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RESEARCH ARTICLE

EFFECT OF ORGANIC FRUITS (BANANA AND PAPAYA) ON LOCOMOTOR ABILITY AND RESISTANCE TO EITHERIZATOIN IN *DROSOPHILA MELANOGASTER*

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ABSTRACT

The amount and quality of diet taken by an organism have a strong impact on almost all activities of organisms. In the present study flies of *D. melanogaster* fed on organic, and non organic and normal food media were subjected to crawling and climbing assay and resistance to etherization. It was found that flies grown on organic fruits had consumed greater quantity of food and had a significantly greater locomotor ability over flies grown on non organic and in normal food media. Similar recent was also noted in flies resistance to etherization. Thus these studies in *D.melanogaster* suggest organic fruits had beneficial effect on locomotor ability.

INTRODUCTION

Locomotion is an important activity of an organism through this activity organism can locate their mate., search food, shelter, escape from predators (Katherine W. jordon *et al.*,). Further the health of an organism is also depends on locomotor activity. Energy required for locomotion comes from its diet. Therefore, diet is one such factor known to effect fitnes of an organism through its nutrients composition (Sisodia, 2012). Diet effect can be divided in to two approaches such as qualitative, which is dependent on nutritional constituents while quantitative effect depends on quantity of food taken in to the body of an organism (Wafa and Krishna, 2015). Day by day consuming organic food over non-organic food is increasing because of the belief that organic foods are free of pesticides and no side effect compared to non-organic food. It has also been shown that individuals exposed to high levels of pesticides face increased risks of developing prostate cancer. In recent times using *Drosophila melanigaster* Chabbra *et al.*, 2013 and Wafa and Krishna, 2015 have shown Benefit of organic fruits and vegetables. However the effect of organic and non organic fruit on locomotory activity has not been studied . since *D. melanogaster* is one of the very good model organism to study nutritional requirements of an organism and it also being used as model system for many of the human disorders because of the mechanisms involved in metabolism are very much conserved between *Drosophila* and humans therefore present study has been undertaken to study effect of organic fruits on locomotor ability and resistance to etherization.

MATERIALS AND METHOD

Establishment of Stock

The experimental flies used for the present experiment was established from the progenies of 150 naturally inseminated females of *D. melanogaster* 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). Twenty flies per culture bottle (10 males and 10 females) were used to culture flies. These flies were maintained at 220º C±10 C with a relative humidity of 70% in a 12 hrs 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 non organic grown fruit based

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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 sallowed 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.

Quantification of Food intake in Larvae using Dye method

Larval feeding rate was measured as fallows. Ten Second instar larvae obtained from normal media were used to study feeding behavior. The larvae were obtained by scooping out from the respective treated media and washed in saline. Each larva was placed individually in a vial containing normal /organic and non organic fruit media containing 2.5% (w/v) blue food dye (FD & C Blue Dye no. 1). The larvae were allowed to feed for 15 minutes. Then the larvae were transferred to Eppendorf tube and frozen. These frozen larvae were homogenized by adding 200 µl of distilled water further 800 µl of distilled water was added. The absorbance was measured at 629 nm using calorimeter. The larvae which were not treated with blue dye used as the blank. The amount of food taken was measured from the standard graph made from serial dilution of a blue dye.

close plastic bowl containing two fies of organic/non organic /normal and time taken for resistance to ether was recorded (in sec). A total of ten trails were made for each of organic, non organic and normal food. The above data was subjected to a one way ANNOVA followed by Tukey's post has test showed significant variation.

Climbing assay

To study climbing ability, ten mated/unmated flies were placed in the bottom of the measuring cylinder and they were given 20sec to climb up. At the completion of 20sec, the number of flies that climbed up to a vertical distance of >8cm was recorded climbing ability was carried on the 10th day of flies they total three trials were made for flies obtained from the organic and non organic fruit media (Banana and Papaya).

Larval crawling assay

Second instar larvae were collected separately from the different fruit media with the help of brush individual larva was transferred to a 15cm petri dish containing 2% agarose (previously poured and allowed to harden) over graph paper with a 0.2cm grid, the number of grid lines crossed by a larvae in 1 minute was recorded under a dissection microscope.

Table 1 One way ANOVA of 'organically grown fruits (Banana and Papaya) effect on different parameters of *D melanogaster*

Dependent variable	Fruits	Source	Sum of squares	Df	Mean square	F-Value
Larval feeding in(µ g)	Banana	Media	0.068067	2	0.034033	133.1739**
		Error	0.001533	6	0.000256	
		Total	0.0696	8		
	Papaya	Media	0.0672	2	0.0336	26.52632**
		Error	0.0076	6	0.001267	
		Total	0.0748	8		
Crawling assay Distance travelled by flies	Banana	Media	84.02867	2	42.01433	88.03511**
		Error	41.52033	87	0.477245	
		Total	125.549	89		
	Papaya	Media	77.78756	2	38.89378	109.8243**
		Error	30.81067	87	0.354146	
		Total	108.5982	89		
Ether test	Banana	Media	240.067	2	120.03	51.52464**
		Error	62.9	27	2.3296	
		Total	302.967	29		
	Papaya	Media	475.464	2	237.73	97.40212**
		Error	65.9	27	2.4407	
		Total	541.367	29		

**P<0.001 level

Table 2 Two way ANOVA of mated and virgin Male flies effect on climbing assay

Source	Type III Sum of Squares	df	Mean Square	F
gender	106.09	1	106.09	9.810532*
source	424.065	4	106.0163	9.803712*
gender *source	216.435	4	54.10875	5.003635*
Error	973.25	90	10.81389	
Total	22861	100		

**P<0.001 level

Table 3 Two way ANOVA of mated and virgin Female flies effect on climbing assay

Source	Type III Sum of Squares	df	Mean Square	F
gender	256.9609	1	256.9609	27.0071**
source	261.9266	4	65.48165	6.882252*
gender *source	33.1386	4	8.28465	0.870733
Error	856.311	90	9.514567	
Total	22366.69	100		

**P<0.001 level

Ether test

To study the effect of organic food on fly resistance to eatherisation. Five ml of ether was soaked in cotton was used to

A total of thirty trails were run made separate experiments were done for flies obtained from organic and non organic fruit media (Banana and Papaya).

RESULT

Different letters on the bar graph indicate significance at 0.05 level by Tukey's Post Hoc Test

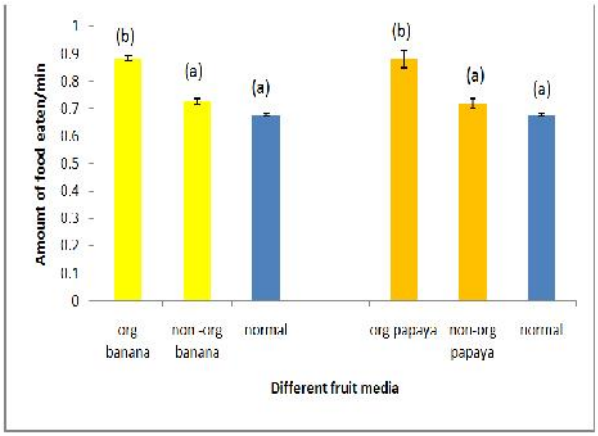

Figure 1 Organically grown fruits' (Banana and Papaya) effect on larval feeding of *D. melanogaster* using dye method

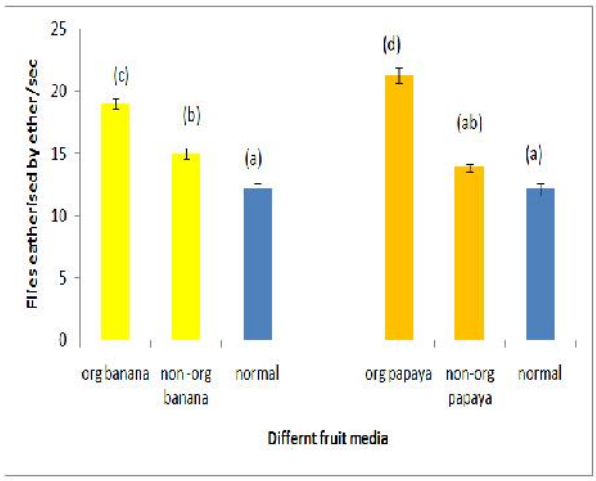

Figure 2 Organically grown fruits' (Banana and Papaya) effect on resistance to etherization of *D. melanogaster*

Different letters on the bar graph indicate significance at 0.05 level by Tukey's Post Hoc Test

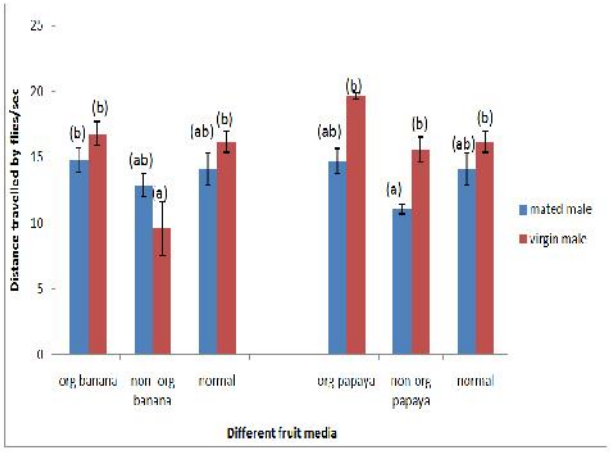

Fig 3 Effect of organic and non organic climbing ability of 10th day old mated males and unmated males flies of *D. melanogaster*

Different letters on the bar graph indicate significance at 0.05 level by Tukey's Post Hoc Test

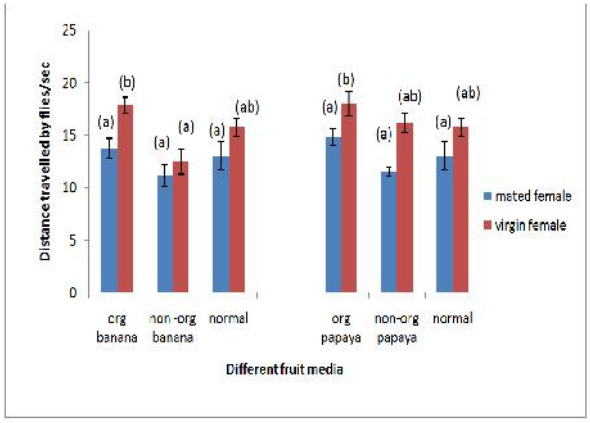

Fig 4 Effect of organic and non organic climbing ability of 10th day old mated females and unmated females flies of *D. melanogaster*

Different letters on the bar graph indicate significance at 0.05 level by Tukey's Post Hoc Test

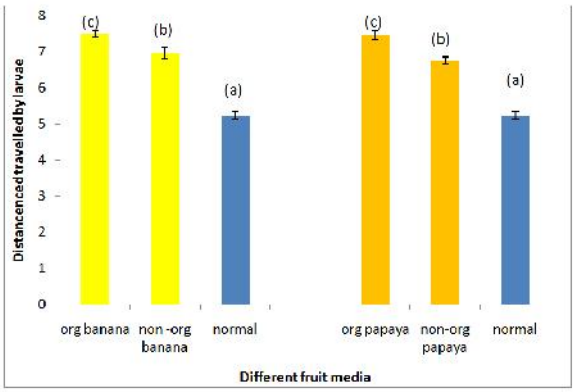

Fig 5 Effect of organic and non organic larval crawling ability of *D. melanogaster*

Different letters on the bar graph indicate significance at 0.05 level by Tukey's Post Hoc Test

Figure 1 show larval feeding rate in different types of diet. It was found that the larvae which are grown in organic fruit media (Banana and Papaya) based media have consumed more amount of food compared to larvae which are grown in non organic fruit based media and wheat cream agar media. One-way ANOVA followed by Tukey's Post Hoc test carried out using SPSS version 14.0 on the above data showed significant variations in feeding rate between different types of media. Tukey's Post Hoc test showed larval feeding rate was significantly greater in larvae fed on organic fruit than larvae fed on non organic and in normal media

Data of organic and non organic fruit (Banana and Papaya) effect on ether is provided in Fig 2 organic grown flies took longer time to under go etherization compared to flies grown on non organic and normal media. One way ANNOVA followed by Tukey's Post Hoc test carried out on above data showed significant variation in recistance to etherization between flies obtained between different media. Tukey's Post

Hoc test also showed that recistance to etherization was significantly higher in flies grown on organic fruits than those flies grown in non organic and in normal media

Data of organic and non organic (Banana and Papaya) effect on larval crawling ability is provided in figure 5. It was found that larval crawling ability as found to be greater in flies grown in organic fruit media (Banana and Papaya) and least crawling ability was notieced in flies obtained from non oraganic and normal media. One way ANNOVA followed by Tukey's Post Hoc test carried out on above data showed significant variation in larval crawling ability between flies obtained between different media. Tukey's Post Hoc test also showed that crawling ability of flies grown organic fruit media had significantly greater ability than those flies grown in non organic fruit and normal media.

Figure 3-4 shown that climbing ability of 10 days old flies grown in organic and non organic (Banana and Papaya) and normal media. It was noticed that both mated and unmated flies of males and females grown on organic fruit media (Banana and Papaya) had greater climbing ability over flies from of non organic and normal media. Further unmated flies had greater climbing ability than those of mated flies in all the media studied. Data of climbing assay was subjected to Two way ANNOVA followed by Tukey's Post Hoc test. Significant variation in climbing ability between flies obtained from different fruit based media and also between mated and unmated flies. Tukey's Post Hoc test also showed that flies grown on organic fruit media had significantly greater climbing ability than those thus obtained from non organic and normal media.

DISCUSSION

To study effect of organic fruits (Banana and Papaya) on locomotory ability flies of *D. melanogaster* was fed with a diet made out of organic fruits obtained from organic, non organic and normal food media were then subjected to study locomotory ability by performing crawling assay, climbing assay and also resistant to authorization. Fig. 1 and table revealed that larval feeding rate varied in different fruit based media. This suggests that diet used by an organism has significant influence on larval feeding. It was shown in species of *Drosophila* that larvae of *Drosophila* show on the inhibition threshold when consuming new or foul taste foods (Melcher *et al.*, 2007). In our study, such inhibition threshold has not been observed when flies fed on organic fruits based media when compared flies fed on non organic fruit and normal media. This is because larval feeding rate was found to be highest among larvae fed on organic fruits.

Locomotion is an integral component of most animal behavior and many human diseases and disorders are associated with locomotor activity. Fig. 3-5 and table1-3 crawling ability assay revealed that larva and flies grown on organic fruits had greater locomotor ability over flies grown on non organic fruits and in normal media. This shows that organic fruit had a beneficial effect on locomotor ability. Our study also support the work of Chabbra *et al.*, (2013) Wafa and Krishna (2015) who while working organic fruits using *D. melanogaster* have also shown beneficial effect of organic fruits on pre adult development, mating latency, copulation duration, fecundity and longevity. This is because organic fruit had fewer pesticide residues compared to non organic fruits. In addition to this flies grown in organic fruits may also obtained greater neutrients than those flies which fed on non organic food and in normal media. In the present study we also measured effect of organic fruits on resistance to etherization fig and table revealed that flies grown in organic fruit had significantly greater resistance to etherization when compared to flies grown on non organic fruits and in normal media. Thus, these studies suggest that organic fruits significant influence on locomotor ability of an organism thereby it had fitness benefit.

Although ether has long been used as anesthetic agent, little is known about variation between organism in their resistance to it. In *Drosophila* for both class work and research work eatherization causes death of the fly where as mild etherization causes no effect in them therefore optimization eatherization is very important in the experiment of *Drosophila*. It was noticed that individual variationdo occur for etherization. In the present study Fig 2 and table revealed that organic grown flies took longer time to under go etherization compared to flies grown on non organic and normal media. This suggests that diet has signific influence on resistance to etherization.

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