

Figure 1), and Argentina (1 record) (Bächli, 2017). Despite this, Robe *et al.* (2013) emphasize this may reflect a bias of the sampling efforts. In this article, we report the first record of *D. incompta* in the state of Minas Gerais.

The flowers of *Cestrum* were collected in the municipality of Belo Horizonte (19°48'S, 43°57'21"W), taken to the laboratory and kept until the hatch of adult flies. These were captured using an entomological aspirator (Machado *et al.*, 2014) and immediately fixed in absolute ethanol. The flies were separated by sex through their external morphology and further identified by the internal male genitalia morphology, as described by Wheeler *et al.* (1962). A total of 61 flies were collected, from which 27 were male. All male individuals were identified as *D. incompta*, according to their internal genitalia morphology patterns.

This report is congruent with the predictions based on Environmental Niche Modeling strategies performed by Robe *et al.* (2013), according to which the potential distribution of *D. incompta* would extend from the southern region of Brazil, in which it can be found in sympatry and even syntopy with *D. cestri*, *D. corderoi*, and *D. flavopilosa*, to the central region of the country. Nevertheless, although this report extends the known distribution range of *D. incompta* in Brazil, the wide area of unsuitable habitats that was projected to follow to the north of this area (Robe *et al.*, 2013) remains to be further assessed.

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Effects of three common orange flavored drinks on survival and phenotype of *Drosophila melanogaster*.

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Abstract

In this study, *Drosophila melanogaster* flies were exposed to three local and common orange flavored 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, and 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 number of deaths of parent flies and phenotypic defects in F₁ flies were noted. It was observed that the drinks caused the death of some parent flies and that females were more affected than males. Phenotypic defects of the wings and abdomen were also observed in F₁ flies. Food products containing chemicals should be adequately tested before release into the market. Further research should be carried out to determine the mode of action of these substances on *D. melanogaster* and on mammalian test systems. **Keywords:** *Drosophila melanogaster*, F₁ flies, fruit drinks, survival, phenotypic defect

Introduction

Environmental toxins pose a constant challenge to the survival of living organisms. These toxins enter the body by physical contact, inhalation, or ingestion and can originate from a wide range of sources (Misra *et*

al., 2011). There are more than 80,000 chemicals in commercial use today, and approximately 2,000 new chemicals are introduced yearly (Rand, 2010). These chemicals comprise food additives, drugs, narcotics, antibiotics, pesticides, cosmetics, contraceptives, air pollutants, water pollutants, and others.

Genetic manipulability and ease of detecting phenotypes made *Drosophila* the model of choice for mutagenesis screens of the 1980's and 1990's (Rand, 2010). *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 (Dillon *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 (Dillon *et al.*, 2009). *D. melanogaster* is uniquely useful in genetic toxicology and mutation research due to extensive knowledge of its genetics and biology together with genetic homology to mammals. Many hundreds of thousands of offspring can be generated in a relatively short period of time (2 weeks at 25°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).

Orange flavored drinks are a common part of the Nigerian diet and are consumed especially by children because of their sweetness and color. There has been no study done to investigate the toxicity of these drinks. This study aims to investigate the effects of three common orange flavored drinks in the Nigerian market on morphology and survival of *Drosophila melanogaster*.

Materials and Methods

Flies were collected from specific sites in the University of Lagos, Akoka campus. These were separated into different sexes after etherization based on morphological differences as described by Parvathi *et al.*, 2009. The culture medium was the Banana-Garri medium containing Banana (250 g), agar (10 g), propionic acid (5 ml), garri (50 g), and distilled water (1 L) (Williams and Akpabio, 1993). The three orange flavored drinks were sourced from a local market in the Lagos metropolis. 10 ml of different concentrations (1, 2, 5, 10, 25, 50, and 100%) of these drinks (Nutri-C, Sari-C, and Eve) were added to 50 ml of the culture medium and poured into a vial. The drinks were applied to eggs, larvae, and adults 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 (27°C). These were left to mate and lay eggs for six days. The parent flies which were alive were then removed after six days. The F₁ offspring were observed and phenotypic changes recorded using a stereomicroscope. This observation was done until no adult eclosion was observed in media.

Results

Survival of parent flies

The number of deaths recorded is presented in Table 1. Some parent flies died but this was not in a linear relationship with concentration. No parent fly died in the control as shown in Table 1. It was observed that 28.57% of parent flies, 33.33% of female parent flies, and 14.29% of male parent flies died in Nutri-C- and Sari-C-containing medium. 35% of parent flies, 40% of female parent flies, and 20% of male flies died in Eve-containing medium. It was obvious that more females died than did males.

Phenotypic changes

Abnormal phenotypes were observed in F₁ progeny exposed to the test media as shown in Table 2. These changes in phenotype were not observed in F₁ flies exposed to the control medium. Nutri-C had the least incidence of phenotypic effects on the phenotype of F₁ flies while more incidences were observed in F₁ flies exposed to Sari-C and Eve.

Table 1. Survival of parent flies.

Media	Parent Flies	Total Dead (%)	Dead (female) (%)	Dead (male) (%)
Nutri-C	28 (21 females, 7 males)	28.57 (8/28)	33.33 (7/21)	14.29 (1/7)
Sari-C	28 (21 females, 7 males)	28.57 (8/28)	33.33 (7/21)	14.29 (1/7)
Eve	20 (15 females, 5 females)	35 (7/20)	40 (6/15)	20 (1/5)
Control	4 (3 females, 1 male)	0	0	0

Table 2. Abnormal phenotypes in F₁ flies.

	Media		
	Nutri-C	Sari-C	Eve
Number of flies	2	9	5
Defect	Curly Wings	Folded wings Rough and short wings Curly wings Smashed abdomen Splattered wings & curved abdomen Short wings	Short wings & smashed abdomen Short bent wings Abnormal wing Rough & raised wings Short wings

Discussion

It was observed that the media containing high concentrations (50, 100%) of Eve did not solidify. This suggests the possibility of chemicals at high concentration that could degrade the Banana-Garri medium. It also indicates that the Banana-Garri medium can be modified and thus provides the basis for further research into the effects of such modifications on several aspects of the biology of *Drosophila melanogaster*.

Parent flies were fed on media containing different concentrations of the three test substances. These parent flies died more in the media containing Eve (35%) than in media containing Nutri-C (28.57%) and Sari-C (28.57%). This is important as it raises issues on the frequent and continuous consumption of these drinks. These results indicate that these substances (Eve, Nutri-C, and Sari-C) particularly at high concentrations are toxic to parent flies of *Drosophila melanogaster*. Such strong toxic effects observed at the highest concentration of exposure were also observed by Tripathy *et al.* (1996), Ding and Wang (2009), and Uysal *et al.* (2013).

The female fly was found to be more susceptible to the toxic effects of the chemicals than males. This may be due to sexual variation and differences in feeding activity, which is at higher levels for female adults. Wong *et al.* (2009) found that females fed more than males over a 30-minute period, because they spent a greater proportion of time with the proboscis extended (2.8-fold more on average) than males. This higher intake by female flies is presumably related to their nutrient usage in egg production. A similar result was obtained by Gayathri and Krishnamurthy (1980) who found that the viability of females is more affected, indicating that female *D. melanogaster* are more susceptible to toxic effects of 1-amino-2-naphthol-4-sulphonic acid. Such toxic effect on adult females than adult males was also reported by Obeidat (2008) following exposure of *D. melanogaster* to Jordanian *Bacillus thuringiensis* isolates.

These substances also effected several morphological changes as indicated. Pre-adult stages of *D. melanogaster* are particularly susceptible as this is the time of active growth and development. Nutri-C showed the least effect on morphology. This was not the case for Sari-C and Eve exposed flies, which showed more abnormal phenotypes. Wing and abdominal defects were the morphological defects observed in F₁ flies. Haq *et al.* (2012) observed morphological changes to wings, abdomen, and color when *D. melanogaster* larvae were fed with lead acetate. Wing alterations were also observed when *D. melanogaster* was exposed to ethidium bromide (Ouchi *et al.*, 2011). Some flies exposed to Eve were also seen to have orange colored abdomen probably indicating indigestion.

These substances thus have the ability to affect development in pre-adult stages and induce detrimental changes to the eclosed adult. The toxicological effect of Eve may be due to its much higher concentration and its composition of dyes (Sunset yellow) and artificial sweeteners (Aspartame). Sayed *et al.* (2012) demonstrated the mutagenic action of sunset yellow also showing an increase of morphological abnormalities in spermatozooids of mice.

The toxicological effect of Nutri-C and Sari-C may also be due to its composition of aspartame, tartrazine, colorings, and acesulfame-K in Sari-C. Gomes *et al.* (2013) found that tartrazine yellow dye has anti-proliferative activity action and potential to cause cellular aberrations using the *Allium cepa* test.

Conclusion

This study demonstrated signs of toxicity of the orange flavored drinks on *D. melanogaster*. A reduction in survival of parent flies as well as morphological changes in F₁ progenies was observed. At the very least, this has shown that these substances can affect some aspects of the biology of fruit flies. Further research should be carried out to determine the mode of action of these substances on *D. melanogaster* and on mammalian test systems.

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How nutritive conditions determine life-history traits in *Drosophila melanogaster*?

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Drosophila melanogaster uses various fruits and vegetables in different manners: as a food, egg-laying sites, or for reproduction (Shorrocks, 1972). Since flies are often exposed to different quality, quantity, and availability of nutritional resources, adjustment to new nutritional environment induces adaptive plastic responses, which include changes in morphological, physiological, life-history, and behavioral traits (Djawan