

Organically grown fruits' effect on reproductive fitness of *Drosophila melanogaster*

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Abstract: Quality of food consumed by an organism plays a very strong environmental impact on the organism's health and fitness. A factor which is easily and quickly influenced by the type of food consumed is reproductive fitness. In the present study *D. melanogaster* were reared in wheat cream agar media as well as media prepared from organically and conventionally grown fruits. These flies were then subjected to tests to study their reproductive fitness. The results obtained showed that consumption of organic fruits increased the reproductive capacity of the fly however no significant beneficial effect was observed when longevity was considered as a parameter.

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1. Introduction

An organism's growth, development, reproduction and survival are influenced by numerous internal and external environments. External environment has been shown to affect the fitness of an organism through the variations in nutrients that have been consumed by the organism (Sisodia and Singh, 2012).

The diet consumed by an individual can be grouped broadly into quantitative, which is dependent on food availability and qualitative, which is dependent on nutritional constitution of the food (Sisodia and Singh, 2012). A study conducted on the food consumed by colobus has shown that there is a significant disparity in the nutritional content of food depending on the external environment (Chapman *et al.*, 2003).

In modern times the popularity and consumption of organic fruits and vegetables is growing at an exponential rate due to a belief that such foods are healthier and free of pesticides. It is believed that consumption of food exposed to pesticides has detrimental effect on the health of an individual. It has been shown that individuals exposed to high levels of pesticides face increased risks of developing cancers such as prostate cancer (Alavanja *et al.*, 2003). On the other hand studies on comparison between organically and conventionally grown foods have shown that organic samples have fewer pesticide residues when compared to conventionally grown food (Baker *et al.*, 2002).

To study the beneficial effects of organic food on reproduction, *D. melanogaster* was selected as a model system since it has been used successfully as a model organism in study of a wide array of subjects ranging from muscular and neuronal disorders to understand diseases like muscular dystrophy (Shcherbata *et al.*,

2007) to understand neurodegenerative diseases like Parkinson's disease (Feany and Bender, 2000), as well as to learn the relation between disease and oxidative stress in the body (Llorens *et al.*, 2007).

A recent study has shown positive effects of organic foods on the health of *D. melanogaster* where flies reared in organically grown food showed overall better health (Chhabra *et al.*, 2013). Studies however were not carried out on the effect of organic foods on reproductive parameters such as mating latency, copulation duration and ovariole number. Hence present study has been undertaken in *D. melanogaster* to study the effect of organically grown fruits on reproductive performance.

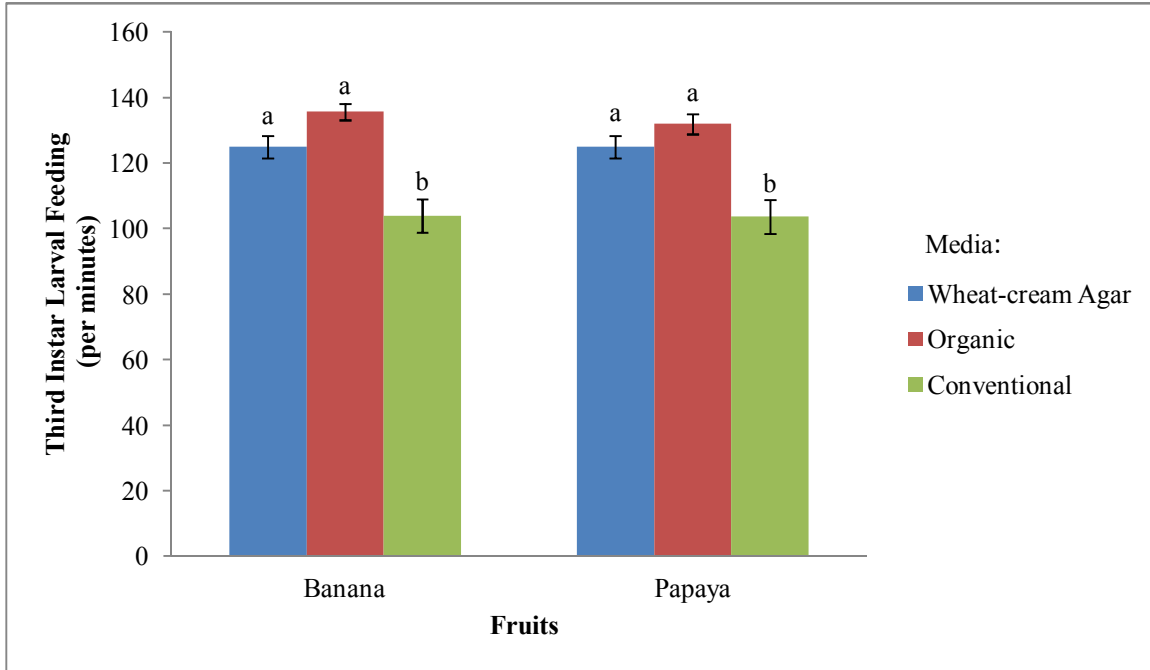
2. Materials and Methods

2.1. Establishment of Stock

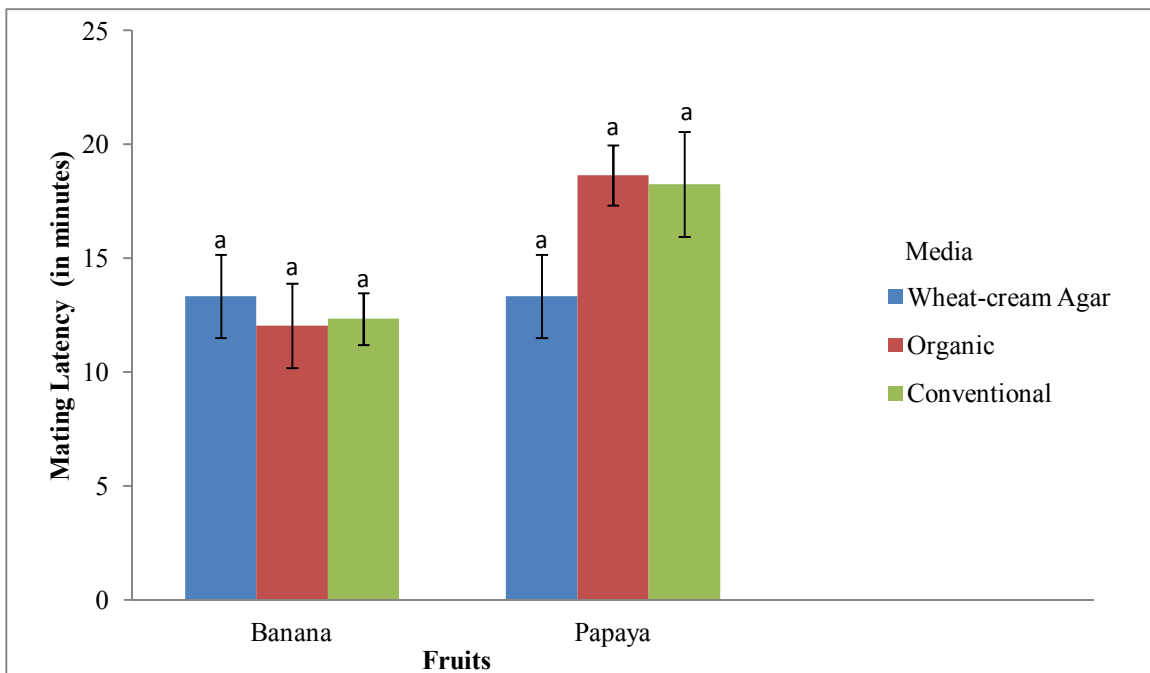
The experimental stock of *D. melanogaster* was established from the progenies of 150 naturally inseminated females which were collected at Chamundi Hills, Mysore, India. In each generation flies obtained from these culture bottles were mixed together and redistributed to 20 different culture bottles containing wheat cream agar media (100g of jaggery, 100g of wheat powder, 8g of Agar was boiled in 1000ml of double distilled water and 7.5ml of Propionic acid was added) each with 20 flies (10 males and 10 females). These flies were maintained at 22°C±1°C with a relative humidity of 70% in a 12 hours dark: 12 hours light cycle. This procedure was carried out for three generations to acclimatize flies to lab condition. At fourth generation, eggs were collected using Delcour's procedure (1969). Eggs (100) were seeded to each culture bottle containing wheat-cream agar media / organically grown fruit based media / conventionally grown fruit based media (1kg of fine paste of organically / conventionally

grown banana or papaya was boiled in 1l of double distilled water containing 10% *Agar*. To this 7.5 ml of Propionic acid was added). When pupae were formed, females and males were isolated and were, allowed to eclose and aged for five days to test for virginity.

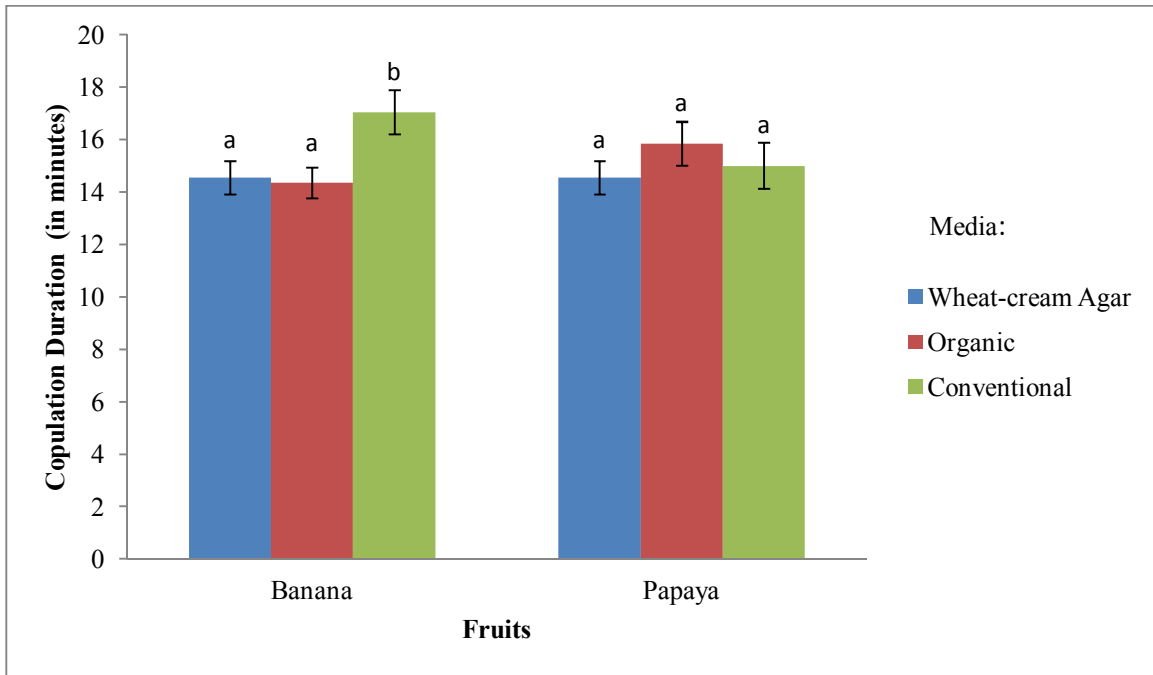
These flies were used for present experiments. Organic fruits were purchased from organic food product distributors, *Hasiru Organic Shop*, Mysore which is a unit of Karnataka State Natural Farming Movement.



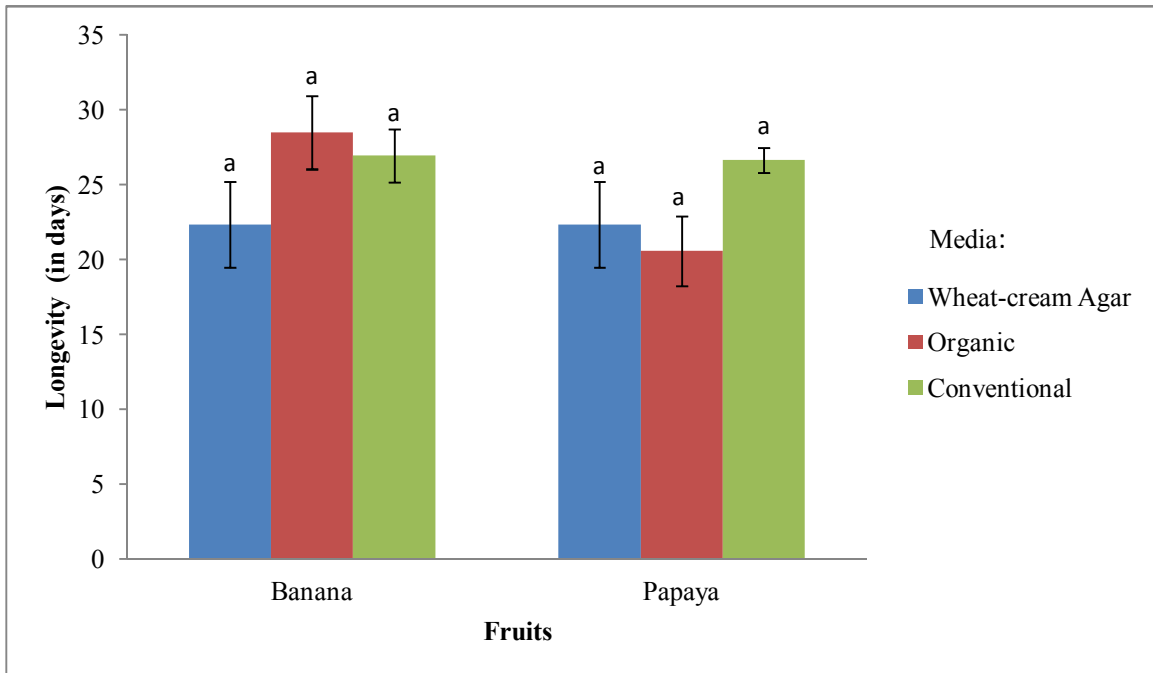
Different letters on the bar graph indicate significance at 0.05 level by Tukey's Post Hoc Test
Figure 1. Organically grown fruits' (Banana and Papaya) effect on larval feeding of *D melanogaster*



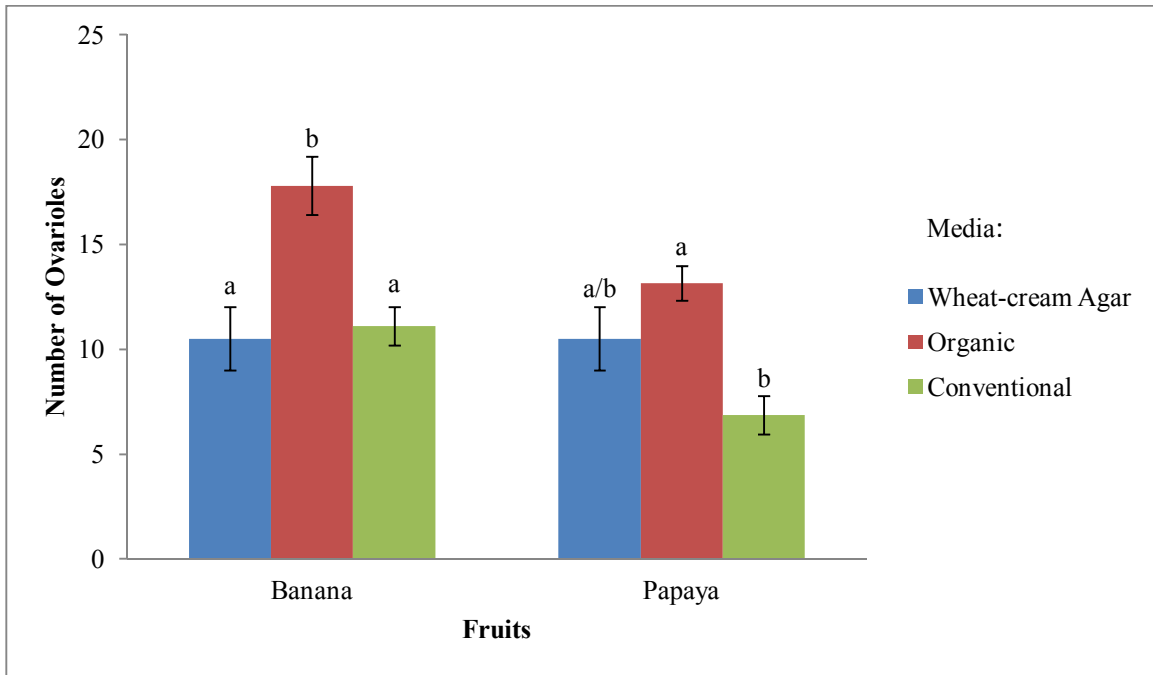
Different letters on the bar graph indicate significance at 0.05 level by Tukey's Post Hoc Test
Figure 2(a). Organically grown fruits' (Banana and Papaya) effect on mating latency in *D melanogaster*



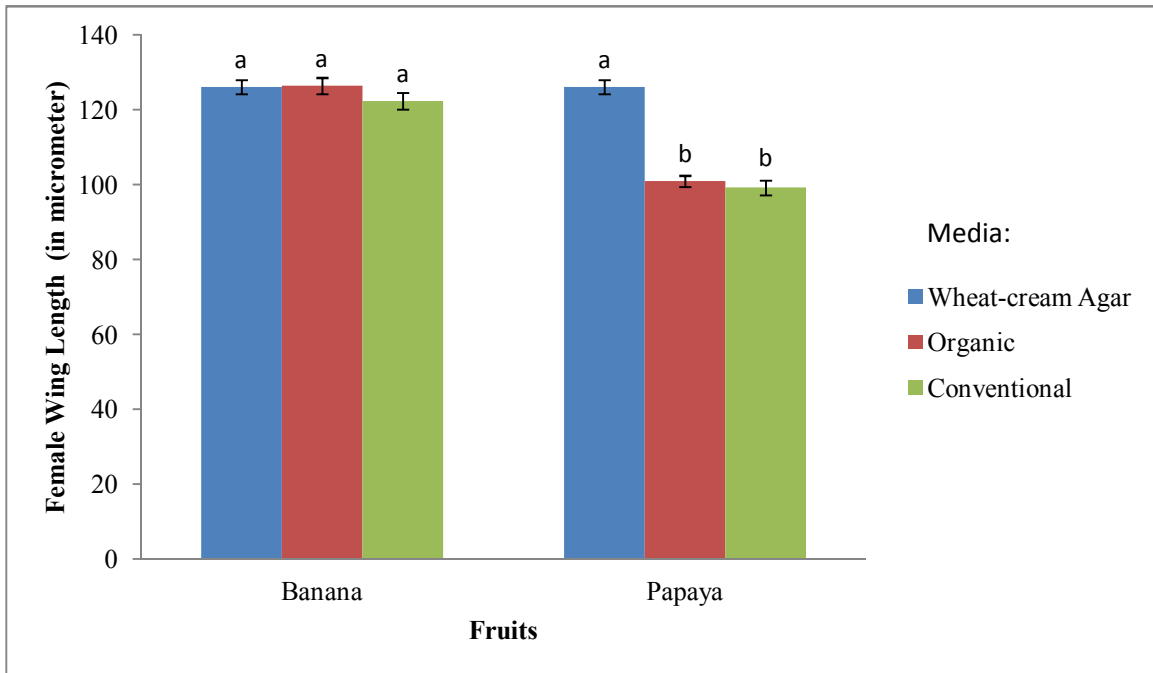
Different letters on the bar graph indicate significance at 0.05 level by Tukey’s Post Hoc Test Figure 2(b). Organically grown fruits’ (Banana and Papaya) effect on copulation duration in *D melanogaster*



Different letters on the bar graph indicate significance at 0.05 level by Tukey’s Post Hoc Test Figure 3. Organically grown fruits’ (Banana and Papaya) effect on longevity of *D melanogaster*



Different letters on the bar graph indicate significance at 0.05 level by Tukey’s Post Hoc Test
 Figure 4(a). Organically grown fruits’ (Banana and Papaya) effect on ovariole number in *D melanogaster*



Different letters on the bar graph indicate significance at 0.05 level by Tukey’s Post Hoc Test
 Figure 4(b). Organically grown fruits’ (Banana and Papaya) effect on female wing length in *D melanogaster*

Table 1. One way ANOVA of organically grown fruits' (Banana and Papaya) effect on larval feeding, longevity, and reproductive fitness and wing length in *D melanogaster*

Dependent Variable	Fruit	Source	Type III Sum of Squares	Df	Mean Square	F-Value	Sig
Larval feeding (minutes)	Banana	Media	10444.633	2	5222.317	17.555	.000
		Error	16956.100	57	297.475		
		Total	914108.000	60			
	Papaya	Media	8733.233	2	4366.617	13.199	.000
		Error	18857.500	57	330.833		
		Total	894954.000	60			
Mating Latency (minutes)	Banana	Media	18.533	2	9.267	.173	.842
		Error	3056.050	57	53.615		
		Total	12575.000	60			
	Papaya	Media	348.400	2	174.200	2.526	.089
		Error	3930.850	57	68.962		
		Total	21113.000	60			
Copulation Duration (minutes)	Banana	Media	90.533	2	45.267	4.705	.013
		Error	548.450	57	9.622		
		Total	14715.000	60			
	Papaya	Media	17.433	2	8.717	.700	.501
		Error	709.500	57	12.447		
		Total	14468.000	60			
Longevity	Banana	Media	409.233	2	204.617	1.772	.179
		Error	6582.500	57	115.482		
		Total	47344.000	60			
	Papaya	Media	387.700	2	193.850	2.034	.140
		Error	5431.900	57	95.296		
		Total	38114.000	60			
Ovariole Number	Banana	Media	656.933	2	328.467	9.782	.000
		Error	1914.000	57	33.579		
		Total	12920.000	60			
	Papaya	Media	400.233	2	200.117	7.910	.001
		Error	1442.100	57	25.300		
		Total	8044.000	60			
Wing Length (micrometer)	Banana	Media	201.433	2	100.717	1.140	.327
		Error	5034.500	57	88.325		
		Total	943236.000	60			
	Papaya	Media	9042.233	2	4521.117	69.224	.000
		Error	3722.750	57	65.311		
		Total	723229.000	60			

2.2. Organically Grown Fruits' (Banana and Papaya) Effect on Larval Feeding in *D melanogaster*

Third instar larvae obtained from eggs collected (± 2 hours) from wheat-cream agar media grown flies using Delcour's procedure (1969) were used to study feeding behavior. Each larva was placed in a vial containing wheat-cream agar / organically grown fruit based media / conventionally grown fruit based media and observed under a stereomicroscope. The back and forth movement of the proboscis was recorded for a minute. A total of 20 replicates were

run separately for each of organic / conventional / wheat-cream agar media grown larvae. Separate experiments were carried out for organic / conventional banana / papaya.

2.3. Organically Grown Fruits' (Banana and Papaya) Effect on Reproductive Performance in *D melanogaster*

Four days old virgin female and unmated male from the wheat-cream agar / organic fruit based media / conventional fruit based media were aspirated into an Elens-Wattiaux chamber (Elens and Wattiaux, 1964). Each pair was observed for an hour.

The pairs which did not mate within this time limit were discarded. Mating latency (time between introduction of a pair of male and female flies into the Elens-Wattiaux chamber until the initiation of copulation between each pair) and copulation duration (time between initiation and termination of copulation) were recorded with the units taken in minutes. Mated pairs were transferred to vials containing 5 ml of their respective media and they were screened for longevity (number of days survived by a fly from day of eclosion to death). A total of 20 replicates were performed separately for wheat-cream agar media / organically / conventionally grown flies. Separate experiments were carried out for organic banana and papaya.

2.4. Organically Grown Fruits' (Banana and Papaya) Effect on Wing Length and Ovariole Number in *D melanogaster*

Four days old virgin females were etherized and killed. The thorax of these flies was dissected out using a pair of fine dissection needles in physiological saline under a binocular stereomicroscope. The ovaries were separated and the total number of ovarioles in either the right or the left ovary was noted. The wings of the same fly were measured following the procedure of Hegde and Krishna (1997)

2.5. Statistical Analysis

Mean, standard error, One-way ANOVA and Tukey's Post-Hoc test were carried out on the obtained data using SPSS version 19.0 (IBM Corp. Released 2010. IBM SPSS Statistics for Windows, Version 19.0. Armonk, NY: IBM Corp).

Results

Figure 1 provides the larval feeding rate in different diets. It was found that highest larval feeding rate occurred in flies grown in both the organic fruit based media compared to conventional and wheat cream agar media. Flies fed on conventional media performed poorly in comparison. One-way ANOVA followed by Tukey's Post Hoc test carried out using SPSS version 19.0 on the above data showed significant variations in feeding rate depending on whether organic or conventional fruits were used (Table 1).

Mating latency of flies grown in different diets is provided in Figure 2(a). It was observed that flies grown in organic papaya based media had highest mating latency closely followed by flies grown in conventional papaya based media. Flies fed on wheat cream agar based media performed the best with lowest mating latency. In case of banana based media, flies grown in organic banana based media had the lowest mating latency whereas mating latency of flies reared in wheat cream agar media was

the highest. One way ANOVA followed by Tukey's Post Hoc test carried out on the above data using SPSS version 19.0.0 however showed no significant variation as shown in Table 1.

Copulation duration (Figure 2b) of flies reared in conventionally grown banana media was found to be higher than those flies reared in organically grown banana based media and wheat-cream agar based media. In case of papaya copulation duration in organic fruit media fed flies was longer than those fed on conventional fruit media. One-way ANOVA followed by Tukey's Post Hoc test carried out using SPSS version 19.0.0 (Table 1) showed significant variations in copulation duration of flies raised in banana based media but no significant variation was observed in case of flies raised in papaya based media.

Figure 3 depicts the number of days survived by the adult flies grown in different diets. It was found that flies grown in organic banana based media survive the longest closely followed by flies fed on conventional banana based media. Flies reared in wheat cream agar media lived for fewer days when compared to flies grown in banana based media. Flies grown in organic papaya based media had the least life span when compared to wheat cream agar media reared flies that performed intermediately and conventional papaya based media reared flies that survived for the longest time span. One-way ANOVA and Tukey's Post Hoc test conducted using SPSS version 19.0.0 on the longevity data revealed no significant variation between organic and conventional media as represented in Table 1.

There was a significant increase in ovariole number of female flies reared in organic media when compared to both conventional media as well as wheat-cream agar media in both fruits. The data is represented in Figure 4(a). A significant variation between organic media and conventional and wheat cream agar media in case of both the fruits was also observed when the data was subjected to one way ANOVA and Tukey's Post Hoc test using SPSS version 19.0.0 as shown in Table 1.

Wing lengths of female flies are represented in Figure 4(b). It was seen that female flies fed on organic banana based diet had wings slightly larger than those fed on wheat-cream agar media and conventional banana based media. Female flies fed on organic papaya based media showed slightly larger wing length when compared to those fed on conventional papaya based media. However when wing length of flies fed on papaya based media was compared with wheat-cream agar media it was found to be substantially shorter. One-way ANOVA followed by Tukey's Post Hoc test carried out using SPSS version 19.0.0 (Table 1) showed highly

significant variations in wing length of flies raised in papaya based media and insignificant variations were observed in wing lengths of flies raised in banana based media.

Discussion

To study the beneficial and detrimental effects of organically grown fruits on reproductive traits of *D. melanogaster*, fruit flies were fed a diet made out of fruits. The flies grown in these cultures were then subjected to experiments to determine their fitness.

As shown in Figure 1 and Table 1 experiments on feeding rate suggest that diet type had a significant influence on larval feeding. During the larval stages, *Drosophila* show an inhibition threshold when consuming new or foul tasting foods (Melcher *et al.*, 2007). Such inhibition threshold is not observed in larvae fed on organic fruits when compared to conventional fruits since the rate of larval feeding was highest among larvae fed on organic fruits.

Mating latency in the present study as represented in Figure 2(a) and Table 1 showed slight decrease by the consumption of organic banana based diets and slight increase by consumption of organic papaya based diets when compared to conventional fruit based diet, although the results were not significant. Another parameter which was observed was duration of copulation. Variation was evident in copulation duration wherein flies consuming conventionally grown banana based media performed significantly better than their organic counterparts. However flies consuming organically produced papaya media fared slightly better than flies consuming conventional papaya media but the values proved insignificant on comparison (Figure 2(b) and Table 1). This variation maybe caused due to varying factors that affect the fruits during cultivation and processing leading to variation in nutrient levels (Crinnion, 2010).

Longevity (Figure 3 and Table 1) is an important life history trait of an animal. Consumption of organically grown banana based media increased the lifespan of flies slightly compared to those grown in conventional media. This co-relates with the study conducted by Chhabra *et al.*, (2013) who proved that flies raised on an organic banana diet had similar longevity to flies raised on conventional banana food. The longevity of flies grown in organic papaya based media decreased when compared to conventional papaya based media however the results were found to be insignificant.

Figure 4(a) and Table 1 represent the ovariole number in female flies. In female flies organic food proved to be highly beneficial in increasing ovariole number. This is supported by a review conducted by Williams (2002), who has stated that organic food

may have a beneficial effect on reproduction and pregnancy however the reason for this remains inconclusive and data inconsistent. Wings as shown in Figure 4(b) and Table 1 also showed a slight increase in size in flies grown in organic media. Difference was observed in the wing length of flies reared in banana media when compared with those raised in papaya media indicating difference in the amount of nutrition provided by different fruits.

Thus we can conclude from this study that organic fruits do provide health benefits to *D. melanogaster*. However the effect may vary from one fruit to another.

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