EFFECT OF THREE COMMON ORANGE FLAVORED DRINKS ON PROGENY COUNTS AND SEX RATIO OF *Drosophila melanogaster* FLIES

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Abdulazeez Giwa[1]*, Khalid Adekoya[2] and Olatunji Abanikannda[1]
[1]Department of Zoology and Environmental Biology, Lagos State University, Ojo, Lagos, Nigeria
[2]Department of Cell Biology and Genetics, University of Lagos, Akoka, Lagos, Nigeria
*Corresponding author:abdulazeez.giwa@lasu.edu.ng

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Abstract: Fruit drinks are a perfect fast food for today's eat on the run lifestyle. In this study, *Drosophila melanogaster* flies were exposed to three local and common orange flavoured drinks (Nutri-C, Sari-C and Eve). The *Drosophila melanogaster* flies were fed on a Banana-Garri medium containing the test substances in varying concentrations (1, 2, 5, 10, 25, 50, 100%). The flies were bred in the media in the ratio of a male to three females, left to mate and lay eggs for six days. The numbers of offspring were counted and sex recorded. This was done until no adult eclosion in the media. There was a significant decrease ($P<0.0001$) in the progeny counts due to the medium and treatment. Also observed was the significant alteration in the sex ratio ($P<0.05$). Food products containing chemicals should be adequately tested before release into the market.

Keywords: *Drosophila melanogaster*, food products, fruit drinks, progeny, sex ratio

Introduction

Fruit Juices are a perfect fast food for today's eat-on-the-run lifestyle. They contain all the goodness of the whole product in a condensed form. 100% juices are a convenient way for adults and children to get a part of their recommended 4.5 or more cups of fruits and vegetable each day (Farid & Enani, 2010). 100% fruit juices can play an important role in a healthy diet because they offer great taste and a variety of nutrients found naturally in fruits. Orange drink refers to a sweet, sugary, sometimes fizzy orange flavoured drink. Typically such beverages contain little or no orange juice and are merely composed of water, sugar or sweeteners, flavour, colouring and additives, sometimes in that order. As such they are low in nutritional value, although they may be fortified with vitamin C. It is known that the food industry is one of the fastest growing economic interfaces in the world generating a high competitiveness among producers that strive to meet new consumers' demands. As a result, they try to produce foods attractive from the hygienic, nutritional and sensory point of view (Gomes *et al*., 2013). However, with the objective of becoming competitive, they use more and more food additives, which are considered any ingredient intentionally added to the foods to modify their physical, chemical, biological or sensorial characteristics without a nutritional purpose.

Genetic manipulability and ease of detecting phenotypes made *Drosophila* the model of choice for mutagenesis screens of the 1980's and 90's (Rand, 2010). These same features make *Drosophila* ideal for toxicological screens. *Drosophila* is a genus of small flies, belonging to the family *Drosophilidae* whose members are often called 'Fruit flies'. The genus is diverse phylogenetically, geographically and ecologically (Dilon *et al*., 2009). Many species are easily reared in the laboratory. One species of *Drosophila* in particular, *Drosophila melanogaster* has been used a lot in genetic research and is a common model organism in developmental biology. *Drosophila melanogaster* has an abundance of molecular and genetic tools and is a leading model system for investigating metazoan biology (Dilon *et al*., 2009). Many hundreds of thousands of offspring can be generated in a relatively short period of time (2 weeks at 25^{o}C), easy to maintain and the complex spectrum of somatic and heritable alterations can be detected under the microscope with low power magnification (Deepa *et al*., 2012). The extensive knowledge of the genetics of *D. melanogaster* and the long term experimental experience with this organism together with extensive genetic homology to mammals has made it uniquely useful in mutation research and genetic toxicology. We thus, aim to study the effect of three common orange flavoured drinks in the Lagos metropolis on number and sex ratio of F_1 flies of *Drosophilamelanogaster*.

Materials and Methods

Flies were collected from specific sites in the university campus. These were separated into different sexes after etherization. The culture medium was the Banana-Garri medium containing Banana, agar, propionic acid, garri, and distilled water (Williams and Akpabio, 1993). The three orange flavoured drinks were sourced from a local market in Lagos metropolis. Different concentrations (1, 2, 5, 10, 25, 50 and 100%) of these drinks (Nutri-C, Sari-C and Eve) were added to the culture medium and poured into a vial. The drinks were applied to eggs, larva and adult by means of nutrition, adding it to the culture medium. The control culture was the Banana-Garri medium without the addition of the drinks.

Flies in the ratio of 1 male to 3 females were bred on the culture media containing different concentrations of the test substances and reared at room temperature (27ºC). These were left to mate and lay eggs for six days at which they were removed and transferred to a new vial of same concentration of test substance. These parent flies were then removed, observed and sacrificed after six days. The numbers of offspring (F_1) were counted and their sex recorded. This counting and sexing was done until no adult eclosion was observed in medium. Data preparation entailed entries of data into excel worksheet. Statistical analysis including Chi-square test and analysis of variance was carried out using SPSS statistical software.

Results and Discussion

Progeny counts

The number of F_1 offspring ranged from 148 in 1% concentration to 60 in 25% concentration of Nutri-C. There was a linear relationship between the number produced and concentration up to 25% (Fig. 1). At all concentration the numbers were significantly different ($P<0.001$) from control as shown in (Table 2). Lower concentrations produced higher numbers than higher concentrations (25, 50 and 100%). (Fig. 2) showed the number of F_1 offspring produced from Sari-C containing media. The number ranged from 113 at 10% concentration to 29 at 100% concentration. At low

concentrations of 1, 2 and 5% there was no linear relationship between number of progeny and concentration. However at 10% to 100% concentration the number of progeny decreased as concentration increased. At all concentrations, the progeny counts decreased significantly (P<0.001) as compared to the control as shown in (Table 2).

(Fig. 3) showed the number of F_1 offspring produced from the Eve containing media. The number ranged from 123 in 5% concentrations to 63 in 2% concentrations. The numbers produced at all concentrations decreased significantly (P<0.001) as compared to the control as shown in (Table 2). At 25% there was no egg hatching and no progeny was therefore produced. Comparing the number of F_1 progeny between all media (Fig. 4), the effect of the medium and treatment was significant (P<0.0001) as indicated in (Table 1).

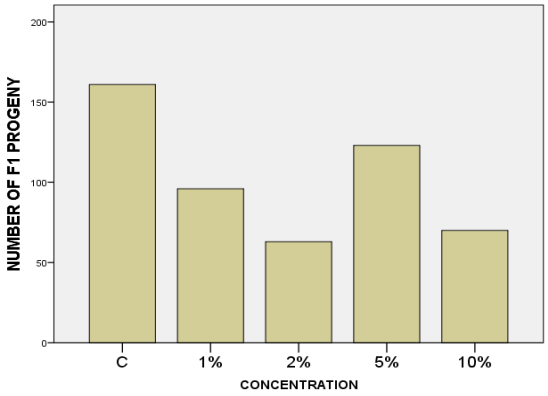

Fig. 3: Number of F_1 progeny from Eve containing medium

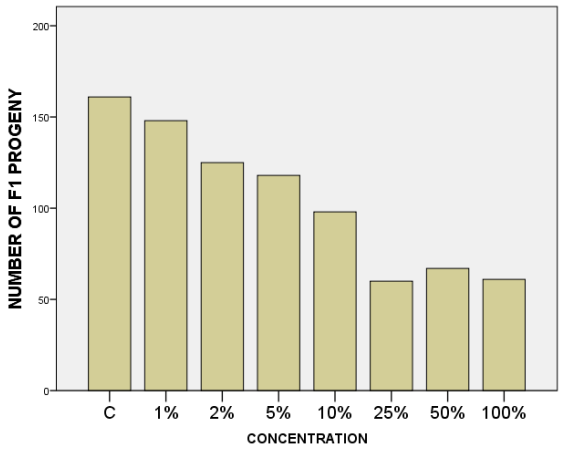

Fig. 1: Number of F_1 progeny from Nutri-C containing media

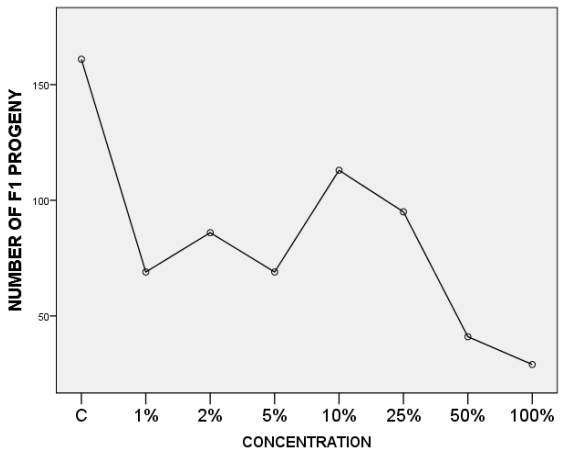

Fig. 2: Number of F_1 progeny produced from Sari-C containing medium

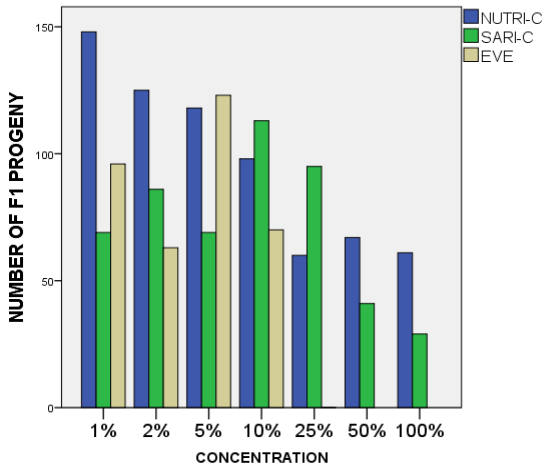

Fig. 4: Number of F_1 progeny at all concentrations in all test substances

There were more progenies at lower concentrations of Nutri-C. This showed that higher concentrations of Nutri-C affected adult eclosions of *D. melanogaster*. Gayathri & Hariri (2013) also observed reduced adult eclosions when *D. melanogaster* were exposed to Phenytoin. The numbers of progenies were also reduced at higher concentrations of Sari-C containing media. At 100%, viability was greatly affected showing that higher concentrations of Sari-C hindered larval development or adult eclosion. This could also be a result of reduction in egg laying by parents feeding on such high concentrations. Dhananjaya & Naik (2009) observed that number of adult eclosions was reduced to lowest at highest concentration of sevin, a pesticide tested on *D. melanogaster*. These observations might be attributed to Sari-C's composition of Acesulfame-K. At all concentrations, the numbers of progenies were lesser than in control in the eve containing media. There was no progeny at 25, 50 and 100%. At 25% this was due to non-hatching of the few eggs laid. This indicates signs of toxicity and adverse effect on viability.

Generally, Nutri-C containing media produced more F_1 offspring than Sari-C and Eve containing media. This showed that Nutri-C is less toxic than Sari-C and Eve on viability of *D. melanogaster*. The numbers of progenies of both (Sari-C + Nutri-C) reduced as concentration increased except at 10%. These results show that these substances adversely affect viability of *D. melanogaster* and this is dose-dependent.

Table 1: Analysis of variance (ANOVA) of progeny counts of Nutri-C, Sari-C and eve groups

Source of variation	Sum of Squares	df	Mean Square	F	P Value
Interaction	20040	14	1431	4294	P<0.0001*
Treatment	78880	7	11270	33800	P<0.0001*
Growth medium	13060	2	6531	19590	P<0.0001*
Residual	8	24	0.3333		

*Significant P<0.05

Table 2: ANOVA Post Hoc Test (Bonferroni)

Medium	0%	1%	2%	5%	10%	25%	50%	100%
NutriC	161	148*	125*	118*	98*	60*	67*	61*
Sari-C	161	69*	86*	69*	113*	95*	41*	29*
Eve	161	96*	63*	123*	70*	0*	0*	0*

*Significant compared to control (P<0.05)

Table 3: Chi-square analysis of F_1 progeny

Concentration	Nutri-C			Sari-C			Eve		
(%)	Male	Female	P-Value	Male	Female	P-Value	Male	Female	P-Value
0	77	84	0.5	77	84	0.5	77	84	0.5
1	53	95	0.01*	29	40	0.20	42	54	0.20
2	50	75	<0.05*	40	46	0.5	20	43	0.01*
5	31	87	0.01*	20	49	0.01*	34	89	0.01*
10	23	75	0.01*	36	77	0.01*	29	41	0.20
25	39	21	0.01*	43	52	0.5	-	-	-
50	25	42	<0.05*	23	18	0.5	-	-	-
100	14	47	0.01*	6	23	0.01*	-	-	-

*Significant (P<0.05), df = 1

Sex ratio

Chi-square analysis to check for deviations in expected sex ratio of 1:1 is presented in (Table 3). The control flies had non-significant probability value of 0.5. At all concentrations flies exposed to Nutri-C had significant probability values while flies exposed to Sari-C had significant probability values at 5, 10 and 100% concentrations. Flies exposed to Eve had significant probability values at 2 and 5%. Sex ratio is one of the adaptive traits of any population which determines the rate of increase or decrease of a sexual population in an environment (Choudhary, 2003).

Environmental factors are known to affect the sex ratio (West *et al*., 2002). Nutri-C was seen to significantly increase the female to male ratio at all concentrations studied except at 25%. This means that female germ cells compared to males are more resistant in exposure to this substance. Alteration in sex ratio of *D. melanogaster* was also reported by Gayathri & Krishnamurthy (1980). Sari-C also significantly altered sex ratios at the highest concentration. This was also the case at some lower concentrations. A similar situation was observed in Eve exposed flies where sex ratios was altered at some concentrations. No alteration in sex ratio was observed in flies exposed to control media. Fakoorziba *et al*. (2012) also reported similar alteration in sex ratio when *D. melanogaster* were exposed to paroxetine.

Conclusion

The three orange flavoured drinks caused a significant decrease in the number of F_1 flies eclosed. The toxic effect was more pronounced at the highest dose. These drinks also altered sex ratios of the F_1 flies. Food products containing chemicals should be adequately tested before release into the market.

Conflict of Interest

There is no conflict of interest whatsoever in this research.

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