

**BIOMORPHOMETRICS AND EFFECT OF NEEM OIL ON
THE FOOD CONSUMPTION OF LEMON BUTTERFLY,
Papilio demoleus L. (LEPIDOPTERA: PAPILIONIDAE)**

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**A THESIS
BY
SAROTHI KARIM
Student No. 0505006
Thesis Semester: July-December, 2006**

**Master of Science (M.S.)
in
Entomology**



**DEPARTMENT OF ENTOMOLOGY
HAJEE MOHAMMAD DANESH SCIENCE AND TECHNOLOGY UNIVERSITY
DINAJPUR**

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November 2006

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**Submitted to the Department of Entomology,
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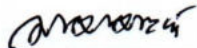
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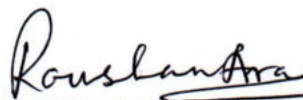
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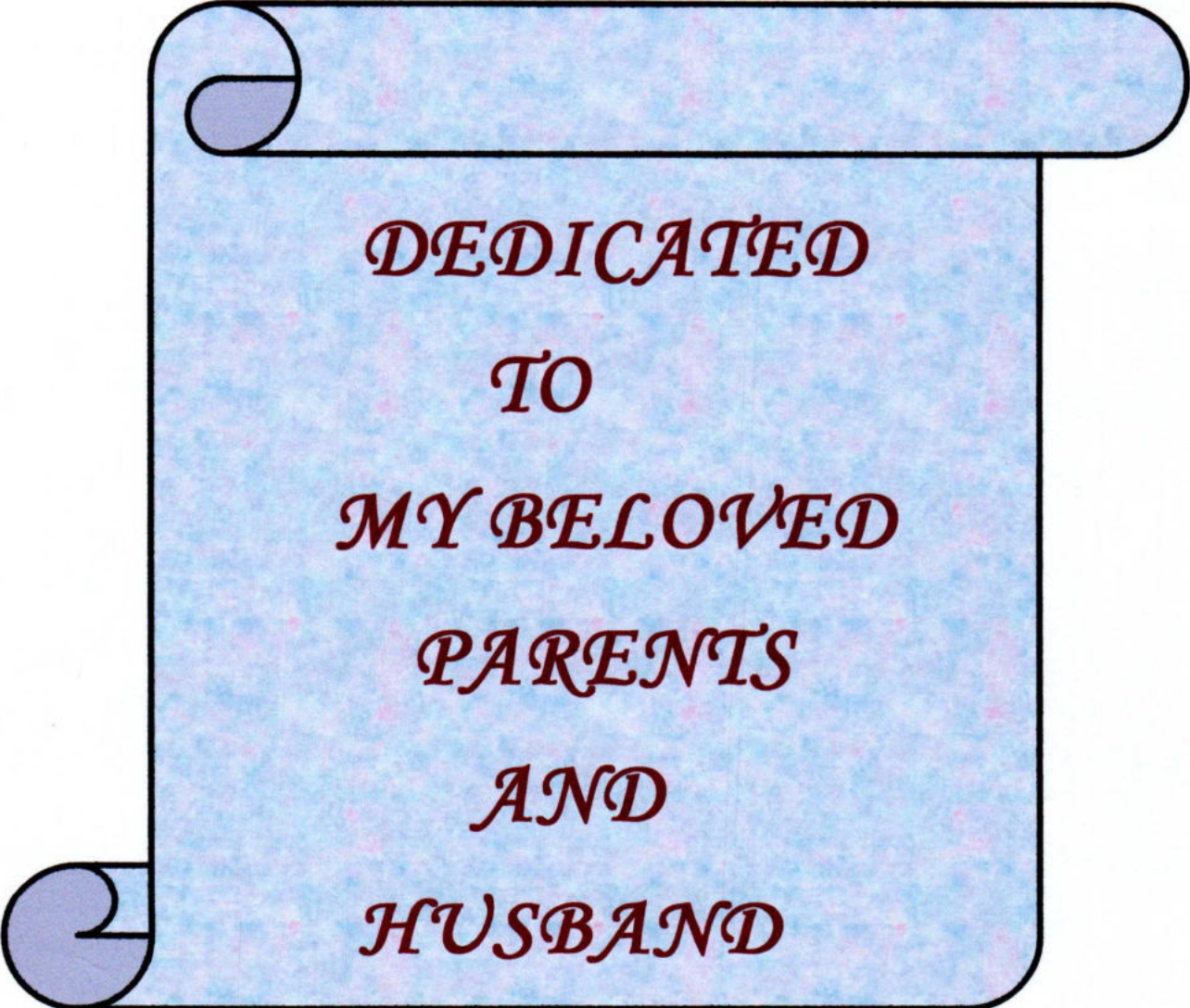
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DEPARTMENT OF ENTOMOLOGY
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DINAJPUR.

November 2006



*DEDICATED
TO
MY BELOVED
PARENTS
AND
HUSBAND*

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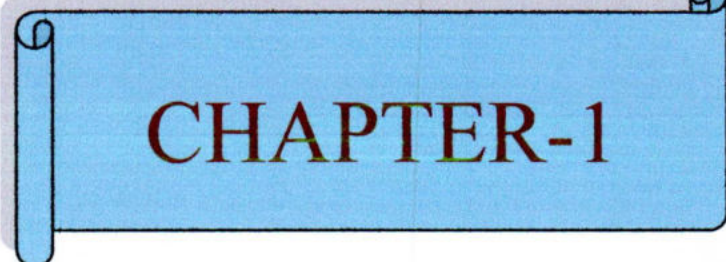
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ABSTRACT

Biology and effect of neem oil on the feeding behaviour of lemon butterfly, *Papilo demoleus* L. were studied in the Laboratory, Department of Entomology, Hajee Mohammad Danesh Science and Technology University, Dinajpur, during September to December 2005. In this study it was found that the mating generally took place 2 to 2.35 days after the emergence of an adult and it continued for 1.50 to 3.30 hours. The pre-oviposition period varied from 1 to 2 days, while the oviposition period was 2 days. The eggs were laid singly. A female laid 22.1 ± 1.41 eggs and 97.52% of the eggs were hatched. The larvae passed through five instars in 16.90 to 20.30 days. The first, the second, the third, the fourth and the final instar larvae were 2.32, 8.15, 16.73, 22.09 and 37.45 mm in lengths, respectively while breadths were 0.73, 1.49, 4.26, 6.58 and 8.82 mm, respectively. The weights of the 3rd, the 4th and the final instar larvae were 0.72, 1.65 and 3.47 g, respectively. The pupal periods ranged from 10 to 11 days. The development periods from egg to adult were 30.5 to 37 days. The females were larger than the males. The average length and breadth of the male butterflies were 24.6 and 4.82 mm (on resting position), whereas the female butterfly was 26.4 and 5.14 mm (on resting position), respectively. The lengths of the antenna of the male and the female butterflies were 16.16 mm and 16.57 mm, respectively. The length and the breadth of the thorax of the male were 10.00 and 6.34 mm, respectively, whereas in the female it was 10.81 and 6.79 mm. The average length and breadth of the forewings of the male butterflies were 39.33 and 23.3 mm and the hind wings were 26.28 and 23.04 mm, whereas the forewings of the female butterflies were 41.74 and 27.25 mm in length and breadth and the hind wings were 27.26 and 23.4 mm in length and breadth, respectively. The longevity of the male adult was only 4.2 days and in the female it was 6.3 days.

When three concentrations of neem oil viz. 0.5, 1.0 and 1.5% were used in the experiment and the 1st, the 2nd, the 3rd, the 4th and the 5th instar larvae were reared on the treated food the food consumption significantly reduced compared to the control one. The highest amount of food consumption was recorded at control (0%) and the lowest one at 1.5% by all stages of larvae, i.e. the results showed that the tested neem oil concentrations had significant effect on the feeding behaviour of the larvae of lemon butterfly.



CHAPTER-1

INTRODUCTION

INTRODUCTION

Citrus fruits are the members of three genera, viz. *Citrus*, *Fortunella* and *Poncirus* of the family Rutaceae. They are one of the leading fruit crops of the orb. The fruits are very popular among the extensively grown tropical and sub-tropical fruits and after grapes and olives they are cultivated in larger areas than other fruits (Hayes, 1957). At present the United States has the largest area- about 40% of Her total acreage. Citrus plantations in Israel cover more than 40,000 hectares and in Japan it occupies the first place among fruit crops. Other vital citrus growing areas are Algeria, Argentina, Brazil, China, Central America, Egypt, Italy, India, Japan, Mexico, New Zealand, Palestine, Paraguay, Russia, Spain, and Uruguay (Ghosh, 1981). So, these fruits are cosmopolitan. Nevertheless, in Bangladesh they are also important fruits comprising of various *Citrus* spp, e.g. lime, lemon, shaddock, mandarin, shatkora, ada jamir and rough lemon.

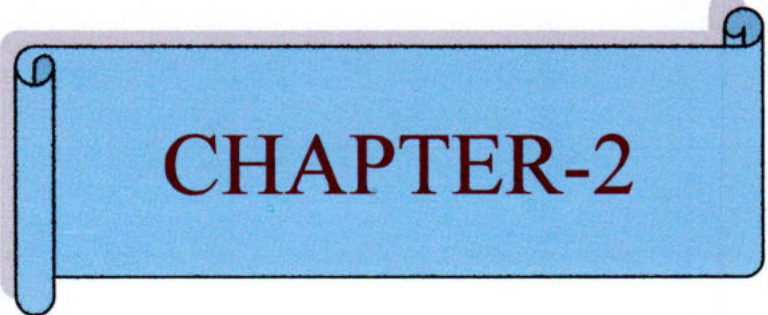
These fruits are not only delicious and refreshing, but also provide vitamins, minerals and many other essential substances required for humans. They are especially important for vitamins C and P, which keep the blood vessels normal (Singh, 1969). Their importance in diet is now well proven. *Citrus* spp. such as mandarin, sweet orange, pummelo and grapefruit are mostly consumed as fresh. Lemon and limes being acidic are not generally consumed as fresh but are commonly used for flavoring vegetable dishes, fish, meat and salads. They are also utilized in making delicious and refreshing cold drinks. In the orient

pummelos are used primarily for culinary and medicinal purposes. Citrus pulps are exploited as cattle feed. Pectin is extracted from its peel. Their flowers, leaves and rinds contain quality essence and used for flavoring different industrial products. One ton of lemon fruit yields 5-9 kgs of essential oil (Kesterson and Braddock, 1975). Moreover, these fruits are available year-round. These multifold nutritional, medicinal and other values make the citrus fruits so imperative. It is a cheap fruit (except orange) too. So, people of the cross section can easily consume this fruit to meet up their essential vitamins and minerals.

Nearly 250 insect species are reported to infest various citrus crops (Fletcher, 1921; Pruthi and Main, 1945; Wadhi and Batra, 1964; Nayar *et al*, 1976). But only a few are major causing regular and heavy damages. Eight species of these are Papilionids and reported feeding on citrus leaves of which lemon butterfly, *Papilio demoleus*, is the foremost one. This insect, originally described by Linnaeus (1758), is widely distributed from North Australia to Arabia including Iran, Pakistan, India, Sri Lanka, Bangladesh, Burma, China, Taiwan and Southeast Asia. It is also known as citrus butterfly and orange dog in Florida (Hume, 1957). Its caterpillars are the worst leaf destroyer (Bindra, 1967), especially in nurseries (Ayyar, 1963; Pruthi and Main, 1945). It is a serious pest in growing-up and matured orchards too (Ayyar, 1963). The caterpillars cause severe defoliations and young plants may become completely leafless (Butani, 1979). They prefer shiny, light yellowish green and tender leaves (Atwal, 1964). Generally they start feeding from margins inwards reaching the midrib (Rehman,

1946; Bindra, 1957, 1967 and 1969; Butani and Jotwan 1975). In severe case of infestation, plants may be completely defoliated (Butani and Jotwan, 1975). Even they feed pedicel causing fruit drops. Sontakay (1943) reported serious outbreak in Madhya Pradesh in July 1943 when caterpillars defoliated the entire orchards of Nagpur.

For the proper management of any dreadful insect it is utmost necessary to know its biology. Moreover, nowadays environment friendly botanical, e.g. neem (*Azadirachta indica* L.) oil is used as an effective remedy for various appalling insects. But biological studies about this terrible insect are nix in Bangladesh. Even, work with neem oil against this hurtful pest is also zilch. The present works were, therefore, undertaken to study the biology of lemon butterfly and its feeding behaviour using lemon leaves treated with different concentrations of neem oil, which are of paramount need for the proper management of this scandalous pest.



CHAPTER-2

REVIEW OF LITERATURE

REVIEW OF LITERATURE

The available literature pertaining to the present study are critically reviewed and harmonically presented here under different suitable headings.

2.1 Biology

Egg

Radke and Kandalkar (1988) studied the biology of this insect in Maharashtra, India and narrated that 14-22 eggs were laid by its each female. In another study Radke and Kandalkar (1989) cited that the females laid 15 and 22 eggs.

Singh and Gangwar (1989) reported that the duration of egg stage of *Papilio demoleus* on khasi mandarins were 4.7days and a female laid a maximum of 18 eggs.

Singh (1995) experienced that the female laid eggs exclusively on lemon twigs having new growth, sprouting buds and some times on branches, petioles and fruits too. Fecundity of females varied between 11-14 and 11-13 eggs in the first and the second generations, respectively. The average egg stage was 2.1days in the first generation, while 2.3 days in the second one.

Rajinder *et al.* (1997) described that the *Papilio demoleus* completed nine generations when reared on *Citrus reticulata* under laboratory and also in field

conditions. The duration of egg period ranged from 2.13-3.50 days. The females laid a maximum of 18 eggs. The average size of an egg was 1.08 mm.

Deepak *et al.* (1998) cited that the females preferred babchi (*Psoralea corylifolia*) more than lemon for egg laying purposes.

Patel *et al.* (2004) observed that *Papilio demoleus* laid eggs on the tender leaves of citrus plants.

Larva

Patel *et al.* (2004) observed that *Papilio demoleus* larvae fed on leaves of *Desmodium gangeticum* weed having medicinal values in India during August 2003. The level of infestation reached 93.33% and larval population ranged from 1 to 4 per plant.

Singh and Gangwar (1989) reported that the duration of 1st - 5th larval instars of *Papilio demoleus* on khasi mandarins averaged 5.3, 5.1, 4.2, 4.2 and 3.5 days, respectively. From a comparative development and survival study of papilionid on 10 different cultivated forms of citrus, the percentage of successful development as recorded were 90, 80, 70, 80, 40, 70 50, 80 and 50 on khasi mandarin, mandain king theipi, shuntala, calmondin, citron, rough lemon, sweet lime, khasi papeda, citrange quat and pink fleshed, respectively.

Radke and Kandalkar (1989) pointed out that the total larval period of this insect was 18 days.

Radke and kandalkar (1988) studied the biology of *Papilio demoleus* in India in a laboratory in August-September and observed that the larval period lasted 15-22 days.

Rajinder *et al.* (1997) observed that the larval period ranged from 9.31-37 days. The larva passed through 5 instars lasting for 1.57- 3.27, 1.52- 3.45, 1.55- 4.33, 1.92- 8.70 and 1.98-17.47 days, respectively. The average sizes of the 5 larval instars were 2.89, 5.60, 7.50, 11.60 and 26.50 mm, respectively.

Deepak *et al.* (1998) showed the biology of *Papilio demoleus* on lemon and babchi. The larval periods were longer on babchi than on lemon and larvae took longer time in completing their life cycle on babchi than lemon. Furthermore, larvae consumed more lemon leaves compared to babchi ones.

Singh (1995) observed the biology of the lemon shoot butterfly (*Chilades tajus*) in the laboratory of the Department of Entomology, University of Agriculture and Technology, Faizabad, India during 1993 and observed that larval periods were 21.4, in the first while 22.3 in the second generation.

Srivastava *et al.* (1986) recorded that the sequential degeneration of the segmental muscles seemed to be associated with the degree of complexity of the adult structure that replaced the larval ones after metamorphosis. It was speculated that the greater the degree of remodelling necessary, for instance from the biting/chewing mouthparts of the larvae to the nectar feeding mouth parts of

the adult, the longer was the time required to achieve it and the earlier its commencement in metamorphosis. Hence, the cranial segments were first degenerated, followed by the thoracic and genital segments. However, the loss of spiracular muscles was not complexity-based but seemed to be related to the position of the spiracular closing apparatus in the larvae and adults.

Tiwari *et al.* (1989) pointed out that nerve cord sectioning in the early fifth instar of *Papilio demoleus* inhibited or delayed pupation depending on the site of sectioning. That might be due to the interruption in feeding, leading to interruption in the proprioceptive input reaching the brain. Sectioning of the peripheral nerves N5 and N8 prevented shorting of the thoracic segment of the ventral nerve cord in the adult.

Sukla *et al.* (1993) investigated the role of proprioception in endocrine activity in *Papilio demoleus*. It was observed that the removal of either the metathoracic or any one of the two abdominal ganglia from early and partially fed fifth instar larvae induced swallowing of air, prolongation of larval span and inhibition of pupation. Removal of any one ganglion between the abdominal segments 1-5 induced distension of the larval body and delayed pupation or yielded larval-pupal intermediates. A great deal of variations in food consumptions following ganglionectomy was also recorded.

Tiwari *et al.* (2002) observed that ventral nerve cord in fifth instar larva comprised of 11 ganglia (a suboesophageal, three thoracic and seven abdominal)

but in adult, it is composed of only six ganglia (a suboesophageal, a composite thoracic ganglionic mass and the four abdominals). During metamorphosis suboesophageal ganglion fused with the tritocerebral part of brain while meso- and metathoracic ganglia along with first and second formed a thoracic ganglionic mass. Likewise, seventh abdominal ganglion fused with the sixth one. The neural lamella, which formed a thin regular lining of interganglionic connectives in fifth instar larva wrinkled, broke and disappeared in those connectives, which were shortened during metamorphosis. The larval nervous system attained the imaginal state in 72 hours old pupa and reduced about 45% in the initial length. Sectioning of peripheral nerves and cauterization of thoracic ganglia revealed that both the humeral as well as the nervous factors played their role in nerve cord condensation.

Pupa

Singh and Gangwar (1989) concluded that the duration of the pupal stage of *Papilio demoleus* on khasi mandarins averaged 19.1 days.

Radke and Kandalkar (1988) studied the biology of *Papilio demoleus* in Maharashtra and observed that the prepupal and the pupal periods lasted for 20-24 hours and 8-12 days, respectively.

After studying the biology of *Papilio demoleus* reared on *Citrus reticulata* in laboratory conditions Rajinder *et al.* (1997) narrated that the pest overwintered

as a pupa. The pupal period ranged from 8.32- 128.94 days. The green colored pupae turned pale and subsequently to brown just one day before emergence as an adult. The adult emerged by dorsally splitting the pupal case. The pupa measured 28.86mm in length while 9.04 mm in breadth.

Deepak *et al.* (1998) studied the biology of *Papilio demoleus* on lemon and babcli leaves and found that the pupal periods were longer on babcli leaves compared to the lemon leaves.

Singh (1995) observed the biology of the lemon shoot butterfly (*Chilades tajus*) in the laboratory and showed that the average pupal periods were 3.1 and 3.2 days in the first and the second generation, respectively.

Adult

Singh and Gangwar (1989) cited that the durations of the male and female adult life spans of *Papilio demoleus* on khasi mandarins averaged 5.1 and 5.8 days, respectively.

Rajinder *et al.* (1997) observed that the female lemon butterfly lived longer, i.e. 5.10-7.05 days than the male (2.95-4.35 days). Moreover, the wing expanse of an adult male was 8.39 while that of a female was 9.02 cm.

Singh (1995) observed the biology of the lemon shoot butterfly and reported that the average longevity of females was 6.1 and 7.2 days in the first and the second generations while males survived for an average of 4.1 days in both the cases.

Divender and Gupta (2000) pointed out that lemon butterfly was a serious pest of citrus in India where it was distributed countrywide. It had also been found on *Aegle marmelos* and *Zizyphus* spp. That note further cited about their occurrence on *Psoralea corylifolia*, an important medicinal herb in the herbal garden of the Regional Horticultural Research Station at Jach, Himachal Pradesh in September 1993. That was is a new record, but the infestation was quite low.

Ram-pratap *et al.* (2000) assessed the incidence of *Papilio demoleus* on lime in the Bundelkhand region of Utter Pradesh, India from July 1992 to July 1996. Finally, it was concluded that the maximum damage by that pest occurred from August to September.

Radke and Kandalkar (1988) studied the biology of *Papilio demoleus* in Maharashtra, India and observed that mating lasted 1.5-2 hours. Moreover, the pre-oviposition and oviposition periods were 1 and 2 days, respectively.

Rajinder *et al.* (1997) observed that the pre-copulation period of lemon butterfly ranged from 0.28 to 1.14 and oviposition period ranged from 2.42-4.52 days when reared on *Citrus reticulata* Blanco under laboratory conditions.

2.2 Food consumption behaviour relating to concentrations of neem oil sprayed on leaves

Direct literature on leaf consumption of lemon butterfly sprayed with neem oil was unavailable. So, cross literature on this aspect is carefully reviewed here.

Neerja and Saroj (2003) studied the efficacy of fresh neem oil in controlling mustard sawfly insect on Indian mustard under laboratory conditions. The leaves were dipped into fresh neem oil at 0.125, 0.25, 0.5, 1.0 and 2.0%. Then the larvae were allowed to feed on them. Mortality was observed after 24 hours of exposure. Pupation was the minimum at 2 while that was the maximum with the 0.125% oil. The adult emergence was also decreased with increasing concentrations.

Haque and Parvin (1996) used 0.25, 0.50, 1.0 and 2.0% of the neem oil for the feeding response and survival of the epilachna beetle (*Epilachna dodecastigma*). They observed that all the first instar larvae died at all the concentrations investigated without feeding any food when the third instar larvae and the adult beetles were reared on the treated food, the food consumption were significantly reduced. The highest larval mortality was recorded at the two higher doses.

Ahmed and Haque (1993) tested the efficacy of oils of neem, linseed, safflower, sunflower and sesame against the pulse beetle, *Callosobruchus chinensis* (L) on mungbean seeds. Four concentrations viz. 0.5, 1.0, 2.0 and 4.0% (v/v) of those oils were tested and found that neem oil at the 4.0% concentration completely

protected the pulse seeds from the attack of the pulse beetle. Moreover, only 2.00, 11.27, 2.08 and 1.96% seed-loss were recorded at the same strengths of linseed, safflower, sunflower and sesame oils, respectively.

Dreyer (1987) observed the effect of neem aqueous extracts and neem oil of 25 or 50 g pulverized neem seed kernels/ likes or with 5 or 10 litter neem oil/ha (ULV) in Togo on 3 April 1984 and examined for damage or infestation, or both, by various pests during May-July and for effects on plant reproductive phases and fruit yield compared to those in untreated plots. The aqueous extract with neem oil gave good reductions in infestations and damages, especially at the high concentrations in case of *Scrobipalpa ergasmia*, *Empoasca facialis* (larvae and adults), *Phycita melongenae*, *Selepa docilis* (larvae) and grasshoppers.

In a laboratory trial, Mishra *et al.* (1989) feed females of Coccinellid *Henosepilachna sparsa* (*Eplilachna sparsa*) brinjal leaves treated with 0.025 and 0.05% neem oil to determine its effect on ovipositional behaviour. The pre-oviposition period of the females fed on leaves treated with 0.05% oil was 21.09% longer than that of the insects fed on untreated leaves. Females fed leaves treated with both the cases had shorter oviposition periods, reduced number of egg laying days, smaller number of egg masses /female and a smaller number of eggs/ female compared to the untreated controls.

Schmutterer *et al.* (1993) observed the effect of extracts of the kernels of neem on nymphs of locusts (*Locusta migratoria*) and grasshoppers (*Schistocerca*

gregaria) in a laboratory and in field trials using *Nomadacris septemfasciata* on maize. There was a very strong feeding deterrent effect of neem oil on *Schistocerca gregaria* and *Nomadacris septemfasciata*. However, *Locusta migratoria* was repelled from the food treated with neem oil to a much lesser extent. Morphogenetic defects on antennae, legs and wings were often observed on nymphs and adults of *Schistocerca gregaria* and to a lesser extent on *Locusta migratoria*. Slight effects occurred on *Nomadacris septemfasciata*. The highest mortality rate after topical application was recorded in *Schistocerca gregaria* and after intake of neem contaminated food in *Locusta migratoria*. Finally, it was concluded that neem oil could be used for controlling some important locust and grasshopper in fields with good success.

Sontakke *et al.* (1994) pointed out that the application of neem oil and the insecticides monocrotophos, chlorpyrifos, carbaryl and ainalphos either alone or in combination reduced the number of *Sogatella furcifera* compared to the untreated plots.

Pathak *et al.* (1993) studied the changes in postembryonic development and reproduction in *Corcyra cephalonica* as a function of neem oil vapour action during rearing or on adults and cited that the larvae of the *Corcyra cephalonica* of the parental generation caused a significant reduction in the total postembryonic developmental period of males and females of the progeny, although reproduction was not markedly affected. The breeding potential of the

insects was again severely depressed when adult males or females of copulating pairs were subjected, prior to mating to the vapors of the oil for 5 minutes. Neem oil present in the rearing environment for the first 20 or 25 days led to a delay in the emergence of adults only in the latter situation.

Venkates *et al.* (1993) tested the effect of neem oil of 1.25, 0.50, 0.75 1.0, 1.25% concentrations on the growth and development of *Lipaphis erysimi* insects. At concentrations of 1.0, 1.25 and 1.5% all the nymphs reared on the treated Indian mustard leaves died before reaching the adult stage. At the lower concentration nymphal survival, fecundity and growth index of the aphid decreased and developmental period increased.

Rajendran (1992) investigated the effect of neem oil to control *Nilaparvata lugens* on rice in Tamil Nadu, India in 1987- 88. He experienced that neem oil was effective against the studied insect.

Anjaneyulu *et al.* (1999) tested the acute toxicity of neem oil against *Cirrhinus mrigala* fingerlings under laboratory conditions. Fish mortality was recorded for 4 days at 24h intervals. *Azadirachta indica* oil was highly toxic to the fish as the LC₅₀ values ranged from 0.245 to 0.109 ml/ litres for 24-96 hours.

Ban *et al.* (2000) examined the effects of the volatile ingredients in neem oil on oviposition, survival and repulsion of the azuki bean weevil *Callosobruchus chinensis*. The number of eggs laid and survival rates were assessed in airtight

jars filled with volatile ingredients of castor, sesame, and sunflower or neem oils. The volatile ingredients of neem oil as well as of the other oils had insignificant effects on oviposition behavior or the survival of the grubs. The beetles notably avoided the oil-treated jar compared to the control Jar.

Swaran *et al.* (2002) noted that neem oil microemulsion was found to be more bioactive than its macroemulsion against *Helicoverpa armigera*. It reduced the larval and pupal weight gains, pupal developments, adult emergences, increased the larval mortalities, larval-pupal intermediates and the pupal deformities in the third instar in the treated larvae. Its relative effectiveness in inhibiting adult emergence compared to the macroemulsion (1.0) was 1.68. The effect was generally dose dependent in both the emulsions.

Guddewar and Chandra (1993) observed that the neem oil and leaf extract of *Tabernaemontana coronaria* reduced aphid populations compared to the control treatment but they were not as effective as their combinations with endosulfan or monocrotophos alone. Combinations of the plant extracts with endosulfan reduced aphid populations by 96.83-100%.

CHAPTER-3

MATERIALS AND METHODS

MATERIALS AND METHODS

Two separate experiments were conducted with the scandalous pest lemon butterfly. The first one was on the biology while the second one was on the feeding behaviour of this insect on lemon leaves treated with 0% (control), 0.5, 1.0 and 1.5% of neem (*Azadirachta indica* L.) oil. Both the experiments were conducted at the Laboratory, Department of Entomology, Hajee Mohammad Danesh Science and Technology University, Dinajpur during September to December 2005 with 10 replications although.

3.1 Experimental Insect

Lemon butterfly *Papilio demoleus* L. was the investigational insect in this study.

3.2 Some Important Aspects of the Experimental Insect:

3.2.1 Systematic position

Phylum: Arthropoda

Class: Insecta

Sub-class: Pterygota

Division: Endopterygota

Order: Lepidoptera

Family: Papilionidae

Genus: *Papilio*

Species: *Papilio demoleus*

3.2.2 Distribution

Lemon butterfly, originally described by Linnaeus (1758), is found in Africa and the greater parts of Asia, as far as Formosa (Atwal, 1964) and Japan. It is widely distributed from North Australia to Arabia including Iran, Pakistan, India, Sri Lanka, Bangladesh, Burma, China, Indonesia, the Philippines and South East Asia.

3.2.3 Morphology

Eggs are smooth, spherical, pale yellow when fresh, turning to brown after a few days. The young caterpillar is brown to black with white markings, and resembles like bird droppings (Gandhi, 1959; Pruthi, 1969; Atwal, 1964 and 1976). When full grown it changes to yellowish green and possesses a horn like structure on the dorsal side of the last abdominal segment (Gandhi, 1959; Atwal, 1976) with a hump like appearance in the front. The adult is a large beautiful butterfly. The entire body is covered with small black and yellow hairs. Its head and thorax are black with creamy yellow streaks on both the sides while abdomen and legs are black. Antennae are black and club shaped. The fore and hind wings are dull black and spotted with yellow markings (Atwal, 1964 and 1976; Pruthi, 1969; Chitriv, 1970; Butani, 1973). The forewings are triangular while the hind wings are round.

3.2.4 Host range

Beside the cultivated *Citrus* spp., the caterpillars can feed and breed on all wild *Citrus* spp. The pest is also recorded on other species belonging to the family

Rutaceae (Fletcher, 1914; Bindra, 1957; Jandu, 1942; Atwal, 1964 and 1976). Thakare and Borle (1969) from Maharashtra reported that caterpillar also feeds on Bael (*Aegle marmelos*), Kadhu limb (*Murrya koenigi*), Bawachi (*Psoralea corylifolia*) and Bhira (*Chloroxylon sweitenia*).

3.3 Mass culture:

To have sufficient number of the adult butterflies for the study, firstly, different available stages of the larvae of this insect were collected from lemon plants of the campus, Hajee Mohammad Danesh Science and Technology University, Dinajpur. Then they were placed singly in 15 petri dishes (11 cm diameter) and young lemon leaves (var. Elachi lebu) were supplied as food. A small amount of wet cotton was used at the petiole to keep the leaves fresh. The leaves were replaced with new leaves at an interval of 24 hours. On the other hand, 10 young lemon gootees (two years old) of the variety "Elachi lebu" were collected from a local nursery. They were potted in 10 earthen tubs (30x20x15 cm) for used as the ten replications. Well rotten cow dung and loamy soil, at the rations of 1:1, were used as the pot mixture. Each tub was placed on an earthen plate to hold excess water. Each tub with the plant was properly veiled with a mosquito net (height 2, length 1.5 and breadth 1.5 M). When the larvae reached at the final instar, they were transferred on to those netted lemon plants for pupation. After adult emergence they were sexed in pairs on lemon plants for mating and laying eggs. Later on the eggs were allowed to hatch on the potted plants and adequate numbers of first instar larvae were obtained for the experimental purpose.

3.4 Biological Studies:

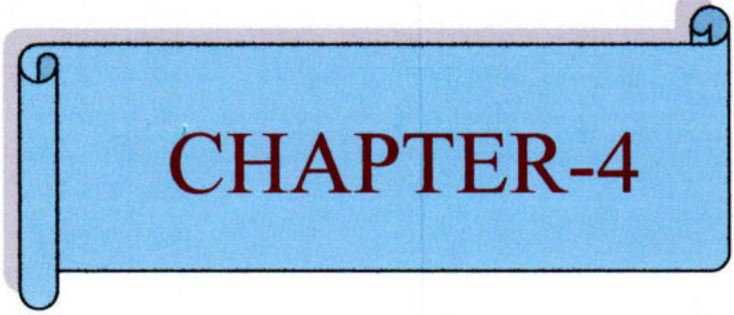
From the laboratory culture, the newly hatched first instar larvae were placed singly in to 10 petri dishes on the weighted lemon leaves. Those 10 petri dishes were used as the 10 replications. The leaves were supplied as food for the larvae and changed after each 24hours. Wet cotton was used to keep them fresh as was used earlier. Data on the body weights, the lengths and the breadths of the caterpillars were taken. Measurement of the antennae, the thoraxes, the wings and the abdomens of the adults were also recorded. The durations, the sizes, and the number of moultings of each larval instar were noted too. The larval instars were determined on the basis of exuviae. As they reached the final instar, they were placed on twigs of lemon plants. Finally, those were set in 10 glass jars (14cm high, 8cm dia.) to assist in pupation. The base of the twig was set in a narrow mouthed bottle filled with water to keep that fresh. The size of the pupa was measured and the days required for the completion of the pupal stages were also recorded. When the adults emerged from the pupa then a male and a female butterfly were released in pair in each netted lemon plant used earlier and they were physically observed constantly to watch the pre-mating, mating periods. After that pre-oviposition, oviposition periods and fecundity of eggs were recorded. Incubation period was also noted.

3.5 Feeding behaviour on lemon leaves treated with neem oil:

For this purpose, 0.5, 1.0 and 1.5% of neem oil were used. At first, 10 lemon leaves were weighted and then sprayed with the oils using a hand sprayer as used by Haque and Parvin (1996) at the Bangladesh Agricultural University, Mymensingh. In control treatment leaves were treated with distilled water. Four hand sprayers were used for each treatment to avoid any admixture of the oils used. Then the leaves from the plants were placed in those 10 petri dishes. Later on, from the laboratory culture, the newly hatched larvae were individually placed in each petri dish on the leaves sprayed with the oil and control. A small amount of wet cotton was used at the bottom of the leaves to keep them fresh. The leaves were renewed after the change of each instar and later on the previously used leaf blade was measured. Data on weights (g) of food consumed by the different larval instars were recorded with the help of an electronic balance. All the measurement was continued upto pupation.

3.6 Statistical Analyses:

The experiments were conducted using Completely Randomized Design (CRD). The data obtained from the study of biology were statistically analyzed. On the other hand the parameters relating to feeding behaviour were statistically analyzed and the differences between means were tested by DMRT (Duncan's Multiple Range Test).



CHAPTER-4

RESULTS AND DISCUSSIONS

RESULTS AND DISCUSSION

In the present study, the biology and the feeding behaviour of lemon butterfly were investigated. In that connection various data were collected. They are statistically analyzed; the results are harmonically presented in different tables and plates and critically discussed in this chapter.

4.1 Mating period:

In *Papilio demoleus* mating took place within two days after the emergence of the adults (Table 1). Before mating the male came towards female with an excited motion and increased wing beat. Sometimes the male moved with the above behavior around the female until they started mating. The female also helped in mating by extends her abdomen from side ways to meet with the male genital organ. The duration of mating varied from 1.50-3.30 hours with an average of 2.30 ± 0.18 hours (Table 1). Radke and Kandalkar (1988) observed that mating lasted 1.5- 2 hours in this insect.

Both the male and the female butterflies remained silent during mating but on disturbances they dispersed. At the time of mating the head region of the male and the female remained in the opposite directions though the posterior abdominal segments of both the sexes were firmly attached. The mating took place on the leaf of the plant or on the net in which they were allowed for mating. They did not require frequent mating rather mate only once. Mating took place both at day and night. However, it was observed that normally mating lasted

longer at night. It might be due to the fact that at night time, there was no disturbance for them.

4.2 Pre-oviposition period:

It was found that the females did not lay eggs just after attaining the adult stage. The pause between the date of the emergence of an adult and the first egg deposition was the pre-oviposition period. A very short pre-oviposition period was found in it as it was noted to vary from 1-2 days with an average of 1.30 ± 0.15 days (Table 1) only. Radke (1998) also observed that pre-oviposition period of lemon butterfly lasted for 1-2 days. Atwal (1964) showed a female accepts a male after a lapse of 24 hours known as pre-oviposition period. During that period the female became less active and preferred taking rest on the ventral surfaces of the leaves.

4.3 Oviposition period:

During the oviposition period the fertilized female visited citrus plants and laid eggs on the ventral surface of the leaves by bending the abdomen so that the tip touched the leaf surface and the eggs came out by the rhythmic muscular activity of the posterior abdominal segment. They generally laid eggs on tender shoot and young leaves. The eggs were laid singly. The maximum number of eggs (15-19 eggs) was laid during the first few days of oviposition and then the rate decreased rapidly. During that period the movement of the female butterfly became slow.

After the completion of the oviposition period, the female lived for very short time i.e. 17-20 hours.

The oviposition period was considered between the period of the first and the last egg laid by the female. This period was found 2 days and the mean was 2 ± 0 days (Table 1).

Rajindar (1997) reported that the oviposition period ranged from 2.42 - 4.52 days on *Citrus reticulata* under laboratory conditions but Radke (1988) reported that it was 2 days.

Table 1. Pre-mating, mating, pre-oviposition and oviposition periods of lemon butterfly as recorded in the study

No. of Observations	Various periods			
	Pre-mating (days)	Mating (hours)	Pre-oviposition (days)	Oviposition (days)
1	2.30	2.30	1.0	2.0
2	2.00	2.20	2.0	2.0
3	2.00	2.30	1.0	2.0
4	2.00	1.50	1.0	2.0
5	2.15	3.30	2.0	2.0
6	2.15	3.00	1.0	2.0
7	2.00	1.50	1.0	2.0
8	2.00	2.00	1.0	2.0
9	2.35	2.50	1.0	2.0
10	2.35	2.40	2.0	2.0
Mean	2.13	2.30	1.30	2.00
±	±	±	±	±
SE	0.04	0.18	0.15	0.00

4.4 Eggs:

Morphological features

The egg was smooth and round. A freshly laid egg was pale yellow but changed to brown during its development. The non-viable egg began to shrink slowly after one day of laying. The fertilized egg changed its color to brown due to the embryonic development inside the egg and was more transparent than the unfertilized egg. The egg hatched into a young larva after it reached its full development. Then the young larva came out of the transparent egg case.

Number of eggs laid and percentage of hatching

A single female laid 15 to 29 eggs in her whole life with an average of 22.1 ± 1.41 (Table 2).

Singh (1995) observed that the fecundity of *Papilio demoleus* varied between 11-14. Radke and Kandalkar (1989) found that the females laid 15-22 eggs. The hatching percentage of eggs varied from 93.75-100.0 with an average of 97.52 ± 1.33 (Table 2).

4.5 Incubation period:

The incubation period is the duration between the dates of laying egg to its hatching. In the study, the incubation period varied from 4-5 days with an average of 4.8 ± 0.11 days (Table 2). Nair (1975) reported that the incubation period varied from 3-7 days.

Table 2. Fecundity of lemon butterfly as noted in the study

No. of observations	Eggs laid (No./female)	Hatching of eggs (%)	Incubation period (Days)
1	29.0	87.50	5.0
2	25.0	93.75	5.0
3	17.0	100.0	5.0
4	15.0	100.0	5.0
5	19.0	100.0	5.0
6	20.0	100.0	4.5
7	27.0	95.00	4.0
8	25.0	98.95	4.5
9	23.0	100.0	5.0
10	21.0	100.0	5.0
Mean	22.1	97.52	4.8
±	±	±	±
SE	1.41	1.33	0.11

4.6 Larva:

The larvae hatched from the small, round and yellow eggs laid on the top most shoots of the plants. They were at first dark colored with patches and the head was provided with two processes. When nearly full grown, they were brilliantly rich green, smooth, velvety (without tubercles) and had dusky brown oblique bands on the sides of the body that did not meet on dorsum. There was a horn like structure on the dorsal side of the last abdominal segment. Moulting of larvae took place four times and thus the larvae developed through five instars. Their body had the usual three parts: the head, the thorax and the abdomen. The larvae had a peculiar habit of feeding on the moulded skin after each moulting. That scavenging habit made rather impossible to trace any exuviae on the leaf surface. Prior to each moulting, the larva stopped feeding. The length and the breadth increased after each moulting and segmentations became clearer in the 5th

instar. The habits, morphological features, morphometric measurements and durations of those five instars are described here

23
16.4.07

4.6.1 First Instar

After hatching, the young larvae first ate the empty eggshells and then started feeding voraciously on leaves. The first instar larvae, as their mandibles were not so hard, preferred only young shiny leaves.

The freshly hatched larva was spiny, cylindrical and light brown or brownish black with a dirty white mark on the dorsal side of the abdomen and resembled bird droppings. Its thoracic region was thicker than the rest of the body.

Morphometric measurements

Morphometric measurements were made for the body length, breadth and weight, and are presented in the Table 3. The results highlighted that the length of the 1st instar larvae measured from 2.02 to 2.50 with an average of 2.32 ± 0.05 and the breadth varied from 0.71 to 0.76 with a mean of 0.73 ± 0.01 mm (Table 3).

Rajinder (1997) mentioned that the standard size of the first instar larvae was 2.89 mm.

Duration

The duration of the 1st instar larvae varied from 4.50 to 6.0 with an average of 5.45 ± 0.17 days (Table 3). The duration of the first instar larvae of *Papilio demoleus* averaged 5.3 days, respectively (Singh and Gangwar, 1989). Rajinder

(1997) observed that the larva passed through five instars and the first instar larvae lasted for 1.57- 3.27 days. ✓

4.6.2 Second Instar

The second instar larvae came out by leaving the exuviae of the first instar larvae. Just before moulting the larva stopped feeding and remained silent on the leaf. The 2nd instar larvae became less spiny and darker. After moulting they fed voraciously. At this instar, the larvae increased in size, whereas all other parts of the body were as usual. The habit and general appearances were more or less same like the previous instar. ✓

Morphometric measurements

The length of the 2nd instar larvae ranged from 7 to 9 with an average of 8.15 ± 0.25 mm and the breadth measured from 1 to 1.95 with a mean of 1.49 ± 0.09 mm (Table 3). Rajinder (1997) pointed out that the average sizes of the second instar larvae were 5.60 mm. ✓

Duration

The results revealed that the duration of the 2nd instar larva varied from 4 to 5 with an average of 4.56 ± 0.13 days (Table 4). Singh and Gangwar (1989) reported that the duration of the second instar larvae was 5.1 days.

Garg (1978) reported that the second instar larvae lasted for 5 days ranging from October to November. ✓

4.6.3 Third Instar

After the second moulting, the 3rd instar larva came out leaving the black exuviae. They were more active and fed greater amount than the previous instars. They were larger than the 2nd instar and became less spiny compared to the 2nd instar. The dirty white line was oblique along the pleurite with a break on the dorsal surface.

Morphometric measurements

The length of the third instar larvae was 15.4 to 18 with an average of 16.73 ± 0.33 mm; the breadth measured from 3.5 to 4.6 with a mean of 4.26 ± 0.11 mm and the body weight was measured from 0.50 to 0.89 with an average of 0.72 ± 0.04 g (Table 3). Rajinder (1997) mentioned that the average size of the third larval instar was 7.50 mm.

Duration:

The results indicated that the duration of the 3rd instar larvae lasted from 2 to 3 with an average of 2.61 ± 0.11 days (Table 4). Singh and Gangwar (1989) noted that the third instar larvae lasted for 4.2 days. Rajinder (1997) pointed out that the duration of the third instar larvae was 1.55-4.33 days. Pruthi (1969) showed that the duration of the third instar larvae was 3 days.

4.6.4 Fourth Instar

After third moulting, the fourth instar larvae came out of the exuviae of the third instar larvae. Following emergence, the 4th instar larva turned almost black though the color reflected little brownish tinge. The larvae were longer than the 3rd instar and fed more voraciously. The white mark on the dorsal side became large too.

Morphometric measurements

The body length of the 4th instar larvae was measured 21 to 25 with an average of 22.09 ± 0.37 mm; the breadth was measured 6.5 to 6.9 with an average of 6.58 ± 0.03 mm and the body weight was measured 1.60 to 1.75 with a mean of 1.65 ± 0.01 g (Table 3). Rajinder (1997) cited that the average size of the fourth instar larva was 11.69 mm.

Duration

Observations made on the larval duration of the fourth instar larvae varied from 2 to 3 with an average of 2.34 ± 0.12 days (Table 4). Singh and Gangwar (1989) pointed out that the duration of the fourth instar larva was 4.2 days. Rajinder (1997) observed that the fourth instar larvae lasted from 1.92-8.70 days.

4.6.5 Fifth Instar

After the 4th moulting, the 5th instar larvae came out from the exuviae of the 4th instar and that was the final instar. The fifth instar larvae were completely different from the previous four instars. In 5th instar larvae turned yellowish green

and became spineless. They were green with brownish stripes on the sides of the body. There was a horn like structure on the dorsal side of the last abdominal segment. At that stage larvae possessed two reddish to purple colored sacs, which were at the posterior position of the head- the osmeterium. When disturbed, those sacs are pushed out from the top of its pro-thorax; that organ is bifid and emits an irritating smell. That function is done to drive away its enemies, which might be due to smell or a kind of warning. In addition, black dots appeared on the dorsal side of the abdomen. At that instar the larvae became more stout and stronger than the previous instars and attained the greatest size. They fed voraciously on whole leaf tissues except the midrib.

Morphometric measurements

The results pointed out that the body length of the 5th instar varied from 35 to 40 with an average of 37.45 ± 0.80 mm; breadth from 8.1 to 10 with an average of 8.82 ± 0.22 mm and the body weight from 2.5 to 4.2 with an average of 3.47 ± 0.22 g (Table 3). Rajinder (1997) mentioned that the average size of the fifth instar larvae was 26.50 mm.

Duration

The duration of the 5th instar larvae lasted for four days with an average of 4 ± 0 days (Table 4). The duration of the fifth larval instar of *Papilio demoleus* averaged 3.5 days, respectively (Singh and Gangwar, 1989). Rajinder (1997) also observed that the duration of the final instar larvae was 1.98-17.47 days.

Table 3. Morphometric measurements of the larva of lemon butterfly as experienced in the study

No. of obser- vations	Various instars														
	1 st		2 nd		3 rd			4 th			5th/Final				
	Length (mm)	Breadth (mm)	Length (mm)	Breadth (mm)	Length (mm)	Breadth (mm)	Weight (g)	Length (mm)	Breadth (mm)	Weight (g)	Length (mm)	Breadth (mm)	Weight (g)		
1	2.50	0.75	7.5	1.5	15.5	3.5	0.50	22	6.6	1.64	35.0	8.2	2.6		
2	2.40	0.76	9.0	2.0	16.0	4.0	0.60	22	6.6	1.63	35.0	8.1	2.5		
3	2.02	0.72	9.0	1.5	16.0	4.0	0.60	22	6.6	1.65	35.0	8.2	3.4		
4	2.06	0.73	8.0	1.0	18.0	4.0	0.60	21.5	6.5	1.63	40.0	8.9	3.95		
5	2.26	0.75	7.0	1.3	17.0	4.5	0.85	21.5	6.5	1.70	41.0	10	4.15		
6	2.23	0.71	8.0	1.5	17.5	4.6	0.84	21.9	6.5	1.60	35.5	8.1	2.5		
7	2.30	0.73	8.0	1.5	17.9	4.5	0.89	21.0	6.6	1.63	40.0	10	4.2		
8	2.50	0.72	7.0	1.30	15.4	4.5	0.84	23.0	6.5	1.75	39.0	8.9	2.2		
9	2.50	0.73	9.0	1.40	16.0	4.5	0.85	25.0	6.9	1.65	39.0	8.9	3.6		
10	2.50	0.74	9.0	1.95	18.0	4.5	0.70	21.0	6.5	1.65	35.0	8.9	3.6		
Mean	2.32	0.73	8.15	1.49	16.73	4.26	0.72	22.09	6.58	1.65	37.45	8.82	3.47		
±		±	±	±	±	±	±	±	±	±	±	±	±		
SE	0.05	0.01	0.25	0.09	0.33	0.11	0.45	0.37	0.03	0.01	0.80	0.22	0.222		

4.7 Total larval period:

The mean of the total larval period was 18.96 ± 0.29 with the minimum of 18.6 and the maximum of 20 days (Table 4). Radke (1989) cited that the total larval period of *Papilio demoleus* was 18; while Singh (1995) observed that larval period was 21.4 days. Similarly Atwal (1976) reported 3 weeks during November to December.

Table 4. Measurements of the larval period of lemon butterfly

No. of observations	Larval periods of different instars (days)					
	1 st	2 nd	3rd	4th	5th	Total
1	6.00	5.00	2.30	3.0	4.0	20.3
2	5.08	4.50	3.00	2.0	4.0	18.58
3	5.30	4.60	3.00	2.0	4.0	18.9
4	5.60	4.90	3.00	2.0	4.0	19.5
5	4.90	5.00	3.00	2.0	4.0	18.9
6	6.00	4.00	2.50	2.30	4.0	18.8
7	5.15	5.15	2.70	3.00	4.0	20.0
8	6.00	4.30	2.30	2.60	4.0	19.2
9	6.00	4.20	2.30	2.10	4.0	18.6
10	4.50	4.00	2.30	2.40	4.0	16.9
Mean	5.45	4.56	2.61	2.34	4.00	18.96
±	±	±	±	±	±	±
SE	0.17	0.13	0.11	0.12	0.00	0.29

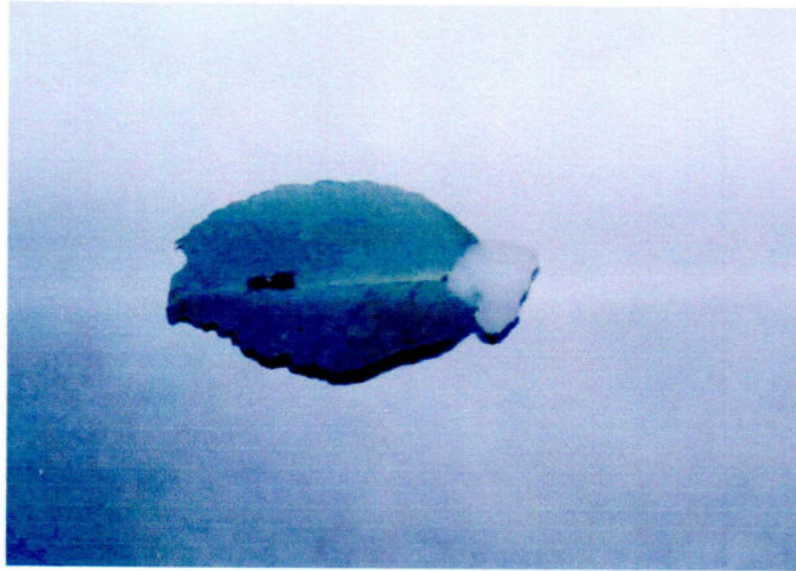


Plate 2. The first instar larva of lemon butterfly on a lemon leaf

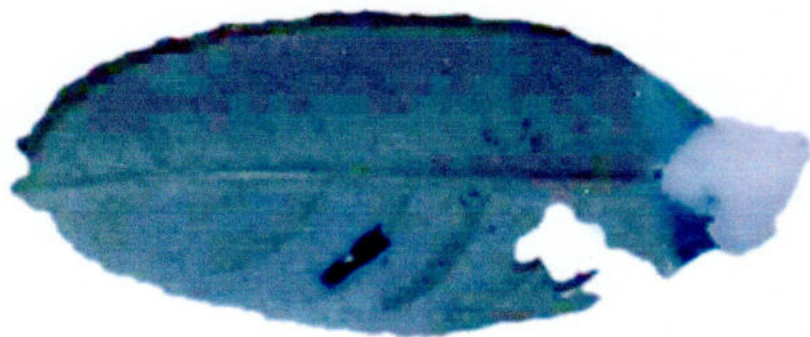


Plate 3. The second instar larva of lemon butterfly on a lemon leaf



Plate 4. The third instar larva of lemon butterfly



Plate 5. The fourth instar larva of lemon butterfly on the midrib of a lemon leaf

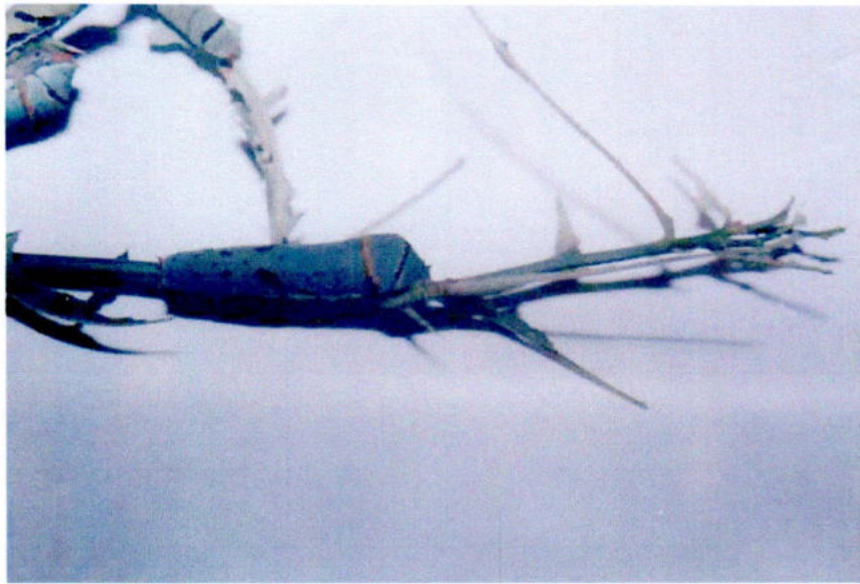


Plate 6. The fifth instar larvae of lemon butterfly on a lemon shoot



Plate 7. The pre-pupa of lemon butterfly on a lemon shoot

4.8 Pupal stage:

Before going to pupal stage, there was a pre-pupal stage; that was recognized by their conspicuous segmentations as well as by the lengthening and straightening processes of their normally curved larval forms.

The pupa was the ultimate transformation of pre-pupa. The pupa was a non-feeding stage. So, after completion of feeding, the final instar larva prepared a rough silken padding and girdled on stems and leaves of the host plant before pupation where as the chrysalis was attached to the plant by its tail and was held with a silken girdle. At that stage, all larval characteristics, sizes and shapes were completely changed. Pupa had three different colors- newly emerged pupa was parrot green; after few days that became yellowish brown (dry straw) and before adult emerging that again became dark grey.

Morphometric measurements

Pupa was widest at the thoracic region. The length of the pupa was measured 30 to 32 mm with an average of 31.2 ± 0.24 mm; the breadth was 10 to 12 with an average of 11 ± 0.25 mm and the weights measured from 5.5 to 5.7 g with an average of 5.63 ± 0.02 g (Table 5). Rajindar (1997) experienced that the pupa measured 28.86 in length while 9.04 mm in breadths. Atwal (1964) also showed that the pupa was 29.5 mm in length and 9.3 mm in breadth.

Duration

The results clarified that the duration of pupal period lasted from 10 to 11.10 with an average of 10.69 ± 0.14 days (Table 5). Radke and Kandalkar (1988) studied the pupal period and experienced that the period lasted for 8-12 days. Nair (1975) also pointed out that the average pupal period was 7 to 24 days.

Table 5. Morphometric measurements and durations of the pupal stage of lemon butterfly as observed in the study

No. of observations	Length (mm)	Breadth (mm)	Weight (g)	Period (days)
1	31.0	10.0	5.6	11.0
2	31.0	11.0	5.6	11.0
3	32.0	12.0	5.6	11.0
4	30.0	11.0	5.7	11.0
5	31.0	10.0	5.7	11.0
6	32.0	10.0	5.7	10.5
7	32.0	11.0	5.7	11.1
8	31.0	11.0	5.5	10.3
9	32.0	12.0	5.6	10.0
10	30.0	12.0	5.6	10.0
Mean	31.20	11.00	5.63	10.69
±	±	±	±	±
SE	0.24	0.25	0.02	0.14

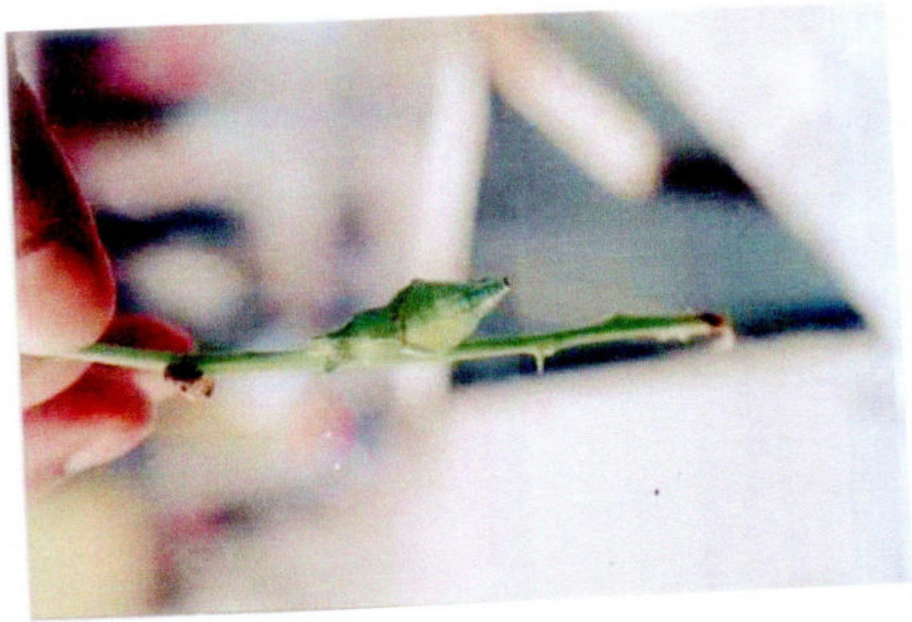


Plate 8. The pupa of lemon butterfly on a lemon shoot

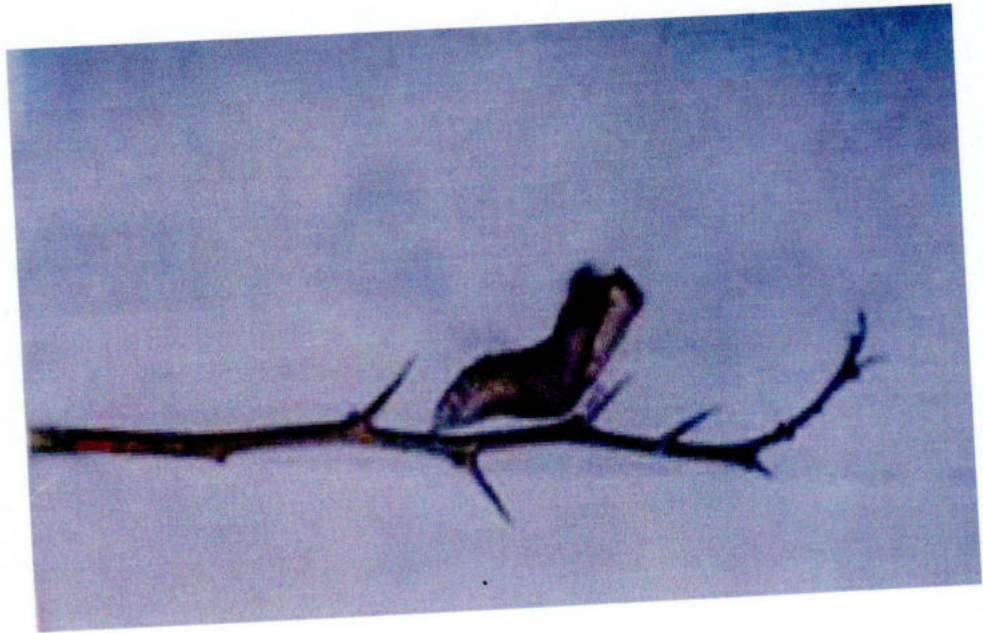


Plate 9. The pupal case of lemon butterfly after adult emergence

4.9 Adult:

The adult emerged by dorsally splitting the pupal case. Before emerging, the butterfly tore the pupal cover with its body. The newly emerged butterfly possessed weak wings and was unable to fly. It took rest under the sun for 1 to 3 hours and became capable to fly. The adult butterflies were beautifully patterned; the body was ventrally yellow and the wings were ornamented too. However, the female was slightly larger (1-3 mm in length and 0.5-1 mm in breadth) than the male. The head, the thorax, the abdomen and other appendages were covered with numerous scales.

Morphometric measurements

Measurements on different aspects of the male butterfly are presented in the (Table 6) and that of the female in the (Table 7). The body weight of male was 1.22 to 1.30 g with an average of 1.24 ± 0.01 g. The female butterfly was 1.32 to 1.43 g with an average of 1.36 ± 0.01 g.

The body length of the male measured 24 to 25 mm with an average of 24.6 ± 0.14 mm and the female body length measured 25 to 28 mm with an average of 26.4 ± 0.30 mm. The breadth of the body of the male at the resting position measured 4 to 5.3 mm with an average of 4.82 ± 0.16 mm and at the expanding position the distance between the tips of the forewings and the hind wings of the male was 80 to 82.50 mm with an average of 80.89 ± 0.37 mm and 50 to 53 mm with an average of 51.35 ± 0.26 mm, respectively.

The breadth of the body of the female at the resting position measured 4.50 to 5.4 mm with an average of 5.14 ± 0.08 mm and at the expanding position the distance between the tips of the forewings and the hind wings of female was 82 to 90.30 mm with an average of 86.06 ± 0.87 mm and 51 to 53 with an average of 51.95 ± 0.32 mm, respectively.

Singh (1995) described that the wing of an adult male at the expanded position was 83.9 mm and that of a female 90.2 mm.

Table 6. Morphometric measurements of the adult male of lemon butterfly as recorded in the study

No. of observations	Body length (mm)	Breadth (mm)			Body weight (g)
		On rest	Tip to tip of forewing	Tip to tip of hind wing	
1	24.5	4.0	80.0	50.0	1.22
2	24.0	4.3	81.0	51.0	1.20
3	24.0	5.0	83.0	51.0	1.20
4	25.0	4.0	80.0	50.5	1.22
5	25.0	5.0	82.0	52.0	1.30
6	25.0	5.0	80.0	51.0	1.30
7	25.0	5.3	80.1	51.5	1.30
8	24.0	5.1	80.1	51.5	1.22
9	24.5	5.2	80.2	52.0	1.22
10	25.0	5.3	82.5	53.0	1.30
Mean	24.6	4.82	80.89	51.35	1.24
±	±	±	±	±	±
SE	0.14	0.16	0.37	0.26	0.01

Table 7. Morphometric measurements of the adult female of lemon butterfly as noted in the study

No. of observations	Body length (mm)	Breadth (mm)			Body weight (g)
		On Rest	Tip to tip of forewing	Tip to tip of hind wing	
1	26	5.1	85.0	52.0	1.35
2	27	5.3	87.3	53.0	1.40
3	25	4.5	83.2	51.0	1.30
4	27	5.4	88.9	53.0	1.40
5	27	5.3	90.3	52.5	1.42
6	25	5.0	82.0	50.0	1.32
7	27	5.2	85.1	51.0	1.35
8	28	5.4	89.3	53.0	1.43
9	26	5.1	85.0	52.0	1.35
10	26	5.1	84.5	52.0	1.32
Mean	26.4	5.14	86.06	51.95	1.364
±	±	±	±	±	±
SE	0.305	0.083	0.871	0.320	0.014

4.9.1 Antennae

A pair of antennae was present at the head region. The colors of the antennae were black and type of the antennae was clubbed.

The length of the antennae of the male butterflies measured from 16 to 16.4 mm with an average 16.16 ± 0.04 mm and of the female butterflies measured from 16 to 17 mm with an average of 16.57 ± 0.12 mm, respectively (Table 8).

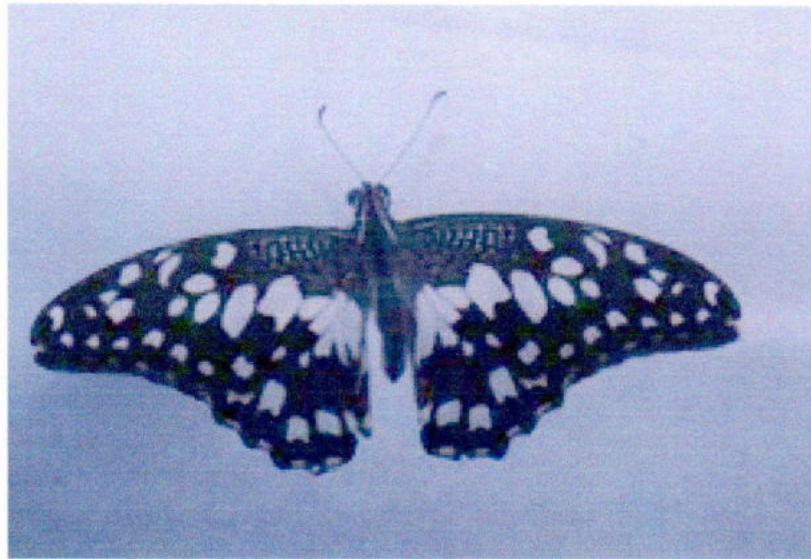


Plate 10. The adult male of lemon butterfly (dorsal view)



Plate 11. The adult female of lemon butterfly (dorsal view)

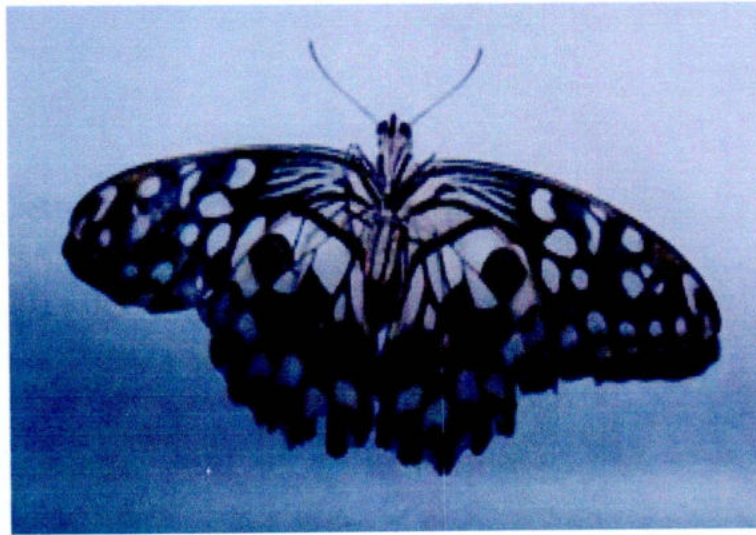


Plate 12. The adult female of lemon butterfly (ventral view)

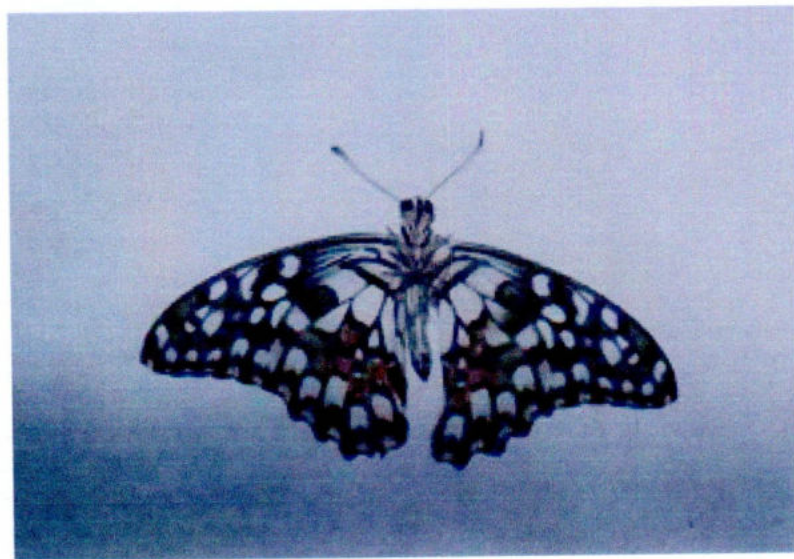


Plate 13. The adult male lemon butterfly (ventral view)

Table 8. Measurements of the antenna of the male and the female lemon butterflies

No. of Observations	Length (mm)	
	Male	Female
1	16.1	16.4
2	16.0	17.0
3	16.0	16.0
4	16.3	17.0
5	16.3	17.0
6	16.4	16.3
7	16.3	16.4
8	16.0	17.0
9	16.1	16.3
10	16.1	16.3
Mean	16.16	16.57
±	±	±
SE	0.04	0.12

4.9.2 Thorax

The thorax was densely covered with scales and that was small. The dorsal side was black while the ventral side was yellow. The thorax was provided with three pairs of legs, which were black and walking type. The forelegs were the shortest while the hind legs were the longest ones. ~

In the male the length of the thorax was 9.40 to 10.5 mm with an average of 10.00 ± 0.18 mm. The breadth was 6.30 to 6.55 mm with an average of 6.34 ± 0.04 mm. The length of the thorax of the female was 9.50 to 12.30 mm with an average of 10.81 ± 0.34 mm and the breadth was 6.40 to 7.2 mm with an average of 6.79 ± 0.08 mm respectively (Table 9).

Table 9. Measurements (mm) of the thorax of the male and the female lemon butterfly

No. of observations	Male		Female	
	Length	Breadth	Length	Breadth
1	09.50	6.30	10.0	6.70
2	10.30	6.40	12.0	7.00
3	09.40	6.10	09.5	6.40
4	10.50	6.50	12.3	7.20
5	10.50	6.50	12.1	7.10
6	09.45	6.10	09.6	6.50
7	10.40	6.40	11.0	6.90
8	11.00	6.55	11.4	6.95
9	09.50	6.30	10.1	6.60
10	09.50	6.30	10.1	6.60
Mean	10.00	6.34	10.81	6.79
±	±	±	±	±
SE	0.18	0.04	0.34	0.08

4.9.3 Wings

Two pairs of wings were originated from the meso-thorax. The forewings were broader than the hind wings. The wings were ornamented with yellow and black markings and covered with numerous scales. Forewings were triangular but the hind wings were more or less round. Hind wings were without tail like projection- the papilla; but had a brick-red oval spot near the upper margin and a blue spot closer to the lower margin.

The length of the forewings of the male were measured 39 to 40 mm with an average of 39.33 ± 0.09 mm. The breadth were measured from 23 to 24.50 mm with an average of 23.3 ± 0.13 mm. Again, the length of the hind wings were measured from

26 to 27.10 with an average of 26.28 ± 0.13 mm and breadth were measured from 23 to 23.20 with an average of 23.04 ± 0.02 mm (Table 10). Oppositely, the length of the forewings of the female were measured from 39.80 to 42.30 mm with an average of 41.74 ± 0.29 mm. The breadth measured from 27 to 28 mm with an average of 27.25 ± 0.09 mm. Again, the length of the hind wings were 27 to 28.10 mm with an average of 27.29 ± 0.10 mm and the breadth was 23 to 24 mm with an average of 23.4 ± 0.07 mm, respectively (Table 11).

Table 10. Measurements (mm) of the wings of the male of lemon butterfly

No. of Observations	Forewing		Hind wing	
	Length	Breadth	Length	Breadth
1	39.0	23.0	26.0	23.0
2	39.4	23.3	26.2	23.0
3	40.0	24.5	27.1	23.2
4	39.0	23.0	26.0	23.0
5	39.5	23.4	26.3	23.1
6	39.1	23.1	26.0	23.0
7	39.2	23.1	26.1	23.0
8	39.2	23.1	26.0	23.0
9	39.3	23.2	26.1	23.0
10	39.6	23.3	27.0	23.1
Mean	39.33	23.30	26.28	23.04
$\pm$	$\pm$	$\pm$	$\pm$	$\pm$
SE	0.09	0.13	0.13	0.02

4.9.4 Abdomens

The abdomen was black on the dorsal side but yellow with black strips on the ventral side. The male butterfly was distinguishable from the female one by their sizes and abdominal segments. The main distinguishing feature was that the last 3-4 abdominal

segments of the female butterflies were broader than the male one and that was due to fact that the abdomen was totally filled in with their egg masses. The lengths of the abdomens of the male and the female were measured from 14.40 to 15 mm with an average of 14.72 ± 0.09 mm and 14.50 to 15.50 mm with an average 15.04 ± 0.11 mm, respectively. On the contrary, the breadths of the male and the female butterflies were measured from 3 to 3.5 mm with an average of 3.23 ± 0.07 mm and 5 to 5.7 mm with an average of 5.33 ± 0.08 mm, respectively (Table 12).

Table 11. Measurement (mm) of the wings of the female lemon butterfly

No. of Observations	Forewing		Hind wing	
	Length	Breadth	Length	Breadth
1	41.6	27.1	27.0	23.3
2	42.0	27.3	27.2	23.4
3	41.0	27.0	27.0	23.3
4	42.3	27.5	27.1	23.0
5	43.0	27.0	27.0	23.5
6	39.8	27.0	27.3	23.4
7	41.6	27.2	27.3	23.4
8	43.0	28.0	28.1	24.0
9	41.6	27.2	27.4	23.3
10	41.5	27.2	27.5	23.4
Mean	41.74	27.25	27.29	23.40
±	±	±	±	±
SE	0.29	0.09	0.10	0.07

Table 12. Measurements (mm) of the abdomens of the male and the female lemon butterfly

No. of observations	Male		Female	
	Length	Breadth	Length	Breadth
1	14.5	3.10	14.5	5.00
2	14.4	3.00	15.3	5.50
3	15.0	3.00	14.6	5.00
4	15.0	3.50	15.3	5.50
5	15.0	3.50	15.3	5.50
6	15.0	3.40	14.6	5.00
7	15.0	3.40	15.3	5.50
8	14.4	3.00	15.5	5.70
9	14.5	3.00	15.0	5.30
10	15.0	3.40	15.0	5.30
Mean	14.72	3.23	15.04	5.33
±	±	±	±	±
SE	0.09	0.07	0.11	0.08

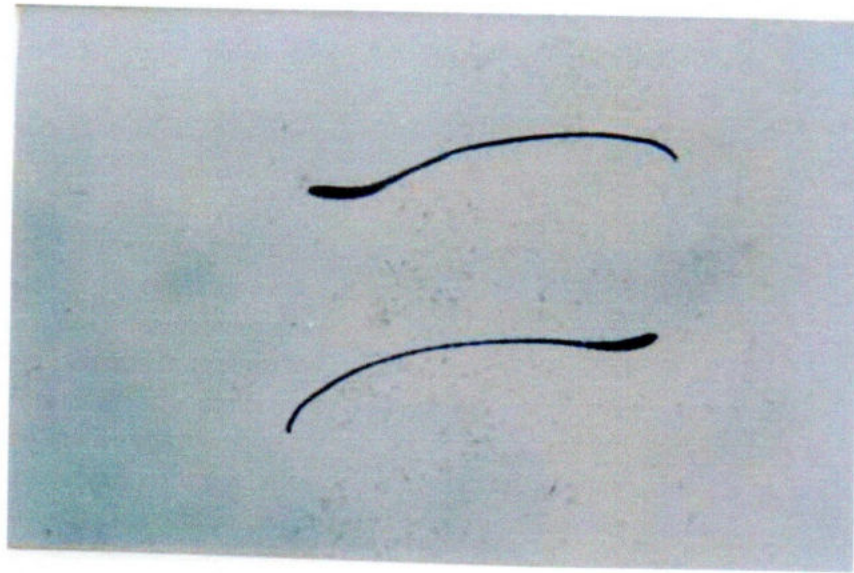


Plate 14. The antennae of lemon butterfly

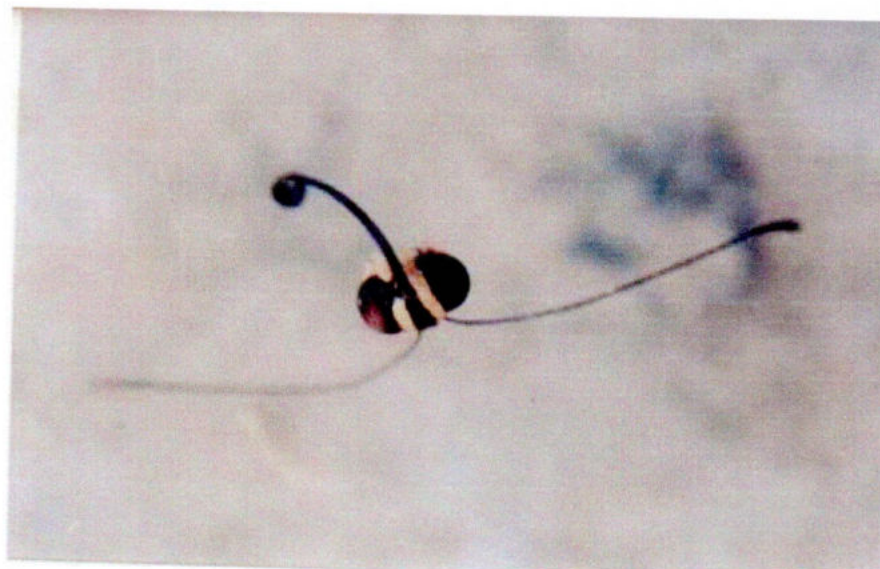


Plate 15. The head region of lemon butterfly



Plate 16. The thorax of lemon butterfly

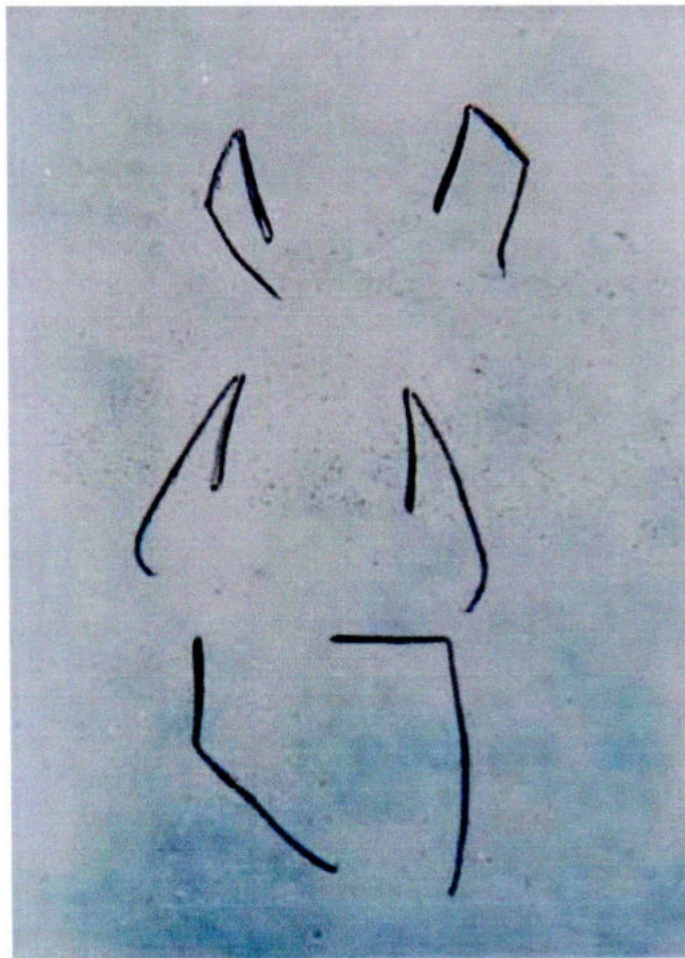


Plate 17. The legs of lemon butterfly



Plate 18. The forewings of lemon butterfly



Plate 19. The hind wings of lemon butterfly



Plate 20. The abdomen of lemon butterfly

4.9.5 Adult longevity

The longevity of the adult butterflies was counted from the emergence of the adult to its death. In general, the longevity of the males was shorter than the female butterflies.

The result pointed out that the longevity of the adult butterfly varied from 4 to 5 days with an average of 4.2 ± 0.13 days. Oppositely, the longevity of the female varied from 6 to 7 days with an average of 6.3 ± 0.15 days (Table 13). Singh (1995) observed that average longevity of the female was 6.1 days and that of the male was 4.1 days. Singh and Gangwar (1989) reported that the longevities of the male and the female averaged between 5.1 and 5.8 days, respectively. Similarly Atwal (1964) showed that the male lived from 3-4 and the females from 5 to 8 days.

Table 13. Adult longevity (days) of lemon butterfly as recorded in the study

No. of observations	Male	Female
1	4	6
2	4	6
3	4	6
4	4	6
5	4	7
6	4	6
7	5	6
8	4	6
9	5	6
10	4	7
Mean	4.20	6.30
$\pm$	$\pm$	$\pm$
SE	0.13	0.15

4.10 Life cycle:

The length of the lifecycle was counted from the laying egg to the emergence of the adult. The total longevity of lemon butterfly in the laboratory conditions varied from 30.5 to 37 with an average of 34.45 ± 0.80 days (Table 14).

Table 14. Summary of the life cycle of lemon butterfly as noted in the study

Different stages of life	Period (days)		Mean $\pm$ SE
	Minimum	Maximum	
Incubation period	04.0	05.0	4.8 ± 0.11
Larval instars:			
1 st Instar	04.5	06.0	5.45 ± 0.17
2 nd Instar	04.0	05.0	4.56 ± 0.13
3 rd Instar	02.0	03.0	2.61 ± 0.11
4 th Instar	02.0	03.0	2.34 ± 0.12
5 th Instar	04.0	04.0	4 ± 0
Total larval period	16.9	20.3	18.968 ± 0.29
Pupal period	10.0	11.0	10.69 ± 0.14
Total developmental period (egg to adult)	30.5	37.0	34.45 ± 0.80
Pre-mating period	02.0	2.35	2.13 ± 0.04
Mating period	01.5	2.40	2.30 ± 0.18
Pre-oviposition period	01.0	2.00	1.30 ± 0.15
Oviposition period	02.0	2.00	2 ± 0
Adult longevity:			
Male	04.0	5.00	4.20 ± 0.13
Female	06.0	7.00	6.30 ± 0.15

4.11 Feeding behaviours of lemon butterflies on lemon leaves treated with neem oil having different concentrations :

Larva generally started feeding from the margin reaching inwards of the midrib of the leaf. The larva voraciously fed the lemon leaves in the control treatment (0% of neem oil). Oppositely, they fed at decreasing rates when the leaves were treated with the 0.5, 1 and 1.5% neem oil (Tables 15 to 19 and Figures 1 to 5). When the 1st instar larvae were reared on the untreated food, the mean consumption of food by the first instar larvae were 0.573 ± 0.067 g and when reared on the treated food, the mean consumption of food were 0.288 ± 0.064 , 0.088 ± 0.029 and 0.029 ± 0.023 g, respectively. The food consumption gradually decreased with the increase of concentration of neem oil. Statistical analysis showed that the food consumption was significantly lower in treated food as compared to control. The lowest food consumption (0.029 g) was recorded at 1.5% of neem oil. This was significantly different from 0.5% (0.288 g), but there was no significant difference treated at 1% (0.088 g) and 1.5% (0.029 g) of neem oil (Table 15).

Later on, by the 2nd instar larvae the food consumption was significantly highest in the control (1.622 g) and the lowest one at 1.5% (0.128 g) and these were significantly different from 0.5% (0.793 g) but no significant differences were observed when reared on 1% (0.231 g) and 1.5% (0.128 g) of neem oil (Table 16).

Afterward, when the 3rd instar larvae were reared on the treated food the results showed that the food consumption was significantly lowest at 1.5% (0.430 g) and the

highest (2.898 g) in the control (0%), which varied significantly from 0.5% (0.1.250 g) but no significant difference was found between 1% (0.600 g) and 1.5%.

Then the weights of lemon leaves consumed by the 4th instar larvae were highest in the control (3.750 g) and the lowest at 1.5% (1.62 g) and these were significantly different from 0.5% (2.630 g). Though the food consumption gradually decreased with the increase of concentration, there were insignificant differences among 1% and 1.5% (Table 18).

Finally, data of the food consumption by the final instar larvae were obtained from 0.0 (control), 0.5, 1.0 and 1.5% neem oil were significantly different from each other. Statistical analyses showed that the food consumption was significantly lower in treated food as compared to the control. Highest (4.264 g) food consumption was recorded in the control (0%) and then at 0.5 (3.755 g), 1 (2.820 g) and 1.5% (2.570 g) of neem oil (Table 19).

In an experiment Haque and Parvin (1996) also used 0.25, 0.50, 1.0 and 2.0% of the neem oil on the brinjal leaf discs for the feeding response and survival of the 1st and 3rd instar larvae of epilachna beetle (*Epilachna dodecastigma*). They observed that all the first instar larvae died at all the concentrations investigated without feeding any food. On the other hand when the third instar larvae and the adult beetles were reared on the treated food, the food consumption were significantly reduced. The highest larval mortality was recorded at the two higher doses.

Table 15. Weights (g) of lemon leaves consumed by the first instar larvae of lemon butterfly in different concentrated neem oil

Neem oil conc. (%)	Food Consumption (g)
	Mean $\pm$ SE
0.0 (control)	0.573 $\pm$ 0.067 a
0.5	0.288 $\pm$ 0.064 b
1.0	0.088 $\pm$ 0.029 c
1.5	0.029 $\pm$ 0.023 c

Means bearing the same letter (s) are not significantly different at 1% level of probability.

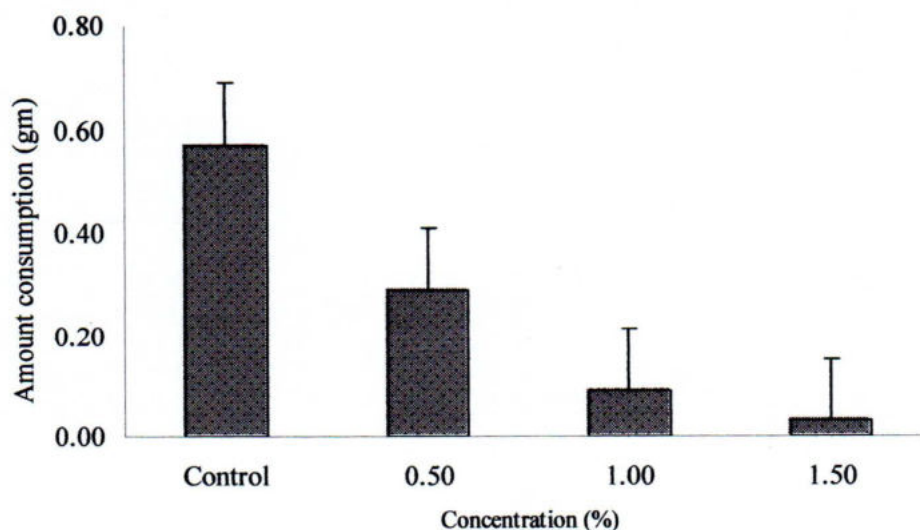


Fig 1: Weights (g) of lemon leaves consumed by the first instar larvae of lemon butterfly in different concentrated neem oil

Table 16. Weights (g) of lemon leaves consumed by the second instar larvae of lemon butterfly in different concentrated neem oil

Neem oil conc. (%)	Food Consumption (g)
	Mean $\pm$ SE
0.0 (control)	1.622 $\pm$ 0.117 a
0.5	0.793 $\pm$ 0.040 b
1.0	0.231 $\pm$ 0.090 c
1.5	0.128 $\pm$ 0.010 c

Means bearing the same letter (s) are not significantly different at 1% level of probability.

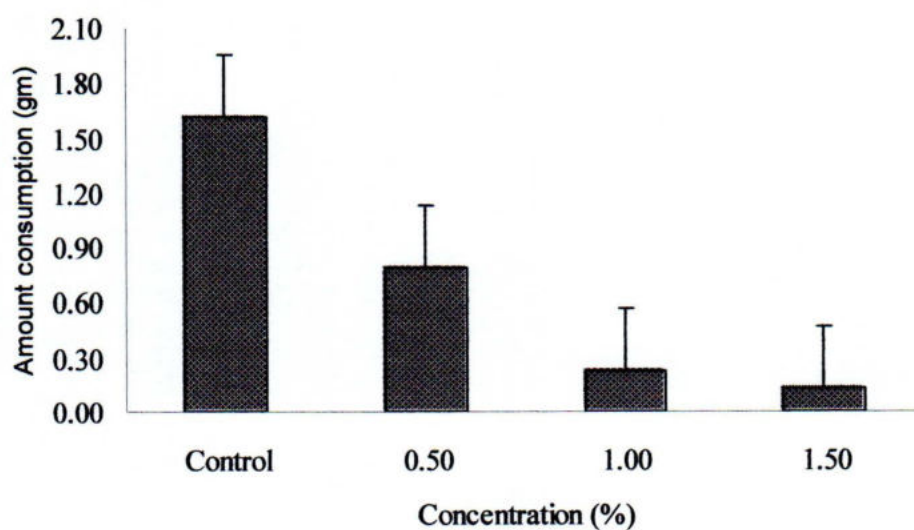


Fig 2: Weights (g) of lemon-leaves consumed by the second instar larvae of lemon butterfly in different concentrated neem oil

Table 17. Weights (g) of lemon leaves consumed by the third instar larvae of lemon butterfly in different concentrated neem oil

Neem oil conc. (%)	Food Consumption (g)
	Mean $\pm$ SE
0.0 (control)	2.898 $\pm$ 0.167 a
0.5	1.250 $\pm$ 0.054 b
1.0	0.600 $\pm$ 0.024 c
1.5	0.430 $\pm$ 0.015 c

Means bearing the same letter (s) are not significantly different at 1% level of probability.

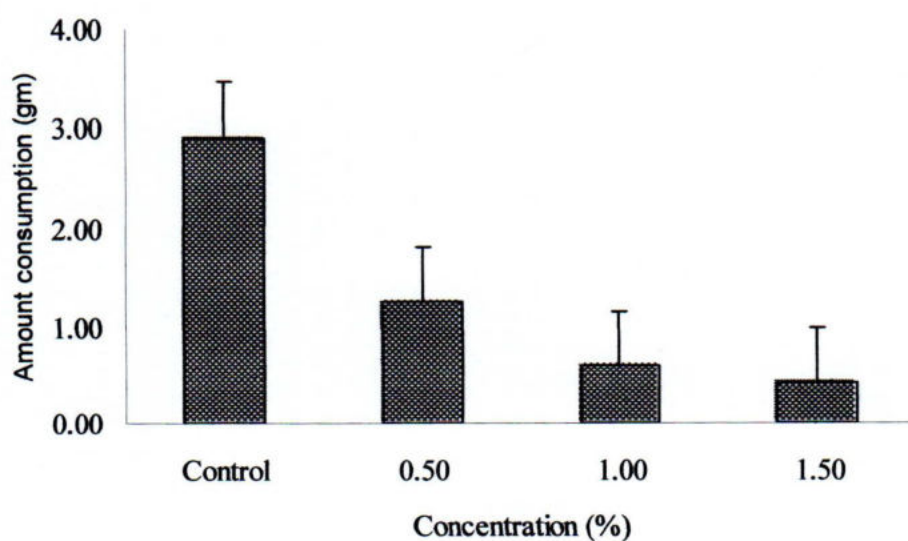


Fig 3: Weights (g) of lemon leaves consumed by the third instar larvae of lemon butterfly in different concentrated neem oil

Table 18. Weights (g) of lemon leaves consumed by the fourth instar larvae of lemon butterfly in different concentrated neem oil

Neem oil conc. (%)	Food Consumption (g)
	Mean $\pm$ SE
0.0 (control)	3.750 $\pm$ 0.103 a
0.5	2.630 $\pm$ 0.076 b
1.0	1.878 $\pm$ 0.018 c
1.5	1.620 $\pm$ 0.046 c

Means bearing the same letter (s) are not significantly different at 1% level of probability.

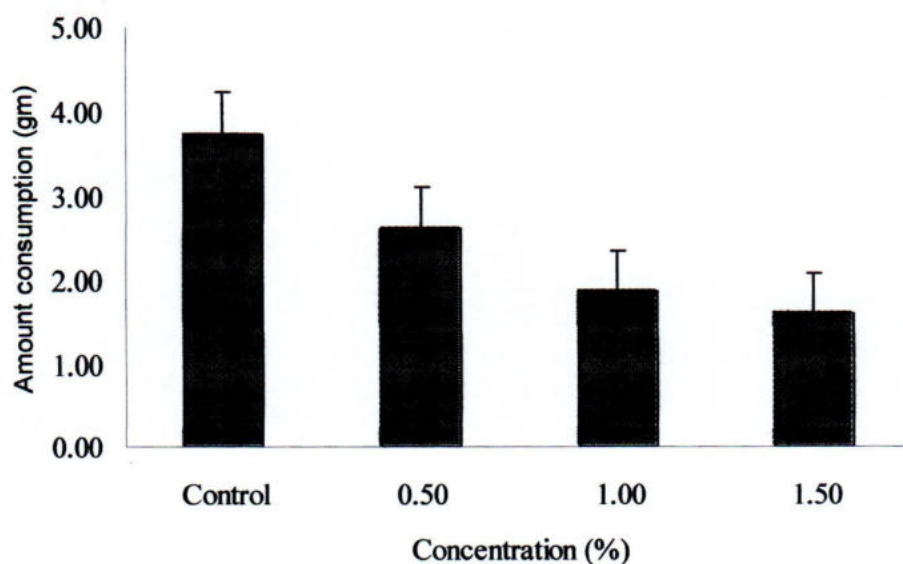


Fig 4: Weights (g) of lemon leaves consumed by the fourth instar larvae of lemon butterfly in different concentrated neem oil

Table 19. Weights (g) of lemon leaves consumed by the final instar larvae of lemon butterfly in different concentrated neem oil

Neem oil conc. (%)	Food Consumption (g)
	Mean $\pm$ SE
0.0 (control)	4.264 $\pm$ 0.102 a
0.5	3.755 $\pm$ 0.048 b
1.0	2.820 $\pm$ 0.055 c
1.5	2.570 $\pm$ 0.026 d

Means bearing the different letter (s) are significantly different at 1% level of probability.

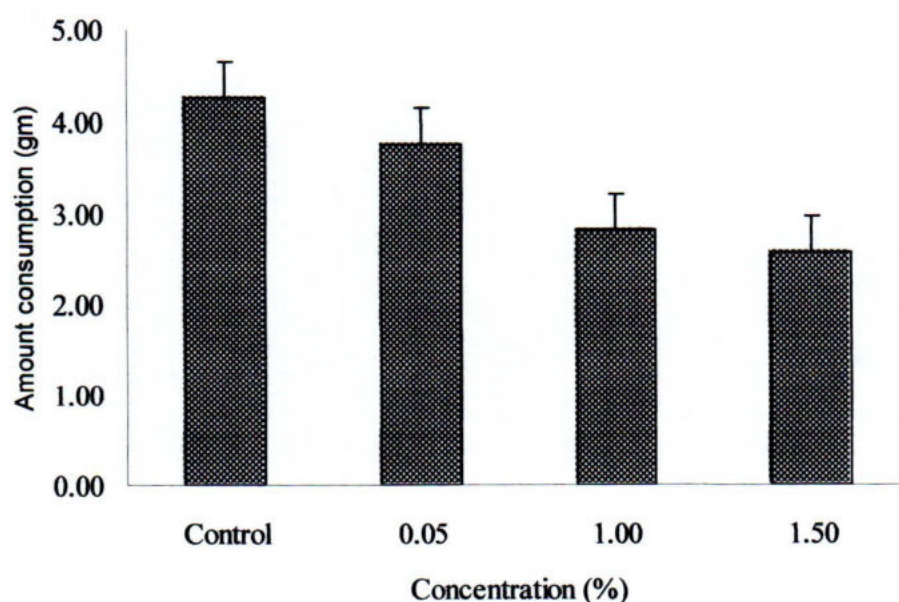
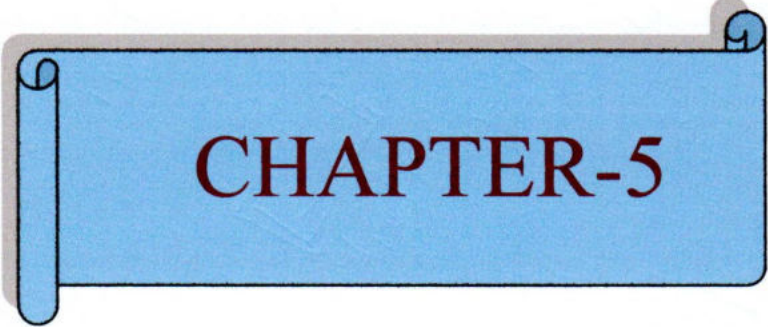


Fig 5: Weights (g) of lemon leaves consumed by the fifth instar larvae of lemon butterfly in different concentrated neem oil



CHAPTER-5

SUMMARY AND CONCLUSIONS

SUMMARY AND CONCLUSIONS

The biology and feeding behaviour of lemon butterfly, *Papilio demoleus* L. were studied at the Department of Entomology, Hajee Mohammed Danesh Science and Technology University, Dinajpur during September to December 2005. For the biological study, all the stages from mating to adult longevity were in depth investigated. Oppositely, to study the feeding behaviour, lemon leaves were first treated with different concentrations of neem oil, namely 0.5, 1.0 and 1.5%. Then the larvae were allowed to feed on them and on that leaves, which were sprayed with, distilled water (0% or control). Finally, amounts of leaf consumptions (g) were measured.

The newly emerged butterfly took part in mating after 2 to 2.30 days of emergence and their mating lasted for 1.5 to 3 hours. The male took active part in mating. Mating took place both at night and daytime. The average pre-oviposition and oviposition periods were 1.3 ± 0.15 and 2 ± 0 days, respectively. The female laid eggs on the dorsal sides of the leaves. Furthermore, 15 to 29 eggs were laid singly by each female. Nonetheless, on an average number of eggs deposited by each female was 22.1 ± 1.41 in her oviposition period. Freshly laid eggs were round, smooth and pale yellow, which gradually turned brown to black. The eggs hatched within 4.8 ± 0.11 days and the hatching percentage were 97.52 ± 1.33 .

The larva passed through five instars. The freshly hatched larva was spiny, cylindrical and light brown with dirty white marks on the dorsal side of the abdomen and the fifth/final instar larvae turned yellowish green and became spineless. The average duration of the first, the second, the third, the fourth and the final instar larvae were 5.45 ± 0.17 , 4.56 ± 0.13 , 2.61 ± 0.11 , 2.34 ± 0.12 and 4 ± 0 days, respectively. The average length of the first instar larvae was 2.32 ± 0.05 and the breadth was 0.73 ± 0.01 mm. As the growth and development proceeded, the larvae increased in length and breadth too. The second instar larvae remained same in color and shape but measured on an average 8.15 ± 0.25 mm in length while 1.49 ± 0.09 mm in breadth. The third instar larvae became longer than the second instar larvae and the body size of the third instar larvae measured 16.73 ± 0.33 mm in length and 4.26 ± 0.11 mm in breadth; the body weight was 0.72 ± 0.04 g. The fourth instar larvae were further elongated and feed more voraciously; the body length was 22.09 ± 0.37 mm and the breadth 6.58 ± 0.03 mm while the weight was 1.65 ± 0.01 g. The final instar larva was completely different in color, shape and more active than all the previous instars. The body length of the final instar larva was 37.45 ± 0.80 mm, the breadth was 8.82 ± 0.22 mm while the body weight was 3.47 ± 0.22 g.

Pupal period ranged from 10 to 11 days with an average of 10.69 ± 0.14 days. There were three different colors in the pupal stage. The average length and breadth of the pupa were 31.2 ± 0.24 mm and 11 ± 0.25 mm while the weight

was 5.63 ± 0.02 g. The insect completed its life cycle in 30.5 to 37 days. The adult male and female butterflies were same in color but in terms of the length the females were slightly longer than the male. The average length of the male butterflies was 24.6 ± 0.14 mm and the breadth on rest was 4.82 ± 0.16 mm, tip to tip of the forewings 80.89 ± 0.37 mm, tip to tip of the hind wings 51.35 ± 0.26 mm and the body weight was 1.24 ± 0.01 g. The average length of the female butterflies was 26.4 ± 0.30 mm and the breadth on rest was 5.14 ± 0.08 mm, tip to tip of the forewings was 86.06 ± 0.87 mm and tip to tip of the hind wings was 51.95 ± 0.32 mm and the body weight was 1.36 ± 0.01 g, respectively. The average longevities of the male and the female butterflies were 4.2 ± 0.13 days and 6.3 ± 0.15 days, respectively. The average length and breadth of the antenna of the male and the female butterflies were 16.16 ± 0.04 mm and 16.57 ± 0.12 mm, respectively. The thorax was black in the dorsal side while yellowish at the ventral side and measured on an average of 10.00 ± 0.18 mm in length and 6.34 ± 0.04 mm in breadth in the male and 10.81 ± 0.34 mm in length and 6.79 ± 0.08 mm in breadth in the female. The abdomen of the male was measured and the mean length and breath were 14.72 ± 0.09 mm and 3.23 ± 0.07 mm, that of the female were 15.04 ± 0.11 mm and 5.33 ± 0.08 mm, respectively. The average length and breadth of the forewings of the male was 39.33 ± 0.09 mm and 23.3 ± 0.13 mm and of the hind wings were 26.28 ± 0.13 mm, respectively. The average length and breadth of the forewings of the female was 41.74 ± 0.29 mm and

27.25 $\pm$ 0.09 mm and length and breadth of the hind wings were 27.26 $\pm$ 0.10 mm and 23.4 $\pm$.07 mm, respectively.

From the food consumption test it was clearly revealed that the larvae preferred lemon leaves treated with distilled water (0%), i.e. fresh leaves. Oppositely, their feeding rate decreased gradually as the concentrations of the neem oil used on the leaves were also increased. So, the finding paved the way to conclude that neem oil could be used in managing this notorious insect. However, proper dose (s) and other related issues must be standardized through further investigations.

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APPENDICES

APPENDICES

APPENDIX I. Weights (g) of lemon leaves consumed by the first instar larvae of lemon butterfly in different concentrated neem oil

No. of observations	Concentrations %			
	0.0 (control)	0.5	1.0	1.5
1	0.57	0.28	0.09	0.04
2	1.00	0.32	0.10	0.03
3	0.91	0.29	0.08	0.02
4	0.58	0.31	0.09	0.02
5	0.41	0.30	0.09	0.03
6	0.39	0.28	0.08	0.03
7	0.38	0.28	0.10	0.04
8	0.50	0.30	0.09	0.03
9	0.54	0.27	0.07	0.02
10	0.45	0.25	0.09	0.03
Mean	0.573	0.288	0.088	0.029
±	±	±	±	±
SE	0.067	0.064	0.029	0.023

APPENDIX II. Weights (g) of lemon leaves consumed by the second instar larvae of lemon butterfly in different concentrated neem oil

No. of observations	Concentrations %			
	0.0 (control)	0.5	1.0	1.5
1	1.58	0.83	0.25	0.17
2	1.25	0.70	0.25	0.09
3	1.25	0.55	0.20	0.10
4	1.89	0.90	0.25	0.15
5	2.00	0.90	0.21	0.12
6	1.75	0.80	0.25	0.15
7	1.80	0.70	0.22	0.12
8	0.90	0.70	0.20	0.10
9	1.80	0.90	0.20	0.10
10	2.00	0.95	0.28	0.18
Mean	1.622	0.793	0.231	0.128
±	±	±	±	±
SE	0.117	0.040	0.090	0.010

APPENDIX III. Weights (g) of lemon leaves consumed by the third instar larvae of lemon butterfly in different concentrated neem oil

No. of observations	Concentrations (%)			
	0.0	0.5	1.0	1.5
1	2.80	1.30	0.60	0.40
2	2.89	1.50	0.60	0.50
3	2.05	1.00	0.55	0.40
4	2.10	1.30	0.60	0.50
5	3.50	1.20	0.80	0.50
6	2.70	1.20	0.55	0.40
7	3.50	1.50	0.60	0.40
8	3.50	1.30	0.60	0.40
9	2.89	1.20	0.50	0.40
10	3.05	1.00	0.60	0.40
Mean	2.898	1.250	0.600	0.430
±	±	±	±	±
SE	0.167	0.054	0.024	0.015

APPENDIX IV. Weights (g) of lemon-leaves consumed by the fourth instar larvae of lemon butterfly in different concentrated neem oil

No. of observations	Concentrations (%)			
	0.0	0.5	1.0	1.5
1	3.50	2.60	1.90	1.30
2	3.80	2.50	1.80	1.50
3	3.90	2.70	1.90	1.70
4	3.50	2.30	1.90	1.60
5	4.00	3.00	1.80	1.70
6	3.50	2.40	1.90	1.70
7	3.80	2.80	1.90	1.70
8	4.50	3.00	1.98	1.80
9	3.50	2.50	1.80	1.70
10	3.50	2.50	1.90	1.50
Mean	3.750	2.63	1.878	1.62
±	±	±	±	±
SE	0.103	0.076	0.018	0.046

APPENDIX V. Weights (g) of lemon-leaves consumed by the final instar larvae of lemon butterfly in different concentrated neem oil

No. of observations	Concentrations (%)			
	0.0	0.5	1.0	1.5
1	4.10	3.80	3.00	2.70
2	4.20	3.90	3.00	2.60
3	4.50	3.70	2.80	2.60
4	4.30	3.85	2.70	2.50
5	5.10	3.50	2.90	2.50
6	4.10	3.70	3.00	2.50
7	4.09	3.80	2.90	2.50
8	4.04	3.90	2.50	2.60
9	4.10	3.50	2.60	2.70
10	4.11	3.90	2.80	2.50
Mean	4.264	3.755	2.820	2.570
±	±	±	±	±
SE	0.102	0.048	0.055	0.026