axis and the optical density on the Y-axis.

Estimation of total carbohydrates: To 0.1 ml of the leaf sample in a clean glass test tube 0.05 ml of 80% phenol was added. Then 5ml of concentrated sulphuric acid was added rapidly and the stream of acid being directed against the sample surface to obtain good mixing. The tubes were allowed to stand for 10 minutes, shaken and then placed for 20 minutes. Optical densities of samples were taken at 790mm. Glucose (AR) served as a standard.

Estimation of lipids: The lipids were extracted with chloroform: Methanol mixture (2:1 v/v) and the proteolipid bonds were broken by extraction with 4% water in chloroform: methanol mixture (2:1 v/v) 0.05N potassium chloride was used to dissolve glycolipids and finally the total lipids were extracted with chloroform solution. Tissues were homogenized in chloroform methanol (2:1 v/v) mixture in a Porter Elvehjen homogenizer and centrifuged. The process was repeated thrice, supernatants pooled and then concentrated in vacuum at 45^0C in a rotary evaporator. The proteolipid bonds were broken resuspending the residue thrice in 20-30ml of chloroform: methanol (2:1 v/v) mixture containing 4% water and evaporating it under reduced pressure. Finally, the residue was quantitatively taken in 100ml of chloroform: methanol (2:1 v/v) in the separatory funnel, 0.05M potassium chloride solution (20ml) was added to it and the funnel swirled gently for a few minutes and allowed to stand for 4-6 hrs. The lower organic phase was then collected and evaporated at 40-50^0C in vacuum. The final residues of the total lipids were taken up quantitatively in 10ml of chloroform, in a glass stoppered tube, sealed on the top with adhesive plaster and kept in a deep freezer at 15^0C. Suitable aliquot of total extract (0.5 ml) was taken in a pre-weighed planchet in the oven at 60^0C over night. This was repeated over 2-3 days interval till a constant weight was obtained. The difference in the weight between the planchet plus lipid and the empty planchet gives the total lipid content. The total lipid concentrations were expressed mg/g wet tissue.

Estimation of nitrogen: 0.2 gm of the samples were taken in a dry kjeldhel flask, 5 gms of potassium sulphate 0.25 gm of copper sulphate and 25 ml of concentrated sulphuric acid were added into the flask in such a way as to wash down any solid adhering in the neck. Then the flask was heated in heating mantle. When flaming has ceased the temperature was increased until the mixture boils gently. This was continued for 30 minutes after the solution has become clear. Then the contents were transferred completely to the ammonia distillation apparatus and 50% sodium hydroxide was added in excess. The amount of ammonia produced was cooled over 2% solution of boric acid to which 2 drops of mixed indicator (0.2%) alcoholic solution of methylene red and methylene red and methylene blue in the ratio of 2:1 respectively was added. The solution became pink in colour and distillation was continued until about 20-25 ml of distillation was collected. As the distillate was being collected over boric acid, pink colour gradually, disappeared and this distillate was then titrated - against O/N hydrochloric acid.

Estimation of total amino acid: 0.1 ml of sample was made to 1 ml with 4 N sodium citrate buffer of pH 5.0 and 1 ml of ninhydrin was added. Tubes were placed in a boiling water bath for 15 minutes. It is taken cooled, diluted with 3 ml of water. Optical density was taken at 570 nm. DL-alanine served as a standard.

Estimation of moisture content: For estimating moisture content of leaves 2 gms of fresh plant material was weighed and kept in an incubator at 37^0C till a constant weight was obtained. The difference between initial and final weight was calculated and water content was represented in percentage.

Statistical Analysis: All the data were statistically analysed and expressed at standard error of mean. Data were subjected to analysis of variance and the means were repeated using Duncan's Multiple Range Test.

Results and Discussion

Insects searching for an acceptable host plant must first locate and identify an appropriate plant species[10]. Accurate selection of host taxa and proper assessment of individual plant quality should also be achieved with minimum time under most ecological conditions. A subject of these compounds seems to be of great importance for identification of the host. Feeding is an active, dynamic process with numerous feedback interactions with considerable effects on growth, reproduction and dispersal[11]. The amount, rate and quality of food consumed by adult insects influence that fecundity, movement and survival, while in the case of larval insects, they influence the growth rate, development time, final body weight and survival[12]. Insect plant relationship therefore has been a very intrinsic one governed by diverse factors involving the plants and insects concerned, but also the micro as well as the macro environment. The establishment of insects on plants is governed by a number of factors involving responses of insects to plants as well as plant characters eliciting these responses[13]. The ability of insects of discriminate the nature of food is largely governed by the physical and chemical characteristics of the food. Hence the present study particularly deals on the influence of host plants on feeding and life history parameters of *D.chrysippus.*

Life cycle of *D.chrysippus* reared on *C.gigantea*, shown in table 1. Total larval duration was shorter in larvae reared on young leaves (10.53 days), and considerably increased inflorescent (11.40 days), mature leaves (9.91 days) and on senescent leaves

(12.25 days). Pre-pupal and pupal duration were also considerably shorter in insects reared on young leaves (2.10,9.5 days) and inflorescence (2.65,10.7 days) than on senescent leaves fed insects (3.20,11.04 days). Pre-oviposition period was longer in larvae reared on senescent leaves (4.05h). Oviposition and post oviposition periods were increased when fed on young leaves (24.05,5.12h) of *C.gigantea*. Fecundity was maximum on young leaves reared insect groups (15.7 eggs) and on senescent leaves it was 4.9 eggs only.

Longevity of male and female was considerably increased on young leaves reared insects than those on other groups. Longevity of male was 9.50 days in insects reared on young leaves but in insects fed on mature leaves it was 5.34 days, and on senescent leaves it was 3.30 days only. Female longevity was also higher in young leaves reared groups than the other leaves reared insects. Based on larval duration,fecundity and longevity, the host parts could be arranged in order of their suitability as, young leaves > inflorescence > mature leaves and senescent leaves.

Food utilization efficiency measures *D.chrysippus* (on different parts of *C.gigantea*). Table 2 provides the food utilization efficiency, CI measures of I instar larvae of *D.chrysippus* on young, mature and senescent leaves were 0.9561g, 0.7526g, and 0.6223g, respectively. Similarly, the RGR on young, mature and senescent leaves as well as inflorescence were 0.2569g, 0.1793g, 0.1125g and 0.1672g, respectively. The nutritional efficiency measures (ECI and ECD) were also decreased on mature (25.00% and 29.63%) and senescent leaves (17.20% and 21.05%) than on young leaves (40.89% and 49.24%).

Table 3 gives the food utilization efficiency measures of II instar of *D.chrysippus* fed on different parts of *C.gigantea*. The RGR was considerably reduced on senescent leaves (0.2218g) and on young leaves it was 0.3225g. The AD of II instar on young leaves 80.15% whereas, larvae fed on inflorescence was 68.25% only. Nutritional efficiency measures were considerably decreased on senescent leaves fed groups when compared to other leaves fed groups.

Table 4 gives the food utilization efficiency measures of III instar of *D.chrysippus* fed on different parts of *C.gigantea*. CI of III instar fed on young leaves was 0.7694g and mature leaves reared insects it was 0.5411g. The RGR was minimum on senescent leaves (0.2693g). Food utilization of IV instar of *D.chrysippus* is depicted in table 5. CI of IV instar on young leaves was 0.5327g; whereas larvae reared on senescent leaves was 0.3010g. Efficiency of conversion of ingested food was 58.5% on young leaves reared larvae and 24.03% on senescent leaves fed groups.

Food utilization values of V instar are presented in table 6. Relatively higher CI was recorded (0.4173) on young leaves and it was (0.1191g) on senescent leaves fed larvae. The RGR, AD, ECI and ECD profiles were diminished in senescent leaves fed V instar of *D.chrysippus* larvae than those when fed on other host parts.

Table 7 and 8 provides the various biochemical nutrient components such as total proteins, total carbohydrates, total lipids, total nitrogen, total amino acids, moisture content of *Calotropis gigantea*. Mature leaves have higher concentration of protein, carbohydrates, lipid and nitrogen when compared to young leaves and senescent leaves. Water content was more in young leaves of *C.gigentea* (81) than the senescent leaves (29).

Table-1
Biological parameters of *Danaus chrysippus* fed on C*alotropis gigantea*

Biological parameters (days)	Host Plant Parts			
	Inflorescence	**Young leaves**	**Mature leaves**	**Senescent leaves**
Total larval duration	11.40[a]	10.53[a]	9.91[d]	10.25[c]
Pre-pupal duration (days)	2.65[c]	2.10[d]	2.90[b]	3.20[a]
Pupal duration (days)	10.7[b]	9.5[d]	10.4[c]	11.4[a]
Pre-oviposition eriod(hrs)	3.65[b]	3.10[d]	3.40[c]	4.50[a]
Oviposition period hrs)	18.25[b]	24.05[a]	14.72[c]	7.19[d]
Post-oviposition period (hrs)	4.10[c]	5.12[a]	4.35[b]	3.72[d]
Longevity Male longevity(days)	7.35[b]	9.56[a]	5.34[c]	3.25[d]
Female longevity(days)	8.72[b]	10.48[a]	4.75[c]	3.30[d]
Fecundity (No of eggs)	14.3[a]	15.7[b]	8.6[c]	4.9[d]

Within a column means followed by a same letter is not significantly different 5% level of DMRT.

Table-2
Food utilization efficiency measures of first instar larva of *Danaus chrysippus* fed on *Calotropis gigantea*

Host Plant parts	CI (g)	RGR (g)	AD (%)	ECI (%)	ECD (%)
Young leaves	0.9561 [d]	0.2569 [e]	75.05 [a]	40.89 [c]	49.24 [b]
Mature Leaves	0.7526 [a]	0.1672 [e]	70.02 [a]	25.00 [c]	29.63 [b]
Senescent leaves	0.6223 [d]	0.1125 [e]	62.15 [a]	17.20 [c]	21.05 [b]
Inflorescence	0.8327 [d]	0.7793 [e]	69.11 [a]	29.32 [c]	34.03 [b]

Within a column means followed by a same letter is not significantly different 5% level of DMRT. CI - Consumption index, RGR- Relative growth rate, AD- Appropriate digestibility, ECI- Efficiency of conversion of ingested food and ECD - Efficiency of conversion of digested food.

Table-3
Food utilization efficiency measures of second instar larva of *Danaus chrysippus* fed on *Calotropis gigantea*

Host Plant parts	CI (g)	RGR (g)	AD (%)	ECI (%)	ECD (%)
Young leaves	0.9905 [d]	0.3225 [e]	80.51 [a]	39.75 [b]	43.12 [b]
Mature Leaves	0.7664 [d]	0.7286 [d]	58.09 [a]	36.07 [c]	37.33 [b]
Senescent leaves	0.5509 [e]	0.2218 [f]	55.27 [a]	26.27 [c]	37.55 [b]
Inflorescence	0.7430 [d]	0.3209 [e]	68.25 [a]	35.32 [c]	39.15 [c]

Within a column means followed by a same letter is not significantly different 5% level of DMRT. CI - Consumption index, RGR- Relative growth rate, AD- Appropriate digestibility, ECI- Efficiency of conversion of ingested food and ECD - Efficiency of conversion of digested food.

Table-4
Food utilization efficiency measures of third instar larva of Danaus chrysippus fed on Calotropis gigantea.

Host Plant parts	CI (g)	RGR (g)	AD (%)	ECI (%)	ECD (%)
Young leaves	0.7694 [d]	0.3952 [e]	76.35 [a]	41.22 [c]	48.61 [b]
Mature Leaves	0.5411 [d]	0.3225 [e]	55.27 [a]	26.27 [c]	45.42 [b]
Senescent leaves	0.505 [d]	0.2693 [e]	51.01 [a]	21.32 [c]	32.55 [b]
Inflorescence	0.6530 [d]	0.3502 [e]	57.01 [a]	32.15 [c]	45.55 [b]

Within a column means followed by a same letter is not significantly different 5% level of DMRT. CI - Consumption index, RGR- Relative growth rate, AD- Appropriate digestibility, ECI- Efficiency of conversion of ingested food and ECD - Efficiency of conversion of digested food.

Table-5
Food utilization efficiency measures of fourth instar larva of *Danaus chrysippus* fed on *Calotropis gigantea*

Host Plant parts	CI (g)	RGR (g)	AD (%)	ECI (%)	ECD (%)
Young leaves	0.5327 [d]	0.4360 [d]	62.94 [a]	58.56 [a]	59.01 [a]
Mature Leaves	0.5001 [d]	0.4915 [e]	45.32 [c]	27.09 [c]	46.31 [c]
Senescent leaves	0.3010 [e]	0.3520 [d]	43.61 [b]	24.03 [c]	39.33 [b]
Inflorescence	0.3210 [e]	0.4932 [e]	49.92 [b]	25.01 [c]	54.64 [a]

Within a column means followed by a same letter is not significantly different 5% level of DMRT. CI - Consumption index, RGR- Relative growth rate, AD- Appropriate digestibility, ECI- Efficiency of conversion of ingested food and ECD - Efficiency of conversion of digested food.

Table-6
Food utilization efficiency measures of fifth instar larva of Danaus chrysippus fed on Calotropis gigantea

Host Plant parts	CI (g)	RGR (g)	AD (%)	ECI (%)	ECD (%)
Young leaves	0.4173 [e]	0.5572 [d]	58.06 [a]	41.29 [b]	60.05 [a]
Mature Leaves	0.2011 [e]	0.4935 [d]	40.36 [c]	21.06 [c]	51.72 [a]
Senescent leaves	0.1191 [e]	0.4791 [d]	37.06 [b]	19.37 [c]	42.06 [b]
Inflorescence	0.4055 [e]	0.5432 [d]	41.21 [b]	24.72 [c]	59.72 [a]

Within a column means followed by a same letter is not significantly different 5% level of DMRT. CI - Consumption index, RGR- Relative growth rate, AD- Appropriate digestibility, ECI- Efficiency of conversion of ingested food and ECD - Efficiency of conversion of digested food.

Table-7
Biochemical components of *Calotropis gigantea*

Biochemical parameters	Young Leaves	Mature Leaves	Senescent leaves	Inflorescence
Protein (mg/g)	111.06[c]	125.27[a]	101.32[d]	122.13[b]
Carbohydrates (mg/g)	125.42[a]	105.61[c]	98.07[d]	117.54[b]
Lipids (mg/g)	28.10[c]	38.25a	21.14[d]	33.22[b]
Total aminoacids (mg/g)	0.350[d]	0.632[b]	0.830[a]	0.545[c]
Phenolics (mg/g)	7.34[d]	13.42[b]	29.32[a]	8.32[c]
Nitrogen (%)	1.34[c]	2.38[a]	1.03[d]	2.61[b]
Water Content (%)	81.00[a]	73.00[c]	39.00[d]	76.00[b]
Phosphorous (%)	0.29[c]	0.38[a]	0.24[c]	0.32[b]
Potassium (%)	1.25[d]	1.36[b]	1.28[c]	1.42[a]
Cardiac glycosides (ug/100mg dry weight)	123.74[d]	270.56[b]	302.81[a]	208.39[c]
Fibre (%)	5.05[c]	9.81[b]	17.93[a]	3.02[d]

Within a column means followed by a same letter is not significantly different 5% level of DMRT.

In the present study, mature milkweed leaves possessed comparatively higher amounts of nitrogen than young and senescent leaves. However, *Danaus chryssipus* feed on young leaves where food intake and RGR were higher. *Danaus chryssipus* fed on young leaves had higher growth and consumption rates and increased efficiencies. Higher water content of food leads to volumetric constraints in moth[14]. Relatively low water content food can lead to increased growth, survivorship and fecundity. A lower level of body hydration increases the efficiency with which water is used for growth, because it permits the hydration of more new tissue with a given amount of water[15].

Dietary nitrogen strongly affects growth, consumption and food utilization of insects[16]. The significant reduction in food consumption and weight gain by *Danaus chryssipus* larva may be due to the higher amount of nitrogen, cardiac glycosides and lower water content in the mature leaves. Higher water /nitrogen ratio and lower level of cardiac glycosides (limiting nutrient) in the young leaves were found to increase the suitability of leaves as food for *Danaus chryssipus*and also in their digestibility[17].

Milkweed has been extensively analyzed chemically, ever since milkweeds were determined to be a possible source of rubber and oil[18]. It contains various compounds including the isoprene derivatives α and β- amyrin and their acetates[19]. Interspecies difference in concentrations and kinds of cardenolides occur[20]. CG levels changed seasonally and also differs in interleaves of the same plant. In this study also the cardenolide content varied in different aged leaves and inflorescence of *Calotropis gigantea.*

Since the mature leaves of *C.gigantea* contain more nutrients and moderate amount of allelochemicals content than the young and senescent leaves, the larva of *Danaus chryssipus* prefer the mature leaves[21]. By consuming the mature leaves, the larva can chemically modify the allelochemicals to other active forms. Chemical modification of absorbed allelochemicals to other active forms and the use of nutrients to synthesize indigenous allelochemicals are common especially among chemically defended species[22]. Being a chemically defined species, *Danaus chryssipus* confirms the above said fact by particularly feeding on the preferred mature leaves of *C.gigantea.* Because ingested plant materials is the most common source of nutrients and allelochemicals for herbivore nutritional ecology we must first be aware of the patterns of allelochemicals and nutrient occurrence in plants, and of the feeding behaviour[23]. The host plant *C.gigantea* as a fast-growing species generally associated with resource-rich and early success ional sites often contain a variety of more chemically complex compounds like alkaloids and glucosinuslates[24].

Insects feeding on protein rich host plants will be more successful than those that consume plant material that is less protein enriched for the majority of insects studied nutritionally. Carbohydrates is found or required at least for optimal growth[25]. Early in star larvae of *Danaus chryssipus* mainly prefer the inflorescence and young leaves of *C.gigantea* and avoid mature and senescent leaves. This may be due to the amount of lipid, carbohydrates, protein, nitrogen and water content of the host plant parts. In *C.gigantea*, cardiac glycoside content was higher in senescent leaves and lower in young leaves.

Conclusion

This study has shown a positive relationship between oviposition preference and larval developmental performance of *Danaus chryssipus* The host plant oviposition preference hierarchy of *Danaus chryssipus* corresponds to nutritive value of the potential host plant selected for oviposition. The data presented reveals the availability of extensive information on the feeding biology, dietary utilization and reproduction of lepidoptera, interaction of host plant biochemical and nutrients with the defensive mechanism of the Lepidoptera.

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