Behaviour of some selected butterflies and its influence on nutritional and medicinal values of host plants

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Dedicated
to
My beloved brother
Mr. Md. Khorshed Alam

Statement of Authorship

I, **Md. Maksudul Alam**, hereby declare that this thesis is my own work and that all sources quoted, paraphrased or otherwise referred to have been properly acknowledged in the references at the end of each respective chapter. To the best of my knowledge, this thesis neither contains material previously published or written by another person, nor material which to a substantial extent has been accepted for the award of any other degree or diploma of the university or other institutes of higher learning, except where due to acknowledgement it has been made clear in the text.

This thesis has been categorized into eight chapters in total and themes of each chapter is stated in brief:

Chapter 1 dealt with general introduction which consists of research concept and objectives of the research work. Research concept was made on the basis of butterfly diversity, behavioural aspects, abundance, distribution, biology, ecology of butterfly and butterfly related nectar, host and supportive plants. The objectives of research work mainly focused on behavioural activities of butterfly in relation to abundance, developmental stages of butterfly, feeding potentiality of larval host-plants and adult nectar plants, medicinal importance of butterfly related host plants.

Chapter 2 dealt with review of literature which was made on the basis of four sub-topics, viz. behavioural strategies of butterfly; biology of *Danaus chrysippus* butterfly; biotic interaction between *Pachliopta aristolochiae* butterfly and its host plant; antioxidant, toxicity and antimicrobial activity of butterfly related host plant. Review of literature was studied and followed from 2018 to 1983 regarding the contents of this thesis.

Chapter 3 described behavioural activities of butterfly through its colonization in the Butterfly Research Park at Bhawal National Park, Gazipur. This chapter was sub-divided into development of butterfly related host and nectar plants for butterfly colonization; the visits of butterfly on nectar plants; behavioural strategies; population fluctuation; effect of abiotic and biotic factors; taxonomic position of butterfly; butterfly species richness, abundance and diversity regarding the butterfly colonization for biodiversity conservation and bio-resource management.

Chapter 4 stated biology of the plain tiger butterfly, *Danaus chrysippus* L. (Lepidoptera: Danaidae) in relation to larval host-plants and adult nectar plants. This chapter was sub-divided into development of the host plants, *Asclepias curassavica;* ovipositional behavior of *D. chrysippus* butterfly; morphological changes of larvae; duration and length variation of larvae; morphological changes of pupa; morphological features of *D. chrysippus* butterfly; enhancement of plant population sustenance; morpho-phenology of the plant; co-evolution between butterfly and plants regarding the determination of butterfly plant host specificity.

Chapter 5 investigated biotic interaction between the *Pachliopta aristolochiae* F. (Lepidoptera: Papilionidae) and its host plant, *Aristolochia indica* (Aristolochiaceae). This chapter was sub-divided into development of the host plant, *A. indica*; egg-laying strategy of *P. aristolochiae,* host-plant utilization strategy of larvae; variation in length, feeding potential and faeces of larvae; pupation strategy*;* morphological features; behavioural status of *P. aristolochiae* butterfly regarding the synchronization of coincidences between the life stages of *P. aristolochiae* butterfly and the different phenological stages of the host plant *A. indica* for assessing the nutritional values of butterfly related host plants.

Chapter 6 analyzed antioxidant, toxicity and antimicrobial activity of the leaves extract of *Aristolochia tagala* (the host plant of *P. aristolochiae* butterfly). This chapter was sub-divided into evaluation of free radical scavenging activity (antioxidant); brine shrimp lethality bioassay (toxicity); antimicrobial screening of the host plant, *A. tagala* for determining the medicinal values of butterfly related host plants in nature in view to biodiversity conservation and bio-resource management.

Chapter 7 dealt with summary and conclusion of the research work which envisaged on the findings of Ph. D research and its significance. This chapter mainly focused on the importance of ethological aspects of butterfly for butterfly colonization; biology of *D. chrysippus* butterfly in relation to larval host plant and adult nectar plants; biotic interaction of *P. aristolochiae butterfly* in relation to host plants; antioxidant, toxicity and antimicrobial activity of the host plant, *A. tagala.*

Chapter 8 dealt with literature cited which was followed alphabetically as per style of Bangladesh Journal of Zoology. Authors name were written in capital letters. Literature cited was emphasized mainly on behavioural activities of butterfly; biology and ecology of butterfly; butterfly diversity, species richness, abundance and distribution; utilization of larval-host plant and adult nectar plants; ecosystem management and biodiversity conservation; antioxidant, toxicity, antimicrobial activity and chemical constituents isolation of medicinal plants. All literature cited were included in the text of this thesis. Finally appendices were included to justify the findings of the research work.

Among eight chapters of the Ph. D. thesis, Chapters 3, 4, 5 and 6 were written as independent manuscripts intended for publication, and in all cases, the manuscripts were written by M. M. Alam. For all the chapters, Professor Dr. M. A. Bashar supported the fieldwork and provided advice throughout the project, contributing to the editing of all the thesis chapters. Professor H. R. Khan and Professor Dr. R. M. Shahajahan guided and contributed to the editing of all the manuscripts especially Chapter 3, 4 and 5. Dr. Md. Abdur Rashid contributed to the editing of Chapter 6, hosting me in the Pharmaceutical Chemistry Laboratory, Faculty of Pharmacy, University of Dhaka.

9th January 2019

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Certificate

This is to certify that the Ph. D. thesis entitled **"Behaviour of some selected butterflies and its influence on nutritional and medicinal values of host plants"** which is the record of authentic research carried out in the Environmental Biology and Biodiversity Laboratory (**EBBL**), Department of Zoology and Phytochemical Laboratory, Department of Pharmaceutical Chemistry, University of Dhaka submitted under the supervision of Prof. Dr. M. A. Bashar, Professor Humayun Reza Khan, and Prof. Dr. Md. Abdur Rashid. All the figures, plates and data presented in this thesis are based on his own observations and no portion thereof has been used in any other thesis for a degree. He has fulfilled all the requirements of the regulations relating to the nature and prescribed period of research for submission of thesis for the award of Doctor of Philosophy degree.

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Abstract

An attempt was made to investigate entitled "**Behaviour of some selected butterflies and its influence on nutritional and medicinal values of host plants**" in both field and laboratory conditions. To establish Butterfly Research Park through butterfly colonization, the developmental process and survival rate of butterfly related host plants and nectar plants were examined. The dynamism of butterfly population in relation to nectar plants and behavioural patterns of butterfly, viz. foraging, mating, egg laying, resting, searching and puddling in relation to population dynamics were observed. The effect of abiotic and biotic factors on the butterfly abundance, species richness, species diversity in the forest ecosystems were also studied and recorded.

To study the biology of *Danaus chrysippus* butterfly in relation to larval host plants and adult nectar plants, ovipositional behavior, length variation, feeding potential and weight of excreta of different larval stages of *D. chrysippus* were observed in both field and laboratory experiments. The utilization of nectar plants by visits of *D. chrysippus* as nutritional requirements for their development were also recorded during the experimentation.

To study biotic interaction between *Pachliopta aristolochiae* butterfly and its related host plants, successive development of the host plants, *Aristolochia indica and A. tagala,* egg laying strategy, host plant utilization by larvae as nutritional sources and pupation strategy were examined in the field. Length variation, feeding potentiality of larvae and weight of larval excreta were analyzed in laboratory experiment. Different behavioural strategies of *P. aristolochiae* butterfly, viz. foraging, mating, egg laying, resting, searching and puddling in relation to population fluctuation were also studied and recorded in the experimental field.

To detect the medicinal values of butterfly related host plants through butterfly behavioural activities, different partitionates of leaves extract of the host plant, *Aristolochia tagala* i.e. Tert-butyl-1-hydroxytoluene (BHT), Ascorbic acid (AA), Methanolic extract (ME), Petroleum ether (PE), Carbon tetrachloride soluble (CTE), Chloroform soluble (CHE) and Aqueous soluble partitionate (AQE) were analyzed for free radical scavenging activity (antioxidant), brine shrimp lethal bioassay (toxicity) and antimicrobial activity. Here, *tert*-butyl-1-hydroxytoluene (BHT) and ascorbic acid (ASA) were used as reference standard.

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Abbreviations

BRP-Butterfly Research Park

EBBL-Environmental Biology and Biodiversity Laboratory

Ap-Asclepias

Hl-Harina lata

Lt-Lantana

Cm-Cosmos

Bt-Botamful

Cd-Chandramallika

Jb-Jaba

Hs-Hatisur

Gd-Gadha

Mt-Marhatitiga

Dp-Dopati

Dk-Dondokolos

Sd-Setodron

Mk- Monkata

Pn-Panika

Wd-Weedelia

NpNilpetunia

Rg-Rongon

Km-Katamehedi,

e.g.-for example

Tl-Tara lata

Pap-Papilionidae

Dan-Danaidae

Nym-Nymphalidae

Pier-Pieridae

Sat-Satyridae

Lyc-Lycaenidae

Hesp-Hesperiidae

Acr-Acraeidae

Riod-Riodinidae

Amathu-Amathusiidae

Av-Available

Rr-Rare

Nt-Near Threatened

Tr-Threatened

Ct-Critically Threatened

En-Endangered

L1-1st instar

L2-2nd instar

L3-3rd instar

L4-4th instar

L5-5th instar

et al.-and others (Author)

Pr-Prepupa

Pp-Pupa

Af-Adult female

Am-Adult male

ME-Methanolic extract

CTE-Carbon tetrachloride soluble partitionate

PE-Petroleum ether soluble partitionate;

CHE-Chloroform soluble extract

AQE-Aqueous soluble partitionate

Dt-Date

T-Temperature

RH-Relative Humidity

Fg-Foraging

Rt-Resting

El-Egg-laying

Mt=Mating

Sr-Searching

Pl-Puddling

g-gram

mm-milimiter

viz.-videlicet

Chapter 1. General Introduction

Many animals have good relations with plants; and even have got good commensalistic association with some related plants. In addition to that, only the butterflies have got very vital biotic-biotic interactions with plants in large species volume. This term 'volume' is equally applicable both for the butterflies and also for the related plants. A butterfly species remains associated with a plant species at very specific level. The 'specific level-retention' is based on trophic relations at the question of energy transformation (in the living forms) from one state to another and vice-versa. In this case, a specific plant supplies food as host plant to a butterfly. The butterfly depends on that plant in its larval stage. Then the butterfly acts as a gene-flow carrier for the host plant together with other plants in wide range. The gene-flow carrier stands as an agent for the plant's existence. Such situation establishes multiplication of "Plant-Butterfly" association in nature. Such establishment is the 'natural tool' for healthy sustenance of both flora and faunal volume in any area especially in a wild state of a terrestrial ecosystem. Advancement to the knowledge in such an adaptive field could provide the best tool for biodiversity conservation and also for the conservation of nature. Some experiments of different modes could accommodate forays to advance to the fact of 'materialization-use' of such association between two biotic aspects, the plant (specific) and the animal (the related butterfly).

This chapter is made up of two parts, viz. research concept and objectives of the research work. Research concept has been made on the basis of butterfly diversity, behavioural aspects, abundance, distribution, biology, ecology of butterfly and butterfly related nectar, host and supportive plants, insect-plant interaction, ecosystem management and biodiversity conservation. The objectives of research work mainly focused on behavioural activities of butterfly in relation to abundance, developmental stages of butterfly, feeding potentiality of larval host-plants and adult nectar plants as nutritional supports, medicinal importance of butterfly related host plants in nature

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1.1 Research Concept

It is estimated that there are more than 17,500 butterfly species in the world and 90 percent of them have been described (Robbins and Opler 1997). Seven families of butterflies, such as Papilionidae, Pieridae, Nymphalidae, Danaidae, Satyridae, Lycaenidae and Hesperiidae are found in Bangladesh (Bashar *et al.* 2005). Nymphalidae is a dominant butterfly family including 5000 described species (Fres 1989). Of the families, Pieridae and Nymphalidae have been taxified into group. They were identified up to species level on the basis of wing-venation in Bangladesh (Bashar *et al.* 2005, 2006 a, b). Wynter-Blyth in his classic "Butterflies of the Indian Region" listed all butterfly species recorded from the sub-continent (Roberts 2001). At least 20 different plant families are selected by different nymphalid species (Roberts 2001). Pieridae is the third largest butterfly family of the world with 2000 described species (Fres 1989). Butterflies are truly the 'Jewels of creation' and amply justify the attention paid to their colours (Fres 1989). The Pieridae of Bangladesh have been taxified into grouping and identified up to species level on the basis of wing-venation (Bashar *et al.* 2005).

Flowers are important feeding sources for insects and are involved in intricate ecological relationships with them. The morphological and chemical characteristics of flowers provide the flower-specific information and play important roles in locating flowers by insects. The adults of most butterfly species typically feed on flower nectar. As they show strong preference for particular coloration, flower colours are regarded as essential visual cues in their foraging (Miyakawa 1976, Scherer and Kolb 1987a, b, Lewis 1989, Kandori and Ohsaki 1996, 1998). In addition, butterflies preferentially respond to several floral volatiles (Myers and Walter 1970, Honda 1973, 1976, Pellmyr 1986). It has been shown that particular floral components (e.g. 2-phenylethanol and phenyl-acetaldehyde) stimulate the foraging behaviour of *Pieris rapae* (Pieridae) (Honda *et al.* 1998, Omura *et al.* 1999a) and *Luehdorfia japonica* (Papilionidae) (Omura *et al.* 1999b).

Floral nectar is highly variable because different plant species produce different quantities and qualities of nectar (Baker and Baker 1983). These differences concern sugar composition, amino acid concentration and composition, contents of lipids, vitamins, and alkaloids (Baker and Baker 1975, 1982, 1983). In spite of variation in amount and concentration of nectar constituents due to environmental factors, considerable constancy of sugar proportions and amino acid concentration of nectar within species has been found (Baker and Baker 1977, 1982, Kradolfer and Erhardt 1995). Demands for nectar may vary due to different energy and nutritional needs in different butterfly species (Boggs 1997).

Different behaviours and different nutritional requirements for the synthesis of eggs and spermatophores could also cause sex-specific flower preferences and foraging patterns in conspecific males and females (Wiklund and Ahrberg 1978, Pivnick and McNeil 1987). Several studies have demonstrated that nectar sugar feeding affects longevity and reproduction of male and female butterflies (Murphy *et al.* 1983, Hill and Pierce 1989, Hill 1989). The nutritional role of amino acids in the adult diet is less clear. In some species, no effects of amino acids on longevity and reproduction have been found (Hill and Pierce 1989, Hill 1989), whereas in other species, clear positive effects have been observed (Gilbert 1972, Dunlap-Pianka *et al.* 1977, Lederhouse *et al.* 1990). Corresponding differences were also observed in nectar preference experiments, in which the response of different butterfly species to nectar amino acids was tested (Alm *et al.* 1990, Erhardt 1991b, 1992, Erhardt and Rusterholz 1998).

Investigations of feeding behaviour and flower visit patterns of butterflies are important not only for understanding the significance of adult feeding for reproductive success and longevity of butterflies, but also its feeding habits are of particular interest for conservation (Thomas 1984). Only the males of *Papilio polytes* take part in mud puddling, usually in cool shaded spots rather than in open areas. They have been known to collect on saline soils to extract minerals (Pola and Garcia-Paris 2005). The swallowtail butterfly is not particularly fond of mud puddling although it prefers sunshine (Haribal 1992). In certain parts of Sri Lanka, the males of *Pachliopta aristolochiae* are known to congregate and form a beautiful sight while mud-puddling (Harris 1996).

As with many flying insects, male mate location in butterflies can be divided into two broad and generally species-specific categories: 'perching' and 'patrolling' (Scott 1974, Wiklund 2003). In patrolling species, males spend the major part of their life actively searching for females, but in perching species the roles are reversed and females assume the role of actively searching for a mate. In the latter case, males are typically faithful to their perching sites and attempt to exclude other males from their site (i.e. they are 'territorial'). Since butterfly territories typically do not contain an abundance of larval or adult resources, the consensus is that they serve as rendezvous places where the sexes meet. In a system in which the females actively search for males, male mating success is conceivably influenced by at least two factors: (i) the degree to which male perching sites (i.e. territories) coincide with female dispersion and (ii) any female preferences for particular male character traits. The hind wings, which are coupled mechanically to the forewings and flap in synchrony with the forewings (flight in lepidopterans is anteromotoric, being driven primarily by the forewings) (Dudley 2000).

Among the diversity of insects, butterflies are ideal subjects for ecological study in landscapes (Thomas and Malorie 1985, Pollard and Yates 1993). In temperate regions, butterfly taxonomy and natural history are relatively well understood and most species can be reliably identified in the field (Simonson *et al.* 2001). Butterflies are particularly sensitive to environmental variation (Scoble 1992). Positive relations have been found between butterfly diversity and environmental variables, such as plant diversity (Erhardt and Thomas 1991, Leps and Spitzer 1990, Spitzer *et al.* 1997). The rapid deterioration of the forest stocks in Bangladesh was caused mainly due to the conversion of forest vegetation to agriculture as a result of high population growth. Forest stocks are also destroyed to meet the demand of rural fodder, fuel and timber supply (Bashar *et al*. 2001, 2002). According to present biodiversity information in the country, certain groups of plants are particularly at risk, notably of medicinal importance (Bashar *et al.* 2002).

Deforestation remains one of the major environmental issues in South Asia, and the flora and fauna of the region are more threatened now than ever before (UNEP 1997). The countries experiencing the fastest deforestation are Bangladesh, Pakistan, the Philippines and Thailand (FAO 1993). Insects are particularly useful in the evaluation of forests for biological resource conservation (Kim 1993, Samways 1994). In the various protected area-management systems, personnel and other forest management agencies have expressed interests in butterflies and their potential use in the evaluation and management of natural areas like forests (Riepe and Toborg 1996). Bell (1909) reported drumming behaviour in female butterflies prior to oviposition in order to check the physical and chemical properties of the host plants. Ilse (1956) also recorded such behaviour in many female butterflies in India and suggested that this behaviour helps them in selecting correct food plant.

The food plants of the common mormon, *Papilio polytes* used the various species of Family Rutaceae including *Aegle marmelos* (Bael), *Atalantia racemosa,* Citrus spp - *C. aurantifolia, C. grandis, C. limon, C. medica, C. sinensis, Glycosmis arborea*, *Murraya* koenigi (Curry leaf), *Murraya paniculata* (Kunte 2006). The larval food plants of the lime butterfly is cultivated lime, cultivated oranges, *Glycosmis pentaphylla,* cultivated citrus, *Ruta graveolens*, *Aegle marmelos*, *Murraya koenigii*, *Chloroxylon swietenia,* Malay Glycosmis, *Acronychia pendunulata* (Gay *et al.* 1992). The foliage of citrus species is used as a food plant by the larvae of Lepidoptera (butterfly and moth) species, such as the *Geometridae, Hemithea aestivaria* (common emerald) and *Gymnoscelis rufifasciata* (double-striped pug), or the Arctiidae, *Hypercompe scribonia* (giant leopard moth), *H. eridanus, H. icasia* and *H. indecisa*. The citrus leafminer (*Phyllocnistis citrella*) has been a pest in California boring meandering patterns through leaves (Mollina *et al.* 2000).

The larval food plants of *Pachliopta aristolochiae* included *Aristolochia bracteolate, A. indica, A. tagala, A. griffithi* and *Thottea siliquosa* (Tian-shung *et al.* 2000). Rearing of this species and releasing the same in the wild will help restocking its slowly depleting population, and also serve as a measure for its conservation (Gay. *et al.* 1992). The caterpillars of the common jay, *Graphium doson* feed on plants of the families Annonaceae, Lauraceae, Magnoliaceae such as *Annona lawii, Cinnamomum macrocarpum, Magnolia grandiflora, Michelia champaca, Milliusa tomentosum* and *Polyalthia longifolia* (Evans 1932). The leaves of *Polyalthia longifolia* are larval food of the kite swallowtails; the leaves are good for ornamental decoration and used in festivals. Methanolic extracts of *Polyalthia longifolia* have yielded 20 known and two new organic compounds, some of which show cytotoxic properties (Chung-yi *et al.* 2000).

The larval food plants of *Danaus chrysippus* are *Asclepias curassavica* (red-head cotton-bush), *Gomphocarpus* (*Asclepias*) *fruticosus* (swan plant), *G. physocarpus* (balloon cotton-bush), *G. cancellatus* (rotundifolia) (broad-leaved cotton-bush), *Calotropis spp., C. procera* (king's crown), *Cynanchum* species including *C. floribundum* (desert cynanchum or native pear), *Marsdenia* (Leichhardtia) *australis* (native pear), *Rhyncharrhena* (Pentatropis) *linearis* (bush bean or cotton vine), *Orbea* (Stapelia) *variegata* (carrion flower), *Sarcostemma spp.* (Asclepiadaceae) (Wynter-Blyth 1957). The common crow, *Euploea core* is known to feed on five plant species belonging to Asclepiadaceae and seven species of Moraceae. From Apocynaceae it is known to feed on three species of *Nerium, Holarrhena pubescens, Holarrhena antidysenterica* and *Ichnocarpus frutescens* (Wynter-Blyth 1957). The common crow, *Euploea core* usually has some preference for certain species in a given area. The more commonly used plants are *Ficus racemosa, Nerium oleander, N. odorum* and *Cryptolepis buchananii. Ficus pumila* a cultivated garden plant which climbs on walls has also been noted (Aravind 2005). Along with other danaids, such as the tigers, the common crow is one of the most common migrating butterfly species (Aitken 1898, Reuben 1961). Males and females in equal proportions have been seen to migrate (Kunte 2005).

1.2 Objectives of the Research Work

The Ph. D. research programme was emphasized on establishment of Butterfly Research Park (BRP) for butterfly colonization; behavioural studies of butterfly; biology of *Danaus chrysippus* butterfly; feeding potentiality of butterfly larvae; nutritional and medicinal importance of butterfly related plants in nature . The prime aims of research work are to:

- study behavioural activities of butterflies through its colonization in the Butterfly Research Park (BRP) at Bhawal National Park, Gazipur
- examine biology of the plain tiger butterfly, *Danaus chrysippus* in relation to larval host plants and adult nectar plants
- investigate biotic interaction between the common rose butterfly, *Pachliopta aristolochiae* and its host plant, *Aristolochia indica*
- analyze antioxidant, toxicity and antibacterial activity of the leaf extracts of *Aristolochia tagala* (host plant of the *Pachliopta aristolochiae* butterfly)

The Ph. D thesis was completed by following above mentioned objectives in rest part of the thesis especially Chapter 3, 4, 5 and 6 where the findings of thesis were discussed in detailed.

Chapter 2. Review of Literature

Review of Literature was made on the basis of four sub-topics, viz. behavioural strategies of butterfly; biology of *Danaus chrysippus* butterfly; butterfly-plant interaction; medicinal importance of butterfly related plants. Review of Literature was followed from 2018 to 1983 which were published in national and international journal regarding the objectives of this thesis.

2.1 Behavioural strategies of butterfly

This part of Review of Literature emphasized on butterfly related host and nectar plants; the utilization of host and nectar plants by butterfly; behavioural activities; population fluctuation; effect of abiotic and biotic factors; taxonomic status of butterfly; butterfly species richness, abundance and diversity, ecology, ecosystems and biodiversity conservation. Review of Literature was included from 2018 to 1997 regarding this sub-topic.

Kamrunnahar *et al.* (2018) studied on the basking behaviour of some nymphalid butterflies in the fields of Bhawal national park, Rema-Kalenga, Zoological and Botanical Gardens of the University of Dhaka. The time budget and the wing-posture activities of butterflies during basking period were studied butterfly species under the family Nymphalidae. The experimental species were *Junonia atlites, J. almana, J. iphita, Neptis soma, Labadea martha, Ergolis ariadne, Phalantha phalantha, Hypolimnas bolina* and *Athyma perius.* Different types of wing postures (viz. appressed, horizontal, angled and closed type) were also recorded. It is found that butterflies take more time for their basking during winter season. Most of them prefer the month of November and December for their basking. The observations reveal that thermal basking increases the temperature in the butterfly body. It directly implies how thermoregulation associated with behavioural activities in different abiotic conditions. The results also showed the importance of wing postures for thermoregulation.

Ahmed *et al.* (2016) studied the host-range and food preferences of butterflies, encountered in and adjacent to the Gir National Park, Gujarat, India. The larval host plants of 67 butterfly species were identified and their host specificity, abundance, perennation were recorded. Out of 74 host-plants, 22 were identified as annuals, 3 bi-annual and 49 perennials. These plant species are further categorized into 21 trees, 22 herbs, 24 shrubs, 6 Climbers and one species of plant parasite. The findings revealed that the plant species belonging to families Memosaceae, Capparaceae and Caesalpiniceae were found to provide most suitable food for butterfly species belonging to the four different families of butterflies in GNP. In addition, a number of significant differences between butterfly families and their host use patterns, such as perination, host specificity etc. was studied and identified. Correlation coefficient ($r = 0.785$) confirms a strong correlation between host plants and butterflies, and was found significant at 1% level ($p = 0.01$). Hence, more number of host-plant species attracts more species of butterflies significantly.

Gideon *et al.* (2016) investigated butterfly diversity which greatly depends on the availability of host plants. The study was carried out from August 2014 to July 2015 in Pachamalai hills of Eastern Ghats in Tamilnadu, India. In the ecological sites of the Eastern Ghats the native plant species especially *Vitex negundo, Ehretia pubescens, Premna serratifolia, Pavetta indica* and *Prosopis juliflora,* were observed as invasive plant species. This study revealed that some of the native nectar host plants played a vital role in attracting butterflies and increasing the diversity of butterflies. *Vitex negundo,* which acts as a primary nectar host plant for a variety of species was studied in detail.

Shihan (2016) attempted to describe the fruit feeding behavior of butterflies from different areas of Bangladesh. The study was conducted from June 2014 to September 2015. A total 53 individuals of 11 species butterflies under two families feeding on the fruits of 11 species under 11 families were reported.

Bashar *et al.* (2015) made an experiment on establishment of butterfly open colonization can create an ecologically 'furnished' open site where species richness becomes enriched. In Bhawal National Park of Bangladesh, a natural butterfly park has been used as the study area to investigate how lively ethological behaviours of butterfly interact. The three-acre area, jungle-bush hedges area and multi-morphic beds-area. The areas provide both sheltering plants and host-plants for butterflies. The Jungle bush ensures safe pupation. The accumulation and well-planned arrangement of these four area-components facilitate the system mechanism of butterfly colonizing process. The colonization process has been made functional by applying some procedural methods and also by safe guarding butterfly-activities in the experimental area. To exercise promoting of butterfly-activities in the assessment of interaction between the phenological stages of related plants and the developmental stages of the butterflies, the entire experiment has been divided into two sections. First one is to examine the criteria responsible for determining the establishment of butterfly colonization process. Second one is to examine the impacts of butterfly colonization on the enhancement of seed production capacity of the target plants in the centre premises. Results of the first and second sections indicated that butterfly colonizing mechanism enhances the multiplication of plant population and protection of successive trophic levels, means the wildlife.

Shihan and Kabir (2015) investigated the diversity of butterflies in relation to *Chromolaena odorata* (Asteraceae) in two selected geographically different areas, namely Kaptai National Park and Jahangirnagar University Campus during the period of December 2011 through January 2015. A total of 55 species of butterflies belonging to 6 families was recorded during this survey period of which 35 species of 28 genera were recorded from Kaptai National Park and 26 species of 23 genera were from Jahangirnagar University Campus. Six different species, viz. which include *Acraea violae, Catopsilia pomona, Papilio demoleus, Eurema hecabe, Cethosia cyane* and *Tagiades japetus* were identified in the both areas.

Kharouba and Vellend (2015) stated variation among species in their phenological responses to temperature change suggesting that shifts in the relative timing of key life cycle events between interacting species are likely to occur under climate warming. However, it remains difficult to predict the prevalence and magnitude of these shifts given that there have been few comparisons of phenological sensitivities to temperature across interacting species. They used a broad-scale approach utilizing collection records to compare the temperature sensitivity of the timing of adult flight in butterflies vs flowering of their potential nectar food plants (days per °C) across space and time in British Columbia, Canada. On average, the phenology of both butterflies and plants advanced in response to warmer temperatures. However, the two taxa were differentially sensitive to temperature across space vs across time, indicating the additional importance of non-temperature cues and/or local adaptation for many species. Across butterfly–plant associations, flowering time was significantly more sensitive to temperature than the timing of butterfly flight and these sensitivities were not correlated. The results indicate that warming-driven shifts in the relative timing of life cycle events between butterflies and plants are likely to be prevalent, but that predicting the magnitude and direction of such changes in particular cases is going to require detailed, fine-scale data.

Patil and Shende (2014) described the Gorewada International Bio-park in India; it is a good habitat for biodiversity studies of butterflies. Its geographical location is 21011'N 7902'E. Butterfly watching and recording was done in such a way that there should be least one visit in each line transect during a week with the aid of a binocular and a digital camera. Total 92 species of butterflies were recorded belonging to 59 genera and five families. Out of total 92 butterfly species, 48.92%, 38.04% and 13.04% were common, occasional and rare species, respectively. Nymphalidae family consisted of maximum number of genera and species. Maximum species richness was reported from July to January and its number declined from late March to last week of June.

Nimbalkar *et al.* (2014) reported that floral attributes influence nectar feeding butterflies. The present study was carried out from Bhor Tahsil of Pune District, Maharashtra, India, from August 2007 to August 2009. A total of 64 butterfly species was recorded. Family Nymphalidae dominates in the study area, followed by Lycaenidae, Pieridae, Hesperiidae and Papilionidae. Nineteen nectar food plants were identified belonging to 10 plant families. Plants of the Asteraceae family are more used by butterflies as nectar food plants. Visits of butterflies were more frequent to the flowers with tubular corollas than to non-tubular ones, to flowers coloured red, yellow, blue and purple than those coloured white and pink and to flower sources available for longer periods in the year. Species abundance reached the peak in the months from August to November. A decline in species abundance was observed from the months from December to January and continued up to the end of May. The findings obtained were important with respect to monitoring butterfly and plant diversity, and defining conservation strategies in the Bhor Tahsil.

Deepika *et al.* (2014) studied the nectar host plants of butterflies at Visakhapatnam, Andhra Pradesh, India. At Visakhapatnam a total of 43 butterfly species under eight families was recorded. Of the 43 species of butterflies recorded at Visakhapatnam, five species, viz. *Elymnias caudata, Mycalesis visala subdita, Melanitis leda ismene, Euthalia garuda,* and *Neptis hylas* seldom foraged on the nectars of flowers. The butterflies fed on over ripe or rotten fruits, sap oozing from wounds and tree trunks. Among the remaining species *Papilio polymnestor, P. poltyes polytes,* and *Princeps demoleus* were seen to feed on mud in addition to foraging on different flowers and three species, viz. *Euthalia nais, Papilio crino,* and *Colotis danae* could not found to feed on any flower during the study period. The remaining 35 species were found taking nectar at the flowers of one or the other 54 plant species. The mutualisitic relationship between plants and insects is widely explained. The plants evolved floral structures for the production of the nectar which was collected by the insects that were in the process of pollinating flowers. Also, the flowering period and colour of the flower were described.

Begum *et al.* (2014) investigated the nectar feeding behaviour of butterflies belonging to the families Nymphalidae, Danaidae, Pieridae, Lycaenidae and Papilionidae in the Botanical garden of Dhaka University. The highest and lowest duration of searching time was 39 ± 2 and 36 ± 5 seconds, respectively for *Catopsila pomona* and *Zizina otis.* The highest and lowest duration of feeding was 13.0 ± 1 and 9.9 ± 0.9 seconds, respectively for *Danaus chrysippus* and *Zizina otis.* The longest proboscis (12.6 ± mm) was recorded in *Danaus chrysipus.* The deepest corolla (22 ± 5mm) was found in the flower of *Cosmos bipinnatus* plants. The proboscis of four butterfly species, namely *Eurema hecabe, Junonia almana, Catochrysopes strabo* and *D. chrysipus* was highly correlated with the corolla tube of *Cosmos bipinnatus, Tephrosia purpurea* and *Tagetes erecta,* respectively.

Gillespie and Wratten (2013) reported that nectar is an important factor influencing the level and persistence of butterfly populations, but particular sources of nectar may not be optimal for all species. In a farmland context, it is not always clear whether nectar sources used by butterflies are good quality species. They may be used opportunistically in the absence of true preferences, therefore possibly limiting maximal reproduction. This study investigated the use of nectar by adults of the endemic New Zealand butterfly, the common copper *Lycaena salustius*, in two ways: (1) a choice experiment in the field using a replicated design of different plant species, and (2) a greenhouse no-choice bioassay examining fitness enhancement by different flower species. In the field experiment, only *Lycaena salustius* males were observed in large numbers, and they spent a significantly longer time on flowers of Veronica 'Youngii' and *Fagopyrum esculentum* than on species already available in vineyards. In the laboratory, *Veronica salicifolia* and *Fagopyrum esculentum* flowers significantly enhanced the fitness of females over *Achillea millefolium* and the water control. These findings together imply that superior and preferred floral resources are not yet available to adult *Lycaena salustius* in vineyard landscapes. The no-choice greenhouse experiment suggests that the plant group with which the butterfly may have co-evolved is more beneficial than other exotic species, and that such plants could enhance populations in vineyards. The conservation of other butterfly populations in farmland and other ecosystems may benefit from similar investigations.

Soler *et al.* (2010) described root-feeding insects can affect the performance of above ground insect herbivores when they are forced to feed on the same host plant. Here we explored whether the oviposition behaviour of two closely related herbivorous species (cabbage butterflies; Lepidoptera: Pieridae) is influenced by root feeding insects, when they are given the chance to choose between host plants with and without root herbivores. Considering that egg load is an important physiological factor influencing the foraging behaviour of insects, they also examined whether root-feeding insects differentially influence oviposition preference in butterflies with low and high egg loads. Oviposition preference in both butterfly species with low and high egg loads was monitored using host plants with and without root herbivores. To ascertain the status of butterfly age with low and high egg loads, the oviducts of a separate group of butterflies was dissected to record the number of immature and mature eggs in the butterflies of various ages. *Pieris brassicae* L. butterflies with low egg loads preferred plants without root herbivores over plants with root herbivores, and laid more egg clutches on the leaves of plants that were not attacked by root herbivores. Butterflies with comparatively high egg loads also selected a larger proportion of plants without root herbivores, but laid a similar number of egg clutches on the plant shoots independent of the presence or absence of root herbivores belowground. Independent of the age and egg load, *Pieris rapae* L. butterflies selected a larger proportion of plants not attacked by root herbivores to lay eggs, but the number of eggs laid was similar in plants with and without root herbivores. This study shows that belowground insects can influence behavioural decisions of aboveground insect herbivores. Interestingly, the strength of these interactions depends on the physiological state of the insects which is probably correlated with their perception of environmental quality.

Bartel *et al.* (2009) evaluated whether ecosystem engineers can accomplish two conservation goals simultaneously: (1) indirectly maintain populations of an endangered animal through habitat modification and (2) increase riparian plant diversity. They tested the effects of a prominent ecosystem engineer, the beaver *Castor canadensis*, on populations of St. Francis' satyr butterfly *Neonympha mitchellii francisci* and plant species richness and composition. They performed the test by surveying riparian vegetation communities in all stages of beaver-influenced wetland succession.

They found that beavers created the wetland habitats that supported plant species not found elsewhere in riparian zones and increased plant species diversity across the landscape by creating a novel combination of patch types. Their results confirmed what others have found about engineering effects on plant diversity, but these results further demonstrated a case where ecosystem engineers indirectly maintain populations of rare animals by modifying the composition and diversity of plant communities within wetlands. Their research demonstrates how an ecosystem engineer can influence habitat availability and composition of plant communities important for an endangered insect, and maintain overall plant species diversity by increasing habitat heterogeneity.

Winarni (2007) observed rapid assessment for butterfly diversity particularly in the tropics which is sometimes confounded by the lack of trained personnel in identification skill and lack of field identification guide for particular sites. In the other hand, detectability is also an important issue in biodiversity survey. A better sampling design is needed to provide better estimates and a better snapshot of the present communities. Modification of Pollard walk using line transect and point count methods was evaluated in assessing diversity of different sites and evaluating detection probability. Patterns of butterfly community based on detection cue by the two methods were also evaluated. Studies were conducted at 6 different sites in Lambusango forest, Buton, Southeast Sulawesi. In overall, the results showed that both line transect and point count produced similar patterns of diversity. The line transect method generated higher species richness than point count method. However, detection probability of ten most common species using point count is significantly higher than line transect method. Point count method allows flexibility in detecting object than line transect. Morphological cues are more important in point count while quantified cues are more important for line transects. Refinement of survey method is important to increase the probability of detection.

Kunte (2007) reported nectar-feeding butterflies and their body size and proboscis length showing an allometric relationship. The butterflies that deviate from this relationship and have disproportionately long proboscis can access nectar from deep flowers, which is inaccessible to the species of similar or larger body size, but with shorter proboscis. Despite this selective advantage, few species possess disproportionately long proboscis for their body size, which indicates that there may be developmental, functional or other ecological constraints on very long proboscis.

They hypothesized that the species with disproportionately long proboscis had a functional cost in terms of higher handling time (amount of time spent per flower); therefore, they were at a competitive disadvantage compared to butterflies that had shorter proboscis and lower handling times. They tested this hypothesis using Costa Rican butterflies. They measured body length, proboscis length and handling time on Lantana and Wedelia, two nectar plants with generalist pollination systems which attract large numbers of nectar-feeding butterfly species. There was a strong positive relationship between 'relative proboscis length' (proboscis length in relation to body size) and handling time per flower on both nectar plants. The species with greater relative proboscis length had up to three time's longer handling time per flower. Thus, the butterflies with relatively long proboscis should harvest less nectar per unit time from the same flower than the butterflies with normal proboscis. Reduced foraging efficiency in the face of competition from other nectarivores may thus be a functional constraint that limits the evolution of disproportionately long proboscis in generalist nectar-feeding butterflies.

Bakowski and Boroñ (2005) presented flower visit patterns of four species of Lycaenidae, observed in meadows near the city of Poznañ (western Poland) in 2001-2002. *Polyommatus icarus* (Rott.) and *Plebeius argyrognomon* (Bgstr.) used broad ranges of flowers as nectar sources: 19 and 14 plant species, respectively. These butterflies fed most frequently on flowers of *Lotus corniculatus*. The univoltine species *Polyommatus semiargus* (Rott.) and *P. amandus* (Schn.) generally directed their foraging activities towards a limited number of available plants. *P. semiargus* visited five species of plants, most frequently *Lathyrus pratensis* and *Vicia* spp. *P. amandus* visited 4 plant species, most frequently *Vicia cracca.* A relationship between adults of all four butterfly species studied and the plants belonging to the family Fabaceae has been confirmed.

Fitzherbert *et al.* (2005) sampled butterflies in six different habitat types in and around Katavi National Park, a remote reserve consisting primarily of miombo woodland and seasonal lakes in western Tanzania. Blendon traps set for 531 trap days and 143 h of butterfly netting at 35 sites yielded 186 species from five families over a 4-month period during the wet season. Eight of these species constituted possible range extensions. Butterfly abundance and species richness were low in cultivated habitats, but high in open riverine habitats; many butterfly species were found only in seasonally flooded grassland. This study constitutes the first butterfly species inventory from this poorly-known national park, shows that protection of dry season water sources provides an important conservation service for invertebrates as well as large mammals, and that increased cultivation outside miombo parks can reduce local butterfly diversity.

Boonvanno (2001) reported fifty-three genera and 98 species of butterflies (Lepidoptera) collected at Ton Nga Chang Wildlife Sanctuary, Songkhla Province, from September 1999 to August 2000. The specimens were collected with aerial nets and hanging baited-traps along transects. Nymphalidae and Satyridae were the best represented families. The most abundant species was *Melanitis leda* (Linnaeus) (Satyridae). The highest diversity was found in April (Shannon-Weiner index, H =3.41), and the lowest in November (H = 1.08).

There were no significant correlations among physical factors (humidity, rainfall and temperature) and the total number of individuals or species. Moreover, butterfly numbers were not related to rainfall in any family. However, humidity was negatively correlated with the individual numbers of Nymphalidae, and temperature was positively correlated with the individual numbers of Pieridae and Lycaenidae.

Kinoshita and Arikawa (2000) observed that the Japanese yellow swallowtail butterfly *Papilio xuthus* uses colour vision when searching for food. In the field, these butterflies feed on nectar provided by flowers of various colours not only in direct sunlight but also in shaded places and on cloudy days, suggesting that they have colour constancy. They trained newly emerged *Papilio xuthus* to feed on sucrose solution on a paper patch of a certain colour under white illumination. The butterflies were then tested under both white and coloured illumination. Under white illumination, yellow- and red trained butterflies selected the correctly coloured patch from a four-colour pattern and from a colour Mondrian college. Under four different colours of illumination, they obtained the results that were fundamentally similar to those under white illumination. Moreover, they performed critical tests using sets of two similar colours, which were also correctly discriminated by trained butterflies under coloured illumination. Taken together, they conclude that the butterfly *Papilio xuthus* exhibits some degree of colour constancy when searching for food.

Goulson *et al.* (1997) described many insects foraging for nectar or pollen exhibiting flower constancy, a learned fidelity to a particular species of plant that previously provided a reward. Constancy may persist even when alternative flowers are available that provide a greater or less variable reward. This strategy entails more travelling time than one of generalization (visiting all suitable flowers as they are encountered). The consensus at present is that this increase in travelling time is offset by decreases in handling time; switching between flower species incurs a cost in time spent learning to 'handle' the new flower species that is avoided by remaining constant. If this is so, then the optimal strategy should depend upon the density of flower species (and thus the travelling time), with switching occurring below a threshold density of the target flower species. This prediction is tested using the butterfly, *Thymelicus flavus*, by analysing foraging patterns under natural conditions. This species exhibited constancy: of 465 visits to flowers 85% were to the same species as last visited. As predicted switches between flowers species occurred in response to low encounter rates of the flower species on which the individual had previously fed. However, butterflies ignored the vast majority of suitable flowers that they encountered, even when they were of the species to which they were constant. This casts doubt on explanations for flower constancy as an adaptive strategy that minimizes handling time and maximizes resource acquisition per unit time within learning constraints.

2.2 Biology of *Danaus chrysippus* butterfly

This part of Review of Literature emphasized on egg-laying behaviour of *D. chrysippus* butterfly; morphological changes of larvae; duration and length variation of larvae; morphological changes of pupa and adult, *D. chrysippus* butterfly; medicinal importance of the host plants, co-evolution between butterfly and plants (*Danaus chrysippus* butterfly and *Asclepius curassavica* host plant). Review of literature was included from 2016 to 2007 regarding this sub-topic.

Rao *et al.* (2016) examined the larval performance and life cycle of *Danaus chrysippus* at Andhra University campus using the leaves of *Asclepias curassavica* as the larval host both in the laboratory and the natural conditions. The behavior and morphological characters of eggs, caterpillars, pupae and adult emergence were observed in the laboratory at 28-30 ^{o}C. The life cycle was completed in 17-18 days, with egg hatching in 3, larvae in 7-8, and pupae in 7-8 days. The values of consumption index (CI), growth rate (GR), and approximate digestibility (AD) across the instars decreased as the larvae aged. The average values of the CI and GR were 0.97, 0.22, respectively, and that of AD was 74.43. But, the values of both efficiency of conversion of digested food (ECD) and efficiency of conversion of ingested food (ECI) either increased or decreased from instar to instar.

Weeramanthri *et al.* (2016) investigated on changes in the abundance and spatial distribution of *D. chrysippus* across Australia over the past 128 years. Their study hypothesises that there will be a decrease in the abundance of *D. chrysippus* over time, as well as, a change in distribution in response to rising average air temperatures. Historical records of *D. chrysippus'* distribution across Australia, spanning from 1889 to 2016, were retrieved from the Atlas of Living Australia. Climate Watch maps contained data collected by citizen scientists from the past five years (2011 to 2016). Climate Watch maps were compared with a graph containing historical data on the mean annual temperature in Australia, retrieved from the Bureau of Meteorology. A graph containing the number of Climate Watch sightings of *D. chrysippus* over the past 5 years was also created.

Their results found that *D. chrysippus* is no longer widely distributed across most of Australia, but primarily concentrated in the cities of Perth and Sydney. Overall, their findings rejected the hypothesis that the abundance of *D. chrysippus'* decreased overtime, but supported a change in distribution across Australia. The limitations of a citizen science program renders the Climate Watch data too unreliable, and thus, the results obtained cannot be ruled as conclusive.

Das *et al.* (2015) investigated on the antioxidant evaluation and cytotoxic activity of *Asclepias curassavica* Linn (Aselepiadacea) which has been known since ancient times for its curative properties and has been utilized for the treatment of various ailments, such as tumor, asthma, fever, homeostasis, inflammation, diarrhea, and warts. In recent times a great number of chemical and pharmacological studies have been done on *A. curassavica*. In the present study hexane, chloroform and methanol extracts of *A. curassavica* were evaluated for their antioxidant and cytotoxic activities. In vitro antioxidant activity was conducted by determining the total phenolic content by using Folin-ciocalteu regent and DPPH free radical scavenging activity. In all the three solvents highest total phenolic content and highest DPPH free radical scavenging activity percentage was shown by chloroform extract of *A. curassavica* i.e. 105.6 GAE mg/g dry material and 93.8%, respectively which are nearly equal to Ascorbic acid and Gallic acid standard. The cytotoxic activities of *A. curassavica* was also studied in vitro by using two assay, i.e. ATP chemi-luminescent and flow cytometry on two cell lines, namely HeLa and MDA-MB-231. *Asclepias curassavica* exhibited potential cytotoxic activity. Thus, the results indicate that this plant might be used as a potential source of natural antioxidant and as anti-cancer agent.

Al-snafi (2015) reviewed the chemical constituents and pharmacological effects of *Asclepias curassavica* which was used traditionally in different populations for many medical complains. It contained a wide range of chemical constituents including flavonols, flavonol glycosides, amino acids, carbohydrates, triterpenes, cardenolides and many other biologically active compounds. The cardenolides isolated from the plant included calactin, calotropin, calotropagenin, coroglaucigenin, asclepin, asclepain CI, asclepain CII, asclepine (asclepiadin), uscharidin, uzarin, uzarigenin, corotoxigenin, asclepogenin, curassavogenin, calotroposide, clepogenin, desglucouzarin, kidjolanin, and uscharidin.

The previous studies showed that the plant exerted many pharmacological activities including antimicrobial, anticancer, cardiovascular, analgesic and antipyretic, and many other pharmacological activities. This paper is a step ahead to open a new insight for the therapeutic efficacy of *A. curassavica.*

Mary *et al.* (2015) focused on the population dynamics and pattern of seasonal migration of *Danaus chrysippus* at Bishop Heber College, Tiruchirappalli, India during the year 2009. The study was conducted to find the population of plain tiger butterfly in a 25 acre campus in four different seasons. During the study period it was observed that the numbers were relatively high in June, October and November which are the months of seasonal migration with the average sighting being 159 per study compared to 61 in the other months. The results revealed that there were significant relation between rainfall and population of butterfly.

Akand *et al.* (2015) studied the biology of the gram blue butterfly, *Euchrysops cnejus* (Fabricius) (Lycaenidae: Lepidoptera) and its relationship with the phenology of host plant cowpea, *Vigna unguiculata* L. (Fabaceae). Eggs were reared under the laboratory conditions at 28 ± 2˚C and 74 ± 3 % RH. The incubation period of the eggs was 2.33 ± 0.51 days, larval developmental period 14.65 ± 0.51 days, pre-pupal period 0.30 ± 0.04 day and pupal period 5.66 ± 0.51 days. The species took 22.94 ± 0.55 days for development from egg to adult under the laboratory condition. The length of 1^{st}, 2^{nd}, 3^{rd} and 4^{th} instar larvae was 3.66 ± 0.40, 6.16 ± 0.51, 12.16 ± 0.51 and 15.33 ± 0.40 mm, respectively. The pre-pupal length was 9.16 ± 0.61 mm and the pupal length was 9.08 ± 0.37 mm. The host-plant occurs in the field from February to July. The butterfly appeared in March. The coincidence of the gram blue butterfly to its host-plant occurred between April and early July. The oviposition behaviour, incubation and immature stages were found to be profoundly related with host plant-phenological phases.

Bala *et al.* (2014) studied the biology of common castor butterfly under the field and laboratory conditions during the year 2013. Field studies indicated that *Ariadne merione merione* Cramer was in continuous flight and reproduction, with highest densities of early and adult stages occurring during June–September, the time of the entire North-West monsoon. Mating which usually takes place once, twice, or thrice, is observed on the same day or a day after emergence. Female starts laying eggs on the same day or a day after mating. Fresh eggs of common castor butterfly were collected from the host plants. Fresh eggs were transported and kept in the laboratory for rearing. Hatched larvae were individually reared on the leaves of common castor, *R. communis* for studying the morphology and life history of the butterfly. The complete life cycle from egg to adult took 22-32 days and usually has 8-9 generations per year. The biology and developmental periods are mainly dependent on the climate, location and plant species on which it feeds.

Golestaneh *et al.* (2009) studied *Danaus chrysippus* L. (Lep.: Nymphalidae), which is the most important pest on *Calotropis procera* in Bushehr-Iran. The larvae feed on the leaves and make some damages and losses on host. This study carried out on *D. chrysippus* life cycle in Bushehr from the years 2006 and 2007. For the life cycle studies, the eggs were collected from the nature and were developed in Petri dishes and 10×12 plastic dishes from egg to adult at under laboratory condition (25±2 ºC and 22±2 ºC, %60±10 RH and 16/8 L:D). The egg, larval and pupal periods in 25 ºC and 22 ºC at laboratory were 3.4 ± 0.1, and 4.5 ± 0.1, respectively; 12.5 ± 0.2 and 19.1 ± 0.4, respectively; 9.8 ± 0.3 and 14.6 ± 0.7 days, respectively. The total period from egg to adult was in 25 ºC and 22 ºC at laboratory condition 26.07± 0.8 and 37.08± 0.5 days, respectively.

Pisciotta *et al.* (2008) reported that *Danaus chrysippus* (L.) is a wide-ranging migrant species; it has considerably increased and extended its range in the North African coastal regions in the past two decades, and from there has colonized parts of the south coast of Spain, Corsica, Sardinia, Italy, Malta and Greece. Although it is a polyphagous species its larvae feed on plants which contain cardenolides especially Asclepiadaceae, Apocynaceae and Moraceae.

They reported a butterfly species in Lampedusa Island where larvae feed on *Caralluma europaea* (Guss.) N.E.Br. (Asclepiadaceae). Observations were carried out from April 2006 to November 2007, during a multi-year research project on the ecology of *C. europaeain* Lampedusa. The larvae of the population found in Lampedusa Island feed on *Caralluma europaea* (Guss.) N.E.Br. (Asclepiadaceae). Larvae and eggs were collected to observe development and feeding preferences under controlled conditions. The strict relation between *Danaus chrysippus* and Asclepiadaceae is confirmed and a strong preference for the follicles versus stems was observed.

Deshmukh (2007) described plain tiger or African monarch or *Danaus chrysippus* which is one of the well distributed species in the Indian subcontinent after Africa, China, Chile, Sri Lanka & other Asian countries including Bangladesh. In the college of Agriculture, Baramati, India a study on this well distributed African monarch was carried out. *Danaus chrysippus* feeds on different species of Milkweed (*Asclepias*). Two most common species *Calotropis giganta* (purple flowers) and *Calotropis procera* (white flowers) are commonly found hosts for *Danaus chrysippus*. In this study, the larvae (caterpillar) of plain tiger monarch at different stages and corresponding population of it on different species of its host plant were collected. Different phases of life cycle i.e. caterpillar, chrysalis, and adult of *Danaus chrysippus* were observed in Natural and laboratory conditions. Mimicry by other insects and chemical (cardiac fluid) defense mechanisms are some of the distinctive study characteristic's about monarch butterfly. Hence this study revealed that there are many threats to the population of *Danaus chrysippus* as in case of *Danaus plexippus* species of Monarch butterfly (An endangered Migratory species) found in Mexico and other American countries. Keeping in mind that, study of biodiversity of different species of living organisms on the earth is important, we found some conservative practices that can be followed for *Danaus chrysippus.* Also, study of its host species is carried out for efficient conservation practices. Conservation of milkweed helps the plant life by serving as important host for "Monarch butterfly, Bees (major pollinators)" and insect carnivorous beetles which feeds on aphids. As a part of conclusion, conservation of biology and life cycle of one species is important to better place the biology of other species living on earth.

2.3 Butterfly-Plant Interaction

This part of Review of Literature emphasized on egg-laying strategy of *P. aristolochiae*, host-plant utilization strategy of larvae; variation in length, feeding potential and faeces of larvae; pupation strategy and morphological changes of adult; behavioural status in relation to abundance of *P. aristolochiae* butterfly . Review of Literature was included from 2017 to 1983 regarding this sub-topic.

Alam *et al.* (2017) studied carried out an experiment on colonization of the butterfly *Pachliopta aristolochiae* (Fabricius, 1775) (Lepidoptera: Papilionidae) in the Zoological Garden, Curzon Hall, University of Dhaka and Bhawal national park, Gazipur from 2010 to 2011, and butterfly host-plants and nectar-plants were identified for this purpose. The field observations and identification confirmed that the host plant species *Aristolochia indica* is a creeper plant. *A. indica* was cultivated for the butterfly oviposition behaviour and to continue developmental stages. The oviposition behaviour of gravid female, hatching, feeding and moulting behaviour of the four larval instars, and pupation behaviour of *P. aristolochiae* were recorded. Both laboratory and field observations revealed that while there was availability of food, 1st and 2nd instar larvae preferred tender leaves, whereas the 3rd and 4th instar larvae fed both on young and mature leaves. However, mature larvae were also observed to feed on the stems, flower and fruits of the host-plant in absence of suitable succulent leaves. The feeding time was recorded for each larval instars and it was relatively low for 1st and 2nd instar larvae, remarkably higher in 3rd instar larvae and highest in the case of ultimate and penultimate larval instars. The egg, larval and pupal mortalities were counted during the study period. Their survivability rate was 80% or more. This result was found to stand good for a successful colonization process of the butterfly *P. aristolochiae*.

Rajeswari and Jeyabalan (2017) studied the development of larvae, pupae and adult of the swallowtail butterflies (Papilionidae) in captivity in Nilgiri hills, India. The eggs on the host plants were collected from the field and reared in the laboratory under optimum conditions of temperature and humidity. The larvae were reared in a temporary laboratory and fed on respective host plants (*Troides minos* :*Aristolochia indica, Pachliopta aristolochiae: Aristolochia indica, Pachliopta hector: Aristolochia indica, Pachliopta pandiyana: Thottea siliquosa, Graphium sarpedon:Cinnamomum camphora, Graphium agamemnon: Annona squamosa, Graphium doson: Polyalthia longifolia, Graphium nomius : Polyalthia longifolia, . Graphium antiphates: Annona zeylanica, Papilio demoleus: Glycosmis pentaphylla, Papilio polytes: Murraya koenigii, Papilio polymnestor: Citrus limon, Papilio Buddha: Clerodendrum paniculatum, Papilio clytia: Cinnamomum camphora, Papilio liomedon: Acronychia pedunculata, Papilio dravidarum: Glysosmis arborea, Papilio helenus: Glycosmis arborea, Papilio paris: Toddalia asiatica* and *Papilio crino: Chloroxylon swietenia*). The egg laying behaviour of the gravid female, hatching, feeding and moulting behaviour of the 5 larval instars were recorded on the respective host plants. The duration of the larval instars on the respective host plants were also recorded. The various stages of pupation up to the emergence of the adult from the chrysalis were recorded. The fecundity of individual butterflies was also recorded.

Aich *et al.* (2016) suggested that the butterfly *Pachliopta aristolochiae* was found to be closely associated with its host plant *Aristolochia indica.* The plant was found to grow synchronously with emergence of the related butterfly's new generation-arrival in the experimental ecosystem. The butterfly was colonized in the laboratory as well as in the natural condition. The adopted colonizing technique has shown that some developmental stages in the host plant were effective in giving high rate of adult production. The feeding potential of the host plant's phenological stages was found to be significant for some of the developmental stages (particularly 3rd and 4th instar larval stages).

Sixteen compounds were isolated from the leaves of the host plant and structures of five compounds were characterized which indicated the role of host plant and the feeding potential in the developmental process. Among those five compounds three were the derivatives of Aristolochic acids, namely Cepharanone-A-N-β-D-gluco-5,13"-O, 4"icosyl-aristolenone (1), Cepharanone-A-N-β-D-gluco-5,13"-O,4"-ethyl-aristolenone (2), and Cepharanone-A-2hydroxy-N-β-D-gluco-5,13"-O-4"-icosyl-aristolenone (3). This record suggests the presence of high amount of Aristolochic acids in the leaves of the host plant that made the butterfly toxic and unpalatable both in adult and immature stages to its predators. This toxicity may suggest that the presence of warning coloration results in the adults via the biochemical (metabolic) changes in the larval stages.

Torres (2016) described the life history, host plant use, and myrmecophily of the Neotropical riodinid butterfly *Adelotypa annulifera* (Godman, 1903) in Tambopata, Peru. Eggs of *A. annulifera* are laid at the tips of new growth bamboo culm sheaths bearing extrafloral nectary sites where adult butterflies and ants gather to feed. *Adelotypa annulifera* larval stages are actively tended by multiple species of ants and were observed feeding on the extrafloral nectaries of the bamboo. Pupation of *Adelotypa annulifera* occurs on the host plant near the base of the bamboo. We also document the potential kleptoparasitic behavior of adult butterflies on ant species that tend the caterpillars. To our knowledge, this is the first account describing the immature stages and life history of a species belonging to the genus *Adelotypa* and the first account of adult riodinid butterfly kleptoparasitism on ants.

Alam *et al.* (2014) studied the biology of *Pachliopta aristolochiae* on its host plant *Aristolochia indica;* the female butterfly singly laid eggs on the host plant. These were collected from the field and reared in the laboratory under optimum conditions of temperature (28 ± 3 $^{\circ}$C) and relative humidity (70 ± 5% RH). Incubation period of the egg was 5.0 ± 0.6 days, larval developmental period was 11 ± 0.3 days, pre-pupal period was 0.87 ± 0.08 day, and the pupation took 12 ± 0.63 days. The length of 1st, 2nd, 3rd and 4th instar larvae were 4.0 ± 0.63, 9 ± 0.63, 22.6 ± 5.2 and 38.2 ± 4.70 mm, respectively. The feeding potential rate of 1st, 2nd, 3rd and 4th instar larvae were 11.4 ± 5.04, 29.6 ± 5.12, 51.4 ± 6.0 and 72.8 ± 4.9%, respectively. The weight of the faeces of 1st, 2nd, 3rd and 4th instar larvae were 0.012 ± 0.004, 0.047 ± 0.018, 0.0114 ± 0.023 and 0.274 ± 0.045 g, respectively.

Barua and Slowik (2007) studied the biology of *Pachliopta aristolochiae aristolochiae* on its host plant *Aristolochia tagala*. Singly laid eggs on the host plant (*Aristolochia tagala*) were collected from the field and reared in the laboratory under optimum conditions of temperature and humidity. The egg laying behaviour of the gravid female, hatching, feeding and moulting behaviour of the four larval instars were recorded on the creeper host-plant *A. tagala*. The feeding potential of all the larval instars on the various leaf maturity stages was also recorded. The various stages of pupation up to the emergence of the adult from the chrysalis were recorded. The laboratory study revealed an incubation period of ± 4 days, larval duration of ± 20 days, pupation period of ± 12 days. The study revealed a total life cycle of ± 30 days in the monsoon season under conditions of laboratory rearing. The larval feeding potential was determined by both the maturity and availability of suitable leaves although the mature larvae were observed to feed on the stems of the host-plant in the absence of suitable leaves. Field observations and laboratory study established that *Pachliopta aristolochiae aristolochiae* endemic to Southeast Asia is on the wings throughout the year with a higher density during the wet season (May-October). It is multivoltine with 7-8 generations yearly. The species displayed single egg-laying habit, which coupled with host-plant specialization with larvae feeding on *A. tagala* and *A. indica* allowed efficient utilization of the food resources.

Munir *et al.* (2005) examined the consumption of ten different varieties of citrus plantation by butterfly *Papilio demoleus* and its effects on its larval and post-larval development at Tando Mohammad Khan, Lower Sindh. Different types of food showed significant effects on growth rate, food utilization and reproductive potential of this pest. It was revealed that the growth index value was highest being 13.84 in *Citrus aurantifolia* (kaghzi lime) while the lowest value was reported to be 4.0 in *Citrus sinensis* (orange Washington). On the basis of ovipostion preference of adult, larval survival, percentage of pupation, emergence and survival of adults, and the sequence of difference citrus varieties was determined.

Weingartner (2005) described the ability of insects to utilize different host plants which has been suggested to be a dynamic and transient phase. During or after this phase, species can shift to novel host plants. Expanding the range of host plants might also be a factor leading to higher levels of net speciation rates. In this paper, the authors have studied the possible importance of host plant range for diversification in the genus *Polygonia* (Nymphalidae, Nymphalini). The authors have compared species richness between sistergroups in order to find out if there are any differences in number of species between clades including the species that utilize only the ancestral host plants ('urticalean rosids') and their sisterclades with a broader (or in some cases potentially broader) host plant repertoire. Four comparisons could be made, and although these are not all phylogenetically or statistically independent, all showed clades including butterfly species using other or additional host plants than the urticalean rosids to be more species-rich than their sisterclade restricted to the ancestral host plants. These results are consistent with the theory that expansions in host plant range are involved in the process of diversification in butterflies and other phytophagous insects, in line with the general theory that plasticity may drive speciation.

Stefanescu (2004) described seasonal change in pupation behaviour and pupal mortality in a swallowtail butterfly - phenotypic plasticity in pupal colour has evolved to render cryptic pupae. Apart from characteristics of the pupation site, the photoperiod experienced by larvae is important in determining pupal colour, long and short photophases eliciting the formation of green and brown pupae, respectively. This seasonal polyphenism is often correlated with developmental pathway, green pupae developing directly and brown pupae entering into diapause. From 1996 to 2000, immature stages of *Iphiclides podalirius* were monitored on natural host plants in NE Spain. Larvae were followed to the pupation site and pupal colour, characteristics of the pupation site and the fate of pupae were recorded. Before August, pupae were non–diapausing green while in early August they were dimorphic, after which, they were brown and overwintered. As theory predicts, differences in pupation sites in successive generations were found in relation to pupal colour. Green pupae occurred on the host plants and brown pupae were found among the leaf litter.

Mortality ranged from 14.3 to 100%. Bird predation was the major mortality factor for green pupae and was also important for brown pupae. Results suggest that preference for pupation sites in the litter in diapausing broods evolved to avoid strong bird predation on the host plants. Preference for sites above ground level in summer generations may have evolved in response to both non–visual (small mammals) and visual (avian) predators.

Hellmann (2002) described the herbivores butterflies that forage on resources that change over time may be strongly affected by environmental factors that alter their temporal overlap with host plants. The magnitude of these effects may be mediated by the availability of alternative hosts and by behavioural adaptations for foraging on temporal food resources. This study examines the temporal interaction of a butterfly species and its two host plants to determine how larvae utilize their host resources and are affected by conditions of accelerated host senescence. By changing host use over time, this butterfly may track temporal declines in host quality and buffer the impacts of environmental variation and change. At the same time, it is hypothesized that host declines and changes in the host environment affect larval survivorship and hence butterfly population size. With three sets of field and greenhouse experiments, the following were examined: (i) larval host plant use and the dependence of larval diet on oviposition, (ii) nutritional differences between hosts, and (iii) the impact of conditions that accelerate host plant death (i.e. temperature) on larval survivorship and growth. Larvae were observed to forage widely, vary their diet through time, and use hosts independently of their natal plant. Larvae tracked changes in host quality by steadily increasing their use of the longer-lasting, but nutritionally variable, host plant. Temperature conditions that accelerated host death actually conferred a survivorship advantage when larvae were able to utilize this host. These results suggest that larval diet choice and movement may be an adaptive strategy for foraging on declining food resources. It also suggests that the net effect of environmental extremes on larvae is strongly mediated by host plant use. Differences in larval survivorship when one or both hosts are available suggest that the long-lasting host is essential to butterfly population persistence. By comparing this mobile taxa to species with fewer host-switching opportunities, we better understand the diversity of foraging strategies of food-limited insects and the effects of environmental change.

Bergman (2000) examined on oviposition, host plant choice and survival on different plants of a grass-feeding butterfly, *Lopinga achine* in the field and laboratory. Grass-feeding butterflies are generally thought to be nonspecific in their host plant choice. This seems not to be true for *L. achine*. Females were selective in their host plant choice and preferred to oviposit near *Carex montana*, although they do not attach their eggs to any plant. *Carex montana* was also generally preferred by the larvae in laboratory experiments among the plants available in the field. However, the larvae preferred three species that they seldom encounter in the field (*Agrostis capillaris, Phleum pratense* and *Poa pratensis*) before *C. montana* when they were offered these four species. Most of the larvae found in the field (>80%), were found on *C. montana*. The larvae survived significantly better on *C. montana* than on six other species in rearing experiments. The results indicate that host plant choice occurs in two steps in *L. achine*: 1) the females choose a patch to drop the egg to the ground, usually in the vicinity of a *C. montana* plant 2) the newly hatched larva moves to the host plant. The apparent dependence of the Swedish mainland *L. achine* population on a single host plant has important conservation implications.

Janz and Nylin (1998) made a database on host plant records from 437 in group taxa has been used to test a number of hypotheses on the interaction between butterflies and their host plants using phylogenetic methods (simple character optimization, concentrated changes test, and independent contrasts test). The butterfly phylogeny was assembled from various sources and host plant clades were identified according to Chase *et al.*'s rbcJ--based phylogeny. The ancestral host plant appears to be associated within a highly derived rosid clade, including the family Fabaceae. As fossil data suggest that this clade is older than the butterflies, they must have colonized already diversified plants. Previous studies also suggest that the patterns of association in most insect-plant interactions are more shaped by host shifts, through colonization and specialization than by cospeciation. Consequently, we have focused explicitly on the mechanisms behind host shifts.

The experimental results confirm, in the light of new phylogenetic evidence, the pattern reported by Ehrlich and Raven that related butterflies feed on related plants. We show that host shifts have generally been more common between closely related plants than between more distantly related plants. This finding. together with the possibility of a higher tendency of recolonizing ancestral hosts, helps to explain the apparent large-scale conservation in the patterns of association between insects and their host plants, patterns which at the same time are more flexible on a more detailed level. Plant growth form as an even more conservative aspect of the interaction between butterflies and their host plants than plant phylogeny. However, this is largely explained by a higher probability of colonizations and host shifts while feeding on tree than on other growth forms.

Veenakumari *et al.* (1997) stated the larval food plants of the butterflies of the Andaman and Nicobar islands have not been studied, although the butterfly fauna *per se* is fairly well known. For the first time they reported the food plants of the larvae of 120 species of butterflies from these islands on the basis of laboratory rearing and field studies. This information is essential for the formulation of management programmes for butterfly conservation on these islands which are known to harbour critical swallowtail and (possibly) danaine faunas.

Rausher and Papaj (1983) reported that individual females of the pipevine swallowtail butterfly, *Battus philenor*, exhibit different search modes when searching for host plants on which to oviposit. However, an alternative explanation for the results of that study exists: apparent differences in searching behaviour may simply represent differences in the composition of the vegetation over which females fly. The results from the present study rule out this alternative explanation and indicate that apparent differences in search mode reflect underlying differences among females in response to leaf shape.

2.4 Medicinal importance of butterfly related plants

This part of Review of Literature focused on evaluation of antioxidant activity, brine shrimp lethality bioassay, antimicrobial activity, isolation of different chemicals from different medicinal plants especially *Aristolochia* spp. (larval food plants of *Pachliopta aristolochiae* butterfly). Review of Literature was studied and included from 2013 to 2000 regarding this sub-topic.

Ogu *et al.* (2013) studied in vitro antioxidant and antimicrobial activities of methanolic leaf extract of *Dialium guineense* in order to provide a pharmacological basis for their ethomedicinal applications. Antioxidant activity was measured using 2,2-diphenyl-1-picrylhydrazyl (DPPH) free radical scavenging activity and reducing power assay. In addition, the total phenolic content was also analyzed. Antimicrobial activity was tested against clinical isolates of *Staphylococcus aureus, Streptococcus mutans, Escherichia coli, Bacillus cereus, Pseudomonas aeruginosa, Klebsiella pneumoniae, Proteus mirabilis, Salmonella typhi, Candida albicans, Microsporium gypseum, Trichophyton mentagrophytes* and *Trichophyton rubrum* using agar well diffusion method. The results of the DPPH scavenging activity of the extract showed a concentration dependent antioxidant activity with maximum scavenging activity (85.35%) observed at 250 µg/ml concentration and comparable to those of ascorbic (95.75%) and gallic acids (93.67%). The reducing potential of the extract (0.069 ± 0.003 nm) was also comparable to that of gallic acid (0.078 ± 0.022 nm), while the total phenolic content was 69.45 ± 0.002 mg/g gallic acid equivalent. The antimicrobial inhibition zone and minimum inhibitory concentration values of the extract ranged between 10.2 to 25.9 mm and 7.81 to 62.5 µg/ml respectively, with the Gram positive bacteria generally being most sensitive, followed by the fungi and the Gram negative bacteria. This study indicates that *D. guineense* leaf extract has significant antioxidant and antimicrobial properties, and thus substantiates its popular and wide traditional applications in diverse ailments. The plant may therefore be exploited as a potential preservative in the pharmaceutical and food industries.

Angalaparameswari *et al.* (2012) investigated the anti-microbial activity of aristolochic acid from the root of *Aristolochia bracteata.* From the methanolic and ethyl extracts of *A. bracteata* aristolochic acid I was isolated and conformed through IR, NMR and MS. The percentage purity of aristolochic acid I was determined by UV and HPLC method. Antibacterial activity of extracts of *A. bracteata* and the isolated compound was determined by disc diffusion method. The results revealed that the isolated aristolochic acid from methanolic extract was more pure than the compound from ethyl acetate extract. The various extracts (500µg/disc) of *A. bracteata* showed moderate antibacterial activity with the average zone of inhibition of 7-18 mm by disc diffusion method. Among the extracts, ethyl acetate and methanol extracts were shown good anti-microbial activity and the growth of *E. coli* (18 mm) was strongly inhibited. Microbial assay of isolated compound (Aristolochic acid I) from ethyl acetate and methanol extracts were shown good antimicrobial activity and the zone of inhibition of both at higher concentration 50 µg/ml was similar with the standard aristolochic acid. It may be concluded that the isolated compound of aristolochic acid I has good anti-bacterial activity.

Kumar *et al.* (2011) evaluated the antimicrobial activities of ethanolic extract of *Aristolachia indica* L. which was a creeper used as traditional folk medicine for the treatment of different infectious diseases and disorders. The antimicrobial activities of the extract against 12 strains belong to bacterial and fungi species were tested by using agar diffusion method. The results showed that ethanolic extract of *A. indica* had moderately significant antibacterial and significant antifungal activity. It inhibited the growth of both bacterial and fungal species dose dependently. The inhibition of growth was highest at 100mg/ml as compared to the controls. Ethanolic extract showed stronger antimicrobial activity against the fungi than that of the bacteria's. Thus it may be concluded that *A. indica* was a potent antimicrobial agent which can be tried as a Novel anti-fungal agent.

Roy *et al.* (2011) investigated the phytochemical screening, cytotoxicity and antibacterial activities of ethanolic extracts of leaves of two medicinal plants, *Aglaonema hookerianum* Schott (Family: Araceae) and *Lannea grandis* Engl. (Family: Anacardiaceae) available in Bangladesh. The brine shrimp lethality bioassay showed that the ethanolic extracts of *A. hookerianum* and *L. grandis* possessed cytotoxic activities with LC_{50} 5.25 (µg mL^{-1}) and 5.75 (µg mL^{-1}) and LC_{90} 10.47 (µg mL^{-1}) and 9.55 (µg mL^{-1}), respectively. Two extracts obtained from leaves were examined for their antibacterial activities against some gram positive bacteria such as *Bacillus subtilis*, *B. megaterium* and *Staphylococcus aureus*, also gram negative strains of *Pseudomonas aeruginosa*, *Escherichia coli*, *Shigella dysenteriae*, *Salmonella typhi*, *S. paratyphi* and *Vibrio cholerae*. Agar disc diffusion method was applied to observe the antibacterial efficacy of the extracts. Results indicated that both plant extracts (500 µg $disc^{-1}$) displayed antibacterial activity against all of the tested microorganisms. These results were also compared with the zones of inhibition produced by commercially available standard antibiotic, Amoxicillin at concentration of 10 µg $disc^{-1}$. Observed antibacterial properties of the ethanolic extract of *A. hookerianum* and *L. grandis* showed that both plants might be useful sources for the development of new potent antibacterial agents.

Apu *et al.* (2010) investigated the crude methanolic extract of *Dillenia indica* Linn. (Dilleniaceae) leaves for the evaluation of antimicrobial and cytotoxic activities. Organic solvent (*n*-hexane, carbon tetrachloride and chloroform) fractions of methanolic extract and methanolic fraction (aqueous) were screened for their antimicrobial activity by disc diffusion method. Besides, the fractions were screened for cytotoxic activity using brine shrimp (*Artemia salina*) lethality bioassay. Among the four fractions tested, *n*-hexane, carbon tetrachloride, and chloroform fractions showed moderate antibacterial and antifungal activity compared to standard antibiotic, kanamycin. The average zone of inhibition was ranged from 6 to 8 mm at a concentration of 400 µg/disc. But the aqueous fraction was found to be insensitive to microbial growth. Compared to vincristine sulfate (with LC_{50} of 0.52 µg/ ml), *n*-hexane and chloroform fractions demonstrated a significant cytotoxic activity (having LC_{50} of 1.94 µg/ml and 2.13 µg/ml, respectively).

The LC_{50} values of the carbon tetrachloride and aqueous fraction were 4.46 µg/ml and 5.13 µg/ ml, respectively. The study confirms the moderate antimicrobial and potent cytotoxic activities of *D. indica* leaves extract and therefore demands the isolation of active principles and thorough bioassay.

Kavitha and Nirmaladevi (2009) focused on the antibacterial and antifungal activity of the medicinal plant *Aristolochia bracteolata.* Aqueous, methanol and chloroform extracts of this plant were evaluated against the bacterial strains *Escherichia coli, Bacillus subtilis, Staphylococcus aureus, Klebsiella pneumoniae, Pseudomonas fluorescens, Shigella flexneri, Proteus vulgaris* and the fungal strains like *Aspergillus niger, A. terreus, Penicillium notatum* and *Rhizopus stolonifer.* Among the three extracts assessed, methanol extract was found to have the significant activity followed by the chloroform extract against certain bacteria. Water extract did not have any activity against bacteria. Antifungal activity assessment indicated that the tested fungal strains are more susceptible to aqueous extract followed by methanol extract and chloroform extract.

Rahman *et al.* (2008) investigated the cytotoxic activity of the methanolic extracts of 35 plant species, including 28 traditionally used plants of Bangladesh was evaluated by the brine shrimp lethality bioassay technique. Among these, 19 plant extracts exhibited significant toxicity to brine shrimps with LC_{50} less than 10 µg/ml.

Jahan *et al.* (2008) reported on the preliminary screenings of the antimicrobial susceptibility and cytotoxicity of the plant extract *Quisqualis indica* (Compositae). The extractives of the plant were subjected to screening for inhibition of microbial growth by the disc diffusion method. The zones of inhibition demonstrated by the n-hexane, carbon tetrachloride, chloroform and aqueous soluble partitionates of the methanolic extract ranged from 8 - 15 mm, 8 - 18 mm, 12 - 20 mm and 10 - 16 mm, respectively at a concentration of 400 µg/disc. All the extractives were also subjected to brine shrimp lethality bioassay for primary cytotoxicity evaluation. The carbon tetrachloride soluble materials demonstrated the highest cytotoxicity with LC_{50} of 0.826 µg/ml, while n-hexane, chloroform and aqueous soluble partitionates of the methanolic extract revealed the LC_{50} of 1.254, 3.866 and 5.366 µg/ml, respectively.

Al-busafi *et al.* (2004) reported on the isolation and structural elucidation of aristolochic acid-A and aristolochic acid-D from Omani *Aristolochia bracteolata* plant. Antioxidant activities of these two natural products were evaluated for their capacity to reduce Mo (VI) to Mo (V). The study revealed that aristolochic acid-D is more active than vitamin C while aristolochic acid-A has activity similar to vitamin C.

Wu *et al.* (2003) investigated the bioactive compounds from *Aristolochia* species, 28 compounds, including three new constituents, demethylaristofolin E (1), aristomanoside (2), and dehydrooxoperezinone (3), were isolated from an extract of the stems of *Aristolochia manshuriensis.* The structures of these compounds were established by extensive 1D and 2D NMR spectral studies. Among these compounds, dehydrooxoperezinone (3) was found to inhibit the replication of HIV, with an EC_{50} value of 17.5 µg/mL and a therapeutic index of 1.43.

Wu *et al.* (2002) extracted four new tetralones, aristelegone-A (1), aristelegone-B (2), aristelegone-C (3), and aristelegone-D (4); one new isoquinoline, pericampylinone-A (5); four new biphenyl ethers, aristogin-A (6), aristogin-B (7), aristogin-D (8), and aristogin-E (9); three new lignans, aristelegin-A (10), aristelegin-B (11), and aristelegin-C (12); and a new dimer, aristolin (13), were isolated from the root and stem of *Aristolochia elegans.* The structures were established on the basis of 1D and 2D NMR and mass spectral data. This was the first report of isoquinolones and biphenyl ethers from this plant which may be representative units for the formation of bisbenzylisoquinoline alkaloids that were common metabolites of *Aristolochia* species. Aristolin (13) is also the first report of a diterpene linked with an aristolochic acid.

Sime *et al.* (2000) reported that the North American pipevine swallowtail, *Battus philenor* (L.) (Papilionidae, Troidini), is protected from natural enemies by aristolochic acids sequestered from its *Aristolochia* food plants. This study confirmed that populations of *B. philenor* from Virginia and east Texas sequester these compounds. A comparison of the aristolochic acid profiles of the Virginia butterflies and their *A. macrophylla* food plants revealed several differences. The aristolochic acid fraction of the foliage was dominated by aristolochic acids I and II, whereas the insects had a much lower proportion of aristolochic acid II and contained, in addition, substantial amounts of aristolochic acids Ia and IVa, which were not detected in the plants. The eggs, larval integument, osmeterial glands, pupal cuticle, and adults (wings and bodies) all contained aristolochic acids. These findings help to explain the abundant ecological data indicating that both immature and adult *B. philenor* are unpalatable and protected from natural enemies.

Wu *et al.* (2000) isolated constituents from the leaves of *Aristolochia elegans.* One new biphenyl ether, aristogin C (1), and two new porphyrins, aristophylls A (2) and B (3), as well as 11 known compounds, were isolated from the leaves of *A. elegans.* Their structures were elucidated according to the spectroscopic (NMR and MS) analyses or by comparison with literature values.

The research work was completed by following and gathering knowledge of above mentioned Review of Literature. The findings of Ph. D thesis were discussed in Chapter 3, 4, 5 and 6 by following the sub-topics of Review of Literature.

Chapter 3. Behavioural activities of butterfly through its colonization in the Butterfly Research Park at Bhawal National Park, Gazipur

3.1 Abstract

To detect the nutritional and medicinal plants in nature through behavioural activities of butterfly, total 15,411 plants (different stages) were planted for butterfly colonization in Butterfly Research Park (BRP) at Bhawal National Park, Gazipur, Bangladesh. Among them 10,740 plants (69.69%) were survived to colonize butterflies in the Butterfly Research Park. Total 5,365 individuals of host plants (different stages) under 13 species were planted; among them 3,849 plants (71.74%) survived and developed in the BRP. Total 10,046 nectar plants were planted, among them 6,891 plants (68.59%) under 15 species developed and survived for sustaining the butterfly colonization in the BRP.

The dynamism of butterfly population was assessed in relation to the nectar plants in the BRP from January 2012 to December 2012. Total 6,767 individuals of butterfly were recorded belonging to seven families on the 20 species of nectar plants. The butterfly population in relation to foraging behaviour on the different nectar plants viz., Asclepias, Harina lata, Lantana, Cosmos, Botamful, Chandramallika, Jaba, Hatisur, Gadha, Marhatitiga, Dopati, Dondokolos, Setodron, Monkata, Panika,Weedelia,Nilpetunia, Rongon, Katamehedi and Taralata were 16%, 1.1%, 10, 5.7%, 0.1%, 0.1%, 0.6%, 9.7%, 1.7%, 25.4%, 0.07%, 0.48%, 1.5%, 0.51%, 3.0%, 8.0%, 6.6%, 1.1%, 0.3% and 7.3%, respectively.

Total 9,588 butterfly individuals were observed under six behavioral patterns at 31.1±4.9 (ºC) and 62.03±15.7% RH. The population density of butterfly in relation to foraging, resting, egg-laying, mating, searching and puddling were 70.6%, 5.3%, 2.0%, 1.4%, 19.2% and 1.3%, respectively. Butterflies belonged to seven family viz. Papilionidae, Danaidae, Nymphalidae, Pieridae, Satyridae, Lycaenidae and Hesperidae were recorded 8.8%, 30.7%, 31%, 16.4%, 0.32%, 1.7% and 25.6%, respectively.

3.1 Abstract

The population fluctuation of butterfly in relation to seasonal variation were recorded at 31.1±4.9 (ºC) and 62.03±15.7% RH. The highest density of butterfly was recorded in May (15.32%) and lowest in October (3.29%), respectively. Total no. of genera (n=57) and species (n=92) of butterfly in Butterfly Research Park, Bhawal National Park during the experimental periods were identified under 10 families and recorded. The identified butterflies were categorized into major and minor families on the basis of availability. The major families are Papilionidae, Danaidae, Nymphalidae, Pieridae, Satyridae, Lycaenidae and Hesperiidae, and minor families are Acraeidae, Riodinidae and Amathusiidae. Out of 92 butterfly species, the status of butterfly is Available (83.69%), Rare (13.04%), Near Threatened (2.17%), Critically Threatened (1.08%) and Endangered (0%).

Key words: Colonization, butterfly, behavioural activities, host plants, nectar plants, population density, Butterfly Research Park (BRP)

3.2 Introduction

The EBBL, Department of Zoology, University of Dhaka has been working since 1999 on the southeast areas of Bangladesh on the biotic-biotic interactions (plant-butterflies interactions and their associations) and found significant result that the butterfly-colonization processes have great role in assessment of the impact of climate changes on the forest and biodiversity. The research work of the laboratory has been practiced in different experimental stations. These are under the greater districts of Chittagong and Sylhet. The EBBL's day to day experimental areas are: Zoological garden, Curzon Hall, University of Dhaka; Bhawal National Park (experimental place allotted by Forest Department, GoB), and Satchari National Park of the Sylhet division. Other than day to day experimental stations, more forest areas are taken under the grand research programme of biodiversity conservation. These are Satchari, Noorjahan, Chautali, Lawachara, Phoolbari, Anarasbari and Rema-kalenga under the greater Sylhet district; Karerhat, Mirsarai, Sitakunda, Tongabati, Chunati, Fashiakhali and Eidgaon are under the greater district of Chittagong. Among the experimental stations, Satchori forest (Research Experimental Station), Zoological Garden (Germplasm Centre) and Butterfly Research Park (Butterfly Colonization Centre) were selected to conduct Ph. D research entitled "**Behaviour of some selected butterflies and its influence on nutritional and medicinal values of host plants**".

Butterfly Research Park is a place where butterfly-colonization is established with all biotic and abiotic requirements in interactive dynamism. This interactive mechanism must be in open place without any artificial boundary where the butterflies can have their freedom of movements, migration, niche selection, vulnerability to the predation, facing disastrous situation, progeny production, pre-mating and mating playing supports, sheltering assurances, pupating supports, nutrition availabilities and pollution free atmosphere (both abiotic and biotic atmosphere). Courtship behaviours, pre-mating and mating stages, egg-laying strategies, larval feeding habits and plant specificity, pupal resting and hibernation, adult-activities and behaviours require a sound colonizing ecosystem. This sound ecosystem-sustenance is indicated first by the soundness of courtship behaviour in butterflies (Bashar 2014a).

It is important to study some ethological aspects in butterflies on the subject of daily activities and daily weather fluctuations. They warm up by basking in the sun, usually with the wings out-spread and the body orientated so that the maximum area of the wing is exposed to the sun. The colour patterns on the wings may assist in heat absorption; those species with large black patches are particularly efficient heat gatherers (Bashar 2014a). Butterflies cool themselves by seeking the shade, or if shelter is not available they may close their wings together and face the sun so that the smallest possible surface is exposed to the sun-rays. It has been suggested that cooling of the body may also take place by the evaporation of water, but this idea is not fully accepted (Fres 1989).

According to the forest researchers it is found that, other phenomenon such as forest fires may have a serious effect on a butterfly population; the adults are usually able to fly to another suitable area, but their eggs, larvae and pupae risk total destruction by fire. It is evident that, butterflies have characteristic and attractive relationships with other species of plants and animals. The success of population sustenance in butterflies depends on their relations with other species and also with other individuals of their own species. The effects of these intra and inter-relationships are usually correlated with the density of the population in a particular area. If the area is a forest ecosystem then the example stands most appropriate for interpretation (Bashar 2014a).

Butterflies are one of the most interesting and fascinating insect groups. Butterflies widely appreciated for their aesthetic value are important as ecological indicators (Chakravarthy *et al.* 1997). The butterflies are selective in their choice of flowers and plants they visit. There is an intimate association between butterflies and plants (Uniyal and Mehra 1996). The rate of visit of butterflies to a flower depends on color, odor and the shape of the flower. Butterflies and their caterpillars are dependent on specific host plants for foliage, nectar and pollen as their food. Thus, butterfly diversity indirectly reflects overall plant diversity, especially that of herbs and shrubs, in the given area. Change in land use pattern leads to landscape changes that can reflect change in butterfly diversity and distribution (Padhye *et al.* 2006). Adult butterflies are considered opportunistic foragers that visit a wide variety of available flowers (Courtney 1986).

Butterflies are often considered to be opportunistic foragers that visit a wide variety of available flowers (Sharp *et al.* 1974 and Dosa 1999). However, their choice of flowers is not random and they often exhibit distinct flower preferences, which can differ between species (Jennersten 1984). The choice of plants as nectar sources by butterflies depends on various factors, including innate colour preferences (Ilse 1932, Jolivet 1986, Weiss 1997, Pariset and Racheli 1998, Dósa 1999). The effectiveness of butterfly foraging depends in part on corolla depth, clustering of flowers, but also on proboscis length, which limits the range of flowers from which nectar can be extracted (Porter *et al.* 1992 and Corbet 2000).

The floral scent is an important cue signal used by butterflies initially to identify and subsequently to recognize and distinguish among rewarding plants (Andersson 2003). Several authors have shown that nectar quality, quantity and concentration affect the longevity and reproduction of butterflies (Murphy *et al.* 1984, Hill and Pierce 1989, Erhardt and Rusterholtz 1998 and Rusterholz and Erhardt 2000). It has been also documented that different generations of bivoltine or multivoltine species may change their preferences due to seasonal variations in available flowers (Loertscher *et al.* 1995, Pariset and Racheli 1998, Rusterholz and Erhard 2000).

Butterflies require species specific host-plants, selective egg-laying supports, pupating and resting plants; they need to remain within the choice of their range of behavioural activities and adaptabilities (Bashar 2012b). But, foraging behaviours are many and highly characteristics in the different families of butterflies (Bashar 2014a). They need large volume of plant variety in connection with suitable ecological conditions. These requirements stand essential for the normal life-building of butterflies (Bashar 2010a). The maintenance of such combinations provides an optimum colonizing environment as “in-situ” conditions for the butterflies, i.e. the sound “home-place” for them.

3.2 Introduction

During the past century numerous researches have been conducted and findings have been published on insect host plant interactions by earlier researchers. These have been primarily dealt with natural history, but many are theoretical as well (Ackery 1991, Brues 1920 and Gilbert 1972). Due to high degree of host-specificity, most of the butterflies appear to select their host plants on the basis of secondary products chemistry rather than on the basis of general ecological consideration. Other groups of insects are fewer hosts specific, and with these insects ecological theories have progressed (Gilbert 1972). Insects which feed on a definite few host plant species and those which feed upon a wide variety of host plant species are called oligophagous and polyphagous, respectively. The chemicals are characteristic of the host plant used by butterfly, this causes the butterfly to oviposit on the correct type of host plants (Schoonhoven 1973).

The butterfly colonization is a process of establishing butterfly-plant interaction in an open area under the presence of all required necessaries (plants, butterflies, water-channel, optimum humidity, temperature, light and other abiotic factors) in a channel which can play as a vital role model for the sustenance of an ecosystem. In this ecosystem, the biotic-biotic interactive mechanism (butterfly-plant interaction) maintains a synchronization of coincidences between the life stages of associated plants (Bashar 2012a).

Through the establishment of colonization, the dynamism in it ensures the conservation of biodiversity in an ecosystem. The establishment and sustenance of butterfly open colonization create such an ecologically ‘functioned’ open ‘situ’ where the species richness of plant and butterflies becomes enriched (Islam *et al.* 2014). Conservation ensures the rational optimum use of the biological resources in accordance to the demand and need to protect them for future use. Insect-plant relationship and host-plant selection strategies are based on insect’s plant-recognition abilities and adaptations in an ecological condition suitable for both of them (Jermy 1988).

3.2 Introduction

The butterfly colonization has been established in an open ecosystem in the Bhawal National Park of Bangladesh. The butterfly colonization has been tested by examining different butterfly activities like foraging, resting, egg-laying, pre-mating, mating, searching, puddling, gene-flow activities, territorialities, larvae and larval activities, pupating patterns, emerging behaviours, prey-predator activities and life cycle of butterflies (Bashar 2014a).

The present experiment was undertaken in view to study behavioural activities of butterfly through accommodation of butterfly related host and nectar plants for butterfly colonization especially emphasizing on the establishment of Butterfly Research Park (BRP) at Bhawal National Park, Gazipur.

3.3 Material and Methods

The EBBL deals with establishment of butterfly colonization centres in the Butterfly Research Park (BRP) at Bhawal National Park, Gazipur. Practice of methodologies for colonizing butterflies and its application that deals with three major facts, viz. the plant selection, the adaptation strategy of fauna (butterflies), the activity-outcome relationships. For materialization of the butterfly-colonization mechanism above facts are to be processed successively in the question of species assemblage both of fauna (the butterflies) and the related flora (the respective plants). Successful butterfly colonization incorporates three different types of plants such as the host or larval plants; the nectar producing plants; the shade plants; and the plant-butterfly maintenance. Maintenance and culture of these plants make a suitable arrangement (compartmental habitat) for preparing a butterfly park. Colonization of butterflies needs very smoothly running powerful management system. This system includes seed banking, seedling, plantation, good nursing system, pest management practice and maintenance of the host plants. Their seasonal and phenological coincidences are to be made accessible to the butterflies at the different life stages. Identification of the host plant is another vital work for colonizing butterflies in certain place. As per design of the butterfly colonizing centres, plans are to be envisaged very carefully. Up to the present day, the EBBL has identified about 150 such plants (wild) to be used for establishing of butterfly colonization centre (Bashar 2014a).

3.3.1 Procedure of butterfly colonizing centre frame-work

The forest Department of the Government allotted ten-acres of land to the Department of Zoology, University of Dhaka for forest conservation technique innovation. Three-acre area of the allotted ten acres were selected for doing research experiments on the establishment of an open butterfly park (Plate 3.2). This area was designed with four area-components as hedge-boundary (10%), canopy-tree area (30%), jungle-bush hedges (30%) and multimorphic beds area (30% of total experimental area) which is shown in Plate 3.1. The hedge boundary was prepared with the composition of approximate 30±5 essential different natural floral species (Plate 3.6D).

The Canopy-tree was designed and prepared with tall-trees and their associated vines and climbers. It is a typical area with canopy covering and man-height supportive bushes. The Jungle-bush was a bushy area that was prepared with biotic composition of vines, herbs, shrubs, climbers, trees, grasses and also with the canes population. The area ensures safe pupation and quick sheltering (due to sudden extreme changes in weather) for the butterflies (Plate 3.1). Different kinds of soil beds were prepared for the growth and maintenance of host, nectar and shelter plants. The plantation in these areas provides with various blooming flowers in relation to the seasonal variations. In addition to that, the area components show a decorative value that attracts social visitors also. The above biotic conditions constitute a suitable assemblage for the survival of considerable number of fecund butterflies in the park area. This type of biotic mechanism creates vital factors for butterfly colonizing system.

3.3.2 Land preparation and plantation by organic farming practices

An accommodation of plants in the colonizing centre was exercised by following some techniques to provide nutrition, sheltering, safe pupation and egg-laying support during the developmental stages and for the life cycle of butterflies. These techniques consisted of various successive stages for land preparation, viz. bed modeling preparation, sand analysis, sand collection, and application, organic manure preparation & application, and plant culture & plantation.

3.3.2.1 Bed preparation

Different sizes and shapes of beds were prepared for plantation in the colonization centre. The bed preparation depends on the type of plants. Normally three types of plants were planted for butterfly colonization; these were host-plants, nectar-plants and shelter-plants. The bed preparation techniques for different types of plants varied from one type to another (Plate 3.3 A-F).

3.3.2.2 Sand analysis, sand collection and application

Sand analysis was dealt with accommodation and mixing of sand with hard soil in the forest area for making the soil ecology-aerable for seedlings and plantation. The collection of sand and its application had been maintained continuously for five years till the colonizing centre was made suitable for the target uses.

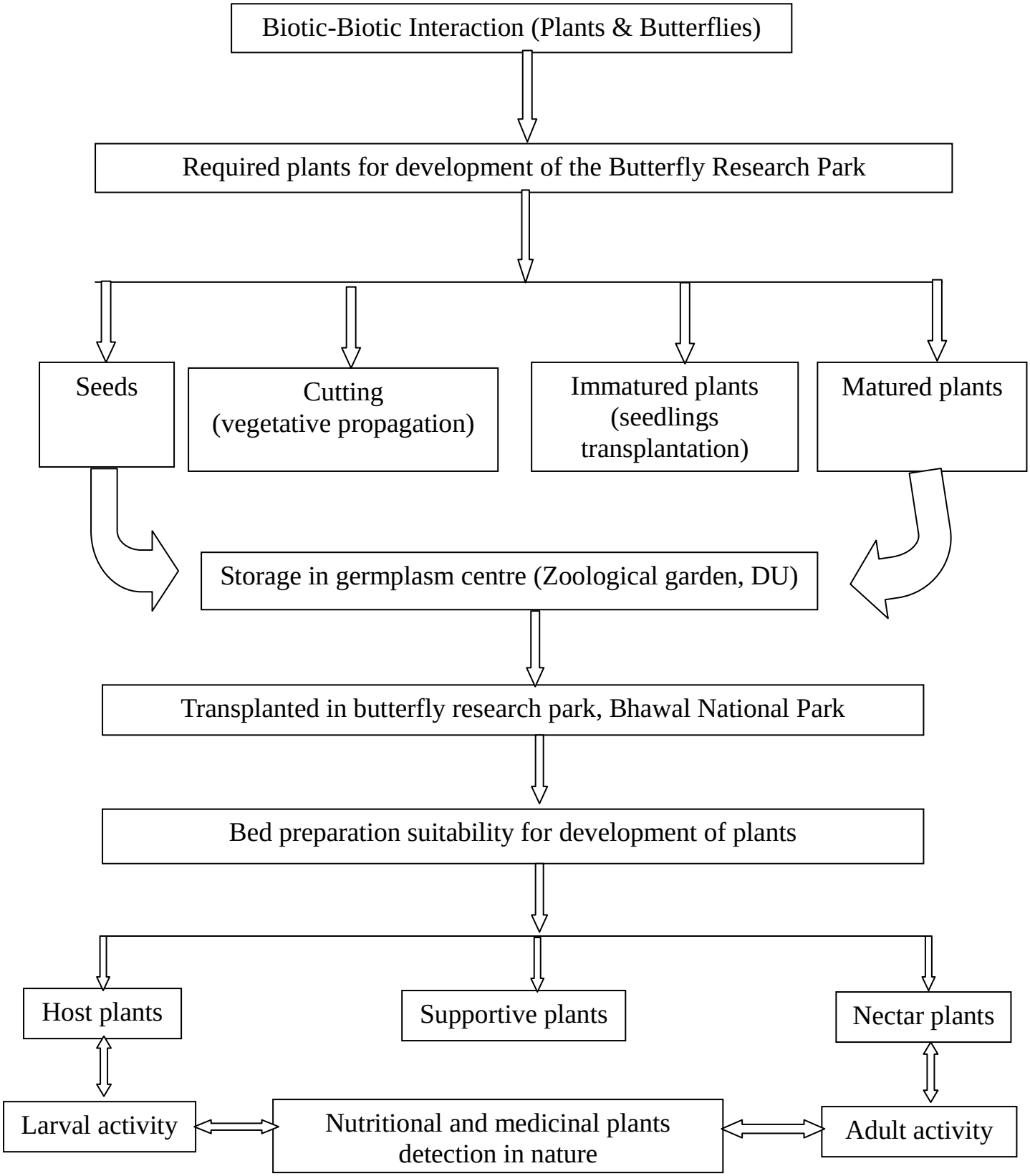

Fig. 3.1. Schematic representation of development of the Butterfly Research Park at Bhawal National Park, Gazipur.

3.3.2.3 Organic manure preparation and application

A green manure crop was grown for a specific period, and then ploughed under soil and incorporated into the soil. Cow dung was applied to the soil in an appropriate proportion before plantation and after plantation. The dung was provided and maintained for keeping the plants of the park in a healthy reproductive condition. Compost was decomposed and recycled as a fertilizer and soil conditioner. The process of composting required supply piling up waste outdoors and waiting for the materials to be broken down in six weeks or more (Plate 3.4 A-F).

3.3.2.4 Plant culture and application

The plant culture was grossly done by adopting two processes as seed bank and vegetative propagation. A seed bank was maintained in the Germplasm Centre, Zoological Garden at Curzon Hall, University of Dhaka. The seed bank was established for the storage of seeds as a source for planting in case our natural seed reserves were destroyed. The healthy seed production process was innovated in the park premises (Plate 3.5A-F). The vegetative propagation was essential for the colonizing of plants to establish the butterfly farming. The EBBL developed a technique for vegetative propagation and it was practiced in the target forests. The plant culture and plantation were followed by some steps for the butterfly colonization procedures. These were arrangement and management of plant, phyto-fencing, water-canalization and water-bodies maintenance, and target area determination.

3.3.3 Colonization process-success and butterfly activities

Butterflies require a strong and successful association with their plants, but they stand difficult to examine whether the relationship occurs successfully or not. The establishment of a sound relationship between butterflies and their related plants can be assessed by studying different activities among themselves. The activities of butterflies are: foraging, puddling, resting, gene-flow activities, territorialities, pre-mating, mating, egg-laying behaviours, larvae and larval activities, pupating strategy, emerging behaviours, predator activities and life cycle of butterflies (Bashar 2014a).

Different behavioural strategies of butterfly in relation to host plants and nectar plants were studied after colonization success at Bhawal National Park. A major requirements for butterfly colonization is accumulation of host, nectar and shelter plants in a correct proportion in a particular ecosystem (Plate 3.6 A-F). Pre-experiment was conducted once per month in a Satchari forest to study on butterfly distribution, abundance, behavioural activities and biology in relation to host, nectar and shelter plants. Developmental stages, viz. eggs, larvae, pupae and adults were collected from Satchari forests by following the methodology of Ek-Amnuay (2006), Braby (2000), Dover (1989) and Wynter-Blyth (1957). The butterfly related host, nectar and shelter plants were also collected for further identification and development at Germplasm Centre, Curzon hall, University of Dhaka in view to butterfly colonization at Bhawal National Park, Gazipur. The identification of butterfly and their status, associated host and nectar plants were studied regarding butterfly colonization in the Butterfly Research Park according to the guide of Kunte (2006) and Bashar (2015a).

Data recording and photography were started from 9.00 am to 13.00 pm and 14.00 pm to 17.00 pm after 1 hour break at noon in the Butterfly Research Park, Bhawal National Park. The population density of butterfly was recorded on the basis of different ethological aspects, viz. foraging, resting, egg-laying, mating, searching and puddling regarding the butterfly colonization according to Mamun *et al.* (2008), Young (2007), Courtney and Parker (1985). Factors influencing nectar plant resource visits by butterflies were studied by following the methodology of Ashish *et al.* (2006), Tudor *et al*. (2004) and Erhardt (1991a). Environmental factors including seasonal variation, temperature, relative humidity and their effects on the fluctuation of butterfly population were studied and recorded during the experimental period by following the methodology of Pollard (1988), Pollard *et al.* (1993) and Moss and Pollard (1993).

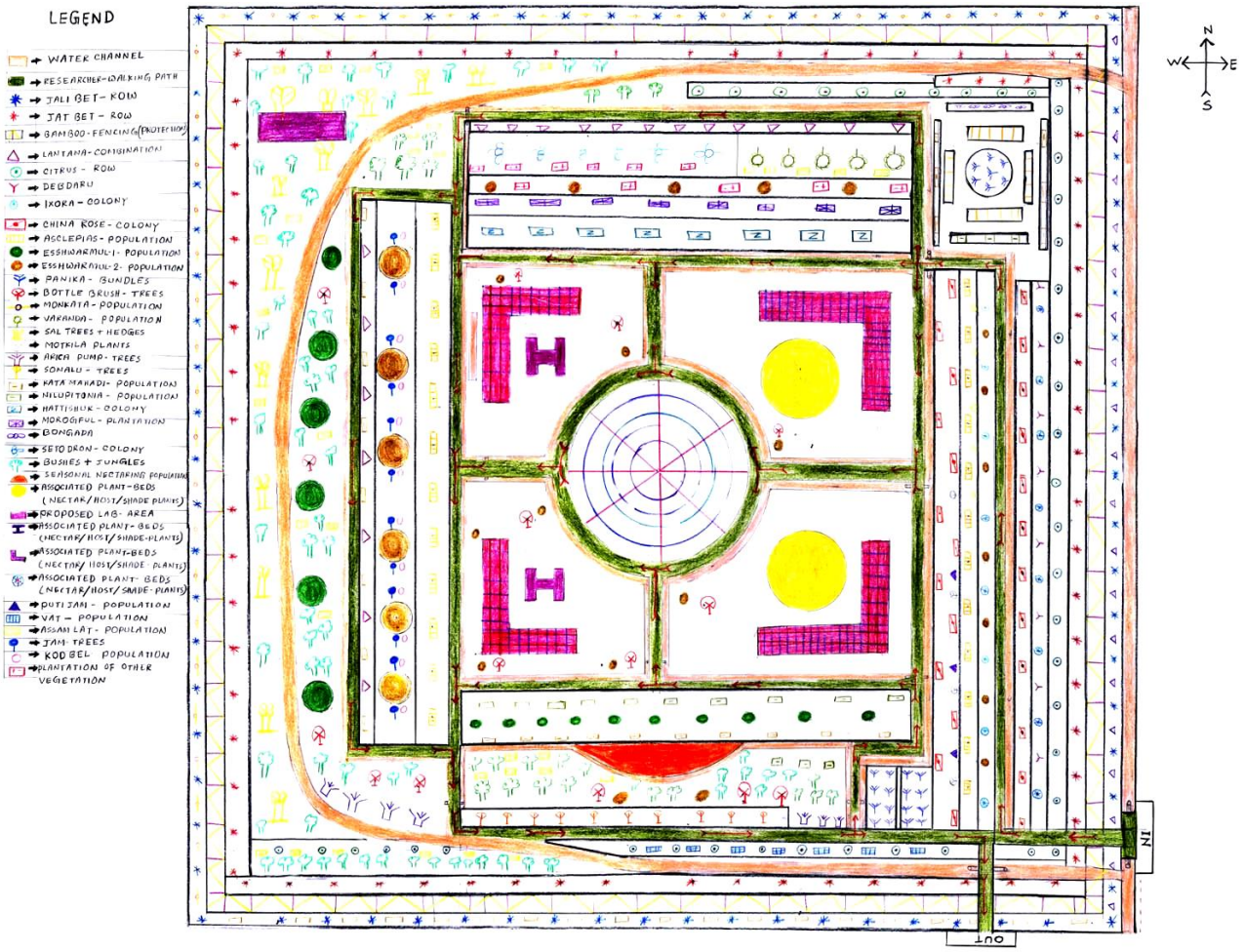

Plate 3.1. A model sketch of Butterfly Research Park (BRP) for establishing sustainable Butterfly Colonization Centre at Bhawal National Park, Gazipur.

Plate 3.2. The open field was allotted to the EBBL, Department of Zoology, University of Dhaka by Forest Department, Government of the People's Republic of Bangladesh for establishing Butterfly Research Park (Photo: 2007).

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Plate 3.3. Bed suitability and management for butterfly colonization in the Butterfly Research Park, Bhawal National Park. A. The Guide supervised field workers to prepare suitable bed B. The researcher prepares bed itself by changing the soil structure C. T-shaped bed was prepared for nectar plants plantation D. Nectar plants were planted in L-shaped bed E. Vegetative propagation of nectar plants through cutting method F. The Guide spraying water for growth and development of nectar plants.

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Plate 3.4. Green and organic manure preparation and application in the Butterfly Research Park (BRP), Bhawal National Park. A. Green manure bed B. Fallen dry leaves were deposited C. Deposition of cow dung D. Weeds were stored E. The researcher was spraying water on dry leaves. F. Weeds, dry leaves, green manure and dead plant materials were pressed and covered by soil to be rotten for preparing compost.

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Plate 3.5. Establishment of seed banking process in the Butterfly Research Park (BRP), Bhawal National Park. A. Collection of seeds B. Preservation of collected seeds in laboratory C. Sample of seeds D. Researchers were sowing seeds in seed bed E. Seed beds of *A. indica* F. Seed beds of *Asclepias curassavica.*

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Plate 3.6. Successive development of Butterfly Research Park (BRP), Bhawal National Park. A. Initiation of plantation of host plants (*Citrus spp.*) B. Development of *Citrus spp.* from immatured seedlings C. The Guide is preparing hedge boundary D. Development of natural hedge boundary E. The researcher is preparing artificial food supply technique for adult butterfly. F. Colonization of satyrid butterflies in an artificial food supply.

Plate 3.7. Establishment of the Butterfly Research Park (BRP) for butterfly colonization as a part of biodiversity conservation and bio-resource management at Bhawal National Park, Gazipur.

3.3.4 Diversity Indices: Shannon's H and E

The Shannon diversity index (*H*) is used to characterize species diversity in a community. Shannon's index accounts for both abundance and evenness of the species present. The proportion of species *i* relative to the total number of species (p_i) is calculated, and then multiplied by the natural logarithm of this proportion ($\ln p_i$). The resulting product is summed across species, and multiplied by -1:

$$H=\sum[(pi)\times\ln(pi)]$$

Where −

pi = proportion of total sample represented by species i. Divide no. of individuals of species i by total number of samples.

H_{max} = ln(S) = Maximum diversity possible

S = number of species, = species richness

$$E = \text{Evenness} = \frac{H}{H_{max}}$$

Shannon's equitability (E_H) can be calculated by dividing *H* by H_{max} (here $H_{max} = \ln S$). Equitability assumes a value between 0 and 1 with 1 being complete evenness.

3.4 Results and Discussion

Butterfly colonization is a natural process of initiation and the process for maintenance of species richness and species assemblage in an ecosystem. But practice of butterfly colonization is the mechanization to establish the natural processes of species assemblage both for plants and animals. This colonization enhances richness of biodiversity significantly and ensures natural safeguard for biodiversity conservation. But this is not usual for all regions of the world. Establishment of this process of species richness is mostly exercisable and practicable in the tropical and sub-tropical regions. Butterfly requires minimum three categories of plants (shelter plant, nectar plants and host plants) together with availability of optimum humidity. This situation stands more applicable for the maintenance of an ecosystem with species richness. The EBBL aims to colonize butterfly in the natural way for making it as a permanent key factor for biodiversity conservation as a whole. This is an attempt to justify the value-judgment practice to give environmental services to the ecotourism and to the nature conservation. And this is the "sagesse" of the EBBL in Dhaka University (Bashar 2014a).

3.4.1 Development of plants

To study the behavioural activities of butterfly and to examine the nutritional and medicinal importance of the plants in relation to butterfly, total 15,411 plants (different stages) were planted for butterfly colonization in Butterfly Research Park at Bhawal National Park. Among them 10,740 plants (69.69%) were survived to colonize the butterflies in the Park. All planted plants were categorized into two types, host plants and nectar plants (Fig. 3.2).

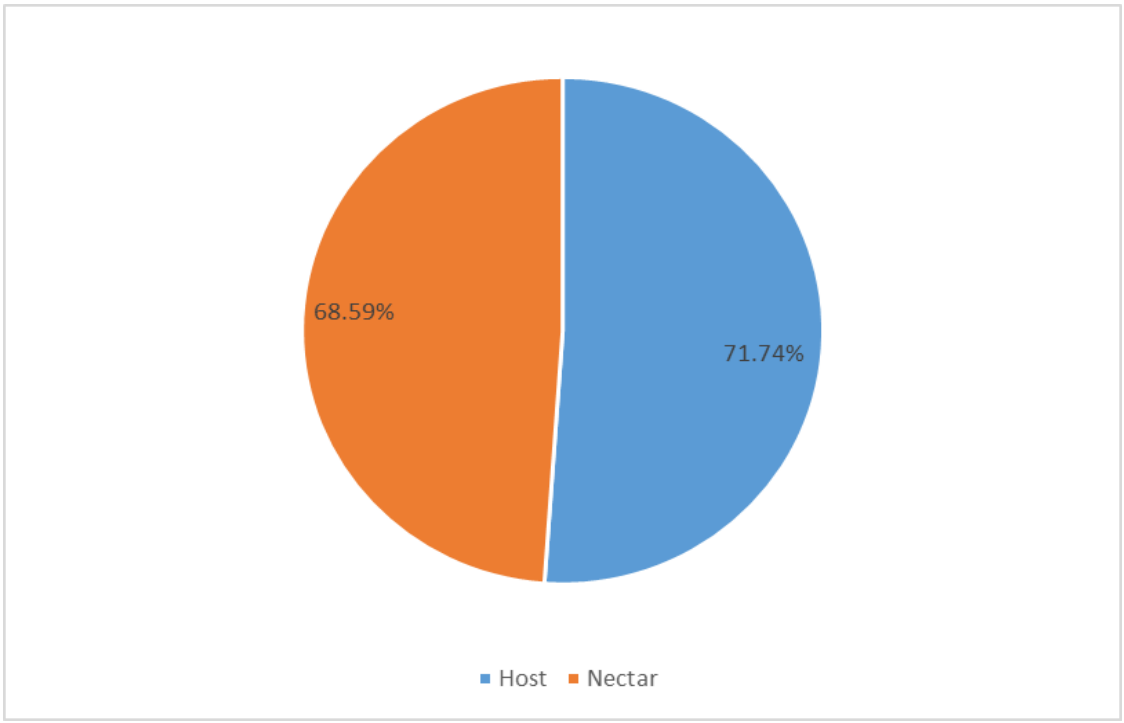

Fig. 3.2. The survival rate of butterfly related plants for butterfly colonization in Butterfly Research Park, Bhawal National Park, Gazipur.

3.4.2 Development of host plants

Total 5,365 individual of host plants (different stages) under 13 species have been planted; among them 3,849 plants (71.74%) have been survived and developed in the BRP. The survival rate of the host plants are *Aristolochia indica* (69.14%), *A. tagala* (82%), *Asclepias curassavica* (68.74%), *Citrus* spp. (71.57%), *Polyalthia longifolia* (78.88%), *Cassia tora* (90%), *Glycosmis pentaphylla* (68.57%), *Feronia limnia* (84%), *Ricinus communis* (78.46%), *Cassia alata* (74%), *Cassia occidentalis* (83.3%), and *Scaparia duleis* (84.76%) (Fig. 3.3. and Appendix 1). The host plants are the key factors for the colonization of butterflies especially regarding the development of larval stages of butterfly as nutritional sources. Larval activity in relation to host plants indicates the chemical value regarding the medicinal importance of the host plants in nature.

It is easy to detect the medicinal plants in nature by studying the larval behavioural activities with the host plants in nature. Butterflies are highly host specific insect that means a particular species of butterfly utilizes a particular type of plant as nutritional source for its development. It has been found that the host plants can be used tremendously for mankind as medicinal and nutritional agents. It has been observed that the butterfly related host plants have highly aesthetic demand as different products.

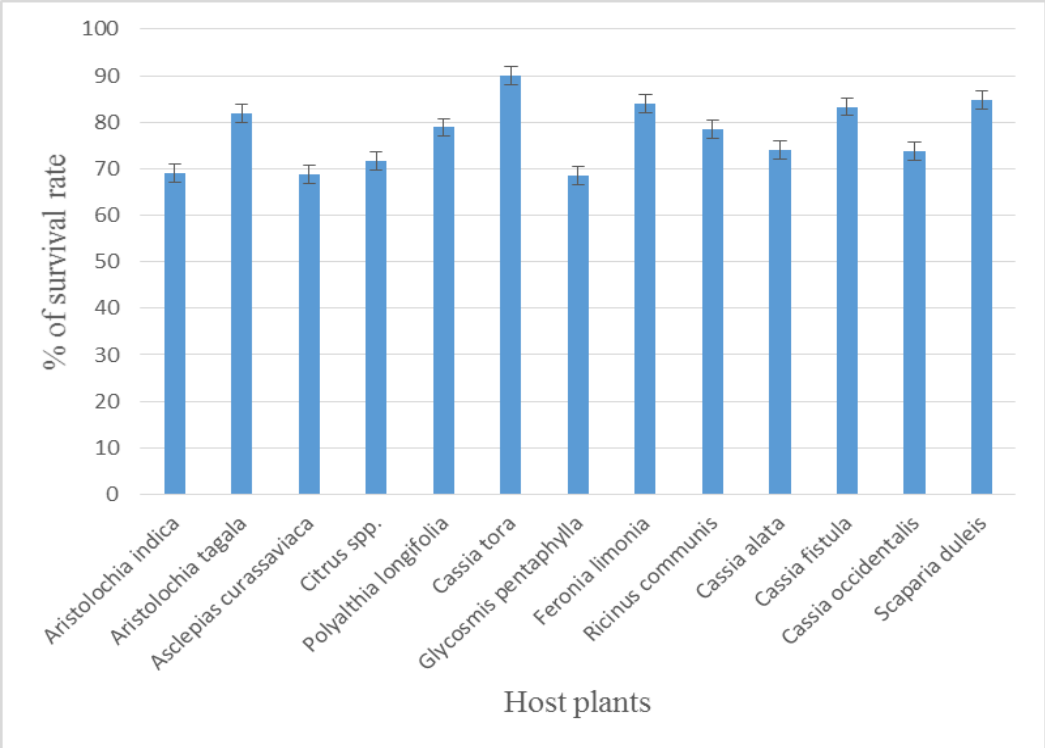

Fig. 3.3. The survival rate of host plants (n=13 spp.) for butterfly colonization in Butterfly Research Park.

3.4.3 Development of nectar plants

To study the behavioural activities of adult butterfly, total 10,046 nectar plants have been planted, among them 6,891 plants (68.59%) under 15 species have been developed and survived for sustaining the butterfly colonization in the BRP. The nectar plants are *Lantana camara* (63.72%), *Duranta plumeri* (69.84%), *Hibiscus rosa sinensis* (81.25%), *Duranta repens* (71.73%), *Tagetes patla* (90%), *Ixora chinensis* (94.89%), *Heliotropium indicum*

(61.17%), *Cosmos bipinnatus* (52.03%), *Weedelia calendulacea* (91.34%), *Punica hybrida* (88.57%), *Spilanthes calva* (92.23%), *Leucas linifolia* (71.15%), *Helianthus annus* (77.77%), *Euphorbia pulcherrima* (88.57%) and *Gomphera globosa* (83.44%) (Fig. 3.4. and Appendix 2). The nectar plants are the key factors to develop and to colonize the adult butterfly in the BRP. The nectar plants provide nutrition for adult butterfly. It has been found that nectar in the flower is a good source of sugar and amino acids which are usually taken by butterflies for their development. The utilization of nectar plants by butterfly highly varied from species to species. It depends on the length of proboscis adaptability of butterfly in relation to the size of corolla length to take the nectar from the nectar guide. So, butterflies do not select the nectar plants randomly, the choice of nectar plants by butterfly is species-specific regarding their nutritional sources (Bashar 2014a).

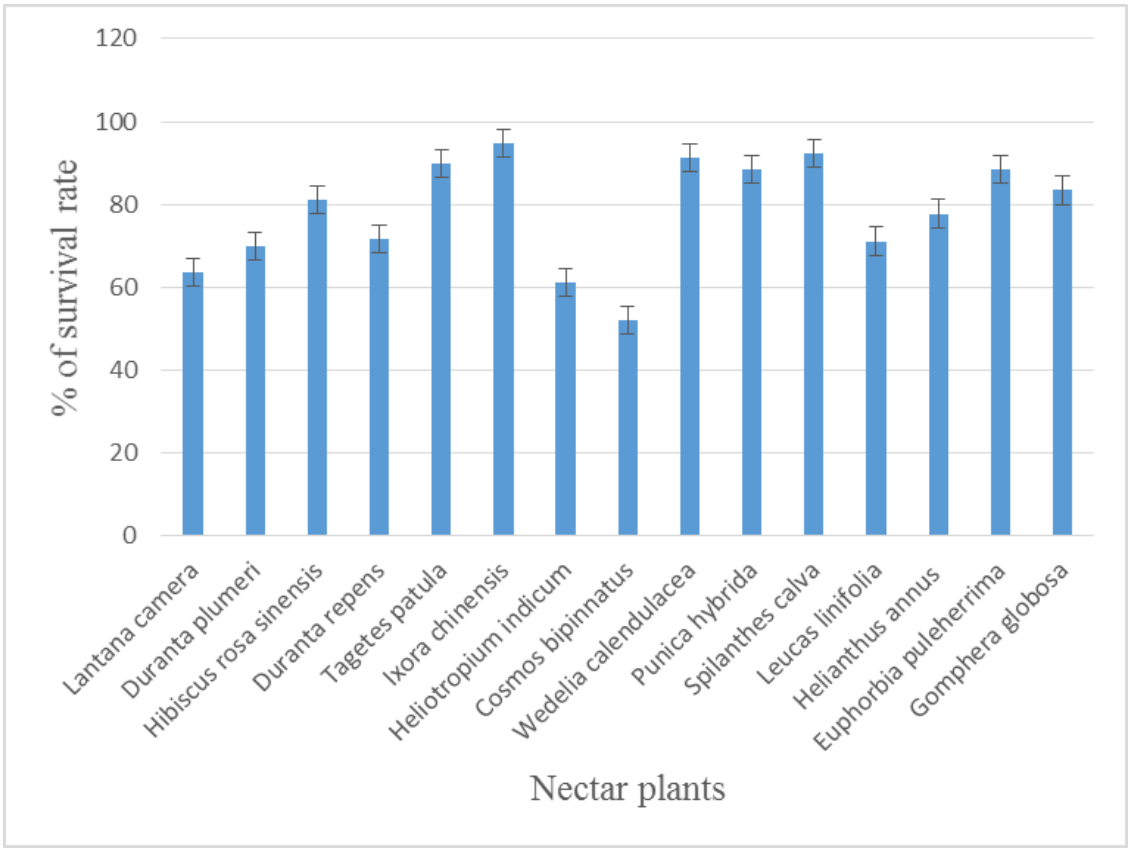

Fig. 3.4. The survival rate of nectar plants (n=15 spp.) for butterfly colonization in Butterfly Research Park, Bhawal National Park.

3.4.4 The visits of butterfly on nectar plants

The dynamism of butterfly population have been assessed in relation to the nectar plants in the BRP from January 2012 to December 2012. Total 6,767 butterflies have been recorded belonging to seven families on the 20 species of nectar plants at the mean temperature and mean relative humidity of 31.1±4.9 ºC and 62.03±15.7%. The butterfly population in relation to foraging behaviour on different nectar plants, viz., Ap, Hl, Lt, Cm, Bt, Cd, Jb, Hs, Gd, Mt, Dp, Dk, Sd, Mk, Pn, Wd, Np, Rg, Km and Tl are 16%, 1.1%, 10%, 5.7%, 0.1%, 0.1%, 0.6%, 9.7%, 1.7%, 25.4%, 0.07%, 0.48%, 1.5%, 0.51%, 3.0%, 8.0%, 6.6%, 1.1%, 0.3% and 7.3%, respectively (Fig. 3.5. and Appendix 5).

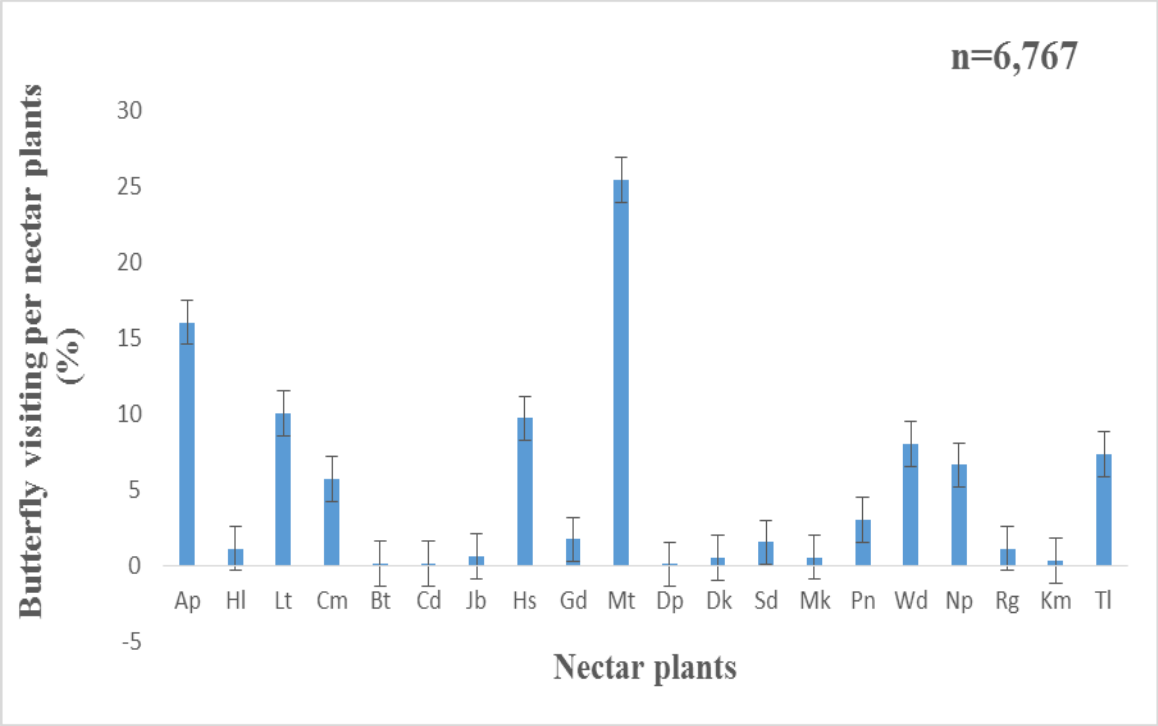

Fig. 3.5. Different nectar plants used (in foraging) as nutritional support for the development of adult butterflies in the field condition from Jan 2012 to Dec 2012.

****** Ap=Asclepias, Hl=Harina lata, Lt=Lantana, Cm=Cosmos, Bt=Botamful, Cd= Chandramallika, Jb=Jaba, Hs=Hatisur, Gd=Gadha, Mt=Marhatitiga, Dp=Dopati, Dk=Dondokolos, Sd=Setodron, Mk= Monkata, Pn=Panika, Wd=Weedelia, Np=Nilpetunia, Rg=Rongon, Km=Katamehedi, Tl=Tara lata.

The individual numbers of butterfly population have been recorded the highest in marhatitiga (25.4%) and lowest in kata mehadi (0.3%). No butterfly has been recorded to visit botamful, chandramallika and dopati nectar plants during the experimental period (Fig. 3.5). The visits rate of butterfly depends on the availability of nectars in the flowering plants. The nectar producing ability of plants varied from species to species. The nectar plants are highly seasonal that means the seasonal effects on the dynamism of butterfly population are significant. Beside the seasonal effect, the temperature, relative humidity, and photoperiod's are highly significant effects in producing nectar in the flowering plants that determine the availability of butterfly in an ecological niche. The visit rates of butterfly population are varied from one species of nectar plants to another species of nectar plants.

Recognition and selection of the best quality foods available by ovipositing females is crucial for optimal and successful larval performance (Liu *et al.* 2006, Ngu *et al.* 2008, Talsma *et al.* 2008a). Due to the low dispersal ability of the juvenile stages compared to adults, especially during the larval instars, the selection of optimal quality host plants and suitable habitats are critical steps in the life cycle of all Lepidoptera (Fartmann and Timmermann 2006, Eichel and Fartmann 2008). Knowledge of butterfly host plants and the relationship of plant-butterfly are pre-requisites for any butterfly conservation as well as biodiversity conservation programme (Bashar 2015a). Success of colonizing process has been assessed through field level experimentation on the analyses of behavioural significance of butterfly with their related plants, viz. host, nectar and shelter plants (Berry *et al.* 2002). Success of colonizing process in the butterfly behaviour has been resulted through accommodating significant stages of life cycle and their functional activities. These behaviours evidenced the ways in which butterflies carry important role in the gene-flow mechanism of the plant kingdom and create good microclimatic condition in different ecosystems, especially in the forest ecosystem (Bashar 2015a).

3.4.5 Behavioural strategies of butterfly

In respect to the ethological aspects, butterflies have the most fascinating behaviours. The timing of adult emergence in butterflies is linked with warmer weather in temperate areas. Mobility and availability of butterflies depend on larval food plants, nectar producing plants and stability of abundance of flowers for adults. The adult butterflies come to copulate soon after emergence. The males being usually more mobile, seek out the females, though this is by no means always the case. Once mating has taken place the females seek out food plants, using all their senses to ensure that suitable ones are selected. This is crucial to the survival of most species; if the eggs are laid on wrong food plant, the young caterpillars die: the females are rarely mistaken in their choice, and the whole cycle starts again. The behavioural fact of selecting such plant is well evidenced, but biochemical fact is not discovered as it required till today. Sometimes scientists guess that adult butterflies feed, not to grow, but to maintain their energy levels for breeding, egg-laying and flying activities. It is in its adult phase that the butterfly colonizes new sites (of plants), and energy and mobility are critical to the accomplishment of this task.

The butterfly population have been estimated on the basis of behavioural activities in the BRP. Six types of behavioural activities of butterfly have been observed and recorded in the BRP from January 2012 to December 2012. Six behavioural activities of butterfly belonging to seven families are foraging, resting, egg-laying, mating, searching and puddling. Total 9,588 butterflies have been observed under six behavioural patterns of butterfly at the mean temperature and mean relative humidity of 31.1±4.9 ºC and 62.03±15.7%, respectively. The population density of butterfly in relation to foraging, resting, egg-laying, mating, searching and puddling are 70.6%, 5.3%, 2.0%, 1.4%, 19.2% and 1.3%, respectively (Fig. 3.6. and Appendix 4). Behavioural activities are key factors to assess the abundance, distribution, habit and habitat of butterflies in any particular ecosystem. Butterfly beahvioural strategies are the indicators to detect the nutritional and medicinal plants in nature.

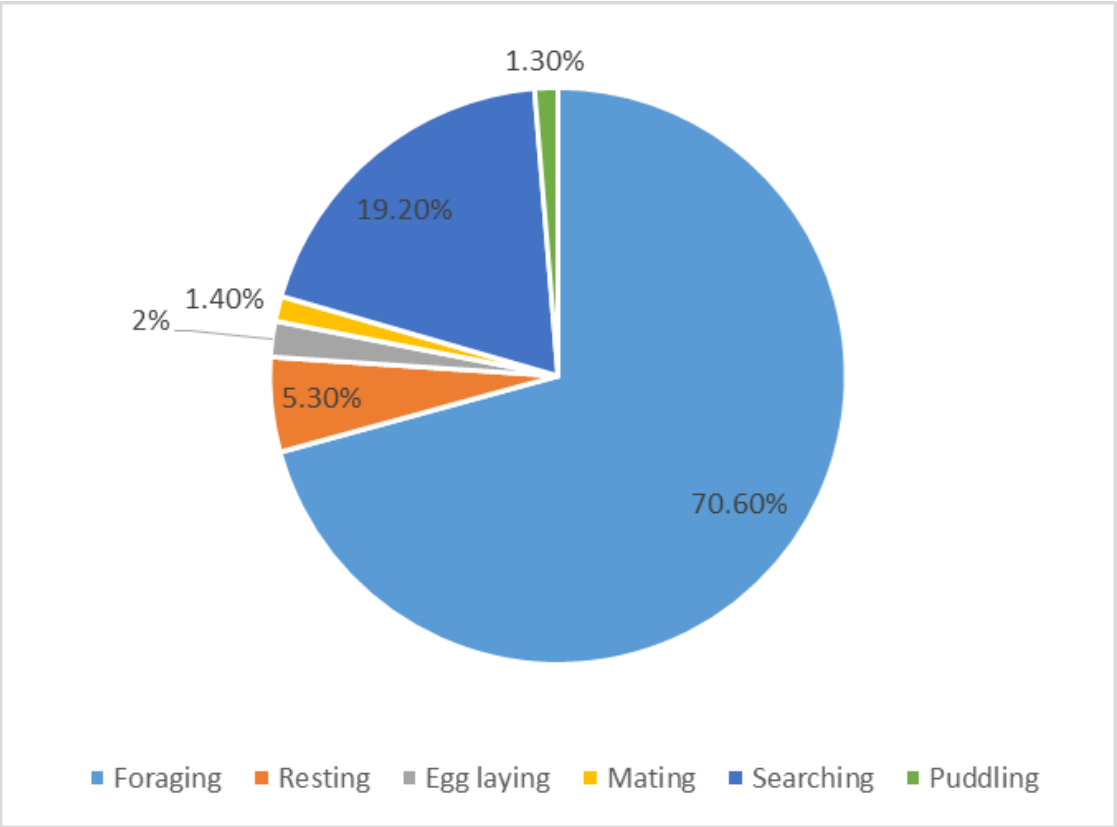

Fig. 3.6. The abundance of butterfly in relation to various behavioural aspects in Butterfly Research Park, Bhawal National Park (Jan 2012-Dec 2012).

Scientists believe that species richness in flora and nectar producing plants stands as the key factor for establishing a butterfly-colonization centre. The butterfly colonization is one of the principal tools in nature for establishing an ecotourism industry. In the present day, the facts of forest bio-resource management and conservation are exercised by using the key factors. Many butterflies are quite sedentary and do not move a few hundred yards in the course of their adult life. Such butterflies tend to form discrete colonies which may evolve into slightly different races and which are very vulnerable to extinction if conditions are to suddenly change. In contrast, there are certain types of butterflies which migrate for hundreds, or even, thousands of miles when conditions are right. Such species have less variation as they are constantly intermixing; they are also less likely to die out because they always are able to find suitable new sites (Bashar 2014a).

Feeding habit of butterfly is bisidal in function and characteristic in its lifestyle. In one side it helps in taking energy directly from the flowers. In other side, it carries great role in the gene-flow of the plant to which it is related. Butterflies feed primarily on nectar from flowers (Gilbert 1972, Singer and Parmesan 1993, Singer 1984). During foraging a single species is often found to visit different flowers of different plants in different pattern and posture (Bashar 2015a). The butterflies have to visit plants because of their nutritional requirements (flowers) in the adult stage, for egg-laying supports of copulated females, and host plants for larval food materials. Proboscis in butterflies is very adaptive and vital organ in the adult. It is used by the adult strategically in relation to the flower structure in different butterfly families (Plate 3.8A). The modifications and interrelations in between the nectar containing flowers and the nectar-sucking organ the proboscis itself in the butterflies are the most important biotic-biotic adaptabilities (Boppre 1984).

Both mating and courtship behaviours in butterflies are characteristic and attractive. Total period of mating can be categorized in chronological steps as pre-mating, mating fact and post mating stages. All the stages are included in the mating as a whole (Bashar 2015a). The butterflies that produce scents possess different shaped of scales in their wings which are variable both within and between different species (Silbergield 1984). These specialized scales are called androconia. It has been found that the males of danaid species also possess a brush of special modified scales at the tail tip which is extrusible and is used for disseminating volatile scents. Very significant results have been obtained on the facts of mating and related plant multiplication in the colonizing centre (Plate 3.8B).

Many butterfly species possess diffuse smells not confined to one sex, and these are derived from chemicals sequestered from the larval food plants (Visser 1986, Vukusic *et al.* 2000). And that is why, butterfly females are seen to search for the right oviposition sites just after mating is completed (Fartmann 2006). The females perform activities settling on the leaves and by drumming on the surface with their front pair of prolegs. According to Bell (1919) in many neotropical satyrids and oriental nymphalids these activities are noticed when the females are in searching for right larval food plant. Egg-laying support is carefully chosen. Sight, smell, touch and taste are involved in its selection (Arnyas *et al.* 2006). Eggs are laid on particular part of the plant like leaves, young stems, flower heads, or in crevices in the bark (Rabasa *et al.* 2005) which is shown in plate 3.8C.

Resting plants are primarily trees and hedges. On the behavioural aspects of butterflies, it is revealed by our experiments that, in summer the butterflies take complete rest, starting from 1:30 pm to 3.30-4.30 pm (Bashar 2015a). During this resting time they do not move and do not feed anything, but resting place need to be with high humidity and comparatively comfortable for them (Plate 3.8D). In comparison to the density of nectar plants and food plants area, the shade / resting plant area must be more dense and with assemblage of high species composition. And this is why the Butterfly Park directly and indirectly is very helpful for biodiversity conservation and establishment of species richness in an ecosystem. This total arrangement could be the shade – tree place in the Butterfly Park described by Bashar (2015a).

Butterflies are cold-blooded creatures. They may need the sun to warm their wing muscles so they can fly. They fly best when air temperatures range from 25 to 35 ℃; so when it's cooler, they bask, using the sun's heat to warm their bodies (Bashar 2015a). When temperature rise occurs in the forest areas where water-bodies are not available nearby then butterflies need some water-supply in different sources of organic matters or in the watery or dump soils (Bashar 2015a). It is found that some butterflies need to maintain ionic balance, particularly to replace lost sodium salts in the case of males (Mollamen *et al.* 2005) which is shown in Plate 3.8E-F. These males are capable of mating with a number of different females. In some pierid butterflies, the males have abdominal brushes to produce mate attracting scents (*Appias* spp.), others have plume like scales (*Delias* and *Pieris* spp.), whilst the clouded yellows (*Colias* spp.) have bands for the same purposes.

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Plate 3.8. Behavioural strategies of butterfly through colonization process in the BRP, Bhawal National Park, Gazipur. A. Danaid butterflies are taking nutrition from *Heliotropium indicum* B. Mating of *Papilio demoleus* on supportive plants *C. Egg laying strategy of a Papilio polytes* on citrus host plant *D.* Resting behaviour of pierid butterflies on supportive plants E-F. Danaid and pierid butterflies are taking mineral ions from mud surface through puddling strategy.

Danaid butterflies in the butterfly colonizing centre of Bhawal Nartional Park are found to show attractive behaviours in cluster formation on some rotten plant-parts available in the colonizing centre area. These butterflies are members of the genus *Danais, Euploea* and *Tirumala* under the family Danaidae (Plate 3.8E). It is to be noted that the Butterfly Colonizing Centre in the Bhawal National Park is made rich in plants required for colonizing the butterflies. It needed four years long to make it suitable for the status of colonizing centre. This cluster formation takes place extensively during the months of May to August for the butterflies in Bangladesh. This occasion is evidenced in our colonizing centre in dense appearance on their host plants and related shelter plants that are available in plenty within the premises of the centre. In the behaviours of such butterflies, it is found that to acquire a mate, male butterflies have to first gather resources to become most desirable and then find a mate. Therefore, newly hatched males often gather in large members to mud-puddle and take in salts. Later these salts-an essential requirement of healthy eggs get concentrated as nutrients in compact capsules that form part of the spermatophores which are passed on to the female during mating (Bashar 2014a).

Alkaloid is one of a large group of nitrogenous bases of vegetable origin, many used as drugs, e.g. morphine, quinine, strychnine etc. These nitrogenous bases are of powerful pharmacological effects in influencing the quality of medicine also. They are naturally used in initiation of production of sex-hormones in many animals like in insects. They are plant-origin chemical substances. In the milk-weed butterflies like Crows and Tigers, the males cluster around alkaloid-rich plants like *Crotalaria*, *Heliotropium* and *Ageratum*. The males swarm over the damaged parts of the plants to suck the oozing sap rich in pyrrolizidine alkaloids that are essential as precursors for the production of mate-attracting chemicals or sex-pheromones. This is a mechanism of the key factor that causes cluster formation in butterflies on the place where the plants are available. A trophico-microclimatic situation is being created in the plants-abundant area that becomes the source place of courtship behaviours in the butterflies (Bashar 2014a).

3.4.6 The population fluctuation of butterfly

Seven families of butterfly have been colonized and studied in the BRP from the January 2012 to December 2012. The butterflies belonging to seven families are Papilionidae, Danaidae, Nymphalidae, Pieridae, Satyridae, Lycaenidae and Hesperidae. Total 9,588 butterflies have been observed and recorded under the seven families at the mean temperature and mean relative humidity of 31.1±4.9 ºC and 62.03±15.7%, respectively in the BRP. The population fluctuation of butterfly belongs to 7 family's viz., Papilionidae, Danaidae, Nymphalidae, Pieridae, Satyridae, Lycaenidae and Hesperidae are to be recorded 8.8%, 30.7%, 31.0%, 16.4%, 0.32%, 1.7% and 25.6% respectively (Fig. 3.7. and Appendix 3). The abundance of butterfly population depends on the combination and accumulation of three types of plants, viz. host plants, nectar plants and shelter plants. The three levels of vegetation like undergrowth, man-height and canopy are also the facts for the significant abundance of butterfly population in a particular ecosystem. The seasonal, temperature, relative humidity and light intensity highly affect the abundance of butterfly population in any ecosystem.

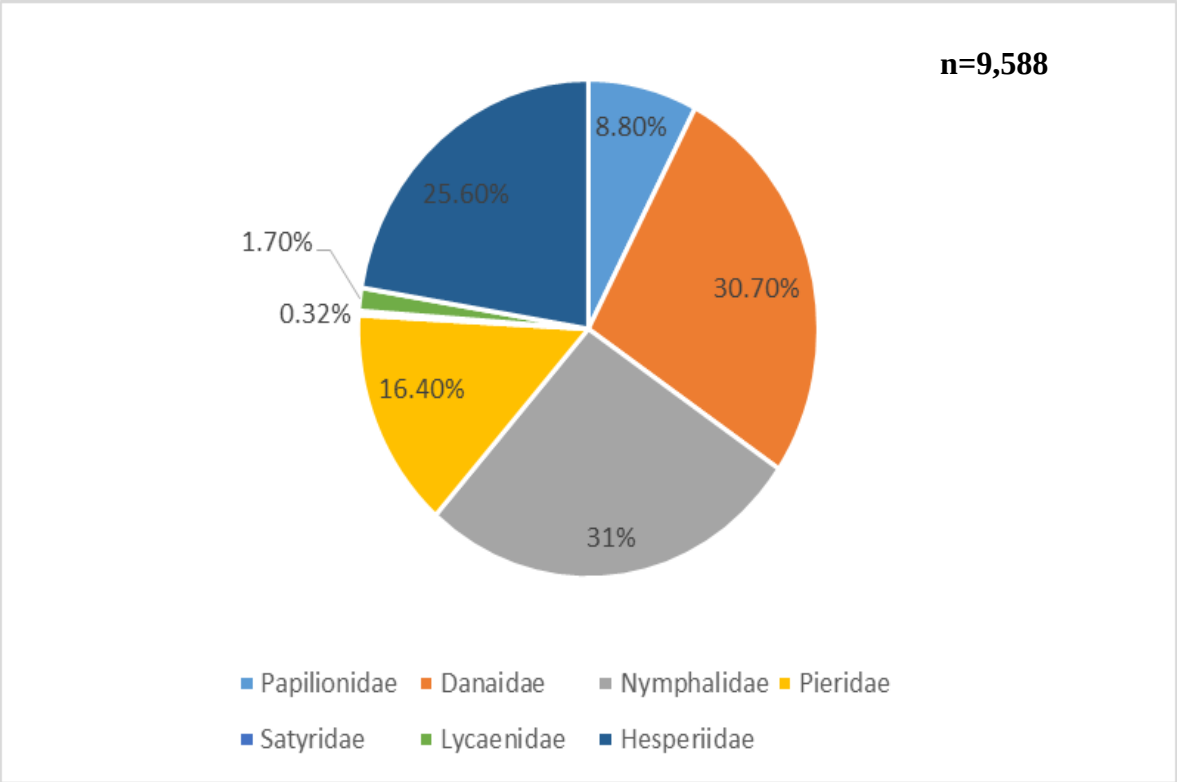

Fig. 3.7. The population density of butterfly under 7 families in the Butterfly Research Park, Bhawal National Park (January 2012-December 2012).

3.4.7 The effect of abiotic factors on butterfly population

The fluctuation of butterfly population in relation to seasonal variation has been studied and recorded in the BRP from the January 2012 to December 2012. Total 9,588 individuals of butterfly have been recorded at the mean temperature and mean relative humidity of 31.1±4.9 ºC and 62.03±15.7% in the BRP during the experimentation. The population fluctuation of butterfly in relation to seasonal variation were 9.84%, 11.18%, 9.97%, 12.32%, 15.32%, 14.6%, 7.65%, 5.69%, 4.54%, 3.29%, 7.63% and 6.04% in January, February, March, April, May, June, July, August, September, October, November and December, respectively (Fig. 3.8. and Appendix 3, 4, 5). The highest abundance of butterfly has been recorded in May (15.32%) and the lowest in October (3.29%).

It has been found that the effect of seasonal variation, temperature, relative humidity and light intensity on the abundance of butterfly population are highly significant during the study period. The interaction of biotic-biotic, biotic-abiotic and abiotic-abiotic equally important for sustainable development of the butterfly colonization in ecological and ecotouristic approach regarding the biodiversity conservation and bio-resource management in nature (Bashar 2014a).

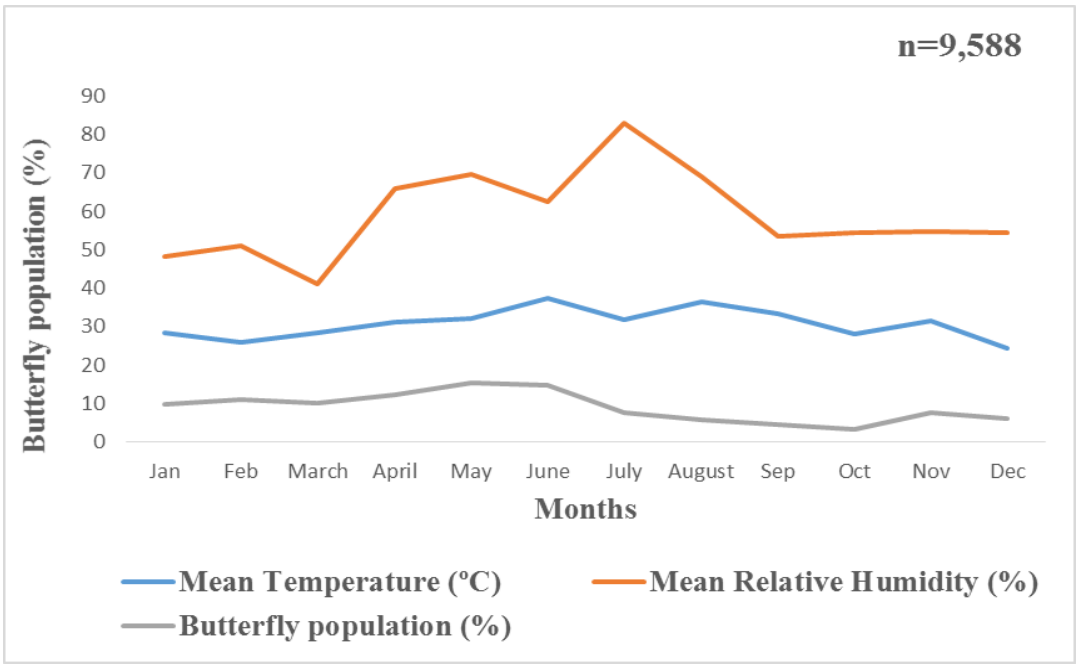

Fig. 3.8. The effects of seasonal, temperature and relative humidity on butterfly population in the Butterfly Research Park, Bhawal National Park (January 2012-December 2012).

None of the physical factors (temperature and humidity) is significantly correlated with the total number of individuals or the total number of species richness (r_1 = - 0.10274 and r_2 = - 0.10274). Humidity and rainfall are significantly and negatively correlated and temperature is significantly and positively correlated with the individual numbers of butterflies and species richness (Table 3.1). There is no evidence of correlation between rainfall and butterfly numbers for any family reported by Boonvanno (2001).

Table 3.1. Correlation between butterfly abundance and species richness and physical factors, using the Spearman rank correlation coefficient (r_s) stated by Boonvanno (2001).

Physical factor: r_s - value	Humidity (%)	Rainfall (mm)	Temperature (°C)
Total individuals	–0.50 (P=0.10)	–0.47 (P=0.13)	0.43 (P=0.16)
Species	–0.21 (P=0.51)	–0.28 (P=0.38)	0.52 (P=0.08)
No. individuals in			
Papilionidae	–0.53 (P=0.08)	–0.17 (P=0.61)	0.22 (P=0.49)
Nymphalidae	**–0.69** (P=0.01) *	–0.27 (P=0.40)	0.41 (P=0.18)
Danaidae	–0.38 (P=0.22)	–0.31 (P=0.33)	0.44 (P=0.15)
Amathusiidae	0.05 (P=0.88)	0.09 (P=0.78)	0.34 (P=0.28)
Satyridae	–0.37 (P=0.24)	–0.41 (P=0.18)	0.31 (P=0.33)
Pieridae	–0.22 (P=0.50)	–0.17 (P=0.59)	**0.58** (P=0.05) *
Riodinidae	–0.08 (P=0.80)	–0.05 (P=0.88)	0.18 (P=0.58)
Lycaenidae	–0.17 (P=0.59)	–0.41 (P=0.19)	**0.69** (P=0.01) *
Hesperiidae	0.25 (P=0.43)	–0.31 (P=0.33)	0.03 (P=0.93)

*Correlation is significant at the 0.05 level (2-tailed)

This result is consistent with that of Boonvanno *et al.* (2000), but it contrasts with Young (1982), Pollard (1988), Pollard *et al.* (1993) and Moss and Pollard (1993). It may reflect the differences between tropical and temperate climate patterns. Environmental fluctuation in temperate countries is relatively greater and may have more severe effects on the abundance and species richness of butterflies.

When a diverse fauna is sampled it is always found that a few species are represented by a lot of individuals and a large number of species are represented by one or a few individuals. These relative abundances are considered in the diversity index. According to Boonvanno (2001) the monthly species index value is maximum in April. It may depend on the flowering of the host plants that affect butterflies feeding on nectar and other biotic factors. November had the lowest species index. Perhaps during this time most butterflies are in the immature rather than adult stages.

3.4.8 Biotic-biotic relationship (butterfly and wildlife)

Wild vertebrate population was regularly observed from the year and it was estimated in four groups, such as amphibians, reptiles, birds and mammals. In 2011, size of population of amphibians, reptiles, birds and mammals were 47±5, 122±9, 970±21 and 194±11, respectively. In 2014, size of population of amphibians, reptiles, birds and mammals were found to increase up to 158±12, 277±13, 2333±21 and 372±9 respectively, in the Butterfly Research Park at Bhawal National Park, Gazipur (Appendix 24). It was observed the concomitant increase of wild vertebrates with the progressive success of the butterfly colonizing mechanism in the BRP from 2011 to 2014 (Bashar *et al.* 2015b). It is evident that the insect interaction with plants (especially the phytophagous pollinating insects and the flowering plants with entomophilous pollens) establishes strong gene-flow mechanism in the forest ecosystem. Then the ecosystem becomes healthy and gave more functional services. Consequently the ecosystems become suitable and compact home for the successive trophic levels which provided fruitful services to all the wild animals living in the forest ecosystem. The fact remains that the forest can serve as a home of "in-situ" conservation site for wildlife fauna (Bashar 2011).

3.4.9 The effects of biotic factors on butterfly

During recording data on the butterflies some peculiar and unusual situations are faced and experienced by the researchers in the forests. Butterflies have some predators. They may be large insects like mantids (raptorial Orthoptera), birds of different kinds, spiders, some amphibians, snakes, lizards, chameleons, varanus and other reptiles. Different mammals also act as vital predators for butterflies. Among them most dangerous are the spiders and arboreal snakes. The arboreal snakes are dangerous for researchers also. They generally remain at the man-height level in the forest vegetation and they camouflage with plant colouration. The researchers cannot see them if they do not move. These snakes are very poisonous. It is to be noted that in Bangladesh Satchari forest still maintains status of natural forest and rich in highest species assemblage both for flora and fauna. The largest butterfly birdwings are of highest population in the forest. Some rare snakes are also seen in the forest. Spiders and snakes as the predators of butterflies are also very often seen in the forest. For collection of butterfly host-plants and for collection of butterflies this station is very vitally important. But risks are there also during collection of both plants and butterflies reported by Bashar (2014a).

3.4.10 The taxonomic position of butterfly

Total number of genera (n=57) and species (n=92) of butterfly under 10 families have been identified and recorded in the Butterfly Research Park, Bhawal National Park during the experimental periods. The identified butterflies have been categorized into major and minor families on the basis of availability. The major families are Papilionidae, Danaidae, Nymphalidae, Pieridae, Satyridae, Lycaenidae, Hesperiidae and minor families are Acraeidae, Riodinidae and Amathusiidae (Fig. 3.9. and Appendix 6).

Papilionid butterflies are the largest and some of the splendid of all butterflies. Largest butterflies of the world birdwings are the members of the family Papilionidae. This family is not too large in its population-size, but most attractive and beautiful butterflies belong to this family. Danaids are brightly coloured, usually brownish with black and white marked; generally medium to large sized butterflies (Fres 1989). Nymphalid butterflies are decorated with various colours.

The colouration may be black with yellow striped, reddish-brown with black marked, brownish with black markings; small, medium and large sized butterfly (Bashar 2015a). Pierids are usually white or yellowish in colour with black marginal marks in the wings. They are small to medium sized butterflies.

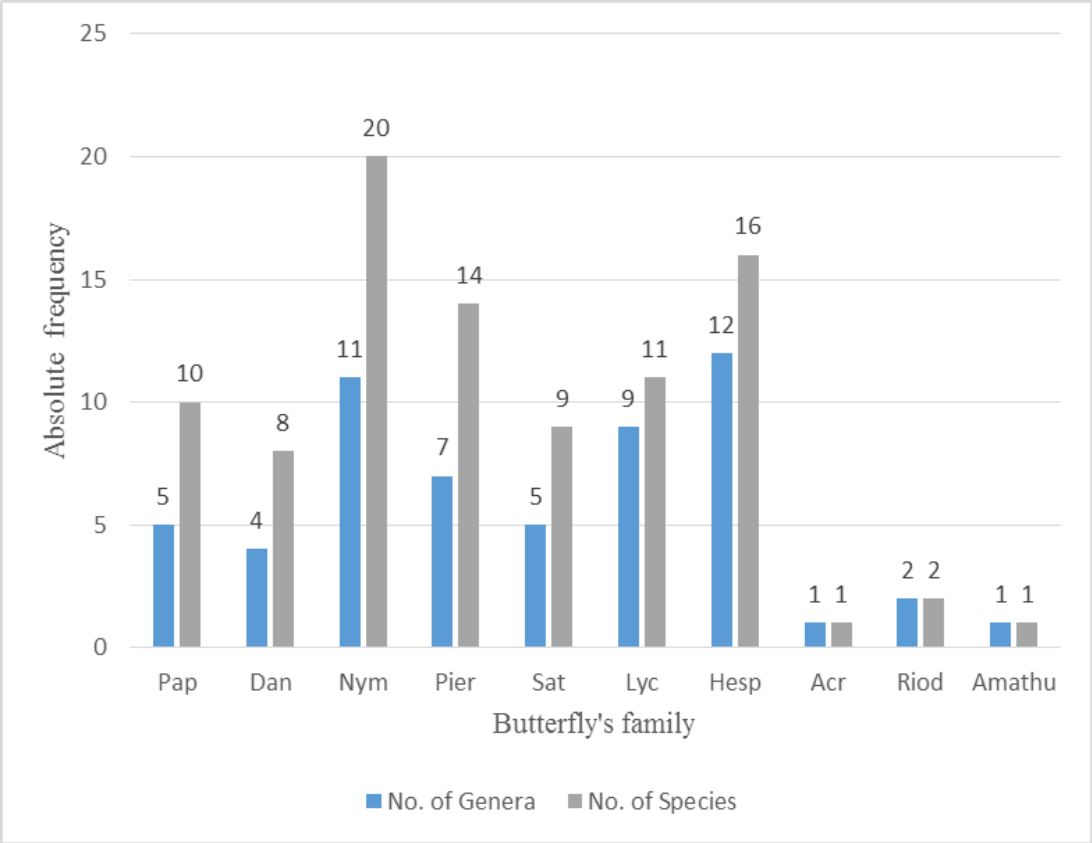

Fig. 3.9. Total no. of genera (n=57) and species (n=92) of butterfly under 10 families in the Butterfly Research Park, Bhawal National Park.

** Pap=Papilionidae, Dan=Danaidae, Nym=Nymphalidae, Pier=Pieridae, Sat=Satyridae, Lyc=Lycaenidae, Hesp=Hesperiidae, Acr=Acraeidae, Riod=Riodinidae, Amathu=Amathusiidae

Satyrids are usually grayish or brown in colour and have eyelike spots on the wings. They are small to medium sized butterflies. The lycaenid butterflies are brightly coloured. They are small sized with slender body and lovely appearance. The hesperiid butterflies are generally small in size by having narrow and short wings but thick body, mostly brown, yellow with black and white margins (Evans 1957). According to Ek-Amnuay (2006), the family Acraeidae comprises small to medium sized butterflies, generally with narrow wings and long slender abdomen. Members of Riodinidae are known as metal mark butterflies that refers to the small metallic-looking spots commonly found on their wings. In Amathusiidae, both wings are generally broad and some tinted with brilliant colours, very often with metallic blue.

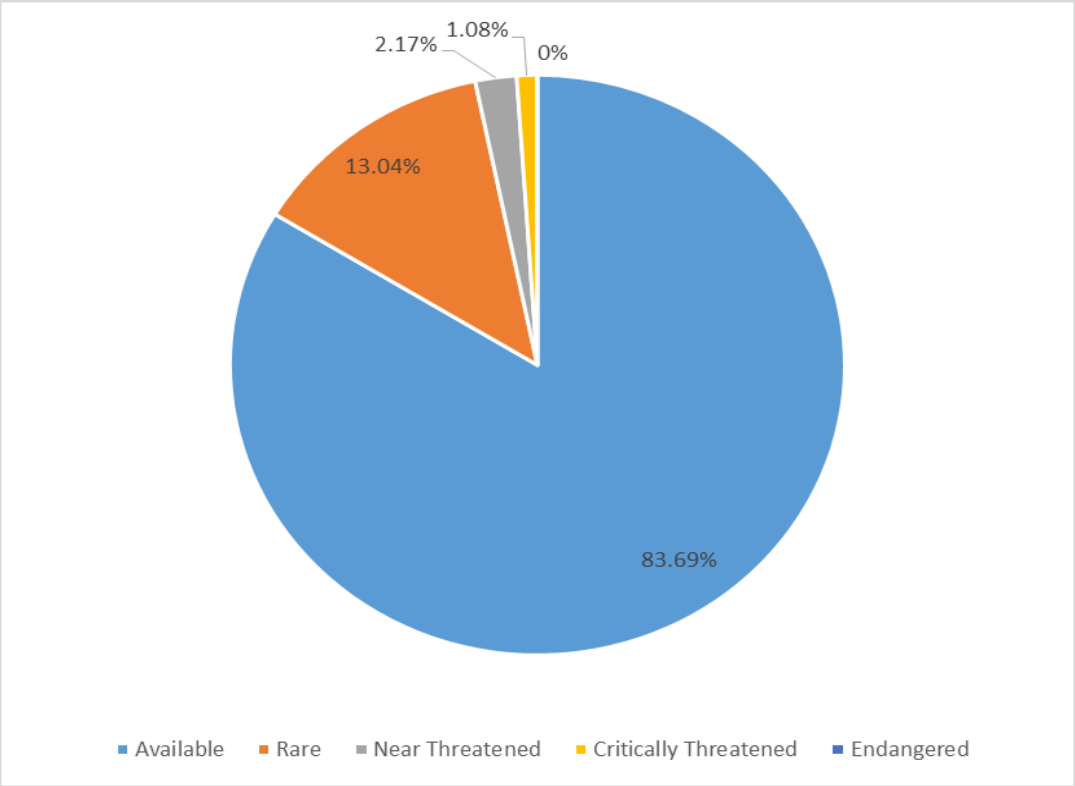

Fig. 3.10. The status of butterfly species (n=92) under 10 families in the Butterfly Research Park, Bhawal National Park.

Six categories have been suggested by the EBBL in the context of categorizing 'vulnerability stages' to prepare the Red List by using all collected identified species from the experimental stations. The researchers suggested the categories are Available (Av), Rare (Rr), Near Threatened (Nt), Threatened (Tr), Critically Threatened (Ct) and Endangered (En) according to Bashar (2015a). Total 92 butterfly species have been identified under 10 families in the Butterfly Research Park. Out of 92 butterfly species, the status of butterfly are Available (83.69%), Rare (13.04%), Near Threatened (2.17%), Critically Threatened (1.08%) and Endangered (0%). (Fig. 3.10 and Appendix 6).

3.4.11 Butterfly species richness, abundance and diversity

The EBBL lab has been dealing with a hypothesis that "butterfly-colonization and colonizing centres are the tools that can determine and assess proper soundness of a healthy forest." Steadiness of the healthy forest could ensure biodiversity conservation. The laboratory has discovered more than hundred host-plant species for Bangladeshi butterflies on which they lay eggs, develop larval stages, go for pupation and use the host-plant hedges as adult emergence supports. As the butterflies of different families have access to plants at different height levels in a forest, they can cause gene-flow in plants at different height levels also. And that is the "fact of happening" how butterflies maintain healthiness of a forest. This healthiness contains natural assemblage of not only the plant populations but also of all trophic levels. The butterfly-colonization and the release of butterflies to the forests can maintain the status of a forest for harvouring wildlife-sustenance and then the term conservation stands valid. The EBBL has established the technique for maintaining the species richness and species assemblages of maximum number of trophic levels and also sustenance of interactions among the trophic levels; and that is required for keeping dynamism in the conservation of biodiversity (Bashar 2015a).

The species diversity index and evenness was calculated using the Shannon-Weiner equation where H = 2.045 and E = 0.89. The Shannon-Weiner Index indicates that butterfly species richness and diversity are found significantly, but species are not equally distributed in the experimental field (Table 3.2).

Table 3.2. Shannon diversity index (H) for butterfly species diversity belonging to 10 families in the BRP, Bhawal National Park, Gazipur.

Butterfly's Family	No. of species	pi	Ln(pi)	pi×ln(pi)
Pap	10	0.109	-2.216	-0.242
Dan	8	0.087	-2.442	-0.213
Nym	20	0.217	-1.528	-0.332
Pier	14	0.152	-1.884	-0.286
Sat	9	0.098	-2.323	-0.228
Lyc	11	0.120	-2.120	-0.254
Hesp	16	0.174	-1.749	-0.304
Acr	1	0.011	-4.600	-0.051
Riod	2	0.022	-3.817	-0.084
Amathu	1	0.011	-4.600	-0.051
S=10	**Sum = 92**	**…**	**…**	**Sum= -2.045**

H=2.045

$$E = \frac{H}{H_{max}} = \frac{2.045}{2.303} = 0.89$$

Shannon diversity index (H) =2.045
Evenness (E) = 0.89

Out of 92 butterfly species, 10 species have been identified and colonized belonging to the family Papilionidae. Among 10 spp. of papilionid butterflies 2 rare, 2 Near Threatened and 6 are Available (Table 3.3). They are usually found to utilize 8 species of plants as host plants belonging to the families Aristolochiaceae, Annonaceae, Lauraceae and Rutaceae. 12 species of plants belonging to the families Asclepiadaceae, Malvaceae, Verbenaceae, Rubiaceae, Euphorbiaceae, Nyctaceae and Rutaceae have been utilized as nectar plants by papilionid butterflies during the experimentation (Fig. 3.11. and Appendix 6).

Table 3.3. Butterfly species richness, status, associated host and nectar plants under the family-Papilionidae in the Butterfly Research Park, Bhawal National Park.

Sl. No.	Butterfly species	Common name	Butterfly status	Host plants	Nectar plants
1	*Pachliopta aristolochiae* Fabricius, 1775	Common rose	Av	*Aristolochia indica* *Aristolochia tagala* F-Aristolochiaceae	*Asclepias curassavica* (Asclepiadaceae) *Hibiscus rosa sinensis* (Malvaceae) *Duranta plumeri* (Verbenaceae)
2	*Graphium agamemnon* Linnaeus, 1758	Tailed Jay	Rr	*Polyalthia longifolia* F-Annonaceae	*Hibiscus rosa sinensis* (Malvaceae) *Ixora chinensis* (Rubiaceae) *Asclepias curassavica* (Asclepiadaceae)
3	*Graphium doson* Felder, 1864	Common Jay	Av	*Polyalthia longifolia* F-Annonaceae	*Ixora chinensis* (Rubiaceae) *Hibiscus schizopetalus* (Malvaceae) *Asclepias*

					curassavica (Asclepiadaceae)
4	*Graphium sarpedon* Linnaeus, 1758	Common bluebottle	Nt	*Polyalthia longifolia* F-Annonaceae	*Micromelum pubescens* (Rutaceae) *Hibiscus rosa-sinensis* (Malvaceae)
5	*Chilasa clytia* Hampson, 1889	Common Mime	Av	*Litsea glutinosa* F-Lauraceae	*Lantana camara* (Verbenaceae) *Bougainvillea spectabilis* (Nyctaceae)
6	*Chilasa paradoxa* Zinken, 1831	Great Blue Mime	Nt	*Litsea glutinosa* F-Lauraceae	*Lantana sellowiana* (Verbenaceae) *Bougainvillea spectabilis* (Nyctaceae)
7	*Troides helena* Rothsch, 1895	Common Birdwing	Rr	*Aristolochia indica* *Aristolochia tagala* F-Aristolochiaceae	*Euphorbia puleherrima* (Euphorbiaceae) *Lantana aculeate* (Verbenaceae) *Hibiscus rosa sinensis* (Malvaceae)
8	*Papilio polytes* Linnaeus, 1758	Common Mormon	Av	*Aegle marmelos* *Citrus spp.* *Glycosmis arborea* F-Rutaceae	*Lantana camara* (Verbenaceae) *Clerodendrum inerme* (Verbenaceae) *Duranta plumeri* (Verbenaceae)

9	*Papilio demoleus* Linnaeus, 1758	Lime Swallowtail	Av	*Aegle marmelos* *Citrus spp.* *Glycosmis arborea* F-Rutaceae	*Lantana camara* (Verbenaceae) *Clerodendrum serratum* (Verbenaceae) *Duranta plumeri* (Verbenaceae)
10	*Papilio polymnestor* Cramer, 1775	Blue Mormon	Av	*Citrus grandis* F-Rutaceae	*Asclepius curassavica* (Asclepiadaceae) *Hibiscus rosa sinensis* (Malvaceae) *Ixora chinensis* (Rubiaceae)

Eight species of butterfly have been identified and colonized belonging to the family Danaidae. Among eight species of danaid butterflies one is Rare and seven are Available (Table 3.4). They are usually found to utilize seven species of plants as host plants belonging to the families Asclepiadaceae, Apocynaceae and Moraceae. 10 species of plants belonging to the families Asclepiadaceae, Verbenaceae, Rubiaceae, Boraginaceae, Ebenaceae and Asteraceae have been utilized as nectar plants by danaid butterflies in the Butterfly Research Park (Fig. 3.11. and Appendix 6).

Table 3.4. Butterfly species richness, status, associated host and nectar plants under the family-Danaidae in the Butterfly Research Park, Bhawal National Park.

Sl. No.	Butterfly species	Common name	Butterfly status	Host plants	Nectar plants
1	*Tirumala limniaceae* Cramer, 1775	Blue Tiger	Av	*Calotropis procera* F-Asclepiadaceae	*Heliotropium indicum* (Boraginaceae) *Asclepias curassavica* (Asclepiadaceae) *Duranta plumeri* (Verbenaceae)
2	*Parantica aglea* Stoll, 1782	Glassy Tiger	Av	*Tylophora indica* F-Asclepiadaceae	*Heliotropium indicum* (Boraginaceae) *Chromolaena odorata* (Asteraceae) *Asclepias curassavica* (Asclepiadaceae)
3	*Parentica agleoides* Felder, 1860	Dark Glassy Tiger	Av	*Gymnema spp*. F-Asclepiadaceae	*Chromolaena odorata* (Asteraceae) *Heliotropium indicum* (Boraginaceae) *Asclepias curassavica* (Asclepiadaceae)
4	*Euploea core* Cramer, 1780	Common Crow	Av	*Ichnocarpus frutescens* F-Apocynaceae	*Adina cordfolia* (Rubiaceae) *Heliotropium indicum* (Boraginaceae) *Diospyros Montana*

					(Ebenaceae) *Duranta plumeri* (Verbenaceae)
5	*Euploea mulciber* Cramer, 1777	Stripped Blue Crow	Av	*Ficus microcarpa* F-Moraceae	*Diospyros montana* (Ebenaceae) *Adina cordfolia* (Rubiaceae) *Heliotropium indicum* (Boraginaceae) *Duranta plumeri* (Verbenaceae)
6	*Euploea klugii* Moore, 1858	Brown King Crow	Rr	*Ficus hispida* F-Moraceae	*Heliotropium indicum* (Boraginaceae) *Diospyros Montana* (Ebenaceae)
7	*Danaus genutia* Cramer, 1779	Common Tiger	Av	*Asclepius curassavica* F-Asclepiadaceae	*Calotropis gigantae* (Asclepiadaceae) *Lantana camara* (Verbenaceae) *Duranta plumeri* (Verbenaceae) *Asclepias curassaviaca* (Asclepiadaceae)
8	*Danaus chrysippus* Linnaeus, 1758	Plain Tiger	Av	*Asclepius curassavica* F-Asclepiadaceae	*Calotropis procera* (Asclepiadaceae) *Lantana camara* (Verbenaceae) *Asclepias curassaviaca* (Asclepiadaceae)

Twnty butterfly species have been identified and colonized belonging to the family Nymphalidae. Among 20 spp. of nymphalid butterflies two are Rare and eighteen are Available (Table 3.5). They are usually found to utilize 15 species of plants as host plants belonging to the families Flacourtiaceae, Euphorbiaceae, Anacardiaceae, Melastomaceae, Rhamnaceae, Bombacaceae, Fabaceae, Passifloraceae, Verbenaceae, Acanthaceae, Mimosaceae, Malvaceae and Menispermaceae. 16 species of plants belonging to the families Verbenaceae, Fabaceae, Asteraceae, Balsaminaceae, Malvaceae, Lythraceae and Compositae have been utilized as nectar plants by nymphalid butterflies in the Butterfly Research Park (Fig. 3.11. and Appendix 6)).

Table 3.5. Butterfly species richness, status, associated host and nectar plants under the family-Nymphalidae in the Butterfly Research Park, Bhawal National Park.

Sl. No.	Butterfly species	Common name	Butterfly status	Host plants	Nectar plants
1	*Phalantha phalantha* Drury, 1770	Common Leopard	Av	*Flacourtia spp.* F-Flacourtiaceae	*Cosmos bipinnatus* (Compositae) *Spilanthes calva* (Asteraceae)
2	*Parthenos gambrisius* Fabricius, 1775	Clipper	Av	*Melothria heterophylla* F-Menispermaceae	*Lawsonia inermis* (Lythraceae) *Spilanthes calva* (Asteraceae)
3	*Pantoporia nefte* Cramer, 1782	Colour Lascar	Av	*Glochidion spp.* F-Euphorbiaceae	*Chromolaena odorata* (Asteraceae) *Spilanthes calva* (Asteraceae)
4	*Pantoporia cama* Moore, 1857	Orange Lascar	Av	*Archidendron spp.* F-Fabaceae	*Duranta repens* (Verbenaceae) *Spilanthes calva* (Asteraceae)

5	*Hypolimnas bolina* Linnaeus, 1758	Great Egg Fly	Av	*Sida rhombifolia* F-Malvaceae	*Duranta plumeri* (Verbenaceae) *Spilanthes calva* (Asteraceae) *Duranta plumeri* (Verbenaceae)
6	*Junonia iphita* Cramer, 1869	Chocolate Pansy	Av	*Hygrophila auriculata* F-Acanthaceae	*Lantana camara* (Verbenaceae) *Wedelia calendulacea* *Duranta repens* (Verbenaceae)
7	*Junonia lemonias* Linnaeus, 1758	Lemon Pansy	Av	*Barleria spp.* F-Acanthaceae	*Chromolaena odorata* (Asteraceae) *Wedelia calendulacea*
8	*Junonia orithya* Linnaeus, 1758	Blue Pansy	Rr	*Mimosa pudica* F-Mimosaceae	*Lantana camara* (Verbenaceae) *Wedelia calendulacea* *Duranta repens* (Verbenaceae)
9	*Junonia hierta* Fabricius, 1775	Yellow Pansi	Av	*Barleria spp.* F-Acanthaceae	*Vitis lanceolaria* *Wedelia calendulacea*
10	*Junonia atlites* Johanssen, 1763	Grey Pansi	Av	*Hygrophila auriculata* F-Acanthaceae	*Lantana camara* (Verbenaceae) *Wedelia calendulacea*
11	*Junonia almana* Linnaeus, 1758	Peacock Pansy	Av	*Phyla nodiflora* F-Verbenaceae	*Lantana camara* (Verbenaceae) *Wedelia calendulacea*

12	*Athyma perius* Linnaeus, 1758	Common Sergeant	Rr	*Glochidion lanceolarum* F- Euphorbiaceae	*Abutilon darwini* (Malvaceae) *Hibiscus rosa sinensis* (Malvaceae) *Duranta repens* (Verbenaceae)
13	*Cethosia cyane* Drury, 1770	Leopard Lacewing	Av	*Adenia pierrei* F-Passifloraceae	*Callicarpa macrophylla* (Verbenaceae) *Eupatorium odoratum* (Asteraceae) *Duranta repens* (Verbenaceae)
14	*Neptis mahendra* Moore, 1872	Himalayan Sailor	Av	*Canavalia ensiformis* F-Fabaceae	*Impatiens balsamina (*Balsaminaceae*)* *Eupatorium odoratum* (Asteraceae) *Wedelia calendulacea* *Spilanthes calva* (Asteraceae)
15	*Neptis jumba* Moore, 1857	Chestnut-Streaked Sailor	Av	*Bombax ceiba* F-Bombacaceae	*Eupatorium odoratum* (Asteraceae) *Clerodendrum infortunatum (Verbenaceae)* *Wedelia calendulacea* *Spilanthes calva* (Asteraceae)
16	*Lebadea Martha* Fabricius, 1775	Knight	Av	*Ziziphus attopoensis* F-Rhamnaceae	*Spilanthes calva* (Asteraceae) *Wedelia calendulacea*

17	*Euthalia lepidea* Butler, 1868	Grey Count	Av	*Melastoma malabathricum* F- Melastomaceae	*Clitoria tarnatea (Fabaceae)* *Lantana camara* (Verbenaceae)
18	*Euthalia julii* Bougainville, 1837	Common Earl	Av	*Melastoma malabathricum* F- Melastomaceae	*Clitoria tarnatea (Fabaceae)* *Lantana camara* (Verbenaceae)
19	*Euthalia jahnu* Moore, 1857	Plain Baron	Av	*Mangifera indica* F-Anacardiaceae	*Clitoria tarnatea* (Fabaceae) *Lantana camara* (Verbenaceae)
20	*Ergolis ariadne* Johanssen, 1764	Angled Castor	Av	*Ricinus communis* F-Euphorbiaceae	*Clerodendrum infortunatum* (Verbenaceae) *Lantana camara (Verbenaceae)*

Out of 92 butterfly species, 14 species have been identified and colonized belonging to the family Pieridae. Among 14 spp. of pierid butterflies three are Rare, one is Critically Threatened and 10 are Available (Table 3.6). They are usually found to utilize 10 species of plants as host plants belong to the families Capparaceae, Viscaceae, Loranthaceae, Lamiaceae, Caesalpiniaceae, Mimosaceae, Cruciferae and Cleomaceae. 14 species of plants belonging to the families Verbenaceae, Asteraceae, Cruciferae, Capparaceae, Euphorbiaceae, Anacardiaceae, Lauraceae, Myrtaceae and Malvaceae have been utilized as nectar plants by pierid butterflies in the Butterfly Research Park (Fig. 3.11. and Appendix 6).

Table 3.6. Butterfly species richness, status, associated host and nectar plants under the family-Pieridae in the Butterfly Research Park, Bhawal National Park.

Sl. No.	Butterfly species	Common name	Butterfly status	Host plants	Nectar plants
1	*Parerona hippie* Bingham, 1907	Common Wanderer	Av	*Capparis zeylanica* F-Capparaceae	*Lantana camara* (Verbenaceae) *Wedelia calendulacea*
2	*Appias lyncida* Boisduval, 1836	Chocolate Albatross	Av	*Crataeva adansonii* F-Capparaceae	*Gomphrena globosa* (Malvaceae) *Wedelia calendulacea*
3	*Delias eucharis* Drury, 1773	Common Jezebel	Av	*Viscum spp.* F-Viscaceae	*Callistemon citrinus* (Myrtaceae) *Wedelia calendulacea*
4	*Delias pasithoe* Linnaeus, 1767	Redbreast Jezebel	Rr	*Dendropthoe spp.* F-Loranthaceae	*Chromolaena odorata* (Asteraceae) *Spilanthes calva* (Asteraceae)
5	*Delias descombesi* Boisduval, 1836	Red Spot Jezebel	Rr	*Clerodendrum spp.* F-Lamiaceae	*Chromolaena odorata* (Asteraceae) *Spilanthes calva* (Asteraceae)
6	*Catopsilia pomona* Fabricius, 1775	Lemon Emigrant	Av	*Cassia fistula* F-Caesalpiniaceae	*Alseodophne petiolaris* (Lauraceae) *Lantana camara* (Verbenaceae)
7	*Catopsilia crocale* Cramer, 1775	Common Emigrant	Av	*Cassia fistula* F-Caesalpiniaceae	*Anacardium occidentale* (Anacardiaceae)

8	*Catopsilia pyranthe* Linnaeus, 1758	Mottled Emigrant	Av	*Cassia fistula* F-Caesalpiniaceae	*Antidesma acidum* (Euphorbiaceae) *Lantana camara* (Verbenaceae)
9	*Eurema anderson*	One Spot Grass Yellow	Ct	*Cassia tora* Caesalpiniaceae	*Chome rutidosperma* (Capparaceae) *Lantana camara* (Verbenaceae)
10	*Eurema blanda* Boisduval, 1836	Tree Spotted Grass Yellow	Rr	*Pithecellobium dulce* F-Mimosaceae	*Chromolaena odorata* (Asteraceae) *Raulwolfia serpentine* *Panica indica*
11	*Eurema hecabe* Linnaeus, 1758	Common Grass Yellow	Av	*Pithecellobium dulce* F-Mimosaceae	*Raulwolfia serpentine* *Chromolaena odorata* (Asteraceae) *Panica indica*
12	*Pieris canidia* Linnaeus, 1768	Indian Cabbage White	Av	*Nasturtium spp.* F-Cruciferae	*Brassica napus* (Cruciferae) *Lantana camara* (Verbenaceae)
13	*Pieris brassicae* Linnaeus, 1758	Large Cabbage White	Av	*Brassica spp.* F-Cruciferae	*Brassica juncea* (Cruciferae) *Lantana camara* (Verbenaceae)
14	*Leptosia nina* Fabricius, 1793	Psyche	Av	*Cleome viscose* F-Cleomaceae	*Lanta camara* (Verbenaceae) *Wedelia calendulacea* *Panica indica*

Nine butterfly species have been identified and colonized belonging to the family Satyridae. Among nine species of satyrid butterflies all are Available (Table 3.7). They are usually found to utilize five species of plants as host plants belonging to the families Palmae and Poaceae, but host plants of *Mycalesis* spp. are not identified. Nine species of plants belonging to the families Euphorbiaceae, Crassulaceae, Orchidaceae, Amaranthaceae, Ranunculaceae, and Palmae have been utilized as nectar plants by satyrid butterflies in the Butterfly Research Park (Fig. 3.11. and Appendix 6).

Table 3.7. Butterfly species richness, status, associated host and nectar plants under the family-Satyridae in the Butterfly Research Park, Bhawal National Park.

Sl. No.	Butterfly species	Common name	Butterfly status	Host plants	Nectar plants
1	*Elymnius hypermnistra* Linnaeus, 1763	Common Palmfly	Av	*Cocos nucifera* F-Palmae	*Ricinus communis* (Euphorbiaceae) *Panica indica*
2	*Ypthima hueberin* Kirby, 1871	Common Four Ring	Av	*Setaria spp.* F-Poaceae	*Bryophallum calycinum* (Crassulaceae)
3	*Ypthima baldus* Fabricius, 1775	Common Five Ring	Av	*Pogonatherum crinitum* F-Poaceae	*Arundina graminifolia* (Orchidaceae) *Panica indica*
4	*Melanitis leda* Linnaeus, 1758	Common Evening Brown	Av	*Setaria palmifolia* F-Poaceae	*Amaranthus faciata (*Amaranthaceae)
5	*Melanitis phedima* Cramer, 1780	Dark Evening Brown	Av	*Setaria palmifolia* F-Poaceae	*Pteris cretica*

6	*Mycalesis thailandica* Aoki and Yamaguchi, 1983	Thai Bushbrown	Av	Not identified	*Delhinium ajacis* (Ranunculaceae) *Panica indica*
7	*Mycalesis preseus* Fabricius, 1775	Common Bushbrown	Av	Not identified	*Chrysalidocarpus lutescens* (Palmae) *Panica indica*
8	*Mycalesis intermedia* Moore, 1892	Intermediate Bushbrown	Av	Not identified	*Chrysalidocarpus lutescens* (Palmae) *Panica indica*
9	*Lethe europa* Fabricius, 1775	Bamboo Treebrown	Av	*Bambusa spp.* F-Poaceae	*Panica indica* *Wedelia calendulacea*

Out of 92 butterfly species, eleven species have been identified and colonized belonging to the family Lycaenidae. Among eleven spp. of lycaenid butterflies all are Available (Table 3.8). They are usually found to utilize 10 species of plants as host plants belonging to the families Combretaceae, Sapindaceae, Mimosaceae, Rubiaceae, Fabaceae, Rhamnaceae, Dioscoreaceae, and Rutaceae. Fifteen species of plants belonging to the families Rubiaceae, Verbenaceae, Rutaceae, Asteraceae, Euphorbiaceae, Compositae, Capparaceae and Asclepiadaceae have been utilized as nectar plants by lycaenid butterflies in the Butterfly Research Park (Fig.3.11. and Appendix 6).

Table 3.8. Butterfly species richness, status, associated host and nectar plants under the family Lycaenidae in the Butterfly Research Park, Bhawal National Park.

Sl. No.	Butterfly species	Common name	Butterfly status	Host plants	Nectar plants
1	*Arhopala amantes* Doubleday, 1847	Large Oakblue	Av	*Terminalia catappa* F-Combretaceae	*Ixora chinensis* (Rubiaceae) *Duranta repens* (Verbenaceae)
2	*Arhopala pseudocentaurus* Doubleday, 1847	Common Oakblue	A	*Terminalia catappa* F-Combretaceae	*Panica indica* *Glycosmis pentaphylla* (Rutaceae)
3	*Rapala pheretima* Hewitson, 1863	Copper Flash	Av	*Dimocarpus longan* F-Sapindaceae	*Chrysanthemum cinerariifolium* (Compositae) *Cosmos bipinnatus* (Compositae)
4	*Rapala manea* Hewitson, 1863	Slate Flash	Av	*Acacia pennata* F-Mimosaceae	*Cosmos bipinnatus* (Compositae) *Chrysanthemum cinerariifolium* (Compositae)
5	*Remalana jangala* Horsfield, 1829	Chocolate Royal	Av	*Ixora coccinela* F-Rubiaceae	*Chromolaena odorata* (Asteraceae) *Spilanthes calva* (Asteraceae)
6	*Euchrysops cnejus* Fabricius, 1798	Gram Blue	Av	*Butea monosoerma* F-Fabaceae	*Spilanthes calva* (Asteraceae) *Chromolaena odorata* (Asteraceae)
7	*Castalius rosimon*	Common Pierrot	Av	*Zizyphus mauritiana*	*Wedelia calendulacea*

	Fabricius, 1775			F-Rhamnaceae	*Cynodon dactylon*
8	*Pseudozizeeria maha* Kollar, 1848	Pale Grass Blue	Av	*Oxalis corniculata* F-Oxalidaceae	*Antidesma ghasembilla* (Euphorbiaceae)
9	*Loxura atymnus* Stoll, 1780	Yamfly	Av	*Dioscorea pentaphylla* F-Dioscoreaceae	*Citrus spp.*(Rutaceae) *Wedelia calendulacea*
10	*Chilades lajus* Stoll, 1780	Lime Blue	Av	*Citrus spp.* F-Rutaceae	*Chome gynandropsis* (Capparaceae) *Panica indica*
11	*Lampides boeticus* *Linnaeus, 1767*	Pea Blue	Av	*Lupinus polyphyllus* F-Fabaceae	*Asclepius curassavica* (Asclepiadaceae) *Duranta repens* (Verbenaceae)

Sixteen butterfly species have been identified and colonized belonging to the family Hesperiidae. Among 16 spp. of hesperiid butterflies three are Rare and 13 are Available (Table 3.9). They are usually found to utilize 15 species of plants as host plants belonging to the families Poaceae, Arecaceae, Malvaceae, Zingiberaceae and Dioscoreaceae. Sixteen species of plants belonging to the families Verbenaceae, Asteraceae, Compositae, Cucurbitaceae, Malvaceae and Amaranthaceae have been utilized as nectar plants by hesperiid butterflies in the Butterfly Research Park (Fig. 3.11. and Appendix 6).

Table 3.9. Butterfly species richness, status, associated host and nectar plants under the Hesperiidae in the Butterfly Research Park, Bhawal National Park.

Sl. No.	Butterfly species	Common name	Butterfly status	Host plants	Nectar plants
1	*Matapa druna* Moore, 1866	Gray-branded Redeye	Av	*Dendrocalamus giganteus* F-Poaceae	*Lantana camara* (Verbenaceae) *Panica indica*
2	*Hyarotis adrastus* Stoll, 1780	Tree Flitter	Av	*Phoenix acualis* F-Arecaceae	*Chromolaena odorata* (Asteraceae) *Wedelia calendulacea*
3	*Parnara naso* Fabricius, 1793	African Straight Swift	Av	*Colocasia esculenta* F-Arecaceae	*Cucurbita moschata* (Cucurbitaceae) *Cucurbita maxima* (Cucurbitaceae)
4	*Parnara apostata* Snellen, 1880	Dark Straight Swift	Rr	*Oryza sativa* F-Poaceae	*Cucurbita maxima* (Cucurbitaceae) *Cucurbita moschata* (Cucurbitaceae)
5	*Parnara ganga* Evan, 1937	Continental Swift	Av	Not identified	*Cosmos bipinnatus* (Compositae) *Wedelia calendulacea*
6	*Spialia galba* Fabricius, 1793	Indian Skipper	Av	*Sida rhombifolia* F-Malvaceae	*Vernonia cinerea* (Asteraceae) *Spilanthes calva* (Asteraceae)
7	*Iambrix salsala* Moore, 1866	Chestnut Bob	Av	*Bambusa spp.* F-Poaceae	*Cosmos bipinnatus* (Asteraceae) *Wedelia calendulacea*

8	*Saustus gremius* Fabricius, 1798	Indian Palm Bob	Av	*Cocos nucifera* F-Arecaceae	*Helianthus annus* (Asteraceae) *Cosmos bipinnatus* (Asteraceae) *Spilanthes calva* (Asteraceae)
9	*Oriens gola* Moore, 1877	Common Dartlet	Av	*Paspalum conjugatum* F-Poaceae	*Wedelia calendulacea* *Cynodon dactylon* *Spilanthes calva* (Asteraceae)
10	*Udaspes folus* Cramer, 1775	Grass Demon	Av	*Zingiber officinale* F-Zingiberaceae	*Benincasa hispida* (Cucurbitaceae) *Cucurbita maxima* (Cucurbitaceae)
11	*Notocrypta curvifascia* Felder, 1862	Restricted Demon	Av	*Costus speciosus* F-Zingiberaceae	*Cucurbita maxima* (Cucurbitaceae)
12	*Borbo cinnara* Wallace, 1866	Rice Swift	Rr	*Setaria pumila* F-Poaceae	*Spilanthes calva* (Asteraceae) *Wedelia calendulacea*
13	*Tagiades japetus* Stoll, 1781	Common Snow Flat	Av	*Dioscorea oppositifolia* F-Dioscoreaceae	*Celosia cristata* (Amaranthaceae) *Wedelia calendulacea* *Spilanthes calva* (Asteraceae)

14	*Tagiades litigiosus* Moschler, 1878	Water Snow Flat	Av	*Dioscorea hispida* F-Dioscoreaceae	*Celosia cristata* (Amaranthaceae) *Wedelia calendulacea* *Spilanthes calva* (Asteraceae)
15	*Pelopidas agna* Moore, 1866	Bengal Swift	Av	*Paspalum conjugatum* F-Poaceae	*Hibiscus rosa sinensis* (Malvaceae) *Spilanthes calva* (Asteraceae)
16	*Pelopidas mathias* Fabricius, 1798	Small Branded Swift	Rr	*Saccharum officinarum* F-Poaceae	*Hibiscus rosa sinensis* (Malvaceae) *Spilanthes calva* (Asteraceae)

Four species of butterfly have been identified and colonized belonging to the three minor families (Acraeidae, Riodinidae and Amathusiidae). Among four spp. of butterflies one is Rare and three are Available (Table 3.10). They are usually found to utilize four species of plants as host plants belonging to the families Passifloraceae, Myrsinaceae and Poaceae. Five species of plants belonging to the families Verbenaceae and Asteraceae have been utilized as nectar plants by butterflies of three minor families in the Butterfly Research Park (Fig. 3.11. and Appendix 6).

Table 3.10. Butterfly species richness, status, associated host and nectar plants under the 3 minor families (Acraeidae, Riodinidae, Amathusiidae) in the Butterfly Research Park, Bhawal National Park.

Sl. No.	Butterfly species	Common name	Butterfly status	Host plants	Nectar plants
1	*Acraea violae* Fabricius, 1775 F-Acraeidae	Tawny Coster	Av	*Passiflora edulis* F- Passifloraceae	*Spilanthes calva* (Asteraceae) *Cosmos bipinnatus* (Asteraceae) *Lantana camara* (Verbenaceae)
2	*Zemeros flegyas* Cramer, 1780 F-Riodinidae	Punchinello	Av	*Maesa indica* F-Myrsinaceae	*Lantana camara* (Verbenaceae) *Panica indica* *Wedelia calendulacea*
3	*Abisara echerius* Stoll, 1790 F-Riodinidae	Plum Judy	Av	*Embelia robusta* F-Myrsinaceae	*Lantana camara* (Verbenaceae) *Panica indica* *Spilanthes calva* (Asteraceae)
4	*Discophora sondaica* Boisduval, 1836 F-Amathusiidae	Common Duffer	Rr	*Saccharum spp.* F-Poaceae	*Wedelia calendulacea* *Cosmos bipinnatus* (Asteraceae) *Spilanthes calva* (Asteraceae)

** Av = Available; Rr = Rare; Nt = Near Threatened; Tr = Threatened; Ct = Critically Threatened; En = Endangered

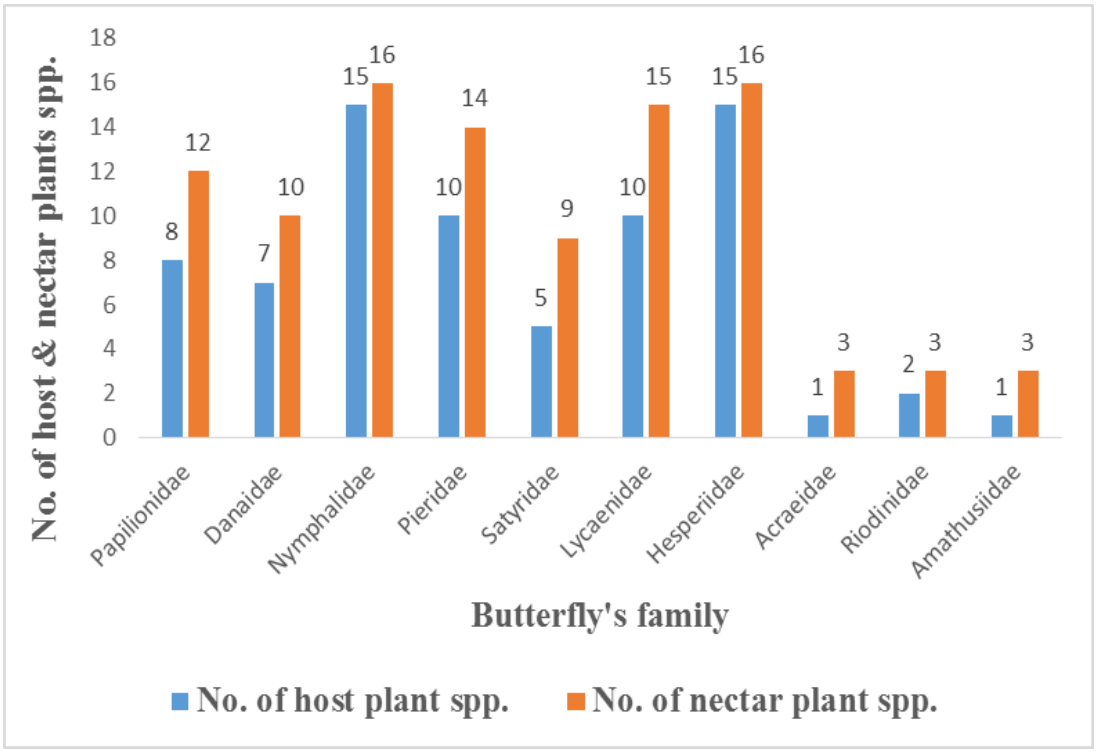

Fig. 3.11. Host and nectar plants were utilized by different developmental stages of butterfly as nutritional supports in the Butterfly Research Park, Bhawal National Park.

Correlation coefficient between butterfly species and number of host and nectar plant species have been found (r = 0.96) and is significant at 1% level (p = 0.01), shows strong correlation between butterfly diversity in relation to host and nectar plant. Hence, more number of host and nectar plant species attract significantly more species of butterflies (Fig. 3.11). Similarly, correlation coefficient (r = 0.454) between number of host plant species and butterfly species richness have also been found significant at 1% level (p = 0.01) and also shows that more butterfly families were attracted significantly the host plant species as their number increases. Whereas, correlation coefficient between butterfly species and butterfly family calculated as (r = 0.394) which shows medium correlation between these two, but was significant at 0.5% level (p = 0.05) according to Ahmed *et al.* (2016).

The study has focused on collecting fundamental information of butterfly resources in the Butterfly Research Park, Bhawal National Park. Data on the other vital consumer resource, nectar flowers (Tudor *et al.* 2004) have already been reported (Tiple *et al.* 2006, 2009). Basic information has been collected on host plant life forms, basic biotopes, perennation, abundance, and host plant distribution. Galetto and Bernardello (2003) reported that hexose nectars are characteristic of Asteraceae. Baker and Baker (1983) also stated that hexose sugars dominate in the nectars of Asteraceae and the nectars are also relatively strong in amino acids to compensate for the low sucrose-hexose ratio in the members of this family which attract butterflies. Butterflies get a source of large amount of nutrition from nectar plants and pollinate the flowers heavily, which is the key success of its high invasiveness in different parts of the world as reported by Shihan and Kabir (2015).

The results and observations of the present research has brought some ideas as to how to deal with the fact of using "butterfly colonization" as a biotic tool for nature conservation as well as forest conservation. It is indicated that ecotourism industry could be framed by keeping practice of the colonizing process as a biotic epicenter (especially in the tropical forest ecosystem). This experiment brings some evidences that species richness and species assemblages are highly related with the sustainability of the colonizing process. Butterfly colonization is a process by which assemblage of butterfly host plants and 'ensure' of butterfly life cycle sustenance. It requires the knowledge of butterfly life cycle and its associated plants because colonization occurs whenever any one or more species populate in an area (Bashar 2006a). Butterfly colonization plays a vital role as a permanent tool for conservation of biodiversity of an ecosystem. Therefore, it is necessary to know the exact needs of the immature stages to make conservation successful (New *et al*. 1995). Under the technique of the establishment of open butterfly park, conservation of nature and conservation of biodiversity could be attempted in a new step to be materialized.

For the questions of butterfly colonization, it stands essential that accumulation of a situation of having assemblage of plant-species richness is the principal biotic mechanism. In connection with this, arrangement of species richness and abiotic requirements is the vital pre-requisite for establishing of butterfly colonizing centre. If the colonizing centre is successfully established then it comes in the way to advance towards the

question of establishing of an open butterfly park. An open butterfly park can ensure the accommodation of habitats and free niche establishment for all other animals (Bashar 2014a).

It is to be noted that butterfly is very sensitive animal. It is too sensitive to sound pollution, air pollution, water pollution, chemical pollution and pesticide pollution. This insect is very sensitive also to human body-smell. Butterfly acts as strong indicator of any pollution in an ecosystem. And that is why normal mating and pre-mating conditions for butterflies are to accommodate and ensure suitable environmental situation. This accommodation is being occurred here because a sound environmental situation persists both for flora and fauna by having no pollution at all. The area becomes suitable in a way which is required by the plants and animals for their survivals and progeny production. When this will be established in natural condition without any hard-boundary (still-bricks boundary) it will be sustained as a sound ecosystem. When persistence of soundness is ensured in an ecosystem, the ecosystem becomes suitable for biodiversity conservation. This may happen for ex-situ conservation or for in-situ conservation. The entire process is to be maintained and administered under the system is called ecotourism in the eco-biological sense (Bashar 2014a).

There are many other plants under the natural phenomenon that could be protected and enhanced for their species richness. Bangladesh ecology is so favourable for establishing the mechanism that the newly experimental idea could be taken into practice to go further study. This could be applied in discovering a device to protect the plant population (first trophic level) and successive trophic levels (animals).

Further research is needed to determine the effect of seasonal, temperature, relative humidity and rain fall on the fluctuation of butterfly abundance and distribution in nature. It may be suggested that more research is required to find out the relationship between butterfly larval-host plant and adult butterfly-nectar plants adaptability to detect medicinal and nutritional plants in nature through biological studies of butterfly in detailed.

Chapter 4. Biology of *Danaus chrysippus* L. (Lepidotera: Danaidae) in relation to larval host plants and adult nectar plants

4.1 Abstract

The development of *Asclepias curassavica* was examined in different five beds, viz. composite-1, children corner, exclusive milkweed, master bed-3 and T-shaped in the Butterfly Research Park at Bhawal National Park. Total 928 seedlings of *A. curassavica* were transplanted in the five beds. The survival rate of *A. curassavica* in these beds are 63.33, 70.0, 72.0, 70.0 and 58.66 percent, respectively. The life stages and development of *Danaus chrysippus* on its host-plant *A. curassavica* were studied. Singly laid eggs on the host-plant were collected from the field and reared in the laboratory under optimum conditions of temperature (25 ± 3 °C) and relative humidity (70 ± 5%). The life stages (viz. egg, larva, pre-pupa, pupa and adult) of the butterfly were described. Incubation period of the egg was 4.6 ± 0.8 days; stage duration of 1st, 2nd, 3rd, 4th and 5th instar larvae were 1.7 ± 0.2, 2.2 ± 0.2, 2.5 ± 0.3, 2.7 ± 0.2 and 3.1 ± 0.4 days, respectively; pre-pupal period was 1.4 ± 0.4 days; pupation took 8.6 ± 1.1 days; adult female period was 7.8 ± 0.3 days, and adult male was 10.4 ± 0.7 days.

The lengths of each of the larval instar were 3.7 ± 0.84, 8.7 ± 1.09, 14.3 ± 1.20, 23.2 ± 2.36 and 38.5 ± 2.54 mm, respectively. The feeding potential rate of the five instar larvae were 5.5 ± 1.11, 15.8 ± 2.86, 30.7 ± 2.90, 46.8 ± 3.03 and 71.2 ± 12.9%, respectively. The weight of the excreta of five instar larvae were 0.06 ± 0.02, 0.13 ± 0.03, 0.24 ± 0.03, 0.36 ± 0.03 and 0.42 ± 0.04 g, respectively. Total 187 individuals of *D. chrysippus* butterfly were recorded in the utilization of 16 nectar plants as nutritional support for their development in the Butterfly Research Park (BRP) for butterfly colonization. Out of 16 nectar plants, *D. chrysippus* visited the highest (16.58% of total individual) in *Lantana camara* and lowest (1.60% of total individual) in *Leucas linifolia*. No *D. chrysippus* butterfly visited the plants *Helianthus annus* and *Euphorbia pulcherrima* during the experimental period.

Keywords: Asclepias curassavica, Danaus chrysippus, Life stages, Feeding potential, Host plants, Nectar plants

4.2 Introduction

Colonization of an animal is a natural process of its multiplication materialization in a certain area of an ecosystem. If the animal is phytophagous, then its colonization needs floral assemblage together with other abiotic factors that are essential to be interacted within that place where the colonization is to be established. Butterfly colonization does require different categories of plant species together with the combination of optimum temperature, light and water bodies. It needs plants for larval food-sources, sheltering purposes and nectar-producing plants for adults. The butterflies require different phenological stages of the related plants in different seasons within the range of tolerance-suitability for their survivals in different stages of the life cycle. Butterflies have got strong coincidental synchronization between their life cycle and the phenological stages of the related plants. Knowledge of butterfly host plants and the relationship of plant-butterfly is a pre-requisite for any butterfly conservation as well as biodiversity conservation programme. Butterfly colonization is a process by which assemblage of butterfly host plants and 'ensure' of butterfly life cycle established. It requires the knowledge of butterfly life cycle and its associated plants because colonization occurs whenever any one or more species populate in an area. Butterfly colonization plays a vital role as a permanent tool for conservation of biodiversity of an ecosystem. Therefore, it is necessary to know the exact needs of the immature stages to make conservation successful (New *et al.* 1995).

The larval host plants of the butterfly belong to the family Asclepiadaceae. The recorded oviposition host plants of this butterfly include *Asclepias curassavica, Calotropis gigantea, Calotropis procera, Cryptostegia grandflora, Pentropis atropurpurea,* and *Mmarsdenia leichnardtiana.* The plant used for ovipositing by this butterfly in the study area was *Asclepias curassavica* (L.) (Asclepiadaceae). It is evergreen perennial sub shrub that grows up to 1 m (3.3 ft) tall and has pale gray stems. The leaves are arranged oppositely on the stems and are lanceolate or oblong-lanceolate shaped ending in acuminate or acute tips. Like other members of the genus, the sap is milky. The flowers are in cymes with 10-20 flowers each. They have purple or red corollas and corona lobes that are yellow or orange. Flowering occurs nearly year round. Moreover, the flowers are necteriferous (Rao *et al.* 2016).

Almost all butterflies are herbivores in their larval stages, majority of them are host specific and have close relationships with their host-plants (Price *et al.* 1991). The phytophagous insects like butterflies are closely related with the plants and provide economic and ecological benefits to the human society. Butterflies are dependent on vegetation both as adults and larvae and involve themselves in complex feeding relationships with green plants. As larvae, they feed chiefly on the foliage of plants and they are typically host specific and often show a botanical instinct in that closely related plant. The larva feeds on the leaves and makes some damage and losses on the host. The larva attacks on young shrubs and causes decline and death at last (Abaii 1999).

If the requirements of the butterfly species in the wild are thoroughly understood, it is possible to conserve them in captivity or wild. A suitable habitat for butterflies should include mating site(s) for the adults, nectar sources for adults and, larval food plants for oviposition. As butterflies are holo-metabolous with distinct developmental stages as egg-larva-pupa-adult, their reproductive output is dependent on the combined effect of larvae-derived and adult-derived nutrients or energy. These findings require a study of adult nectar resources, larval food plants, and food consumption and utilization by the larvae (Bashar 2014a).

The adult, *Danaus chryssipus* are shiny, orange in color with black and white spots on their wings (Borror *et al.* 1989). Its common name is plain tiger in Asia and African Monarch in Africa. *D. chrysippus* was found on an Egyptian tomb about 3500 B.C. and becomes the first record of butterflies in the world. *Danaus chrysippus* is a one of the well distributed species of Monarch butterfly in the Indian subcontinent after Africa, China, Chile, Sri Lanka and other Asian countries including Bangladesh (Larsen 1994).

The plain tiger butterfly is widespread and common throughout Southeast Asia and scarce in southern Honshū, Japan (Evans 1932 and Aravind 2005). In Africa, *D. chrysippus* is found in natural lowland biomes ranging from wooded savanna through grassland to semi-desert, closed-canopy forest, high mountains and sand deserts (Migdoll 1988).

The species is well adapted to human disturbance (Owen 1970), since it is most abundant in human made habitats, such as farms, gardens, waste land and roadsides. *Danaus chrysippus* does not form discrete populations and the long-lived adults range widely in search of nectar, pyrrolizidine alkaloid sources, mates and food-plants. Moreover, it is a migratory butterfly which undergoes both short term and long term movements (Smith and Owen 1997).

Danaus chrysippus is polyvoltine and prefers bushy, rocky places, and coastal gullies, usually near gardens and cultivated areas (Perkovi 2006). The biology of this species is influenced by the availability of larval food plants. Altough it is polyphagous, its larvae feed on plants which contain cardenolides, especially Asclepiadaceae, Apocynaceae and Moraceae (Ackery and Vane-Wright 1984).

The primary host plants of *D. chrysippus* are the indigenous milkweed species, Cynanchum and Marsdenia, as well as the introduced milkweed species, such as the curassavica, and fruiticose (Climate Watch 2012), all of which belong to the genus *Asclepias.* The larval milkweed host-plants of *D. chrysippus* often contain poisons, which the larva is able to isolate and retain in its body as a protection against vertebrate predation; these poisons can be passed on to the pupa and the adult butterfly (Bruyns 2000).

The theme of present experiment was undertaken to examine life stages and development of the plain tiger butterfly, D. chrysippus in both field and laboratory conditions with a view to analyze the association of the butterfly with its related plants to determine the butterfly-plant host specificity.

4.3 Material and Methods

The present experiment was conducted entitled "**Biology of *Danaus chrysippus* L. (Lepidoptera: Danaidae) in relation to larval host plants and adult nectar plants**" in the Zoological Garden, Curzon Hall of Dhaka University and Butterfly Research Park, Bhawal National Park, Gazipuer from December 2011 to January 2012). During the experimental period, successive development of *A. curassavica*, morpho-phenology of the plant, different developmental stages, viz. eggs, larvae, pupae and adults of *D. chrysippus* butterfly were maintained and examined in both laboratory and field conditions.

4.3.1 Experimental field

The Zoological garden of Dhaka University (ZGDU) was made as a germplasm centre to develop butterfly related host plants and nectar plants primarily. After development of immatured seedlings, these were transferred to the Butterfly Research Park (BRP), at Bhawal National Park, Gazipur for studying the coincidence between the phenological stages of the host plants and the life stages of butterflies. The different behavioural studies of butterflies, viz. foraging, mating, egg laying, searching, resting and puddling, and overall breeding biology of butterflies were studied and recorded in the BRP. The different developmental stages of butterfly, viz. eggs, larvae, pupae and adults were collected, preserved and identified according to the guide of Ek-Amnuay (2006), Kunte (2006) and Bashar (2015a) in the Environmental Biology and Biodiversity Laboratory (EBBL), Department of Zoology, University of Dhaka.

4.3.2 The host plant, Asclepias curassavica

Different types of seed beds were made to study the germination rate of *A. curassavica* in the Zoological garden of Dhaka University (ZGDU). After certain time, the immatured seedlings of *A. curassavica* were transferred to reach the maturation in the Butterfly Research Park. Various types of beds, viz. Composite-1, Children corner, Milkweed, Master bed-3 and T-shaped were prepared for development of the host plant in the Butterfly Research Park for sustaining butterfly colonization process (Plate 4.1A-F).

The soil of these seed beds were prepared by mixing with sand, cow dung, green manure, decomposed materials, cultivated plant under soiling and regular water supply in a correct proportion for the development of matured *A. curassavica.* This plant was developed by following different methods, viz. seed sowing, seedling transplantation, nodal explant, vegetative propagation and wind pollination. The survival rate of this plant was maximum through seedling transplantation than any other technique. Seeds were collected from the mature fruits at regular interval for preservation as a part of seed banking process in the laboratory. The nature of the seeds of matured *A. curassavica* was explosive and the seeds become burst out from the fruits. The seeds are usually found to be pollinated by wind as these were very light. Seed germination rate from seed sowing on the soil surface area was better than depth sowing of the soil and that's why wind pollination is the effective technique for the growth and development of the host plant, *A. curassavica.*

The larvae of *Danaus chrysippus* were found to damage this plant tremendously. Net covering was used to protect the host plant, *A. curassavica* from larval infestation during the developmental period temporarily. The beds of *A. curassavica* were opened for the development of *D. chrysippus* after reaching the maturation of the plant up to the expected level. The hand picking of larvae were also practiced to protect and develop this plant for establishing the butterfly colonization at Bhawal National Park.

The larval activities in relation to host plants utilization as nutritional supports were analyzed and recorded by following the methodology of Barua and Slowik (2007) and Rao *et al.* (2006). The utilization of nectar plants as nutritional supports *by D. chrysippus* was studied following Ashish *et al.* (2006) and May (1992).

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Plate 4.1. Development of the host plant, *A. curassavica.* A. Seeds B. Seed beds C. Net covering of immature seedlings D. Seedling transplantation E. Colonization of respective butterfly was observed by a visitor on matured host plants F. Explosive seeds from matured fruits.

4.3.3 Morpho-phenology of the plant

The size, shape, colour of stem, leaf arrangement and type of plants were studied during the experimental period. The arrangement of different parts of inflorescence, flowers, fruits and seeds was described by following the methodology of LeRoy (1997). The traditional uses of *A. curassavica* were described according to Oliver-Bever (1986) and Kalidas *et al.* (2009).

4.3.4 Life history of Danaus chrysippus

The life stages of *D. chrysippus* were studied in the field and in an ambient environment (25 ± 3 °C and 70 ± 5% RH) laboratory conditions during the monsoon season in the years 2011 and 2012.

4.3.4.1 Eggs

Butterflies are 'host-specific' means that females usually lay eggs on the plant on which the larva feeds. The egg-laying happens especially on or with young leaves and stems. Egg-laying support is carefully chosen. Sight, smell, touch and taste are involved in its selection. Eggs are laid on particular part of the plant like leaves, young stems, flower heads, or in crevices in the bark. The eggs are fixed to a leaf (or any substrate on which they are laid) with a special glue which hardens them very rapidly. As the glue hardens it contracts. Very often deformation of egg-shape is seen. This glue is easily seen surrounding the base of every egg forming a meniscus. The nature of the glue is unknown and is an interesting subject for doing research. The egg-laying is very characteristic in butterflies; and that is why plant-species composition according to their neediness' is necessary to be accommodated.

Butterfly eggs are protected by a hard-ridged outer layer of shell, called the chorion. This is lined with a thin coating of wax which prevents the egg from drying out before the larva has had time to fully develop. Each egg contains a number of tiny funnel-shaped openings at one end, called micropyles. Purpose of these holes is to allow sperm to enter and fertilize the egg. Butterfly eggs vary in size from species to species, but they are all spherical or ovate in shape. In the crevices the eggs are protected from rain and sunshine and to some extent from predators.

Female *D. chrysippus* lays eggs on the leaves of the host plant *A. curassavica.* The plants were planted in the 'Butterfly Research Park' of the Bhawal National Park at Gazipur. The female butterfly laid eggs singly, usually on the underside of the leaves of the host plant; sometimes she laid eggs on the other softer parts of the plant. The eggs of the butterfly were collected along with the leaves of the plant on which the eggs were laid; these were then brought to the EBBL for rearing (Plate 4.2A-F). The eggs were kept in a 3-layered larval rearing plastic cage, the description of which was given by Alam *et al* (2014). The life stages of *D. chrysippus* were observed following the methods of Golestaneh (2009).

4.3.4.2 Maintenance of caterpillars

After mating, female butterflies lay their eggs under surface of the young leaves of their selected host plants. On close observation the butterflies have been seen to lay their eggs on the host plants grown in the butterfly-colonizing areas. Before hatching these eggs could be collected and sometimes caterpillars of different instars are also collected to be reared in the insectaries of the laboratory to take necessary adult butterfly population for the new colonizing centres and for regular release in the fields where it stands necessary. Usually small plastic aquariums are used to grow caterpillars in the laboratory but sometimes one-gallon jars are also found to be suitable. The top of the container should be covered by a piece of cheesecloth and fastened securely by a rubber band.

Caterpillars were provided some sticks that fit securely into the cage for them to pupate on. Also have to keep the container with caterpillars in a light, airy space, but not in direct sunlight. The most difficult part in raising butterflies is to provide the caterpillars with fresh cuttings from the host plant appropriate for the species of caterpillar. Caterpillars are very picky eaters. Each species will only eat very specific plants. Therefore, in order to take care of a caterpillar, it is important to know what kind of caterpillar it is, and what that kind of food it eats. Caterpillars will starve to death before they will eat the wrong food. Caterpillars must always have fresh food. Caterpillars will not eat old or dry leaves. The easiest way to feed the caterpillars is to provide them with a live, potted plant in their cage.

For assessing the feeding potential rate of the larvae of the butterfly, 10 fresh young leaves of the plant were provided to the larvae hatched from the eggs and the same supply of leaves was provided daily to the subsequent larval stages of the butterfly. Sometimes, water was sprayed on the leaves to prevent these from desiccation. The larval excreta were collected every day and weighed with an electronic balance. The feeding potential rates of the larvae were calculated by following the formula used by Alam *et al.* (2014):

$TLSA = l_1 + l_2 + \ldots\ldots\ldots\ldots\ldots\ldots + l_n$

Where, TLSA = Total surface of leaf per 24 hours,

l_1 = Single leaf's surface area = 10% (was supposed),

l_2 = Double leaf's surface area = 20% and

l_n = n number leaf's surface area = 10 leaves (constant) = 100% (provided per 24 hours).

4.3.4.3 Maintenance of pupae

At some point the caterpillars will have to stop with eating. They will then crawl up to the top of the container and pupate. It is best to carefully remove them at this stage to another suitable container where they can pupate easily without any obstacle for going pupation. Newly-emerged butterflies must be able to hang high enough so that the tips of its wings will not touch the ground when they are fully expanded. Pupae do not need food or water. Most butterflies will emerge from their pupa after around two weeks. Most butterfly pupae will either turn dark or become clear when the butterfly is ready to emerge. Sometimes pupae from the natural host-plants have to be collected to rear in the laboratory.

4.3.4.4 Maintenance of adult butterfly

Butterflies can be found just about everywhere imaginable: forests, mountains, deserts, jungles, grasslands, marshes, and even the Arctic regions. Butterflies are adapted to their environments. Adaptations are inherited abilities that help butterflies carry out their life functions and survive in the natural world. Adaptations include physical structures (size, shape, and special body features), as well as internal processes (egg production, food digestion and protection

against hazards). Butterflies have adaptations for mating, feeding, escaping enemies, and environmental changes.

It is already stated that butterflies require three categories of plants for their colonization. The host plants, the nectar plants, and the shelter/shade plants. These plant categories comprise all plants at the soil surface layer of vegetation, undergrowth layer of vegetation and the canopy layer of vegetation in the forest(s), especially in our tropical rain forests. Assemblage of these three layers of vegetation in a forest gives the sound status of the forest(s). The butterflies have equal access to the plants of all the three layers of vegetation; they are very much actively related with gene-flow and diversification of plant populations in the forest.

The experiments were repeated five times and all stages were analyzed statistically following mean and standard deviation. The life stages of *D. chrysippus* were examined following the methods of Barua and Slowik (2007). Factors influencing nectar plant resource visits by butterflies were studied by following the methodology of Ashish *et al.* (2006), Tudor *et al*. (2004) and Erhardt (1991a).

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Plate 4.2. Rearing technique of *D. chrysippus* in EBBL Lab. A. Rearing cages B. Collected eggs with leaves C. Development of larva on the host plants D. Attachment of pupa with larval cage E. Emergence of butterfly from pupa F. Emerged butterfly was provided with sugar solution in a cotton wool.

4.4 Results and Discussion

Butterflies are phytophagous insects and they are very selective to their larval host plants. Relationships between life-stages of butterflies and their host-plants are most vital interactive factor in forest ecosystem. This type of interaction has got special significance in the forests of tropics and sub-tropics. Life cycle-study stands essential not only to know about butterflies but also to know forest ecosystem functioning and magnification of its healthiness. The life cycle of butterflies vary in duration with seasonality, host plant availability, host plant phenology, nectar plants availability and shelter plant availability. Some strictly host-specific species show long hibernation period in developing stages. This chapter has been dealt with development of the host plants, oviposition behaviour of *D. chrysippus* butterfly, morphological changes of larvae, duration and length variation of larvae, feeding potential of larvae, morphological changes of pupae and adults, nectar plants utilization strategy of the adult butterfly, and coevolution between butterfly and plants.

4.4.1 The host plants

Asclepias curassavica is the host plant where *Danaus chrysippus* butterfly is usually developed. The plain tiger butterfly, *D. chrysippus* lays eggs on different parts of the host plant, *A. curassavica* to maintain and sustain its breeding. It is important to develop this plant for studying the breeding biology of *D. chrysippus* butterfly as a part of butterfly colonization process. The development of *A. curassavica* has been examined in different five beds, viz. composite-1, children corner, exclusive milkweed, master bed-3 and T-shaped in the Butterfly Research Park at Bhawal National Park. Total 928 seedlings have been transplanted in the five beds. The survival rate of *A. curassavica* in composite-1, children corner, exclusive milkweed, master bed-3 and T-shaped are 63.33%, 70.0%, 72.0%, 70.0% and 58.66%, respectively (Fig.4.1 and Appendix 1)).

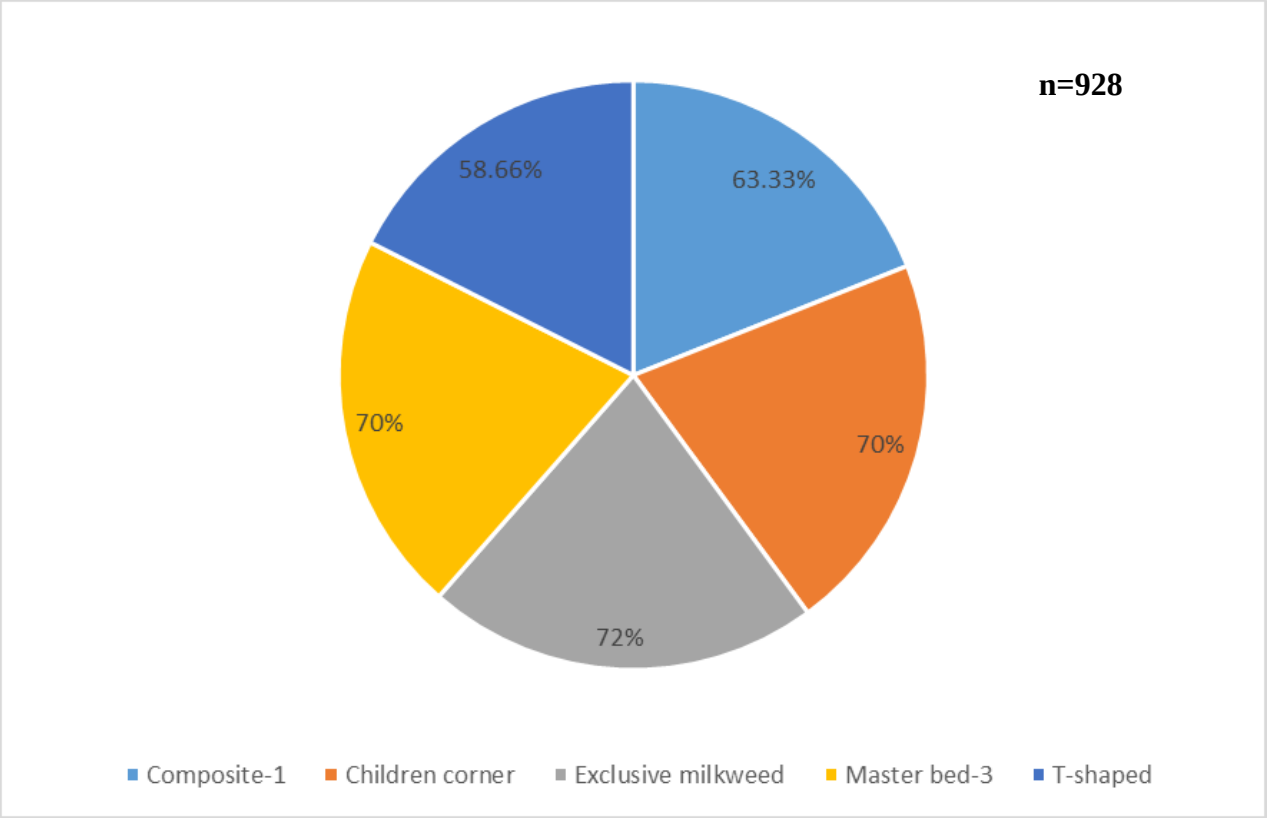

Fig. 4.1. The survival rate of the host plant, *A. curassavica* in different beds at Butterfly Research Park, Bhawal National Park, Gazipur.

The germination rate of *A. curassavica* was observed to vary in different plots. The survival rate of the seedlings of *A. curassavica* varied in different plots slightly. The structure of soil quality and seed viability are the key factor for the successive development of the plants. During the experimentation period, it was observed that correct proportion of sand, cow dung, decomposed materials and regular water supply are essential requirements for seed germination. The proper management and maintenance of ecological as well as environmental factors in a particular ecosystem are very much important for optimum growth and development of the host plant, *A. curassavica.*

4.4.2 Ovipositional behaviour of D. chrysippus butterfly

Mating of *D. chrysippus* was observed mostly during morning and evening and the copulating pair stayed at a place. The breeding female laid eggs mostly during 7.30-11.30 a.m. in the morning and 14.30-1700 p.m. in the evening. The freshly laid egg was white shiny in color, then gradually changed into creamy colour and in the end it became brownish in colour. The egg was dome shaped, with 20-22 longitudinal ridges, and with numerous indistinct lateral ridges. The egg pits were rectangular shaped. Each egg was 1.7 ± 0.5 mm in length and 0.5 ± 0.1 mm in diameter (Plate 4.3A). During oviposition, a butterfly first settled on a leaf, then turned its abdomen to underside and laid one egg on one leaf of the host plant.

In the field, it was observed that a gravid female laid 10-12 eggs at a time on different leaves of the host plant and took 5-6 minutes for egg laying at each occasion. The females were found to visit the host-plants repeatedly, to probe leaves for examining, to ascertain the tender nature of the leaves, and to search for the availability of shade for egg laying (Plate 4.3K). After flying repeatedly around the host plant for about 5-8 minutes, a female was observed to lay one egg in each of the tender leaves. During egg laying, the forewings were observed to be continuously fluttering and it took about five seconds to lay a single egg. The female under observation laid only two eggs within a time span of 30 seconds. The larva immediately after emerging started to consume its eggshell. It passed through five instars over a period of 17-18 (17.54 ± 0.54) days. Similar oviposition behaviour of the butterfly has been reported by Rao *et al.* (2016). The incubation period is 4.6 ± 0.8 days (Fig. 4.2. and Appendix 7).

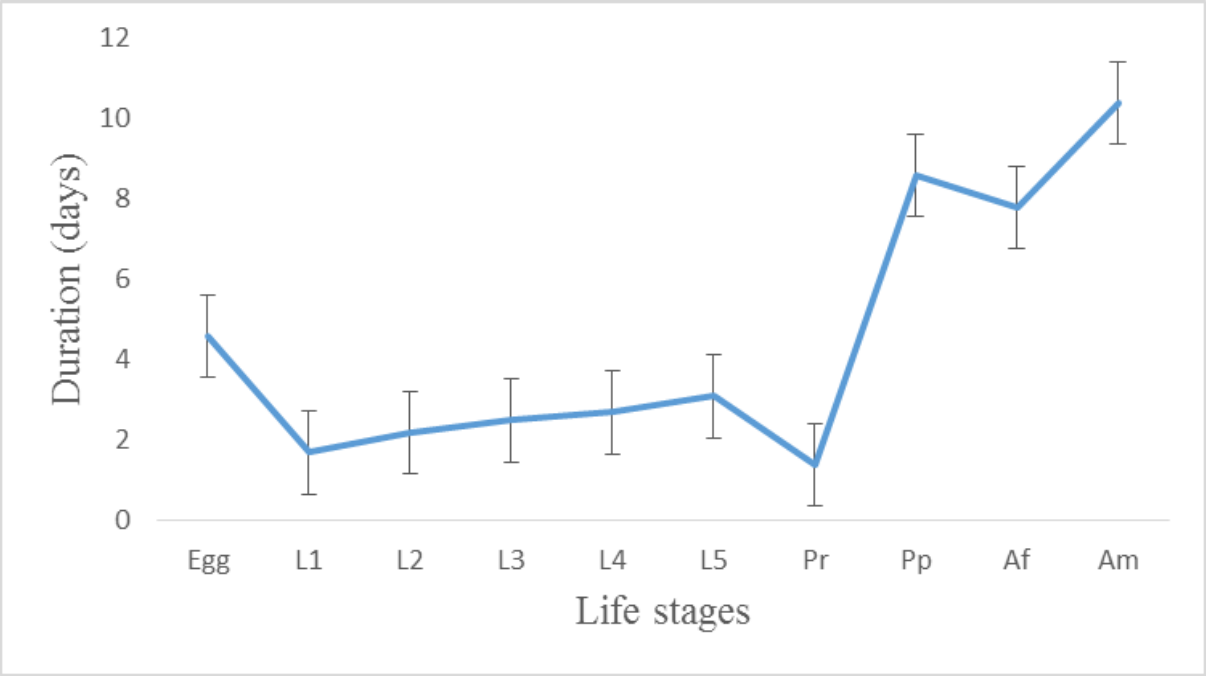

Fig. 4.2. Duration of different life stages (egg-adult) of *D. chrysippus* butterfly on the host plant, *A. curassavica* in the Environmental Biology and Biodiversity Laboratory (EBBL), Department of Zoology, University of Dhaka (2011-2012).

L1=1st instar, L2=2nd instar, L3=3rd instar, L4=4th instar, L5=5th instar, Pr=Pre-pupa, Pp=Pupa, Af=Adult female, Am=Adult male

4.4.3 Morphological changes of larvae

1st instar: Its body was yellow, with minute hairs on head and body. Head was black and head wide, was 1.20 – 1.30 (1.22 ± 0.04) mm with a pair of black horns. Yellow lines were present on the dorsal side longitudinal to the body (Plate 4.3B).

2nd instar: Body became totally green with black square shaped head, 2 (2.00 ± 0.00) mm wide. Anal spines were black. There were well-developed longitudinal yellow lines dorsally, and a pair of thinner yellow lines present on each lateral side of the body. Body and head were rough and hairy (Plate 4.3C).

3rd instar: Head was black, hairy, with two forked horns. It had white marks. Head wide, was 3.4 – 3.5 (3.48 ± 0.04) mm. There were well-developed dorsal and lateral yellow lines on the body, the dorsal pair extending up to the black anal spines. Segmentation was clear. There were no changes in other characters from the previous instar (Plate 4.3D).

4th instar: Head grew to 4.30 – 4.6 (4.6 ± 0.13) mm width and turned to reddish brown in colour along with the head horns. The white markings on head turned to cream in colour, well developed and triangular in shape. Anal spines developed orange colour dorsally. There were no changes in other characters from the previous instar (Plate 4.3E).

5th instar: Head grew to a width of 5.80 – 6.70 (6.48± 0.38) mm. Anal spines were orange coloured with black tips. Body was completely hairy. It was rough dorsally and ventrally soft and light green in colour. Orange and dark blue to green coloured spots (three pairs each) were seen on dorsal yellow pair of lines. There were no changes in other characters from the previous instar (Plate 4.3F).

4.4.4 Duration and length variation of larvae

The durations of 1st, 2nd, 3rd, 4th and 5th instar larvae were 1.7 ± 0.2, 2.2 ± 0.2, 2.5 ± 0.3, 2.7 ± 0.2 and 3.1 ± 0.4 days, respectively (Fig. 4.2). The lengths of the five instar larvae were 3.7 ± 0.84, 8.7 ± 1.09, 14.3 ± 1.20, 23.2 ± 2.36 and 38.5 ± 2.54 mm, respectively (Fig. 4.3. and Appendix 7).

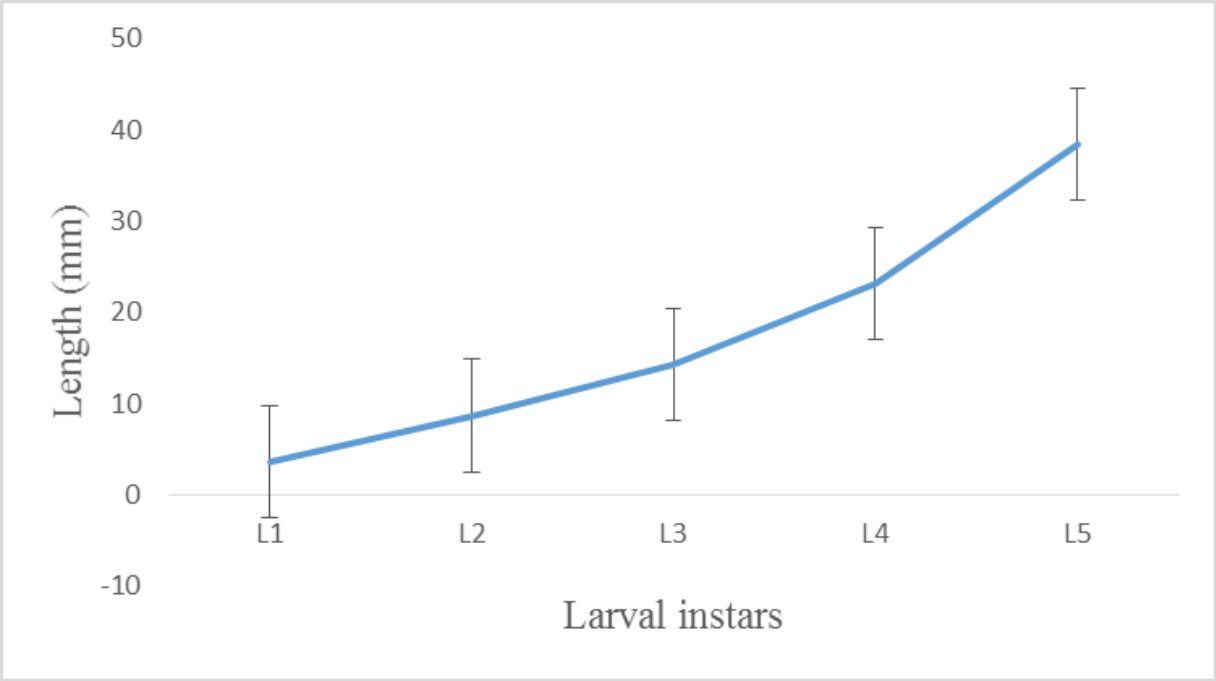

Fig. 4.3. Length of different larval instars of *D. chrysippus* butterfly on the host plant, *A. curassavica* in the Environmental Biology and Biodiversity Laboratory (EBBL), Department of Zoology, University of Dhaka (2011-2012).

The larval size depends on the availability of food sources. The later instar larva quickly metamorphosed into pre-pupa when foods are not available to them. In this condition, the larvae cannot make pupal covering (cocoon) properly and take a longer time than required, and the emergence rate of adult from pupa is poor. The size of larval instar is also reduced in such

condition. Environmental factors, moderate supply of larval food and different host plants caused the variation of larval length as reported by Alam *et al.* (2014).

4.4.5 Feeding potential of larvae

The feeding potential rate of five instar larvae were 5.5 ± 1.11, 15.8 ± 2.86, 30.7 ± 2.90, 46.8 ± 3.03 and 71.2 ± 12.9%, respectively (Fig. 4.4. and Appendix 7).

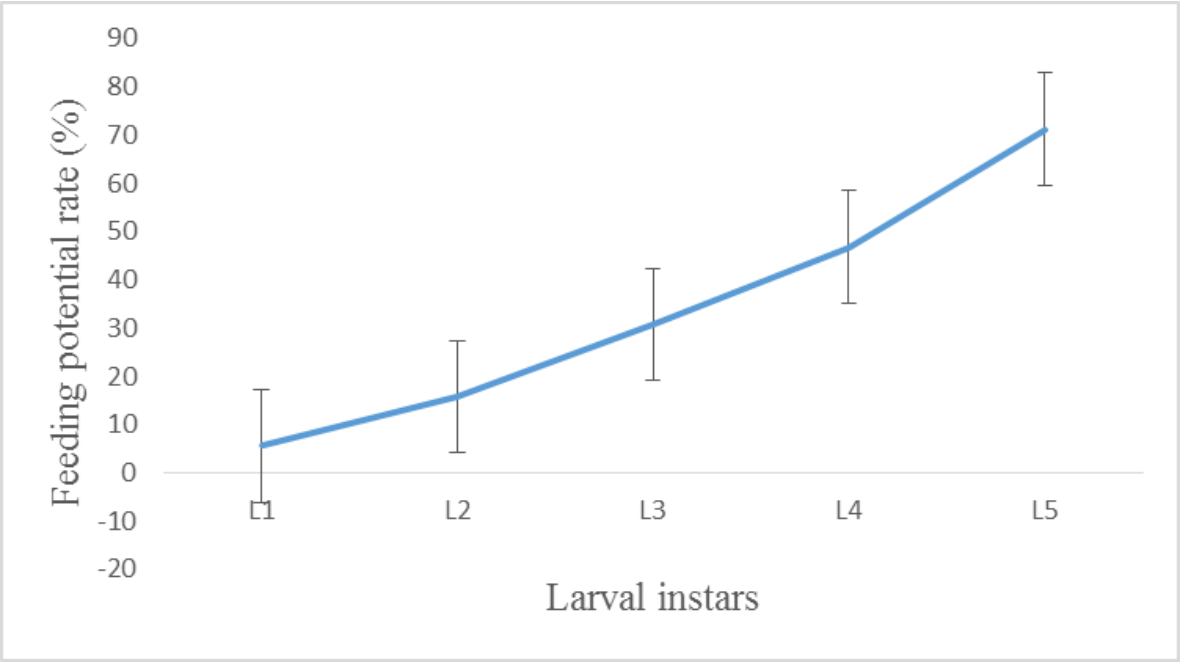

Fig. 4.4. Feeding potential rate of different larval instars *of D. chrysippus* butterfly on the host plant, *A. curassavica* in the Environmental Biology and Biodiversity Laboratory (EBBL), Department of Zoology, University of Dhaka (2011-2012).

The 1st, 2nd and 3rd instar larvae usually prefer to feed on the tender parts of *A. curassavica* (Plate 4.3B-D); the 4th and 5th instar larvae usually prefer young and mature leaves, flowers and fruits of the host plants (Plate 4.3E-F). The feeding potential of the early instar larvae is very less than later instar larvae, which are, in fact, voracious feeders. More energy is

required to be metamorphosed into pupa and that's why 90-100% supplied leaves are consumed by later instar larvae according to Barua and Slowik (2007).

4.4.6 Measurement of larval faeces

The weight of the excreta of all instar larvae were 0.06 ± 0.02, 0.13 ± 0.03, 0.24 ± 0.03, 0.36 ± 0.03 and 0.42 ± 0.04 g, respectively (Fig. 4.5. and Appendix 7).

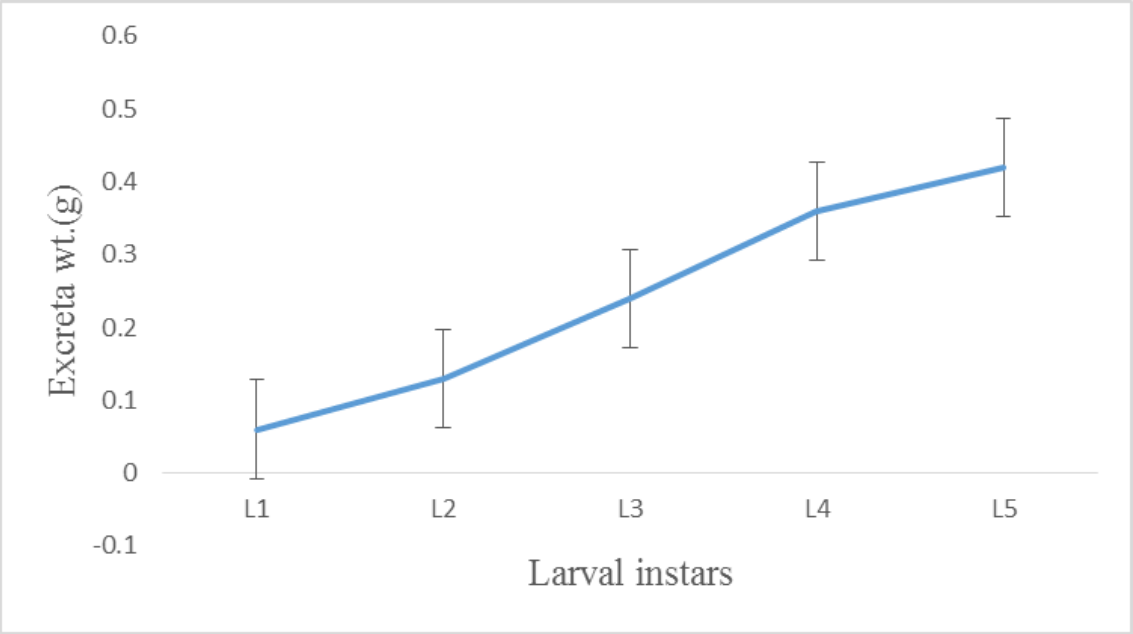

Fig. 4.5. Amount of excreta of different larval instars *of D. chrysippus* butterfly on the host plant, *A. curassavica* in the Environmental Biology and Biodiversity Laboratory (EBBL), Department of Zoology, University of Dhaka (2011-2012).

The excretory product of the larvae is proportionate to the feeding potential rate of the larvae. It has been calculated that the excretory products of the early instar larvae are less compared to later instar larvae, because the feeding potential rate of 3^{rd,} 4th and 5th instar larvae are more in comparison to 1st and 2nd instar larvae. Before pre-pupation, last instar larvae usually

excreted very large sized droppings and more amount than previous larval instars. Larval excreta are in liquid form primarily at hatching, but became solid after 2-3 days as reported by Rao *et al*. (2016).

4.4.7 Morphological changes of pupa

In pre-pupal period, the larva stopped feeding and settled down motionlessly. Its color changed from grey to brown (Plate 4.3G). The pre-pupal duration was 1.4 ± 0.4 days (Fig. 4.2. and Appendix 7). The pre-pupa is moulted into a pupa which is large, stout, mostly smooth, about 20 mm long (including the cremaster), the posterior end is rounded and slightly rugose, and ended in a short black cremaster. Rounded anteriorly, but with a pair of apical protuberances, the wing bases are slightly protuberant, and there is a finely beaded slightly raised transverse ridge dorsally on the abdomen (Plate 4.3H).

The pupae are polymorphic in colour, either pale green, bluish-green, pink or yellowish-brown. There is a series of paired dorsal yellow marks on pupal body. The pupa is 17.4 ± 0.4 mm in length and 7.5 ± 0.4 mm in wide. The pupal duration was 8.6 ± 1.1 days (Fig. 4.2. and Appendix 7). The larval, prepupal and pupal development times are the same as observed by Ramana *et al*. (1998), but different as reported by Swilem and Esmail (1972). The main reasons for these differences could be due to variety in subspecies, hosts and climates. Pupa is found in pale green and pale brown colors; the same colours observed by Smith *et al.* (1988), but different by Sharma and Verma (2005) who observed just one color in the pupal period. The color variation in pupa was controlled by the greening hormone in the larval head according to Smith *et al.* (1988).

4.4.8 The adult butterfly, D. chrysippus

Both sexes of adults are similar, but males could be distinguished by the presence of a single, small raised black sex pouch in the lower-centre of each hindwing. The males had a pair of grey "hairpencils" enclosed near the tip of their abdomens, which they can protrude and expand into a feathery like mop and dispense a characteristic scented pheromone, which is required for successful courtship. The adults are the shiny butterflies with orange and brown colors. The main difference between males and females are the presence of spots on the hind wings. Each hind wing of the males had four black spots while the females had only three black spots (Plate 4.3J). The male and female longevity were 10.4 ± 0.7 and 7.8 ± 0.3 days, respectively. The total development time from egg to adult was 35.9 ± 0.6 days (Fig. 4.2. and Appendix 7). A total of 26 to 37 days are taken for development from egg to adult as reported by Swilem and Esmail (1972) and Sharma and Verma (2005).

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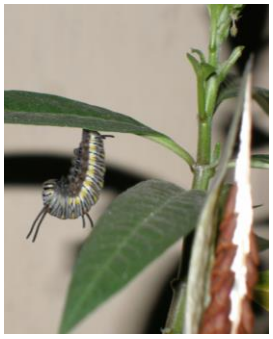

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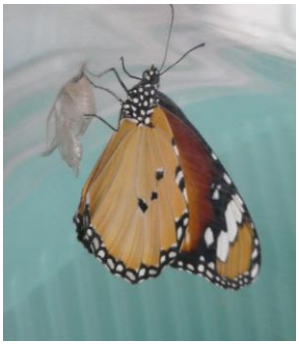

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Plate 4.3. Life stages of *Danaus chrysippus* on its host plant, *Asclepius curassavica*. A. an egg on tender shoot B. 1st instar larva feeds on young leaves; C. 2nd instar larva feeds on stem; D. 3rd instar larva feeds on matured leaves; E. 4th instar larva feeds on flowers; F. 5th instar larva feeds on matured fruits; G. 5th instar larva turned into pre-pupa; H. green colour pupa at 45° on supportive plants; I. after emergence of adult from pupa J. mating on supportive plants; K. Female adult turned its abdomen for egg laying on its host plant.

4.4.9 Nectar plants utilization strategy

The biology of *D. chrysippus* with respect to egg-laying and larval development is dependent on the host-plants *A. curassaviaca.* The food-plant resources of *D. chrysippus* in the field showed that it utilized nearly 10-15 species of flowering shrubs and trees for harvesting nectar and species, of them *Lantana camara, Duranta repens, Cosmos bipinnatus, Wedelia calendulacea, Heliotropium indicum, Duranta plumeri, Adina cordifolia, Spilanthes calva and Clerodendrum infortunatum were* observed in yearlong flowering condition.

Total 187 individuals of *D. chrysippus* butterfly were recorded from 16 nectar plants in the butterfly research park for butterfly colonization. Out of 16 nectar plants, *D. chrysippus* visited the highest (16.58% of total individual) in *Lantana camara* and the lowest (1.60% of total individual) in *Leucas linifolia*. No *D. chrysippus* butterfly visited in *Helianthus annus* and *Euphorbia pulcherrima* during the experimental period (Fig. 4.6. and Appendix 5). The nectar plant's visit of *D. chrysippus* butterfly was recorded more than average number in *Asclepias curassavica* (14.97%), *Duranta plumeri* (10.16%), *Hibiscus rosa sinensis* (7.49%), *Duranta repens* (9.09%), *Ixora chinensis* (8.02%), *Cosmos bipinnatus* (6.95%) and *Wedelia calendulacea* (5.88%), respectively. Below than average nectar sources for *D. chrysippus* were been recorded in *Tagetes patula* (3.74%), *Panica hybrida* (4.81%), *Heliotropium indicum* (2.67%), *Spilanthes calva* (5.35%), and *Gomphera globosa* (2.67%) (Plate 4.4. and Appendix 5).

It has been assumed that butterflies had no specific flower preference, and that their feeding behaviour was governed by the distribution and abundance of available nectar plants (Dosa 1999). Butterflies do not select the nectar plants randomly. The choice of nectar plants by butterfly is correlated with flower structure and their proboscis size and adaptability (Erhardt 1991a). Butterfly population size in selected species increases as the number of special nectar plant diversity increases according to Schultz and Dlugosch (1999). Nectar-feeding mechanism could play a significant role in the reproductive success and longevity for adult butterflies. Floral nectar is highly variable because different plant species produce different quantities and qualities of nectar (Baker and Baker 1983).

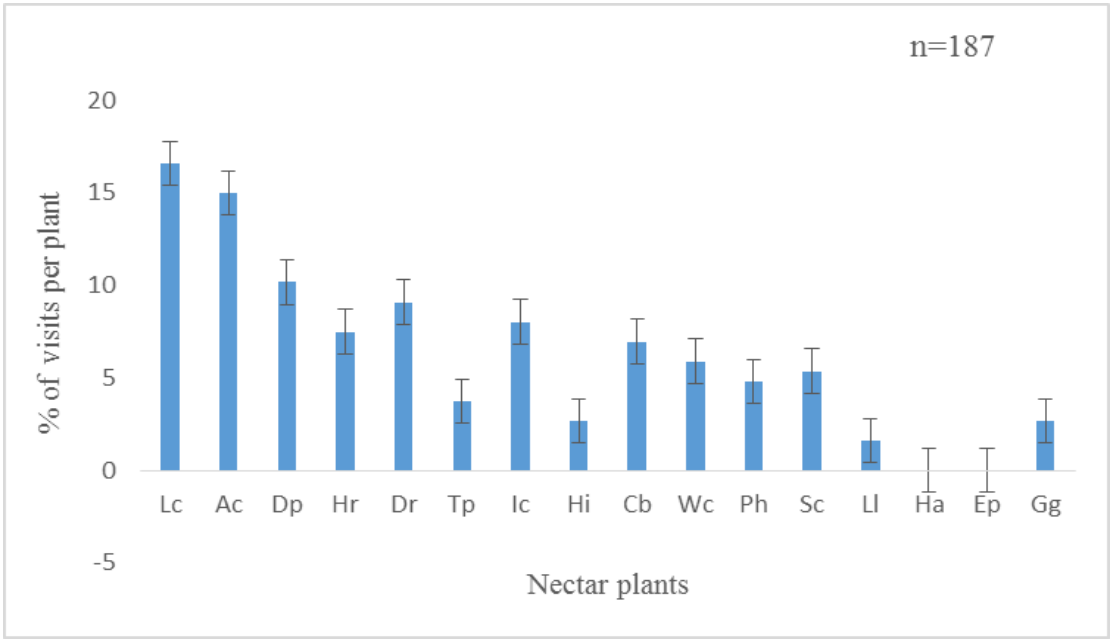


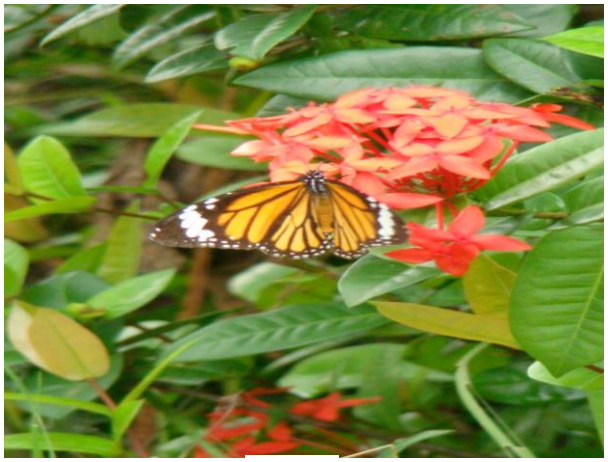
Fig. 4.6. Foraging behaviour of *D. chrysippus* observed on sixteen different nectar plants in the year of 2011-2012 at BRP, Gazipur (as indicated by percent visitors).

Lc*=*Lantana camara*; *Ac*=*Asclepias curassavica*; Dp=*Duranta plumeri*; *Hr*=*Hibiscus rosa-sinensis*; Dr=*Duranta repens*; Tp=*Tagetes patula*; Ic=*Ixora chinensis*; HI=*Heliotropium indicum*; Cb=*Cosmos bipinnatus*; Wc=*Wedelia calendulaca*; Ph=*Punica hybrida*; Sc=*Spilanthes calva*; Ll=*Leucas linifolia*; Ha=*Helianthus annus*; *Ep*=*Euphorbia pulcherrima*; Gg=*Gomphera globosa

Caterpillars are often limited to a single host plant, but adult butterflies utilize a wide variety of plants as nectar sources. For many holometabolous insects, the quality and availability of nutrient resources during the adult stage correlates with fecundity, egg weight, and longevity (Boggs 1997, MeviSchütz and Erhardt 2003, O'Brien *et al.* 2004). Nectar, a primary nutrient source for adult Lepidoptera, varies by plant species in both its carbohydrate and constituent components which can affect fecundity (Romeis and Wäckers 2002). Therefore, nectar resources for adult Lepidoptera influence species occurrence, distribution, and density, thus nectar sources are an important component of butterfly conservation.

A

B



C

D

E

F

Plate 4.4. Foraging strategy of *D. chrysippus* butterfly utilizing different nectar plant species as nutritional supports for their development. A. *Lantana camara* (Verbenaceae) B. *Asclepius curassavica* (Asclepiadaceae) C. *Ixora chinensis* (Rubiaceae) D. Not identified E. *Heliotropium indicum* (Boraginaceae) F. *Chromolaena odoratum* (Asteraceae).

4.4.10 Morpho-phenology of the plant

Asclepias curassavica is a perennial herb, an erect, glabrous, perennial herb that grows up to 1.2m tall. It has a milky exudate throughout. The stem is smooth, round, dull green or suffused with dull red. The leaves are simple, opposite, shortly petioled, lanceolate of oblong-lanceolate, acuminate and measures 7-13cm long and 6-25cm wide. The base is narrowed. Inflorescence in the form of an umbel with 6–15 flowers on terminal or axillary peduncle. The flowers are perfect, radially symmetrical or irregularly shaped, bright red or orange with yellow centers. There are 5 sepals, deeply divided, reflexed, and green. Five petals which are linear with base united into a fused corolla. The corolla lobes are red, reflexed, oblong and approximately 8mm long. The corona scale is orange in colour, 5-lobed and measures 3.5-4.0mm long. The corona is hood-shaped with inwardly curved horns; stamens 5 in number; anthers with two pollen sacs; pollen aggregates into masses called pollinia or pollen sacs. The style filaments are united with pistils 2-carpelled. The fruit is a pair of dry dehiscent, spindle-shaped follicels, measuring 5-15cm long, many seeded, splitting lengthwise on one side at maturity. The seeds are ovate, flat, winged, measures 4-6mm long and 2.2-4.0mm wide, brown in colour, minutely ridged, with a pappus of fine white silky hairs at the apex, measures 23cm long (LeRoy 1997). *Asclepias curassavica* is used in China to disperse fever (clears heat), improve blood circulation and to control bleeding. Entire plant is dried and decocted as a cardiac tonic, for tonsillitis, pneumonia, bronchitis, urethritis and external and internal bleeding (Oliver-Bever 1986 and Kalidass *et al*. 2009).

4.4.11 Enhancement of plant population sustenance

The *A. curassavica* is related with *D. chrysippus* butterfly both as host and nectar plants. The plants exposed to butterflies are found to produce more fruits and seeds than those of the plants not exposed to butterflies. On the other hand, the non-exposed plants produced seeds with low quality and less weight compared to the seeds produced by the plants exposed to the butterflies. Similar results were also obtained when experiments were conducted with other two plants, viz. *Aristolochia indica* and *Duranta plumieri.* It is evident that butterflies can bring significant results to the case of healthy seed production examined by Bashar *et al.* (2015b). The healthy seed production can enhance the production of genetically more viable plants also and can sustain good population size in the ecosystem where the colonizing process is practiced.

As each of the butterfly species was related with each respective plants for foraging, egg-laying, resting and pupating, and other activities, and if through the activities they (butterflies) bring healthiness to the related plants, they can help in the questions of their conservation in the same ecosystem reported by Bashar *et al.* (2015b) and Bashar *et al.* (2006a).

To conserve the plain tiger butterfly, the host-plants and nectar-plants of these species must be protected and conserved in nature according to Wiklund and Ahrberg (1978). A sustainable harvest of the butterfly in breeding houses will not only help in maintaining the recovering populations in the wild, but the dead stock having good commercial trade value will also contribute to the trade in butterflies. Captive breeding will also help in a better understanding of its biology and an effective conservation strategy through the creation of local awareness, particularly amongst school children and local villagers living near protected and unprotected forests, can prove to be the most effective method for the conservation of butterflies as described by Barua and Slowik (2007).

Butterfly-colonizing process is characterized by some special biotic interactions. To enhance species assemblage and species richness both for plants (first trophic level) and animals (successive trophic levels) butterfly-colonization stands as an important key factor in a terrestrial ecosystem (Bashar 2015b). This hypothesis is much more practicable especially in the tropical rain forests like the forests of Bangladesh (Bashar *et al.* 2006a). In compare to the density of nectar plants and food plants area, the shade/resting plant area must be more dense and with assemblage of high species composition for butterfly colonization according to Bashar (2015a).

Further research is required to analyze the chemical and nutritional values of larval stages of *Danaus chrysippus* butterfly and different extractive parts of the host plant, *Asclepias curassavica* to examine the medicinal importance. It may be suggested to analyze chemical values of different nectar plants utilized as nutritional support by the adult butterfly, *D. chrysippus* in nature. To determine the butterfly-host specificity, synchronization of coincidences between larval feeding potential of *Pachliopta aristolochiae* butterfly and different phenological stages of the host plant, *Aristolochia indica* will be discussed in detailed (Chapter 5).

Chapter 5. Biotic interaction between *Pachliopta aristolochiae* F. (Lepidoptera: Papilionidae) and its host plant, *Aristolochia indica* (Aristolochiaceae)

5.1 Abstract

To study biotic interaction between *P. aristolochiae* butterfly and its related plants, successive development of the host plants, *A. indica*, egg laying strategy, host plant utilization of larvae and pupation strategy in the field were examined. Length variation, feeding potentiality of larvae and weight of larval excreta were analyzed in the laboratory experiment. *P. aristolochiae* and its different behavioural strategies, viz. foraging, mating, egg laying, resting, searching and puddling in relation to population fluctuation were also studied and recorded in the experimental field. The survival rate of the seedlings of *A. indica* in plot-1, plot-2, plot-3, plot-4 and plot-5 were 74%, 66%, 76%, 54% and 42%, respectively. The average germination and seedlings rate of total seeds (out of 250) were 73.6% and 62.4%, respectively.

The average egg frequency in young leaves, stems (tender shoots), matured leaves, inflorescence and fruits of *A. indica* (n=10) were 60.5, 30.6, 15.8, 0.7 and 0, respectively. Out of total 269 larvae of *P. aristolochiae*, maximum number (65 out of 84) of the 1st instar larvae in young leaves, minmum (6 out of 84) in matured leaves and no larvae in inflorescence and fruits were recorded; maximum number (47 out of 77) of the 2nd instar larvae in young leaves, minimum (2 out of 77) in inflorescence and no larvae in fruits were observed; maximum number (21 out of 59) of the 3rd instar larvae in matured leaves and minimum (5 out of 59) in inflorescence were found; maximum number (22 out of 49) of the 4th instar larvae in matured leaves and minimum (3 out of 49) in young leaves were recorded during the experimentation.

5.1 Abstract

Out of 10 beds (n=10 plants per bed), 100% of the host plant, *A. indica* were consumed by the larvae of *P. aristolochiae* in bed-2, bed-5, bed-8 and bed-9. 70%, 90% and 80% of the host plant, *A. indica* were utilised as nutritional support by the different larval instars of *P. aristolochiae* in bed-1, 4 and 6, respectively. On the other hand, no plant was consumed in bed -2, 7 and 10 by the larvae of *P. aristolochiae,* as these beds were planted by other species (except *A. indica*) as controlled experiments. Out of 116 pupae, 40% of the pupae emerged as the adult butterfly in early (on time) and 24.2% emerged in late (diapause) in the field condition.

The variation in length, feeding potential rate and faeces of larvae were examined and presented in laboratory experiment. The length of 1^{st} instar, 2^{nd} instar, 3^{rd} instar and 4^{th} instar larvae were 4.0 ± 0.63, 9 ± 0.63, 22.6 ± 5.2 and 38.2 ± 4.70 mm, respectively. The feeding potential rate of 1^{st} instar, 2^{nd} instar, 3^{rd} instar and 4^{th} instar larvae were 11.4 ± 5.04%, 29.6 ± 5.12%, 51.4 ± 6.0% and 72.8 ± 4.9%, respectively. The weights of the faeces (excreta) of 1^{st} instar, 2^{nd} instar, 3^{rd} instar and 4^{th} instar larvae were 0.012 ± 0.004, 0.047 ± 0.018, 0.0114 ± 0.023 and 0.274 ± 0.045 g, respectively.

Keywords: Biotic interaction, Seedlings, Host plant, *A. indica, P. aristolochiae*, Larval activity, Feeding potentiality

5.2 Introduction

Biotic interaction is the direct or indirect interaction between species or individuals and the effect of interaction between two components can be positive, neutral or negative. Indirect interactions are any activity where organisms change the environment whereby conditions for other organisms are altered. Examples of indirect interactions are when the Barn owl eating bank vole thus affecting the food source of the red fox or when the seeds of the Brazil nut germinate and reach maturity thus offering nectar and pollen to its Hymenopteran pollinators, because an Agouti several years earlier distributed and burrowed (and forgot) them. Direct interaction is when individual (of the same or different species) interact. Examples of direct biotic interactions are when the Peacock eats the seeds of plants, when the peacock butterfly uses its colourful eyespots as anti-predation measures against Great tits, or when a male of the peacock spider dances and displays its spectacular abdomen to courtship a female (Begon *et al.* 1996).

The host plant, *Aristolochia indica*, one of the 500 species of the family Aristolochiaceae is distributed throughout the tropical, subtropical and Mediterranean countries. In Indian subcontinent, the plant is found in low hills and plains of India from Nepal and lower Bengal to Chittagong in Bangladesh and Coromondal Coast (Murugan *et al*. 2006, Kanjilal *et al*. 2009). This endangered medicinal plant, locally known as Isharmul (Bengali and Hindi) is a shrub with long twinning stem. The plant is a shrubby or herbaceous vine with a woody root stock (Kanjilal *et al*. 2009).

Flowering and fruiting of this climbing herb are found from December to February (Neelima *et al*. 2011). The leaves are glabrous, very variable, usually obovate-oblong to sub-pandurate entire with undulate margins, cordate acuminate. Flowers are few, in axillary racemes with a perianth up to 4 cm long having a glabrum pale green inflated (Das *et al*. 2010). Co-evolution of *A. Indica* and Papilionid Butterflies have been noted (Sivarajan and Pradeep 1989).

Insects which feed on a definite few host plant species and those which feed upon a wide variety of host plant species are called oligophagous and polyphagous respectively. The chemicals are characteristic of the host plant used by butterfly, this causes the butterfly to oviposit on the correct type of host plants (Schoonhoven 1973). The idea of co-evolutionary balance between host plant resistance and herbivore "virulence" have been used to explain the observed pattern of butterfly/ host plant taxonomic relationship (Ehrlich and Raven 1964).

All butterflies are herbivores in their larval stages, majority of them are host specific and have close relationships with their host-plants (Price *et al.* 1991). The Common Rose Butterfly, *Pachliopta aristolochiae* (Lepidoptera:Papilionidae) is a swallowtail butterfly. It is a common butterfly and abundant across South and South East Asia (Wynter-Blyth 1957). The IUCN status of this species is 'Very Common' and 'Not Threatened' in the Indo-Burma hotspot (Collins and Morris 1985).

The larval food plant *Aristolochia indica* and *A. tagala* are poisonous creepers (vine) belonging to the plant family Aristolochiaceae which are also mostly tropical (Hoehne 1942). Commonly known as the Indian Birthwort (Local name: Iswarimul) which has pharmacological properties (Chen and Zhu 1987) and the plant extract is known to have potent anti-cancer properties (Hynniewta and Baishya 1992).

Butterflies select host plants for oviposition using chemical cues. Females usually oviposit only on those plants, which are suitable for larval growth and survival. They usually select new larval food plants, which are related to their usual host plants (Kunte 2006). The larval stages of all the red-bodied swallowtails are *Aristolochia* feeders and this makes them unpalatable to their vertebrate enemies like birds and reptiles due to the sequestering of aristolochic acids in the larval tissues which are passed on to the adults (Tian-Shung *et al.* 2000, Von *et al.* 1968, Rausher and Feeny 1980).

Butterflies are one of the most studies invertebrate groups (Boggs *et al.* 2003). They represent a special case since adults almost exclusively feed on nectar from flowering plants, limiting the acquirements of nitrogen and other important resources to the larval stage. Since life history consequences may vary extensively among hosts, female oviposition choice to a great extent determines offspring future fitness.

A significant majority of butterflies has strong interactions with flowers, plants and other biotic components of any ecosystem, but the information about butterfly species and their nectar-host plant relationships in Bangladesh are scanty (Ehrlich and Raven 1964, Huffaker *et al.* 1999). Floral attributes are well known to influence nectar-feeding butterflies. The diversity of butterflies for particular habitats is associated with the availability of larval host plants and adult nectar plants. Many of the flowering plants are used by butterflies as nectar plants and support a rich diversity of butterflies. Butterflies have been found to differ in the range of available nectar sources used (Ashish 2006 and Nair *et al.* 2014). The *Chromolaena odorata* (L.) (Asteraceae) florets attract butterflies and an important nectar source for adult butterflies (Lakshmi and Raju 2011).

The experiment envisaged to investigate the synchronization of coincidences between life stages of Pachliopta aristolochiae butterfly and different phenological stages of its host plant, Aristolochia indica especially emphasizing on host plant utilization strategy by larvae for assessing nutritional values of the host plant.

5.3 Material and Methods

The present experiment was investigate entitled "**Biotic interaction between *Pachliopta aristolochiae* butterfly and its host plant, *Aristolochia indica***" in Satchori forest, Zoological Garden and Butterfly Research Park from July 2010 to June 2014. Considering the importance of butterflies from multifaceted directions, the Environmental Biology and Biodiversity Laboratory (EBBL), University of Dhaka has undertaken a grand programme to go through the Plant-Butterfly interaction in nature. Under the programme, the EBBL has envisaged to materialize a vital frame-work for colonizing butterflies in an open forest ecosystem. By this time, the laboratory has innovated 'biotic-biotic interactions' as tools for wildlife conservation and conservation of nature (Bashar 2014a).

5.3.1 Field selection and field sampling

Satchari forest was selected to carry out the experiment on "Biotic interaction between the *Pachliopta aristolochiae* and its related plants. The *P. aristolochiae* butterfly and its related plants especially host plant, *A. indica* and *A. tagala* are available in Satchari forest and that's why this area was select to conduct the research experiment. Site selection was made according to Pollard and Yates (1993).

5.3.2 Host plant selection

P. aristolochia butterfly usually used two species of *Aristolochia* plant; one is *A. indica* and another is *A. tagala* as host plant (Plate 5.1A, B). These two species are wild climber herb in nature and abundant in Satchari forest. *A. indica* and *A. tagala* were detected and identified as a host plant by following egg laying behaviour of *P. aristolochiae* butterfly. These host plants were identified after long time and regular monthly sampling in Satchari forest (Plate-5.2). Interaction between butterfly and related host plants were studied according to methodology of Rausher and Papaj (1983), Ehrlich and Raven (1964) and Price *et al.* (1991).

A. Aristolochia indica bed

B. ***Aristolochia tagala bed***

Plate 5.1. Development of the matured host-plants for colonization of *Pachliopta aristolochiae* butterfly in the Zoological garden at Curzon Hall, University of Dhaka. A. *A. indica* was supported with ladder made of bamboos. B. *A. tagala* was supported with bamboos stick.

5.3.3 Selection of biotic epicentre

Satchari forest is one of the hilly rain forests in Bangladesh. About 20 biotic epicenters were selected and marked to determine the behavioural activities and population density of common rose butterfly and birdwings in relation to host plants. The biotic epicenters were made on availability of *A. indica,* and *A. tagala* in Satchari forests. As these were climber in nature, at least 5-6 species composition of plants were also included as key component of biotic epicentres in addition to host plants (Plate 5.2).

Plate 5.2. The Researcher searching the host plant of *P. aristolochiae* butterfly to select biotic epicenter in the Satchari forest, Hobiganj.

5.3.4 Seed collection

The matured seeds of *A. indica* and *A. tagala* were collected on regular basis in each sampling from the Satchari forest. It was difficult to collect seeds from biotic epicenter. As their height was 100 to 150 ft, seeds were usually collected by ascending on hill top or throwing pieces of brick or rope to the top of the plant where matured fruits were available.

5.3.5 Seed storing and preservation

After the extraction of seeds from matured fruits, these were immediately placed inside a small sized plastic container and finally stored and preserved in the laboratory. The seeds in two week intervals were exposed to sunlight for drying and preventing from fungal infection. The tiny seeds for germination were extracted from the matured fruits and tested for viability by steeping them into a suitable container of water. The floated seeds were discarded as non-viable seeds while the sunken seeds were regarded as viable seeds. These were removed from the water and spread on old news print under room temperature (32ºC)

for three days. The seeds were certified dried when they no longer stick together. After drying, the seeds were kept in plastic containers in the laboratory.

5.3.6 Seed bed preparation

Seed beds were prepared to carry out the experiment on the seeds of *A. indica* and *A. tagala* in germplasm centre at the Zoological garden, Curzon Hall, University of Dhaka (Plate 5.3A). Different size and shape of beds were prepared to measure germination rate, seedling rate and survival rate of the host plants *A. indica* and *A. tagala.* Beds were covered by net after germination of seeds to protect seedlings from larvae of *P. aristolochiae* and any undesirable organism (Plate 5.3B). The beds were prepared by mixing of sand, cow dung, green manure, decomposed materials, cultivated plant under soiling and regular water supply in a correct proportion for the development of matured *A. indica* and *A. tagala plants.* Temperature and humidity were maintained by supplying of water on regular basis according to needs. Seeds for germination experiments were collected in their dried state on the plant species. Germination, seedling and survival rate of the host plants were evaluated by following the methodology of Murugan *et al.* (2006), Ehiagbonare (2004) and Sivarajan and Pradeep (1989).

5.3.7 Seedling transplantation

Immature seedlings were transplanted to establish the colony of *P. aristolochiae* butterfly at Bhawal National Park. Different types of beds were prepared for the development of *A. indica* and *A. tagala* host plants. The soil quality of Bhawal National Park was not suitable for development of these plants. As these plants were wild climber herb and usually developed in hilly rainforest area, so it was needed to change soil structure. 10-20 immature seedlings were planted with bamboo stick support initially with each seedlings (Plate 5.3C). Seedlings were planted in two types of beds like support with shade and support without shades (direct sunlight) to compare the survival rate of *A. indica* and *A. tagala* plants up to maturation of host plants. Support was made by Bamboo ladder, canopy tree or medium tree or combination of other biotic hedge. During the experimentation period, it was observed that correct proportion of sand, cow dung, decomposed materials and regular water supply are essential requirements for seed germination and proper growth and development of *A. indica*. It prefers enough moisture of soil structure to develop properly. The successful growth and

development of *A. indica* depends on the proper management and maintenance of ecological as well as environmental factors in a particular ecosystem (Plate 5.3D-F).

5.3.8 Ethological aspects of P. aristolochiae butterfly

P. aristolochiae butterfly laid eggs on immature seedlings immediately after plantation of *A. indica* and *A. tagala*. Primarily it was difficult to develop the host plant of *A. indica* and *A. tagala* for tremendous attacking of the larvae of *P. aristolochiae* butterfly. Preventive measures were taken to develop the host plant of *P. aristolochiae* butterfly by net covering and hand picking of larvae up to maturation of the host plant, *A. indica* in temporarily. The plantation of *A. indica* and *A. tagala* was increased significantly for colonization of *P. aristolochiae* butterfly in the experimental field. The colonization process of butterfly depends on three types of plant, viz. host plants, nectar plants and shelter plants and these were successfully developed at Bhawal National Park according to requirements of butterfly colonization. The larval activities in relation to host plants utilization in both laboratory and field were analyzed and recorded according to Barua and Slowik (2007), Rao *et al.* (2006), Zalucki *et al.* (2002), Scriber (1984) and Rausher and Feeny (1980). Differenr behavioural strategies of the adult butterfly, *P. aristolochiae,* viz. mating, oviposition, searching, and puddling in relation to abundance were studied and recorded according to Mamun *et al.* (2008), Young (2007), Pola and Garcia-Paris (2005), Elsevier (2003), Brakefield (1982) and Courtney and Parker (1985). Factors influencing nectar plant resource visits by *P. aristolochiae* butterflies were studied by following the methodology of Ashish *et al.* (2006), Tudor *et al.* (2004), May (1992), Jennersten (1984) and Erhardt (1991a).

A

B

C

D

E

F

Plate 5.3. Successive development of phenological stages of the host plant, *A. indica* at the Zoological garden, Curzon Hall, DU. A. Seed germination B. Seedlings covered by net to protect from larvae of *P. aristolochiae* C. Seedlings transplantation to be matured D. Matured *A. indica* with bamboo stick support E. Inflorescence of *A. indica* with supporting plants F. Matured fruits of *A. indica with* support.

5.4 Results and Discussion

Environmental Biology and Biodiversity Laboratory (EBBL), Department of Zoology, University of Dhaka very recently made a study on the strategy of larval damage caused by a butterfly species (*Pachliopta aristolochiae*) on its host plant (*Aristolochia indica*). The insect is host specific and in the larval stages it does not eat plants other than the Indian birthwort. The study has revealed that after larval-damage caused on the young leaves and stems, the rest parts (stems) of the damaged plant get proliferated. During the infestation, damage caused by the insect is supposed to be colossal to the plant. On the contrary, branching occurred on the host plant after damage is made by the insect. The plant's propagation was found generated even after damage had caused. The association is very much commensalistic among different plant species and at the same time in between the plant and insect species related to them. In accordance with different seasonal variations, commensalism of such type carries significant and important role in natural species equilibrium. This is a process of natural equilibrium diversity (NED) for the plant through interaction with the related butterflies (Bashar 2011). The EBBL finds interesting aspects in butterflies to conduct such scientific investigations. This chapter has been emphasized on development of the host plant, *Aristolochia indica*, egg laying strategy, host plant utilization strategy of larvae, variation in length, feeding potential, faeces of larvae, pupation strategy, morphological and behavioural status of *Pachliopta aristolochiae* butterfly in both field and laboratory conditions during the research period.

5.4.1 The host plant, Aristolochia indica

Total 250 viable seeds of the host plant *A. indica* were sown in different five seed beds at Germplasm centre. Fifty seeds were sown in each seed beds. The survival rate of seedlings in plot-1, plot-2, plot-3, plot-4 and plot-5 were 74%, 66%, 76%, 54% and 42%, respectively. The average germination and seedling rates of total seeds were 73.6% and 62.4%, respectively (Fig. 5.1. and Appendix 1).

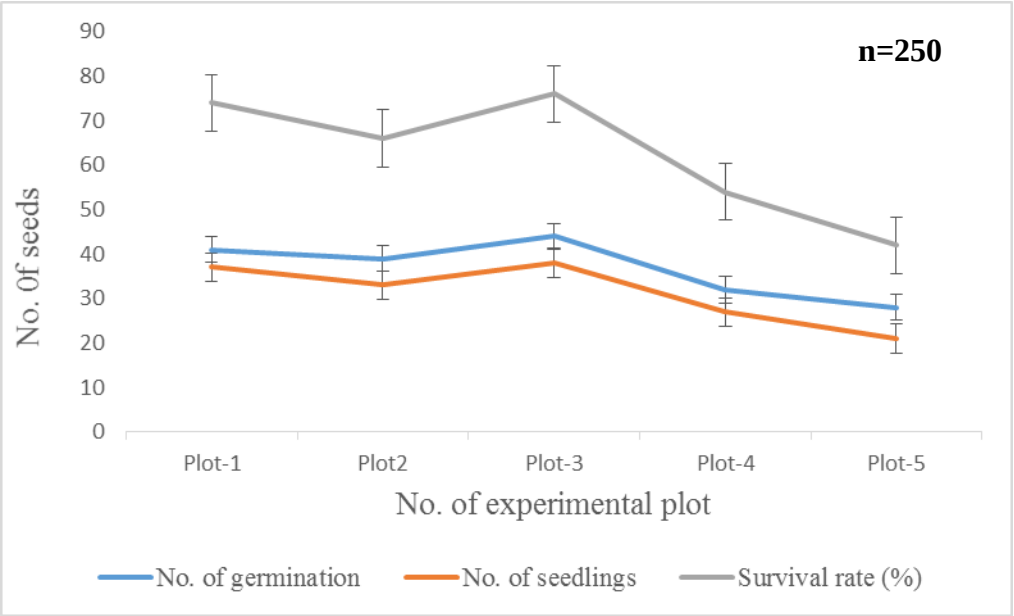


Fig. 5. 1. Development of *A. indica* seedlings in germplasm centre at Zoological Garden, Curzon Hall, University of Dhaka.

Sufficient temperature, water and oxygen are required for germination of seeds. The germination rate of *A. indica* varied in different plots. It was found that the seedlings rates of *A. indica* varied in different plot significantly. The survival rate of seedlings of *A. indica* gradually decreased more than the germination rate. Actually *A. indica* is a wild plant in nature. It is usually developed in hilly area, but my experiment was practiced in urban area. The successful germination rate of seeds depend on the maintenance of environmental factors. The structure of soil quality is the key component for the successful germination. It may vary from one soil ecology to another soil ecology. It was observed that the survival rate of seedlings depended on the proper management and maintenance of ecological values.

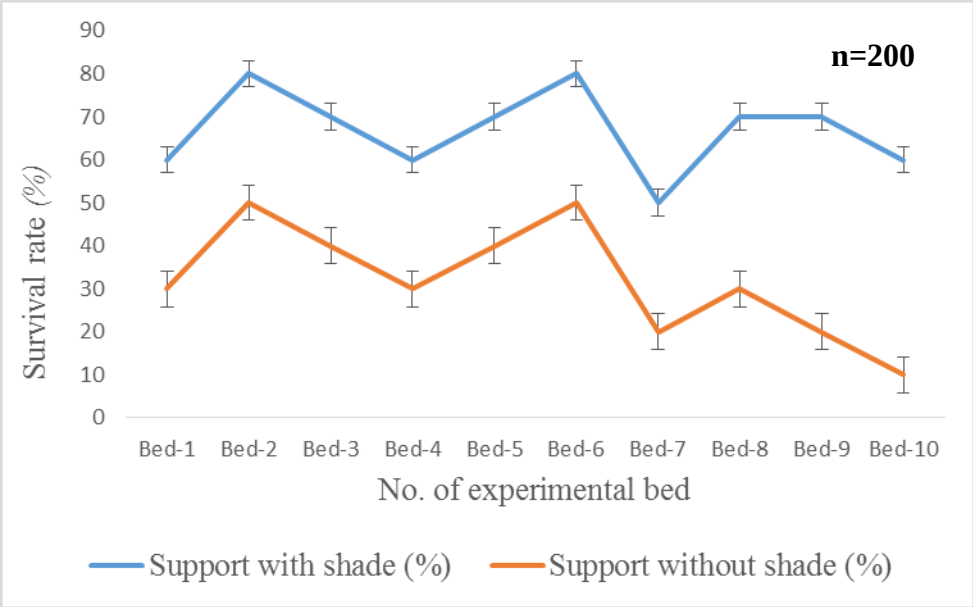


Fig. 5.2. Survival rate of matured *A. indica* for colonizing *Pachliopta aristolochiae* butterfly in Zoological Garden of Dhaka University and Butterfly Research Park at Bhawal National Park, Gazipur (2010-2011).

Total 200 healthy seedlings of *A. indica* were transplanted in two category of beds, supported with shade and without shade. Each category consisted of 10 beds and 10 seedlings were sown in each of the beds of each category to examine the growth rate and development of matured *A. indica*. The host plant, *A. indica* can grow very well in shade than direct sunlight. Because this plant is one kind of wild climber herb. It can grow up to 150-200 feet high with support. A fruitful result was found when the seedlings of *A. indica* were transplanted around the large tree than the small tree as climber support. The average growth and survival rate of *A. indica* is significantly higher in support with shade (67%) than support without shade (32%) as shown in Fig. 5.2. and Appendix 8. It was found that the plant growth rate was moderate from August to November, the rate became dormant from November to February and rapidly increase in the months of March and April for reproductive and fruitification (Aich *et al.* 2016).

Significant differences exist in germination and seedling growth as influenced by pre-sowing treatment, depth of sowing and type of soil. Seed sown at a depth of one cm had the highest value of 95 ± 2.8% germination in sand, garden soil, sphagnum and cowdung (SGSC) while 75 ± 2.8% was obtained from the soil garden (SG) and 70 ± 2.8% in sand. The surface sowing had 90 ± 2.8% germination in SGSC medium, 65 ± 2.8% in SG medium and 65 ± 2.8% in sand. The seedling growth was of the same pattern as reported by Sarma *et al.* (2015).

One of most usual causes of failure of seed to germinate and emerge is sowing too deeply. Usually a seed has only food reserve for limited period of growth and when sown deeply more than necessary, it soon expend that food or energy and dies before it reaches the surface (Ehiagbonare 2004). Furthermore from field experimentation it has been found out that depth of seed sowing is species specific. It is therefore of utmost importance to determine optimum sowing depth before embarking on a large scale macropropagation. Ehiagbonare (2007) also observed one cm depth of sowing to be the best for *Ocimum gratissimum.* Further, optimization of suitable macropropagation method would be very much important from the conservation point of view of this endangered plant species (Sarma *et al.* 2015). A single method has been standardized for the micropropagation and in vitro conservation of *A. indica* an important medicinal plant and this will augment the overall conservation strategy to protect this species (Prabha 2008).

5.4.2 Egg laying strategy of Pachliopta aristolochiae

Total 10 individual host plants of *A. indica* were taken for examination of the frequency of eggs distribution of *P. aristolochiae.* It has been found that the average number of egg frequency in young leaves, stems (tender shoots), matured leaves, inflorescence and fruits of *A. indica* are 60.5, 30.6, 15.8, 0.7 and 0, respectively (Fig. 5.3. and Appendix 9). The eggs were laid on a particular part of the plant, like leaves, young stems, flower heads, or crevices in the bark (Rabasa *et al.* 2005).

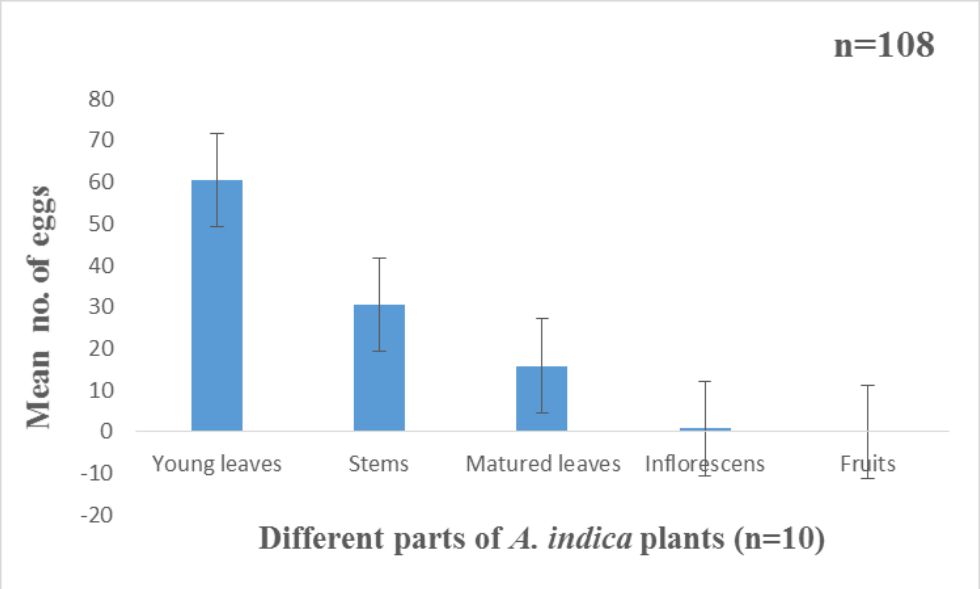


Fig. 5.3. Oviposition of *Pachliopta aristolochiae* butterfly in phenological stages of the host plant, *A. indica* in the Zoological Garden of Dhaka University (2010-2011).

In field conditions, it was observed that a gravid female laid 8-10 eggs at a time on different leaves of the host plant and within a time span of five minutes. The females were observed to repeatedly visit the host-plants and tried to probe the leaves for ascertaining their suitability for egg laying like the tender nature of the leaves and availability of shade. After repeatedly flying around the host-plant for about 5-8 minutes, a female was observed to lay one egg each of the tender leaves (Plate 5.4B).

During egg laying, the forewings were observed to be continuously fluttering and it took about five seconds to lay a single egg. The female under observation laid only two eggs within a time span of 30 seconds. The female repeatedly tested similar-shaped leaves before finally selecting the underside of suitable tender leaves in a shady damp place for egg laying. Similar behavior was reported by Stamp (1980) and Davies and Gilbert (1985). A large number of eggs are laid by a single female to ensure that at least some will hatch successfully (Chew and Robbins 1984). In Bangladesh, in case of poison eater butterflies, *P. aristolochiae*, lacking of coincidence between post hibernation adult-emergence and new leaf-appearance of their host plants was recorded. In this case, the hibernation takes place in winter. Because of such coincidental lacking, eggs are laid very thickly on the early

appearing leaves and it is found that percentage of successful hatch is very poor. This happens in Bangladesh area during the period of March especially in forest areas while the host plants are wild (Bashar 2015a).

In the laboratory study, it was observed that just 5-10 minutes before hatching, the apical portion of the egg became dark brown in colour while the remaining portion of the egg was bright yellow. In the absence of suitable tender leaves, the female preferred to oviposit on tender shoots. The incubation period was 5 ± 0.6 days (Fig. 5.4. and Appendix 23).

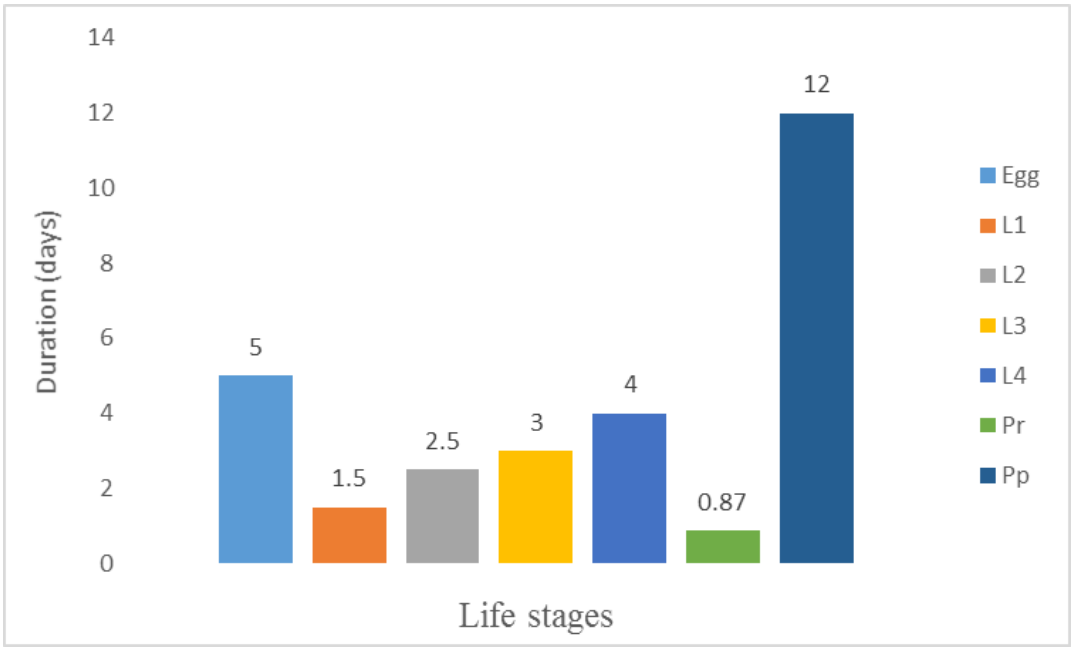


Fig. 5.4. Duration of different life stages of *P. aristolochiae* butterfly on the host plant, *A. indica* in the Environmental Biology and Biodiversity Laboratory (EBBL), Department of Zoology, University of Dhaka (2010-2011).

L1=1st instar; L2=2nd instar; L3=3rd instar; L4=4th instar; Pr=Pre-pupa; Pp=Pupa

5.4.3 Host plant utilization strategy of larvae

Total 10 individuals of *A. indica* were examined to determine the larval distribution of *P. aristolochiae* in different parts of *A. indica*. Total 269 larvae were recorded in different parts of *A. indica*. During the examination period, among the 1st instar larvae, maximum number (65 out of 84) was found in young leaves, minimum (6 out of 84) in matured leaves and no larva in inflorescence and fruits. Among the 2nd instar larvae, maximum number (47 out of 77) was observed in young leaves, minimum in inflorescence (2 out of 77) and no larva in fruits. Among the 3rd instar larvae, maximum number (21 out of 59) was recorded in matured leaves and minimum (5 out of 59) in inflorescence. Among the 4th instar larvae, maximum number (22 out of 49) was observed in matured leaves, and minimum (3 out of 49) in young leaves (Fig. 5.4. and Appendix 9-10).

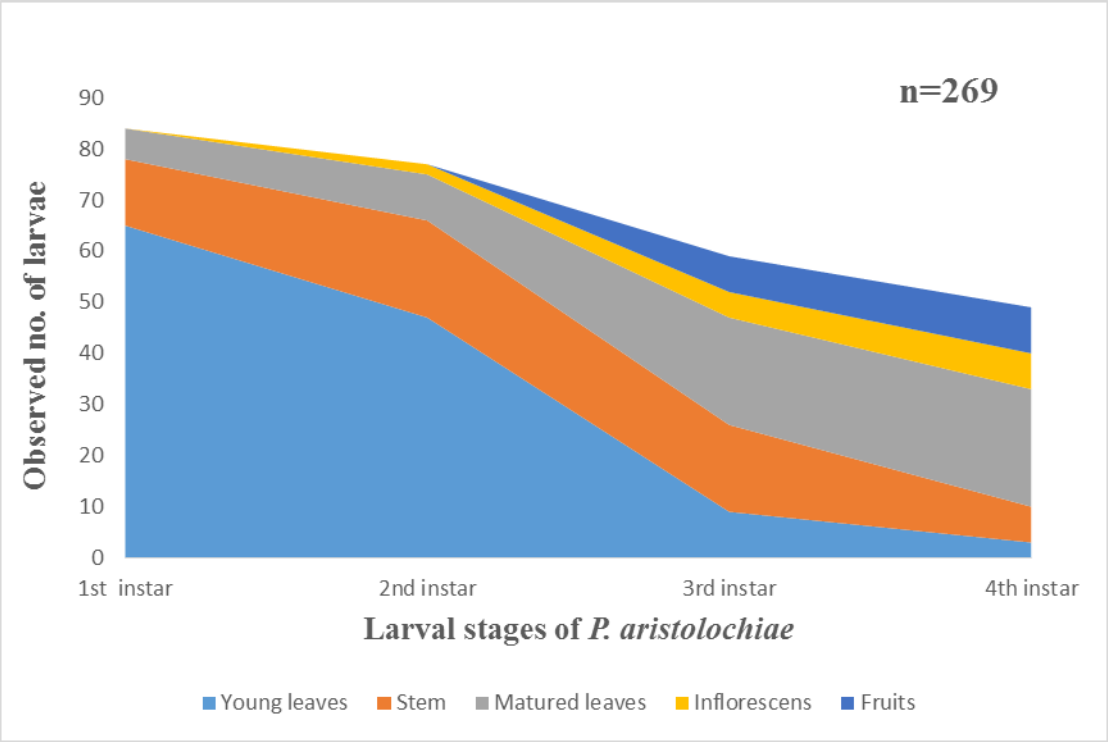


Fig. 5.5. Coincidence between the larvae of *P. aristolochiae* and phenological stages of the host plant, *A. indica* in the Zoological Garden of Dhaka University from January 2012 to December 2012.

The 1st instar larvae slowly emerged by splitting open the egg case at the apical tip. The time taken for hatching was 25-30 minutes. The freshly hatched larvae started feeding on the yellow coloured empty egg case only after about 10 minutes. The larvae were very sluggish in movement. They defoliated the early tender leaves by making small irregular shaped holes (Plate 5.4C). The feeding time was recorded 1-2 minutes followed by a resting period of 80-100 minutes. This larval duration was found to be 1.5 ±0.3 days (Fig. 5.4. and Appendix 23).

The 2nd instar larvae also preferred to feed on the tender leaves and defoliated along the sides of the leaf margin (Plate 5.4D). The feeding duration was 2-3 minutes followed by a resting period of 1-2 hours. While feeding, it was observed that the larvae defoliated the leaves from the lower side and therefore remained least exposed to their predators. The larvae took rest on the underside of the leaves. The 2nd larval duration was found to be 2.5±0.3 days (Fig. 5.4. and Appendix 23).

The mature 3rd instar larvae are voracious feeders, which defoliated tender, young and matured leaves (Plate 5.4E). The feeding time was recorded 10-12 minutes followed by a resting period of 45 minutes to one hour. The larvae preferred to take rest on the underside of leaves. In the absence of suitable leaves, they were also observed to feed on the tender shoots and flowers of the host-plant (Plate 5.3b-G, H). Larval duration was recorded 3±0.3 days (Fig. 5.4. and Appendix 23).

The robust 4th instar larvae were also found to be voracious feeders, which preferred to defoliate the mature leaves and fruits (Plate-5.4F, I and J). The feeding time recorded was 20-25 minutes which was followed by a resting period of 1-2 hours. When disturbed, they ejected out a pair of orange-coloured osmateria and quickly moved away to a different site. In the absence of suitable leaves the larvae were found to consume both young and mature shoots as well as fruits of the host-plant. Larval duration was found to be 4 ± 0.3 days (Fig. 5.4. and Appendix 23).

If a suitable food plant is not available then the larvae will starve to death rather than eat something else, this phenomenal habit has been established in the case of *Pachliopta aristolochiae* (a poison-eater butterfly). The experimental larva strictly maintains to eat the leaves of *Aristolochia indica* (the host plant of the species). A larva recognizes its food plants by certain aromatic vegetable oils which they contain. It is thought that selection may depend upon the detection of chemical attractants in the food species and of repellents in other plants according to Bashar (2015a).

Total 10 experimental beds were selected to measure the feeding potential rate of the larvae of *P. aristolochiae* butterfly in the field. Among them seven beds were prepared by planting by *A. indica* and rest of the beds were used for control experiment. Each bed contained 10 individual of plants and hence total 100 plants were planted in 10 beds. It has been found that 100% of the host plant, *A. indica* were consumed by the larvae of *P. aristolochiae* (Plate 5.3K) in bed-2, bed-5, bed-8 and bed-9. 70%, 90% and 80% of the host plant, *A. indica* were utilised as nutritional support by the different larval instars of *P. aristolochiae* in bed-1, 4 and 6, respectively. On the other hand, no plant was consumed in bed - 2, 7 and 10 by the larvae of *P. aristolochiae,* as these beds were planted by other plant species ((Fig. 5.6. and Appendix 10). It has been found new proliferation of leaves was grown from the residual portion of the host plant after 100% consumption in natural condition (Plate 5.4L).

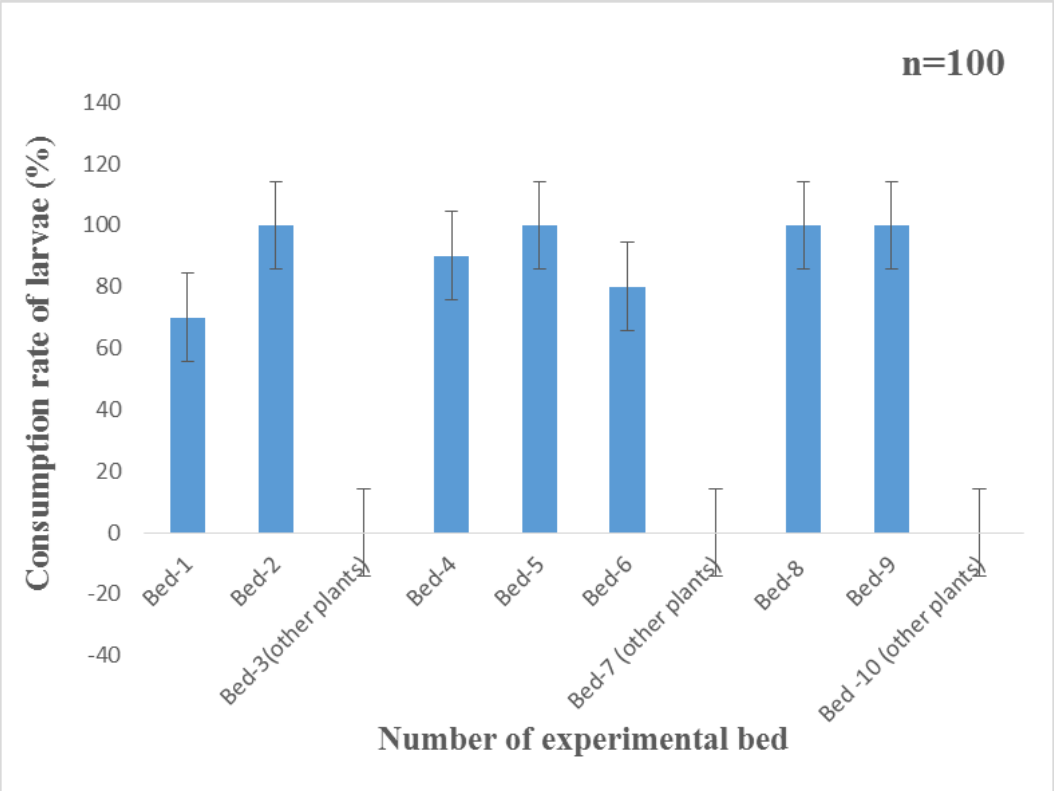
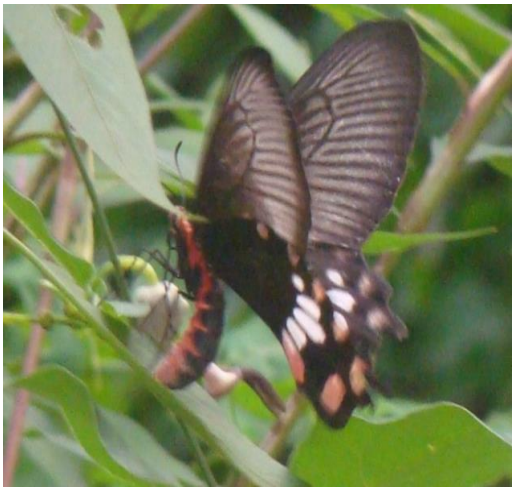


Fig. 5.6. Feeding potential rate of the host plant, *A. indica* as nutritional support for the larval development of *P. aristolochiae* butterfly in the Zoological Garden of Dhaka University from January 2012 to December 2012.

The 1st instar and 2nd instar larvae usually prefer to feed on tender parts of *A. indica* (Plate 5.4C, D). The 3rd instar and 4th instar usually prefer to feed on young and mature leaves, flowers and fruits (Plate 5.4G, H). During the infestation, the damage caused by the insect is supposed to be colossal to the plant. On the contrary, branching occurred on the host plant after damage was made by the insect. The plant's propagation was found generated even after damage had caused. The association is very much commensalistic among different plant species and at the same time in between the plant and insect species related to them. In accordance with different seasonal variations, commensalism of such type carries significant and important role in natural species equilibrium. This is a process of natural equilibrium diversity (NED) for the plant through interaction with the related butterflies (Bashar 2015a).

A



B

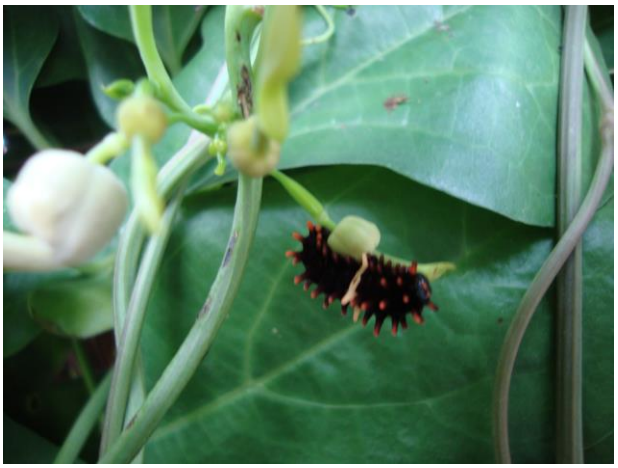
C

D

E

F

Plate 5.4. Feeding potentiality of the larvae of *P.* aristolochiae on its host plant, *A. indica* as nutritional supports for their development in the experimental field. A. Untouched host plant, *A. indica* with bamboo supporting B. Egg laying strategy of *P. aristolochiae* C. 1st instar larva feeding on young leaf D. 2nd instar larvae feeding on young leaves in colony form E. 3rd instar larvae feeding on matured leaves F. 4th instar larva feeding on matured leaves voraciously.



G

H

I

J

K

L

Plate 5.4 (Contd.). G. 3rd instar larva feeding on flower H. 4th instar larva feeding on flower I. 4th instar larva feeding on matured fruits voraciously. J. 4th instar larva feeding on matured fruits in colony form K. 100% consumed of the host plant by larvae. L. New proliferation of leaves from 100% damaged of host plant.

The host plant was found to grow synchronously with emergence of the related butterfly's new generation-arrival in the experimental ecosystem. The adopted butterfly colonizing process has shown that some developmental stages in the host plant were effective in giving high rate of adult production. The feeding potential of the host plant's phenological stages was found to be significant for some of the developmental stages (particularly 3rd and 4th instars larval stages). It could be concluded from the larval activity with the phenological stages of the host plant *A. indica*, *P. aristolochiae* butterfly are highy host specific. During the experimental period, it was found that two species of plants *A. indica* and *A. tagala* were utilised by the larvar of *P. aristolochiae* butterfly as nutritional support.

5.4.4 The variation in length, feeding potential and faeces of larvae

The length of 1st, 2nd, 3rd and 4th instar larvae were 4.0 ± 0.63, 9.0 ± 0.63, 22.6 ± 5.2 and 38.2 ± 4.70 mm, respectively in laboratory rearing (Fig. 5.7. and Appendix 23).

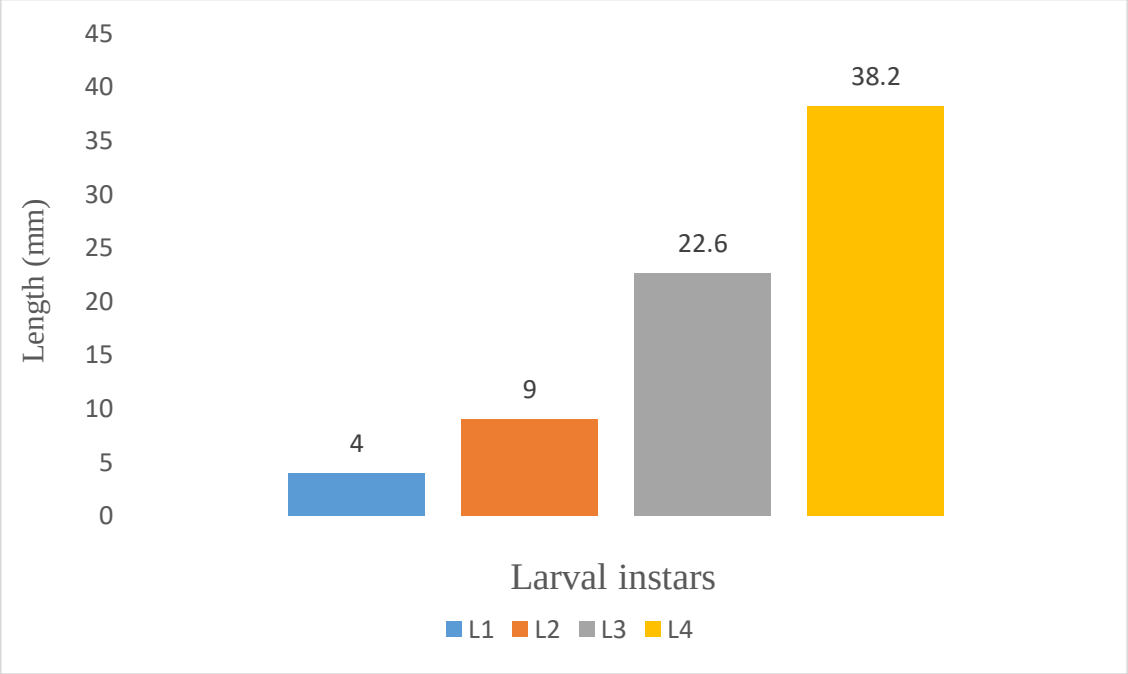


Fig. 5.7. Length of different larval instars of *P. aristolochiae* butterfly on the host plant, *A. indica* in the Environmental Biology and Biodiversity Laboratory (EBBL), Department of Zoology, University of Dhaka (2010-2011).

The feeding potential rate of 1st instar, 2nd instar, 3rd instar and 4th instar larvae were found to be 11.4 ± 5.04, 29.6 ± 5.12, 51.4 ± 6.0 and 72.8 ± 4.9 %, respectively in laboratory condition (Fig. 5.7. and Appendix 23).

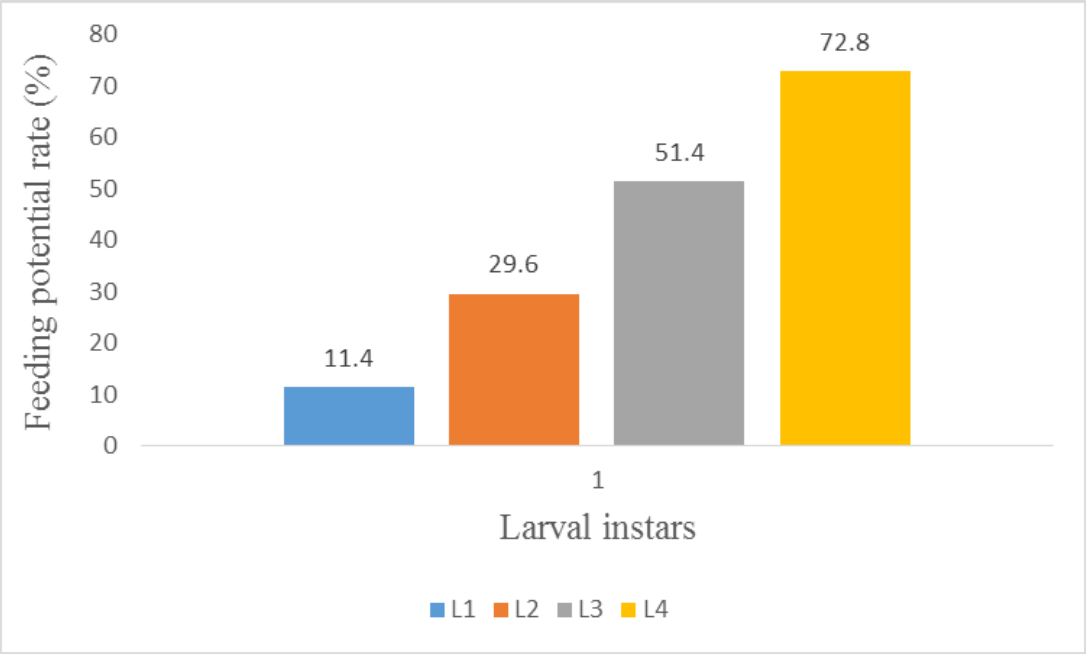


Fig. 5.8. Feeding potential rate of different larval instars *of P. aristolochiae butterfly* on the host plant, *A. indica* in the Environmental Biology and Biodiversity Laboratory (EBBL), Department of Zoology, University of Dhaka (2010-2011).

In laboratory experiment, the weight of faeces (excreta) of 1st instar, 2nd instar, 3rd instar and 4th instar larvae were 0.012 ± 0.004, 0.047 ± 0.018, 0.0114 ± 0.023 and 0.274 ± 0.045 g, respectively (Fig. 5.9. and Appendix 23).

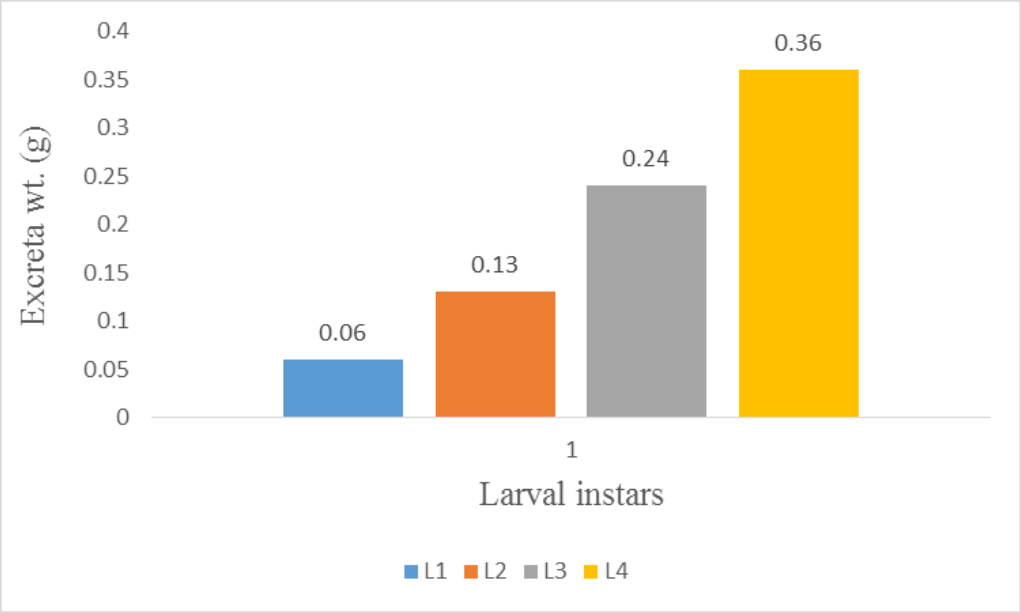


Fig. 5.9. Amount of excreta of different larval instars *of P. aristolochiae butterfly* on the host plant, *A. indica* in the Environmental Biology and Biodiversity Laboratory (EBBL), Department of Zoology, University of Dhaka (2010-2011).

The larval size depends on the availability of food sources. The 4th instar larva quickly metamorphosed into pre-pupa when foods were not available to them. In this condition, the larvae could not make pupal covering (cocoon) properly and took a longer time than required and the emergence rate of adult from pupa was poor. The size of larval instar is also reduced in such condition. Barua and Slowik (2007) reported that the average lengths of these larval instars of *P. aristolochiae* were 4.0, 7.5, 19.0 and 35.0, mm respectively on their host plant, *A. tagala* in laboratory condition. Environmental factors, moderate supply of larval food and different host plants caused the variation of larval length according to Davies and Gilbert (1985).

The 1st instar and 2nd instar larvae usually prefer to feed on tender parts of *A. indica*. The 3rd instar and 4th instar usually prefer to feed on young and mature leaves. Feeding potential of 1st and 2nd instar larvae are very less than 3rd and 4th instar larvae reported by Barua and Slowik (2007). Later instar larvae become voracious feeder to store more nutrients. More energy is required to be metamorphosed into pupa and that's why 90-100% supplied leaves were consumed by later instar larvae (Alam *et al.* 2014).

The excretory product of larvae is proportionate to the feeding potential rate of larvae. It was measured that the excretory products of 1st and 2nd instar larvae were less compared to 3rd and 4th instar larvae, because the feeding potential rate of 3rd and 4th instar larvae were more in comparision to 1st and 2nd instar larvae. It was found that before pre-pupation, 4th instar larvae usually excreted large sized droppings and more amount than previous instars. Larval faeces were in liquid form primarily at hatching but become solid after 2-3 days reported by Alam *et al.* (2014).

5.4.5 Pupation strategy of the P. aristolochiae

Total 116 pupae in different 10 individual of *A. indica* have been examined to find the emergence rate of the adult butterfly *P. aristolochiae* in the field condition. It was found that 40% of the pupae (n=116) were emerged into the adult butterfly in early (on time) and 24.2% emerged as adult in late (diapause) during the experimentation (Fig. 5.10. and Appendix 9).

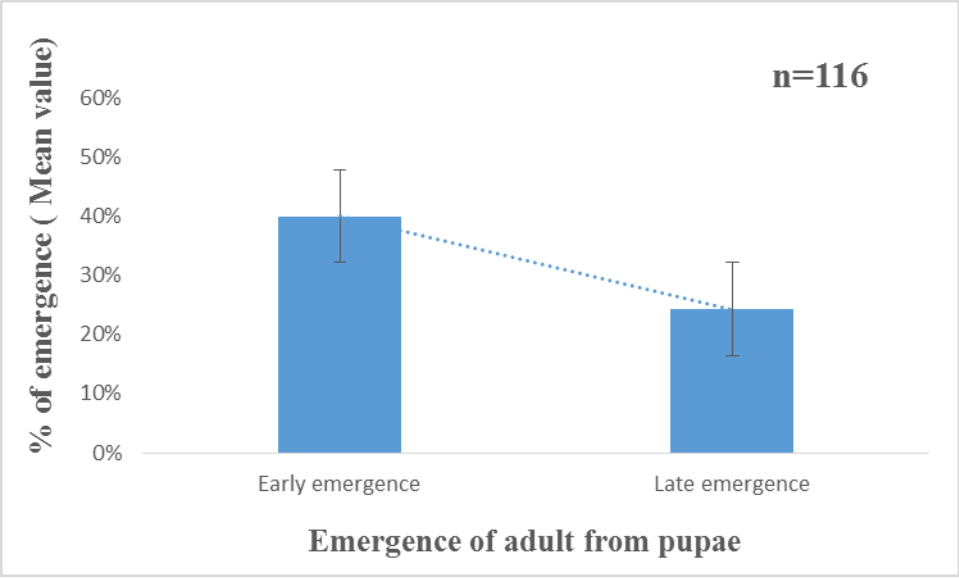


Fig. 5.10. Emergence rate of the adult butterfly, *P. aristolochiae* from the pupae in the Zoological Garden of Dhaka University from January 2012 to December 2012.

The early emergence rate is higher than late emergence in the pupae of *P. aristolochiae*. Overwinter is the factor for diapause of the pupae of *P. aristolochiae* butterfly. Diapause duration for the pupae was recorded from mid-October to mid-February in most cases. Lack of food supply before pupation and attack of predators after pupation are major causes for decreasing adult emergence rate. Fourth instar larvae become shorten to form pre-pupae. During this stage feeding becomes stopped (Plate 5.5A, B). Gradually it forms arch shape and thread like structures through which are attached hard supporting to be modified into pupa (Plate 5.5C-F). The entire process of pupation completed within 14-15 hours. In the field condition, pupation took place among dense and low-growing vegetation, and this observation was in conformity with the records of Vane-Wright and Ackery (1984).

The pupa is brownish with various shades of brown and pink markings. It is attached to the twig or stick by the tail and held at an angle by a body band. The distinguishing feature of the pupa is the presence of large semi-circular projections on the back of the abdomen, thorax and head. It is light brown in colour with a mixture of white, orange and dark brown patterned markings on the dorsal side (Barua and Slowik 2007). In the papilionid butterflies, pupa is suspended horizontally or vertically, head upwards, along a plant stem or twig, with a stout silken band over the thorax (Plate 5.5E, F) and its tail claspers firmly embedded in a pad of silk according to Bashar (2015a).

The ventral side of the pupae is light brown with faint white stripes. The anterior end of the chrysalis is produced into a frontally flattened broad projection, which further has a pair of flattened flaps on either lateral side. The 2nd pair of dorso-ventrally flattened flaps was present in the mid-anterior region. Between these two pairs, there is a pair of markings having a mixture of white and dark brown colouration. The pupal size was recorded as 25 x 15 mm and the period was 12±0.63 days (Fig. 5.4. and Appendix 23) reportedby Alam *et al.* (2014).

The life cycle of butterflies vary in duration with seasonality, host plant availability, host plant phenology, nectar plants availability and shelter plant availability. Some strictly host-specific species show long hibernation period in developing stages. In our experiment it is found that, a papilionid butterfly *Pachliopta aristolochiae* is highly specific to its host plant and it passes through a long period of hibernation in its pupal stage. The hibernation starts at the end of September and ends at the end of February in Bangladesh. The pupal hibernation lasts for about five months long in a year. It is to be noted that in Bangladesh ecology, the host plant of the butterfly remains dormant during this period in the forests. Appearance of new leaves and flowers in the plant shows strong coincidental relation with the termination of the hibernation. More or less similar cases are seen in some pierid butterflies with the appearance of their host plants, especially in the forest ecosystems (Bashar 2014a).

A

B

C

D

E

F

Plate 5.5 (A-F). Pupating strategies of *P. aristolochiae* butterfly. Pupa pupating the dead or live parts of other plants rather than host plants.

5.4.6 Morphological features of the Adult

The extended tail of the hind wings is comparatively pointed in females and more or less rounded in males. The upper side of male is velvety black. Fore wing with well-marked pale adnervular streaks on the discal area that do not reach the terminal margin, the latter broadly velvety black; the streaks beyond the end of cell extended inwards into its apex. Hind wing with elongate white discal markings. In females, the abdomen is seen distinctly larger than that of the males as described by Collins and Morris (1985). The inner margin of the hind wings has tufts of hair in the males, which are actually the scent scales from where the pheromones are released during the time of mate selection described by Kemp (2002b). Haribal (1992) reported that the total life cycle of *P. aristolochiae* was completed within ± 30 days in laboratory conditions. In the field study, it was found that the common rose completed 7-8 generations in a year. The first generation emerged in March-April and the last generation was completed in December.

5.4.7 Behavioural status of the adult butterfly

Out of total 168 individuals of *P. aristolochiae* butterfly, the highest (41.90%) was recorded in foraging and the lowest (4%) in puddling condition. The population density of *P. aristolochiae* was observed in mating, egg-laying, resting and searching during the experimental period were 8.4%, 9.7%, 12.1%, and 23.7%), respectively (Fig. 5.11. and Appendix 3, 4).

Butterflies activities deal with the determination of status of butterflies in relation to their associated plants-abundance, status of forests and the various aspects of environmental soundness (Grinnell 1924, Wiklund and Fridberg 2008). Butterflies require three types of plants like host plants, shelter plants and nectar plants, and these plants together enrich the species assemblage in an ecosystem that makes suitable habitats for conserving wildlife according to Bashar (2011).

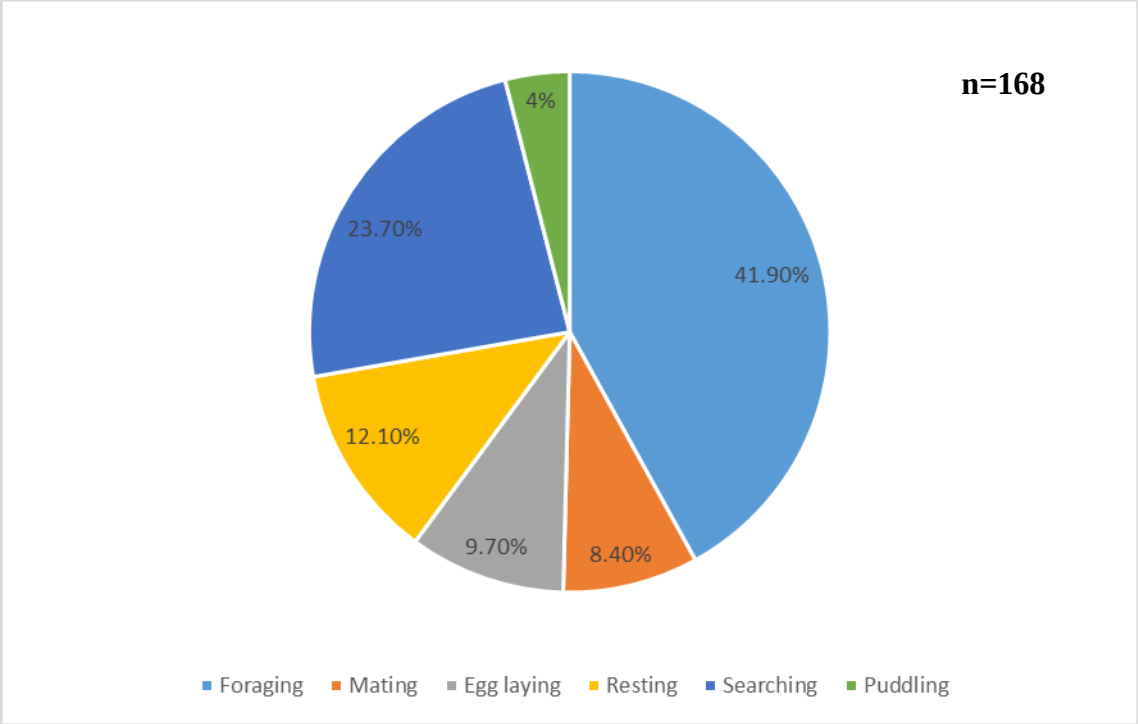


Fig. 5.11. The population fluctuation of *P. aristolochiae* butterfly in different behavioural conditions at Butterfly Research Park, Bhawal National Park from January 2012 to December 2012.

Most species of butterflies use flower nectar as their primary food source (Plate 5.6A) and this sugar-rich material is required for energy used in flight (Krauss *et al.* 2005). Nectar, a primary nutrient source for adult Lepidoptera, varies by plant species in both its carbohydrate and constituent components which can affect fecundity (Romeis and Wäckers 2003). Therefore, nectar resources for adult Lepidoptera influence species occurrence, distribution, and density, thus nectar sources are an important component of butterfly conservation.

Nectarivorous butterfly species rely on the sugars and water in nectar for optimal longevity and reproduction (Baker and Baker 1975, Boggs 1997, Fischer and Fiedler 2001). In the field butterfly species vary widely, however, in their preferences for flower species (Watt *et al.* 1974, Faegri and Pijl 1979 and May 1992), and in farmland conservation of butterflies, catering for this variation in nectar preferences has been attempted through a general approach, albeit with some positive results. 'Wild flower mixes' can increase

populations of butterflies, bumblebees, and other invertebrates spatially and temporally (Feber *et al.* 1996), and conserving non-native species in field margins contributes to increasing abundance of butterfly species on farmland (Dover 1990, 1996, 1997 and Feber *et al.* 1996). In other ecosystems, research to determine the importance of certain nectar sources for butterfly habitat conservation is based on observed feeding patterns in the field (Brakefield 1982, Thomas 1984 and Tudor *et al.* 2004) or the effect of nectar availability on butterfly populations (Shepherd and Debinski 2005, Nelson and Wydoski 2008).

The plants are not only used as the nutritional sources for animals, but they are also used as ecological sources and as the ecological niche-sources. The bird seven-sisters are found to take characteristic rest in the trees, but these trees are not found to supply them food materials. Without these trees they cannot take their characteristic rest in the forest. If they do not take the rest properly they cannot survive soundly in the forest. So needs of the trees is multidimensional, many of the needs have yet not been identified and discovered (Bashar 2015a).

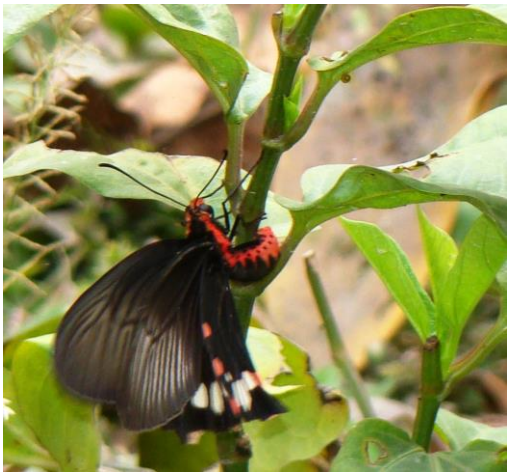
Another interesting example of the plant association of birdwing butterfly (*Troides* is scientifically named) with the specific plant (the Indian birthwort: *Aristolochia*). It is to be noted that birdwings are the largest butterflies in the world. Some of the butterfly species are available in Bangladesh. They do not found to copulate without the *Aristolochia* plant. When the mating behaviour is studied it is found that their characteristic mating takes place in hanging condition with the members of the genus *Aristolochia*. If the specific plant is not available, the butterfly does not go for successful copulation. For progeny-maintenance of this species association with the plant is absolutely necessary. This peculiar mating behavior (Plate 5.6B) is the simple example of an interaction of the biotic-biotic factors in an ecosystem reported by Bashar (2014a).

Mostly males get together in puddling during which they feast on extra salts and other nutrient found in the water around sand. Butterflies require these extra salts and other nutrients to mate successfully. The swallowtail butterfly is not particularly fond of mud puddling although it prefers sunshine (Plate 5.6F) reported by Haribal (1992). In certain parts of Sri Lanka, the males of *Pachliopta aristolochiae* are known to congregate and form a beautiful sight while mud-puddling (Harris 1996).

Female butterflies recognize host plants through visual cues, such as leaf shape and colour. A female butterfly never lay her eggs on the wrong plant. The laid eggs hatch to a larva and the larva develops on feeding the host plant parts like leaves and young stems. It has been reported egg-laying behaviour in female butterflies prior to oviposition (Plate 5.6C) in order to check the physical and chemical properties of the host plants and such behaviour also recorded in many female butterflies in India and help them in selecting correct food plant (Bell 1909 and Ilse 1956).

A

B



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F

Plate 5.6. Different behavioural activities of *P. aristolochiae* butterfly. A. taking nectar from *Lantana camara* B. mating condition C. egg-laying on the shoot by bending of abdomen D. resting condition E. searching nectar on the *Euphorbia pulcherrima* F. mud-puddling condition.

Butterflies are phytophagous insects and they are very selective to their larval host plants. Relationships between life-stages of butterflies and their host-plants are most vital interactive factor in forest ecosystem. This type of interaction has got special significance in the forests of tropics and sub-tropics. To understand all the above points and facts, life cycle-study stands essential not only to know about butterflies but also to know forest ecosystem functioning and magnification of its healthiness. Success of colonizing process has been assessed through field level experimentation on analyses of behavioural significances of butterfly with their related plants (host, nectar and shelter plants) according to Berry *et al.* (2002). Success of colonization process in the butterfly behavior was resulted through accommodating significant stages of life cycle and their functional activities. These behaviors evidenced the ways in which butterflies carry important role in the gene-flow mechanism of the plant kingdom and create in different ecosystem, especially in the forest ecosystem.

It was found that *P. aristolochiae* significantly utilized the host plant, *A. indica* as nutritional sources especially from its larval feeding behavior and that's why it could be said this butterfly is highly host specific as it does not take any nutrition from another plant species in larval stages. It was studied that butterfly related host and nectar plants are used as traditional herbal medicine to prevent from difficult disease in human in different countries. To confirm the medicinal importance of butterfly related host plants, antioxidant, toxicity and antimicrobial activity of the *Aristolochia tagala* (another host plant of *Pachliopta aristolochiae* butterfly) were analyzed in the laboratory (Chapter 6). Further research is suggested to analyze the larval fat body for examining toxic substances aristolochic acid which was fed by larvae of *P. aristolochiae* butterfly from the host plant *A. indica* and *A. tagala* as defense and protection in larval stages.

Chapter 6. Antioxidant, toxicity and antimicrobial activity of the leaf extracts of *Aristolochia tagala* (the host plant of *Pachliopta aristolochiae* butterfly)

6.1 Abstract

Different leaf extracts of *Aristolochia tagala* with different solvents, such as Tert-butyl-1-hydroxytoluene (BHT), Ascorbic acid (AA), Methanolic extract (ME), Petroleum ether (PE), Carbon tetrachloride soluble (CTE), Chloroform soluble (CHE) and Aqueous soluble partitionate (AQE), were subjected to free radical scavenging activity, brine shrimp lethal bioassay and antimicrobial activity. Here, *tert*-butyl-1-hydroxytoluene (BHT) and ascorbic acid (ASA) were used as reference standard.

Free radical scavenging activity of methanolic extract (ME) partitionate of *A. tagala* showed the highest free radical scavenging activity with IC_{50} value 4.67 μg/ml. At the same time the PE and AQE also exhibited strong antioxidant potential having IC_{50} value 4.09 and 7.45 μg/ml, respectively. Free radical scavenging activity of CTE and CHE are 9.17 and 17.45 μg/ml, respectively. The LC_{50} values of ME, PE, CTE, CHE and AQE of *A. tagala* were 5.77 μg/ml, 0.84 μg/ml, 1.52 μg/ml, 7.61 μg/ml and 7.99 μg/ml, respectively.

The antimicrobial activity of CHE of *A. tagala* demonstrated strong against *Sarcina lutea* and *Vibrio parahemolyticus* and mild against *Salmonella paratyphi.* The antimicrobial activity of the AQE exhibited the strong against *Shigella boydii* and mild against *Salmonella typhi*. Growth having zone of inhibition ranged from 7 to 21 mm. No antimicrobial activity was recorded for ME, PEP and CTE of *A. tagala.*

Keywords: Antioxidant, Brine shrimp lethal bioassay, Antimicrobial activity, *A. tagala*

6.2 Introduction

The synchronization of coincidences between life stages of *Pachliopta aristolochiae* butterfly and different phenological stages of *Aristolochia indica* and *A. tagala* were studied in both field and laboratory experiment. Both *A. indica* and *A. tagala* are used as host plants by *P. aristolochiae* butterfly in its developmental stages. This butterfly are highly host specific. It was observed that *P. aristolochiae* butterfly is a poison eater butterfly. Larval stages of *P. aristolochiae* butterfly feeds on different phenologica stages such as young leaves, mature leaves, stem, inflorescence, fruits of *A. indica* and *A. tagala* as nutritional sources which was discussed in detailed (Chapter 5). It was found that *A. indica* and *A. tagala* were used as egg-laying support and larval food by *P. aristolochiae* butterfly in the field level experimentation. These two host plants contain toxic substances aristolochic acid which is fed by larvae of *P. aristolochiae* butterfly and that's why this butterfly is called poison eater butterfly (Bashar 2015a).

Aristolochia Linn (Aristolochiaceae) is a large genus of herbs or twinning plants comprising about 300 species found in the tropical and temperate regions of the world (Brazil, Texas, Europe etc.) (Mathew *et al.* 2011). Eight species are known to occur in India of which *Aristolochia braccteolata, A. indica,* and *A. tagala* are of medicinal importance (Mathew *et al.* 2011). They generally contain alkaloids and are reported to be useful in the treatment of snakebites (Mathew *et al.* 2011). The genus *Aristolochia* is a source of aristolochic acid, which has been evaluated in China for the treatment of wounds and infectious diseases; it was found to be useful for promoting wound healing in ulcers, burns, and scalds (Jiangsu 1977). Recently, aristolochic acids, present in *Aristolochia* plants, have been shown to be nephrotoxic in rats (Matsuo *et al.* 2003). A family of aristolochic acids have been isolated from *Aristolochia argentina* (Priestap 1987) and *Aristolochia indica* (Kupchan *et al.* 1968).

The assumption that aristolochic acids (AAs, henceforth) protect insects as well as plants from natural enemies inspired the search for these compounds in the tissues of butterflies. Sequestration of aristolochic acids (AAs) was first confirmed for *Pachliopta aristolochiae* (Fabricius) and since then for 13 more troidine and three zerynthiine species (von Euw *et al.* 1968). However, knowledge of the defensive roles of AAs lags behind the chemical investigations; unpalatablity is more often assumed than documented. Among the

species, only for *P. aristolochiae* (Uesugi 1996), *Troides aeacus* (Rothschild *et al.* 1970), *Battus polydamas* (L.) (Chai 1986, Pinheiro 1996), and *B. philenor* (L.) does some experimental evidence for unpalatability complement the chemical data.

6.2.1 Free radical scavenging activity (Antioxidant)

Antioxidants have been found to play a major role in protecting the human body against damage induced by reactive free radicals (Halliwell and Gutteridge 1990, Mates *et al.* 1999, Omale and Omajali 2010) by reacting with free radicals, chelating and also by acting as oxygen scavenger (Shahidi and Wanasundara 1992, Buyukokuroglu *et al.* 2001). Free radicals or highly reactive oxygen species are formed by exogenous chemicals or endogenous metabolic processes in the human body. These are capable of oxidizing bio-molecules, viz. nucleic acids, proteins, lipids and DNA and can initiate different degenerative diseases like neurological disorders, cancer, emphysema, cirrhosis, atherosclerosis, arthritis etc (Halliwell and Gutteridge 1984, Maxwell 1995).

Antioxidants are the compounds which terminate the attack of free radicals and thus reduce the risk of these disorders (Rice-Evans *et al.* 1996). Almost all organisms are protected up to some extent by free radical damage with the help of enzymes, such as super-oxide dismutase, catalase and antioxidant compounds, viz. ascorbic acid, tocopherol, phenolic acids, polyphenols, flavonoids and glutathione. Prior and Cao (1999) reported that antioxidant supplements or dietary antioxidants protect against the damaging effects of free radicals.

Plants contain different natural products, which have a remarkable role in the traditional medicine in different countries. Nowadays, the prevention of many diseases has been associated with the ingestion of different plants rich in natural antioxidants (Johnson 2001, Virgili *et al*. 2001). It has been found that a higher intake of such compounds is associated with a lower risk of mortality from different diseases (Ajih and Janardhanan 2002, Lim *et al*. 2002, McCune and Johns 2002, Tziveleka *et al*. 2002).

Antioxidants can interfere with the oxidation process by reacting with free radicals, chelating catalytic metals, acting as oxygen scavengers (Shahidi and Wanasundara 1992) and prevent lipid auto oxidation (Brand-Williams *et al*. 1995, Bondet *et al*. 1997). Natural antioxidants like vitamin C and E, carotenoids and polyphenols like flavonoids, are considered to be beneficial components from fruit and vegetables (Gupta and Prakash 2009, Serteser *et al*. 2009). They are responsible for the protective effects against different diseases (Peto *et al*. 1981, Block *et al*. 1992, Diplock 1996, Rietjens *et al*. 2002).

6.2.2 Brine shrimp lethality bioassay (Toxicity)

Plants are the natural reservoir of many antimicrobial and anticancer agents. Bangladeshi people have traditional medical practice as an integral part of their culture. A lot of medicinal plants are available for the treatment of various diseases. However, scientific studies have been conducted only to a limited extent with few medicinal plants (Rashid *et al.* 1997, Nutan *et al.* 1997, Haque *et al.* 2000a, b). In this investigation, 35 locally used plants have been selected and tested to justify their existing bioactivities by the brine shrimp lethality bioassay (Meyer *et al.* 1982). The method utilizes in vivo lethality in a simple zoological organism brine shrimp nauplii as a convenient monitor for screening cytotoxicity of the plant extracts which can be further correlated with its anticancer potentiality and other bioactivities.

The brine shrimp lethality bioassay is rapid (24 h), simple (e.g., no aseptic technique is required), easily mastered, inexpensive, and requires small amounts of test material (2-20 mg or less) (Ghisalberti 1993). The bioassay has a good correlation with cytotoxic activity in some human solid tumors and with pesticidal activity (Ghisalberti 1993, Mclaughlin, *et al.* 1998). Brine shrimp (*Artemia salina*) larvae have been used for many years in toxicological studies. Many researchers are now using brine shrimp as a pre-screen for plant extracts as it provide a quick, inexpensive, and desirable alternative to test on larger animals.

It is known that a positive correlation exists between brine shrimp lethality and 9 KB (human nasopharyngeal carcinoma) cytotoxcity; brine shrimp are therefore used in many pre-screens for potential anti-tumor activity (Meyer *et al.* 1982). In addition, brine shrimp bioassay effectively predicts pesticidal activities and respond to a broad range of chemically and pharmacologically diverse compounds (McLaughlin *et al.* 1998).

6.2.3 Antimicrobial activity

Medicinal plants have long been the subjects of human curiosity and need. It is estimated that there are about 2,500,000 species of higher plants and the majority of these have not been examined in detail for their pharmacological activities (Ram *et al.* 2004). The antimicrobial properties of certain Bangladeshi medicinal plants were reported based on folkloric information and a few attempts were made to study the inhibitory activity against certain pathogenic bacteria and fungi (Rashid *et al.* 1997, Rahman *et al.* 2001). In recent years, multiple drug resistance in human pathogenic microorganisms has developed due to indiscriminate use of commercially available antimicrobial drugs used in the treatment of infectious diseases. This situation forced scientists for searching new antimicrobial substances from medicinal plants, which are the good sources of novel antimicrobial and chemotherapeutic agents (Karaman *et al.* 2003).

The world health organization (WHO) also reports that 80% of world's population depend mainly on traditional medicine and the traditional treatment involve mainly the use of plant extracts (WHO 1993). Plant based antimicrobials represent a vast untapped source for medicines and further exploration of plant antimicrobials needs to occur (Iwu *et al.* 1999). *Aristolochia indica*, commonly known as Ishwari, Nakuli and Gandhanakuli have enormous therapeutic potential and it was found to be effective in the treatment of intermittent fever, malaria, parasitic infestations, various skin diseases, as an aphrodisiac, an anthelmintic and it is also used in oedema, intestinal disorders (Heinrich *et al.* 2009), fungal and bacterial infections (Shafi *et al.* 2002, Kumar *et al.* 2006).

Aristolochia bracteolata is used in traditional medicine as a gastric stimulant and in the treatment of cancer, lung inflammation, dysentery and snake bites (Negi *et al.* 2003). This plant belongs to the family Aristolochiaceae and known as kidmar. It has insecticidal properties. Its roots and leaves are bitter and anti-helmintic, and are medicinally important. Almost every part of the plant have medicinal usage. Identifying bioactive compounds and establishing their health effects are active areas of scientific enquiry (Etherton *et al.* 2004).

Majority of the researchers uses one of the three following methods for the assessment of antimicrobial activity: Disc diffusion, agar dilution, and broth dilution/micro-dilution method. The disc diffusion method (also known the zone of inhibition method) (Bauer *et al.* 1966) is probably the most widely used of all methods used for testing antibacterial and antifungal activity (Wilkinson 1966). It requires only small amounts of the test substance (10-30 µl), can be completed by research staff with minimal training, and as such may be useful in field situations (Wilkinson 1966). Several researchers have used the method to identify the antibacterial and antifungal activities of the plant extracts (Belboukhari and Cheriti 2006), compounds isolated from plants (Khan *et al.* 2008), and also to find out the antimicrobial resistant strains of microorganisms (Hallander and Laurell 1972, Vedel 2005). It is important to note that the disc diffusion method demonstrated activity *in vitro* does not always translate to activity *in vivo* (Wilkinson 1966).

The present study was envisaged to analyze the free radical scavenging activity (antioxidant), brine shrimp lethality bioassay (toxicity) and antimicrobial activity of the leaves extract of A. tagala for assessing the medicinal importance of butterfly related host plants in the nature.

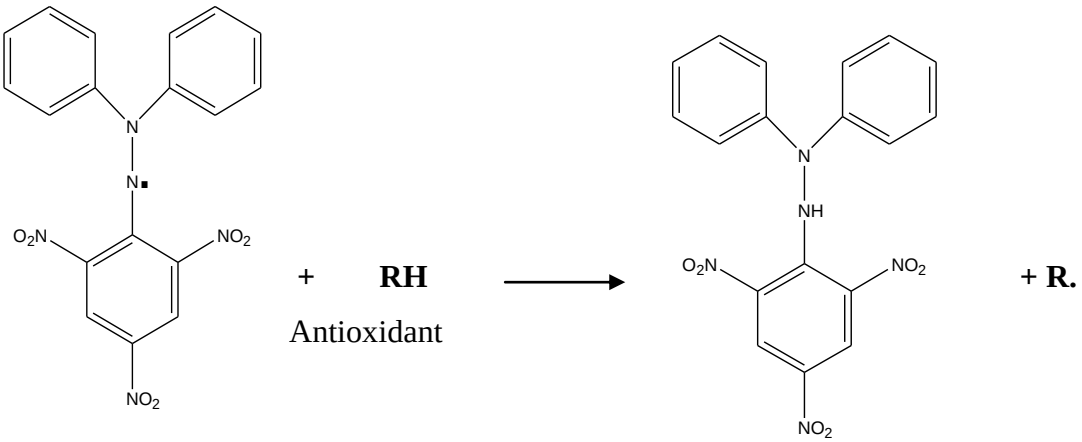
6.3 Material and Methods

The present experiment was conducted entitle "Antioxidant, toxicity and antimicrobial activity of the leaf extracts of *Aristolochia tagala* (host plant of *P. aristolochiae* butterfly)" in the phytochemical laboratory, Faculty of Pharmacy, University of Dhaka in the year of 2013-2014.

6.3.1 Antioxidant activity: DPPH assay

6.3.1.1 Principle

The free radical scavenging activities (antioxidant capacity) of the plant extracts on the stable radical 1, 1-diphenyl-2-picrylhydrazyl (DPPH) were estimated by the method of Brand-Williams *et al.* (1995). Two ml of a methanol solution of the extract at different concentration were mixed with 3.0 ml of a DPPH methanol solution (20μg/ml). The antioxidant potential was assayed from the bleaching of purple colored methanol solution of DPPH radical by the plant extract as compared to that of *tert*-butyl-1-hydroxytoluene (BHT) and ascorbic acid (ASA) by UV spectrophotometer.



* DPPH = 1, 1-diphenyl-2-picrylhydrazyl

DPPH was used to evaluate the free radical scavenging activity (antioxidant potential) of various compounds and medicinal plants (Shahidi and Wanasundara, 1992; Bondet *et al.* 1997).

6.3.1.2 Materials

1,1-diphenyl-2-picrylhydrazyl	UV-spectrophotometer
tert-butyl-1-hydroxytoluene (BHT)	Beaker (100 & 200ml)
Ascorbic acid	Amber reagent bottle
Distilled water	Test tube
Methanol	Light-proof box
Chloroform	Pipette (5ml)
Carbon tetra chloride	Micropipette (50-200 µl)
n-hexane	

6.3.1.3 Control preparation for antioxidant activity measurement

Ascorbic acid (ASA) and *tert*-butyl-1-hydroxytoluene (BHT) were used as positive control. Calculated amount of ASA and BHT were dissolved in methanol to get a mother solution having a concentration 1000 µg/ml. Serial dilution was made using the mother solution to get different concentration ranging from 500.0 to 0.977 µg /ml.

6.3.1.4 Test sample preparation

Calculated amount of different extractives were measured and dissolved in methanol to get the mother solution (Conc. 1000 µg/ml). Serial dilution of the mother solution gave different concentration ranging from 500.0 to 0.977 µg /ml which were kept in the marked flasks.

6.3.1.5 DPPH solution preparation

Twenty mg DPPH powder was weighed and dissolved in methanol to get a DPPH solution having a concentration of 20 µg/ml. The solution was prepared in the amber reagent bottle and kept in the light proof box.

6.3.1.6 Assay of free radical scavenging activity

Two ml of a methanol solution of the sample (extractives/ control) at different concentration (500 µg/ml to 0.977 µg/ml) were mixed with 3.0 ml of a DPPH methanol solution (20 µg/ml). After 30 min reaction period at room temperature in dark place the absorbance was measured at 517 nm against methanol as blank by UV spetrophotometer.

Inhibition of free radical DPPH in percent *(I%)* was calculated as follows:

$$(I\%) = (1 - A_{sample}/A_{blank}) \times 100$$

Where A_{blank} is the absorbance of the control reaction (containing all reagents except the test material).

Extract concentration providing 50% inhibition *(IC_{50})* was calculated from the graph plotted inhibition percentage against extract concentration.

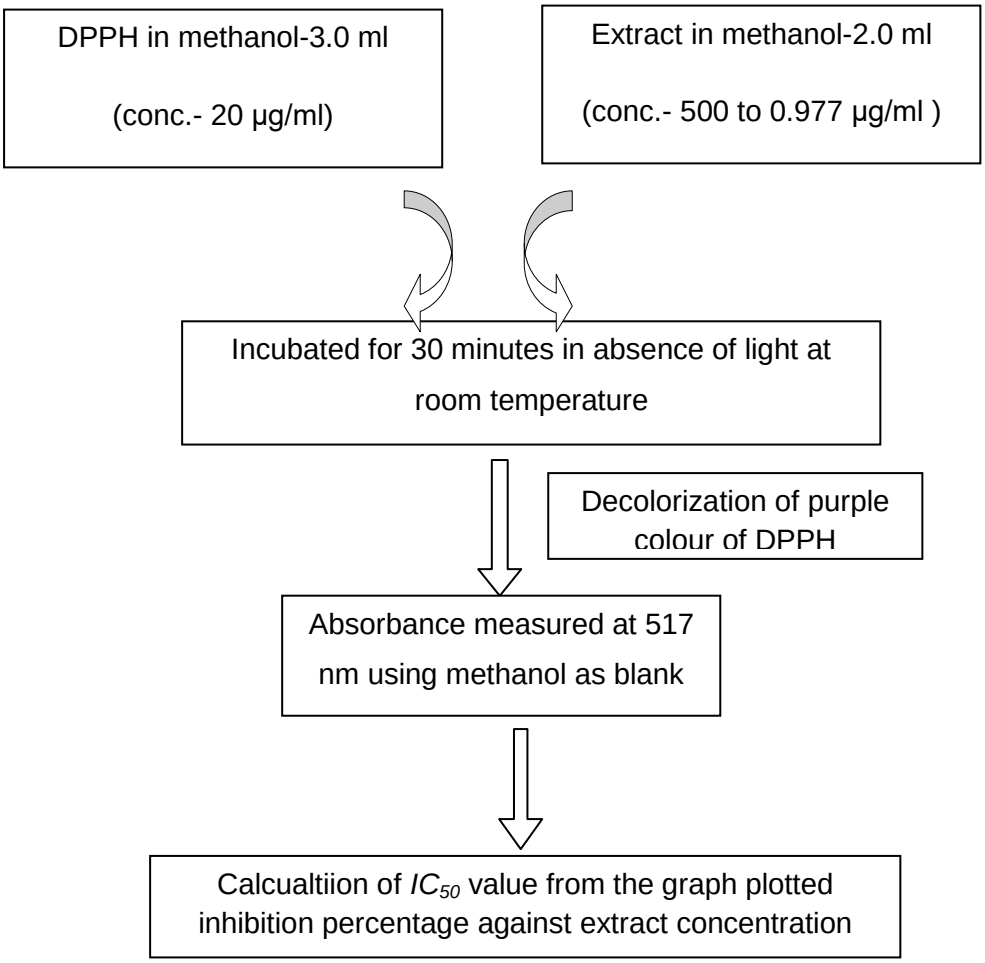


Fig. 6.1. Schematic representation of the method of assaying free radical scavenging activity.

6.3.2 Brine shrimp lethality bioassay

6.3.2.1 Principle (Meyer *et al*. 1982)

Brine shrimp eggs were hatched in simulated sea water to get nauplii. By the addition of calculated amount of dimethylsulphoxide (DMSO), desired concentration of the test sample was prepared. The nauplii were counted by visual inspection and were taken in vials containing 5 ml of simulated sea water. Then samples of different concentrations were added to the pre-marked vials through micropipette. The vials were then left for 24 hours. Survivors were counted after 24 hours.

6.3.2.2 Materials

- *Artemia salina* leach (brine shrimp eggs)
- Sea salt (NaCl)
- Small tank with perforated dividing dam to hatch the shrimp
- Lamp to attract shrimps
- Pipettes
- Micropipette
- Glass vials
- Magnifying glass
- Test tubes
- Test samples of experimental plants

Table 6.1. Test samples of experimental plants.

Plant part	Sample code	Test Sample	Calculated amount (mg)
Leaves extract of *A. tagala*	PESF	Petroleum ether partitionate	4.0
	CTCSF	Carbon tetrachloride soluble partitionate	4.0
	CSF	Chloroform soluble partitionate	4.0
	MEF	Methanolic Extract partitionate	4.0
	AQSF	Aqueous soluble partitionate	4.0

6.3.2.3 Experimental Procedure

6.3.2.3.1 Preparation of seawater

Thirty eight g sea salt (pure NaCl) was weighed, dissolved in one litre of distilled water and filtered off to get clear solution.

6.3.2.3.2 Hatching of brine shrimps

Artemia salina leach (brine shrimp eggs) collected from pet shops was used as the test organism. Seawater was taken in the small tank and shrimp eggs were added to one side of the tank and then this side was covered. One day was allowed to hatch the shrimp and to be matured as nauplii. Constant oxygen supply was carried out through the hatching time. The hatched shrimps were attracted to the lamp through the perforated dam and they were taken for experiment. With the help of a pasteur pipette 10 living shrimps were added to each of the test tubes containing 5 ml of seawater.

6.3.2.3.3 Preparation of test samples of the experimental plant

All the test samples (Table 6.3.1) were taken in vials and dissolved in 100 µl of pure dimethyl sulfoxide (DMSO) to get stock solutions. Then 50 µl of solution was taken in the first test tube containing 5ml of simulated seawater and 10 shrimp nauplii. Thus, final concentration of the prepared solution in the first test tube was 400 µg/ml. Then a series of solutions of varying concentrations were prepared from the stock solution by serial dilution method. In every case, 50 µl samples were added to test tube and fresh 50µl DMSO were added to vial. Thus different concentrations were found in the different test tubes (Table 6.2).

Tabl 6.2. Test samples with concentration values after serial dilution.

Test Tube No	Concentration (µg/ml)
1	400.0
2	200 .0
3	100 .0
4	50 .0
5	25 .0
6	12.5
7	6.25
8	3.125
9	1.5625
10	0.78125

6.3.2.3.4 Counting of nauplii

After 24 hours, the vials were inspected using a magnifying glass and the number of survivors were counted. The percent mortality was calculated for each dilution. The concentration-mortality data were analyzed statistically by using linear regression using a simple IBM-PC program. The effectiveness or the concentration-mortality relationship of plant product is usually expressed as a median lethal concentration (LC_{50}) value. This represents the concentration of the chemical that produces death in half of the test subjects after a certain exposure period.

6.3.3 Antimicrobial screening

6.3.3.1 Principle of disc diffusion method

In this classical method, antibiotics diffuse from a confined source through the nutrient agar gel and create a concentration gradient. Dried and sterilized filter paper discs (6 mm diameter) containing the test samples of known amounts are placed on nutrient agar medium uniformly seeded with the test microorganisms. Standard antibiotic (Ciprofloxacin) discs and blank discs are used as positive and negative control. These plates are kept at low temperature (4°C) for 24 hours to allow maximum diffusion of the test materials to the surrounding media (Bauer *et al.* 1966). The plates were then inverted and incubated at 37°C for 24 hours for optimum growth of the organisms. The test materials having antimicrobial property inhibit microbial growth in the media surrounding the discs and thereby yield a clear, distinct area defined as **zone of inhibition**. The antimicrobial activity of the test agent is then determined by measuring the diameter of zone of inhibition expressed in millimeter (Bauer *et al.* 1966, Wilkinson 1966). In the present study the crude extracts as well as fractions were tested for antimicrobial activity by disc diffusion method.

6.3.3.2 Experimental description

6.3.3.2.1 Apparatus and reagents

- Filter paper discs
- Nutrient Agar Medium
- Petridishes
- Sterile cotton
- Micropipette
- Inoculating loop
- Sterile forceps
- Screw cap test tubes
- Autoclave
- Laminar air flow hood
- Spirit burner
- Refrigerator
- Incubator
- Chloroform
- Ethanol
- Nose mask and Hand gloves

6.3.3.2.2 Test organisms

The bacterial and fungal strains were used for the experiment were collected as pure cultures from the Institute of Nutrition and Food Science (INFS), University of Dhaka. Both gram positive and gram-negative organisms were taken for the test and they are listed in the Table 6.3.

Table 6.3. List of organisms.

Gram positive Bacteria	**Gram negative Bacteria**
Bacillus cereus	*Escherichia coli*
Bacillus megaterium	*Salmonella paratyphi*
Bacillus subtilis	*Salmonella typhi*
Sarcina lutea	*Shigella boydii*
Staphylococcus aureus	*Shigella dysenteriae*
	Pseudomonas aeruginosa
	Vibrio mimicus
	Vibrio parahemolyticus

6.3.3.2.3 Test materials

Table 6.4. List of test materials.

Plant part	**Sample code**	**Test Sample**
Leaves extract of *A. tagala*	PEP	Petroleum ether soluble partitionate
	CTE	Carbon tetrachloride soluble extract
	CHE	Chloroform extract
	ME	Methanolic extract
	AQE	Aqueous soluble extract

6.3.3.2.4 Composition of culture medium

The following media were used normally to demonstrate the antimicrobial activity and to make subculture of the test organisms.

a) Nutrient agar medium

Ingredients	**Amount**
Bacto peptone	0.5 g
Sodium chloride	0.5 g
Bacto yeast extract	1.0 g
Bacto agar	2.0 g
Distilled water q.s.	100 ml
pH	7.2 + 0.1 at 25 ℃

b) Nutrient broth medium

Ingredients	**Amount**
Bacto beef extract	0.3 g
Bacto peptone	0.5 g
Distilled water q.s.	100 ml
pH	7.2 + 0.1 at 25 ℃

c) Muller – Hunton medium

Ingredients	**Amount**
Beef infusion	30 g
Casamino acid	1.75 g
Starch	0.15 g
Bacto agar	1.70 g
Distilled water q.s.	100 ml
pH	7.3 + 0.2 at 25 ℃

d) Tryptic soya broth medium (TSB)

Ingredients	**Amount**
Bacto tryptone	1.70 g
Bacto soytone	0.30 g
Bacto dextrose	0.25 g
Sodium chloride	0.50 g
Di potassium hydrogen Phosphate	0.25 g
Distilled water q.s	100 ml
pH	7.3 + 0.2 at 25℃

Nutrient agar medium is the most frequently used and also used in the present study for testing the sensitivity of the organisms to the test materials and to prepare fresh cultures.

6.3.3.2.5 Preparation of the medium

To prepare required volume of this medium, calculated amount of each of the constituents was taken in a conical flask and distilled water was added to it to make the required volume. The contents were heated in a water bath to make a clear solution. The pH (at 25 ^{0}C) was adjusted at 7.2-7.6 using NaOH or HCl. Ten ml and five ml of the medium was then transferred in screw cap test tubes to prepare plates and slants respectively. The test tubes were then capped and sterilized by autoclaving at 15-lbs. pressure at 121^{0}C for 20 minutes. The slants were used for making the fresh culture of bacteria and fungi that were in turn used for sensitivity study.

6.3.3.2.6 Sterilization procedure

In order to avoid any type of contamination and cross contamination by the test organisms the antimicrobial screening was done in Laminar Hood and all types of precautions were highly maintained. UV light was switched on one hour before working in the Laminar Hood. Petridishes and other glassware were sterilized by autoclaving at a temperature of 121ºC and a pressure of 15-lbs/sq. inch for 20 minutes. Micropipette tips, cotton, forceps, blank discs etc. were also sterilized by UV light.

6.3.3.2.7 Preparation of subculture

In an aseptic condition under laminar air cabinet, the test organisms were transferred from the pure cultures to the agar slants with the help of a transfer loop to have fresh pure cultures. The inoculated strains were then incubated for 24 hours at 37^0C for their optimum growth. These fresh cultures were used for the sensitivity test.

6.3.3.2.8 Preparation of the test plate

The test organisms were transferred from the subculture to the test tubes containing about 10 ml of melted and sterilized agar medium with the help of a sterilized transfer loop in an aseptic area. The test tubes were shaken by rotation to get a uniform suspension of the organisms. The bacterial and fungal suspension was immediately transferred to the sterilized petridishes. The petridishes were rotated several times clockwise and anticlockwise to assure homogenous distribution of the test organisms in the media.

6.3.3.2.9 Preparation of discs

Measured amount of each test sample (specified in table 6.3) was dissolved in specific volume of solvent (chloroform or methanol) to obtain the desired concentrations in an aseptic condition. Sterilized metrical (BBL, Cocksville, USA) filter paper discs were taken in a blank Petri dish under the laminar hood. Then discs were soaked with solutions of test samples and dried.

Table 6.5. Preparation of sample discs.

Plant part	**Test Sample**	**Dose** μg/disc	**Required amount for 20 disc (mg)**
Leaves extract of *A. tagala*	Methanolic extract	400	8.0
	Petroleum ether partitionate	400	8.0
	Carbontetrachloride soluble partitionate	400	8.0
	Chloroform soluble partitionate	400	8.0
	Aqueous soluble partitionate	400	8.0

Standard Ciprofloxacin (30 μg/disc) discs were used as positive control to ensure the activity of standard antibiotic against the test organisms as well as for comparison of the response produced by the known antimicrobial agent with that of produced by the test sample. Blank discs were used as negative controls which ensure that the residual solvents (left over the discs even after air-drying) and the filter paper were not active themselves.

6.3.3.2.10 Diffusion and incubation

The sample standard antibiotic control discs were placed gently on the previously marked zones in the agar plates pre-inoculated with test bacteria and fungi. The plates were then kept in a refrigerator at 4^0C for about 24 hours upside down to allow sufficient diffusion of the materials from the discs to the surrounding agar medium. The plates were then inverted and kept in an incubator at 37^0C for 24 hours.

6.3.3.2.11 Determination of the zone of inhibition

The antimicrobial potency of the test agents were measured by their activity to prevent the growth of the microorganisms surrounding the discs which gave clear zone of inhibition (Plate 6.1). After incubation, the antimicrobial activities of the test materials were determined by measuring the diameter of the zones of inhibition in millimeter with a transparent scale (Plate 6.2).

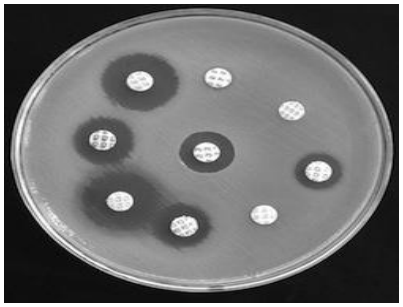


Plate 6.1. Clear zone of inhibition

Plate 6.2. Determination of clear zone of inhibition

6.4 Results and Discussion

Free radical scavenging activity, brine shrimp lethality bioassay and antimicrobial activity of the *Aristolochia tagala* (host plant of *Pachliopta aristolochiae* butterfly) were analyzed in the laboratory for determination of chemical values and the medicinal significance of butterfly related host plants. This part of Ph. D research programme is very innovative key tool to assess medicinal values of butterfly related host plants in nature especially forest ecosystem for biodiversity conservation and bio-resource management.

6.4.1 Evaluation of antioxidant activity

Different partitionates of leaves extract of *A. tagala*, i.e. Tert-butyl-1-hydroxytoluene (BHT), Ascorbic acid (AA), Methanolic extract (ME), Petroleum ether (PE), Carbon tetrachloride soluble (CTE), Chloroform soluble (CHE) and Aqueous soluble partitionate (AQE) were subjected to free radical scavenging activity according to Brand-Williams *et al. (*1995). Here, *tert*-butyl-1-hydroxytoluene (BHT) and ascorbic acid (ASA) were used as reference standard (Table 6.6).

Free radical scavenging activity of methanolic extract (ME) partitionate of *A. tagala* showed the highest free radical scavenging activity with IC_{50} value 4.67 µg/ml. At the same time the PE and AQE also exhibited strong antioxidant potential having IC_{50} value 4.09 and 7.45 µg/ml, respectively. Free radical scavenging activity of CTE and CHE are 9.17 and 17.45 µg/ml, respectively (Fig. 6.2. and Appendix 11-17).

Table 6.6. IC_{50} values of the standard and partitionates of leaves of *A. tagala*.

Plant part	Sample code	Test Sample	IC_{50} (µg/ml)
Leaves of *A. tagala*	**BHT**	Tert-butyl-1-hydroxytoluene (standard)	27.5
	AA	Ascorbic acid (standard)	5.8
	ME	Methanolic extract	4.67
	PE	Petroleum ether soluble partitionate	4.09
	CTE	Carbon tetrachloride soluble partitionate	9.17
	CHE	Chloroform soluble partitionate	17.45
	AQE	Aqueous soluble partitionate	7.45

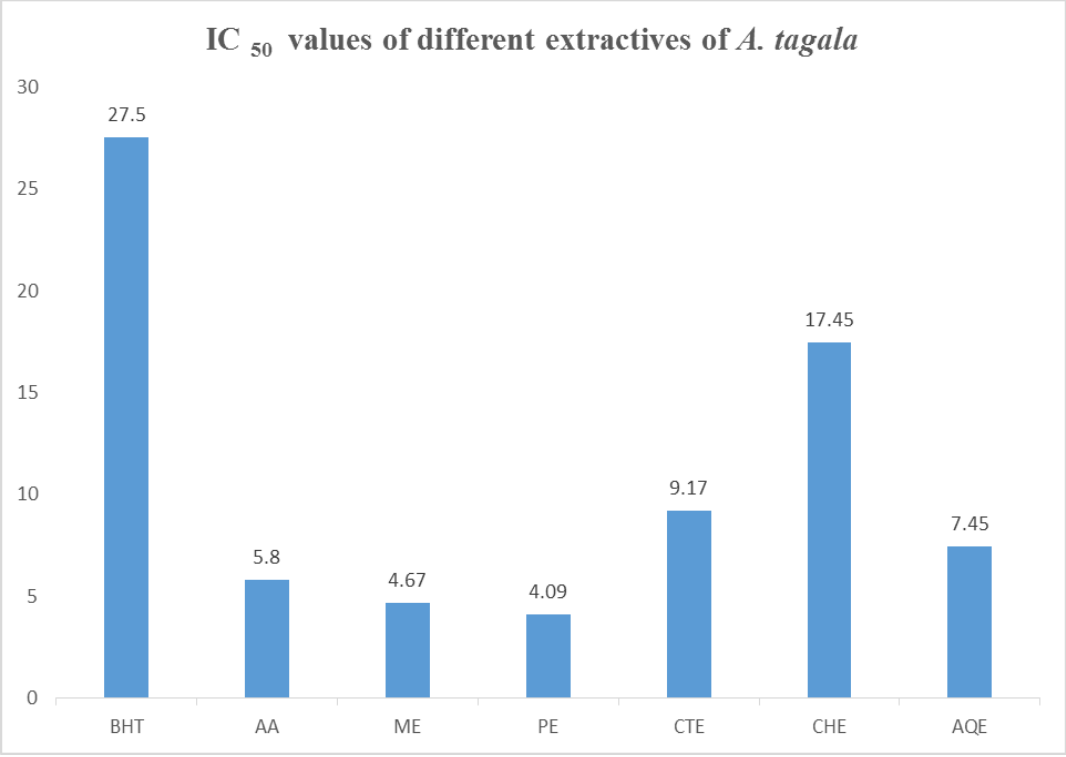


Fig. 6.2. IC $_{50}$ values of the standard and partitionates of *A. tagala*.

6.4.2 Toxicity of A. tagala

Different partitionates of the leaves extract of *A. tagala*, i.e. Methanolic extract (ME), Petroleum ether (PE), Carbon tetrachloride soluble (CTE), Chloroform soluble (CHE) and Aqueous soluble partitionate (AQE) were tested for brine shrimp lethality bioassay following the procedure of Meyer *et al.* (1982). The lethality of the extractives to brine shrimp was determined and the results are given in Table 6.7.

The lethal concentration LC_{50} of the test samples after 24 hr was obtained by a plot of percentage of the shrimps died against the logarithm of the sample concentration (toxicant concentration) and the best-fit line was obtained from the curve data by means of regression analysis.

The LC_{50} values of Methanolic extract (ME), Petroleum ether (PE), Carbon tetrachloride soluble (CTE), Chloroform soluble (CHE) and Aqueous soluble partitionate (AQE) **of *A. tagala*** were 5.77 μg/ml, 0.84 μg/ml**,** 1.52 μg/ml, 7.61 μg/ml and 7.99 μg/ml, respectively (Fig. 6.3. and Appendix 18-22).

Table 6.7. LC_{50} values of the test samples of *A. tagala.*

Test samples	Regression line	R^2	LC_{50} (µg/ml)
ME	y = 24.021x + 31.438	0.9021	5.77
CTE	y = 22.79x+45.84	0.935	1.52
PE	y = 20.489x+51.491	0.9595	0.84
CHE	y = 24.021x+31.438	0.9021	7.61
AQE	y = 24.429x+27.969	0.8892	7.99

ME = Methanolic extract; CTE = Carbon tetrachloride soluble partitionate; PE= Petroleum ether soluble partitionate; CHE = Chloroform soluble extract; AQE = Aqueous soluble partitionate

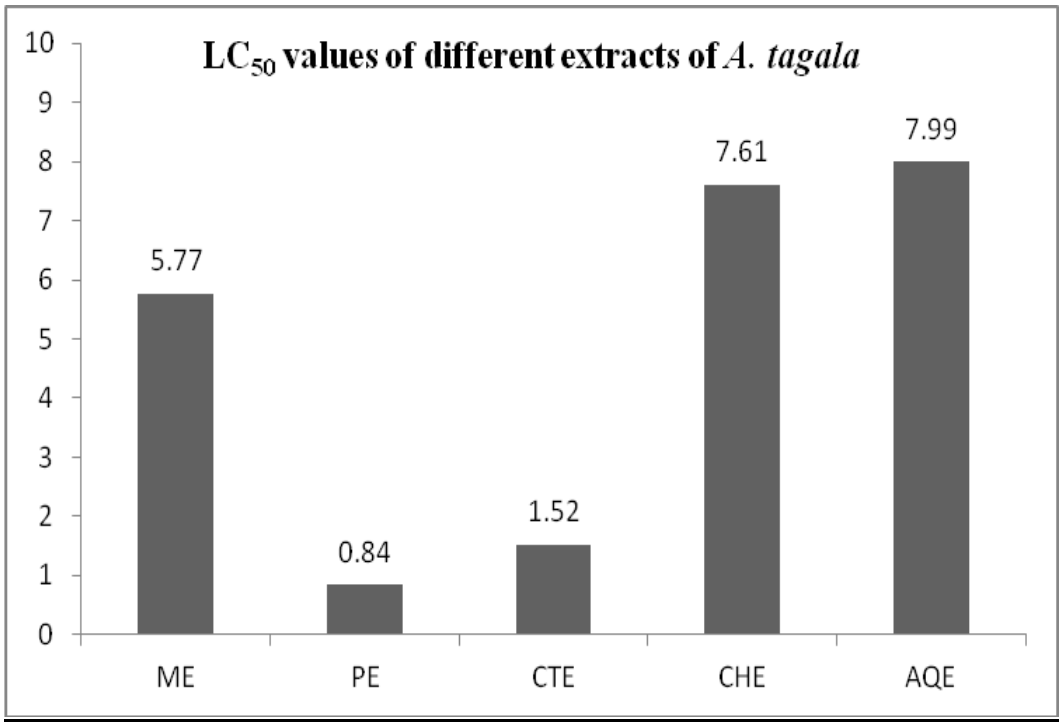


Fig. 6.3. LC_{50} values of the different extractives of *A. tagala.*

6.4.3 Antimicrobial screening of A. tagala

Different partitionates of the leaves extract of *A. tagala*, i.e. Methanolic extract (ME), Petroleum ether (PEP), Carbon tetrachloride soluble (CTE), Chloroform soluble (CHE) and Aqueous soluble extract (AQE) were subjected to antimicrobial screening with a concentration of 400 μg/disc in every case.

The antimicrobial activity of Chloroform soluble extract (CHE) of *A. tagala* demonstrated strong against *Sarcina lutea* and *Vibrio parahemolyticus*, and mild against *Salmonella paratyphi*. The antimicrobial activity of Aqueous soluble extract (AQE) of *A. tagala* exhibited the strong against *Shigella boydii* and mild against *Salmonella typhi* (Table 6.8).

Growth having zone of inhibition ranged from 7 to 21 mm. No antimicrobial activity was recorded for Methanolic extract (ME), Petroleum ether (PEP) and Carbon tetrachloride soluble (CTE) of *A. tagala* (Table 6.8).

Table 6.8. Antimicrobial activity of test samples of *A. tagala.*

Test microorganisms	Diameter of zone of inhibition (mm)					
	ME	PEP	CTE	CHE	AQE	Ciprofloxacin
Gram positive bacteria						
Bacillus cereus	-	-	-	13	10	38
B. megaterium	-	-	-	14	-	46
B. subtilis	-	-	-	9	11	36
Staphylococcus aureus	-	-	-	11	10	41
Sarcina lutea	-	-	-	21	12	37
Gram negative bacteria						
Escherichia coli	-	-	-	14	11	40
Pseudomonas aeruginosa	-	-	-	11	9	40
Salmonella paratyphi	-	-	-	8	8	32
S. typhi	-	-	-	11	7	34
Shigella boydii	-	-	-	16	13	37
Sh. dysenteriae	-	-	-	12	10	33
Vibrio mimicus	-	-	-	13	8	34
V. parahemolyticus	-	-	-	21	11	36
Fungi						
Candida albicans	-	-	-	12	8	40
Aspergillus niger	-	-	-	-	-	36
Saccharomyces cerevisiae	-	-	-	11	11	39

The present results showed that medicinal plants which were used in traditional medicine against infections may have some antimicrobial activity. This is true for *Aristolachia* indica L. methanolic extract. These results were consistent with traditional uses of the plant leaves *Aristolachia tagala* and pharmacological actions of its leaves. The concentrations of methanolic extract needed for bacteristasis were 10000 times higher than the concentrations of usual antibiotics (Kumar *et al.* 2011). However, the Soxhlet extracts were very crude preparations, and further purifications may yield more potent compounds. Furthermore, the detection of antimicrobial activities-albeit to varying extents-indicates that the plants may be sources for bactericidal and fungicidal drugs (Kumar *et al.* 2011).

In vitro antibacterial studies of the three different leaf extracts of *A. bracteolata* revealed that the methanol extract had significant activity against most of the organism, while the chloroform extract possessed moderate activity (Kavitha and Nirmaladevi 2009). Methanol extract exhibited the maximum inhibitory effect against *B. subtilis, P. aeruginosa, Salmonella typhi* and considerable inhibitory activity against *E. coli* and *Staphyllococcus aureus.* Chloroform extract had significant inhibitory activity against *E. coli* and moderate activity against *Bacillus subtilis, Shig. flexneri, K. pneumoniae, P. aeruginosa* and *Sal. typhi.* No zone of inhibition was found against *Sal. typhimurium* and *P. vulgaris* using the aqueous and methanolic extracts. *B. subtilis* was found to be the most susceptible bacterium with minimal inhibitory concentration of 39.06 µg/ml for the methanol extract. But chloroform extract and aqueous extract inhibited the same organism at a higher concentration (Kavitha and Nirmaladevi 2009).

The various extracts (500µg/disc) of *Aristolochia bracteata* showed moderate antibacterial activity with the average zone of inhibition of 7-18 mm by disc diffusion method. Among the extracts, ethyl acetate and methanol extracts were shown good anti-microbial activity and the growth of *E.coli* (18 mm) was strongly inhibited. Microbial assay of isolated compounds from ethyl acetate and methanol extracts were shown good antimicrobial activity and the zone of inhibition of both at higher concentration was similar with the standard aristolochic acid I (Angalaparameswari *et al.* 2012).

The toxic effects of aristolochic acids I and II have been inferred from effects seen in patients suffering from kidney nephropathy as a result of consuming herbal mixtures containing *Aristolochia* species, which leads to rapidly progressive fibrosing interstitial nephritis (Pakrashi and Shaha 1971a). Various constituents of *Aristolochia indica* including aristolochic acids-I and aristolochic acid-II (a metabolite) caused termination of pregnancy in female mice, hamsters and rabbits, but not rats. The dose levels used, however, may also lead to general toxicity (Pakrashi and Chakrabarty 1978a).

According to Ehrlich and Raven (1964), the use of toxic plant families, which present a considerable evolutionary barrier to colonization, has arisen many times among butterflies because of two important advantages: exploitation of underused ‘‘adaptive zones,’’ and use of plant defensive chemistry to the herbivore’s own advantage. These benefits compensate for whatever disadvantages are associated with becoming evolutionarily ‘‘trapped’’ on a particular plant family. The Troidini are a classic example: their association with the Aristolochiaceae is considered ancient, and they are nearly alone in exploiting these plants (Feeny 1991).

Their dependence on aristolochic acids for host-plant recognition, established in *B. philenor* (Sachdev-Gupta *et al.* 1993) and *Parides alcinous* (Nishida 1995a), supports the idea of their being ‘‘trapped.’’ Nishida and Fukami (1989a) showed that aristolochic acids are present in all life-history stages of *P. alcinous.* The present study corroborates their work, and, by associating these findings with the abundant ecological data available for *B. philenor*, provides strong support for the hypothesis of defensive advantage in all stages. Haase’s (1893) proposal, partially supported by Rothschild *et al.* (1970) and Rothschild (1972), that toxins acquired from Aristolochia protect *B. philenor* from the natural enemies that burden other Papilionidae, has been confirmed in full.

It has long been assumed that the North American pipevine swallowtail, *Battus philenor* (L.) (Papilionidae, Troidini), is protected from natural enemies by aristolochic acids sequestered from its Aristolochia food plants. This study confirmed that populations of *B. philenor* from Virginia and east Texas sequester these compounds. A comparison of the aristolochic acid profiles of the Virginia butterflies and their *A. macrophylla* food plants revealed several differences (Sime *et al.* 2000). The aristolochic acid fraction of the foliage was dominated by aristolochic acids I and II, whereas the insects had a much lower proportion of aristolochic acid II and contained, in addition, substantial amounts of aristolochic acids Ia and IVa, which were not detected in the plants. The eggs, larval integument, osmeterial glands, pupal cuticle, and adults (wings and bodies) all contained aristolochic acids. These findings help explain the abundant ecological data indicating that both immature and adult *B. philenor* are unpalatable and protected from natural enemies (Sime *et al.* 2000).

The antioxidant, antimicrobial and cytotoxic activities of various fractions of the host plant, *A. tagala* leaves, found in this study, may explain some of the traditional medicinal uses of this plant. These could be of particular interest in relation to find out its unexplored efficacy and can be a potential source of chemically interesting and biologically important drug candidates.

Research is recommended to isolate chemical compositions and their proximate values from different extractive parts, viz. leaves, roots, stems, flowers and fruits of the host plant *Aristolochia tagala* for further confirmation of medicinal significance in the field level as a part of biodiversity conservation and bio-resource management.

Chapter 7. Summary and Conclusion

7.1 Summary of the research work

A research investigation was conducted entitled "**Behaviour of some selected butterflies and its influence on nutritional and medicinal values of host plants"** in the Satchori forest (Research field), Zoological Garden of Dhaka University (Pre-experiment field) and Butterfly Research Park (Application field) at Bhawal National Park, Gazipur. Different developmental stages of butterfly and different phenological stages of butterfly related host, nectar and supportive plants were examined, identified and studied in the Environmental Biology and Biodiversity Laboratory (EBBL), Department of Zoology and Plant Taxonomy Laboratory, Department of Botany, University of Dhaka. The free radical scavenging activity (antioxidant), brine shrimp lethality bioassay (Toxicity) and antimicrobial activity were analyzed in the Phytochemical Laboratory in the Department of Pharmaceutical Chemistry, University of Dhaka.

The Ph. D thesis is comprised of total eight chapters. Among eight chapters, Chapter 3, 4, 5 and 6 are the major parts of Ph. D thesis which have findings of research and Chapter 1 (General Introduction), Chapter 2 (Review of Literature), Chapter 7 (Summary and Conclusion) and Chapter 8 (Literature Cited) are supportive part of thesis for completion of Ph. D. research.

The findings of Ph. D research programme was dealt with 4 chapters in total:

- ***Behavioural studies of butterfly through its colonization in the Butterfly Research Park at Bhawal National Park, Gazipur (Chapter 3);***
- ***Biology of Danaus chrysippus in relation to larval host plants and adult nectar plants (Chapter 4);***
- ***Biotic interaction between Pachliopta aristolochiae butterfly and its host plant, Aristolochia indica (Chapter 5);***
- ***Antioxidant, toxicity and antimicrobial activity of Aristolochia tagala (Chapter 6).***

Total 15,411 host, nectar and supportive plants (different stages) were planted for butterfly colonization in Butterfly Research Park (BRP) at Bhawal National Park, Gazipur, Bangladesh to detect the nutritional and medicinal plants in the field through behavioural activities of butterfly. Association between butterfly and their related host plants, the dynamism of butterfly population in relation to nectar plants, behavioural patterns of butterfly, the effect of abiotic and biotic factors on butterfly abundance, the taxonomic status of butterfly, butterfly species richness and species diversity were studied and recorded in the research fields. Behavioural activities of butterfly were innovated through accommodation of butterfly related host, shelter and nectar plants. The first open butterfly park was established through sustainable butterfly colonization at Bhawal National Park, Gazipur during the research period (Chapter 3).

To understand the biology of butterfly, developmental stages of *Danaus chrysippus* in relation to the host plant, *A. curassavica* and utilization of nectar plants by visits of *D. chrysippus* as nutritional requirements for their development were examined during the experimentation. The life stages and development of the plain tiger butterfly, *D. chrysippus* were studied and recorded in both field and laboratory conditions with a view to analyze the association of the butterfly with its related plants for innovating techniques to establish sustainable butterfly colonization naturally (Chapter 4).

Successive development of the host plants, *A. indica* and *A. tagala*, egg laying strategy, host plant utilization of larvae, pupation strategy of *P. aristolochiae* and its different behavioural strategies, viz. foraging, mating, egg laying, resting, searching and puddling in relation to population fluctuation were investigated during the research period. The synchronization of coincidences between life stages of *Pachliopta aristolochiae* butterfly and different phenological stages of its host plant, *Aristolochia indica* were studied to determine the host-specificity of butterfly through assessing larval feeding potentiality of host plant in the experimental field (Chapter 5).

Different partitionates of leaves extract of *Aristolochia tagala* i.e. Tert-butyl-1-hydroxytoluene (BHT), Ascorbic acid (AA), Methanolic extract (ME), Petroleum ether (PE), Carbon tetrachloride soluble (CTE), Chloroform soluble (CHE) and Aqueous soluble partitionate (AQE) were analyzed during the research period. The free radical scavenging activity (antioxidant), brine shrimp lethality bioassay (toxicity) and antimicrobial activity of the leaves extract of *A. tagala* were examined for assessing the nutritional and medicinal importance of butterfly related host plants as a part of biodiversity conservation and bio-resource management (Chapter 6).

7.2 Conclusion

As larval stages of two butterfly, *Danaus chrysippus* (**host plant-*Asclepius curassavica***) and *Pachliopta aristolochiae butterfly* (**host plants*, Aristolochia indica* and *Aristolochia tagala***) depend on specific host plants for their **nutritional supports and development** and; hence butterflies are **highly host specific** in their larval stages. On the other hand, adult butterfly (***D. chrysippus* and *P. aristolochiae***) depends on **nectar plants** for their **nutritional supports** and single species of butterfly may utilize 10-15 spp. of nectar plants for their **development.**

It has been found that interaction of developmental stages **(larval and adult activity)** of butterfly with their respective **host and nectar plants** indicate that butterfly related host and nectar plants play a vital role **as nutritional sources** to sustain their life cycle. It has been experimentally proved that A*ristolochia tagala* (host plant of *P. aristolochiae* butterfly) showed **significant antioxidant, toxicity and antibacterial activity** which can be used as traditional herbal medicine in humans already being practiced in different countries.

It could be said that **butterfly's behavioural activity** is the best **biotic indicator** for detection of **medicinal plants** from the field which is key factor for **biodiversity conservation and bio-resource management.**

Chapter 8. Literature Cited

Chapter 8 was dealt with Literature Cited. Literature Cited was followed alphabetically as per style of Bangladesh Journal of Zoology. Authors name were written in capital letters. Literature cited was emphasized mainly on behavioural activities of butterfly; biology and ecology of butterflies and their related plants; butterfly diversity, abundance and distribution; utilization of larval-host plants and adult nectar plants; butterfly-plant interaction; ecosystem management and biodiversity conservation; antioxidant, toxicity, antimicrobial activity and chemical constituents isolation of medicinal plants. All Literature Cited were included in the text for enriching the Ph. D. thesis.

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Appendices

Appendix 1. Development of host plants in the Butterfly Research Parks at Bhawal National Park, Gazipur.

Common name of plants	Scientific name of plants	No. of planted plants	No. of survived plants	Rate of survival plants (%)	Types of plant
Indian birthwort	*Aristolochia indica*	1465	1013	69.14	Climber
Indian birthwort	*Aristolochia tagala*	500	410	82	Climber
Milkweed	*Asclepias curassaviaca*	1350	928	68.74	Herbs
Lemon	*Citrus spp.*	950	680	71.57	Shrubs
Debdaru	*Polyalthia longifolia*	90	71	78.88	Tree
Cassia	*Cassia tora*	400	360	90	Herbs
Motkila	*Glycosmis pentaphylla*	70	48	68.57	Shrub
Weedelia	*Feronia limonia*	50	42	84	Herbs
Verenda	*Ricinus communis*	65	51	78.46	Tree
Dadmordon	*Cassia alata*	50	37	74	Shrub
Sonalu	*Cassia fistula*	60	50	83.33	Tree
Junjuni	*Cassia occidentalis*	95	70	73.68	Herbs
Bondhone	*Scaparia duleis*	105	89	84.76	Herbs

Appendix 2. Development of nectar plants in the Butterfly Research Parks at Bhawal National Park, Gazipur.

Common name of plants	Scientific name of plants	No. of planted plants	No. of survived plants	Rate of survival plants (%)	Types of plant
Lantana	*Lantana camera*	2660	1695	63.72	Shrub
Nilpetunia	*Duranta plumeri*	1396	975	69.84	Shrub
Jaba	*Hibiscus rosa sinensis*	112	91	81.25	Shrub
Katamehdi	*Duranta repens*	605	431	71.73	Shrub
Gadha	*Tagetes patula*	430	387	90	Herbs
Rongon	*Ixora chinensis*	98	93	94.89	Shrubs
Hatisur	*Heliotropium indicum*	935	572	61.17	Herbs
Cosmos	*Cosmos bipinnatus*	1080	562	52.03	Herbs
Weedelia	*Wedelia calendulacea*	520	475	91.34	Herbs
Panica	*Panica indica*	770	682	88.57	Herbs
Marhatitiga	*Spilanthes calva*	325	303	92.23	Herbs
Setodron	*Leucas linifolia*	260	185	71.15	Herbs
Sunflower	*Helianthus annus*	135	105	77.77	Herbs
Seeje	*Euphorbia puleherrima*	105	93	88.57	Herbs

Botamful	*Gomphera globosa*	290	242	83.44	Herbs

Appendix 3. Family-wise butterfly population in Butterfly Research Park, Gazipur (Jan 2012-Dec 2012).

Dt	T (ºC)	RH (%)	Butterfly's Family						
			Pap	Dan	Nym	Pier	Sat	Lyc	Hesp
06/01/12	26	54	4	11	3	8	1	1	1
20/01/12	31	50	6	92	22	19	0	2	1
27/01/12	28.4	41	3	38	9	20	0	20	0
10/02/12	26.3	50	9	73	11	28	1	15	2
17/02/12	25.4	52	30	51	21	40	6	5	1
09/03/12	28.2	43	19	25	20	20	0	0	0
23/03/12	28.6	39	32	71	10	38	9	4	0
06/04/12	24.5	59	0	7	0	7	0	0	0
20/04/12	33.2	72	34	222	61	129	0	25	10
27/04/12	35.6	67	26	173	22	76	1	20	28
04/05/12	27.2	80	73	332	21	104	0	1	73
11/05/12	33.4	72	126	234	55	42	5	11	19
18/05/12	35.6	57	53	157	89	79	0	2	26

15/06/12	31.5	83	29	363	15	23	0	11	21
29/06/12	43.3	42	45	71	64	40	3	0	52
01/07/12	27.9	81	7	0	11	4	0	0	0
04/07/12	32.6	85	6	0	2	2	0	0	0
07/07/12	34	81	32	6	25	27	5	2	4
14/07/12	32.2	86	13	7	23	11	0	5	0
20/07/12	33.1	81	39	16	107	32	0	13	0
27/07/12	31.3	83	4	7	3	11	0	2	2
03/08/12	31.8	89	19	22	25	27	0	0	0
31/08/12	40.9	49	10	8	13	15	0	0	7
07/09/12	38.7	64	5	7	16	21	0	0	4
28/09/12	28.2	43	10	11	14	27	0	0	21
02/11/12	34.5	51	31	65	61	75	0	0	58
09/11/12	28.2	59	21	187	137	106	0	0	237
16/11/12	29.9	56	24	277	273	137	0	0	731
23/11/12	29.1	59	0	199	187	175	0	0	562

30/11/12	35.9	48	86	155	180	140	0	0	372
07/12/12	30.2	47	23	55	48	67	0	0	295
28/12/12	18.8	62	0	0	0	5	0	0	0

Dt=Date; T=Temperature; RH=Relative Humidity; Pap=Papilionidae; Dan=Danaidae; Nym=Nymphalidae; Pier=Pieridae; Sat=Satyridae; Lyc=Lycaenidae; Hesp=Hesperiidae;

Appendix 4. Behaviour-wise butterfly population in Butterfly Research Park, Gazipur (Jan 2012-Dec 2012).

Dt	T (ºC)	RH (%)	Butterfly's behaviour					
			Fg	Rt	El	Mt	Sr	Pl
06/01/12	26	54	17	21	2	3	6	14
20/01/12	31	50	118	0	0	9	19	11
27/01/12	28.4	41	37	32	11	0	50	0
10/02/12	26.3	50	78	22	6	6	54	9
17/02/12	25.4	52	21	40	5	12	69	7
09/03/12	28.2	43	25	5	3	5	50	0
23/03/12	28.6	39	71	21	20	1	63	5
06/04/12	24.5	59	0	0	0	0	14	0
20/04/12	33.2	72	356	42	17	13	61	0
27/04/12	35.6	67	167	8	11	17	132	15
04/05/12	27.2	80	451	4	0	0	149	15
11/05/12	33.4	72	325	0	5	3	229	13

18/05/12	35.6	57	239	9	0	9	162	5
15/06/12	31.5	83	229	214	9	0	18	0
29/06/12	43.3	42	232	8	0	11	31	7
01/07/12	27.9	81	9	0	10	4	12	11
04/07/12	32.6	85	1	0	0	0	9	5
07/07/12	34	81	33	16	8	6	33	3
14/07/12	32.2	86	14	20	13	0	25	3
20/07/12	33.1	81	74	9	11	14	122	0
27/07/12	31.3	83	4	13	12	0	10	2
03/08/12	31.8	89	37	3	0	5	53	0
31/08/12	40.9	49	12	9	15	2	32	0
07/09/12	38.7	64	11	9	8	1	23	0
28/09/12	28.2	43	45	0	0	3	38	0
02/11/12	34.5	51	186	5	10	0	75	10
09/11/12	28.2	59	681	0	0	13	4	3
16/11/12	29.9	56	1440	2	15	0	0	0

23/11/12	29.1	59	1096	0	0	3	27	0
30/11/12	35.9	48	758	0	0	2	176	0
07/12/12	30.2	47	437	0	11	0	40	0
28/12/12	18.8	62	0	0	0	0	5	0

Fg=Foraging; Rt=Resting; El=Egg-laying; Mt=Mating; Sr=Searching; Pl=Puddling;

Appendix 5. Butterfly and nectar plants association in Butterfly Research Park, Gazipur (Jan 2012-Dec 2012).

Dt	T (ºC)	RH (%)	Nectar Plants																		
			Ap	Hl	Lt	Cm	Bt	Cd	Jb	Hs	Gd	Mt	Dp	Dk	Sd	Mk	Pn	Wd	Np	Rg	Km	Tl
06/01/12	26	54	8	7	3	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
20/01/12	31	50	8	63	9	28	4	8	-	-	-	-	-	-	-	-	-	-	-	-	-	-
27/01/12	28.4	41	11	9	-	4	4	-	3	6	-	-	-	-	-	-	-	-	-	-	-	-
10/02/12	26.3	50	18	-	-	-	-	-	-	23	7	25	5	-	-	-	-	-	-	-	-	-
17/02/12	25.4	52	6	-	-	3	-	-	2	5	1	4	7	-	-	-	-	-	-	-	-	-
09/03/12	28.2	43	6	-	5	3	-	-	-	7	-	4	-	-	-	-	-	-	-	-	-	-
23/03/12	28.6	39	19	-	16	-	5	-	-	17	-	-	-	6	8	-	-	-	-	-	-	-

06/04/12	24.5	59	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
20/04/12	33.2	72	50	-	45	15	-	-	10	42	-	-	-	-	38	35	37	49	35	-	-	-
27/04/12	35.6	67	47	-	38	-	-	-	-	21	-	-	-	-	-	-	7	37	17	-	-	-
04/05/12	27.2	80	85	-	73	-	-	-	29	73	-	-	-	27	17	-	-	63	71	13	-	-
11/05/12	33.4	72	71	-	66	6	-	-	-	36	-	-	-	-	16	-	-	56	38	15	21	-
18/05/12	35.6	57	84	-	35	-	-	-	-	-	-	-	-	-	26	-	-	52	42	-	-	-
15/06/12	31.5	83	61	-	53	5	-	-	-	44	3	-	-	-	-	-	-	7	49	7	-	-
29/06/12	43.3	42	174	-	21	-	-	-	-	-	-	-	-	-	-	-	-	-	27	10	-	-
01/07/12	27.9	81	-	-	9	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
04/07/12	32.6	85	-	-	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-

07/07 /12	34	81	**7**	-	21	-	-	-	-	-	-	-	-	-	-	-	-	-	-	5	-	-
14/07 /12	32.2	86	5	-	7	-	-	-	-	-	-	-	-	-	-	-	-	-	-	2	-	-
20/07 /12	33.1	81	54	-	13	5	-	-	-	-	**2**	-	-	-	-	-	-	-	-	-	-	-
27/07 /12	31.3	83	2	-	2	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
03/08 /12	31.8	89	13	-	-	11	-	-	-	-	-	-	-	-	-	-	-	-	6	-	-	-
31/08 /12	40.9	49	-	-	-	6	-	-	-	-	-	-	-	-	-	-	-	4	2	-	-	-
07/09 /12	38.7	64	-	-	-	5	-	-	-	-	-	-	-	-	-	-	-	2	-	-	-	-
28/09 /12	28.2	43	-	-	-	-	-	-	-	24	-	21	-	-	-	-	-	-	-	-	-	-
02/11 /12	34.5	51	48	-	31	-	-	-	-	42	-	58	-	-	-	-	-	-	-	7	-	-
09/11 /12	28.2	59	79	-	74	37	-	-	-	55	18	340	-	-	-	-	-	-	47	-	-	49

16/11/12	29.9	56	150	-	87	58	-	-	-	70	30	660	-	-	-	-	-	155	65	-	-	165
23/11/12	29.1	59	75	-	120	59	-	-	-	63	25	351	-	-	-	-	75	112	51	22	-	145
30/11/12	35.9	48	-	-	155	75	-	-	-	125	35	145	-	-	-	-	86	-	-	-	-	137
07/12/12	30.2	47	-	-	257	67	-	-	-	-	-	113	-	-	-	-	-	-	-	-	-	-
28/12/12	18.8	62	-	-	-		-	-	-	-	-		-	-	-	-	-	-	-	-	-	-

Ap=Asclepias; Hl=Harina lata; Lt=Lantana; Cm=Cosmos; Bt=Botamful; Cd= Chandramallika; Jb=Jaba; Hs=Hatisur; Gd=Gadha; Mt=Marhatitiga; Dp=Dopati; Dk=Dondokolos; Sd=Setodron; Mk= Monkata; Pn=Panika; Wd=Weedelia; Np=Nilpetunia; Rg=Rongon; Km=Katamehedi; Tl=Tara lata;

Appendix 6. Butterfly species richness, status, associated host and nectar plants in Butterfly Research Park, Bhawal National Park.

Butterfly's family	Scientific name of butterfly	Common name of butterfly	Status of butterfly	Butterfly related host plants	Butterfly related nectar plants
Papilionidae	*Pachliopta aristolochiae* Fabricius, 1775	Common rose	Available	*Aristolochia indica* *Aristolochia tagala* F-Aristolochiaceae	*Asclepias curassaviaca* (Asclepiadaceae) *Hibiscus rosa sinensis* (Malvaceae) *Duranta plumeri* (Verbenaceae)
	Graphium agamemnon Linnaeus, 1758	Tailed Jay	Rare	*Polyalthia longifolia* F-Annonaceae	*Hibiscus rosa sinensis* (Malvaceae) *Ixora chinensis* (Rubiaceae) *Asclepias curassaviaca* (Asclepiadaceae)
	Graphium doson Felder, 1864	Common Jay	Available	*Polyalthia longifolia* F-Annonaceae	*Ixora chinensis* (Rubiaceae) *Hibiscus schizopetalus* (Malvaceae) *Asclepias curassaviaca* (Asclepiadaceae)

	Graphium sarpedon Linnaeus, 1758	Common bluebottle	Near threatened	*Polyalthia longifolia* F-Annonaceae	*Micromelum pubescens* (Rutaceae) *Hibiscus rosa-sinensis* (Malvaceae)
	Chilasa clytia Hampson, 1889	Common Mime	Available	*Litsea glutinosa* F-Lauraceae	*Lantana camara* (Verbenaceae) *Bougainvillea spectabilis* (Nyctaceae)
	Chilasa paradoxa Zinken, 1831	Great Blue Mime	Near threatened	*Litsea glutinosa* F-Lauraceae	*Lantana sellowiana* (Verbenaceae) *Bougainvillea spectabilis* (Nyctaceae)
	Troides helena Rothsch, 1895	Common Birdwing	Rare	*Aristolochia indica* *Aristolochia tagala* F-Aristolochiaceae	*Euphorbia puleherrima* (Euphorbiaceae) *Lantana aculeate* (Verbenaceae) *Hibiscus rosa sinensis* (Malvaceae)
	Papilio polytes Linnaeus, 1758	Common Mormon	Available	*Aegle marmelos* *Citrus spp.* *Glycosmis arborea*	*Lantana camara* (Verbenaceae) *Clerodendrum inerme* (Verbenaceae)

				F-Rutaceae	*Duranta plumeri* (Verbenaceae)
	Papilio demoleus Linnaeus, 1758	Lime Swallowtail	Available	*Aegle marmelos* *Citrus spp.* *Glycosmis arborea* F-Rutaceae	*Lantana camara* (Verbenaceae) *Clerodendrum serratum* (Verbenaceae) *Duranta plumeri* (Verbenaceae)
	Papilio polymnestor Cramer, 1775	Blue Mormon	Available	*Citrus grandis* F-Rutaceae	*Asclepius curassavica* (Asclepiadaceae) *Hibiscus rosa sinensis* (Malvaceae) *Ixora chinensis* (Rubiaceae)
Danaidae	*Tirumala limniaceae* Cramer, 1775	Blue Tiger	Available	*Calotropis procera* F-Asclepiadaceae	*Heliotropium indicum* (Boraginaceae) *Asclepius curassavica* (Asclepiadaceae) *Duranta plumeri* (Verbenaceae)
	Parantica aglea Stoll, 1782	Glassy Tiger	Available	*Tylophora indica* F-Asclepiadaceae	*Heliotropium indicum* (Boraginaceae) *Chromolaena odorata*

					(Asteraceae) *Asclepias curassaviaca* (Asclepiadaceae)
	Parentica agleoides Felder, 1860	Dark Glassy Tiger	Available	*Gymnema spp*. F-Asclepiadaceae	*Chromolaena odorata* (Asteraceae) *Heliotropium indicum* (Boraginaceae) *Asclepias curassaviaca* (Asclepiadaceae)
	Euploea core Cramer, 1780	Common Crow	Available	*Ichnocarpus frutescens* F-Apocynaceae	*Adina cordfolia* (Rubiaceae) *Heliotropium indicum* (Boraginaceae) *Diospyros Montana* (Ebenaceae) *Duranta plumeri* (Verbenaceae)
	Euploea mulciber Cramer, 1777	Stripped Blue Crow	Available	*Ficus microcarpa* F-Moraceae	*Diospyros montana* (Ebenaceae) *Adina cordfolia* (Rubiaceae) *Heliotropium indicum* (Boraginaceae)

					Duranta plumeri (Verbenaceae)
	Euploea klugii Moore, 1858	Brown King Crow	Rare	*Ficus hispida* F-Moraceae	*Heliotropium indicum* (Boraginaceae) *Diospyros Montana* (Ebenaceae)
	Danaus genutia Cramer, 1779	Common Tiger	Available	*Asclepius curassavica* F-Asclepiadaceae	*Calotropis gigantae* (Asclepiadaceae) *Lantana camara* (Verbenaceae) *Duranta plumeri* (Verbenaceae) *Asclepias curassaviaca* (Asclepiadaceae)
	Danaus chrysippus Linnaeus, 1758	Plain Tiger	Available	*Asclepius curassavica* F-Asclepiadaceae	*Calotropis procera* (Asclepiadaceae) *Lantana camara* (Verbenaceae) *Asclepias curassaviaca* (Asclepiadaceae)
Nymphalidae	*Phalantha phalantha*	Common Leopard	Available	*Flacourtia spp.*	*Cosmos bipinnatus* (Compositae)

	Drury, 1770			F-Flacourtiaceae	*Spilanthes calva* (Asteraceae)
	Parthenos gambrisius Fabricius, 1775	Clipper	Available	*Melothria heterophylla* F-Menispermaceae	*Lawsonia inermis* (Lythraceae) *Spilanthes calva* (Asteraceae)
	Pantoporia nefte Cramer, 1782	Colour Lascar	Available	*Glochidion spp.* F-Euphorbiaceae	*Chromolaena odorata* (Asteraceae) *Spilanthes calva* (Asteraceae)
	Pantoporia cama Moore, 1857	Orange Lascar	Available	*Archidendron spp.* F-Fabaceae	*Duranta repens* (Verbenaceae) *Spilanthes calva* (Asteraceae)
	Hypolimnas bolina Linnaeus, 1758	Great Egg Fly	Available	*Sida rhombifolia* F-Malvaceae	*Duranta plumeri* (Verbenaceae) *Spilanthes calva* (Asteraceae) *Duranta plumeri* (Verbenaceae)
	Junonia iphita Cramer, 1869	Chocolate Pansy	Available	*Hygrophila auriculata* F-Acanthaceae	*Lantana camara* (Verbenaceae) *Wedelia calendulacea* *Duranta repens* (Verbenaceae)
	Junonia lemonias Linnaeus, 1758	Lemon Pansy	Available	*Barleria spp.* F-Acanthaceae	*Chromolaena odorata* (Asteraceae) *Wedelia calendulacea*

	Junonia orithya Linnaeus, 1758	Blue Pansy	Rare	*Mimosa pudica* F-Mimosaceae	*Lantana camara* (Verbenaceae) *Wedelia calendulacea* *Duranta repens* (Verbenaceae)
	Junonia hierta Fabricius, 1775	Yellow Pansi	Available	*Barleria spp.* F-Acanthaceae	*Vitis lanceolaria* *Wedelia calendulacea*
	Junonia atlites Johanssen, 1763	Grey Pansi	Available	*Hygrophila auriculata* F-Acanthaceae	*Lantana camara* (Verbenaceae) *Wedelia calendulacea*
	Junonia almana Linnaeus, 1758	Peacock Pansy	Available	*Phyla nodiflora* F-Verbenaceae	*Lantana camara* (Verbenaceae) *Wedelia calendulacea*
	Athyma perius Linnaeus, 1758	Common Sergeant	Rare	*Glochidion lanceolarum* F- Euphorbiaceae	*Abutilon darwini* (Malvaceae) *Hibiscus rosa sinensis* (Malvaceae) *Duranta repens* (Verbenaceae)
	Cethosia cyane Drury, 1770	Leopard Lacewing	Available	*Adenia pierrei* F-Passifloraceae	*Callicarpa macrophylla* (Verbenaceae) *Eupatorium odoratum* *Duranta repens* (Verbenaceae)

	Neptis mahendra Moore, 1872	Himalayan Sailor	Available	*Canavalia ensiformis* F-Fabaceae	*Impatiens balsamina (Balsaminaceae)* *Eupatorium odoratum* (Asteraceae) *Wedelia calendulacea* *Spilanthes calva* (Asteraceae)
	Neptis jumba Moore, 1857	Chestnut-Streaked Sailor	Available	*Bombax ceiba* F-Bombacaceae	*Eupatorium odoratum* (Asteraceae) *Clerodendrum infortunatum (Verbenaceae)* *Wedelia calendulacea* *Spilanthes calva* (Asteraceae)
	Lebadea Martha Fabricius, 1775	Knight	Available	*Ziziphus attopoensis* F-Rhamnaceae	*Spilanthes calva* (Asteraceae) *Wedelia calendulacea*
	Euthalia lepidea Butler, 1868	Grey Count	Available	*Melastoma malabathricum* F- Melastomaceae	*Clitoria tarnatea (Fabaceae)* *Lantana camara* (Verbenaceae)
	Euthalia julii	Common Earl	Available		

	Bougainville, 1837				
	Euthalia jahnu Moore, 1857	Plain Baron	Available	*Mangifera indica* F-Anacardiaceae	*Clitoria tarnatea* (Fabaceae) *Lantana camara* (Verbenaceae)
	Ergolis ariadne Johanssen, 1764	Angled Castor	Available	*Ricinus communis* F-Euphorbiaceae	*Clerodendrum infortunatum* (Verbenaceae) *Lantana camara (Verbenaceae)*
Pieridae	*Parerona hippie* Bingham, 1907	Common Wanderer	Available	*Capparis zeylanica* F-Capparaceae	*Lantana camara* (Verbenaceae) *Wedelia calendulacea*
	Appias lyncida Boisduval, 1836	Chocolate Albatross	Available	*Crataeva adansonii* F-Capparaceae	*Gomphrena globosa* (Malvaceae) *Wedelia calendulacea*
	Delias eucharis Drury, 1773	Common Jezebel	Available	*Viscum spp.* F-Viscaceae	*Callistemon citrinus* (Myrtaceae) *Wedelia calendulacea*
	Delias pasithoe Linnaeus, 1767	Redbreast Jezebel	Rare	*Dendropthoe spp.* F-Loranthaceae	*Chromolaena odorata* (Asteraceae) *Spilanthes calva* (Asteraceae)
	Delias descombesi	Red Spot	Rare	*Clerodendrum spp.*	*Chromolaena odorata*

	Boisduval, 1836	Jezebel		F-Lamiaceae	(Asteraceae) *Spilanthes calva* (Asteraceae)
	Catopsilia pomona Fabricius, 1775	Lemon Emigrant	Available	*Cassia fistula* F-Caesalpiniaceaes	*Alseodophne petiolaris* (Lauraceae) *Lantana camara* (Verbenaceae)
	Catopsilia crocale Cramer, 1775	Common Emigrant	Available	*Cassia fistula* F-Caesalpiniaceaes	*Anacardium occidentale* (Anacardiaceae)
	Catopsilia pyranthe Linnaeus, 1758	Mottled Emigrant	Available	*Cassia fistula* F-Caesalpiniaceaes	*Antidesma acidum* (Euphorbiaceae) *Lantana camara* (Verbenaceae)
	Eurema anderson	One Spot Grass Yellow	Critically Threatened	*Cassia tora* Caesalpiniaceae	*Chome rutidosperma* (Capparaceae) *Lantana camara* (Verbenaceae)
	Eurema blanda Boisduval, 1836	Tree Spotted Grass Yellow	Rare	*Pithecellobium dulce* F-Mimosaceae	*Chromolaena odorata* (Asteraceae) *Raulwolfia serpentine* *Panica indica*

	Eurema hecabe Linnaeus, 1758	Common Grass Yellow	Available	*Pithecellobium dulce* F-Mimosaceae	*Raulwolfia serpentine* *Chromolaena odorata* (Asteraceae) *Panica indica*
	Pieris canidia Linnaeus, 1768	Indian Cabbage White	Available	*Nasturtium spp.* F-Cruciferae	*Brassica napus* (Cruciferae) *Lantana camara* (Verbenaceae)
	Pieris brassicae Linnaeus, 1758	Large Cabbage White	Available	*Brassica spp.* F-Cruciferae	*Brassica juncea* (Cruciferae) *Lantana camara* (Verbenaceae)
	Leptosia nina Fabricius, 1793	Psyche	Available	*Cleome viscose* F-Cleomaceae	*Lanta camara* (Verbenaceae) *Wedelia calendulacea* *Panica indica*
Satyridae	*Elymnius hypermnistra*	Common Palmfly	Available	*Cocos nucifera*	*Ricinus communis* (Euphorbiaceae)

	Linnaeus, 1763			F-Palmae	*Panica indica*
	Ypthima hueberin Kirby, 1871	Common Four Ring	Available	*Setaria spp.* F-Poaceae	*Bryophallum calycinum* (Crassulaceae)
	Ypthima baldus Fabricius, 1775	Common Five Ring	Available	*Pogonatherum crinitum* F-Poaceae	*Arundina graminifolia* (Orchidaceae) *Panica indica*
	Melanitis leda Linnaeus, 1758	Common Evening Brown	Available	*Setaria palmifolia* F-Poaceae	*Amaranthus faciata (*Amaranthaceae)
	Melanitis phedima Cramer, 1780	Dark Evening Brown	Available	*Setaria palmifolia* F-Poaceae	*Pteris cretica*
	Mycalesis thailandica Aoki and Yamaguchi, 1983	Thai Bushbrown	Available		*Delhinium ajacis* (Ranunculaceae) *Panica indica*
	Mycalesis preseus	Common	Available		*Chrysalidocarpus lutescens*

	Fabricius, 1775	Bushbrown			(Palmae) *Panica indica*
	Mycalesis intermedia Moore, 1892	Intermediate Bushbrown	Available		*Chrysalidocarpus lutescens* (Palmae) *Panica indica*
	Lethe europa Fabricius, 1775	Bamboo Treebrown	Available	*Bambusa spp.* F-Poaceae	*Panica indica* *Wedelia calendulacea*
Lycaenidae	*Arhopala amantes*	Large Oakblue	Available	*Terminalia catappa* F-Combretaceae	*Ixora chinensis* (Rubiaceae) *Duranta repens* (Verbenaceae)
	Arhopala pseudocentaurus Doubleday, 1847	Common Oakblue	Available	*Terminalia catappa* F-Combretaceae	*Panica indica* *Glycosmis pentaphylla* (Rutaceae)
	Rapala pheretima	Copper Flash	Available	*Dimocarpus longan*	*Chrysanthemum cinerariifolium*

	Hewitson, 1863			F-Sapindaceae	(Compositae) *Cosmos bipinnatus* (Compositae)
	Rapala manea Hewitson, 1863	Slate Flash	Available	*Acacia pennata* F-Mimosaceae	*Cosmos bipinnatus* (Compositae) *Chrysanthemum cinerariifolium* (Compositae)
	Remalana jangala Horsfield, 1829	Chocolate Royal	Available	*Ixora coccinela* F-Rubiaceae	*Chromolaena odorata* (Asteraceae) *Spilanthes calva* (Asteraceae)
	Euchrysops cnejus Fabricius, 1798	Gram Blue	Available	*Butea monosoerma*	*Spilanthes calva* (Asteraceae) *Chromolaena odorata* (Asteraceae)
	Castalius rosimon Fabricius, 1775	Common Pierrot	Available	*Zizyphus mauritiana*	*Wedelia calendulacea* *Cynodon dactylon*
	Pseudozizeeria maha Kollar, 1848	Pale Grass Blue	Available	*Oxalis corniculata* F-Oxalidaceae	*Antidesma ghasembilla* (Euphorbiaceae)

	Loxura atymnus Stoll, 1780	Yamfly	Available	*Dioscorea pentaphylla* F-Dioscoreaceae	*Citrus spp.*(Rutaceae) *Wedelia calendulacea*
	Chilades lajus Stoll, 1780	Lime Blue	Available	*Citrus spp.* F-Rutaceae	*Chome gynandropsis* (Capparaceae) *Panica indica*
	Lampides boeticus *Linnaeus, 1767*	Pea Blue	Available	*Lupinus polyphyllus* F-Fabaceae	*Asclepius curassavica* (Asclepiadaceae) *Duranta repens* (Verbenaceae)
Hesperiidae	*Matapa druna* Moore, 1866	Gray-branded Redeye	Available	*Dendrocalamus giganteus* F-Poaceae	*Lantana camara* (Verbenaceae) *Panica indica*
	Hyarotis adrastus Stoll, 1780	Tree Flitter	Available	*Phoenix acualis* F-Arecaceae	*Chromolaena odorata* (Asteraceae) *Wedelia calendulacea*

	Parnara naso Fabricius, 1793	African Straight Swift	Available	*Colocasia esculenta* F-Aracaceae	*Cucurbita moschata* (Cucurbitaceae) *Cucurbita maxima* (Cucurbitaceae)
	Parnara apostata Snellen, 1880	Dark Straight Swift	Rare	*Oryza sativa* F-Poaceae	*Cucurbita maxima* (Cucurbitaceae) *Cucurbita moschata* (Cucurbitaceae)
	Parnara ganga Evan, 1937	Continental Swift	Available		*Cosmos bipinnatus* (Compositae) *Wedelia calendulacea*
	Spialia galba Fabricius, 1793	Indian Skipper	Available	*Sida rhombifolia* F-Malvaceae	*Vernonia cinerea* (Asteraceae) *Spilanthes calva* (Asteraceae)
	Iambrix salsala	Chestnut Bob	Available	*Bambusa spp.*	*Cosmos bipinnatus* (Asteraceae)

	Moore, 1866			F-Poaceae	*Wedelia calendulacea*
	Saustus gremius Fabricius, 1798	Indian Palm Bob	Available	*Cocos nucifera* F-Arecaceae	*Helianthus annus* (Asteraceae) *Cosmos bipinnatus* (Asteraceae) *Spilanthes calva* (Asteraceae)
	Oriens gola Moore, 1877	Common Dartlet	Available	*Paspalum conjugatum* F-Poaceae	*Wedelia calendulacea* *Cynodon dactylon* *Spilanthes calva* (Asteraceae)
	Udaspes folus Cramer, 1775	Grass Demon	Available	*Zingiber officinale* F-Zingiberaceae	*Benincasa hispida* (Cucurbitaceae) *Cucurbita maxima* (Cucurbitaceae)
	Notocrypta curvifascia Felder, 1862	Restricted Demon	Available	*Costus speciosus* F-Zingiberaceae	*Cucurbita maxima* (Cucurbitaceae)

	Borbo cinnara Wallace, 1866	Rice Swift	Rare	*Setaria pumila* F-Poaceae	*Spilanthes calva* (Asteraceae) *Wedelia calendulacea*
	Tagiades japetus Stoll, 1781	Common Snow Flat	Available	*Dioscorea oppositifolia* F-Dioscoreaceae	*Celosia cristata* (Amatanthaceae) *Wedelia calendulacea* *Spilanthes calva* (Asteraceae)
	Tagiades litigiosus Moschler, 1878	Water Snow Flat	Available	*Dioscorea hispida* F-Dioscoreaceae	*Celosia cristata* (Amatanthaceae) *Wedelia calendulacea* *Spilanthes calva* (Asteraceae)
	Pelopidas agna Moore, 1866	Bengal Swift	Available	*Paspalum conjugatum* F-Poaceae	*Hibiscus rosa sinensis* (Malvaceae) *Spilanthes calva* (Asteraceae)

	Pelopidas mathias Fabricius, 1798	Small Branded Swift	Rare	*Saccharum officinarum* F-Poaceae	*Hibiscus rosa sinensis* (Malvaceae) *Spilanthes calva* (Asteraceae)
Acraeidae	*Acraea violae* Fabricius, 1775	Tawny Coster	Available	*Passiflora edulis* Passifloraceae	
Riodinidae	*Zemeros flegyas* Cramer, 1780	Punchinello	Available	*Maesa indica* F-Myrsinaceae	
	Abisara echerius Stoll, 1790	Plum Judy	Available	*Embelia robusta* F-Myrsinaceae	
Amathusiidae	*Discophora sondaica* Boisduval, 1836	Common Duffer	Rare	*Saccharum spp.* F-Poaceae	

Appendix 7: Developmental stages of the plain tiger butterfly, *Danaus chrysippus* in the EBBL (2011-2012).

No. of eggs	Egg-1	Egg-2	Egg-3	Egg-4	Egg-5
Incubation period (days)	4	4	6	4	5
Larval duration in days					
Larval stages	Instar-1	Instar-2	Instar-3	Instar-4	Instar-5
1st instar	1.5	1.5	2	1.5	2
2nd instar	2	2	2.5	2	2.5
3rd instar	2.5	2	2.5	2.5	3.0
4th instar	2.5	2.5	3	2.5	3
5th instar	3	3	2.5	3.5	3.5

Pre-pupal duration in days					
No. of pre-pupa	Pre-pupa-1	Pre-pupa-2	Pre-pupa-3	Pre-pupa-4	Pre-pupa-5
Pre-pupal period	1.5	1.5	1	1	2
Pupal duration in days					
No. of Pupa	Pupa-1	Pupa-1	Pupa-1	Pupa-1	Pupa-1
Pupal period	8	9	7	9	10
Larval length (in mm)					
Larval stages	Instar-1	Instar-2	Instar-3	Instar-4	Instar-5
1st instar	3	3.5	3	5	4
2nd instar	7	8.5	10	9	9

3rd instar	16	14	13.5	15	13
4th instar	20	21.5	24	25.5	25
5th instar	35	39.5	38	42	38
Infestation of leaves in percentage					
Larval stages	Instar-1	Instar-2	Instar-3	Instar-4	Instar-5
1st instar	4	5	5.5	6	7
2nd instar	12	16	15	16	20
3rd instar	28	31.5	28	31	35
4th instar	42	48	48	50	46
5th instar	66	74	58	66	92

Larval faeces in gram					
Larval stages	Instar-1	Instar-2	Instar-3	Instar-4	Instar-5
1st instar	0.03	0.07	0.05	0.07	0.1
2nd instar	0.11	0.08	0.13	0.15	0.18
3rd instar	0.25	0.28	0.25	0.24	0.18
4th instar	0.36	0.38	0.32	0.35	0.42
5th instar	0.44	0.48	0.35	0.44	0.42

Appendix 8. Survival rate of matured *A. indica* in Zoological Garden of Dhaka University and Butterfly Research Park, Bhawal National Park, Gazipur.

Hedge No.	Planted no. of seedlings	Support with shade	Support with shade (%)	Support without shade	Support without shade (%)
1	10	6	60	3	30
2	10	8	80	5	50
3	10	7	70	4	40
4	10	6	60	3	30
5	10	7	70	4	40
6	10	8	80	5	50
7	10	5	50	2	20
8	10	7	70	3	30
9	10	7	70	2	20
10	10	6	60	1	10

Appendix 9. Coincidences between the developmental stages of *P. aristolochiae* butterfly and the host plant *Aristolochia indica* in Zoological Garden of Dhaka University.

Date	T(ºC)	RH (%)	*P. aristolochiae* butterfly				
			Egg	Larva	Pre-pupa	Pupa	Adult
02/01/12	22.9	82	-	-	-	6	3
03/01/12	22.4	81	-	-	-	6	-
05/01/12	-	-	-	2	-	6	2
08/01/12	24.1	76	-	1	-	6	1
09/01/12	20.6	87	-	2	-	6	-
15/01/12	19.8	64	-	2	-	6	2
16/01/12	20.3	70	-	-	-	6	2
19/01/12	21	80	-	1	-	6	-
21/01/12	24.8	80	-	1	-	6	1
24/01/12	20.5	68	-	1	-	6	-
29/01/12	-	-	-	-	-	6	1

31/01/12	21.8	58	-	-	-	6	-
14/02/12	23.7	45	-	-	-	6	-
15/02/12	24.8	50	-	-	-	6	3
24/01/12	20.5	68	-	-	-	-	-
26/02/12	26.7	51	-	-	-	6	-
27/02/12	28.3	33	-	-	-	6	-
29/02/12	-	-	-	-	-	6	-
18/03/12	27.4	63	17	-	-	6	4
22/03/12	28.1	65	4	11	-	6	3
25/03/12	28.5	70	-	13	-	6	3
27/03/12	27.1	63	-	15	-	6	2
30/03/12	29.1	65	-	15	-	-	1
02/04/12	28.5	70	-	13	-	-	3
05/04/12	30.1	75	-	14	-	-	5
07/04/12	28.2	67	-	6	9	-	-
13/04/12	27.3	71	-	-	-	7	2

17/04/12	28.5	75	-	-	-	2	5
21/04/12	28.1	65	-	-	-	9	7
28/04/12	29.2	63	-	-	-	-	7
05/05/12	30.3	59	-	-	-	-	5
12/05/12	31.5	75	-	-	-	-	5
19/05/12	33.4	67	-	-	-	-	3
26/05/12	32.1	69	21	-	-	-	7
30/05/12	33.3	66	4	13	-	-	4
02/06/12	31.3	74	-	17	-	-	5
04/06/12	32.6	68	-	15	-	-	3
06/06/12	30.3	75	-	13	-	-	5
09/06/12	30.1	68	-	15	-	-	3
12/06/18	31.5	79	-	13	-	-	5
14/06/12	31	76	-	14	-	-	5
16/06/12	29.7	87	-	5	9	-	3
24/06/12	28.5	76	-	-	3	13	2

30/06/12	30.3	85	-	-	-	4	8
08/07/12	32	86	-	-	-	-	11
15/07/12	30.1	83	-	-	-	-	7
21/07/12	31.5	89	26	-	-	-	9
24/07/12	28.7	78	5	15	-	-	7
28/07/12	29.2	75	-	19	-	-	5
31/07/12	30.5	70	-	17	-	-	5
04/08/12	30.3	87	-	15	-	-	3
07/08/18	28.2	76	-	17	-	-	3
09/08/12	30.4	71	-	-	5	11	5
14/08/12	35.6	66	-	-	-	11	8
30/08/12	38.8	51	-	-	-	-	7
08/09/12	36.6	61	-	-	-	-	13
18/09/12	34.3	66	-	-	-	-	9
27/09/12	27.5	56	14	-	-	-	6
01/10/12	29.2	61	3	8	-	-	3

03/10/12	32.3	61	-	11	-	-	4
05/10/12	30.1	65	-	9	-	-	4
08/10/12	30.5	67	-	9	-	-	2
11/10/12	31.5	70	-	10	-	-	3
14/10/12	32.2	68	-	11	-	-	1
17/10/12	31.1	67	-	3	8	-	2
21/10/12	29.7	69	-	-	2	7	1
03/11/12	32.3	61	-	-	-	7	3
10/11/12	27.5	71	-	-	-	7	2
17/12/12	28.1	67	-	-	-	7	1
24/11/12	28.5	73	-	-	-	7	3
29/11/12	33.3	59	-	-	-	7	1
08/12/12	28.1	61	-	-	-	7	2
18/12/12	19.5	68	-	-	-	7	-
29/12/12	17.6	71	-	-	-	7	-

Appendix 10. Synchronization between larval development of *P. aristolochiae* butterfly and the phenological stages of the host plant, *Aristolochia indica* in the Zoological Garden of Dhaka University.

Date	T(ºC)	RH (%)	Phenological stages of *A. indica*				
			Yl	Ml	St	Fl	Fr
02/01/12	22.9	82	-	-	-	-	-
03/01/12	22.4	81	-	-	-	-	-
05/01/12	-	-	-	-	-	-	-
08/01/12	24.1	76	3	-	-	-	-
09/01/12	20.6	87	2	-	-	-	-
15/01/12	19.8	64	-	-	-	-	-
16/01/12	20.3	70	-	-	-	-	-
19/01/12	21	80	-	-	-	-	-
21/01/12	24.8	80	-	-	-	-	-
24/01/12	20.5	68	-	-	-	-	-
29/01/12	-	-	-	-	-	-	-

31/01/12	21.8	58	-	-	-	-	-
14/02/12	23.7	45	-	-	-	-	-
15/02/12	24.8	50	-	-	-	-	-
26/02/12	26.7	51	-	-	-	-	-
27/02/12	28.3	33	-	-	-	-	-
29/02/12	-	-	-	-	-	-	-
18/03/12	27.4	63	2	-	-	-	-
22/03/12	28.1	63	L1=10	L1=3	L1=2	-	-
25/03/12	28.5	65	L2=7	L2=5	L2=3	-	-
27/03/12	27.1	70	L3=3	L3=6	L3=4	L3=2	-
30/03/12	29.1	75	L3=2	L3=7	L3=3	L3=2	L3=1
02/04/12	28.5	71	L4=1	L4=5	L4=4	L4=3	L4=2
05/04/12	30.1		L4=0	L4=7	L4=3	L4=2	L4=3
07/04/12	28.2	67	L4=0	L4=3	L4=2	L4=1	L4=0
13/04/12	27.3	71	-	-	-	-	-
17/04/12	28.5	75	-	-	-	-	-

21/04/12	28.1	65	-	-	-	-	-
28/04/12	29.2	63	-	-	-	-	-
05/05/12	30.3	59	-	-	-	-	-
12/05/12	31.5	75	-	-	-	-	-
19/05/12	33.4	67	-	-	-	-	-
26/05/12	32.1	69	-	-	-	-	-
30/05/12	33.3	66	L1= 8	L1=3	L1=2	-	-
02/06/12	31.3	74	L2=7	L2=5	L2=3		
04/06/12	32.6	68	L3=3	L3=6	L3=4	L3=2	
06/06/12	30.3	75	L3=1	L3=8	L3=3	L3=2	L3=1
09/06/12	30.1	68	L4=1	L4=7	L4=4	L4=1	L4=2
12/06/18	31.5	79	L4=0	L4=6	L4=5	L4=2	L4=2
14/06/12	31	76	L4=0	L4=3	L4=2	L4=0	L4=0
16/06/12	29.7	87	-	-	-	-	-
24/06/12	28.5	76	-	-	-	-	-
30/06/12	30.3	85	-	-	-	-	-

08/07/12	32	86	-	-	-	-	-
15/07/12	30.1	83	-	-	-	-	-
21/07/12	31.5	89	-	-	-	-	-
24/07/12	28.7	75	L1=7	L1=5	L1=3	-	-
28/07/12	29.2	87	L2=9	L2=6	L2=3	-	-
31/07/12	30.5	83	L3=3	L3=9	L3=4	L3=1	L3=1
04/08/12	30.3	85	L3=2	L3=7	L3=5	L3=2	L3=1
07/08/18	28.2	88	L4=1	L4=9	L4=3	L4=3	L4=2
09/08/12	30.4	77	L4=0	L4=2	L4=0	L4=1	L4=4
14/08/12	35.6		-	-	-	-	-
17/08/12	35.6	66	-	-	-	-	-
30/08/12	38.8	51	-	-	-	-	-
08/09/12	36.6	61	-	-	-	-	-
18/09/12	34.3	66	-	-	-	-	-
27/09/12	27.5	56	-	-	-	-	-
01/10/12	29.2	61	L1=5	L1=3	L1=1	-	-

03/10/12	32.3	61	L2=4	L2=3	L2=1	L2=1	-
05/10/12	30.1	65	L2=5	L2=2	L2=2	L2=0	-
08/10/12	30.5	67	L3=2	L3=4	L3=2	L3=1	-
11/10/12	31.5	70	L4=0	L4=5	L4=3	L4=0	L4=1
14/10/12	32.2	68	L4=0	L4=4	L4=2	L4=1	L4=2
17/10/12	31.1	67	L4=0	L4=2	L4-0	L4=0	L4=1
21/10/12	29.7	69	-	-	-	-	-
03/11/12	32.3	61	-	-	-	-	-
10/11/12	27.5	71	-	-	-	-	-
17/12/12	28.1	67	-	-	-	-	-
24/11/12	28.5	73	-	-	-	-	-
29/11/12	33.3	59	-	-	-	-	-
08/12/12	28.1	61	-	-	-	-	-
18/12/12	19.5	68	-	-	-	-	-
29/12/12	17.6	71	-	-	-	-	-

T=Temperature, RH=Relative Humidity, Yl=Young leaf, Ml=Mature leaf, St=Stem, Fl=Flower, Fr=Fruit ** Ten hedges were undertaken to examine the synchronization of coincidences between developmental stages of *P. aristolochiae* and phenelogical stages of *A. indica.* Each hedge consists of ten *A. indica* plants. It is to be noted that data of hedge-1 is summarized in Appendix 9 and Appendix 10

Appendix 11. IC_{50} value of tert-butyl-1-hydroxytoluene (BHT) for evaluation of antioxidant activity.

Conc. (µgm/ml)	Absorbance of the extract	% Inhibition	IC_{50}
500	0.018	94.44	27.5
250	0.068	79.01	
125	0.097	70.06	
62.5	0.135	58.33	
31.25	0.159	50.93	
15.625	0.175	45.99	
7.813	0.206	36.42	
3.906	0.225	30.56	
1.953	0.238	26.54	
0.977	0.287	11.42	

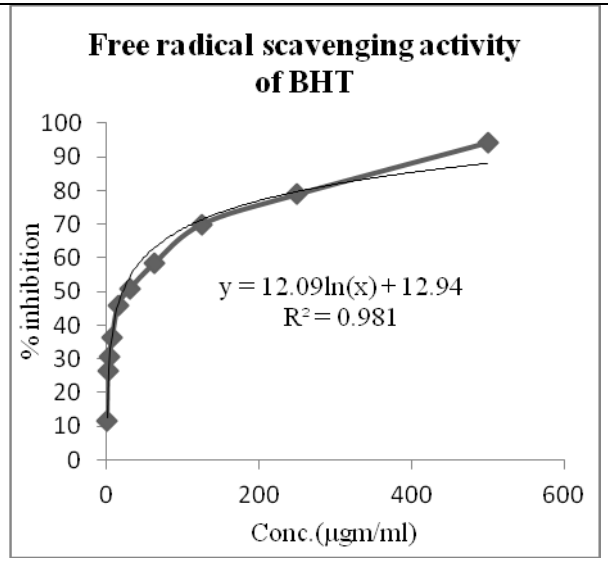

Fig. 1. IC_{50} value of tert-butyl-1-hydroxytolune(BHT)

Absorbance of the blank: 0.325

Appendix 12. IC_{50} value of Ascorbic acid (ASA) for evaluation of antioxidant activity.

Conc. (µgm/ml)	Absorbance of the extract	% Inhibition	IC_{50}
500	0.005	98.46	5.8
250	0.006	98.15	
125	0.015	95.37	
62.5	0.024	92.59	
31.25	0.068	79.01	
15.625	0.098	69.75	
7.813	0.139	57.1	
3.906	0.186	42.59	
1.953	0.175	45.99	
0.977	0.193	40.43	

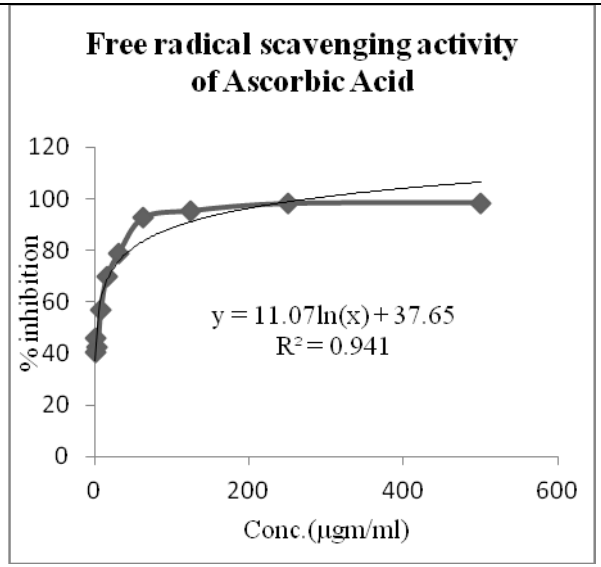

Fig. 2. IC_{50} value of Ascorbic acid

Absorbance of the blank: 0.325

Appendix 13. IC_{50} value of methanolic extract (ME) of leaves of *A. tagala* for evaluation of antioxidant activity.

Conc. (µgm/ml)	Absorbance of the extract	% Inhibition	IC_{50}
500	0.011	96.62	4.67
250	0.021	93.54	
125	0.03	90.77	
62.5	0.067	79.38	
31.25	0.078	76.00	
15.625	0.112	65.54	
7.813	0.136	58.15	
3.906	0.172	47.08	
1.953	0.189	41.85	
0.977	0.24	26.15	

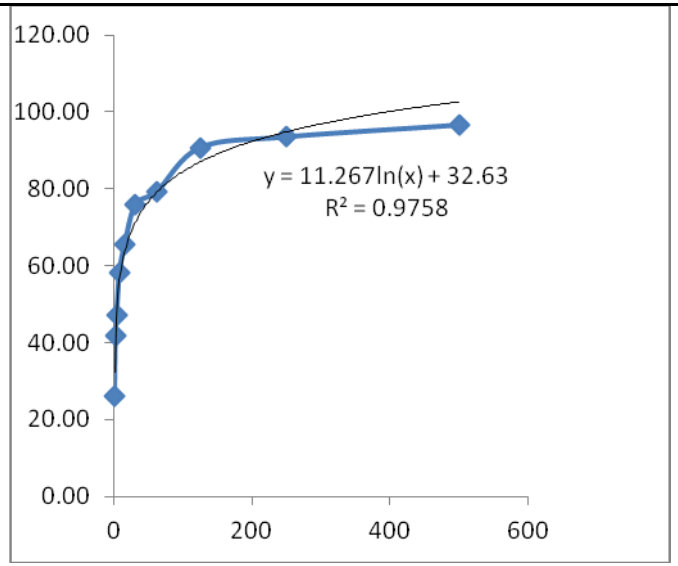

Fig. 3. IC_{50} value of methanolic extracts of *A. tagala*

Absorbance of the blank: 0.325

Appendix 14. IC_{50} value of petroleum ether soluble partitionate (PE) of leaves of *A. tagala* for evaluation of antioxidant activity.

Conc. (µgm/ml)	Absorbance of the extract	% Inhibition	IC_{50}
500	0.023	92.92	4.09
250	0.027	91.69	
125	0.039	88.00	
62.5	0.061	81.23	
31.25	0.074	77.23	
15.625	0.13	60.00	
7.813	0.145	55.38	
3.906	0.166	48.92	
1.953	0.192	40.92	
0.977	0.21	35.38	

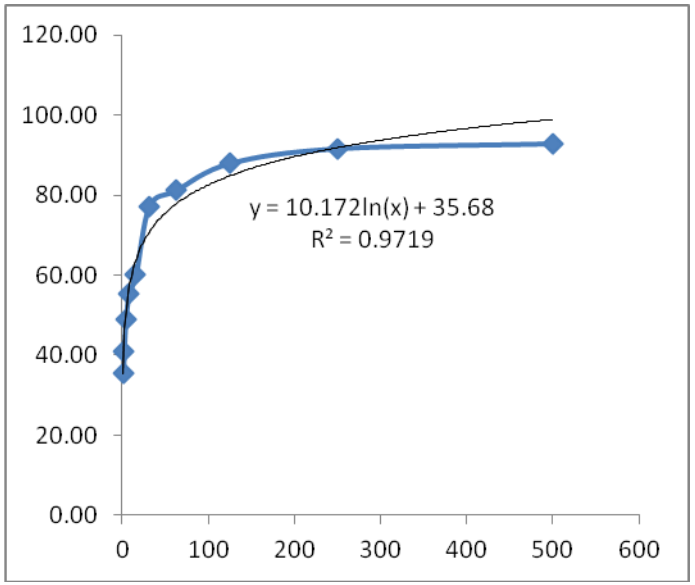

Fig. 4. IC_{50} value of petrolium ether partitionate of *A. tagala*

Absorbance of the blank: 0.325

Appendix 15. IC_{50} value of carbon tetrachloride soluble partitionate (CTE) of leaves of *A. tagala* for evaluation of antioxidant activity.

Conc. (µgm/ml)	Absorbance of the extract	% Inhibition	IC_{50}
500	0.018	94.46	9.17
250	0.037	88.62	
125	0.073	77.54	
62.5	0.091	72.00	
31.25	0.121	62.77	
15.625	0.148	54.46	
7.813	0.157	51.69	
3.906	0.192	40.92	
1.953	0.225	30.77	
0.977	0.243	25.23	

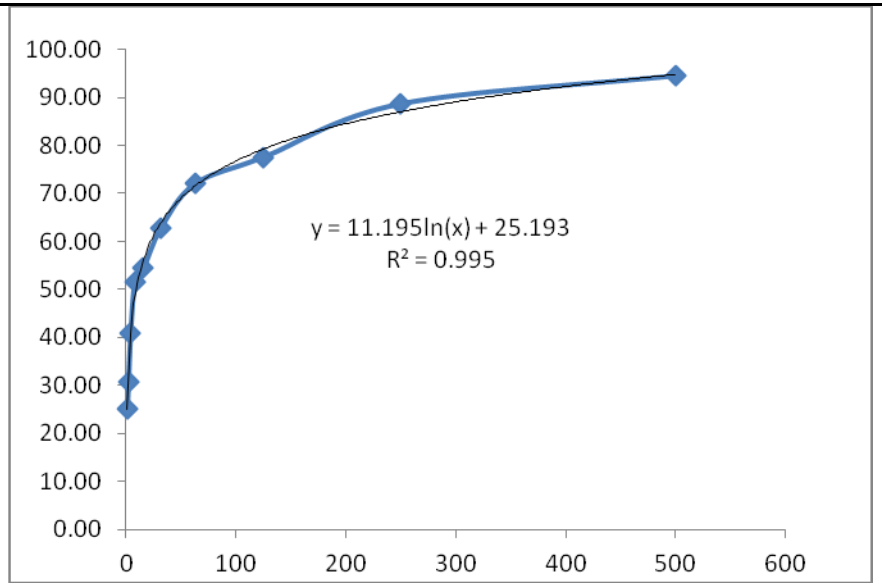

Fig. 5. IC_{50} value of carbon tetrachloride partitionate of *A. tagala*

Absorbance of the blank: 0.325

Appendix 16. IC_{50} value of chloroform soluble partitionate (CHE) of leaves of *A. tagala* for evaluation of antioxidant activity.

Conc. (µgm/ml)	Absorbance of the extract	% Inhibition	IC_{50}
500	0.032	90.15	17.45
250	0.048	85.23	
125	0.084	74.15	
62.5	0.11	66.15	
31.25	0.143	56.00	
15.625	0.172	47.08	
7.813	0.191	41.23	
3.906	0.226	30.46	
1.953	0.253	22.15	
0.977	0.274	15.69	

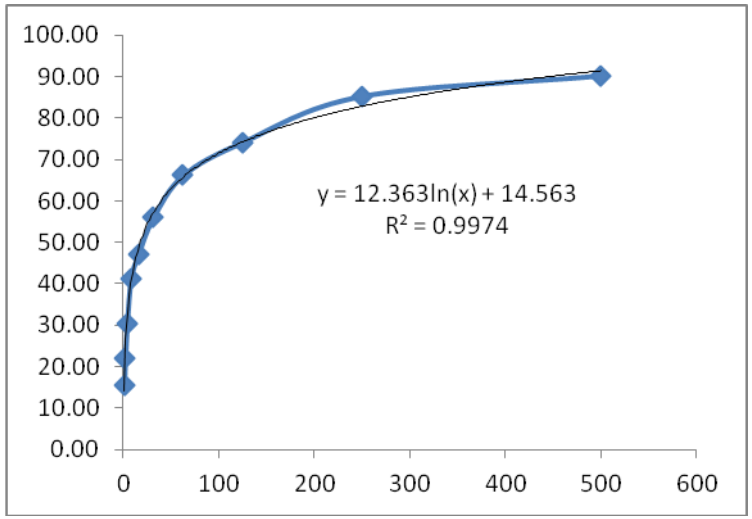

Fig. 6. IC_{50} value of chloroform partitionate of *A. tagala*

Absorbance of the blank: 0.325

Appendix 17. IC_{50} value of aqueous soluble partitionate (AQE) of leaves of *A. tagala* for evaluation of antioxidant activity.

Conc. (µgm/ml)	Absorbance of the extract	% Inhibition	IC_{50}
500	0.021	93.54	7.45
250	0.032	90.15	
125	0.052	84.00	
62.5	0.073	77.54	
31.25	0.096	70.46	
15.625	0.135	58.46	
7.813	0.152	53.23	
3.906	0.187	42.46	
1.953	0.223	31.38	
0.977	0.245	24.62	

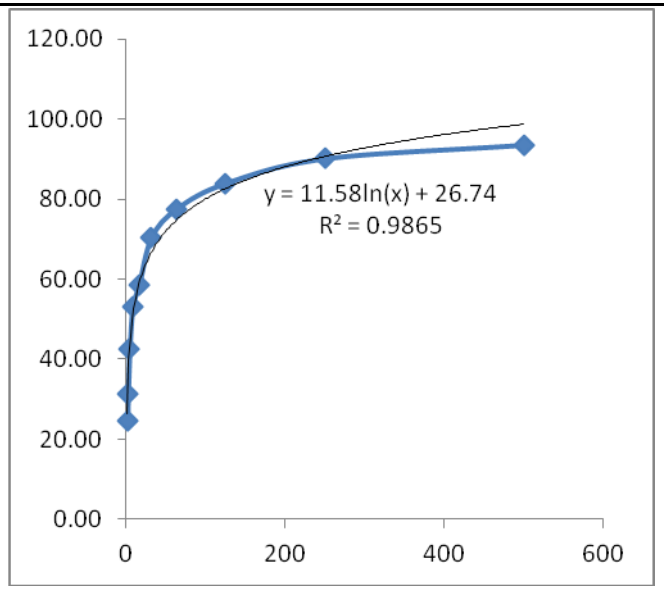

Fig. 7. IC_{50} value of aqueous partitionate of *A. tagala*

Absorbance of the blank: 0.325

Appendix 18. Effect of methanolic extract of leaves of *A. tagala* on shrimp nauplii for determination of toxicity.

Conc. (µg/mL)	Log_{10} Conc.	% Mortality	LC_{50}
0		0	
0.0390	-1.1072	20	
0.078125	0.19382	30	
0.15625	0.49485	40	
0.3125	0.79588	40	
0.625	1.09691	50	5.77
1.25	1.39794	60	
2.5	1.69897	70	
5	2	80	
10	2.30103	100	
20	2.60206	100	

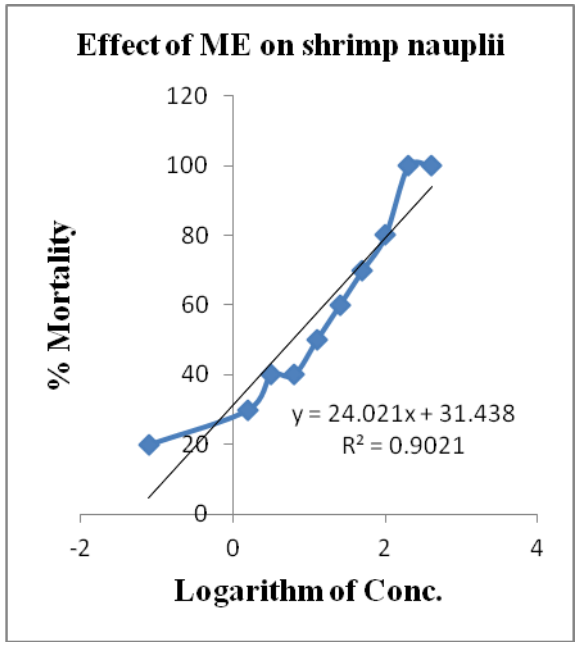

Fig. 8. Plot of % mortality and predicted regression line of ME

Appendix 19. Effect of carbon tetrachloride of leaves of *A. tagala* on shrimp nauplii for determination of toxicity.

Conc. (µg/mL)	Log_{10} Conc.	% Mortality	LC_{50}
0	-	0	1.52
0.0390	-1.1072	30	
0.078125	0.19382	40	
0.15625	0.49485	50	
0.3125	0.79588	60	
0.625	1.09691	70	
1.25	1.39794	80	
2.5	1.69897	90	
5	2	100	
10	2.30103	100	
20	2.60206	100	

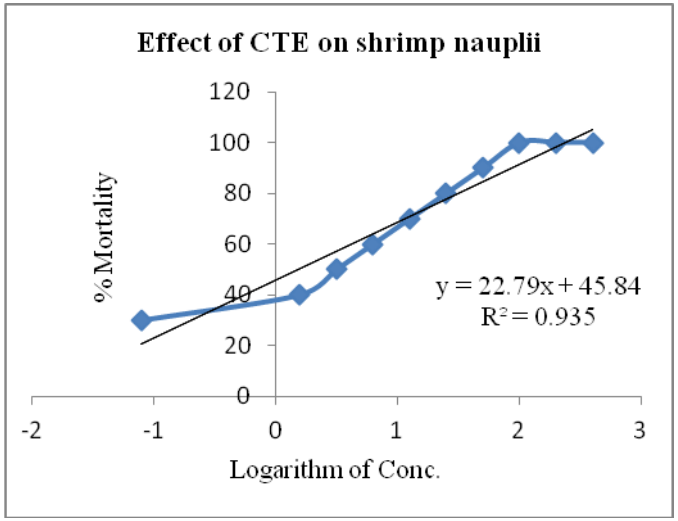

Fig. 9. Plot of % mortality and predicted regression line of CTE

Appendix 20. Effect of Pet-ether extract of leaves of *A. tagala* on shrimp nauplii for determination of toxicity.

Conc. (µg/mL)	Log_{10} Conc.	% of mortality Mortality	LC_{50}
0	-	30	
0.0390	-1.1072	50	
0.078125	0.19382	60	
0.15625	0.49485	70	
0.3125	0.79588	70	
0.625	1.09691	90	0.84
1.25	1.39794	90	
2.5	1.69897	90	
5	2	100	
10	2.30103	100	
20	2.60206	30	

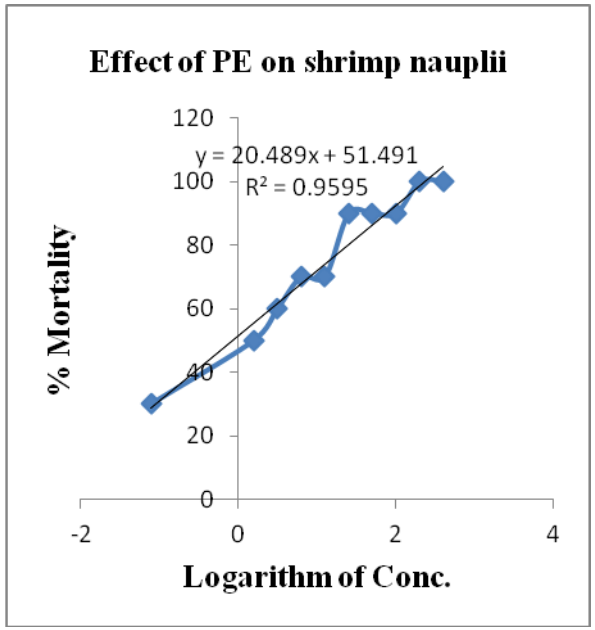

Fig. 10. Plot of % mortality and predicted regression line of PE

Appendix 21. Effect of chloroform extract of leaves of *A. tagala* on shrimp nauplii for determination of toxicity.

Conc. (µg/mL)	Log_{10} Conc.	% of mortality Mortality	LC_{50}
0	-	0	
0.0390	-1.1072	20	
0.078125	0.19382	30	
0.15625	0.49485	30	
0.3125	0.79588	40	
0.625	1.09691	50	7.61
1.25	1.39794	60	
2.5	1.69897	70	
5	2	80	
10	2.30103	100	
20	2.60206	100	

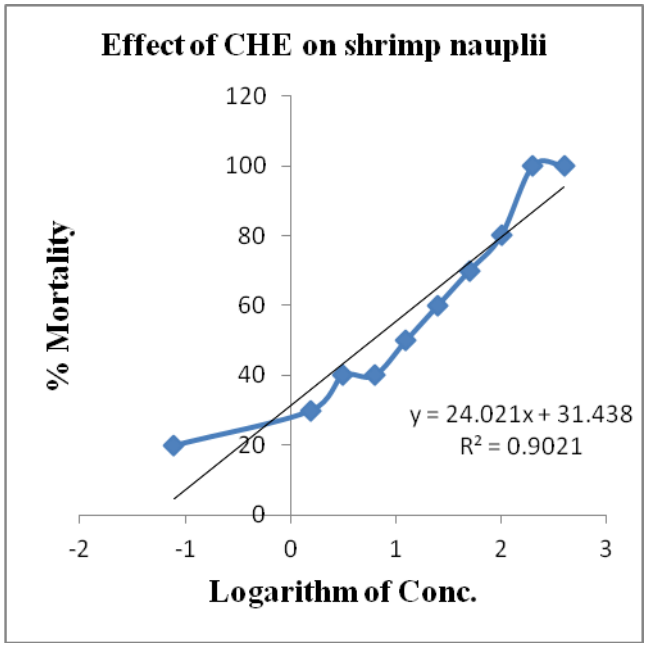

Fig. 11. Plot of % mortality and predicted regression line of CHE

Appendix 22. Effect of aqueous extract of leaves of *A. tagala* on shrimp nauplii for determination of toxicity.

Conc. (µg/mL)	Log_{10} Conc.	% of mortality Mortality	LC_{50}
0	-	0	
0.0390	-1.1072	20	
0.078125	0.19382	20	
0.15625	0.49485	30	
0.3125	0.79588	40	
0.625	1.09691	50	7.99
1.25	1.39794	60	
2.5	1.69897	70	
5	2	80	
10	2.30103	90	
20	2.60206	100	

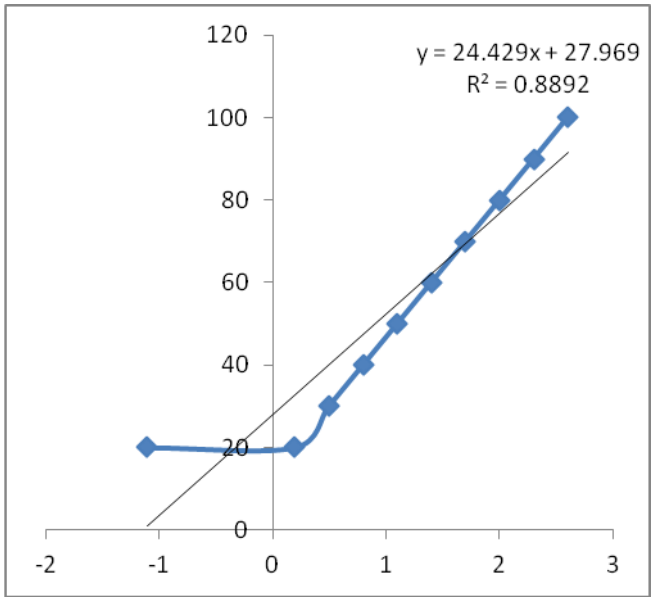

Fig. 12. Plot of % mortality and predicted regression line of AQE

Appendix 23. Biology of common rose butterfly, *Pachliopta aristolochiae* Fabricius (Lepidoptera: Papilionidae) on the host plant, *Aristolochia indica* L. (Aristolochiaceae).

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Appendix 24. Wildlife conservation through butterfly colonization.

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