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Application of Caffeine Consume in High Concentration can be Harmful or Exaggerated in the Central Nervous System of Human and also used when, as like an Effective Pesticide in Agriculture —Whether A Comparative Study using Drosophila Melanogaster as a Model System

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***Abstract*— Using the model system Drosophila melanogaster, Caffeine is an efficient pesticide. Humans and fruit flies have 44% of the same genetic makeup, and 75% of the genes linked to recognized human diseases are also present in fruit flies. A number of human illnesses, including the neurodegenerative conditions Parkinson's, Huntington's, and Alzheimer's disease, are being studied using Drosophila as a genetic model. The fly is also being used to investigate the processes behind drug misuse, reproductive problems, diabetes, cancer, and immunity. When caffeine is given to Drosophila Melanogaster food by preparing the culture medium, primary culture medium and sub-culture medium and maintaining Drosophila in the Genetics Laboratory, after it has been dissolved in water, the flies will either be deterred from eating it or will die thereafter. Exhibit peculiar behavior after consuming coffee. show a decrease in reproductive output after consuming caffeine. The abnormalities will exhibit in the progeny of the exposed person's health. Because the caffeine overloaded the flies' hearts, stomachs, and other organs, it led to their deaths. It considerably slowed down the flies' reproductive cycle. This development was most likely brought about by caffeine's effects on the "ring gland."In Drosophila, the ring gland is an organ of hormonal release; it secretes the hormones that mark the beginning and end of each stage of the drosophila life cycle. Neuron activity influences the ring gland; hence, hyperactive neurons may cause challenges with hormone release and reproductive disorders. These results would favor a slower rate of reproduction. The central nervous system's reaction to typical environmental stimuli was heightened by the increased neuron activity by GABA and serotonin in Drosophila.**

***Keywords*— Drosophila melanogaster, Caffeine, Fly Fatality, Life Cycle Observation, Genetic similarity, Neurogenetive and Reproductive Genetics.**

I. INTRODUCTION

Caffeine is a crystalline, bitter xanthine alkaloid that is taken as a stimulant drink in Coffee. Caffeine is found in varying concentrations in the seeds, leaves, and fruit of some plants, where it acts as a natural insecticide. The pricey powdered caffeine that is abused as a stimulant drug. Caffeine was initially isolated to a somewhat pure condition in 1819 and was called "Kaffebase" (a base present in coffee) by German scientist Friedlieb Ferdinand Runge. Caffeine was isolated in 1821 by French scientist Pierre Jean Robiquet and another pair of French chemists, Pierre-Joseph Pelletier and Joseph Bienaimé Caventou, according to Swedish chemist Jöns Jacob Berzelius's yearly diary. Chemical Make-Up: Formula: $C_8H_{10}N_4O_2$, point of melting: 238 °C. Molar mass: 194.19 g/mol; IUPAC ID: 1,3,7-trimethyl-1H-purine-2,6(3H,7H)2,6-dione 3,7-dihydro-1,3,7 trimethyl-1H-purine, Point of boiling: 178 c° Water-soluble. Present in Confectionaries, Tea, Coffee, Cola, and Chocolate. Actually, coffee is applied as a pesticide in the agricultural sector like plants that are frequently attacked by slugs can be sprayed with a solution made of coffee and water. Leaves might be prevented by up to 62% with a combination that contained just 0.1 percent caffeine. At the proper concentrated dosages, caffeine has been shown to be an efficient insect repellent; the test compounds killed the insects in a matter of hours or days. The scientists also discovered that when caffeine molecules were combined with other natural insecticides, their killing effectiveness increased. By reducing Neurotransmitter activity and expanding blood vessels to improve oxygen delivery, Adenosine aids in the body's preparation for sleep. Brain cell receptors are unable to distinguish between Caffeine (C) and Adenosine (A). Caffeine binds to its receptors and blocks the release (or slowdown) of Adenosine so causing this effect. Our brain activity increases by GABA and serotonin (nor-adrenaline and dopamine) and we become more awake when there is no Adenosine to induce sleep. Caffeine additionally narrows our blood vessels by inhibiting adenosine, which eliminates our headache. But excessive amount of taking coffee can be harmful for the human also. On the other side, among the model organisms used in Genetics, consist of the fruit fly or Drosophila melanogaster, Caenorhabditis elegans, Zebrafish or Danio

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rerio, Arabidopsis thaliana, and Dictyostelium discoideum usage for the investigation by scientists into a wide range of biological topics. In scientific fields like Genetics and Developmental biology, these kinds of species like Drosophila melanogaster are particularly valuable. It additionally has the potential to check for various Reproductive and Neurogenetic diseases. Because Humans and Drosophila share similarities were Humans and flies share 44% genetic similarities. The genetic coding of fruit flies has a discernible match for around 75% of the known human disease genes (Reiter et al (2001). The scientific classification of Drosophila or Taxonomy is as follows: Species group: melanogaster; Species subgroup: melanogaster; Species complex: melanogaster; Species Binomial name: D. melanogaster by Meigen, 1830; Kingdom: Animalia; Phylum: Arthropoda; Class: Insecta; Order: Diptera; Family: Drosophildae; Genus: Drosophila; Subgenus: Sophophora; Species group: Drosophila; Species complex: melanogaster; and so on. Drosophila inhabits and feeds on damaged fruits, slimes, mushrooms, fermenting bananas, and other areas where they gather & reproduces on fruit. These are tiny insects, measuring 3 mm in height and length, with a red eye and a brownish body colour. Their structure encompasses one pair of wings, three pairs of legs, and three body segments. The animals used in the Lab practical are tiny, inexpensive, and simple to raise in big quantities. Their life span is only two weeks. The life cycle of BOD Culture is around 10–12 days at [24°C+/–1]. The complete genome has been sequenced, and the mutant flies have abnormalities in any one of the thousands of genes that are known to exist. Male bodies are often petite in size and have dark skin tones. They have a sex comb on their first leg. A dark black patch is seen at the end of the body. On the other hand, female bodies are larger than men's and are lighter than men's. The female has no sex comb, and there is a light black speck at the end of her body. The body is 1-2 mm in length. When it comes to Drosophila reproduction, species differ greatly in terms of their ability to reproduce, but D. melanogaster, which breeds in big, relatively scarce resources, has ovaries that may produce 10–20 eggs at a time. It is known that the sperm cells of males in this species are the longest of any living thing on Earth. The length of development differs greatly between species. One or more respiratory segments that protrude above the surface of the egg filaments near the anterior end and other tip enable oxygen to reach the developing embryo. The egg's length is around 0.5 millimeters. After fertilization, the embryo takes around a day to mature and hatch into a warm-looking larva. In the Drosophila life cycle, the larvae continually feed and grow before going through three instars—one, two, and four days—after hatching. The larva undergoes one more moult to become an immobile pupa after two days as a third instar. The body fully re-molldes over the course of the following four days to reveal the adult form with wings. Emerges from the pupal case and becomes fertile in around 12 hours (doubling the development time at 18–25 times).

II. USE OF THESE MODEL ORGANISM

Numerous human illnesses, including neurodegenerative disorders, are being researched with Drosophila melanogaster as a genetic model like Alzheimer's, Parkinson's, and Huntington's diseases. The fly is also being utilized for research on misuse of drugs, diabetes, cancer, along with reproductive genetic immunity mechanisms. The D. melanogaster is tiny, simple to keep in laboratory, and has a rapid generation time. That generate a respectable quantity of progeny and has several readily apparent traits. Their significance for human health was recognized by the award in medicine/physiology given to Ed Lewis, Christiane Nusslein-Volhard, and Eric Chaus in 1995, when it was discovered that genes are related to protein and that the principles of genetic inheritance can be studied from that perspective, as well as how a complex organism emerges from a relatively simple fertilized egg. So, we are geneticists or other researchers and scientists who are concerned and care about Drosophila melanogaster. Melanogaster, which stands for "Strong Dark Gut," and Drosophila, which means "The Dew Lover," combined to generate "The Dew Lover with a strong dark gut who has historically been easy to handle and well understood." The diploid genome contains four pairs of chromosomes called "Balancer chromosomes." X/Y, the Y chromosome, and the Autosomes: 2, 3, and 4. Fourth chromosome: quite small and seldom mentioned. The genome is 165 million base pairs in size. has an estimated 14,000 genes, with a viable mutation. Gene size average: 3000 base pairs. 2000 saw the sequencing of the genome. Approximately 50% of the genes shared with humans. The Transparent Chromosome as a Magic Marker: A chromosomal polytene: In Drosophila salivary glands, name Polytene chromosomes—the distinctive identity of Drosophila—are located. Each chromosome divides 100 times as the larva grows, yet all of the strands remain connected to produce new gene product. They are significantly longer than metaphase chromosomes, measuring 2 mm. A chromosome of polytene that is very thick, is clearly visible under a microscope. Polytene Chromosome: The 102 numbered bands of the genome are [(1-20=X), (21-60=second), (61-100=third), & (101-102=fourth)]. In the Neuroscience & Behavioral Genetics: The Biological Clock Drosophila melanogaster mutants, A set of genes and the products they produce that make up a biological or biochemical clock are affected by mutations. Although this clock is present in many different fly cell types, the cells that contain it and regulate activity are a few dozen neurons in the fly's central brain that are powered by cyclic AMP and an ineffective gene. Regular intake of caffeine can result in Humans and Drosophila both have central nervous systems (CNSs), that can have a variety of effects on the reproductive system. For example, the brain or CNS produces hormones which can delay the puberty and the breakdown of eggs and sperm, and chemicals like bisphenol A (BPA) can have an impact on gametogenesis and brain development, both of which can affect fertility. So, effected Central Nervous system can affect reproductive system.

Fig: 1 Drosophila melanogaster. Courtesy: “Flies' daily rhythms differ in lab and outdoors”. Baker, M. Drosophila's outdoor schedule. Nat Methods 9, 529 (2012). https://doi.org/10.1038/nmeth.2048.

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III. OBJECTIVE

My research's hypothesis is that if caffeine is dissolved in water and added to Drosophila Melanogaster meal:

1) The flies will either be discouraged from eating the food or would perish after consuming it.
2) Display strange behaviour following caffeine consumption.
3) Exhibit reduced reproductive production following exposure to the caffeine.
4) The exposed person's progeny will exhibit abnormalities.

IV. MATERIALS AND METHOD REQUIRED FOR MY EXPERIMENT

The Drosophila melanogaster colony Glass vials, an incubator, Distilled water (600ml), Coffee Powder (With preliminary culture (food) preparation, the coffee powder is provided with the components necessary (instead of caffeine), 50g of cornflowers [carbon source], 50g of molasses [carbon source], 5g of agar solid, yeast, a microscope, and a brush The preservative Propanic acid Nipagin powder with Rectified Spirit, Nipagin (in paper form if necessary), and cotton for plugging. Diethyl Ether, BOD Culture Chamber, and First Food Culture Preparation: The Instead of caffeine, the necessary supplies contain coffee powder. REMEMBER: [400ml: Agar] [200ml: Molasses] [200ml: jaggery] [Yeast: 200ml].

- Preparing the culture medium: Maintaining Drosophila in the Genetics Laboratory:

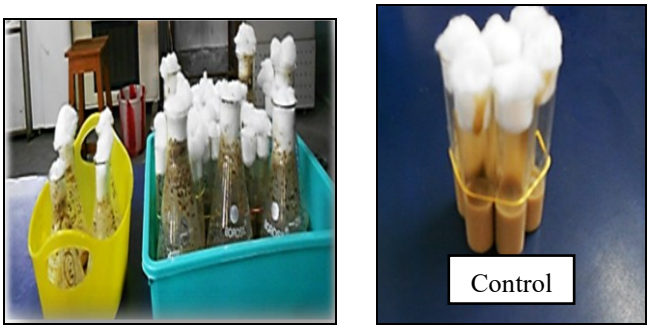
1. Warm water was used to dissolve the agar-agar, which was then heated to ensure total dissolution.
2. Initially, cornflower and molasses were added and cooked for around 15-20 minutes.
3. The temperature was reduced, and the meal was chilled to between 40 and 50°C.
4. After adding the dry yeast, the mixture was heated to 40–50°C for 15–20 minutes.
5. Nipagin, dissolved in rectified spirit, was added together with propionic acid.

- Preparing the Primary Culture Medium:

6. The meal was prepared for consumption when its consistency was rather thick.
7. After that, it was carefully put into a sterile container.
8. Once the meal had dried, a piece of Nipagin-soaked paper was introduced in each bottle using a blunt forceps.
9. After being chilled, the bottles were left open until the moisture evaporated.
10. The bottle's excess moisture was drained before a cotton plug was placed inside.
11. Prior to use, a drop of live yeast suspended in water is applied.

Fig 2 Preparation of food for Drosophila of culture medium Coffee powder used with the food for the experiment of caffeine, instead of any caffeine chemicals. Also used Agar, Cornflower, jaggery, molasses.

Fig 3: The Primary Culture Medium stored in BOD incubator for growing Drosophila life cycle.



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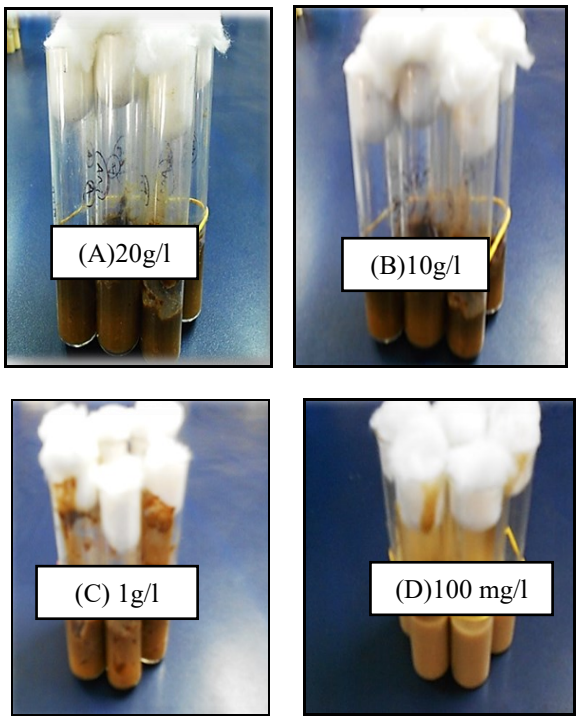

Fig 4: Drosophila melanogaster culture with caffeine solution. Different concentration makes different amount that create different results with compare to Controls.

- In My Experiment:

I. THE FLIES HAVE BEEN OBTAINED:

1. Preparing the Basic Culture Medium
2. Upkeep of Drosophila in the Genetics Lab
3. Take a colony of Drosophila melanogaster out of the culture medium.
4. Until they are ready for subculturing, keep the flies in an incubator at 25 °C.

II. CAFFEINE SOLUTION PREPARATION:

1. Collect some caffeine (in the form of powder).
2. Stir 100 ml of distilled water with two grams of caffeine. Mark "20g/L" on the container.
3. Take a hot bath to rewarm the container. Shake until the caffeine is completely gone.
4. Transfer the mixture to a glass container. Write "10g/L" on the container.
5. Pour 100 millilitres of distilled water into the flask. Give the flask a shake.
6. Transfer the fresh mixture into a glass vial. Indicate on the container "1g/L."
7. Create a 100 mg/L solution by diluting 10 millilitres (of the 1 g/L sample) with 90 millilitres of water. The following caffeine solutions were made: (A) 20 mg/L, (B) 10 mg/L, (C) 1 mg/L, and (D) 100 mg/L.

III THE SUBCULTURES' PREPARATION:

1. Set up the Drosophila medium by adding the appropriate amount of caffeine. (A) 20 g/L, (B) 10 g/L, (C) 1 g/L, and (D) 100 mg/L.
2. Acquired 10 transparent glass observation vials filled with culture media and 10 cotton plugs that fit snugly.
3. Write the following on the tubes: A: 20 g/L, B: 10 g/L, C: 1 g/L, D: 100 mg/L.
4. Designate "Control 1" and "Control 2" for the two extra tubes.
5. Insert a cotton plug into each tube.

IV. HANDLING THE FLY TRANSFER:

1. Obtain the Institute of Genetic Engineering Lab's D. Melanogaster culture.
2. Removed a subculture tube.
3. Tip the subculture tube onto the Drosophila Melanogaster culture tube.
4. Connect the two tubes' open ends as soon as you take out the plugs from them.
5. Permit around ten flies to go inside the subculture tube.
6. Immediately disconnect the tubes and put the cotton plug back in.
7. Confirm using the additional subculture tubes.

Fig 5: Transfer the Male and Female flies which are alive and which are dead that should be generated.

V. OBSERVING THE FLIES:

1. Put the subcultures in a BOD and adjust the temperature to 24 °C.
2. Take one daily look at the flies. Seek out: Observations of fly fatalities-Tally the quantity of live and dead flies. Life cycle observations. Are there any larvae or pupae exist?

- Precaution

1. Food stuff needs to be constantly stirred during preparation to prevent burning.

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2. It is important to adequately sanitize cotton, vials, and bottles.
3. To avoid softening the food, avoid plugging in bottles and vials until the food has cooled.
4. B.O.D. should always be closed; only B.O.D. should be opened if it is necessary to take up or place the culture vials.

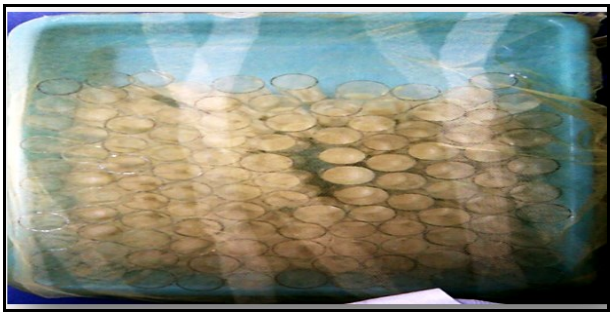

Fig 6: For precaution to make culture media or food for Drosophila, Mosquito Net is very useful.

V. REVIEW OF LITERATURE

According to the journal name: "Is Caffeine an Effective Pesticide Against Drosophila (fruit fly)?" A Science Fair Research Project. (This project won first at PJAS Region 1B, first at PJAS States, second at Montgomery County Science Research Competition, and third at Delaware Valley Science Research Competition.) Coffee contains a molecule called caffeine, which has pesticidal effects on Drosophila. For drosophila, caffeine would be deleterious. In essence, caffeine is an inhibitory neurotransmitter that may replace adenosine. Caffeine stops the "slow down" message of adenosine from being sent between nerve cells and instead sends its own "speed up" message. Numerous hormonal effects of hyperactive neurons include the production of adrenaline. to examine how caffeine affects the flies. Does it eliminate other dangerous insects? Does it degrade in the surrounding environment? Also, "The development of resistance to caffeine in Drosophila prosaltans: productivity and longevity after ten generations of treatment journal by Itoyama, MM. de Campos Bicudo, HEM.; Manzato, AJ" says Caffeine treatment at 50, 100, 1000, 1500, 2000, and 2500 micro g/ml of culture media lowered D. prosaltans fertility during 10 generations (~8 months) in a concentration-dependent manner. However, at the end of the treatment, all flies restored normal productivity except those treated with 2500 micro g/ml. Longevity in the tenth generation was severely decreased in both men and females at the 2500 micro g/ml concentration, with males being much more impacted than females. It is suggested that selection took place over the course of ten generations, recovering productivity and reducing the processes that shorten life spans.

VII. RESULTS

(A) Table I. Fatality Test:

Concentration of Caffeine:	**Flies Setup for The Experiment:**	**No. of Flies are Alive After 8days:**
20g/l	60	9
	60	21
1g/l	60	35
100mg/l	60	60
Control 1	60	60
Control 2	60	60

Table 1: 1) By the fourth day, 20g/l of flies are dead, and very few remain alive. 2) By the eighth day, 10g/l may be lethal, but some flies may still be alive. 3)After eight days, everyone in 1g/l and 100mg/l were still alive.4) Controls 1 and 2 are identical.

The Graphical Representations of Fly Fatalities:

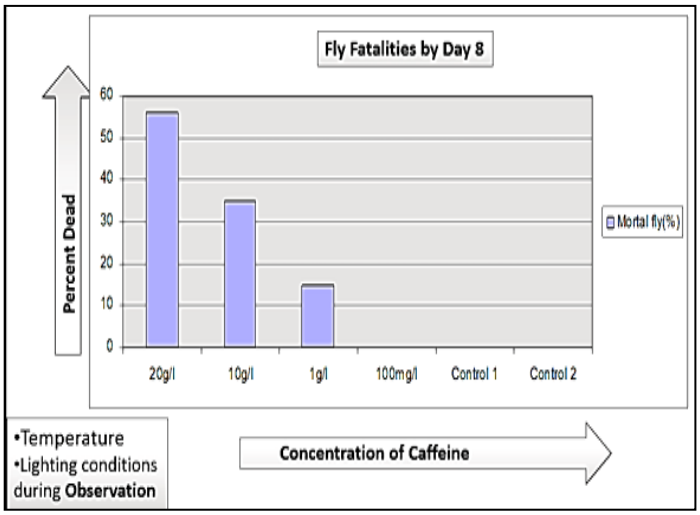

Fig 7: (A) In Fly Fatality Graph: There is Graphical view of the Mortality percent in concentration of caffeine against the percent of dead fly or the fly fatalities by day 8.

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(B) Table II. Life Cycle Observation Test:

Concentration of Caffeine	First Pupae Appeared	New Flies Appeared
20g/l	After 17 days	After 19 days
10g/l	After 13 days	After 17 days
10g/l	After 10 days	After 15 days
10g/l	After 8 days	After 12 days
Control 1	After 8 days	After 12 days
Control 2	After 8 days	After 12 days

Table 2: 1) Caffeine at 20g/l causes higher fatalities; the majority of the flies died, with only 15% remaining alive and their life cycles delayed. 2)10g/l Caffeine causes mortality; 35% of the flies survive, and their life cycle is postponed. 3)1g/l and 100mg/l are not lethal like 1 and 2. In 1g/L, the life cycle is somewhat delayed, but not in 100mg/L. 4) Control flies (1&2) exhibit typical growth.

The Graphical Representations of Life Cycle Observation:

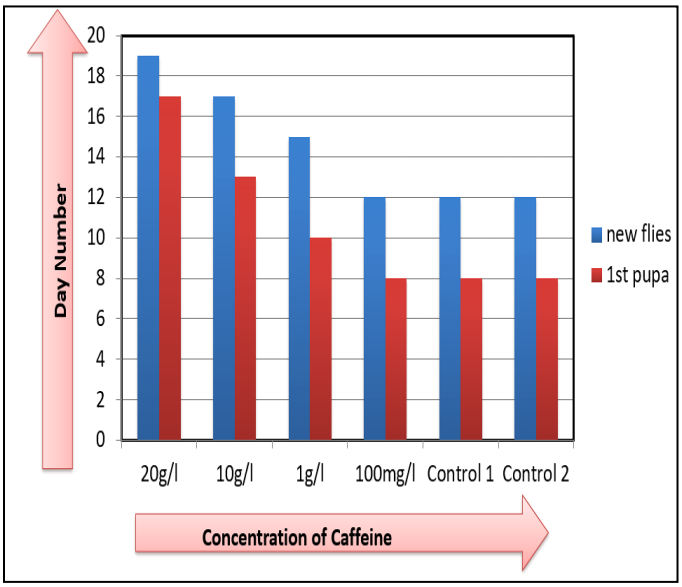

Fig 8: (B)In Life Cycle Graph: There is Graphical view of Life Cycle observation where the new fly comes observation and 1st Pupa comes happens in the Concentration of Caffeine against the Day number.

VIII. FURTHER STUDY:

In the further study we can see the opposite reaction between the Drosophila with Green tea (Green Tea Polyphenols or GTP) where Drosophila life is more and they are alive in high concentration after 8 days test and first pupa has been seen and new fly comes. Green tea has flavonoids epigallocatechin gallate (EGCG), caffeine, theobromine, theophylline, and L-theanine which can help in Drosophila and Human body a good effect though in proper amount concentration. Also, helps in the brain and nervous system and helps in reproduction process. So, it is very good because We use Drosophila as a model organism, we don't have any intention to kill them, it's a research purpose they have got detrimental results. Also, we can do further identification study from POLYTENE CHROMOSOME identification if we dissect them properly, the salivary glands of Drosophila larvae have enormous chromosomes called Polytene Chromosomes. They are created when DNA replicates repeatedly without physically separating the bands, leaving open interbands and thick bands in the wake of the replication. By successively duplicating each chromosomal element (chromatid) without their segregation, it develops from the chromosomes of diploid nuclei. Polytene chromosomes are structures like cables made up of freshly created chromatids that stay linked longitudinally. The dissection of these chromosome can detect the effects of Caffeine about Drosophila melanogaster in neurodegenerative disorder and reproductive disorder by Chromosome organization, Gene expression, Gene mapping and detect the disease comes from Genetics.

CONCLUSION

In my work done in the Lab of Department of Genetics (2013) in Institute of Genetic Engineering I have found the interesting concluded area that should be discussed. There is no doubt that caffeine harms Drosophila. Without a doubt in excessive amount, it detrimental for these tiny insects and also harmful for Human being which can slowly poisonous in its excessive tacking regularly. Because the caffeine overworked the flies' hearts, stomachs, and other organs, it led to their deaths. It considerably slowed down the flies' reproductive cycle. This development was most likely brought about by caffeine's effects on the "ring gland." In Drosophila, the ring gland is an organ of hormonal release; it secretes the hormones that mark the beginning and end of each stage of the drosophila life cycle. Since the ring gland is regulated by neuron activity, hyperactive neurons may cause issues with the reproductive cycle and hormone secretion. These results would favor a slower rate of reproduction. The central nervous system's reaction to typical environmental inputs was amplified by the increased neuron firing. There is no denying that caffeine has a negative effect or impact on Drosophila. My investigation showed that, at high enough doses, caffeine may work as a pesticide against Drosophila. It's evident that caffeine kills insects at high quantities. Furthermore, caffeine is generated naturally by plants and is not as dangerous as manufactured pesticides, giving it several advantages over other pesticides. So, we can use Caffeine as pesticide in a certain amount and also can intake as Coffee or other Caffeine products in a specific amount which does not harm out Central Nervous Systems and Reproductive systems.

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REFERENCES

[1] Is Caffeine an Effective Pesticide Against Drosophila (fruit fly)? A Science Fair Research Project, 2012.

[2] Burrows, Malcolm. The Neurobiology of an Insect Brain. Oxford: Oxford University Press, 1996.

[3] Chatterjee, Camille. "Coffee: Can it kill?" Psychology Today. Nov/Dec 1999.

[4] Cherniske, Stephen. Caffeine Blues. Warner Books, 1998.

[5] Demerec, M., ed. Biology of Drosophila. New York: Hafner Publishing Company, 1965.

[6] Essig, E. O. College Entomology. New York: The Macmillan Company, 1942.

[7] Flagg, Raymond O. Carolina Drosophila Manual. North Carolina: Carolina Biological Supply Company, 1988.

[8] Hocking, Brian. Six-Legged Science. Massachusetts: Schenkman Publishing Co., Inc., 1968.

[9] Itoyama, M.M. "Effects of caffeine on mating frequency and pre-copulation and copulation durations in Drosophila prosaltans." Cytobios, 1995.

[10] Itoyama, M.M. "The development of resistance to caffeine in Drosophila prosaltans: productivity and longevity after ten generations of treatment." Cytobios, 1998.

[11] Little, V. A. General and Applied Entomology. New York: Harper and Brothers Publishers, 1957.

[12] Manning, Gerard. "A quick and simple introduction to Drosophila Melanogaster." Available http://www.ceolas.org/fly/intro.html

[13]"Phosphodiesterase."Available http://www.mblab.gla.ac.uk/tubules/map/pde.html

[14]"Product Information Sheet for CO750 CAFFEINE (ANHYDROUS)."Available http://www.sigma.sial.com/sigma/proddata/c0750.htm

[15] Ross, Herbert H. A Textbook of Entomology. New York: John Wiley and Sons, Inc., 1967.

[16]Shapton, David. Internet Project on Caffeine. Available http://tiger.chm.bris.ac.uk/cm1/DaveS/Welcome.html

[17] Thor, D.H. "Caffeine and copulatory experience: interactive effects on social investigatory behaviour." Physiological Behaviour, 1986.

[18] Weeden, et al., eds. "Insect Biology and Ecology: A Primer." Cornell University.
Available http://www.nysaes.cornell.edu/ent/biocontrol/info/primer.html

[19] Wigglesworth, V.B. Insect Hormones. San Francisco: W.H. Freeman and Company, 1970.

[20] Wu MN, Ho K, Crocker A, Yue Z, Koh K, Sehgal A. The effects of caffeine on sleep in Drosophila require PKA activity, but not the adenosine receptor. J Neurosci. 2009 Sep 2;29(35):11029-37.