



Effects of Dietary L-ascorbic Acid Supplementation (0.5%) on Adult Survival of Pumpkin Fruit Fly *Zeugodacus tau* (Diptera: Tephritidae), Laboratory Conditions

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ABSTRACT

Adult survival is an important life-history trait in fruit flies and can be influenced by dietary factors, including antioxidant nutrients. This study evaluated whether dietary L-ascorbic acid supplementation at 0.5% affects adult survival and lifespan of the pumpkin fruit fly, *Zeugodacus tau*, and whether the response differs between sexes. Newly emerged males and females were maintained on a control diet of yeast hydrolysate and sugar or the same diet supplemented with 0.5% L-ascorbic acid. Three replicate cages of 20 flies were used for each sex-by-treatment group, and survival was monitored for 51 days. Survival probabilities were compared with Kaplan-Meier analysis and the log-rank test. Restricted mean survival time was calculated, and Cox proportional hazards regression with robust standard errors clustered by replicate cage was used to estimate mortality risk. L-ascorbic acid significantly improved male survival by the log-rank test ($p = 0.0422$). Restricted mean survival time was 49.40 days in supplemented males and 47.63 days in control males. The mortality hazard was lower in supplemented males (hazard ratio = 0.449; 95% confidence interval = 0.251-0.804; $p = 0.007$). Female survival did not differ significantly between diets by the log-rank test ($p = 0.4912$), and the mortality hazard was not significantly associated with supplementation. Dietary L-ascorbic acid was associated with improved survival in male *Zeugodacus tau* under laboratory conditions, whereas a statistically significant survival response was not detected in females.

Keywords: Kaplan-Meier analysis; L-ascorbic acid; restricted mean survival time; survival; *Zeugodacus tau*

INTRODUCTION

The pumpkin fruit fly, *Zeugodacus tau* (Walker) (Diptera: Tephritidae), is an important tephritid pest whose biology and management have recently been reviewed (Liu & Ji, 2024).

In Bangladesh, the pumpkin fruit fly, *Zeugodacus tau* (Walker), formerly referred to as *Bactrocera tau* (Walker) in the older literature, is considered an important pest of cucurbit vegetables (Huque, 2006), causing yield losses and reducing the market quality of

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fruits and vegetables. Due to its broad host range, high reproductive potential, and adaptability to diverse environmental conditions, *Z. tau* has become a major constraint to cucurbit production in many agricultural regions.

Adult survival is an important life-history trait that influences insect persistence and population dynamics (Carey, 2001). Dietary factors may influence adult survival through nutritional and physiological effects. Oxidative stress has long been associated with aging (Harman, 1956). Vitamin C (L-ascorbic acid) can interact with ROS-related physiology; in *Bactrocera dorsalis*, Vitamin C ingestion has been used as a ROS-scavenging treatment and has been associated with changes in ROS-related signaling and detoxification enzyme activity (Zeng et al., 2024).

Previous studies in *Drosophila* have reported variable effects of dietary ascorbic acid on longevity (Massie et al., 1976; Massie et al., 1991). The potential effects of dietary Vitamin C should, however, be considered through several distinct pathways. Its antioxidant activity may contribute to cellular protection, whereas its nutritional and direct physiological effects may influence the insect's overall condition independently of antioxidant activity. Dietary supplementation may also be associated with reproductive processes and related resource allocation, which could indirectly influence adult survival. In addition, Vitamin C may potentially influence oxidative stress; however, oxidative stress markers and antioxidant enzyme activity were not measured in the present study. Therefore, any antioxidant- or oxidative stress-related mechanism underlying survival responses cannot be directly established from the present experiment.

Despite the agricultural importance of *Z. tau*, information regarding the effects of dietary L-ascorbic acid supplementation on adult survival remains limited. Therefore, the present study evaluated its effect on the survival of adult *Z. tau* during a 51-day laboratory observation period, with particular emphasis on possible sex-specific responses. We hypothesized that

L-ascorbic acid supplementation would improve adult survival and that the magnitude of the response would differ between males and females.

METHODS

Insect Culture and Experimental Conditions

The experiment was conducted at the Laboratory of the Radiation Entomology and Acarology Division, Institute of Food and Radiation Biology (IFRB), Atomic Energy Research Establishment (AERE), Bangladesh.

The laboratory colony of *Zeugodacus tau* was originally established using approximately 200–300 pupae obtained from naturally infested cucumbers collected from a local vegetable market in Savar, Bangladesh, during the summer of 2016. The colony has been maintained under laboratory conditions for approximately 10 years, corresponding to about 90 generations. The colony had not previously been exposed to insecticides or irradiation.

Adult flies were reared in steel-frame cages covered with nylon mesh net. The rearing room was maintained at $25 \pm 3^\circ\text{C}$ and 60–70% relative humidity under a 12:12 h light: dark photoperiod. Larvae obtained from the parental generation were allowed to pupate in trays containing sawdust. Newly emerged adults were collected daily, separated by sex, and used for the experiment.

Experimental Diet Preparation

The control diet consisted of yeast hydrolysate and sugar mixed at a 1:3 (w/w) ratio. The treatment diet consisted of the same diet supplemented with 0.5% (w/w) L-ascorbic acid (Sigma-Aldrich, 99%). Sodium benzoate (0.05%, w/w) was incorporated into both diets as an antifungal agent. Food and water were provided *ad libitum* throughout the experimental period. The residual diet was removed and replaced with a freshly prepared diet at 2-day intervals to maintain diet quality and hygiene.

Experimental Design

Newly emerged male and female adults were randomly assigned to either the control diet or

the L-ascorbic acid supplementation diet. Flies were maintained in separate cages according to sex and dietary treatment. For each sex-by-treatment combination, three independent replicate cages (R1, R2, and R3) were established, with 20 adults per cage, yielding 60 individuals per group ($n = 60$).

The experiment comprised four groups: male control, male L-ascorbic acid supplementation, female control, and female L-ascorbic acid supplementation. Thus, a total of 12 replicate cages and 240 flies (120 males and 120 females) were included in the study.

Survival was monitored for 15 days, and mortality was recorded daily throughout the observation period. Individuals remaining alive at the end of the 51-day observation period were treated as right-censored observations.

Statistical Analysis

Survival probabilities were estimated using the Kaplan–Meier product-limit method, and survival curves were compared using the log-rank (Mantel–Cox) test. Restricted mean survival time (RMST) up to 51 days was calculated because the median survival was not reached during the observation period.

Cox proportional hazards regression was performed separately for males and females, with dietary treatment as the predictor and the control diet as the reference group. Robust standard errors, clustered at the replicate cage level, were used to account for potential within-cage correlation. Hazard ratios (HRs) with 95% confidence intervals (CIs) were reported. Statistical significance was set at $p < 0.05$.

Kaplan–Meier analyses were performed using GraphPad Prism, whereas Cox regression with cluster-robust variance estimation was performed using Python (statsmodels v0.14.6, PHReg).

RESULTS AND DISCUSSION

The effect of dietary L-ascorbic acid supplementation on the survival of adult *Zeugodacus tau* was evaluated separately for males and females using Kaplan–Meier survival analysis. In male flies, the survival curve of the L-ascorbic acid-supplemented group remained higher than that of the control group during the observation period (Figure 1A). The survival distributions differed significantly between treatments according to the log-rank (Mantel–Cox) test ($\chi^2 = 4.127$, $df = 1$, $p = 0.0422$), indicating improved survival in L-ascorbic acid-supplemented males.

In females, the Kaplan–Meier survival curves for the control and L-ascorbic acid - supplemented groups showed substantial overlap (Figure 1B). The log-rank test showed no significant difference in survival distributions between treatments ($\chi^2 = 0.474$, $df = 1$, $p = 0.4912$). However, the absence of statistical significance should not be interpreted as evidence of no biological effect, particularly given the limited number of replicate cages and relatively few mortality events.

Median survival time was not reached in any treatment group because more than 50% of the flies remained alive at the end of the 51-day observation period. The restricted mean survival time (RMST) over 51 days was 47.63 days for control males and 49.40 days for Vitamin C-supplemented males. For females, RMST was similar between the control (49.65 days) and Vitamin C-supplemented (49.42 days) groups.

Cox proportional hazards regression with robust standard errors clustered by replicate cage showed that Vitamin C supplementation was associated with a significantly lower mortality hazard in males (HR = 0.499, 95% CI: 0.251–0.804, $p = 0.007$). In females, Vitamin C supplementation was not significantly associated with mortality hazard (HR = 1.372, 95% CI: 0.711–2.647, $p = 0.346$) (Table 1).

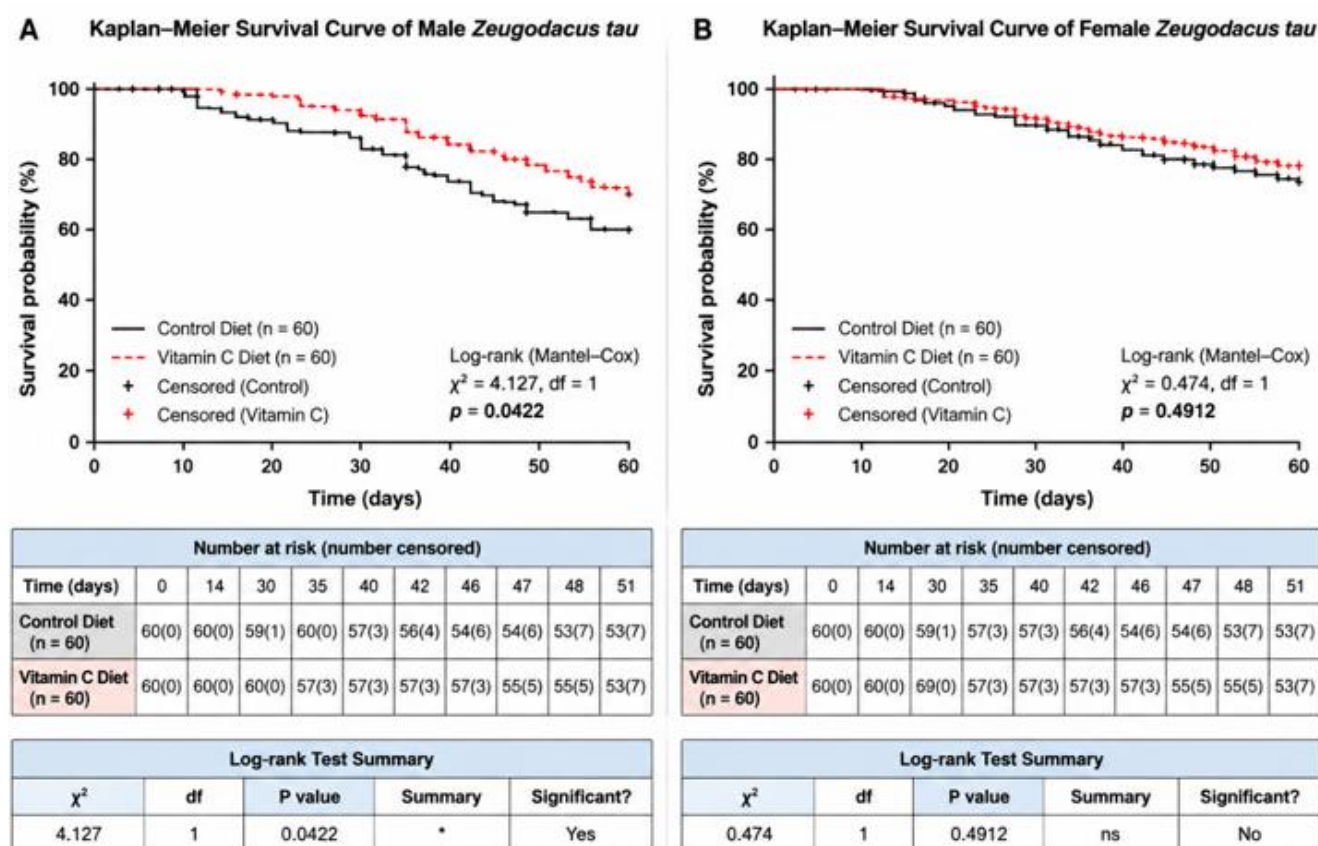


Figure 1. Kaplan–Meier survival curves of adult *Zeugodacus tau* fed control and L-ascorbic acid - supplemented diets. (A) Male flies and (B) female flies. Censoring marks indicate that individuals are still alive at the corresponding observation times. Differences between treatment groups were evaluated using the log-rank (Mantel–Cox) test.

Table 1. Quantitative survival estimates and Cox proportional hazards analysis accounting for replicate-cage clustering.

Sex	Treatment	Deaths	Censored	Median survival	RMST (51 days)	HR (95% CI)	p value
Male	Control	18	42	Not reached	47.63	Reference	—
Male	Vitamin C	9	51	Not reached	49.40	0.449 (0.251–0.804)	0.007
Female	Control	8	52	Not reached	49.65	Reference	—
Female	Vitamin C	11	49	Not reached	49.42	1.372 (0.711–2.647)	0.346

Abbreviations: RMST, restricted mean survival time; HR, hazard ratio; CI, confidence interval. Control was used as the reference category for Cox proportional hazards regression.

The present study demonstrates a sex-specific association between dietary L-ascorbic acid supplementation and adult survival in *Zeugodacus tau* under laboratory conditions. Supplementation with 0.5% L-ascorbic acid significantly improved male survival, as indicated by Kaplan–Meier analysis (log-rank $p = 0.0422$), increased restricted mean survival

time (RMST: 49.40 days in supplemented males compared with 47.63 days in controls), and reduced mortality risk in Cox proportional hazards analysis (HR = 0.449, 95% CI: 0.251–0.804). In contrast, female survival was not significantly affected by supplementation (log-rank $p = 0.4912$; HR = 1.372, 95% CI: 0.711–2.647). These findings indicate that, under the

conditions tested, the survival benefit associated with L-ascorbic acid supplementation was primarily observed in males.

Previous studies investigating dietary L-ascorbic acid have reported variable outcomes. In *Drosophila*, dietary L-ascorbic acid supplementation has produced different responses depending on concentration, strain, and experimental conditions. Earlier work examined dietary ascorbic acid in relation to longevity, whereas a later study reported that lifelong ascorbic acid feeding did not consistently improve lifespan across *Drosophila* strains (Massie et al., 1976; Massie et al., 1991). Oxidative manipulations can also have complex effects on lifespan; for example, early-life antioxidant exposure increased longevity in *Drosophila* in one experimental system (Obata et al., 2018). Together, these findings indicate that antioxidant-related interventions do not universally extend lifespan and may depend on species-specific physiology, dose, timing, and nutritional context. The present study extends this context to a tephritid fruit fly and indicates a sex-specific survival response.

Adult nutritional conditions are important determinants of survival and fitness-related traits in tephritid fruit flies. In *Ceratitis capitata*, post-teneral protein feeding can influence male sexual competitiveness and longevity (Kaspi & Yuval, 2000), while adult dietary composition can affect the longevity of sterile flies (Barry et al., 2007). Post-teneral diets also influence performance-related traits of sterile *Anastrepha* species used in sterile insect technique (SIT) programs (Liedo et al., 2013). In *Bactrocera dorsalis*, diet has been linked to adult fecundity and larval development (Hou et al., 2020), microbiota-associated changes in foraging, fecundity, and survival (Akami et al., 2019), larval fitness and immunity (Hassan et al., 2020), male metabolic rate (Dacre et al., 2024), and mass-rearing performance (Khan et al., 2019; Momen et al., 2025). Probiotic supplementation has also been reported to restore irradiation-related deficits in ecological fitness in *B. dorsalis* (Cai et al., 2018b). In *Zeugodacus cucurbitae*, diet and irradiation can

alter the bacterial symbiome (Asimakis et al., 2019), dietary shifts can affect the gut microbiome (Hendrycks et al., 2022), and gut microbial communities have been associated with adaptability to diet quality and temperature stressors (Hafsi et al., 2024). Additional studies document bacteriome variation and early-adult gut bacterial dynamics in *Z. cucurbitae* and other pest tephritids (Asimakis et al., 2021; Mason et al., 2023). Diet- and irradiation-associated microbiome changes have likewise been reported in *Bactrocera tryoni* (Woruba et al., 2019), while probiotic-based diets can influence immune and stress responses in irradiated *C. capitata* males (Msaad Guerfali et al., 2021). Collectively, these studies show that nutritional interventions can affect multiple survival- and quality-related traits in tephritids, supporting further evaluation of the male survival response observed here in laboratory colony management.

The physiological mechanism responsible for improved male survival remains unresolved. Nutritional and environmental conditions can influence oxidative physiology in tephritid fruit flies. In *Bactrocera dorsalis*, a sugar-only diet reduced several antioxidant enzyme activities and increased malondialdehyde levels, indicating altered oxidative balance (Chen et al., 2017). Radiation and UV exposure have also been associated with changes in antioxidant enzyme activities and physiological stress responses in tephritids (Cai et al., 2018a; Cui et al., 2021). Vitamin C has also been used as a ROS-scavenging treatment in *B. dorsalis* and was associated with changes in ROS-related CncC/Keap1/MafK signaling and detoxification enzyme activity (Zeng et al., 2024). However, ROS levels, oxidative damage markers, and antioxidant enzyme activities were not measured in the present study. Antioxidant protection should therefore be regarded as a possible explanation rather than a demonstrated mechanism.

The absence of a significant female response requires cautious interpretation. Sex-specific differences in nutrient allocation, metabolism, and life-history strategies may contribute to different responses to dietary supplementation

(Carey, 2001). However, reproductive traits such as fecundity, egg production, and mating performance were not measured, so reproductive allocation cannot be established as the explanation for the observed sex difference. In addition, the limited number of replicate cages and mortality events may have reduced statistical power to detect smaller effects in females. The nonsignificant female result should therefore not be interpreted as evidence that Vitamin C has no biological effect in females.

The improved survival observed in males may have potential implications for laboratory colony maintenance and SIT-related nutritional management. Nevertheless, increased longevity alone does not demonstrate improved reproductive quality or field performance. This study did not evaluate mating competitiveness, flight ability, reproductive performance, irradiation tolerance, or post-release survival. Consequently, any application of Vitamin C supplementation in mass-rearing or SIT programs requires validation using appropriate quality-control and reproductive-performance measures.

Several limitations should be considered. Only one Vitamin C concentration was tested; the experiment included three replicate cages per treatment group, and the 51-day observation period resulted in substantial right-censoring. Physiological markers of oxidative stress and reproductive traits were also not measured. Future studies should evaluate dose-dependent responses, antioxidant enzyme activity, oxidative-damage markers, reproductive performance, and SIT-related fitness traits.

CONCLUSIONS

In conclusion, dietary supplementation with 0.5% L-ascorbic acid was associated with improved survival and reduced mortality risk in male *Zeugodacus tau* under laboratory conditions, whereas no statistically significant survival response was observed in females. These findings indicate that adult nutritional supplementation can influence survival in this tephritid, but further physiological and reproductive studies are needed to establish

the underlying mechanisms and practical applications.

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