EFFECT OF TEMPERATURE ON THE DEVELOPMENT OF CITRUS BUTTERFLIES, *PAPILIO DEMOLEUS* AND *PAPILIO POLYTES* ON ACID LIME, *CITRUS AURANTIFOLIA*

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Abstract. The physiological and metabolic response of insects to varied temperature has getting enormous attention in recent times. Insects are poikilothermic organisms; their growth, development, reproduction and survival depend on temperature. The citrus butterflies, *Papilio demoleus* L. and *Papilio polytes* L. are two important butterfly pests on citrus crops. The larval stage of pest inflicts economic damage by defoliating large quantity of foliage. The hypothesis that the development of these citrus butterfly species mainly depends upon surrounding temperature was studied under controlled laboratory condition at constant temperature of 23, 28 and 32°C on leaves of acid lime (*Citrus aurantifolia* Swingle). The results revealed an incubation period of 3.39, 3.06 and 2.82 days respectively, at 23, 28 and 32°C for *P. demoleus* whereas the incubation period was 3.83, 3.61 and 3.08 days respectively at 23, 28 and 32°C for *P. polytes*. The larval duration ranged from 11.18 to 16.49 and 13.15 to 14.88 days, respectively for *P. demoleus* and *P. polytes* at different temperature regimes. The pupal duration of 8.83 to 17.53 and 8.40 to 13.36 days were observed for *P. demoleus* and *P. polytes* respectively. The *P. demoleus* and *P. polytes* took 27.83 to 44.67 and 31.78 to 41.47 days respectively, for completion of their entire life cycle. Overall, development of two citrus butterfly species was significantly affected by temperature and temperature was found to be a key factor that influenced the development of these two citrus butterflies on acid lime.
Keywords: *metabolism, physiology, abiotic factor, incubation period, total life cycle*

Introduction

Temperature is one of the important abiotic factors that influences various biological phenomena in insects *viz*., growth, development, body size, sex ratio, life span, voltinism, fecundity, fertility and survival (Yang et al., 1994; Zeng et al., 2008; Shi et al., 2014; Kobori and Hanboosong, 2017). Thereby temperature greatly affects abundance, distribution, colonization, behavior and fitness of insects (James et al., 2002; Parmesan and Yohe, 2003; Ju et al., 2011). The temperature at which species develop, reproduce and survive can be considered as optimum temperature for particular species at which growth and development will not be affected, above or below this temperature the development can be severely affected (Begon et al., 2006; Du-Plessis et al., 2020). In recent times the physiological response of insects to

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temperature has getting lot of attention worldwide as this information may be useful in developing prediction models thereby to know the life history patterns of insects and to plan for timely intervention measures against harmful insect pests.

The citrus butterflies, *Papilio demoleus* L. and *Papilio polytes* L. are two economically important insect pests of citrus (Singh, 1993). These pests are known to be found throughout Southern and Southeast Asia (Corbet and Pendlebury, 1992). The larvae are the serious defoliator of the plants belongs to family rutaceae and pose potential threat to their production (Patel et al., 2017b). These pests are reported to complete 3 to 12 generations in a year depending on the weather conditions and host plants on which they feed (Alturi et al., 2002; Sarada et al., 2014). The succession of arthropods' development is mostly affected and influenced by temperature and humidity. In warmer temperature and high humidity, insects have a tendency of growing faster. The opposite conditions have also been noted to retard insect growth significantly (Grassberger and Reiter, 2001).

The metabolic theory of ecology opines that temperature and body size are fundamental determinants of development, reproduction, population growth and species diversity of organisms (Brown et al., 2004). At the favorable temperature ranges insects can grow at a rapid rate, as a result insect can pass through the vulnerable life stages very quickly to get protection from natural enemies (Jaworski and Hilszczanski, 2013). So, under changing climatic conditions, it is of paramount importance to determine the favorable range of temperatures for the development of citrus butterflies. Hence, the objective of this study was to determine the developmental rate of two citrus butterfly species namely *P. demoleus* and *P. polytes* at constant temperatures and number of degree-days (°D) required to complete each developmental stage of test insects. The data generated during present study may be useful in predicting seasonal and phenological development, population dynamics, life history patterns and useful in developing forecasting models. Thereby it can help to initiate suitable control measures against these insect pests.

Materials and methods

Field collection and laboratory rearing of test insects

The field culture of *P. demoleus* and *P. polytes* were collected from the acid lime orchards near College of Agriculture, Vijayapura, Karnataka, India (16°49'39.1620" N 75°43'31.1772" E). The collected larvae were reared in plastic containers (12 × 12 × 5 cm) with wire mesh covered at the top and provided with leaves of acid lime, *Citrus aurantifolia* Swingle twice in a day. Every day fresh leaves were provided for growing larva until completion of larval stage. The larvae were reared at 27 ± 1°C, 60-70% RH and 14 L: 10 D photoperiod in the laboratory. Later, pupae were collected and placed in an oviposition cage (50 × 50 × 70 cm) for adult emergence. After emergence, adult butterflies were allowed for mating after sexual identification. Ten pairs of adults were allowed for mating and oviposition. The acid lime seedlings provided as substrate for the oviposition. The cotton soaked in 10% honey solution was provided as food source. The observations were made every day for collection of eggs from each species and taken for further studies.

The effect of temperature on development of egg, larva and pupa of P. demoleus and P. polytes

Acid lime leaves along with freshly laid eggs of respective species of citrus butterflies were collected and placed in controlled chamber at 23, 28 and 32°C with 60-70% RH and

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14 L: 10 D photoperiod. About 30 eggs from each species were placed in petri dish (9 cm diameter and 2 cm height) with a piece of moistened filter paper and kept in controlled chamber at respective temperatures. The eggs were observed daily for hatching and number of days taken for hatching was recorded. The freshly hatched larva was placed in Petri dishes individually (9 cm diameter and 2 cm height) and fed with fresh leaves of acid lime twice in a day until the completion of larval stage. The observation on number of instars and duration of each instar was recorded based on molted skin and head capsule size. Upon formation of pupa, developmental duration of pupa was recorded until the adult emergence at respective temperatures. The freshly emerged adult insects were released into oviposition cages pairwise and provided with 10% honey as food source. The fresh seedling of acid lime was provided as oviposition substrate. The observations on number of eggs laid, longevity of male and female adults, pre-oviposition, oviposition and post-oviposition periods were recorded at respective temperatures.

Statistical analysis

The mean developmental time for completion of egg, larva, pupa and adults for both the species of citrus butterflies at each temperature were computed and standard error was determined using SPSS software. The comparison of developmental duration of each stage at respective temperatures was made using student's t-test. The P value (<0.05) was considered as upper limit for significant difference for developmental duration of each stage. The P value more than 0.05 was considered as non-significant. Later comparisons were made using Duncan's multiple range test ($p < 0.05$). The mean number of degree-days (°D) required for the development of egg, larva, pupa and adults of both the species of test insects were calculated using the formula $(°D) = T(c\text{-}T_{min})$ (Jackson and Elliot, 1988), where T is the number of days taken to complete the development at a constant temperature (c) and T_{min} is the minimum temperature for development of respective life stage of test insect.

Results

The developmental duration of egg, larvae and pupae of P. demoleus

The developmental duration of egg, different larval instars and pupa of *P. demoleus* on acid lime at different constant temperature regime is presented in *Figure 1*. A significant difference in developmental duration was observed for immature stages when reared at three constant temperature regimes ($p < 0.05$). The results indicated that developmental duration was significantly decreased at higher temperature (32°C) whereas it was prolonged at low temperature (23°C). The incubation period was 3.39, 3.06 and 2.82 days, respectively at 23°C, 28°C and 32°C. The eggs of *P. demoleus* were hatched early at higher temperature and took long time for hatching at low temperature. The larva molted four times and consists of five larval instars. The longer duration of larva was observed at 23°C (16.49 days), while the shorter larval duration was noticed at 32°C (11.18 days). The development of pupa was influenced by the different temperatures. The pupal developmental duration was 8.83 days at 32°C, while it was considerably extended to 17.53 days at 23°C (*Fig. 1*).

The overall life cycle duration of *P. demoleus* was significantly prolonged at lower temperature, while it completed early at higher temperature. The male sex of *P. demoleus* was completed its life cycle in 27.83, 33.33 and 42.47 days, respectively at

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32°C, 28°C and 23°C whereas, female sex of *P. demoleus* was completed its life cycle in 29.78, 35.53 and 44.67 days, respectively at 32°C, 28°C and 23°C (*Fig. 2*). The mean number of degree days required for the completion of egg, larva and pupa of *P. demoleus* was 19.18, 327.67 and 154.79 °D, respectively. The number of degree days required for egg to adult male and adult female was 725.30 and 806.24 °D, respectively (*Table 2*). The temperature of 32°C was found to be favorable range for the rapid development of *P. demoleus*.

Table 1. *The fecundity, pre-oviposition, oviposition and post-oviposition period of citrus butterfly, Papilio demoleus L. reared on acid lime at various temperatures*

Sl. No.	Particulars	Duration (days)		
		23°C	28°C	32°C
		Mean ± SE	Mean ± SE	Mean ± SE
1	Fecundity	19.30 ± 2.18	20.45 ± 1.50	18.25 ± 3.31
2	Pre-oviposition	1.36 ± 0.05 a	1.01 ± 0.01 b	1.20 ± 0.04 ab
3	Oviposition	3.75 ± 0.05 a	3.95 ± 0.05 a	3.65 ± 0.06 a
4	Post-oviposition	1.29 ± 0.06 a	1.24 ± 0.04 a	1.19 ± 0.05 a

SE- Standard Error. (Means within the same row followed by a different letter are significantly different at $P < 0.05$ Duncan's multiple range test)

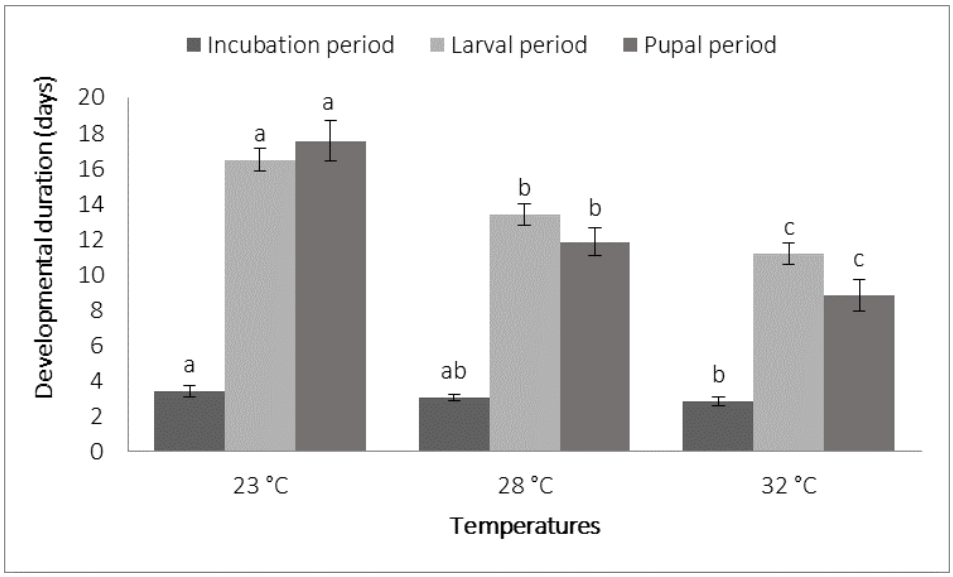

Figure 1. *The development duration of life stages of citrus butterfly, Papilio demoleus L. reared on acid lime at various temperatures. (The same colored bars followed by different letters differ significantly at $p < 0.05$)*

Fecundity and longevity of adults of P. demoleus

The average fecundity of *P. demoleus* was 18.25, 20.45 and 19.30 eggs/female at 32°C, 28°C and 23°C respectively. The longevity of female *P. demoleus* was ranged from 6.04 to 6.38 days and that of male butterfly was varied from 3.99 to 4.06 days at different temperature (*Fig. 3*). The effect of temperature was found to be non-significant on longevity of female and male sex of *P. demoleus*. The pre-oviposition (1.01 to 1.36 days),

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oviposition (3.65 to 3.95 days) and post-oviposition period (1.19 to 1.29 days) were observed at different temperatures for *P. demoleus* when reared on acid lime (*Table 1*).

Table 2. *The mean development time and degree-days (°D) for Papilio demoleus L. at constant temperatures*

Developmental stage	Temperature (°C)	Developmental time (days)	Mean number of degree-days (°D)
Egg	23	3.39	6.17
	28	3.06	20.87
	32	2.82	30.51
Mean			19.18
Larva	23	16.49	327.49
	28	13.39	332.88
	32	11.18	322.65
Mean			327.67
Pupa	23	17.53	149.53
	28	11.83	160.06
	32	8.83	154.79
Mean			154.79
Adult male	23	42.47	721.57
	28	33.33	732.93
	32	27.83	723.30
Mean			725.93
Adult female	23	44.67	801.38
	28	35.53	815.06
	32	29.78	802.27
Mean			806.24

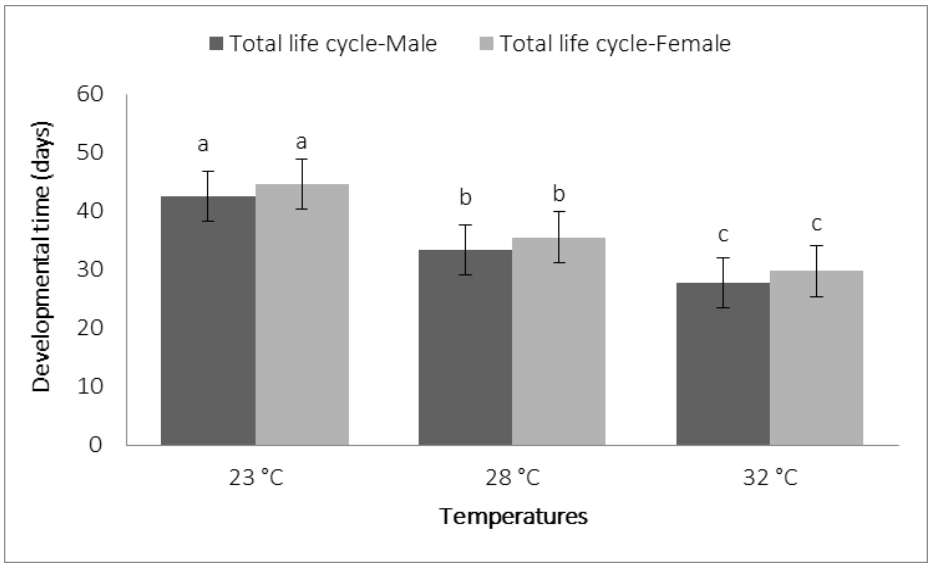

Figure 2. *The effect of temperatures on total development male and female of citrus butterfly, Papilio demoleus L. reared on acid lime. (The same colored bars followed by different letters differ significantly at $p < 0.05$)*

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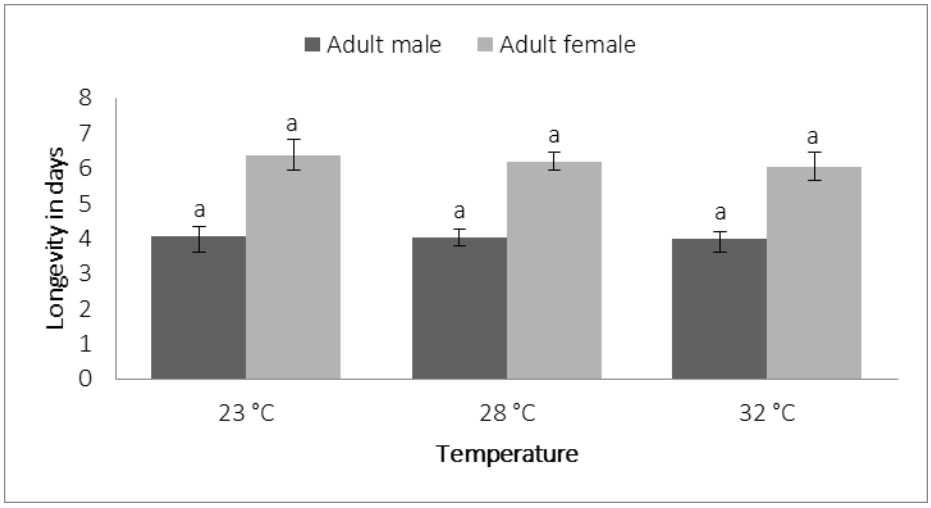

Figure 3. *The longevity of male and female of citrus butterfly, Papilio demoleus L. reared on acid lime. (The same colored bars followed by different letters differ significantly at $p < 0.05$)*

The developmental duration of egg, larvae and pupae of P. polytes

The developmental duration of egg, larva and pupa of *P. polytes* on acid lime at various temperatures is presented in *Figure 4*. The results revealed a significant influence of temperature on developmental duration of immature stages of *P. polytes*. The incubation period was ranged from 3.08 to 3.83 days. The hatching of eggs was delayed at lower temperature (23°C), while it was faster at high temperature (32°C). The larva typically consist of five instars and completed their development in 13.15, 13.69 and 14.88 days, respectively at 32°C, 28°C and 23°C. The temperature was found to significantly influence on larval development ($p < 0.0002$). The pupal duration was ranged from 8.40 to 13.36 days, where the pupal development delayed at low temperature (23°C) while faster developmental rate was noticed at high temperature (32°C) (*Fig. 4*).

Table 3. *The fecundity, pre-oviposition, oviposition and post-oviposition period of citrus butterfly, Papilio polytes L. reared on acid lime at various temperatures*

Sl. No.	Particulars	Duration (days)		
		23°C	28°C	32°C
		Mean ± SE	Mean ± SE	Mean ± SE
1	Fecundity	21.70 ± 1.45	23.05 ± 2.50	20.50 ± 3.10
2	Pre-oviposition	1.18 ± 0.04 a	1.13 ± 0.03 a	1.05 ± 0.02 a
3	Oviposition	5.96 ± 0.07 a	5.98 ± 0.06 a	5.94 ± 0.07 a
4	Post-oviposition	1.20 ± 0.04 a	1.18 ± 0.04 a	1.15 ± 0.04 a

SE- Standard error. (Means within the same row followed by a different letter are significantly different at $P < 0.05$ Duncan's multiple range test)

The rearing temperature influenced significantly on overall development of *P. polytes*. The male sex of *P. polytes* was completed its life cycle in 31.78, 35.54 and 39.42 days, respectively at 32°C, 28°C and 23°C. Whereas, female sex of *P. polytes* was completed its life cycle in 33.73, 37.54 and 41.47 days, respectively at 32°C, 28°C and

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23°C (*Fig. 5*). The number of degree days required for the completion of egg, larva and pupa of *P. polytes* was 45.17, 325.66 and 201.28 °D, respectively. The number of degree days required for egg to adult male and adult female was 921.32 and 966.09 °D, respectively (*Table 4*). The temperature range of 28 to 32°C was found to be suitable for faster completion of life cycle of this pest.

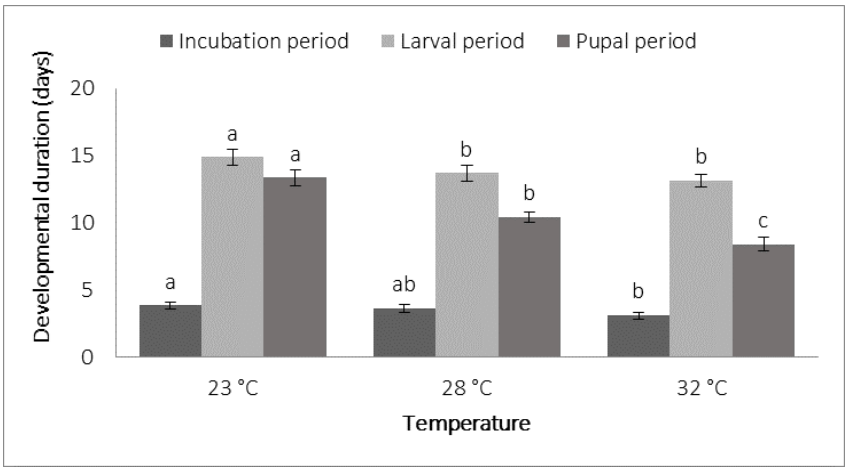

Figure 4. *The development duration of life stages of citrus butterfly, Papilio polytes L. reared on acid lime at various temperatures. (The same colored bars followed by different letters differ significantly at $p < 0.05$)*

Table 4. *The mean development time and degree-days (°D) for Papilio polytes L. at constant temperatures*

Developmental stage	Temperature (°C)	Developmental time (days)	Mean number of degree-days (°D)
Egg	23	3.83	32.67
	28	3.61	48.84
	32	3.08	53.99
Mean			45.17
Larva	23	14.88	281.83
	28	13.69	327.74
	32	13.15	367.41
Mean			325.66
Pupa	23	13.36	197.59
	28	10.43	206.41
	32	8.40	199.84
Mean			201.28
Adult male	23	39.42	849.50
	28	35.54	943.59
	32	31.78	970.88
Mean			921.32
Adult female	23	41.47	885.38
	28	37.54	989.18
	32	33.73	1023.71
Mean			966.09

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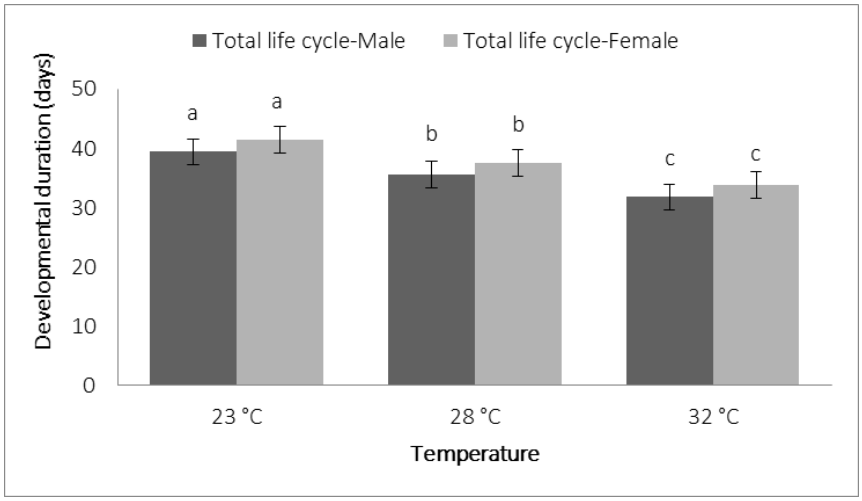

Figure 5. *The effect of temperatures on total development male and female of citrus butterfly, Papilio polytes L. reared on acid lime. (The same colored bars followed by different letters differ significantly p < 0.05)*

Fecundity and longevity of adults of P. polytes

The average fecundity of *P. polytes* was 20.50, 23.05 and 21.70 eggs/female at 32°C, 28°C and 23°C respectively. The longevity of female *P. polytes* was ranged from 8.14 to 8.29 days and that of male was varied from 6.06 to 6.35 days at different temperature (*Fig. 6*). The temperature produced non-significant influence on longevity of female and male sex of *P. polytes*. The pre-oviposition (1.05 to 1.18 days), oviposition (5.94 to 5.98 days) and post-oviposition period (1.15 to 1.20 days) were observed at different temperatures for *P. polytes* when reared on acid lime (*Table 3*).

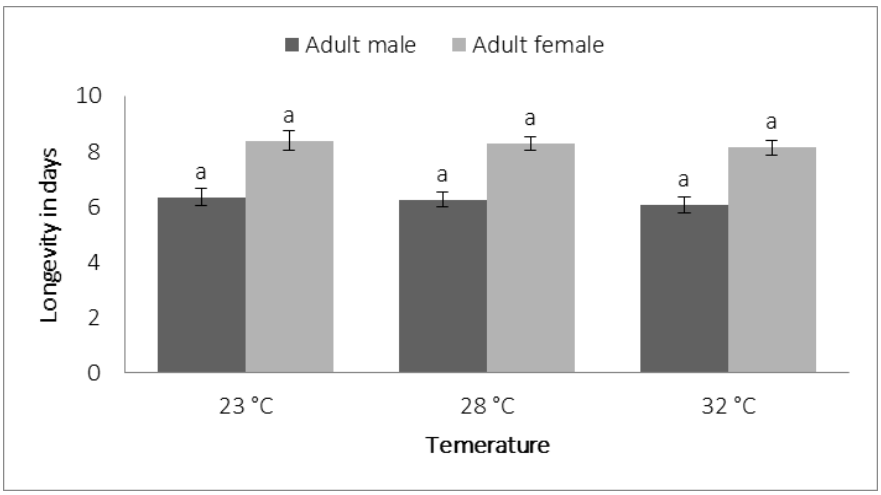

Figure 6. *The longevity of male and female sex of citrus butterfly, Papilio polytes L. reared on acid lime. (The same colored bars followed by different letters differ significantly p < 0.05)*

Discussion

Metabolism is the chemical and biological process occurring in living organisms that convert food into energy and helps organisms to grow. Organisms take up resources and

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converts into useful forms at various parts of the body and same products will be utilized for physiological processes like survival, growth, development and reproduction. Often the metabolism in insects is highly temperature dependent (Brown et al., 2004; Sinclair, 2015).

The development of citrus butterfly species, *P demoleus* and *P polytes* was significantly affected at change in temperatures. The developmental duration of egg, larva and pupa of *P demoleus* was reduced considerably at higher temperature (32°C), whereas it was delayed at lower temperature (23°C). The incubation period was varied from 2.82 to 3.39 days at different temperatures. These findings are supported by Minh et al. (2015) where they reported that incubation period of *P. demoleus* was 2.60 days at 30°C and 3.55 days at 25°C. Similarly, Shrivastava and Rahalkar (2016) reported that incubation period was longer in winter season and shorter in summer season for *P. demoleus*. The larval developmental duration of *P. demoleus* was 11.18, 13.39 and 16.49 days respectively, at 32°C, 28°C and 23°C. Similarly, Minh et al. (2015) and Patel et al. (2017a) recorded larval duration ranging from 11.50 to 18 days at different temperature regimes. The pupa took 8.83 to 17.53 days for eclosion at various temperatures. The pupa of *P. demoleus* were found to be most sensitive to change in temperature and developmental duration may vary from 5 to 20 days (Munir et al., 2007; Minh et al., 2015 Raut et al., 2018). Identically, Islam et al. (2017a) also opined that pupal eclosion extend 6.8 time more during winter months when the daily mean temperature was below 25°C. The longevity of adult butterfly was not affected by prevailing temperature. In similar to present study Karim et al. (2007), Patel et al. (2017a) and Jahnavi et al. (2018) revealed adult longevity of 3.81 to 4.20 and 6.30 to 6.80 days respectively, for male and female butterflies. However, the food source which is rich in proteins may have positive influence on adult life span and fecundity. The overall development from egg to adult was ranged from 27.83 to 42.47 days for male butterfly, whereas female butterfly took 29.78 to 35.53 days for completion of life cycle depending on the temperature. The observations are supported by Islam et al. (2017) where they have reported that, the development of *P. demoleus* was slow at lower temperatures (18-25°C) and faster at higher temperatures (27-30°C). Temperature is the most dominant abiotic factor that directly affects development, survival, range and abundance of herbivore insects, however species with large geographical range is tend to be less affected (Bale et al., 2002). The availability of abundant heat at high temperature may provide efficient thermal budget for growth and reproduction, while absence of abundant heat energy at low temperature may lead to delayed development, sometimes may affect survivability of insect.

The information on influence of temperature on egg hatching duration of *P. polytes* is scanty. However, compared with earlier studies on bionomics of same insect. During present study a significant variation was observed in hatching duration of eggs of *P. polytes* when the test insect was reared at different constant temperatures. The incubation period was ranged from 3.08 to 3.83 days. Similarly hatching duration of 3 to 4 days recorded for *P. polytes* (Suwarno et al., 2007; Jaafar et al., 2014; Islam et al., 2017). The larval developmental duration was 13.15, 13.69 and 14.88 days respectively, at 32°C, 28°C and 23°C. Similar observations were made by Gaikwad and Bhawane (2013) where duration of larval stage was completed early during rainy (20.02 days) and summer months (20.94 days), whereas larval duration was extended up to 22.64 days during winter months. The pupal duration was varied from 8.40 to 13.36 days at various temperatures. Identically, Gaikwad and Bhawane (2013) and Islam et al.

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(2017) opinioned that pupal development was slow during winter months compared to summer months. The overall development of *P. polytes* was significantly affected when the insect was reared at three constant temperatures. The insects require certain amount of temperature in order to complete each life stages in one life cycle that can be called as thermal constant (Begon et al., 2006), so the low temperature may have prolonged reaching of equilibrium of thermal constant for each life stage during development of *P. polytes*. While at high temperature, the life stages may have reached equilibrium of thermal constant very early because of availability of good amount of heat.

The physiology of insect herbivore is sensitive to change in temperature (Regniere et al., 2012). The metabolic rates of insect will be doubled for every 10°C rise in temperature. The increased metabolic rates may have resulted in completion of development early at high temperature while low temperature may have slow down the metabolic rate and arrested development of *P. demoleus* and *P. polytes*. The temperature also plays vital role in metabolism, metamorphosis and behaviors of insects (Skendzic et al., 2021). The change in temperatures has contributed for significant variation in growth and development of each life stage of these test insects.

Temperature is an important factor that exerts enormous influence on development of insect species. The effect of temperature on development may vary from insect to insect, the higher temperature results in increase in rate of development and decrease in time spent in completion of individual life stage, while lower temperature results in decrease in rate of development and increase in time spent on completion of life stages (Ju et al., 2011).

Conclusion

The results from present study clearly demonstrated that the development of various life stages of two citrus butterfly species, *P. demoleus* and *P. polytes* was significantly affected by temperature, where insect completed life cycle early at high temperature and took long time at lower temperature. The temperature range of 28 to 32°C was found to be a favorable range for development of these insect pests, however at high temperature (32°C) these insects complete their life cycle early; it may lead to more number of generations in a year and assumes a severe pest status. This information forms a valuable asset in predicting seasonal development, developing forecasting model and designing control measures against these harmful pests of citrus crops (Regniere and Bolstad, 1994; Campolo et al., 2014; Kim et al., 2017; Agbessenou et al., 2021; Neta et al., 2023).

Conflict of interests. The authors declare that they have no conflict of interests.

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