

Evaluation of Developmental Toxicity of Various Food Preservatives Using *Drosophila Melanogaster*

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Abstract: Food additives are substances added to food to preserve flavor or enhance its taste, appearance, or other qualities. Some additives have been used for centuries; for example, preserving food by pickling (with vinegar), salting, as with bacon, preserving sweets or using sulfur dioxide as with wines. With the advent of processed foods in the second half of the twentieth century, many more additives have been introduced, of both natural and artificial origin. Food additives also include substances that may be introduced to food indirectly (called "indirect additives") in the manufacturing process, through packaging, or during storage or transport. The main objectives of the study was to understand and identify the effects of increased intake of food preservatives such as BHT, Aspartame and MSG in the metamorphosis and development of *Drosophila melanogaster*. The method used to assess the protein concentration was Lowry's and also colourimetry method. In this study the effect of preservatives on demography, Gender, Polytene chromosome, Sexcomb and Protein concentrations of *Drosophila melanogaster* were analysed.

Keywords: *Drosophila melanogaster*, Protein, Metamorphosis, BHT, Aspartame, control and MSG

1. Introduction

Food additives are substances added to food to maintain or improve its safety, freshness, taste, texture, or appearance. Food additives need to be checked for potential harmful effects on human health before they can be used. Food additives can be derived from plants, animals, or minerals or they can be synthetic. They are added intentionally to food to perform certain technological purposes which consumers often take for granted. In this study three food additives are used such as Aspartame, BHT and MSG. Fernstrom et.al., studied neuroendocrine effects in eight Caucasian nonsmoking males following ingestion of a single 12.7g dose of MSG observing that neither plasma prolactin (PRL) levels of any of the other variables studied were significantly elevated compared with those of controls. Olney stated that MSG induced modification of pituitary function seemed unwarranted. Destruction of is not only mechanism by which (glutamate) GLU can have adverse effects on children. Repetitive exposure of immature humans to Glutamate throughout critical stages of development entails potential risk even if brain damage does not occur that hormonal biorhythms may be disturbed with adverse effects on growth and development. Glutamate is important for intestinal tract function and gut development. Decreased intake impairs growth performance due to damaged intestinal villi. Rezaei et al., observed that supplementary upto 4% dietary Monosodium Glutamate (MSG) is safe and improved growth performance in postweaning pigs. Food Colour is known to provoke asthma, uncontrolled hyper agitation and confusion, wakefulness, inhibit metabolism and interfere abdominal pain and allergic reactions. The effect of Butylated Hydroxy Toluene (BHT) on early stages of morphogenesis of *Drosophila melanogaster* is sharply decreased. *Drosophila melanogaster* cultivated with the supplement of BHT decreased superoxide generation. However this was accompanied by impairments of morphogenesis changes in growth rate. This is consistent

with the notion on superoxide anion as a factor regulating mitosis and apoptosis. This also suggests that alteration of normal cellular level of superoxide impairs normal development of *Drosophila melanogaster*.

2. Materials and Method

To experiment the effect of food additives (ASP, BHT, MSG and Colour) in the metamorphosis and development of *Drosophila melanogaster* four cycles of analysis were done. In each cycle 10 bottles were used. Each preservative is added in two bottles containing culture media and two bottles for control. First two cycles were carried out by adding 10 mg of preservatives in 20ml of media in the bottle. Next two cycles were carried out by adding 40 mg of preservatives in 20ml of media.

Parameters such as Demography, pattern of sex comb, polytene chromosome and Protein analysis were done. Demography, pattern of sex comb and polytene chromosome were analyzed using simple and compound microscope. Protein was estimated by Lowry's method, BHT, Aspartame, Colour and MSG were analysed by using colorimetry method.

Culture media preparation:

Culture Media was prepared by using 1 liter of boiled water added 100gms of suji, 100gms of jiggery, once the jiggery dissolved, to the preparation added 3.75ml of propionic acid to avoid fungal growth then 5gms of agar and yeast were added. 10mg of preservatives (BHT, Aspartame, Colour and MSG) weighed and added separately to 100ml of labeled beakers and to which 20ml media was added. Two sets of bottles were prepared for each preservative. There were 10 bottles prepared of which 2 were control. Finally yeast granules were added to all the bottles and introduced 5 male and 5 female flies were added to each bottle. In the cultured

bottles demography, sex comb pattern, polytene chromosomes and protein analysis were done.

Demography

In the process of analysis of demography, the flies were etherized and calculated the total number of flies and also number of males and females in each bottle by direct counting method.

Sex Comb

In *Drosophila melanogaster*, *Scr* expression in the T₁ leg is sexually dimorphic. In males, *Scr* protein levels are highest in the distal part of t₁, around the developing sex comb, whereas no such up regulation is observed in females. This is controlled by *dsx* and given the important role in determining sex comb morphology and position (Loveshia sivanantharajaiah et al., 2009).

To found the pattern of sex comb in male flies, the male flies were identified after etherization by abdominal banding pattern and dissected the sex comb by dissecting fore limbs using dissection microscope. The dissected sex comb containing fore legs were placed on the slide containing glycerin and placed the coverslip- The mounted sex combs were observed under the compound microscope and counted the number of bristles.

Polytene Chromosome

Balbani rings of Polytene chromosome are the site of transcription and translation process. Polytene chromosomes were prepared from the salivary gland of third instar larva by using Carnoy's fixative, LAO stain and observed under the microscope.

Protein Analysis

Protein was estimated using Lowry's method. 20 *Drosophila melanogaster* flies were taken in mortar and crushed with pestle using 5 ml of distilled water. Take 0.2, 0.4, 0.6, 0.8 and 1 ml of sample using pipette in the test tubes. The sample volume of each test tube was made upto 1ml using distilled water. To which added 5ml of freshly prepared Lowry's reagent and incubated the same for 10minutes in dark. Then added 0.5 ml of FC reagent to all the test tubes and keep them in dark for 30 minutes (Incubation). Followed by this OD was taken at 670nm.

3. Results

Demography

First two cycles were carried out by adding 10 mg of preservatives in 20ml of media in the bottle. In these cycles, Aspartame added culture bottles were showing highest demography, the next highest was seen in Red food colour added bottles, third highest demography was seen in the control bottles, the fourth highest concentration was seen in the bottles added with BHT and the least demography was seen in MSG containing bottles.

Third and fourth cycles were carried out by adding 40 mg of preservatives in 20ml of media bottle. In these cycles also, Aspartame added culture bottles were showing highest demography, the next highest was seen in Red food colour added bottles, third highest demography was seen in the

control bottles, the fourth highest concentration was seen in the bottles added with BHT and the least demography was seen in MSG containing bottles.

Gender wise females were found to be more than males. In both the concentrations Polytene chromosomes were found to be well spread in MSG Control, Aspartame and Red food colour, moderate spread was found in BHT and least spread was seen in MSG.

Protein

Highest Protein content was found in MSG, second high concentration of Protein was found in Control, third high concentration of Protein was found in Aspartame and Red food colour containing bottles and the least concentration of Protein was seen in the bottles added with BHT in both the 10mg and 40mg concentration of food additive added bottles.

4. Discussion

Aspartame is an artificial non-saccharide sweetener 200 times sweeter than sucrose, and is commonly used as a sugar substitute in foods and beverages. Canned or bottled fruit and vegetables, jam, chewing gum, cereals, processed fish and fishery products, soups, sauces, dietary foods, beer and malt beverages, soft drinks, soda, desert, snacks in which it acts as preservatives.

The safety of aspartame has been studied since its discovery by EFSA National Experts (May 2010) and is one of the most rigorously tested food ingredients, stated by Mitchell H (2006). Aspartame has been deemed safe for human consumption by over 100 regulatory agencies in their respective countries said Butchko HH et al (April 2002)., including the United States Food and Drug Administration (Henkel J, November–December 1999) UK Food Standards Agency, the European Food Safety Authority (EFSA), Health Canada, (Aspartame". Health Canada. 5 November 2002) Australia, and New Zealand.

As of 2017, reviews of *clinical trials* showed that using aspartame (or other non-nutritive sweeteners) in place of sugar reduces calorie intake and body weight in adults and children ("*Aspartame*". *Health Canada*. 5 November 2002). Caloric contribution is negligible.

Demography was found to be highest in both the concentrations of 10mg and 40mg per 20ml of media. Gender wise females were found to be more than males. Polytene chromosomes were well spread under microscopic view. Protein content was found to be in 3rd place when compared to other four parameters.

BHT:

BHT is used as a preservative ingredient in some foods. With this usage BHT maintains freshness or prevents spoilage; it may be used to decrease the rate at which the texture, color, or flavor of food changes revealed by US Department of Health & Human Services. Household Products Database revealed that BHT is listed under several categories in catalogues and databases, such as food

additive, household product ingredient, industrial additive, personal care product/cosmetic ingredient, pesticide ingredient, plastic/rubber ingredient and medical/ veterinary/ research. Demography was found to be in fourth place and male population was found to be more when compared to female population. Polytene chromosomes were poorly spread. Protein concentration was found to be least. This result indicates that there is correlation between polytene chromosome and protein synthesis as polytene chromosome is responsible for mRNA synthesis.

MSG:

It acts as preservatives in processed cheese, fats, fruit and vegetables, coco and chocolate products, chewing gum, breakfast cereals, bread, fish and fishery products, seasonings, dietary food, nonalcoholic and alcoholic beverages, desserts, meat products etc... In animal studies injecting high doses of MSG into the brains of rats and mice caused them to become obese, weight gain and obesity (Yakuguku et. al., 2014). Increased MSG intake has been linked to weight gain with average intake ranging from 0.33 to 2.2g per day (Am J Chin Nutr, 2011). Demography was found to be least in both the concentrations which indicate MSG has more health hazardous effect. Polytene chromosomes were well spread and protein content was seemed to be highest among other four parameters. This result is supported by the findings of Yakuguku Zasshi et al., 2014. They stated that higher MSG dosage restricts the release of corticosterone (stress hormone) due to which, stored fat get metabolized first instead of protein, resulted in higher protein content while higher dosage were given.

Red Food colour

Food coloring, or color additive, is any dye, pigment or substance that imparts color when it is added to food or drink. They come in many forms consisting of liquids, powders, gels, and pastes. Food coloring is used both in commercial food production and in domestic cooking. Food colorants are also used in a variety of non-food applications including cosmetics, pharmaceuticals, home craft projects, and medical devices (CFR).

Hyperactive behaviour in kids, muscular dystrophy caused due to dystrophin glycoprotein deficiency, can be caused by usage of food colour.

Demography was found to be second highest place and males were more in number when compared to females. Sex combs were well developed. Polytene chromosomes were moderately spread. It indicates Red food colour is one of the agents for chromosomal damage. Protein content was found to be fourth highest place among five parameters.

Table 1

Cycle - I	Culture Bottle with 10mg of preservatives in 20ml of media				
Parameters	Demography	Males	Females	Tooth on Sex Comb	Protein
Control	126	57	69	8	4
Aspartame	167	73	94	9	2
BHT	72	41	31	8	2
MSG	66	32	34	9	5
Red food colour	144	72	72	8	1

Table 2

Cycle - II	Culture Bottle with 10mg of preservatives in 20ml of media				
Parameters	Demography	Males	Females	Tooth on Sex Comb	Protein
Control	101	48	53	11	5
Aspartame	157	76	81	10	4
BHT	81	49	32	10	3
MSG	70	34	36	8	6
Red food colour	151	77	74	8	2

Table 3

Average of both Cycle I and II					
Parameters	Demography	Males	Females	Tooth on Sex Comb	Protein
Control	114	53	61	10	5
Aspartame	162	75	88	10	3
BHT	77	45	32	9	3
MSG	68	33	35	9	6
Red food colour	148	75	73	8	3

Table 4

Cycle - III	Culture Bottle with 40mg of preservatives in 20ml of media				
Parameters	Demography	Males	Females	Tooth on Sex Comb	Protein
Control	126	25	101	9	5
Aspartame	164	43	121	8	3
BHT	120	27	93	8	3
MSG	98	29	69	8	6
Red food colour	161	61	100	9	3

Table 5

Cycle - IV	Culture Bottle with 40mg of preservatives in 20ml of media				
Parameters	Demography	Males	Females	Tooth on Sex Comb	Protein
Control	159	36	123	10	4
Aspartame	187	38	149	9	3
BHT	111	22	89	10	2
MSG	75	21	54	9	4
Red food colour	159	52	107	9	4

Table 6

Average of both Cycles III and IV					
Parameters	Demography	Males	Females	Tooth on Sex Comb	Protein
Control	143	31	112	10	5
Aspartame	176	41	135	9	3
BHT	116	25	91	9	3
MSG	87	25	62	9	5
Red food colour	160	57	104	9	4



Figure 1: Culture bottle with media and preservatives

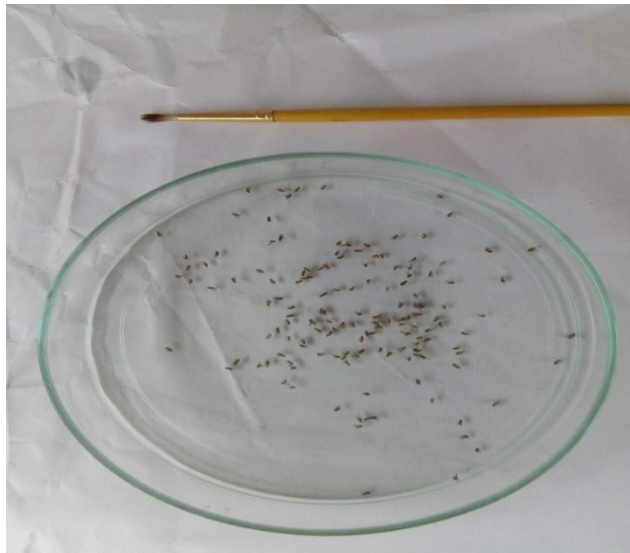


Figure 2: Petriplate with etherized flies



Figure 3: Microscopic view of Male and Female Flies

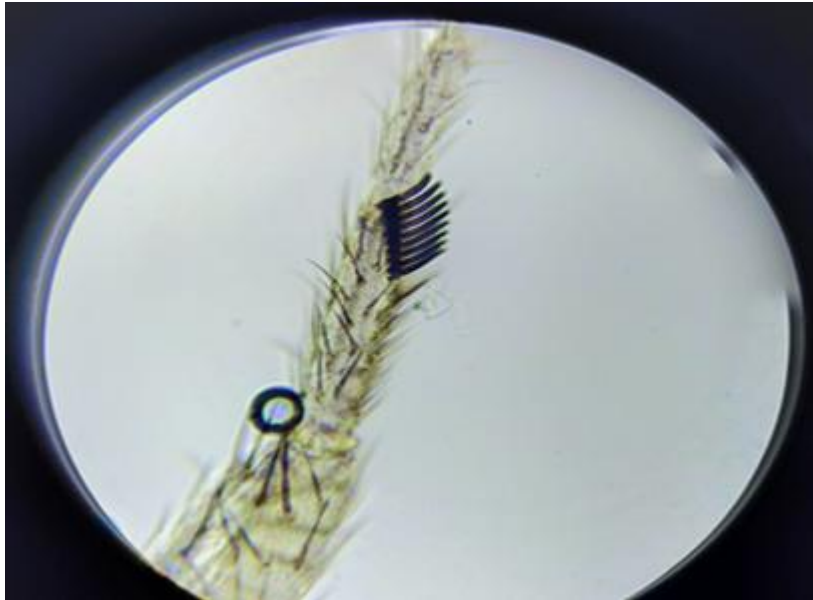


Figure 4: Sex comb of Male fly cultured in Control

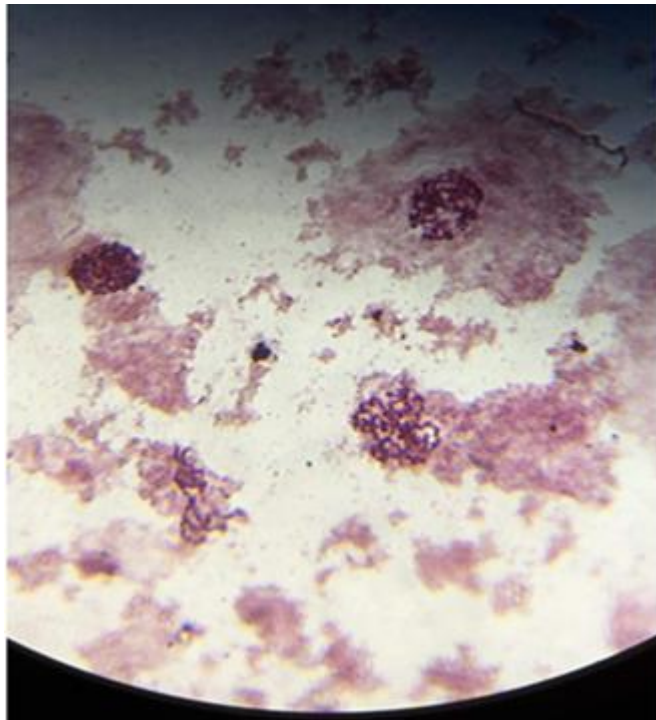


Figure 5: Polytene Chromosome of flies cultured in MSG

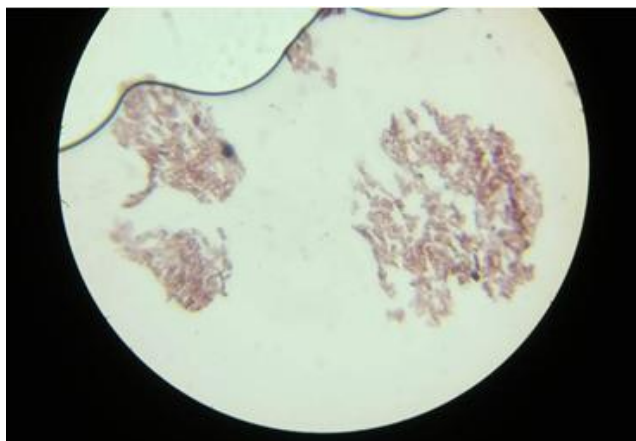
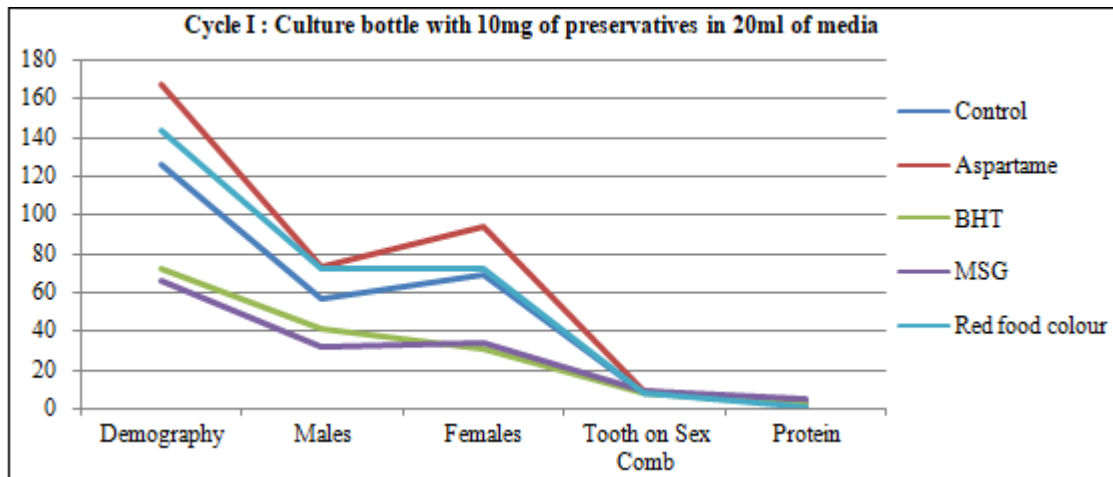
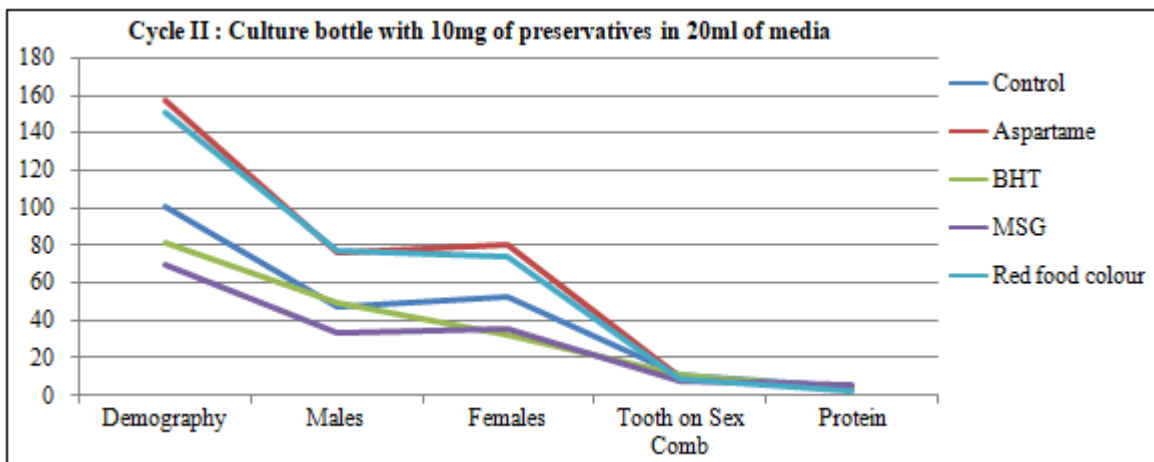


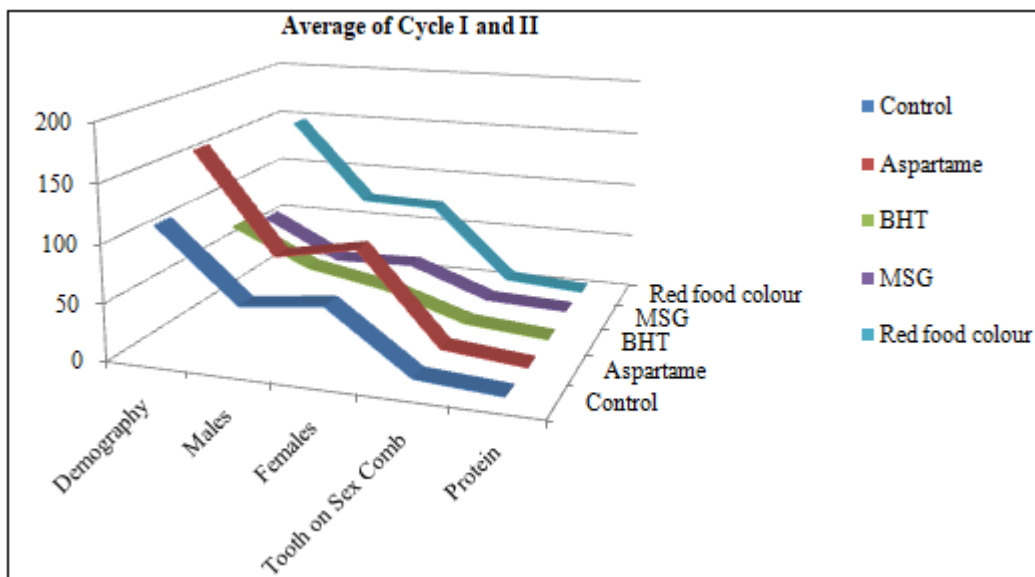
Figure 6: Polytene Chromosome of flies cultured in MSG



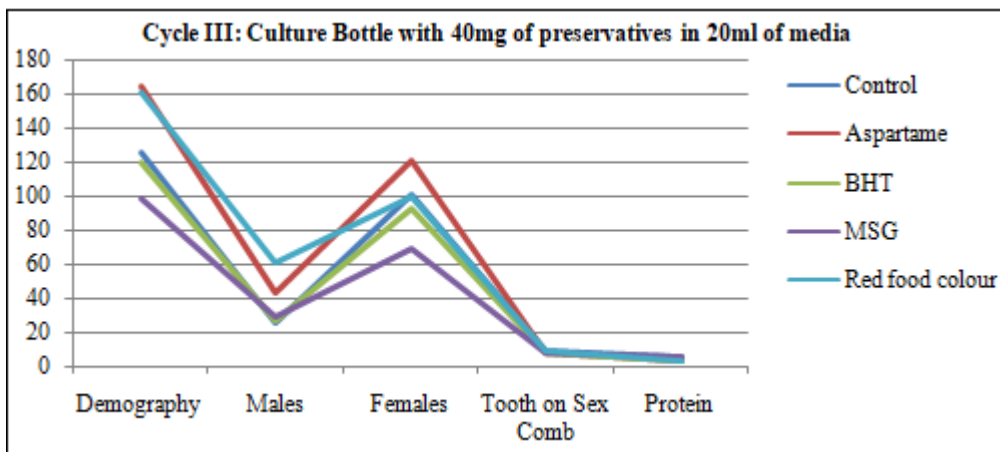
Graph 1



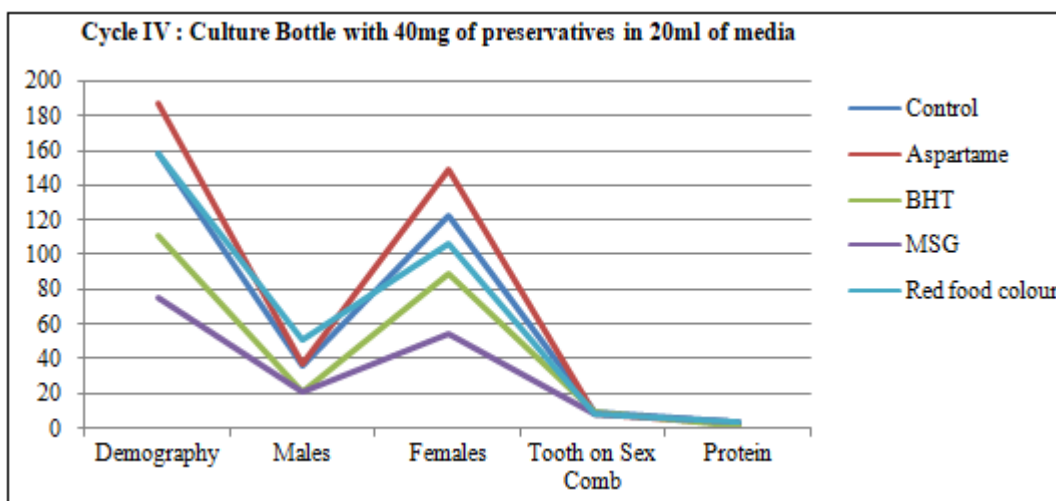
Graph 2



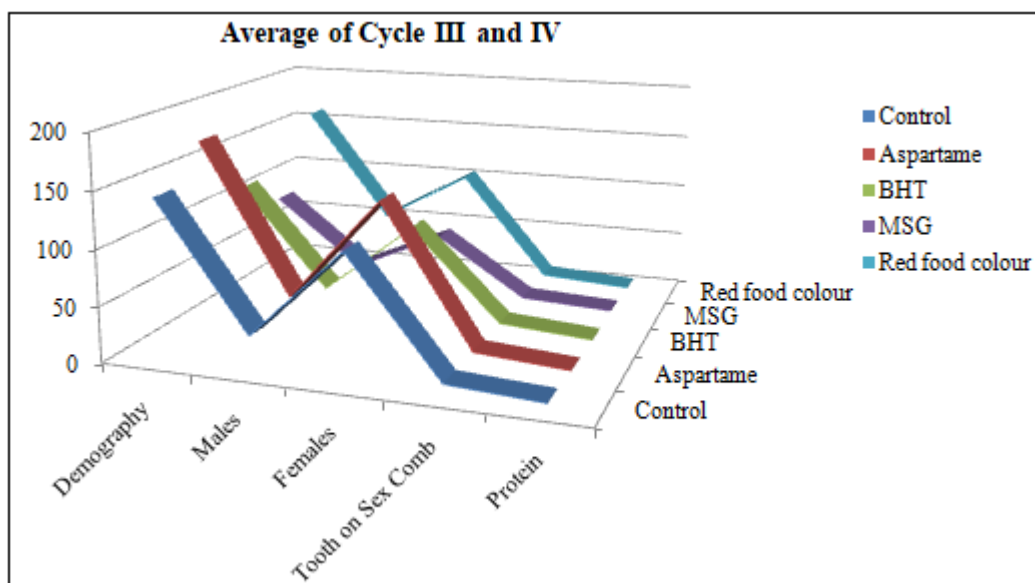
Graph 3



Graph 4



Graph 5



Graph 6

5. Acknowledgment

I acknowledge the lab facilities provided by our College Management Indian Academy Degree College Autonomous to carry out this Experiment.

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