

REVIEW

Developmental Biology of *Zeugodacus cucurbitae* (Diptera: Tephritidae) in Three Cucurbitaceous Hosts at Different Temperature Regimes

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ABSTRACT. Fruit flies are key pests of cucurbits in many parts of the world, including Tanzania. Developmental biology of *Zeugodacus cucurbitae* (Coquillett) has been determined across temperature regimes in some cucurbitaceous hosts, in limited geographies. This study was conducted to determine duration and survival rates of immature stages of *Z. cucurbitae* in three cucurbitaceous hosts, at different temperature regimes. It was hypothesized that temperature and cucurbitaceous hosts influence duration and survival of immature stages of *Z. cucurbitae*. We conducted experiments in the environmental chamber set at $75 \pm 10\%$ RH and a photoperiod of 12:12 (L:D) h, at temperatures of 20, 25, and 30°. Our results showed that duration and survival of immature stages of *Z. cucurbitae* differed significantly among the temperature regimes but not among the hosts. Egg incubation period as well as larval and pupal stages were significantly longer ($P < 0.0001$) at low temperature in all three hosts. Likewise, survival rate of all immature stages were significantly higher ($P < 0.0001$) at higher than lower temperatures. The three hosts, cucumber (*Cucumis sativus*), watermelon (*Citrullus lanatus* (Thunb.) Matsum. and Nakai), and pumpkin (*Cucurbita pepo*) did not significantly affect duration or survival rates of immature stages of *Z. cucurbitae*. The low developmental thresholds were estimated at 15.88, 13.44, and 12.62 for egg, larva and pupa, respectively. These results further confirm that *Z. cucurbitae* is well adapted to warm climate, which dominates many areas of Tanzania.

Key Words: *Zeugodacus cucurbitae*, immature stage, duration, survival, cucurbits

Cucurbitaceous vegetables are cultivated throughout the world, from tropical to sub temperate zones. Cucurbit fruits and vegetables are important sources of food and revenue for exporting countries (Kadio et al. 2011). The major cucurbits, cucumber (*Cucumis sativus* L.), watermelon (*Citrullus lanatus* (Thunb.) Matsum. and Nakai), and pumpkins (*Cucurbita pepo* L., *Cucurbita maxima* Duchesne ex Lam, and *Cucurbita muschata* Duchesne ex Poiret) are commonly grown in Tanzania. Most of these fruit vegetables are consumed within Tanzania although a few are exported to the neighboring countries. According to Rai et al. (2008) cucurbits are consumed in various forms and are good source of vitamin A and C and various vital minerals (Srivastava and Butani 2009).

Erratic weather, pests and diseases, among others, limit production of cucurbits. Fruit flies (Diptera: Tephritidae) are among the major insect pests of cucurbits (Kadio et al. 2011). According to Sapkota et al. (2010), fruit flies damage large quantities of fruits grown in the tropics. Fruit flies cause variable losses depending on species, host fruits, and geographic location (Mwatawala et al. 2009). For example, CABI (2005) reported that the melon fly *Zeugodacus cucurbitae* (Coquillett) can cause up to 100% loss in unprotected crop. Fruit flies puncture the skin of fruits by their stout ovipositor and lay four to ten eggs per fruit each time (Srivastava and Butani 2009). The hatched larvae damage the fruit by feeding and burrowing out to pupate in the soil (Stonehouse et al. 2007). *Zeugodacus cucurbitae* is an important frugivorous pest of cucurbits in the tropics. Losses caused by *Z. cucurbitae* in Tanzania are variable among commercial hosts (Mwatawala et al. 2009). The pest prefers watermelon, pumpkin, and cucumber, grown in low land areas where the pest is abundant (Mwatawala et al. 2006). Vayssières et al. (2008) reported developmental rates of immature stages of *Z. cucurbitae* at different temperatures and hosts. However, the authors did not include watermelon, which is among the key hosts of *Z. cucurbitae*. Furthermore, information on survival of each immature stage of the

pest in cucurbitaceous hosts at different temperature regimes was lacking, hence the focus of this study.

Materials and Methods

Establishment of Cohorts of *Z. cucurbitae*. Colonies of *Z. cucurbitae* originated from infested fruits of *C. sativus*, *C. pepo*, and *C. lanatus* and maintained in the Basic and Applied entomology laboratory of Sokoine University of Agriculture (SUA). Fruits were processed following procedures used by Mwatawala et al. (2006). We collected, sorted and introduced emerged adults into population cages made of perspex glass (30 × 30 × 30 cm), with petri dishes containing adults diet and distilled water. Adult's diet was composed of enzymatic yeast hydrolysate (ICN Biomedical, Aurora, Ohio, USA) and sucrose (sugar) in a ratio of 1:3. The petri dishes with water also contained pumice granules for safe landing of flies (see also Vayssières et al. 2008).

We established cohorts adults *Z. cucurbitae* by first placing into population cages thin slices of *C. sativus*, *C. pepo*, and *C. lanatus* collected from SUA Horticulture Unit. The slices were removed after 24 h and placed into individual plastic rearing cages (30 cm by 15 cm by 10 cm) covered with fine mesh at top for ventilation, and left until emergence of adults. Thereafter, we grouped adults emerging within 24 h into cohorts of 30, in a ratio of 15:15 (male:female).

Establishment of Egg Incubation Period, Larval, and Pupal Stage Duration. We compared developmental times of immature stages of *Z. cucurbitae* in three cucurbitaceous hosts (*C. sativus*, *C. pepo*, and *C. lanatus*) at three temperature regimes (20, 25, and 30°C), in independent factorial experiments, replicated three times.

We introduced a petri dish with a single, fresh slice of each fruit species (30 g) into a rearing cage containing a cohort of 14–21 days old adult *Z. cucurbitae*. The slices were placed into cages with cohorts originating from similar fruits. We used a camel's hairbrush to place, on a moist filter paper (Whatman 541), cohorts of 100 eggs extracted from

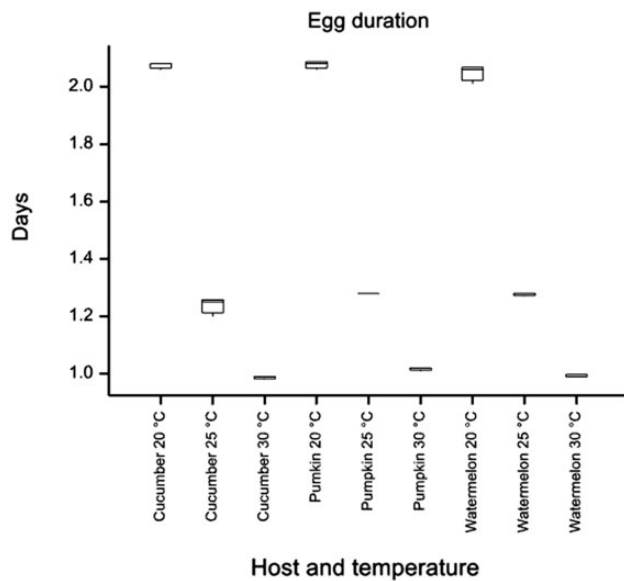


Fig. 1. Egg duration of *Z. cucurbitae* in three cucurbitaceous hosts at three temperature regimes.

slices of similar fruits (Vayssières et al. 2008). We observed hatching eggs under a microscope twice a day, 12 h after extractions.

In a set up similar to above, we collected and placed cohorts of 50 newly emerged larvae onto a fruit slices on a petri dish, in a rearing cage containing sterilized sand as pupation media. We sifted sand and examined for puparia every 24 h, a week after collection. We placed a cohort of 30 collected pupae (24 h after emergence) onto a moist filter paper on a petri dish and observed until adults emergence.

We extracted eggs, larvae, and pupae following procedures described by Vayssières et al. (2008). We recorded at each temperature and for each host, times taken for 50% eggs to hatch, 50% larvae to pupate, and 50% pupae to emerge into adult flies. We also recorded, twice a day, the number of newly emerged adults. We then established the total developmental duration of *Z. cucurbitae*, from egg to adult emergence.

Establishing Survival Rate of Immature Stages of *Z. cucurbitae*.

We compared survival rates of immature stages of *Z. cucurbitae* in a factorial experiment, with three temperature regimes (20, 25, and 30°C) and cucurbitaceous hosts (*C. sativus*, *C. pepo*, and *C. lanatus*) as sources of variation, replicated three times. We extracted and observed cohorts of 100 eggs, larvae (<1-h old) and pupae of *Z. cucurbitae* following procedures described above. We observed eggs, larvae, and pupae for 72, 300, and 640 h respectively, based on results of Vayssières et al. (2008).

We recorded, at each temperature and for each host, numbers of eggs hatched, emerged larvae, pupae, and adults. We computed percentages of hatched eggs, emerged larvae, pupae, and adults. Survival rate was determined as the percentage of individuals in a stage that successfully emerged into a subsequent stage.

Data Analysis. Data on duration and survival of developmental stages of *Z. cucurbitae* were subjected to a two way Analysis of Variance followed by Least Significance Difference (LSD) means separation. Data for survival rate were log transformed to improve normality then de-transformed after analysis. The data were analyzed using SAS software. The relationship between developmental rates of *Z. cucurbitae* and temperature was determined by linear regressions. The number of degrees days above the lower threshold required to complete the development the so called thermal constant (K) for egg, larva, pupa and total development was calculated following the regression equation $y = K / (x - t)$ as reported by Fletcher, (1989). Lower development

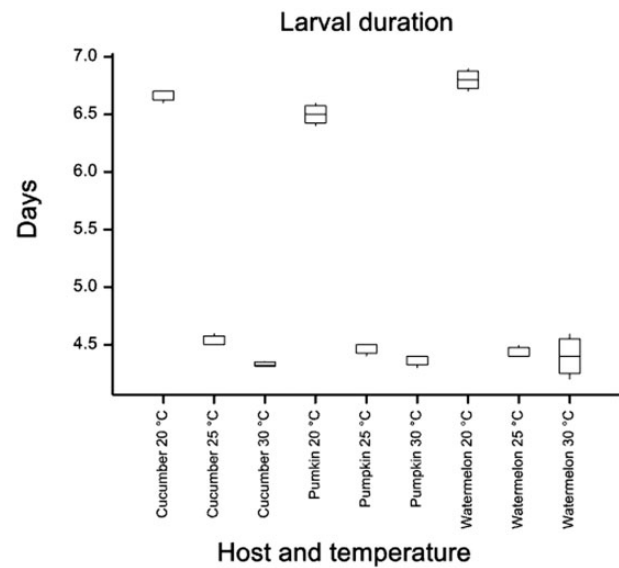


Fig. 2. Larval duration of *Z. cucurbitae* in three cucurbitaceous hosts at three temperature regimes.

thresholds (t , the temperature at which there is no development) (Vayssières et al. 2008) were also calculated.

Results

Developmental Durations of Immature Stages of *Z. cucurbitae*.

Egg incubation period was significantly shorter at higher temperatures as shown in Figure 1 ($F = 9730.92$; $df = 2, 18$; $P < 0.0001$). The incubation period did not differ significantly among hosts ($F = 5.55$; $df = 2, 18$; $P = 0.133$) or interaction between host and temperature ($F = 3.03$; $df = 4, 18$; $P = 0.457$). Egg incubation period was longest in pumpkin at 20°C, and shortest in watermelon at 30°C. Egg incubation period increased with decreasing temperature.

Larval duration varied significantly among temperature regimes ($F = 47.06$; $df = 2, 18$; $P < 0.0001$). Larval stage was longer at low temperatures (see Figure 2). However, the variations were not significant among hosts ($F = 0.17$; $df = 2, 18$; $P = 0.8437$) or interaction between host and temperature ($F = 0.16$; $df = 4, 18$; $P = 0.9560$). Larval stage was longest in watermelon at 20°C and shortest in cucumber at 30°C. Larval stage duration also increased with decreasing temperature.

Pupa stage was significantly longer ($F = 223.42$; $df = 2, 18$; $P < 0.0001$) at lower than at higher temperature regimes (Fig. 3). However there were no significant differences in pupa stage duration among hosts ($F = 1.04$; $df = 2, 18$; $P = 0.372$) or interactions between host and temperature ($F = 2.51$; $df = 4, 18$; $P = 0.078$). Pupa stage was longest in cucumber at 20°C and shortest in the same host at 30°C. Average pupa developmental time increased with decreasing temperature.

Total developmental duration (days) from egg to adult *Z. cucurbitae* varied significantly among temperature regimes ($F = 14178$; $df = 2, 18$; $P < 0.0001$) among hosts ($F = 100.17$; $df = 2, 18$; $P = 0.0001$) and interactions between host and temperature ($F = 24.17$; $df = 2, 18$; $P < 0.0001$). The total developmental time was longest in pumpkin at 20°C (Fig. 4), and shortest in cucumber at 30°C. Total developmental time of *Z. cucurbitae* increased with decreasing temperature.

Relationship Between Developmental Rates of *Z. cucurbitae* and Temperatures. The estimated values of the linear models are presented in Table 1. The low developmental thresholds were estimated to be 15.88, 13.44, 12.62, and 11.41°C for egg, larva, pupa, and development from egg to adult emergence, respectively. Their corresponding thermal constants were 16.21, 42.05, 61.49, and 147.1 degree-days.

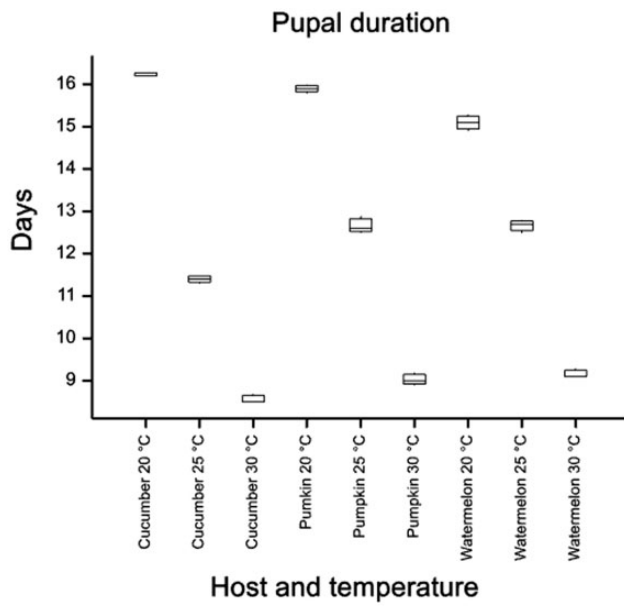


Fig. 3. Pupal duration of *Z. cucurbitae* in three cucurbitaceous hosts at three temperature regimes.

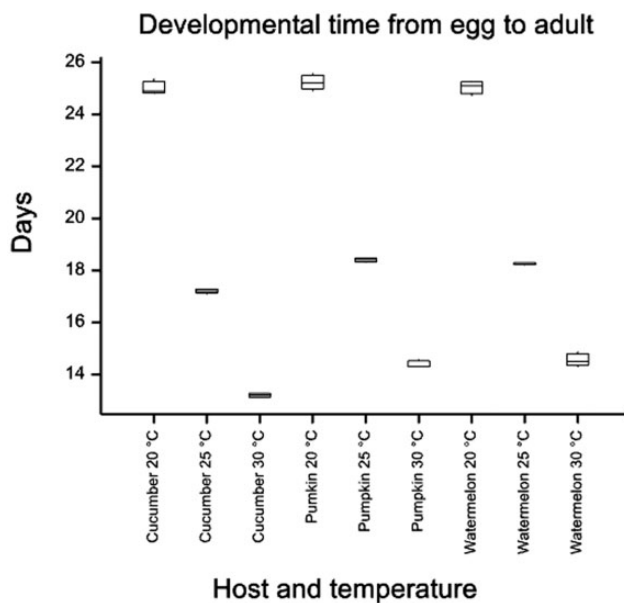


Fig. 4. Developmental time of *Z. cucurbitae* from egg to adult in three cucurbitaceous hosts at three temperature regimes.

Table 1. Lower developmental threshold (t, °C) and Thermal constants (K) estimated by linear regression for *Z. cucurbitae* developmental rates

Developmental stages	Degree of freedom	Regression statistics		t; (°C)	K
		P	R ²		
Egg	25	<0.001	0.921	15.88	16.21
Larval	25	<0.001	0.957	13.44	42.05
Pupal	25	<0.001	0.937	12.62	61.49
Total development	25	<0.001	0.961	11.41	147.1

Survival Rate of *Z. cucurbitae* Immature Stages. Egg hatching rate varied significantly among temperature regimes ($F=176.37$; $df=2$,

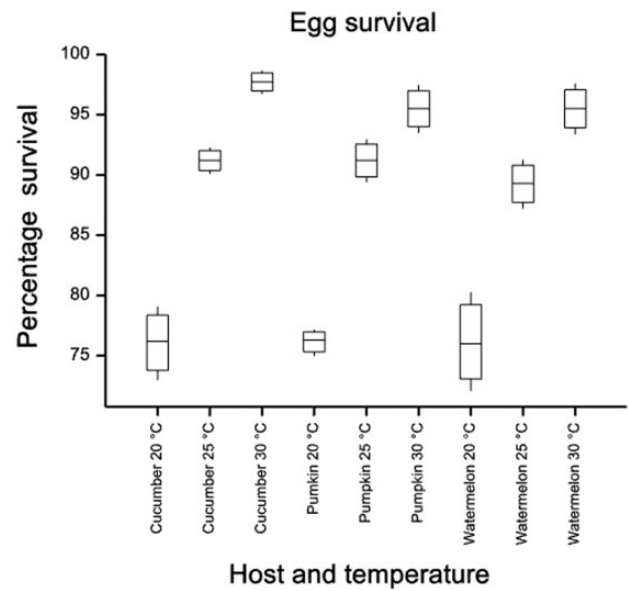


Fig. 5. Egg survival of *Z. cucurbitae* in three cucurbitaceous hosts at three temperature regimes.

18; $P < 0.0001$). The differences on egg hatching rate were not significant among hosts ($F=0.66$; $df=2$, 18; $P=0.5296$) or interaction between host and temperature ($F=0.33$; $df=4$, 18; $P=0.8541$). Hatching rate was highest at 30°C on eggs extracted from cucumber (Fig. 5) and lowest at 20°C on eggs extracted from the same host. Egg hatching rate increased with increasing temperature in all cucurbitaceous hosts.

Temperature significantly affected pupation rate ($F=109.87$; $df=2$, 18; $P < 0.0001$). However, effects of hosts ($F=0.2$; $df=2$, 18; $P=0.8220$) or the interaction between host and temperature ($F=0.14$; $df=4$, 18; $P=0.9635$) were not significant. Pupation rate was highest in pumpkin at 30°C and lowest in watermelon (Fig. 6) at 20°C. Pupation rate increased with increasing temperature regimes in all cucurbitaceous hosts.

Emergence rate of *Z. cucurbitae* was also significantly affected by temperature ($F=223.71$; $df=2$, 18; $P < 0.0001$) but not by hosts ($F=0.21$; $df=2$, 18; $P=0.8107$) or interaction between host and temperature ($F=0.46$; $df=4$, 18; $P=0.7670$). Highest number of emerged flies were recorded on pupae that originated from pumpkin at 30°C (Fig. 7) while low rate was recorded at 20°C from the same host. Emergence rate increased with increasing temperature in all cucurbitaceous hosts.

Discussion

Our results showed significant variations in developmental times of *Z. cucurbitae* at different temperature regimes. Temperature is an important factor affecting developmental life stages of fruit flies (Fletcher 1987). According to Ye (2001) developmental threshold and thermal constant (K) are important factors in the geographic distribution of fruit flies.

Duration of immature stages of *Z. cucurbitae* varied inversely with temperature. Vayssières et al. (2008) reported longest egg, larva, and pupa stages of *Z. cucurbitae* and *Dacus ciliatus* Loew in pumpkin and shortest in cucumber. The authors did not include *C. lanatus* in their study. A study by Vargas et al. (2000) reported similar trend in *Bactrocera dorsalis* Hendel and *Ceratitis capitata* (Wiedemann) (see also a study by Duyck et al. 2004).

Decreased developmental time of immature stages of *Z. cucurbitae* at high temperatures suggested that the species grows and multiplies fast in the areas of high ambient temperatures (low land areas). According to Ekesi et al. (2006) most fruit fly species of genus *Bactrocera* (a former genus of *Z. cucurbitae*) are low land residents

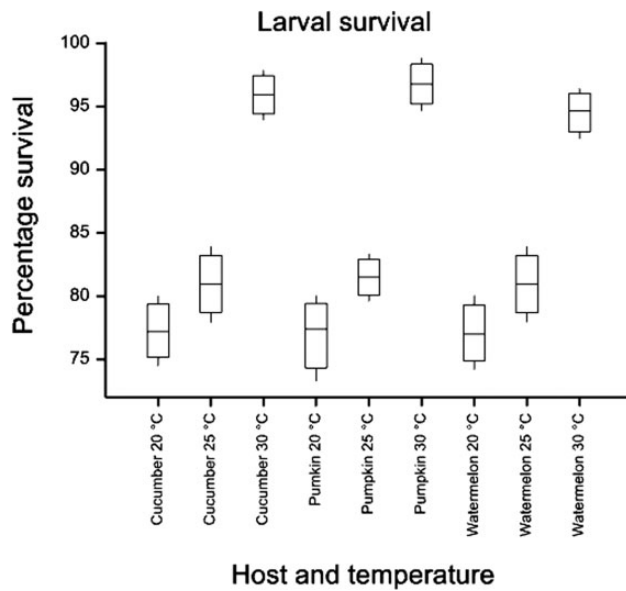


Fig. 6. Larval survival of *Z. cucurbitae* in three cucurbitaceous hosts at three temperature regimes.

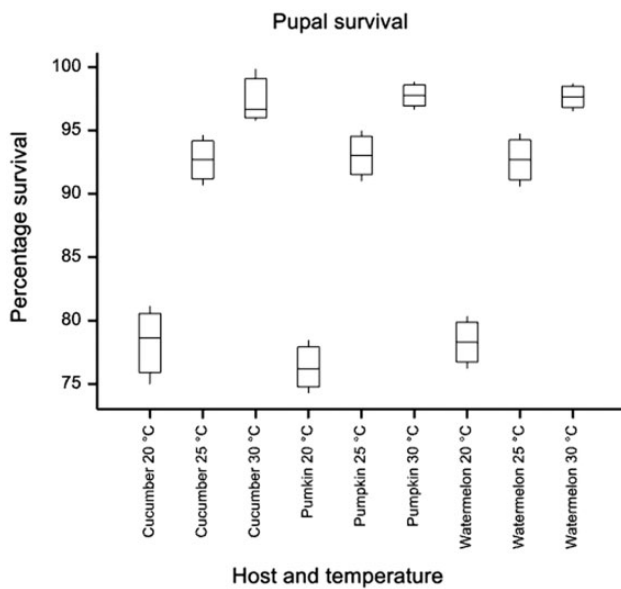


Fig. 7. Pupal survival of *Z. cucurbitae* in three cucurbitaceous hosts at three temperature regimes.

(with warmer climates). In Yunan, *Bactrocera correcta* (Bezzi) occurs in low-altitude areas under 1,500 m altitude, where the annual average temperatures of the areas are all over 15.8°C (Liu and Ye 2009). The low developmental thresholds reported in this study were however below temperature regimes reported for *Z. cucurbitae*, *D. ciliarius* (Vayssieres et al. 2008), *Bactrocera zonata* (Saunders) (Duyck et al. 2004) and *B. correcta* (Liu and Ye 2009).

We did not observe significant effects of cucurbitaceous hosts on durations of immature stages of *Z. cucurbitae*. Cucurbitaceous hosts (cucumber, watermelon, and pumpkin) had similar effects on developmental duration of immature stages of *Z. cucurbitae*. Vayssières et al. (2008) did not observe significant difference on duration of immature stages of *Z. cucurbitae* and *D. ciliarius* reared in pumpkin, cucumber, and squash. Temperature is an important abiotic factors affecting immature development of insects (Wagner et al. 1984). Egg incubation periods 23.8, 29.8, and 50.4 h at 30, 25, and 20°C, respectively recorded

during the study were almost the same as 23.7, 30, 49.7 h reported by Vayssières et al. (2008).

The lowest larva stage duration (4.33 days) of *Z. cucurbitae* at 30°C in cucumber and highest (6.80 days) at 20°C in watermelon are within the range of 3–21 days reported by Hollingsworth et al. (1997). Furthermore the lowest (8.57 days) and highest (16.23 days) pupa stages observed in the present study are within the range (6.5–21.8 days) as reported by Koul and Bhagat (1994) on bottle gourd. Total developmental time (17.2 days) of the pest at 25°C in cucumber was slightly lower than 17.8 days reported by Huang and Chi (2011). The difference could have been caused by level of maturity and weight of the hosts used.

Our study showed significant variations in survival rates of immature stages of *Z. cucurbitae* at different temperature regimes. Survival rates increased with increasing temperature, from 20 to 30°C in all selected cucurbitaceous hosts. This indicated that survival of immature stages of *Z. cucurbitae* was much favored by increase in ambient temperatures. However, there was no significant difference on survival rate among cucurbitaceous hosts. Egg hatch rate in cucumber at 25°C is similar to that recorded by Samalo et al. (1991) at 27 ± 1°C.

Larva (80.97%) and pupa (92.68%) survival rates in cucumber at 25°C were slightly higher than (79%) and (88%), respectively as reported by Koul and Bhagat (1994).

Adult emergence rate (97.77%) at 30°C in *C. pepo* is higher than 97.25% reported by Liu and Ye (2009) for *B. correcta* reared in the artificial diet. Generally, survival of immature stages of *Z. cucurbitae* increased with increasing temperatures from 20 to 30°C. Temperature plays a key role in the insect breeding process (Thierry and Serge 2000). Furthermore ambient temperature is an important abiotic factor that affects developmental biology of immature stages of insects (Vayssières et al. 2008).

We conclude that cucumber (*C. sativus*), watermelon (*C. lanatus*), and pumpkin (*C. pepo*) had almost similar effects on developmental time and survival of immature stages of *Z. cucurbitae*. Developmental time of *Z. cucurbitae* immature stages decreased with increasing temperatures from 20 to 30°C. Survival rate of *Z. cucurbitae* immature stages increased with increasing temperatures from 20 to 30°C. This further confirms that *Z. cucurbitae* is well adapted at warm climates situated almost in each zone namely southern, northern, eastern, and western of Tanzania.

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