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Effects of sucrose in adult diet on survivorship of males of *Bactrocera cucurbitae*

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Abstract
Mortality of adult males of melon flies, *Bactrocera cucurbitae,* fed sucrose and protein was studied in the present study. Flies fed only sucrose plus protein (optimal food) or only sugar from the day of adult eclosion shows long survivorship, or no significant mortality, in the study period. However, flies fed only protein from the day of eclosion or no food shows rapid change of survivorship from 100% to 1.7% and 8.4% respectively within four days. At the same time, flies diet changed from optimal diet to protein or optimal diet to no food on 7th day results survivorship of only 0% and 6.6%, and on 11th day survivorship of 0% in only 96 hours. However, no significant mortality of adults was observed when flies were shifted from the optimal diet to sugar-only on 7th and 11th day onwards. Therefore, the experiment demonstrates that flies have an absolute requirement of carbohydrate in the adult diet. It also indicates that flies have limited capability to convert lipids to metabolic energy.

Keywords: *Bactrocera cucurbitae*, sucrose, mortality

Introduction
Fruit flies (Diptera: Tephritidae) are extremely noxious pests of fruits, vegetables. Most of the species of fruit flies are polyphagous and thus damage a wide range of fruits and vegetables [1, 2]. Feeding by their larvae (maggots) damages the fruit internally, causing it to ripen prematurely and rot. Upto 100% of fruits may damage by fruit fly when lift uncontrolled [3]. As such, their infestation not only reduces the fruit yield but also affects the quality and commercial value of the crop. They are very common insect pests of economic importance in tropical, subtropical and several temperate regions of world [4, 5]. Billions of dollars of agriculture commodity are lost due to these pests infestation every year worldwide [6]. Therefore, their cosmopolitan nature pinnacles them internationally importance in sustainable fruit and vegetable production as well as trade issues.
The fruit flies of the family Tephritidae is considered the main fruit pests worldwide [7]. Out of 5000 documented species in Tephritidae family, 70 species of fruit flies are important agricultural pests on different vegetables and fruits of tropical and subtropical regions [8, 9, 10]. Around 40 species of fruit flies under the *Bactrocera* genera are considered the most significant group of insect pests from the economic point of view.
The melon fruit fly, *B. cucurbitae* (Coquillett) (Diptera: Tephritidae), a noxious pest of vegetable crops [11], is known as *Dacus cucurbitae* [12, 13]. It has 125 plant species as host in Hawaii, while 42 host species in South-east Asia [14]. In Bangladesh, among other fruit flies infestation in different vegetables *B. cucurbitae* represents 74.5% of the total population [15].
Numerous approaches have been implemented to control pests all over the world for years. Among them Sterile Insect technology had been proved as an efficient tool to control this pest and it is a more target-specific and safe for fruit fly control method [16] where a large number of flies are reared in the laboratory. Although SIT has been proved to be the most effective population management tools for insects in isolated areas, one of the biggest pitfalls with this technique is its high cost. At the same time more significant costs of SIT requires for mass rearing of flies for as many as 7 days prior to release so that they have enough time to become sexually mature to meet wild female insects. As a result, cost is still a big concern for effective pest control by this technique. Therefore the efficacy of SIT management protocol should be improved minimizing cost without compromising reproductive capabilities.
Before applying SIT it is essential to ensure biological parameters of the pest and pre-requisite to develop healthy adult. To produce healthy adult it is necessary to maintain the colony with a good dietary supplement where the competitive adults could bring about a successful suppression in the field population [17].
Yeast is considered a major source of nutrition for fruit flies due to its appropriate concentration of protein, sterols, vitamins and minerals. Although, it also has an important components and used to mass rearing of larvae and adult diets for rearing of tephritid flies [18, 19, 20, 21], Scientists are looking for better diet to produce more strong and suitable insect generation for SIT. In line with this object, we prepared different diets and conducted experiments to check their suitability in our region. We observed the diets effect of different biological parameters on *B. cucurbitae*.
The necessity of consumption of both carbohydrate and protein for the reproduction and effective signaling has been studied [22]. Although absolute necessities for either protein or sugar in the adult diet of *Anastrepha suspensa* has recently been studied [23] in USA, no such work on the *B. cucurbitae* has been performed so far, especially in slight different environment of subtropical region. This report justifies the absolute requirement of carbohydrate for adult survival.

Materials and Methods

The present study of melon flies (*B. cucurbitae*) were conducted at the laboratory of Radiation Entomology & Acarolgy Division(READ), Institute of Food and Radiation Biology (IFRB), Atomic Energy Research Establishment (AERE), Savar, Dhaka, during August 2018 to November 2018. Different parameters like survivorship of flies after being fed only protein or sugar or no food, were studied in the study. In this experiment, flies were either provided with protein, sugar or optimal diet, from the day of eclosion or switched from the optimal diet to only protein or sugar, or protein or sugar to no food(only water), from 7th and 11th day onwords. Laboratory cultures of *B. cucurbitae* were housed in steel frame cages (12×10×8 cm) covered with nylon net, maintained at 25 ± 2^0 C temperature and 65-70% humidity and 12:12 L:D at the fruit fly laboratory.

The experiment on adult diets were conducted using the same sizes of rearing cages fencing with nylon net where the adult emerge from the randomly selected 100 pupae from the lab stock were kept. A 50 ml conical beaker full of distilled water with soaked cotton provided as continuous supply of water and for the maintenance of moisture content inside the rearing cages. The residual diets were discarded and fresh diet was provided in every two days interval. The optimal diet (Sugar and Yeast in 3:1) was used as the standard diet. Sugar was supplied in cube form where as protein diet was provided in cake form. Data of mortality of flies were recorded each 24 hours from where survivorship was calculated.

After emergence a certain amount of optimal diets, only sugar or only protein, were served in a petridish in all cages as food source and the effects of these diets on mortality of adult male flies were assessed. Experiments were also conducted in which we maintained the males on the optimal diet after eclosion and then switched to sugar-only or protein-only or no food (only water) diets from 7th day and 11th day onwards, to have effect of individual dietary change on survivorship. In each case, mortality of flies was counted to compare with mortality on optimal diet. ANOVA was employed to study variance for the present study.

Results

Impact of different diet on survivorship males of *Bactrocera cucurbitae* has been confirmed in the present study. Study shows (Table 1-4) mortality profile of 100 flies fed different diet or switched from one diet to another or no food(except water). At the beginning, flies fed only optimum diet from the day of eclosion, shows large survivorship of 88% on 4th day (Table 1).

Table 1: Mean survivorship of flies on each day after being fed only optimum diet (OPD).

Days feeding only optimum diet	Mean survivorship of *B. Cucurbitae* flies
0	100
1	100
2	97.7
3	92.4
4	88

* Mean survivorship of flies fed only OPD was 95.62

However, the experiment ensures very poor survivorship, i.e. large mortality, of only 1.7% flies when they were fed only protein from the day of eclosion (Table 2). It also demonstrates the similar mortality pattern after switching optimal diet to only protein on 7th day or 11th day. Study reveals 100% of mortality for both cases in only 4 days with new food supliment.

Table 2: Mean survivorship of flies on each day after being fed only protein.

Days after feeding only protein	Mean survivorship of flies fed only protein from day of eclosion		Mean survivorship of flies switched to protein on day 7		Mean survivorship of flies switched to protein on day 11	
	Present study	Anastrepha [23]	Present study	Anastrepha	Present study	Anastrepha
0	100	100	100	100	100	100
1	99.1	91.5	96.81	94.8	99.67	92.4
2	70.6	33.0	40.4	39.7	60.67	8.0
3	31.5	8.5	11.1	8.7	6.67	2.4
4	1.7	0	0	1.0	0	0.0

* Means in the same column or row are not significantly different in an ANOVA or Fisher's Least Significant Difference (LSD) test (*P=0.93>0.05, df=2, 12; F=0.072*). Mean survivorship of flies fed protein-only for 4 days from day of eclosion was 60.58%; 4 days from 7th day was 49.66%; and 4 days from the day of 11th day was 53.4%

Similarly, Survivorship decreases dramatically for the flies fed no food (except water). The experiment maintained poor survivorship, or large mortality, of only 8.4% in four days, as they were fed no food (except water) from the day of eclosion (Table 3). It also illustrates similar pattern of mortality while switching from optimal diet to no food on 7th day or 11th day. Experiment shows 6.6% and 0% survivorship respectively, or 93.4% and 100% of mortality respectively, on 4th day since withdrawing of diet.

Table 3: Mean survivorship of flies on each day after all food was withheld:

Days after removing food	Mean survivorship of flies starved from day of eclosion		Mean survivorship of flies switched to no food on day 7		Mean survivorship of flies switched to no food on day 11	
	Present study	Anastrepha [23]	Present study	Anastrepha	Present study	Anastrepha
0	100	100	100	100	100	100
1	98.6	81	76.4	90.7	100	85.6
2	79.5	21.5	68.6	54.6	53.3	8.0
3	55.5	4.0	16.4	5.8	3.0	1.6
4	8.4	0.0	6.6	0.5	0.0	0.0

* Means in the same column or row are not significantly different in an ANOVA or Fisher's Least Significant Difference (LSD) test (P=0.79>0.05, df=2, 12; F=0.235). Mean survivorship of flies fed no food (except water) for 4 days from day of eclosion was 68.4%; 4 days from 7th day was 53.6%; and 4 days from the day of 11th day was 51.2%

However, it shows very high survivorship (97.3%) when flies were fed only sugar from the day of eclosion (Table 4). It also presents the similar mortality diagram while switching from optimal diet to only sugar. Study demonstrates 97% and 98.67% of survivorship on 4th day after switching optimal diet to only sugar on 7th and 11th day respectively.

Table 4: Mean survivorship of flies on each day after being fed only sugar.

Days after feeding sugar only	Mean survivorship of flies fed only sugar from day of eclosion		Mean survivorship of flies switched to sugar on day 7		Mean survivorship of flies switched to sugar on day 11	
	Present study	Anastrepha[23]	Present study	Anastrepha	Present study	Anastrepha
0	100	100	100.0	100	100	100
1	100	100	98.33	100	100	100
2	99.1	100	97.67	99.8	100	100
3	98.6	100	97.3	98.6	100	98
4	97.3	99.0	97	96.6	98.67	94.7

* Means in the same column or row are not significantly different in an ANOVA or Fisher's Least Significant Difference (LSD) test (P=0.06>0.05, df=2, 12; F=3.49). Mean survivorship of flies fed only sugar for 4 days from day of eclosion was 99%; 4 days from 7th day was 98.06%; and 4 days from the day of 11th day was 99.734%

Therefore, flies fed only sucrose plus protein (optimal food) or only sugar from the day of adult eclosion, or fed only sugar switched from optimal diet shows no significant mortality change in the study period. Results also shows good agreement with the experiment with Anastrepha [23].

Discussion

A number of studies have demonstrated the importance of dietary supplements of *Tephritidae, the family that B. cucurbitae also belongs to*. Protein dietary supplement enhance sexual performance of adult male [24] and play positive effects on ovarian maturation and fecundity in female [25] of *Tephritidae* species.

Another research also shows that sugars are necessary dietary requirements [26] and is important for long live of species. Also removal of protein and carbohydrate from adult dietary supplements results rapid mortality of adult insects. However, there is very little information about the role of sugar only and if the protein can be replace of sugar. There has been no such research especially on *B. cucurbitae* so far.

The current research demonstrates clearly the carbohydrate is the mandatory supplement for survival of both sexually matured and immature male insects. The same rate of survival of flies, provided with only protein or switched from carbohydrate to protein after 7 and 11 day, suggest strongly that *B. cucurbitae* flies have limited capacity to convert protein resources, rich in amino acids that are readily convertible by some other insects, into energy. Therefore it can't take the place of sugar.

At the same time the absolute requirement of carbohydrate and inability to live with metabolic energy of protein indicates the physiological adaptation of flies to the environment. Files in regions like Bangladesh, where is abundance of sugar from fruits and nectar, and scarcity of protein, might have been adapted to live with the help of carbohydrate only. Indeed, protein dietary supplements have been shown to have positive effects on ovarian maturation and fecundity of Tephritidae [24, 25] indicating that dietary protein is a critical component for reproductive success for egg production. Thus, the flies have probably developed a physiological strategy in which they utilize the most available food source, sugars, to insure survival, and take advantage of limited protein resources when available to achieve sexual maturity [26]. The present research findings in B *cucurbitae* has good match with the findings in Anastrepha research [23].

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