

BIOLOGY AND MORPHOMETRIC STUDIES OF CITRUS BUTTERFLY *PAPILIO DEMOLEUS* LINNAEUS ON KINNOW MANDARIN (*CITRUS* *NOBILIS* × *CITRUS DELICIOSA*)

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ABSTRACT : The biology of citrus butterfly was studied under laboratory (room temperature 29-35°C; RH 60-75%) and screen house conditions on Kinnow mandarin. The results revealed that pre-oviposition, oviposition and post-oviposition period averaged 1.30±0.48, 4.20±0.79 and 2.44±0.45 days, respectively. A single female laid 100.9±4.04 eggs which hatched in 4.24±0.69 days. The mean duration of 1st, 2nd, 3rd, 4th and 5th larval instar was 2.7±0.48, 2.6±0.52, 2.9±0.31, 3.2±0.42 and 5.2±0.42 days, respectively. The mean longevity of male was 4.07±0.18 days and that of female as 6.84±0.19 days. Morphometric studies on *P. demoleus* revealed that the eggs measured 1.01±0.05 mm in diameter. Average body lengths of 1st, 2nd, 3rd, 4th and 5th instar larvae was 5.30±0.38, 9.18±0.11, 14.00±0.53, 26.21±2.02 and 45.00±2.62 mm, respectively. The average wingspan and body length measured 90.45±1.59 and 27.35±0.97 mm in male, while 90.93±2.13 and 28.58±1.24 mm in female, respectively.

Key words : Morphometrics, citrus butterfly, Kinnow, oviposition.

INTRODUCTION

Citrus (Family: Rutaceae) has originated from tropical and subtropical regions of Southeast Asia, particularly India and China. Northeast India is the native place of many citrus species. India ranks third in the global citrus production after China and Nigeria. The genus citrus includes a large number of species and cultivars, among which, mandarin, sweet orange (*mosambi*, *malta* or *satgudi*), lime and grapefruit are very popular. In citrus group plants, mandarin is the largest cultivated fruit in India and the world, whereas, sweet orange is the second largest growing species among the citrus group fruits. Mandarin (*Citrus reticulata* Blanco) occupies a premier position due to its high vitamin C and juice content with pleasant aroma and flavour. It is being traditionally cultivated in home gardens and modern plantations (Saljoqi *et al*, 2006) and is used in food industries, chemical and pharmaceutical industries, perfumes, cosmetics and aromatherapy. In India, mandarin is mostly grown in Maharashtra, Andhra Pradesh, Punjab and Haryana. In Haryana, the major citrus growing districts are Sirsa, Hisar, Bhiwani, Gurugram and Fatehabad. Mandarin is grown in the area of 429 thousand hectares with a production of 4754 thousand tonnes in India (National Horticulture Board, 2016). The area under citrus is increasing at a faster rate especially under Kinnow, which has emerged as the most remunerative and popular fruit

crop due to its wider range of adaptability, precocious bearing and fruit quality (Devi, 2014).

Despite a significant increase in area and production of citrus, its cultivation is under the severe threat of abiotic and biotic stresses, and among them, insect-pest problem is one of the major constraints. A number of insect-pests attack citrus plants in both the nurseries and orchards, inflicting heavy economic losses. Citrus in India is attacked by more than 250 insect pest species (Bhutani, 1979). Out Among various pests attacking the citrus, the citrus caterpillar [*Papilio demoleus* Linnaeus (Lepidoptera: Papilionidae)] is a serious pest, which is difficult to kill (Sarada *et al*, 2013). Outbreaks of this pest were reported in Nagpur during 1943 and in Maharashtra during 1974 and 1982-83 on citrus nurseries (Thakare and Borle, 1974 and Radke and Kandalkar, 1988).

Citrus caterpillar can feed and breed on all varieties of citrus including wild citrus plants. It was also recorded on various species (*Citrus sinensis*, *C. aurantifolia*, *C. reticulata*, *C. hystrix*, *Murraya koenigii*, *Aegle marmelos* etc.) of plants belonging to family Rutaceae (Atwal, 1964). It is an economically important pest, which at larval stage causes serious damage to citrus plants by devouring large quantity of foliage during later stages of their development. This insect is considered as the most important leaf-feeding insect as it causes extensive

damage to nurseries and young seedlings leaving behind midribs only, which results in defoliation of tree (Butani and Jotwani, 1975) and retardation of plant growth. The larvae prefer 30-60 cm tall young plants and are capable of completely defoliating nursery groves (Narayayanamma *et al*, 2001) in India. The citrus caterpillar can be found throughout the year, with population peaks in rainy days, the period of greatest vegetative flushes in citrus plants. The population peak of caterpillars is usually observed from August to October (Maheswarababu 1988; Mathur and Upadhyay, 1996 and Rampratap *et al*, 2000). Study of biology of an insect provides an opportunity to examine closely its different developmental stages and their survival; weak link in different stages; its behaviour and other parameters, which may help in planning its management in a better way.

MATERIALS AND METHODS

Studies on biology of citrus butterfly were carried out in the Laboratory and Screen House of the Department of Entomology. The observations recorded on biological parameters were tabulated and analyzed through Completely Randomized Design (CRD).

Rearing technique

Rearing was done in the laboratory at 29-35°C temperature and 60-75% relative humidity. To maintain the culture, a large number of *Papilio demoleus* L. larvae were collected from less than one year old Kinnow plants, brought from nursery to laboratory and kept in separate petri-dishes measuring 2.5 cm diameter. These larvae were fed on fresh Kinnow leaves. The mouth of each petri-dish was covered with muslin cloth. The Kinnow leaves were changed daily to avoid the dryness of leaves and the petri-dishes were changed after 2 to 3 days and cleaned with soap water to maintain hygiene. The larvae were allowed to pupate inside the petri-dishes. Most of the larvae pupated on muslin cloth and edge of petri-dishes. Everyday freshly formed pupae were transferred into glass jars for the emergence of adults. One pupa was kept in each glass jar, which was provided with a circular moist filter paper at its base for easy emergence of the butterfly. After adult emergence, ten pairs of newly emerged male and female were caged separately on Kinnow plants in pots covered with mosquito net (1.2 mm mesh size) on the top and tied from all sides. The caged plants were provided with cotton soaked in 10 per cent honey and sugar solution for adults. The eggs thus laid on both sides of tender leaves, twigs and stems were removed gently with the help of camel hair brush and kept in the petri-dishes with leaves for determining incubation period and viability of eggs.

Observations recorded

Observations on biological parameters *viz.*, pre-oviposition, oviposition and post-oviposition period, fecundity, incubation period, per cent egg hatchability, larval period, pre-pupal and pupal period, adult emergence percentage, adult longevity, total life cycle and sex ratio were recorded. Morphometric parameters on different stages of pest *viz.*, eggs (shape, colour and diameter), larvae (length, width and colour) of different instars, pupae (length, width and colour), adult *viz.*, body length (head to tip of abdomen), width and wing span were also measured. Eggs and first instar was observed under ocular microscope, while second instar larvae to adult butterfly were recorded by using a standard graphic paper method (Sukhatme and Amble, 1985).

RESULTS AND DISCUSSION

The results of duration of different stages and morphometric data of *P. demoleus* L. on Kinnow mandarin are presented here.

Egg

Observation taken on egg showed that a single female laid 95 to 109 eggs (average 100.9 ± 4.04 eggs) during her total life time (Table 1). The maximum number of eggs was laid on the second day of oviposition. In the previous studies, Maheswarababu (1988) and Patel *et al* (2017) also found similar egg laying capacity, who recorded 103 to 121 eggs (110.80 ± 4.46 eggs) per female. Eggs were laid singly on the lower surface of tender leaves and shoots of the citrus plant. Similar observations of egg laying behaviour were also reported by Phartiyal *et al* (2012); Rao (2015) and Patel *et al* (2017), who observed that eggs were mostly laid on the underside of the leaves. The diameter of eggs ranged between 0.90 and 1.08 mm with an average of 1.01 ± 0.05 mm (Table 2). The shape, size and colour of eggs was observed to be smooth, spherical, round creamy yellow in colour and colour changed to grayish with brown streaks all over the chorion before hatching. Similar results were made of Atwal (1964); Ganguli and Ghosh (1967); Maheswarababu (1988); Reshma *et al* (1986) and Rao (2015), who reported that the mean diameter of egg varied from 1.04, 1.00 to 1.10, 1.15, 1.20 and 0.90 to 1.07 mm, respectively.

Larva

Observation on duration, number of larval instars and total larval period were made on fifty freshly hatched larvae. The data mention in Table 1 reveals that the larvae passed through five instars before entering the pupal stage, which is in conformity with the observations of Durgesh *et al* (2012); Phartiyal *et al* (2012) and Patel *et*

al (2017). The newly hatched larvae were brownish black in colour, soft body with thorax thicker than rest of the body, cylindrical in shape, had dirty white mark on dorsal side showing resemblance to bird excreta. Similar morphometric characteristics were recorded by Grund (2002); Munir (2004) and Salmah *et al* (2002). The newly emerged caterpillar length and width varied as 2.41 ± 0.16 to 0.44 ± 0.05 mm in Table 2. These observations are in agreement with Reshma *et al* (1986), Maheswarababu (1988) and Rao (2015), who observed the newly emerged caterpillar with 1.0 to 1.5, 2.5 and 2.31 mm length and 0.48, 0.5 and 0.43 mm width.

First instar larva

The duration of first instar larva ranged from 2 to 3 days (2.7 ± 0.48 days) (Table 1). Similarly, it was also reported as 3 days (Ackery *et al*, 1995) and 2.80 days (Patel *et al*, 2017) on citrus. The larvae ranged between 4.58 and 5.70 mm in length, with a mean of 5.30 ± 0.38 mm and 1.52 to 1.71 mm in width, with a mean of 1.64 ± 0.05 mm (Table 2). These findings were in agreement with the report of Rao (2015) who depicted that the larvae ranged from 4.50-5.70 mm length and 1.50-1.70 mm width.

Second instar larva

Second instar larvae morphologically resembled to the first instar larvae, except being larger in size and slightly darker in colour as also reported by Grund (2002). Second instar larvae remained in this stage from 2 to 3 days with an average of 2.6 ± 0.52 days (Table 1). Similarly, Singh and Gangwar (1989) and Patel *et al* (2017) reported duration of second instar larva as 2-4 and 2.72 days, respectively. The data presented in Table 2 show that the larval length and width varied from 8.98 to 9.32 mm (average 9.18 ± 0.11 mm) and 2.79 to 3.00 mm (average of 2.91 ± 0.09 mm). These results were more or less similar to Patel *et al* (2017), who reported that length of second instar larvae was 8.13 ± 0.80 mm and breadth 3.48 ± 0.39 mm. Similar results were also reported by Durgesh *et al* (2012) for length (8.98 and 9.10) and Rao (2015) for width (2.82 mm). The second instar larvae perceived to be dark brown with a dirty white line present on the lateral sides of the abdomen but with a break on the dorsal side. A horn like structure was present on the dorsal side of the last body segment. These results are in agreement with Grund (2002) and Munir (2004).

Third instar larva

Third instar larvae were much longer, darker than preceding instar and took 2 to 3 days (average 2.9 ± 0.31 days) to complete this stage (Table 1). The results are in accordance with Phartiyal *et al* (2012) and Patel *et al*

(2017), who observed the third instar larval period found to be 2-3 and 2-4 days. The third instar larvae resembled the second instar larvae except for size. This is in agreement with respect to findings of Grund (2002) and Munir (2004). The length of third instar larva measured from 12.89 to 14.61 mm (14.00 ± 0.53 mm), while width measured from 3.40-4.12 mm (3.81 ± 0.20 mm) (Table 2). Similar observations were made by Durgesh *et al* (2012) and Rao (2015), who recorded 18.61 ± 0.94 , 13.38 mm length and 4.00, 3.72 mm width.

Fourth instar larva

The fourth instar larval period varied from 3 to 4 days with an average of 3.2 ± 0.42 days (Table 1). The present findings are in confirmation with the previous studies carried by Phartiyal *et al* (2012) and Patel *et al* (2017), who reported larval period as 4.6 and 3.8 days, respectively. The fourth instar larvae appeared black in colour with whitish bands visible on meso and meta thoracic lateral segments and on last anal segments. The description was in agreement with Munir (2004) and Salmah *et al* (2002). The data presented in Table 2 revealed that the average length and width of fourth instar larvae ranged from 22.64 to 28.09 mm and 5.41 to 6.01 mm, the average recorded as 26.21 ± 2.02 mm (length) and 5.80 ± 0.18 mm (width). Similar results were obtained by Maheswarababu (1988) 27.30-29.50 mm length and 5.35-6.00 mm width, Rao (2015) 22-29 mm length and 5.40-6.00 mm width and Patel *et al* (2017) 23.50 to 25.30 mm (average 24.32 ± 0.49 mm) length and 2.70 to 4.70 mm (average 3.82 ± 0.54 mm) width.

Fifth instar larva

The average duration of fifth instar larvae was 5.2 ± 0.42 days and ranged from 4 to 6 days (Table 1). Similar observation with respect to fifth larval instar were made by Ackery *et al* (1995) and Patel *et al* (2017), who recorded a period of 3-5 and 4-6 days, respectively. A fourth and fifth instar larva had an osmeterial gland in the first thoracic segment and was defensive in function. The description was in agreement with the Burger *et al* (1978) and Leslie and Berenbaum (1990). The fifth instar larva varied from green to yellowish green in colour, two eye-like spots were present on second thoracic segment and a horn like structure found on the dorsal side of the last body segment. Brownish stripes were present on eighth and ninth sternite with two semicircular yellowish bands on elevated portion of the body. The description was in agreement with Braby (2000) and Munir (2004). The length and width of fifth instar larva ranged from 40.00 to 48.00 mm (45 ± 2.62 mm) and 6.43 to 7.13 mm (6.79 ± 0.24 mm), respectively (Table 2). More or less

Table 1 : Duration of different stages of *Papilio demoleus* L. on Kinnow.

S. No.	Parameter (Insect stage/Duration)	Number observed	Range	Mean duration (days)
				Mean \pm SD
Egg				
1.	Incubation period (days)	50	3.00-5.00	4.24 \pm 0.69
2.	Hatching (%)	50	73.34-90.00	83.33 \pm 6.23
Larval period (days)				
3.	1 st instar	50	2.00-3.00	2.70 \pm 0.48
4.	2 nd instar	50	2.00-3.00	2.60 \pm 0.52
5.	3 rd instar	50	2.00-3.00	2.90 \pm 0.31
6.	4 th instar	50	3.00-4.00	3.20 \pm 0.42
7.	5 th instar	50	4.00-6.00	5.20 \pm 0.42
Total larval period (days)		50	16.00-19.00	17.60 \pm 0.97
8.	Pre-pupa	50	1.00	1.00 \pm 0.00
9.	Pupa	50	8.00-9.00	8.30 \pm 0.48
Adult (days)				
10.	Adult emergence (%)	10	72.00-85.00	76.00 \pm 6.51
11.	Pre-oviposition period	10	1.20-1.38	1.30 \pm 0.48
12.	Oviposition period	10	4.00-4.35	4.20 \pm 0.79
13.	Post-oviposition period	10	2.00-3.00	2.44 \pm 0.45
Adult longevity (days)				
14.	Female	10	6.40-7.10	6.84 \pm 0.19
15.	Male	10	3.90-4.50	4.07 \pm 0.18
Total life cycle (days)				
16.	Female	10	27.50-31.00	29.30 \pm 1.08
17.	Male	10	25.00-28.00	26.90 \pm 1.10
18.	Pre-mating (h)	10	10.00-13.00	11.97 \pm 0.95
19.	Mating (h)	10	1.60-4.00	3.88 \pm 0.13
20.	Fecundity (eggs)	10	95.00-109.00	100.90 \pm 4.04
21.	Sex ratio	50	1:1.30-1:2.00	1.00:1.20

similar observations were reported by Atwal (1964) 40 mm length and 6.5 mm width, Rao (2015) 38-45 mm length and 6.40-7.10 mm width and Patel *et al* (2017) 46.4-48.3 mm length and 3.1-5.2 mm width.

Total larval period

The total larval development period of *P. demoleus* ranged from 16 to 19 days (17.6 \pm 0.97 days). This is similar to the observations of Yunis and Munir (1972); Rafi *et al* (1989); Durgesh *et al* (2012); Phartiyal *et al* (2012), who recorded a period of 16.50, 16.00, 17.98 and 17-24 days, whereas Patel *et al* (2017) reported as 14 to 18 days. On the other hand, El-Khidir (1968) and Hills (1981) reported a larval period of 23-30 days whereas Mal *et al* (2015) recorded it as 13.52 days, which is different from the findings reported in this study. The variation in the larval period may be because of different host plants.

Pre-pupal and pupal period

Data presented in Table 1 revealed that pre-pupal period sustained for one day, whereas pupal period varied as 8 to 9 days with an average of 8.3 \pm 0.48 days. Similar

observations pertaining to this study were recorded by Rao (2015) as 0.98 days pre-pupal period and 7.99 days pupal period and Patel *et al* (2017) as 6-9 days. Pupae were naked and different in colour from green, straw to brown majority being green in colour with several black markings on the body. At the end of the pupal stage the wings and abdomen of the adult were clearly seen inside the pupal case. Earlier, Reshma *et al* (1986); Braby (2000) and Munir (2004) also reported similar findings. The average pre-pupal length and width was found to be 27.20 \pm 1.67 mm and 7.71 \pm 0.41 mm, respectively (Table 2). The pupa length measured from 27.00 to 34.00 mm (30.60 \pm 2.37 mm), while the width ranged from 8.66 to 9.67 mm (9.13 \pm 0.30 mm). The present findings are in conformity with Patel *et al* (2017), who estimated pupal length from 27.20 to 31.70 mm (30.04 \pm 1.07 mm), while breadth measured from 8.70 to 9.60 mm (9.22 \pm 0.24 mm).

Adult emergence and adult longevity

The per cent adult emergence ranged from 72 to 85 per cent with an average of 76.00 \pm 6.51 per cent. Similar results were also reported by Atwal (1976) and Phartiyal *et al* (2012), *i.e.*, 72 and 70 per cent. Adult

butterflies were large and beautiful with wide wings. The longevity of adult female varied from 6.40 to 7.10 days with an average of 6.84 \pm 0.19 days, whereas in case of male it varied from 3.90 to 4.50 days (average 4.07 \pm 0.18 days). The female adults lived longer than the males. These studies are in accordance with the findings of Atwal (1964); Singh and Gangwar (1989) who reported male and female average longevity of 5.70, 5.80 and 5.00, 5.10 days, and Patel *et al* (2017) reported 6-7 days in females and 3-4 days in males.

Incubation period and hatching per cent

Data pertaining to incubation period (Table 1) indicate that hatching started on third day after egg laying and continued up to fifth day. The incubation period varied from 3-5 days (average 4.24 \pm 0.69 days) and per cent egg hatchability ranged from 73.34 to 90.00 per cent (average 83.33 \pm 6.23%). These studies are approximately in agreement with the findings of other researchers have reported an incubation period of 4.9 (Phartiyal *et al*, 2012), 4.5 (Mal *et al*, 2015) and 1-4 days (Patel *et al*, 2017).

Table 2 : Morphological parameters of *P. demoleus* on Kinnow.

S. No.	Parameter (Insect stage)	Number Observed	Range	Measurement in (mm) Mean±SD
1.	Egg diameter	10	0.90-1.08	1.01±0.05
2.	Newly hatched caterpillar			
a.	Length	10	2.06-2.58	2.41±0.16
b.	Width	10	0.30-0.50	0.44±0.05
3.	First Instar Larvae			
a.	Length	10	4.58-5.70	5.30±0.38
b.	Width	10	1.52-1.71	1.64±0.05
4.	2nd Instar larvae			
a.	Length	10	8.98-9.32	9.18±0.11
b.	Width	10	2.79-3.00	2.91±0.09
5.	3rd instar larvae			
a.	Length	10	12.89-14.61	14.00±0.53
b.	Width	10	3.40-4.12	3.81±0.20
6.	4th instar larvae			
a.	Length	10	22.64-28.09	26.21±2.02
b.	Width	10	5.41-6.01	5.80±0.18
7.	5th instar larvae			
a.	Length	10	40.00-48.00	45.00±2.62
b.	Width	10	6.43-7.13	6.79±0.24
8.	Pre-pupa			
a.	Length	10	24.00-29.00	27.20±1.67
b.	Width	10	7.00-8.32	7.71±0.41
9.	Pupa			
a.	Length	10	27.00-34.00	30.60±2.37
b.	Width	10	8.66-9.67	9.13±0.30
10.	Adult butterfly			
A	Male			
a.	Length	10	25.96-29.00	27.35±0.97
b.	Width (Head, thorax and abdomen)	10	5.90-6.20	6.03±0.11
c.	Wing expansion	10	87.00-92.28	90.45±1.59
B	Female			
a.	Length	10	26.00-30.00	28.58±1.24
b.	Width	10	6.00-6.52	6.33±0.16
c.	Wing expansion	10	87.59-94.00	90.93±2.13

Per cent hatchability observed in the present studies was in agreement with the findings of Patel *et al* (2017), who reported 70.48% egg hatchability, when reared on citrus. The differences in the incubation period and hatching (%) were probably due to variations in the weather factors of different regions.

Sex ratio

The average male and female sex ratio was 1.00: 1.20. The data indicated that females outnumbered males, which is not in agreement with the findings of Patel *et al* (2017) and Rao (2015), who recorded sex ratio of male to female as 1: 2.21, 1: 2.25 and 1: 2.14 on different host plants. The variation in the sex ratio may be due to

different host plants.

Pre-oviposition, oviposition and post-oviposition period

The pre-oviposition, oviposition and post-oviposition period ranged from 1.20 to 1.38 days (average 1.30±0.48 days), 4.00 to 4.35 days (average 4.2±0.79 days) and 2 to 3 days (average 2.44±0.45 days), respectively (Table 1). Earlier pre-oviposition, oviposition and post-oviposition period were reported by Maheswarababu (1988) 1.52-1.82, 3.20-4.76 and 0.11-0.88 days, Rao (2015) 1.22-1.36, 4.12-4.58 and 2.20-2.59 days and Patel *et al* (2017) 1-2, 4-5 and 2-3 days.

Pre-mating and mating period

Mating period of *P. demoleus* adult was observed during early hours of the day on tender twigs. Similar studies were carried out by Atwal (1964); Radke and Kandalkar (1988) and Munir *et al* (2007). The pre-mating and mating period varied from 10 to 13 h and 1.60 to 4.00 h with an average duration of 11.97±0.95 h and 3.88±0.13 h, respectively. Earlier, Rao (2015) observed the mating period varied from 1.90 to 11.75 h.

Total life cycle

The total life cycle of male varied from 25 to 28 days (average 26.9±1.10 days), whereas female life cycle was completed 27.50 to 31.00 days with an average of 29.3±1.08 days (Table 1). Therefore, a total life period of male was smaller than that of female. This was in agreement with the findings of Schmutterer (1969) and Patel *et al* (2017), who observed the total life cycle varied from 29 to 32 days, 23 to 26 days of male and 26 to 29 days of female. The length of adult male varied from 25.96 to 29.00 mm (27.35±0.97 mm) and the width ranged from 5.90 to 6.20 mm (6.03±0.11 mm), whereas wing expansion altered from 87.00 to 92.28 mm (90.45±1.59 mm), respectively. The average length, width and wing expansion of female butterfly found to be 28.58±1.24 (ranged from 26.00-30.00 mm), 6.33±0.16 (varied from 6.00-6.52 mm) and 90.93±2.13 mm (extended from 87.59-94.00 mm), respectively. Similar observations were reported by Rao (2015), according to their study, the length, width and wing expansion of male butterfly was observed as 26.95, 6.02 and 90.57 mm,

while female butterfly length, width and wing expanse was found to be 28.40, 6.30 and 90.78 mm, respectively.

In case of morphological parameter, the abdomen of male butterfly was pointed, whereas in case of female it was observed to be round in shape. Head, thorax and legs were black with creamy yellow streaks on either side of entire abdomen, antennae were black and club shaped. The body was covered with black and yellow hairs and shape of fore wings was triangular, while hind wings were rounded. The wings were black in colour with yellow markings. There were two rows of parallel yellow spots laterally on outer margins of wings of female and a brick red oval patch on posterior angle of the hind wing of male. These findings are in agreement with Maheswarababu (1988), Reshma *et al* (1986) and Poorten (2004)

CONCLUSION

The mean duration of *P. demoleus* egg, larvae and pupa was 4.24, 17.60 and 8.30 days, respectively. The adult male lived for 4.07 days and female for 6.84 days with a total life span of 26.90 and 29.30 days for male and females, respectively.

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