

A STUDY ON FOOD PREFERENCE IN DROSOPHILA POPULATION IN EX-SITU CONDITION

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Abstract: The genus *Drosophila* shows diverse preference of food. To determine food preference in constant laboratory temperature, collected *drosophila* were cultured and they were used for study of food preference in different natural and artificial foods. Three experiments were conducted and it is found that in without yeast medium, larva, pupa never develops. When natural and artificial foods are given at the same time, the cultured *drosophila* in artificial medium still prefers natural food. In course of study it was found that *drosophila* which was collected from wild area of Tiger-Hill forest (26.9967° N, 88.2944° E), could not survive in artificial food medium because of their specificity of food. It was found that, they prefer mango as a seasonal fruit and they can detect the sweetness of artificial sweetener. It was also found that they never come in salted food items.

Keywords: artificial food, sex comb, culture, pupa, Yeast, Propionic acid, ethanol.

1. INTRODUCTION

Small fruit flies which belongs order Diptera, family Drosophilidae are widely found lingering around over ripe or rotten fruits. They are widely used in biology as a tool of research. The entire genus contain more than 1500 species (*Gerhard Bächli, 1999–2006*). Development time varies on environmental factors such as temperature, humidity, and breeding substrate etc. Larva feed on the yeast present on breeding substrate (Therese A. Markow & Patrick M. O’Grady, 2005) *Drosophila* are cultured in laboratory as a popular experimental animal. They have a short generation time. However, some species of *Drosophila* are hard to culture in laboratory may be because they breed on a single specific host in the wild. (Therese A. Markow & Patrick M. O’Grady (2005). *Drosophila: A guide to species identification and use*. London: Elsevier. ISBN 0124730523)

It was found that odourant binding protein OBP 57d and OBP 57e affect taste perception and host plant preference in some *Drosophila* species (Matsuo, T., Sugaya, S., Yasukawa, J., Aigaki, T., & Fuyama, Y. (2007). Odorant-Binding Proteins OBP57d and OBP57e Affect Taste Perception and Host-Plant Preference in *Drosophila sechellia*. *PLoS Biology*, 5(5), e118. Doi:10.1371/journal.pbio.0050118)

Yeasts are common *Drosophila* symbionts and they consistently remain associated with live flies (Chandler, J. A., Eisen, J. A., & Kopp, A. ,2012). Fruit flies have four stages of life cycle- egg, larva, pupa and adult. They undergo metamorphosis . At room temperature *Drosophila* develop into adult within 1-2 weeks(egg and larval stage 8 days and pupal stage 6 days). During the larva’s third instar, it crawls to drier area to pupate. (Therese A. Markow & Patrick M. O’Grady (2005). *Drosophila: A guide to species identification and use*. London: Elsevier. ISBN 0124730523)



Fig-THIRD INSTAR LARVA, IT CRAWLS TO DRIER AREA TO PUPATE

DROSOPHILA COLLECTION SITES AND SITE MAP:

1. Tiger Hill forest (Latitude & longitude-26.9967° N, 88.2944° E, Altitude-2590 meters)
2. Hookar Road Grave Yard area i.e. Darjeeling Memorial Cemetery (Latitude and longitude-27.0526° N, 88.2590° E)
3. Darjeeling Government College (Latitude & longitude-27.0543° N, 88.2580° E, Altitude-2134 meters)

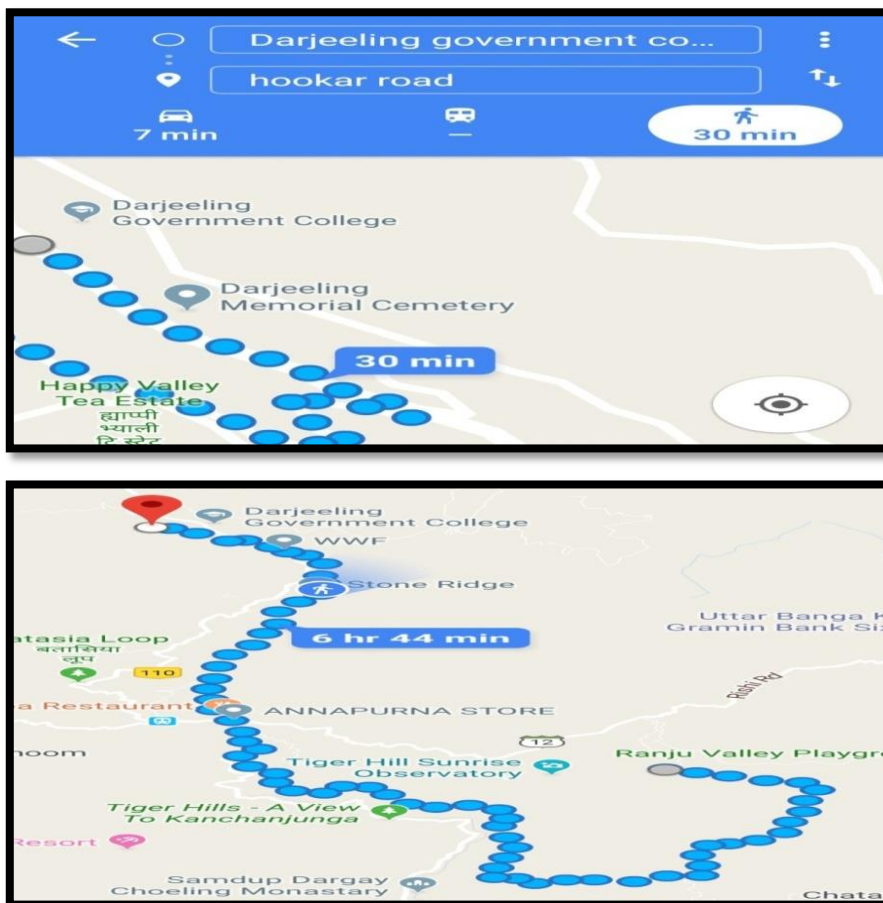


FIGURE-SITE MAPS

AIMS AND OBJECTIVES:

The aims and objectives of the study are as follows in respect of Darjeeling Himalayan Drosophilids:

1. To determine whether wild type Drosophila can grow in artificial food medium
2. To ascertain food preference of Drosophila among natural and artificial foods

2. MATERIAL AND METHOD**TOOLS AND CHEMICALS USED:**

- a. Petri dish
- b. Fine brush
- c. Diethyl ether
- d. Etherizing implement
- e. Drosophila collection trap(using plastic bottle)
- f. Drosophila culture vials
- g. Simple microscope
- h. Binocular microscope
- i. Aquarium (for keeping Petri dishes for observation)
- j. Fine forceps
- k. Non absorbent cotton
- l. Propionic acid
- m. Yeast (commercially available)
- n. Distilled water
- o. Pan for cooking food medium
- p. Ethyl alcohol
- q. Agar
- r. Autoclave for sterilization
- s. Electric lamp
- t. Weighing machine
- u. Blotting paper
- v. Molasses (Veligur).

DROSOPHILA TRAP MAKING AND COLLECTION:

Drosophilas (Fruit flies) are found to be hovering around over ripe or rotten fruits. Hence, to make a Drosophila trap mixed fruit viz., banana, tomatoes and lemon were used as bait. Plastic bottles were used as container of bait. The bottle trap is more preferable than cup trap being inexpensive. Two liter bottles were used for this purpose. The top of 2 lt bottle was cut and it was pushed into the interior of the bottle. The flies entered the bottle trap through the opening at the top. Such traps having mixed fruit was placed in different areas (both Jungle and residential area) and left for 2-3 days. The Drosophila coming inside the trap were collected by using diethyl ether and non absorbent cotton and brush.



FIG. MODIFIED DROSOPHILA BOTTLE TRAP

PREPARATION OF DROSOPHILA FOOD MEDIUM:

Lots of recipes for *Drosophila* are described in Markow and O'Grady's book – *Drosophila: A guide to species identification and use* (1st edition 2005, ISBN 9780124730526). Around the globe mainly three types of recipes are used – Tucson Recipe (used by old Tucson stock centre, the standard corn meal- molasses medium used by Bloomington and third a banana based food (http://www.research_gate.net/post/what_are_the_best_food_for_Drosophila_flies). Self modified corn meal molasses medium were used for my study (modified Bloomington medium) as all the ingredients were easily available in the market and chemicals were available in the laboratory readily. To prepare culture medium for *Drosophila*, 25 gm of maize powder, 25 gm molasses were added to boiling 500 ml of distilled water. The mixture was stirred to dissolve the molasses; 7.5 gm of agar was added thereafter and allowed the mixture to cool down to 40 degree C. 3 gm of commercial baker's yeast was added. Finally 4 drops of propionic acid and 10 drops of ethanol were added. Molasses were added for providing sweetness in the food, agar was added for gel like viscosity of the food. Yeast was added for the purpose of fermentation. Propionic acid is also used as a preservative and flavoring agent. Ethanol as disinfectant. Nepagin, widely used antifungal reagent, however ever was not used in this experiment. For further observation different types of media were prepared such as with artificial sweetener, without yeast, salted, without molasses.



DROSOPHILA TRANSFER TECHNIQUE INTO THE CULTURE VIALS:

The collected *drosophila* was transferred into the culture vials by etherizing and transferring to the culture vials by using fine brush. The etherized *drosophila* were observed under microscope and separated as male or female. To etherize *drosophila* and to collect from trap, non absorbent cotton was applied on the opening of the bottle trap so that no *drosophila* can come out. Then on non absorbent cotton, few drops of diethyl ether were applied. Separating those etherized *drosophila* as males and females, they were transferred to the culture vials using fine brush.



FIG- DROSOPHILA INSIDE CULTURE VIALS

DROSOPHILA CULTURE AND MAINTENANCE:

After transferring into the culture vials, male female drosophila were kept in prepared food medium in laboratory temperature of 17-25 degree centigrade. At night too maintain temperature, electric lamp was used. The male female drosophila copulate and within few days in all vials larvae, pupae and adult drosophila developed. The newly formed drosophila is small in size and pale white in appearance. The third instar larvae is found to stick on dry walls of the vials.

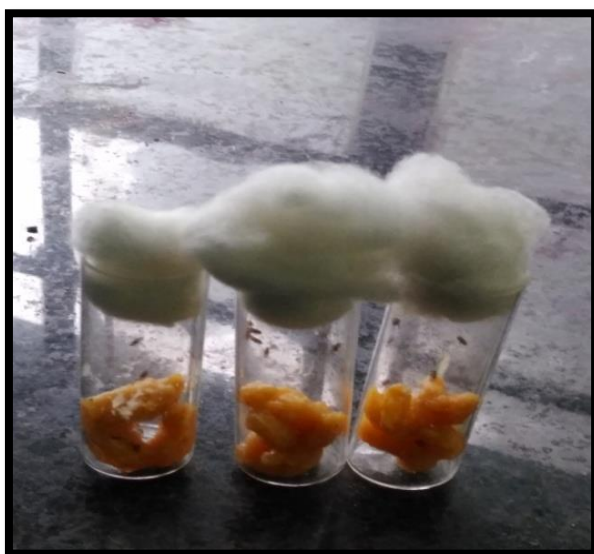


FIGURE- DROSOPHILA CULTURE AND MAINTAINANCE

EXPERIMENT NO: I

Drosophila collected from a. Tiger hill forest and b. forest areas near residential area- hooker road graveyard in Darjeeling were separated into two batch- (A). Collected from wild (B). collected from near to home areas. After preparing food for drosophila, representative sample from each (A) and (B) type were transferred into the vial by etherizing. This experiment was repeated twice to ascertain the actual cause. Total in 7 tubes, total 48 drosophila collected from wild (A) were transferred. Among them, there were 6 male and 42 female. On the other hand total 20 drosophila of (B) type (collected from near the home area) were transferred in vials. Among them 15 were female and 5 were male. The objective of this experiment was to determine whether the drosophila collected from wild areas of the tiger hill forest can survive in artificial food medium.

EXPERIMENT NO: II

Drosophila collected from near the home area were transferred into culture vials containing food medium without yeast. While preparing food incase of these food medium, yeast were not added. Total 10 drosophila 5 male and 5 female and 5 larvae from the stock were transferred in this experiment. Then it was observed for 10 days. The main objective of this experiment was to determine the effect of yeast in the life cycle of drosophila;i.e.- they can grow in yeast free medium or not.



FIGURE- STUDY IN YEAST FREE FOOD MEDIUM

EXPERIMENT NO: V

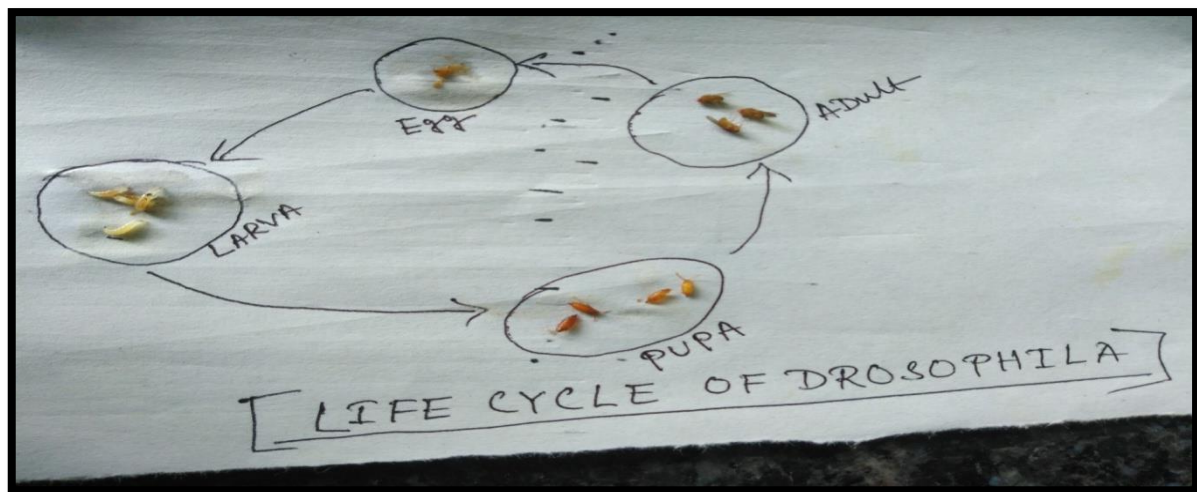
In this experiment, the drosophila which were cultured in prepared food medium were used to determine the preference of food when both natural and artificial food medium were present. Total 30 drosophila were given 4 natural and 4 prepared food in petridishes. All petridishes were kept inside the aquarium and the top of the aquarium were covered by net and papers so that no other drosophila can enter into the experimental set up from outside. Among these 30 drosophila, 6 were male and 24 were female. The main objective of this experiment was to determine food preference of lab cultured drosophila among natural and prepared food.



FIGURE- FOOD PREFERENCE OF DROSOPHILA WHEN BOTH NATURAL AND PREPARED FOOD ITEMS ARE PRESENT

OBSERVATIONS:**LIFE CYCLE OBSERVED IN DROSOPHILA CULTURE:**

As cultured in modified Bloomington medium, life cycle of drosophila was also observed. At laboratory temperature, egg, larvae, pupa, and adult stage was observed and it was found that they took 9 days approximately in eggs and larval stages and took more 6-7 days in pupal stage. Adult fly found to live for several weeks. Larval stages found to undergo moulting stages known as instars. Third instar larval stage found to crawl to drier places to pupate and they were widely found on the wall of the vials. Initially when the adult fruit fly comes out through operculum, they are found to be light in colour. Approximately after two days after coming out from puparia, female become sexually mature. The larval stage is white in colour when tried to culture in yeast free medium, larval stage even adult found not to survive.

**FIGURE- LIFECYCLE OF DROSOPHILA AS OBSERVED IN****LABORATORY CONDITION:****OBSERVATION NO: I**

It was found that greater than 90% of drosophila which were collected from tiger hill forest area (A) died in prepared food medium. However, they were found alive in original stock in natural medium. It was found that even after transferring to the same food medium, the drosophila which were collected from near the home area were still alive. Dead drosophila was collected in 70% alcohol.

Same experiment was repeated to ascertain the actual cause of death of drosophila which were collected from wild (A) in prepared food medium. The observations of the experiment is tabulated below-

TABLE: 1 (for A-type drosophila)

TUBE NO.	MALE	FEMALE	TOTAL	DIED	ALIVE
1.	2	6	8	8	0
2.	0	4	4	4	0
3.	1	7	8	6	2
4.	0	5	5	5	0
5.	1	3	4	3	1
6.	0	7	7	7	0
7.	2	10	12	11	1
TOTAL	06	42	48	44	4

From the above observation, it was found that 44 out of 48 A- type drosophila died in prepared food medium. Those were collected from tiger hill forest. The proportion shows 91.67% mortality among them. Those died drosophila were observed under microscope tube wise and were separated as male and female. It was found that mortality among male were 5 out of 6 and female were 39 out of 42. Almost same was observed (93.25% mortality) when the experiment was repeated once again with same number of drosophila collected from wild.

TABLE: 2

TUBE NO.	MALE	FEMALE	TOTAL	DIED	ALIVE
1	2	6	8	7	1(F)
2	1	3	4	2	2(1M,1F)
3	1	7	8	8	0
4	2	3	5	5	0
5	1	3	4	4	0
6	0	7	7	7	0
7	4	8	12	12	0
TOTAL	11	37	48	45	3

TABLE: 3 (FOR B- TYPE DROSOPHILA SHOWING 0.1% MORTALITY IN PREPARED FOOD MEDIUM)

TUBE NO	MALE	FEMALE	TOTAL	ALIVE	DEAD
1	1	3	4	4	0
2	2	2	4	4	0
3	1	3	4	3	1
4	1	3	4	3	1
5	0	4	4	4	0
TOTAL	5	15	20	18	2

OBSERVATION NO: 3

After 10 days of observation, only 3 out of 10 drosophila were found living; whereas all 5 larval forms were found dead no larval pupal development noted also in these yeast free medium.

TABLE: 4 (OBSERVATION IN YEAST FREE MEDIUM)

TUBE NO	ALIVE	DEAD	MALE	ALIVE	FEMALE	ALIVE
1	0	3	2	0	1	0
2	1	1	1	0	1	1
3	1	1	1	1	1	0
4	1	2	1	1	2	0
TOTAL	3	7	5	2	5	1

OBSERVATION NO: 3

Total 30 drosophila(6 male and 24 female) which were cultured in laboratory under prepared food have shown their preference of food when 4 natural and 4 prepared food items were placed together in separate petridishes. The observation table is as follows-

TABLE: 5 (DATA COLLECTED FROM OBSERVATION NO: 3)

DAY	LEMON	CHILLI	BANANA	TOMATO	PF (1)	PF (2)	PF (3)	PF (4)	TEMP (DEG. C)
1	10	1	12	5	1	0	1	0	21
2	6	1	13	4	2	0	1	0	22
3	11	1	15	1	1	0	1	0	19
4	10	0	14	1	3	0	2	0	18
5	4	0	11	3	8	1	3	0	21
6	5	0	13	3	7	1	3	1	22
7	3	1	12	2	9	0	2	0	22
8	3	0	9	2	10	1	4	1	22
9	5	0	6	3	10	0	7	0	22
10	2	0	7	1	13	1	5	1	19
Total	59	4	112	25	64	4	29	3	

While doing observation during day time, for these 5 experiments, it was found that drosophila was more active during 2-3 pm in the afternoon session.

3. RESULTS

RESULTS AND INTERPRETATION OF OBSERVATION (1):

From the observation 1 it was found that drosophila which was collected from wild areas of tiger hill forest shows more than 90% mortality and they cannot live in prepared food medium.

From the table 1 it was evident that out of 48 flies 44 died in prepared food medium (91.67% mortality).

From the table 2 it was found that there were 93.25% mortality, whereas, table 3 shows only 0.1% mortality for the drosophila which were collected from near residential areas. The following charts interpreted the data for the three tables-

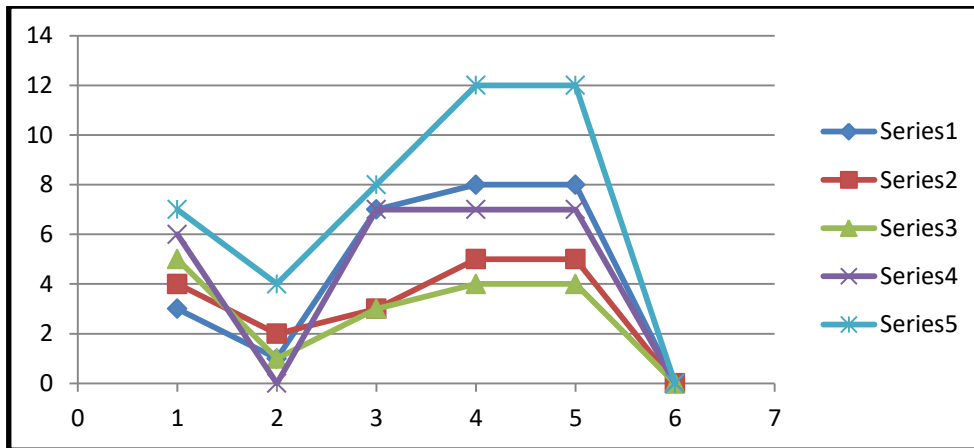


CHART-1 (FOR TABLE-2)

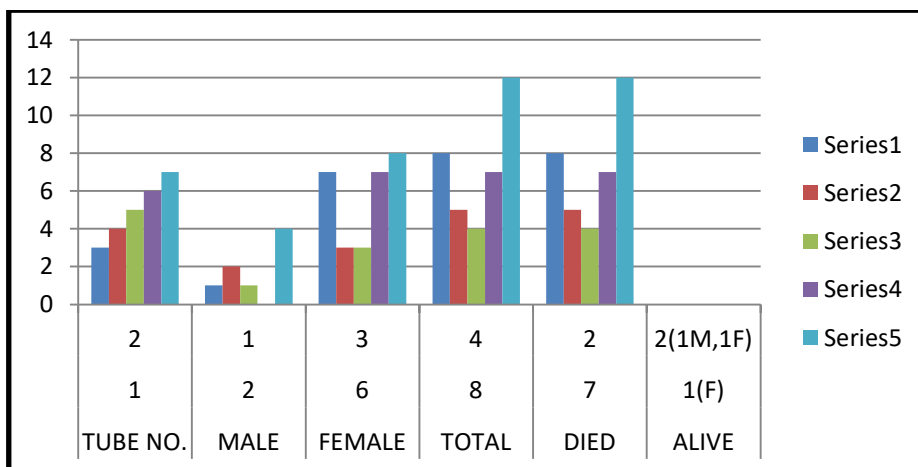


CHART-2 (FOR TABLE-2)

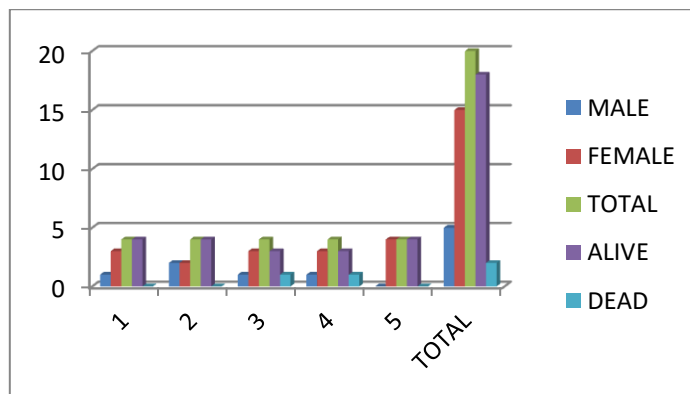


CHART-3 (FOR TABLE-3)

INTERPRETATION:

From the literatures it was found that there were few drosophila species which are specific to particular host and hard to culture in prepared medium in the laboratory (<http://www.eol.org/pages/54522/overview>). The drosophilas which were collected from tiger hill forest may be specific to single host and they cannot survive in prepared food medium when tried to culture in laboratory. Observation table 3 supports this inference as in this same food medium B-TYPE drosophila were cultured and they have shown only 0.1% mortality. This indicates the prepared food was not infected and was suitable for B-TYPE drosophila. Moreover, in the original stock in natural medium, all A-TYPE drosophila were alive.

Hence, A-TYPE drosophila cannot survive in artificial food medium for their host specificity at natural habitat and hard to culture in laboratory.

RESULT AND INTERPRETATION OF OBSERVATION 3:

From the observation 3 it was evident that yeast is an essential component of food in drosophila. No larval, pupal development occurs in absence of yeast and in yeast free medium, larva as well as adult die. The data of table 6 is represented graphically below.

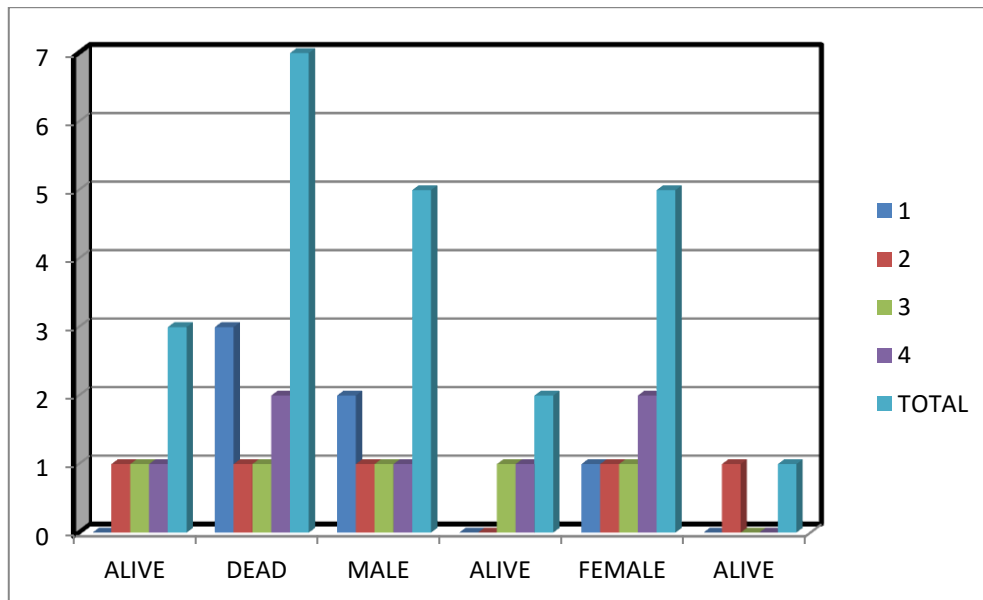


CHART-4 ANALYZING THE DATA OF OBSERVATION-3

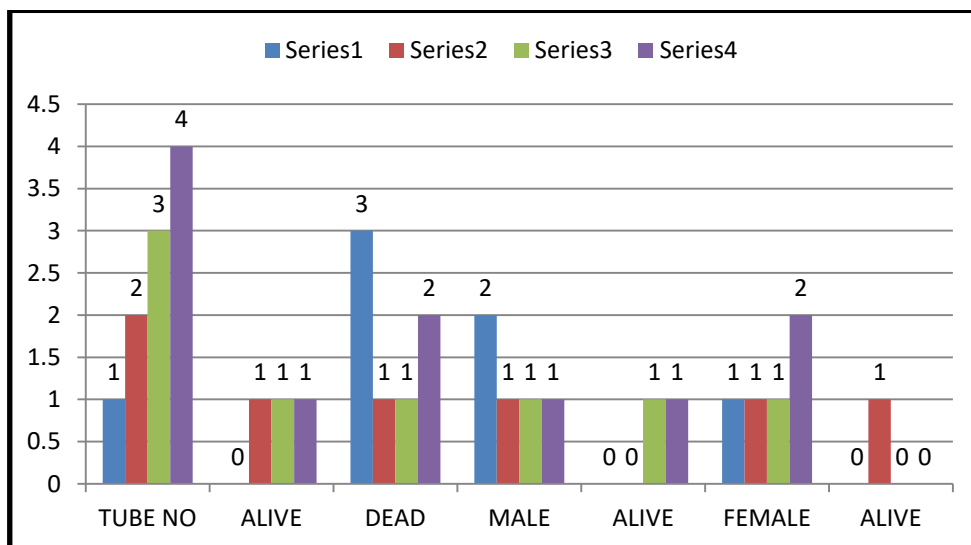


CHART-5-TUBE WISE DATA ANALYSIS

RESULT AND INTERPRETATION OF OBSERVATION 3:

The table 9 data is interpreted using following chart.

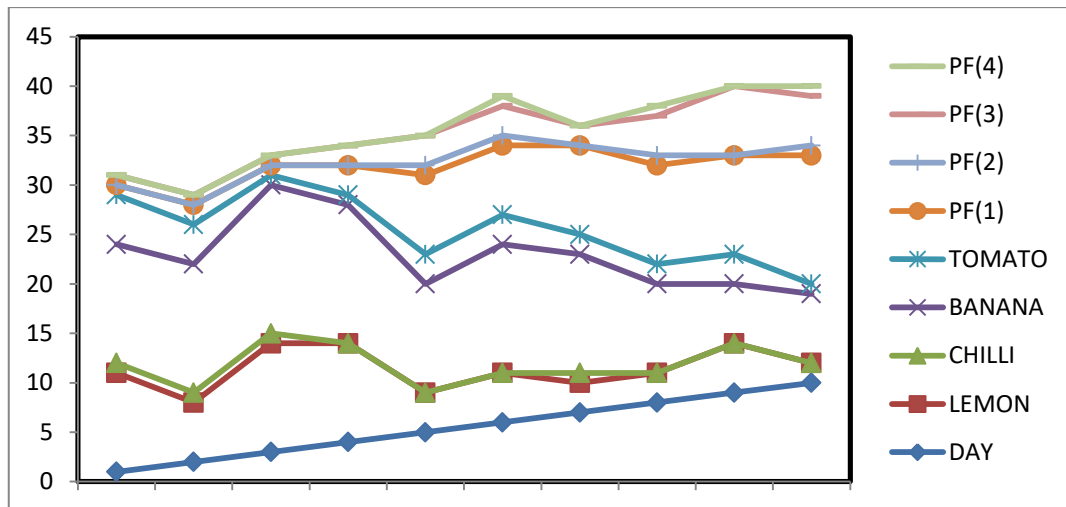


CHART-6: DAY SPECIFIC DATA ANALYSIS OF TABLE-9

The data shows that when both natural and artificial foods are provided, even if the drosophila were cultured in prepared medium, still they prefer natural foods than artificial items. Only when the natural foods get degraded, then only they switch over to prepared food items. They can detect the artificial sweetener containing food also. Among natural and artificial food items, on chili and on salted food, they show most inhibition. Here also a negative correlation is noted with food preference and effect of temperature on it.

4. DISCUSSION

In course of study of food preference in drosophila experiment and its results show that drosophila collected from wild areas of Tiger hill forest cannot survive in prepared food medium in laboratory condition because of their food specificity in their natural habitat. Tiger Hill is located at the top of the Himalayan belt has different floral diversity. Drosophila collected from near the residential area of Darjeeling town develops within 15-16 days from egg to adult in laboratory prepared food medium but they cannot survive in the medium without yeast. Even the larval forms also died showing that yeast is an essential food factor for the development in drosophila. Result shows drosophila is attracted more on odorous, sweet smelling substances. Among artificial food items they prefer mostly the modified Bloomington medium. Experiment showed they also get attracted to artificial sweetener containing food, meaning that they can detect sweetness of artificial sweetener. When both natural and prepared foods are given, even though they were cultured in prepared food medium, still they will prefer natural food items mostly. The temperature and food preference in drosophila in a particular food item shows negative correlation meaning increase in temperature causes number of drosophila to decrease. 22-25 degree cent temperature of the climate seems to be more favourable for its survival.

FURTHER SCOPE OF STUDY:

1. It was found that drosophila are attracted more on odorous natural food items like banana, mango etc. The substance causing this favored smell could not be ascertained as it was beyond purview of my project. However, it is important that we knew the exact source and nature of this sweet smelling agent.
2. Among so many species of drosophila, it may so happen that different varieties may have different preferences. In future exploration in this line is highly suggestive.

5. CONCLUSION

Small fruit fly, drosophila had been a tool of research in the field of biological sciences. Around the globe several research works were conducted throughout the years on their food preference. In course of only 3 months of study it was not possible to observe up to species level food preference, or to study chemical constituent on which they get more attracted. Further works are needed

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EXTERNAL LINKS:

- [19] <http://www.eol.org/pages/54522/overview>
- [20] <http://journals.plos.org/plosbiology/article?id=10.1371/journal.pbio.0050118>.
- [21] <http://www.ncbi.nlm.nih.gov/pmc/articles/pmc3457106>
- [22] <http://www.orkin.com/flies/fruit-fly-metamorphosis/>
- [23] <http://www.nature.com>
- [24] <http://pdfs.semanticscholar.org>
- [25] <http://www.ncbi.nlm.nih.gov/pmc/articles/pmc2467463>
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