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Biology of citrus butterfly *Papilio demoleus* L. (Papilionidae: Lepidoptera)

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Abstract

The present research was carried out to study the biology of the citrus butterfly *Papilio demoleus* L. (Papilionidae: Lepidoptera) under laboratory conditions. Observations on lifespan (days), adult longevity on various diets and leaf area consumed by various larval instars per day (sq. cm). Also, descriptions and measurements of various stages of *P. demoleus* on *Citrus limon* (L.) were carried out *in vitro* at room temperature. Results showed that, mean egg-hatching time was about 3.25 ± 0.91 days and 5.5 ± 1.28 , 4.5 ± 0.57 , 3.75 ± 0.48 , 3.75 ± 0.48 , 3.25 ± 0.70 days for 1st, 2nd, 3rd, 4th and 5th instars respectively. Pupal stage and average adult longevity recorded 6 ± 0.57 and 3 ± 0.57 days respectively. Adult longevity on distilled water, 10% honey, pure honey and 50% sugar + water solution recorded 2, 3, 5 and 3 days respectively. The mean value for egg size is 1 ± 0 mm and 2.4 ± 0.85 , 16.75 ± 1.25 , 21.5 ± 1.73 , 32.0 ± 0.81 , 35.7 ± 1.09 mm for 1st, 2nd, 3rd, 4th and 5th instar respectively. The size of pupa and adult were 26 ± 1.28 and 98.7 ± 2.89 mm respectively. The mean values for leaf area consumed per day were 2 ± 0.34 , 8.87 ± 2.37 , 15 ± 1.41 , 62 ± 4.24 , and 108 ± 3 cm² for 1st, 2nd, 3rd, 4th and 5th instar respectively. The results on life span, adult longevity with various food sources and leaf area consumption provide valuable scientific evidence on building citrus butterfly management practices and helps to access damage.

Keywords: biology, citrus butterfly, *Papilio demoleus*, Papilionidae

Introduction

Many insect pests attack citrus plants both in the nurseries as well as in the orchards inflicting heavy economic losses. Some of the most serious pests of citrus include *Papilio demoleus* L. and *Papilio polytes* L. (Citrus caterpillar), *Diaphorina citri* K. (Citrus psylla), *Phyllocnistis citrella* Stainton (Citrus leaf miner), *Aonidiella aurantii* Maskell and (Citrus red scale) and *Dialeurodes citri* Ash. (Citrus whitefly). Among them *Papilio demoleus* L. (Papilionidae: Lepidoptera), commonly known as orange dog, chequered swallowtail, lemon butterfly, is found throughout tropical and subtropical areas of Southern and Southeast Asia (Guerrero *et al.*)^[1]. Moreover, *P. demoleus* whose caterpillars are a serious insect pest feeding on citrus leaves and blossoms are a potential threat to citrus nursery stocks and other young citrus trees in Asia and the Middle East (Lewis; CABI)^[2, 3].

In India, in terms of area under cultivation, citrus stands third after Banana and Mango. Over the last 30 years, the area and production under citrus cultivation have increased at the rate of 11 and 9 per cent respectively, which shows that the expansion of the citrus industry was quite sustainable. The average yield of citrus fruits in India is alarmingly low (10.1 t/ha) compared to other developed countries like Brazil, USA, China, Mexico and Spain (30-40 t/ha). Globally, to develop improved strategies of managing *P. demoleus*, numerous studies have been conducted in various localities to provide valuable information on its biology (Singh and Gangwar; Ramarethinam and Loganathan)^[4, 5] and ecology (Badawi; Nandni *et al.*)^[6, 7] from sub-tropical to tropical regions. More importantly, a precise understanding of life history parameters of insect pests is essential for monitoring and studies of population dynamics. Considering these facts, the following research studies the life cycle, description of various stages and longevity of the adult insect when provided with different food source under laboratory conditions.

Material and Methods

This study was carried out *in vitro* under room temperature at Regional Agricultural Research Station (RARS), Vijayapur, Karnataka during the year 2019-20. Freshly laid eggs were collected along with plant materials and reared in the laboratory. The leaf petioles of the collected leaves were covered with a cotton swab which was soaked in water to avoid desiccation of the leaves and maintains humid conditions. The eggs were reared in plastic vials of about 8 cm in length. The egg hatched and started to feed on the leaves which were provided two times a day. Later, they were shifted to larger vials to accommodate good space and excreta was removed every day to ensure good hygienic conditions. Each vial was covered by a mesh to provide free ventilation. The time taken for adult emergence starting from the day of incubation was recorded. The adults were reared in large plastic boxes of about 30x15 cm. The longevity was estimated by providing three different food sources *viz.*, distilled water, 10% honey, pure honey and 50% sugar+ water solution with the help of the cotton swab dipped in the food source and hanged in the plastic box which contained adult butterfly. The length of each instar was measured using a milli meter (mm) scale. The area of leaf consumed by each larval instar per day is recorded as square centimetre by measuring the portion of leaf area eaten by the larvae with the help of grid paper or graph sheet.

Results and Discussion

Development time of eggs and immature stages of *P. demoleus*

The developmental periods such as incubation and growth of immature stage of *P. demoleus* on citrus leaves at room temperatures are summarized in Table 1. Mean values for egg hatching time were recorded 3.25 ± 0.91 days. The larvae typically had five stadia with 5.5 ± 1.28 , 4.5 ± 0.57 , 3.75 ± 0.48 , 3.75 ± 0.48 , 3.25 ± 0.70 days for 1st, 2nd, 3rd, 4th and 5th instars respectively, the longest development duration of larval instar observed is 1st in star with 5.5 ± 1.28 days. The mean developmental period for the pupal stage recorded 6.0 ± 0.57 days which was longer than other life stages. In this study, the incubation period was reported to be 3.25 ± 0.91 days which was found to be similar to the studies conducted by Atluri *et al.* [8] who reported a time of about 3 days for eggs in India. Likewise, Chen and Ouyang [9] also reported a hatching time of around 3 days for eggs of this species in Taiwan. In the present study, the total larval duration was found to be 15.4 ± 1.81 days which is slightly less than the larval period

reported by Chen and Ouyang [9]. Munir [10] reported that the duration of the larval stage up to the 5th instar for this species was in the range of 12.5 to 17.5 days in Pakistan. Our results stand intermediate and showed similar results to these studies. We attribute these discrepancies to different climatic conditions and mainly to diet differences. The larvae raised by Chen and Ouyang [9] were fed with leaves of *Citrus sunki* only, those reared by Munir [10] were given a combination of citrus and *Murraya* leaves, while our larvae fed on *Citrus limon* leaves only. The duration of the pupal stage in this study is about 6.0 ± 0.57 days. This is also slightly different from those reported by Chen and Ouyang [9] who reported 10.2 ± 0.1 days and Munir [10] reported a range of 9.5 to 25 days. Again, these variations can be attributed to regional differences brought about by the interplay of climate, weather and diets.

Eggs were mostly oviposited on the underside of host plant leaves, to protect from exposure to high rainfall and also as a precautionary measure from predators (Suwarno) [11]. Freshly laid eggs were 1 ± 0 mm in diameter and were lemon yellow. The eggs are hatched in about 3.25 ± 0.91 days and the newly hatched larva consumes the egg case to retain the nutrients and obtain nourishments (Anon) [12]. The length of the larva at this stage (first instar) is about 2.4 ± 0.85 mm. The larvae fed the leaves immediately after eclosion. The first three instars of larvae were reported to be brown in colour and the next two instars were light and dark green. These results are provided in Table 1. They usually consume the shed skin within few minutes after each moulting process.

Mature fifth instar larvae were ready to form a pupa. At this stage, they excreted soft and watery stools as opposed to dry and grainy stools in the other stages. Later, during the onset of pupation, their body was compressed and silk threads were produced. This was followed by the production of more silk threads from the mouth to fasten and anchor itself to the substratum. This can be termed the pre-pupal stage [Fig. 4]. The larva was in fixed position for 15 to 18 hours and the pupa was formed after shedding dried outer skin.

The pupa of Papilionidae was torpedo-shaped when viewed from the lateral part. Two colours of pupa were present *viz.*, light green and light brown [Fig 4]. This stage lasted for 6.0 ± 0.57 days. In later stages, the pupal skin turned slightly transparent and the dark coloured wing was seen through the semi-transparent skin. At the end of the metamorphosis, the adult butterfly moved and wiggled to break the cocoon and the adult form slowly crawled out of the skin in 10 to 15 minutes.

Table 1: Mean developmental stages of *P. demoleus* showing various parameters.

Parameters involved	Mean developmental stages of <i>P. demoleus</i>							
	Egg Stage	1 st Instar	2 nd Instar	3 rd Instar	4 th Instar	5 th Instar	Pupal stage	Adult
Length (mm)	1 ± 0	2.4 ± 0.85	16.75 ± 1.25	21.5 ± 1.73	32.0 ± 0.81	35.7 ± 1.09	26 ± 1.28	98.7 ± 2.28
Longevity (days)	3.25 ± 0.91	5.5 ± 1.28	4.5 ± 0.57	3.75 ± 0.48	3.75 ± 0.48	3.25 ± 0.70	6.0 ± 0.57	3.0 ± 0.57
Colour	Pale yellow	Brown	Brown	Brown	Green	Green	Green/ Brown	Lemon green

Measurement of various stages of *P. demoleus*

The mean values showed the egg size to be 1 ± 0 mm, and 2.4 ± 0.85 , 16.75 ± 1.25 , 21.5 ± 1.73 , 32.0 ± 0.81 , 35.7 ± 1.09 mm for 1st, 2nd, 3rd, 4th and 5th instars with pupa 26 ± 1.28 mm, and adult with wing span of 98.7 ± 2.89 mm respectively. The studies on measuring the various larval stages showed the similar results (Nandini *et al.*) [7], where larval length was found to be 4.06 ± 0.43 mm, 8.98 ± 0.62 mm, 18.61 ± 0.94 mm, 25.37 ± 0.99 mm and 35.09 ± 1.45 mm during different larval stages.

Longevity of *P. Demoleus* adult when reared on different food source

In this study, longevity of *P. demoleus* was examined on different diets of honey, sugar and water considered as natural food source of the species. Results of life span studies on various diets [Table 2] revealed that the adult butterfly which was reared on pure honey solution has shown high life span (5 days) and the one which was reared on distilled water was found to be the lowest (2 days) and the one which reared on 50% sugar+ water solution and 10% honey was found to be

intermediate (3 days each). Average life span of adult butterfly was reported to be 3 ± 0.57 mean days [Table 1]. A similar study by Dang [13] showed that the longevity of female adult was about 2-5 days on pure honey diet at 28.4 °C and 75.2% RH. For example, Badawi [6] reported that adult longevity on citrus ranged from 4 to 6 days with an average of 5.1 days during spring. Similar observations were made by Singh and Gangwar [4] while studying mandarin plants, where they reported the mean adult lifespan of male and female was about 5.1 and 5.8 days respectively. Additionally, seasonal climatic condition may influence adult longevity of the pest.

Table 2: Longevity of *P. demoleus* L. adult on different diets

Different types of food source	Life span of adult reared on different diets (days)
Distilled water	2
10% honey	3
pure honey	5
50% sugar+ water solution	3

Leaf-feeding capacity of *P. demoleus* larvae at different stages

Larvae of the citrus butterfly have shown a different capacity of food consumption during the larval instars. The leaf area consumed in cm² per day is presented in Table 3. Leaf feeding capacity of *P. demoleus* larvae on citrus leaves had a positive correlation with nymphal stages. Leaf feeding activities of 4th and 5th larvae were rapid in comparison to other larval stages. Our results are consistent with the results of Nandini *et al.* [7] and Dang [13].

Table 3: Mean leaf consumption of age-specific larvae

Age-specific larvae	Leaf consumption (cm ² /day)
1 st larvae	2 ± 0.34 cm ²
2 nd larvae	8.87 ± 2.37 cm ²
3 rd larvae	15 ± 1.41 cm ²
4 th larvae	62 ± 4.24 cm ²
5 th larvae	108 ± 3 cm ²

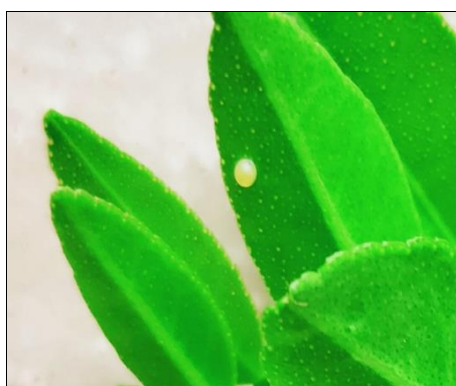


Fig 1: Egg of the lime swallowtail, *Papilio demoleus* L. deposited singly near leaf edge.

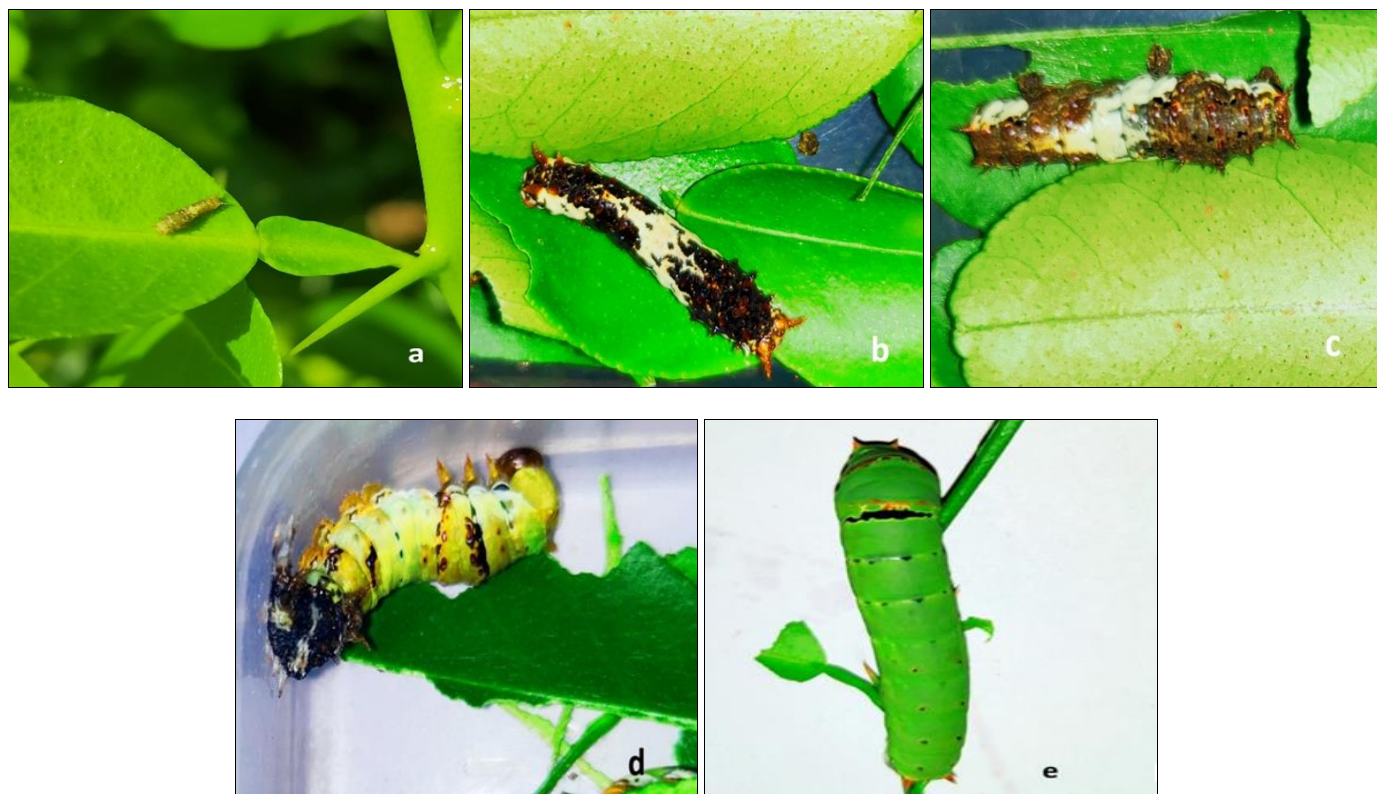


Fig 2: (a-e). a) 1st instar larvae b) 2nd instar larvae c) 3rd instar larvae d) emerging 4th instar after moulting and e) fully mature 5th instar caterpillar.



Fig 3: Caterpillar with osmeteria on thorax, early instar (left) and late instar (right)



Fig 4: left to right: mature fifth instar larvae undergoing pupation (left) and fully formed pupa showing dual colours (right)



Fig 5: a) Dorsal view of an adult butterfly, b) Ventral view of an adult butterfly

Conclusion

This study concluded that, among the different life stages of a citrus butterfly, pupa has shown highest life span with 6.0 ± 0.57 days followed by the first instar with 5.5 ± 1.28 days. Among the other stages 5th instar showed the lowest lifespan of 3.25 ± 0.70 days. The overall larval stage is about 15.4 ± 1.81 days. The results on longevity of adult butterfly fed with different food source showed that, the insect which was fed with the pure honey had higher life span (5 days) followed by 50% sugar+ water solution and 10% honey (3 days each). While, distilled water showed the shortest life span (2 days). Amount of leaf area consumed by the various larval instars was found to be the highest in the case of 4th and 5th instars when compared to the 1st, 2nd and 3rd stages. Results of study on biological traits and food habits of *P. demoleus* would be valuable scientific evidence on building citrus butterfly management programs by applying the insecticides at the susceptible stage of the insect. The leaf consumption data provides insights to estimate the damage loss in large areas.

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