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Rashmi MA
Regional Plant Quarantine Station, HA Farm Post, Hebbal-Bhoopasandra Road, Bengaluru, Karnataka, India

Abraham Verghese
GPS institute of technology, No.1, Techno Industrial Complex, Peenya 1st Stage, Peenya, Bengaluru, Karnataka, India

Rami Reddy PV
Indian Institute of Horticultural Research, Hesserghatta, Bengaluru, Karnataka, India

Subhash Kandakoor
UAS, Dharwad, Karnataka, India

Chakravarthy AK
Indian Institute of Horticultural Research, Hesserghatta, Bengaluru, Karnataka, India

Corresponding Author:
Rashmi MA
Regional Plant Quarantine Station, HA Farm Post, Hebbal-Bhoopasandra Road, Bengaluru, Karnataka, India

Effect of climate change on biology of oriental fruit fly, *Bactrocera dorsalis* hendel (Diptera: Tephritidae)

Rashmi MA, Abraham Verghese, Rami Reddy PV, Subhash Kandakoor and Chakravarthy AK

Abstract
Effect of temperature was studied at six constant temperatures *i.e.* 16, 20, 24, 28, 32 and 36 °C and for four generations. The duration of different developmental stages of *Bactrocera dorsalis* varied as a function of temperature. The developmental time from egg to adult was inversely proportional to temperature. The total duration from egg to adult emergence varied from 96 days to 14 days at 16 to 36 °C respectively. Temperatures less than 16 °C and more than 36 °C were detrimental to adults as they not survive at 36 °C while at 16 °C though adults survived they failed to oviposit. There is a tendency for fruit flies to shift to newer niches of cooler climes that will record a rise in the fruit flies infestation on crops. The fruit flies were not drastically affected up to 570 ppm and so with excess CO_2 no mitigation is warranted but the larvae developed faster with short duration in 380 ppm and 570ppm and there was a change in calling behaviour of either sex due to which the circadian rhythm with respect to time of mating was altered.

Keywords: *Bactrocera dorsalis*, temperature, Carbon dioxide, Biology, Climate change

Introduction
The Oriental Fruit Fly, *Bactrocera dorsalis* (Hendel) (Diptera: Tephritidae) is a major polyphagous pest with diversified host range (more than 100 fruits and vegetables) and geographic distribution. Temperature plays an important role in several functions within an insect. Insect systems function optimally only within a limited range of temperatures in which several enzymes are involved. In temperatures below and above this range, reactions will slow down, but within the range, the rate of reactions will increase with increasing temperatures and vice versa. Therefore, global warming will adversely affect the organisms and ecosystems [1].
Impacts of climate change on insect pest populations include change in phenology, physiology, development, distribution, community composition and ecosystem dynamics. Climate change effects could either be direct, through the influence that weather may have on the insect's physiology and behavior and indirect effects can occur through the influence of climate on the insect's host plants, natural enemies and inter-specific interactions with other insects [2]. The increased temperature and CO_2 associated with climate change coupled with anticipated extreme weather conditions (more and longer droughts, more frequent storms and increased rainfall) are predicted to have an impact on insect population dynamics. Even modest changes to climate are expected to have a rapid impact on the distribution and abundance of pest insects because of their physiological sensitivity to temperature, short life cycles, high mobility and high reproductive potential [3, 4]. Many non-pest insects are already responding rapidly to climate change [5] and expanding northwards [6].
Temperature and Carbon dioxide is probably the most important environmental factor influencing insect behavior, distribution, development, survival, reproduction and pestilence potential [7]. So, a study was initiated to know the effect of temperature and Carbon dioxide on different life stages, survival and molecular level changes in *Bactrocera dorsalis.*

Materials and methods
Temperature studies: The temperatures for the study were fixed based on the preliminary studies. The studies showed that *B. dorsalis* develop between 20 °C and 28 °C, so temperatures lower and upper than these at increments of 4 °C were chosen.

Experiments were carried out with six temperatures *i.e*. 16, 20, 24, 28, 32 and 36 °C (± 1 °C) with five replications each. Insects of a particular stage were tested in environmental chambers (Labtop Instruments Pvt. Ltd. Maharashtra, India) under conditions of L12:D12 photoperiod and 80 ± 10% relative humidity. The time required for 50% of the individuals develop to a particular stage was determined at the above-mentioned temperatures.

Carbon dioxide studies: An experiment was carried out with two constant carbon dioxide levels i.e. 360 ppm and 570 ppm as treatments with seven replications. Insects of a particular stage were kept in environmental chambers (Percival scientific, Perry USA) under conditions of L12:D12 photoperiod, 27 ^{o}C constant temperature and 80 ± 10% relative humidity. The time required for 50% of individuals to achieve development to a particular stage was determined at both carbon dioxide levels mentioned with seven replications.
Test Insect: The test insect *i.e*. 30 laboratory reared pupae (1 day old) were placed in plastic containers (20 cm x18 cm) designed with three windows (6x4 cm) on the sides and one at the bottom covered with a cotton cloth to facilitate ventilation and maintenance of equal temperature. One lateral face bore cotton cloth sleeve through which sugar, yeast and water were provided for adults. Once the adults completed the pre mating period, ripe banana (Local variety yelakki) fruits were provided for oviposition. After 24 hours the banana with eggs (eggs are laid in batches of 1-20) were transferred into the larval rearing containers half-filled with sand. On pupation the pupae were transferred into small Petri plates. The same procedure was continued up to four generations at each temperature in the environmental chambers (Labtop Instruments Pvt. Ltd.). Fifteen adults from the fourth generation reared at six constant temperatures were stored at -20 ^{o}C for further molecular studies. Observations on the pupal, pre mating, egg and larval periods were recorded at six constant temperatures.

DNA Isolation: DNA was isolated from ten adult males using 2% Cetyl trimethylammonium bromide (CTAB) modified method (8). A total of 170 decamer primers randomly selected from OPA - OPZ, OPAA -OPAX, OPBA-OPBD and OPYO series obtained from Operon Technologies Inc., USA was screened. Of which, 51 primers produced reproducible bands and were tested across the DNA samples.

PCR amplification: PCR was carried out to amplify the bands in samples of different locations using decamer primers. PCR amplification was carried out in an Eppendorf master gradient cycler (Eppendorf AG, Germany) in Eppendorf PCR plates. Thermal cycle was programmed for 35 cycles with one cycle of initial denaturation and steps 2-4 were repeated 35 times.

Scoring: All the bands, in the range of resolution were scored, except for very faint and ghost bands. Monomorphic bands were not considered for scoring only polymorphic bands were scored as present (1) or absent (0) for RAPD analyses.

Statistical Analysis: The data were subjected to One-way Analysis of Variance (ANOVA) followed by Least Significant Difference (LSD) test to examine if the means were significantly different (Microsoft corporation version 12.0). To determine the influence of six constant temperatures on different stages of fruit fly, the data were subjected to correlation and linear regression analyses (Minitab Inc. version 16.1.1).

Results and discussion

Effect of different temperatures on development of *Bactrocera dorsalis*

The body temperature of an insect is always a reflection of ambient conditions coupled with any heat that may be produced by metabolic activity. The ambient temperature influences the physiology of the insect to the extent that it affects systems like digestive, respiratory, nervous, muscular and reproductive. Effect of these is reflected in growth and development measurable as duration of each instar, progeny and fecundity. In the present study growth (duration) was negatively affected by temperature [9]. The duration of different developmental stages of *B. dorsalis* varied as a function of temperature. The duration of the embryonic stage decreased with increasing temperatures (Figure 1A). The duration of the larval stage also significantly shortened with increase in the range of 16–36 °C from 53.4 to 6.6 days (Figure 1B). The duration of the pupal stage were 32.4days (at 16 °C), 19 days (20 °C), 13.8 days (24 °C) and 8.6 days (28 °C), 7.4 days (32 °C) and 6.2 days (36 °C) (Figure 1C). Temperatures less than 16 °C and more than 36 °C were detrimental to adults as adults did not survive. At 16 °C adults survived but failed to oviposit (Table 1, Figure 1D).
The linear negative relationship exhibited between the developmental stages and temperature was consistent with the previous studies (10, 11, 12, 13, 14). There was an indication that temperature beyond 36°C is detrimental to the fruit flies. This also explains why fruit flies are not serious problems in high temperature belts where mango is grown, like Lucknow (UP), Ranga reddy (AP), Mehabobnagar (AP) where, maximum ambient temperature is more than 40 °C during mango fruiting. However after rains temperature drops below 35 °C and pupae in soil will emerge and population rises. Therefore late mango varieties like Chausa are prone to fruit fly infestation [15, 16].
In case of *Bactrocera zonata,* Mohamed [17] found that the larval instar took 21 days at 16 °C while in present study for corresponding temperature it was 53 days. This indicated that there can be intrespecific variation within the same genus *Bactrocera*. This is probably why *B. zonata* is common in North India where temperature goes extremely low, while *B. dorsalis* is more dominant in southern region where, the temperatures are higher than 16 °C for most part of the year [16, 18]. However at higher temperatures there was no variation in duration in both the species (7 days vs. 6.6 days).
The study also showed that the temperatures at 36 °C and above are not congenial for the development of adults and pupae but larval mortality was less compared to the pupal and adults because they were concealed in the banana fruit which was used as larval host food in the laboratory. This may have escaped from exposure to the constant high temperatures.
The developmental time from egg to adult was inversely proportional to temperature (Figure 1). This is significant for IPM, in areas of low temperatures as the eggs would take longer period to hatch *i.e*., around 10 days at 16 °C (Figure 1A). The fruit fly infestation close to harvest will probably escape detection if the fruits and vegetables are processed and consumed without much storage on shelf. This can be true in certain fruits which ripen early or climacteric fruits for

example in North India, fruit fly infestation on guava is almost nil during winter seasons, as the egg hatches after long time and to some extent pupae undergo diapause. This study clearly showed that between 28 °C and 32 °C the developmental time of egg, larva and pupa decreases as a result the total life cycle is less than a month. Thus at this window of temperature between 28-32 °C the intergeneration time is shortened and leads to quicker population build up and consequent increase in infestation. Extremes (cooler/warmer) in temperature will have less infestation, extremes in temperatures *i.e.*, 16 °C and 36 °C were biased against ovarian maturation. Therefore, there was no mating or oviposition in the adults at the above-mentioned temperatures. This phenomenon was in line with the studies of Duyck *et al.* [19] on *B. zonata* in which there was no ovarian maturation at 15, 20 and 35 °C. However, Kasana and Aliniazee [20], in their study on the effect of temperature on the pre-oviposition period of *Rhagoletis completa* Cresson (Diptera: Tephritidae), showed that females never laid eggs despite the presence of mature eggs in their ovaries. Similarly Duyck and Quilici [14] also reported that ovarian maturation was obtained only over a very narrow range of temperature (25–30 °C), as observed for *Ceratitis catoirii* Guérin-Mèneville. However, laboratory conditions (constant temperatures, light intensity and photoperiod) may also have influenced the maturation of ovaries, as stated for other tephritid species by Tzanakakis and Koveos [21]. Depending on the severity and duration of the winter, fruit flies may survive cool winter climates by surviving as adults or as larvae in fruit or pupae in north India [22].

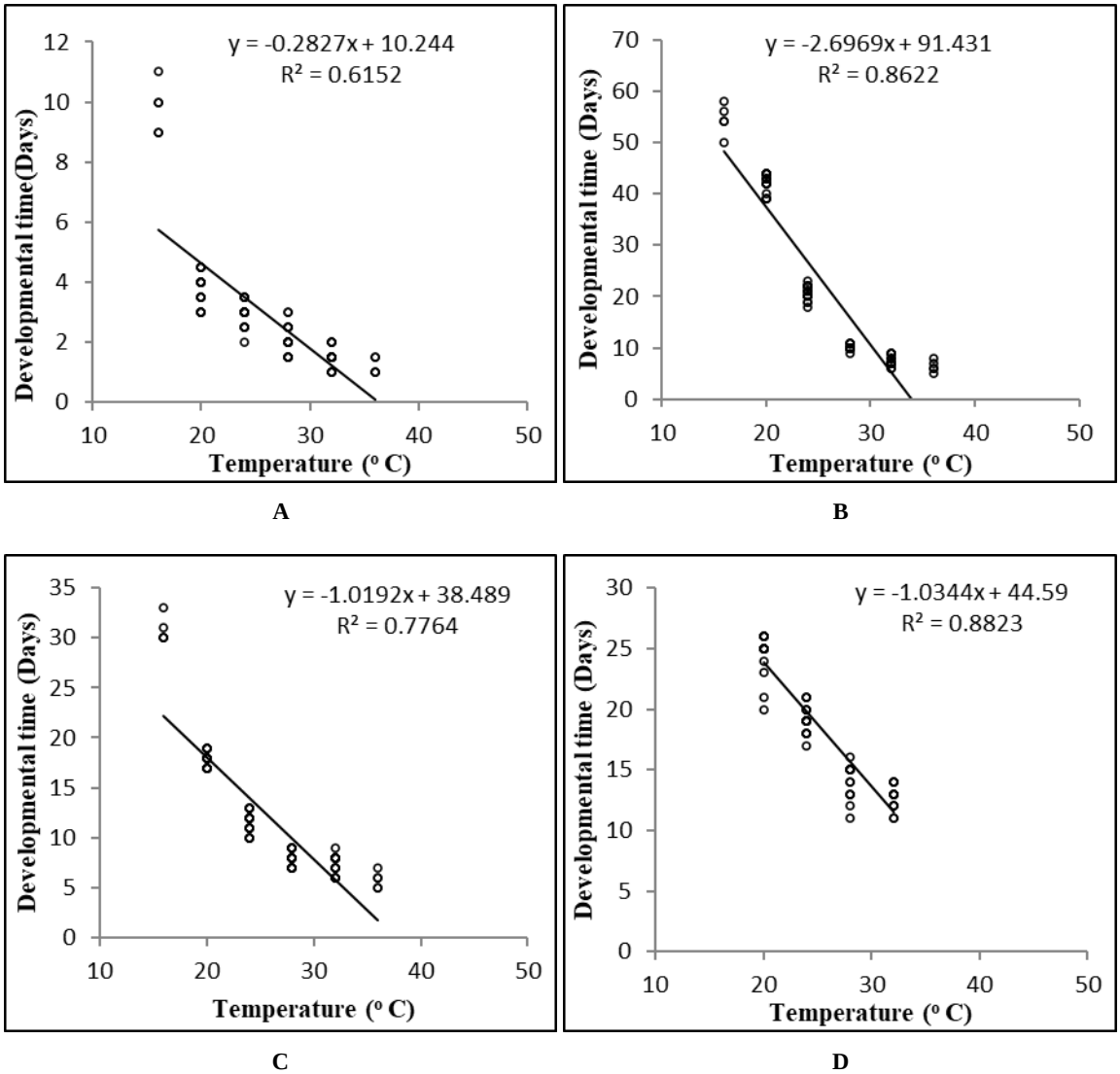

Fig 1: Relationship between *Bactrocera dorsalis* development stages A. Egg, B. Larvae C. Pupae D. Pre-mating and temperature during first to fourth generation

Effect of different temperatures on molecular level changes in adult fruit fly *B. dorsalis* at fourth generation: RAPD-PCR analysis was carried out to quantify the molecular changes among the populations reared at 20, 24, 28 and 32 °C because populations did not survive at 16 and 36 °C. OPBB-17, OPV-13, OPB-09, OPB-05, OPB-20, OPB-13, OPAO-13, OPB-19, OPB-03, OPBB-14, OPQ-15, OPAU-02 were the 12 random primers selected for analysis gave no difference between the treatment as only monomorphic bands were found.

Effect of different levels of carbon dioxide on development from first to fourth generation of *B. dorsalis*

The duration of the embryonic stage during G1 generation was long at higher CO2 level i.e., 570 ppm which was on par with the control population (reared in laboratory). The duration of the larval stage differed significantly across 380 ppm, 570ppm and control, though it did not differ

significantly across 380 ppm and 570ppm ($p < 0.01$). The pupal period and pre-mating period did not vary significantly among the treatments (Table 2). However, there was a change in mating period, which was noticed during the day time (9:00am -10:30am) in 380 ppm and 570ppm CO_2 levels, whereas mating was recorded from evening hours to past mid-night time in control. The same trend was seen in the subsequent generations i.e., G2, G3 and G4 but there was no significant difference among the treatments on developmental stages except for the larval stage during G2 generation. The duration of the larval stage differed significantly across 380ppm (7.57 days), 570ppm (7.43 days) and control, though it did not differ significantly at 380ppm and 570ppm ($p < 0.01$) (Table 2). There was no significant difference between the number of pupae during first generation but in consecutive generations the fecundity in 380 ppm and 570 ppm decreased which was seen in pupal number as it was significantly less compared to control.

CO_2 impacts on insects are thought to be indirect in terms of insect feeding damage resulting from changes in the host crop. Direct effects of higher CO_2 concentrations on insects are basically not investigated. It seems that insects can detect CO_2 sources such as plants and elevated levels might affect the insect's CO_2-sensing system [23]. The results of the present study showed that fruit flies were not drastically affected up to 570 ppm and so with excess CO_2 no mitigation is warranted. The duration of the embryonic stage during G_1 generation was long in control population (reared in laboratory under ambient conditions) whereas, the larvae developed faster with short duration in 380 ppm and 570 ppm. The effects of CO_2 may $_{vary}$ with the insects of different orders were the lepidopteran larval duration took longer time at elevated CO_2 of 550 and 700 ppm on *Spodoptera litura* compared with ambient CO_2 [24].

The reduced input from CO_2 receptor cells strongly interfere with brain processing signals involved in oviposition behaviour of moths which may lead to hindered oviposition [25]. Direct evidence for role of CO_2 in the oviposition behaviour of *B. dorsalis* was noticed where the oviposition was delayed and fecundity was also reduced (pupae from 50 to 20 numbers) at 380ppm and 570ppm in consecutive generations, whereas there was no changes in the control population. However, the decrease in fecundity and delayed oviposition maybe favourable in terms of IPM.

Tephritid flies oviposit into small existing lesions in the skin of fruits which release volatile compounds including respiratory CO_2 which is responsible for the exploratory behaviour and ovipositional activity in the fruit. In *Bactrocera tryoni*, CO_2 acts as a stimulant and attractant in oviposition [26, 27]. This is indicative of increased oviposition and infestation with rise in CO_2, is a future line of study in *B. dorsalis.*

Courtship and mating behaviour are very complex and elaborate in tephritid species and may continue for hours. The *Bactrocera dorsalis*, mating behaviour is rather complicated. Studies have been done to demonstrate that the mating behaviour of the fruit fly is affected by the outside stimulant such as temperature, pheromone, time, nutritional status and diet, and more and more researchers have found that the important roles played by the male fruit fly. Identification of the genes and pathways that might be involved in the mating mechanisms of the male of the *B. dorsalis*, which may be of the benefit of the pest management [28, 29].

The study also showed that the photosensitivity/ olfactory or both of the adult fruit flies probably change as pheromonal changes might occur. Therefore, mating during dusk changed to early morning matings in 380 ppm and 570ppm CO_2 levels. From this, it may be gathered that calling behaviour of either sex or pheromone release altered and thus the circadian rhythm with respect to time of mating. This altered mating phenology at elevated CO_2 levels may lead to competition between the species. This opens new vistas of studies at increased CO_2 in new ethological study.

In the consecutive generations of 380 ppm and 570 ppm the larval stage was more prone to bacterial and fungal infections which were not noticed in the control population. The probable reasons may be the changes in the physiology of insect systems due to the down regulation of the immune system, greater fungal spore production as observed at the higher CO_2 concentrations [30].

Therefore, from the present results on CO_2, major geographical shifts in the *B. dorsalis* may not be expected. In current scenario and in next couple of decades CO_2 may not be a threat to *B. dorsalis* and does not ring an alarm.

Effect of different levels of carbon dioxide on molecular level changes in adult fruit fly *B. dorsalis* at fourth generation: RAPD-PCR analysis was carried out to quantify the molecular changes among the populations reared at different levels of carbon dioxide. OPBB-17, OPV-13, OPB-09, OPB-05, OPB-20, OPB-13, OPAO-13, OPB-19, OPB-03, OPBB-14, OPQ-15, OPAU-02 were 12 random primers selected for analysis gave no difference between the treatment as only monomorphic bands were found and no polymorphic bands were noticed.

Conclusions

Implications of global warming are that there is a tendency for fruit flies to shift to newer niches of present cooler climates resulting in change of distribution of *B. dorsalis* [31]. Studies on the range shifts is necessary for anticipating the fruit fly management programmes, as fruit fly and climate change related dynamics in India is important to keep vigil, as *B. dorsalis* is a serious pest of fruits and vegetables with quarantine implications.

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Table 1: Developmental time of different stages of *Bactrocera dorsalis* at six temperatures from first to fourth generations

G_1 Temperatures (^{0}C)	Egg period	Larval period	Pupal period	Pre-mating period	Total period from egg to adult
	Mean + SD (Days)				Mean (Days)
First generation					
16	10.8^{a}±0.84	53.4^{a} ± 3.65	32.4^{a} ± 1.82	No mating	96.6^{a}
20	4.00^{b}±0.57	43.2^{b} ± 2.17	19^{b} ± 1.22	26.6^{a} ± 0.55	66.2^{b}
24	3.50^{b}±0.35	22.6^{c} ± 1.14	13.8^{c} ± 1.30	20.2^{b} ± 1.30	39.9^{c}
28	1.80^{c}±0.57	11.4^{d} ± 1.34	8.6^{d} ±1.14	15^{c} ± 0.71	21.8^{d}
32	1.60^{c}±0.35	7.8^{e}± 0.84	$7.4d^{d}$ ± 0.55	13.6^{c} ± 0.55	16.8^{e}

36	1.40^{c}±0.27	6.6^{e} ± 1.14	6.2^{de} ± 0.84	No Survival	14.2^{e}
CD (*p*=0.01)	0.90	3.31	2.04	1.45	3.904
Second generation					
20	3.80^{a} ± 0.57	42.4^{a} ± 2.07	17.8^{a} ± 0.84	25.4^{a} ± 0.55	64.00^{a}
24	3.00^{a} ± 0.35	21^{b} ± 1.0	11.6^{b} ± 1.14	19.4^{b} ± 1.14	35.60^{b}
28	2.20^{b} ± 0.57	10.2^{c} ± 0.45	7.8^{c} ± 0.84	14.4^{c} ± 0.89	20.20^{c}
32	1.40^{c} ± 0.42	7.6^{c} ± 0.89	7.4^{c} ± 0.89	13.2^{c} ± 0.84	16.40^{d}
CD (*p*=0.01)	0.84	2.17	1.61	1.52	2.409
Third generation					
20	3.80^{a} ± 0.57	42.6^{a} ± 1.67	17.8^{a} ± 0.84	24.6^{a} ± 1.14	64.16^{a}
24	3.00^{b} ± 0.35	20.6^{b} ± 2.07	11.6^{b} ± 1.14	18.8^{b} ± 0.84	35.20^{b}
28	2.00^{c} ± 0.35	10^{c} ± 0.71	8.2^{c} ± 0.84	13.6^{c} ± 1.67	20.20^{c}
32	1.35^{c} ± 0.42	7.4^{d} ± 1.14	7.4^{c} ± 0.89	12^{c} ± 1.58	16.15^{d}
CD (*p*=0.01)	0.75	2.58	1.62	2.34	2.84
Fourth generation					
20	3.60^{a} ± 0.65	42^{a} ± 1.87	18.2^{a} ± 0.84	23.4^{a} ± 2.70	63.80^{a}
24	2.80^{b}± 0.57	20.4^{b} ± 1.14	11.4^{b} ± 1.14	19^{b} ± 1.41	34.60^{b}
28	1.90^{c} ± 0.42	10.2^{c} ± 0.45	7.8^{c} ± 0.84	13.4^{c} ± 2.07	19.90^{c}
32	1.33^{c} ± 0.43	7.2^{d} ± 1.10	7.6^{c} ± 1.14	12^{c} ± 1.0	16.13^{d}
CD (*p*=0.01)	0.91	2.16	1.73	3.31	2.596

n = 5 replicates Test insects: 30 pupae per replication (1 day old)

Table 2: Developmental time of different stages from first to fourth generation of *Bactrocera dorsalis* at different carbon dioxide levels

G_1		**Egg period (Days)**	**Larval period (Days)**	**Pupal period (Days)**	**Pre-mating period (Days)**	**No. of Pupae**
	380 ppm	1.21^{b}	7.43^{b}	7.93	13.14	58.71
	570 ppm	1.71^{a}	7.71^{b}	8.00	14.43	57.29
	Control	1.86^{a}	9.71^{a}	8.14	14.14	69.29
	CD (*p*= 0.01)	0.42	1.54	NS	NS	NS
G_2		**Egg period (Days)**	**Larval period (Days)**	**Pupal period (Days)**	**Pre-mating period (Days)**	**No. of Pupae**
	380 ppm	1.29	7.57^{b}	7.79	13.43	38.86^{b}
	570 ppm	1.68	7.43^{b}	8.43	14.29	34.43^{b}
	Control	1.86	9.80^{a}	8.43	14.86	70.29^{a}
	CD (*p*= 0.01)	NS	1.85	NS	NS	22.37
G_3		**Egg period (Days)**	**Larval period (Days)**	**Pupal period (Days)**	**Pre-mating period (Days)**	**No. of Pupae**
	380 ppm	1.43	7.43	8.21	13.71	18.71^{b}
	570 ppm	1.82	8.00	8.86	14.43	18.43^{b}
	Control	1.86	9.43	8.14	15.00	72.71^{a}
	CD (*p*= 0.01)	NS	NS	NS	NS	13.73
G_4		**Egg period (Days)**	**Larval period (Days)**	**Pupal period (Days)**	**Pre-mating period (Days)**	**No. of Pupae**
	380 ppm	1.71	7.71	8.50	14.14	20.00^{b}
	570 ppm	1.86	7.86	8.71	15.71	16.57^{b}
	Control	1.86	9.29	8.29	14.86	75.00^{a}
	CD (*p*= 0.01)	NS	NS	NS	NS	12.49

n = 7 replicates; Test insects: 30 pupae per replication (1 day old);

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