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# BIOLOGY OF LEMON BUTTERFLY *PAPILIO DEMOLEUS* (LEPIDOPTERA: PAPILIONIDAE): THE EFFECT OF NEEM OIL ON FOOD CONSUMPTION

**S. Karim[1], M. A. Ahad[1], M. R. Amin[1] and T. M. T. Iqbal[2]**

## ABSTRACT

The study was conducted to know the biology and the effect of neem *Azadirachta indica* oil on the food consumption of lemon butterfly *Papilio demoleus*. The results of the biology of lemon butterfly showed that *P. demoleus* starts mating after emergence of 2.1 ± 0.1 days and mating lasts for 2.3 ± 0.2 hours, and the preoviposition period was found 1.3 ± 0.2 days. A female laid on an average 22.1 ± 1.4 eggs during the lifespan and 97.5% of them were hatched. The ovipostion, incubation, larval and pupal period was found 2.0 ± 0.0, 4.8 ± 0.1, 18.9 ± 0.3 and 10.7 ± 0.2 days, respectively. The male and female longevity were 4.2 ± 0.1 and 6.3 ± 0.2 days, respectively. The 5$^{th}$ instar larvae consumed the highest amount of lemon leaves. Among the treatments, 1.5% neem oil showed strong antifeedant effect on food consumption.

***Keywords:*** *Biology, Papilo demoleus, neem*

## INTRODUCTION

Citrus are one of the leading fruits of the world. The fruits are very popular among the extensive grown tropical and subtropical fruits. They are cultivated in larger areas than other fruits (Hayes, 1957). 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 (Singh, 1969). Nearly 250 insect species are reported to infest various citrus crops (Nayar *et al.*, 1976). But only a few cause regular and heavy damage. Among them lemon butterfly *Papilio demoleus* is the foremost one. It has the potential to become a pest because it shows rapid population growth under favourable circumstances (Chatterjee *et al*., 2000; Pathak and Rizvi, 2003). *P. demoleus* have 5 generations per year in warm temperate China (Chen *et al*., 2004). Pathak and Rizvi (2003) reported that generation time for *P. demoleus* to be just over 30 days. They noted that eggs are laid singly, but may accumulate 8–10 eggs within 2-3 days on the surface of young leaves or twigs, eggs hatch in 4–5 days, and there are five instars over 27-35 days, followed by 10-12 days in pupation and an adult lifespan of 7-12 days. The young larvae feed only on fresh leaves and terminal shoots, the entire plant may be defoliated (Butani, 1979). In severe cases of infestation they feed pedicels and heavily attacked plants bear no fruit (Butani and Jotwan, 1975).

[1]Department of Entomology, Hajee Mohammad Danesh Science and Technology University, Dinajpur, Bangladesh; [2]Department of Horticulture, Hajee Mohammad Danesh Science and Technology University, Dinajpur, Bangladesh

Dispersal ability and capacity for rapid population growth make *P. demoleus* a potentially serious. So, appropriate management of this pest could play significant economic impact on production and profitability of the citrus crop. For proper management of any insect pest, it is important to know the biology and feeding behaviour of the insect. But there is no sufficient information regarding biology of lemon butter fly under Bangladesh condition. Now a days environmental friendly botanical insecticide e.g. neem oil is used as an effective remedy against various destructive insects. So, the present study was undertaken to know the biology of lemon butterfly and to figure out the effect of different concentrations of neem oil on the amount of leaf consumption which might help to formulate proper management of this pest.

## MATERIALS AND METHODS

**Rearing of insect:** The study was conducted in the laboratory of the Department of Entomology, Hajee Mohammad Danesh Science and Technology University, Dinajpur, Bangladesh from September to December 2006. To get sufficient number of the adult butterflies for the study, different available stages of the larvae were collected from lemon plants of the university campus. They were placed singly in 15 petridishes (11 cm diameter) with young lemon leaves (variety Elachi lebu, *Citrus lemon*). A small water socked cotton ball was put around petiole to keep the leaves fresh. The leaves were renewed at an interval of 24 hours. On the other hand, two years old 10 air layered lemon plants of the variety elachi lebu were collected from a local nursery. They were potted in 10 earthen pots (30 cm × 20 cm × 15 cm). Each pot with the plant was veiled with a mosquito net (2 m × 1.5 m × 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 and placed in pair on lemon plants for mating and laying eggs. Later on the eggs were allowed to hatch on the potted plants and adequate number of first instar larvae was obtained for further study.

**Study of biology:** From the above laboratory culture, the newly hatched first instar larvae were placed singly in 10 petridishes. Those 10 petri dishes were used as 10 replications. The leaves were supplied as food for the larvae and were changed every 24 hours interval. Water socked cotton ball was used around petiole to keep the leaves fresh. The durations and the number of moultings of each larval instar were noted. The larval instars were determined by the presence of exuviae. As they reached final instar, larvae were placed on twigs of lemon plants. Finally, those were set in 10 glass jars (14 cm × 8 cm) to assist in pupation. The base of the twig was set in a narrow mouthed bottle filled with water to keep that fresh. Days required for the completion of the pupal stages were also recorded. When the adults emerged, a male and a female butterfly were paired inside the netted lemon plant and they were observed constantly to watch the pre-mating, mating, pre-oviposition, oviposition, incubation periods and fecundity.

**Effect of neem oil on food consumption:** 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). In control treatment leaves were treated with distilled water. Four hand sprayers were used for each treatment to avoid any admixture of the oils. Then the leaves from the plants were placed in 10 petridishes. Later on, the newly hatch larvae were individually placed in each petridish. The leaves were renewed after the change of each instar and later on the previously used leaf blade was measured. The quantity (mg) of leaf consumed by the different larvae was determined by deducting the weight of the healthy leaves from that of fed leaves. In this way the weight (mg) of leaf consumed by different larval instars was recorded with the help of an electric balance.

## RESULTS AND DISCUSSION

**Biology:** The period between the date of mating and the first egg deposition is the pre-oviposition period. A very short pre-oviposition period was found and it varied from 1-2 days with an average of 1.3 ± 0.2 days (Fig. 1). Radke and Kandalkar (1988) observed the pre-oviposition period of lemon butterfly and stated that it lasted for 1-2 days. The female butterfly laid eggs on the ventral surface of the leaves by bending her abdomen and touching the leaf surface. The mean oviposition period was 2.0 ± 0.0 days (Fig. 1). Rajendra *et al.* (1997) reported that the oviposition period ranged from 2.4 - 4.5 days on *C. reticulata* under laboratory conditions. The incubation period ranged from 4 – 5 days with a mean of 4.8 ± 0.1 days (Fig. 1). Nair (1975) reported that the incubation period of lemon butterfly varied from 3-7 days. Moulting of larvae took place four times and thus the larvae developed through five instars. The duration of larval period varied from 16.9-20.3 days with an average of 18.9 ± 0.3 days (Fig. 1).

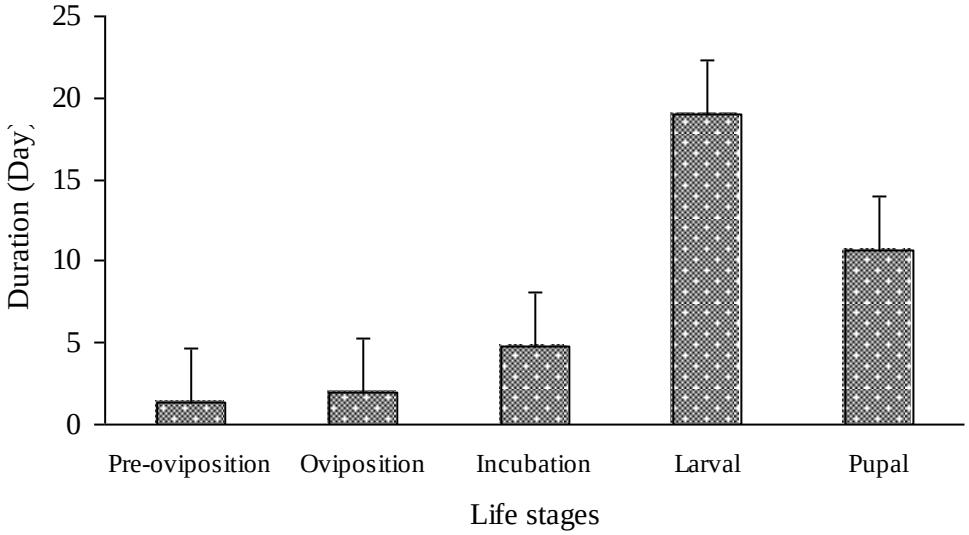


Fig. 1. Duration (mean ± SE) of different life stages of *P. demoleus* feeding on lemon leaves.

Before going to pupal stage, there is a pre-pupal stage that is recognized by their conspicuous segmentations with curved form. The final instar larva prepared a rough silken padding and girdling on stems and leaves of the host plant before pupation where the chrysalis is attached to the plant by its tail and is held with a silken girdle. At that stage, all larval characteristics were completely changed. The pupal period lasted for 10.0 - 11.1 days with a mean of 10.7 ± 0.2 days (Fig. 1).

The larvae hatched from the small, round and yellow eggs laid on the ventral surface of the leaves. Moulting of larvae took place four times and thus the larvae develop through five instars. Newly hatched 1$^{st}$ instar larvae are light brown and its duration ranged from 4.5 - 6.0 days with a mean of 5.5 ± 0.2 days (Fig. 2). The second instar larvae are spiny and darker in color. The duration of the 2$^{nd}$ instar larvae ranged from 4 - 5 days with a mean of 4.6 ± 0.2 days. The 3$^{rd}$ instar larvae are less spiny compared to the 2$^{nd}$ instar. The duration of the 3$^{rd}$ instar larvae lasted for 2 - 3 days with a mean of 2.6 ± 0.1 days. The 4$^{th}$ instar larvae turned into

black though the colour reflected little brownish tinge. It was observed that the larval duration of this instar ranged from 2 - 3 days with a mean of 2.4 ± 0.1 days.

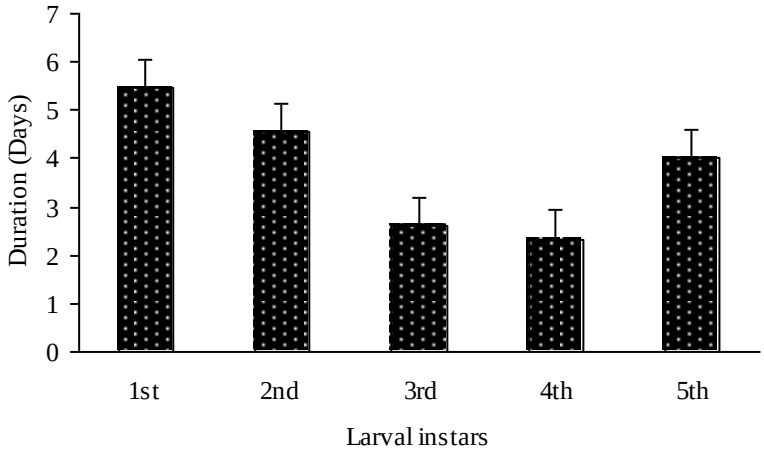


Fig. 2. Duration (mean day ± SE) of different larval instars of *P. demoleus* feeding on lemon leaves.

The fifth instar larvae are completely different from the earlier instars. The 5th instar larvae turned into yellowish green and they were spineless. There are green and brownish stripes on the pleural region of the body. The dorsal side of the last abdominal segment possessed a horn like structure. The duration of the 5th instar larvae lasted for four days. Rajinder *et al.* (1997) observed that the larva of this insect passed through 5 instars and lasted 5.7, 5.5, 2.6, 1.9 and 4.7 days for 1st, 2nd, 3rd, 4th and 5th instars, respectively. Similarly Singh and Gangwar (1989) reported that the duration of 1st-5th instars larvae of *P. demoleus* averaged 5.3, 5.1, 4.2, 4.2 and 3.5 days, respectively.

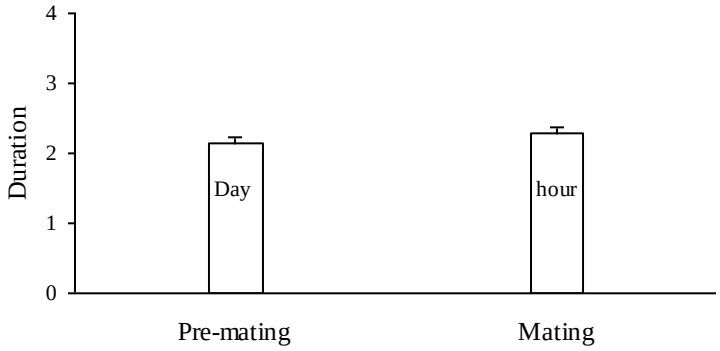


Fig. 3. Pre-mating (mean day ± SE) and mating duration (mean hour ± SE) of *P. demoleus* feeding on lemon leaves.

In *P. demoleus,* mating took place within two days of emergence of the adults (Fig. 3). Before mating the male came towards female with an excited motion and increased beating of wings. The female also helped in mating by extending her abdomen from side ways to touch the male genital organ. Duration of mating ranged from 1.5 - 3.3 hours with an average of 2.3 ± 0.2 hours (Fig. 3). At the time of mating the head region of the male and female remained in

opposite directions keeping tip of the abdomen firmly attached. Mating took place both in day and night. However, it was observed that normally mating lasted longer at night. It might be due to the fact that at night, there was no disturbance. Almost similar mating behaviour of lemon butterfly was observed by Radke and Kandalkar (1988). They also observed that mating lasted for 1.5 - 2.0 hours.

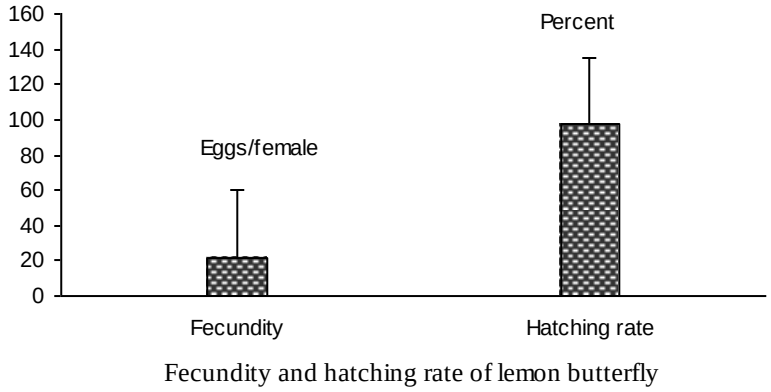


Fig. 4. Fecundity (mean number of eggs laid / female ± SE) and hatching rate (mean% ± SE) of *P. demoleus* feeding on lemon leaves.

The female laid 15 - 29 eggs in her life with an average of 22.1 ± 1.4 eggs (Fig. 4). Singh (1995) observed that the fecundity of *P. demoleus* varied between 11-14 days. Radke and Kandalkar (1989) found that the female laid 15 - 22 eggs. The hatching percentage of eggs varied from 93.8 - 100.0 with an average of 97.5 ± 1.3 (Fig. 4).

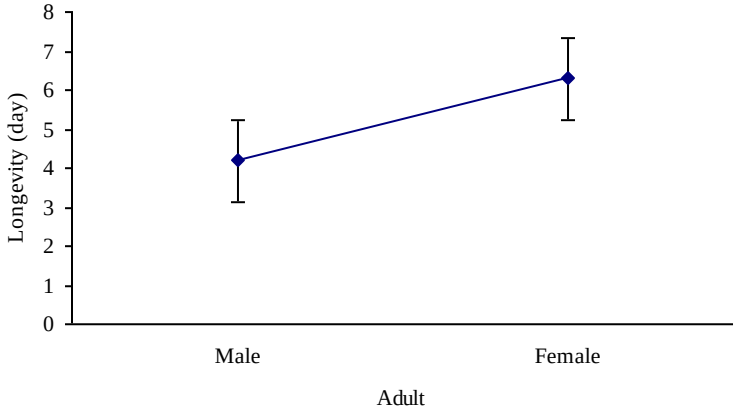


Fig. 5. Longevity (mean day ± SE) of male and female of adult *P. demoleus* feeding on lemon leaves.

The adult butterfly emerged by splitting the pupal case dorsally. Before emerging, the butterfly tears the pupal cover. The newly emerged butterfly possesses weak wings and is unable to fly. The adult butterfly is beautifully patterned; the body is yellow ventrally and the wings are

ornamentally colored. The longevity of the males was shorter than the female butterflies. The result revealed that the longevity of the male butterfly ranged from 4 - 5 days with a mean of 4.2 ± 0.2 days. But, the longevity of the female adults varied from 6 - 7 days with an average of 6.3 ± 0.2 days (Fig. 5). Singh (1995) observed that average longevity of female was 6.10 days and that of the male was 4.15 days.

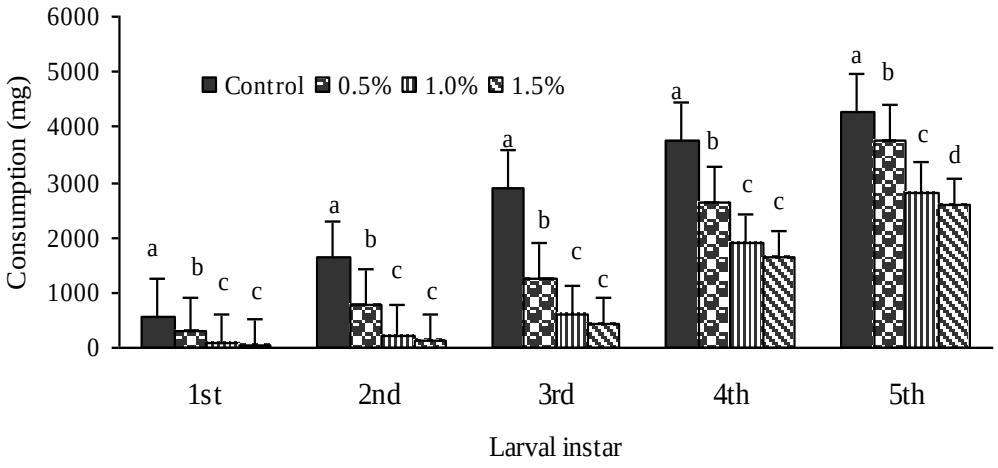


Fig. 6. Effect of neem oil on the amount of lemon leaves (mean g ± SE) consumed by different larval instars of *P. demoleus*.

**Effect of neem oil on food consumption:** Larva generally started feeding from the margin reaching inwards of the midrib of the leaf and voraciously fed the lemon leaves. Whereas, they feed at decreasing rates when the leaves were treated with 0.5, 1.0 and 1.5% neem oil (Fig. 6). When the first instar larvae were reared on untreated leaves, the mean leaf consumption by the larva was 573.0 ± 67.0 mg which was significantly greater than treated leaves. When 1$^{st}$ instar larvae were reared on 0.5, 1.0 and 1.5% oil treated food, the mean consumption of food were 288.0 ± 64.0, 88.0 ± 29.0 and 29.0 ± 23.0 mg, respectively. Same trend was observed on 3$^{rd}$, 4$^{th}$ and 5th instar larvae. But in 5th instar all concentrations showed significant differences.

In an experiment, Haque and Parvin (1996) applied 0.25, 0.5, 1.0 and 2.0% neem oil on brinjal leaf discs to observe the feeding response and survival rate of the 1$^{st}$ and 3$^{rd}$ instar larvae of epilachna beetle, *Epilachna dodecastigma* and found that the 1$^{st}$ instar larvae did not consume any food and all of them died. On the other hand, the 3$^{rd}$ instar larvae and the adult beetles were alive but consumed significantly lower amounts of food in comparison to control. From the food consumption test it was clearly revealed that the larvae did not prefer neem oil treated lemon leaves. Food consumption gradually decreased with the increase of oil concentration. Mordue (Luntz) and Nisbet (2000) reported that neem oil contain a large number of compounds with insecticidal properties and is effective on reducing population of several pest species. Its main active compound azadirachtin is toxic to over 500 insect species and acts mainly as food deterrent and growth disruptor (Flavia *et al.*, 2004). So, the finding paved the way to conclude

that neem oil could be used in managing lemon butterfly. However, proper dose(s) and other related issues must be standardized through further investigations.

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