Role Of TiO_2 Nanoparticle On Growth And Development Of *Drosophila Melanogaster*

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SUBMITTED BY:

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RIGHT TO INFORMATION

CERTIFICATE

This is to certify that the thesis entitled "***Role of TiO_2 nanoparticle on growth and development of Drosophila Melanogaster***" which is being submitted by **Ms. Sibani Moharana**, Roll No. **413LS2039** for the award of the degree of Master of Science from National Institute of Technology, Rourkela, is a record of bonafide research work, carried out by her under my supervision. The result embodied in this thesis are new and have not been submitted to any other university or institution for the award of any degree or diploma.

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DECLARATION

I do hereby declare that the Project Work entitled "***Role Of TiO_2 Nanoparticle On growth and development of Drosophila Melanogaster***", submitted to the Department of Life Science, National Institute of Technology, Rourkela is a faithful record of bonafide and original research work carried out by me under the guidance and supervision of Dr. Monalisa Mishra, Assistant Professor, Department of Life Science, National Institute of Technology, Rourkela, Odisha.

Date: 11.05.2015 Sibani Moharana

Place: Rourkela Roll.No.413LS2039

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Sibani Moharana

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List of Abbreviation:

NP: Nanoparticle

FP: Fineparticle

TiO_2: Titanium Dioxide

CS: Canton-S

ppm: Parts-per-million

ABSTRACT

In modern days the growth and progress of nanotechnology has tremendous. A nanoparticle (or nanopowder or nanocluster) is a microscopic particle with at least one dimension less than 100 nm.Nanoparticle-based technologies are the fastly growing side of our industrial, medical and environmental economies. Titanium dioxide (TiO_2) nano particles (NPs) are synthesized worldwide in huge quantities for accomplishing in a wide range of applications. TiO_2 NPs have many physicochemical properties on the contrary of their fine particle (FP) analogs, which might affect their bioactivity. Titanium oxide (TiO_2) is obtainable in the form of nanocrystals or nanodots having a high surface area. They demonstrate magnetic properties. As one of the assuring nanomaterial TiO_2 has have stimulated a large attention in catalysis ,coating, chemical sensing and a lot of other scientific field due to unusual properties.The fruit fly *Drosophila Melanogaster* is the well known sightseer and an organism of preference in genetics laboratories. The gut is one of the largest organs in the body cavity.. The fruit fly is small and has a simple diet. Therefore, large numbers of flies can be maintained inexpensively in the laboratory. The life cycle is also very short, taking about two weeks, so large-scale crosses can be set up and followed through several generations in a matter of months. Fruit flies also have large polytene chromosomes, whose barcode patterns of light and dark bands allow genes to be mapped accurately.

Here in this study the aim was to check the toxic concentration of TiO_2 on the growth and development of *Drosophila Melanogaster*.

Keywords: TiO_2, *Drosophila Melanogaster*, Canton-S

1. INTRODUCTION:

In modern days the growth and progress of nanotechnology has tremendous. A nanoparticle (or nanopowder or nanocluster) is a microscopic particle with at least one dimension less than 100 nm. The study of nanoparticle is currently an area of deep scientific research, due to a large diversity of potential applications in biomedical, optical, and electronic fields. Nanoparticles are of great scientific interest as they are effectively a bridge between bulk materials and atomic or molecular structures. The application of NPs for drug delivery system, antibacterial materials, cosmetics, sunscreens and electronics is very vast (Kisin *et al 2007*).In October 2011 the European Union defined nanomaterial as an usual, subsidiary or artificial material containing particles which is present in an unbound state or an aggregate or agglomerate state; in which 50% or more of the particles were manifested.

As a result of their undersized dimensions, NPs could be harmful to biological systems, even more so than faintly larger particles in the micron range, known as microparticles (MPs; ≥ 1 μm). Their size can guide them to actin a different way from other potentially hazardous materials contributing to the dispute in accepting their effects. The two main differentiation between NPs and their MPs and bulk forms are those observed in their exterior effects and quantum effects. NPs have an enormously high surface area to volume ratio due to their small size, so that the portion of atoms that are at the surface is much larger than in bulk materials of the same composition (Buzea*et al.*, 2007). Typically, this results in growing reactivity and a reducing melting point of the NP as the particle size gets lesser, which can be accredited to few neighbouring atoms that keep the surface atoms in place (Roduner, 2006; Buzea *et al.,2007).*

1.1 Nanoparticles used in modern days:

Nanoparticle-based technologies are the fastly growing side of our industrial, medical and environmental economies. Their toxicological properties, different from bulk form, have been subject to intensive research (Oberdörster *et al.,* 2005; Nel *et al.,* 2006*;* Rivera Gil *et al.,* 2010).Manufactured, or engineered, NPs can be separated into different material classes, including metals, metal oxides, non-metals, polymer-based, carbon-based as well as those classified as semi-conductor materials, such as quantum dots (Klaine *et al.,*2008).A figure shown below that defines the role of NPs inside the body. Silver and titanium dioxide nanoparticles are recognized to persuade oxidative stress in vitro and in

vivo. Zinc oxide nanoparticles combine with titanium dioxide also encompasses well-known inhibitory and bactericidal effects. It was studied to designed the efficacy of zinc and titanium dioxide nanoparticles against biofilm producing methicillin-resistant *S. aureus*,

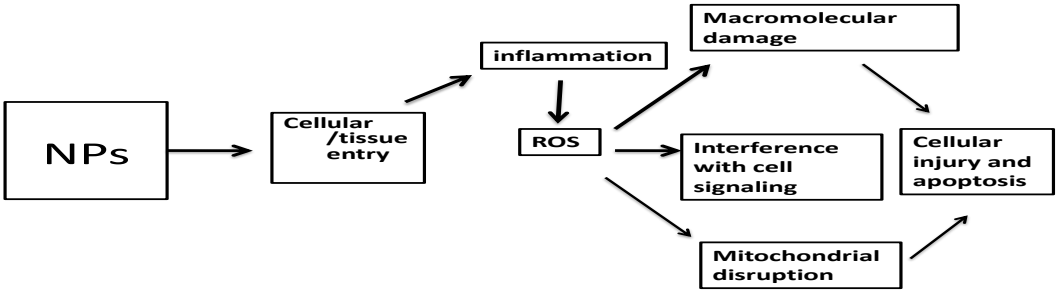


Fig1 :Schematic diagram showing role of NP

but here in this review a special emphasis was given to nanoparticle Titanium dioxide(TiO_2) which affects the growth and development of *Drosophila Melanogaster.*

1.2 TiO_2 used in current days:

Titanium dioxide (TiO_2) nanoparticles (NPs) are synthesized worldwide in huge quantities for accomplishing in a wide range of applications. TiO_2 NPs have many physicochemical properties on the contrary of their fine particle (FP) analogs, which might affect their bioactivity. Titanium dioxide, is an unprocessed natural obtaining oxide commonly known as titanium(IV) oxide or titania with the chemical formula TiO_2.

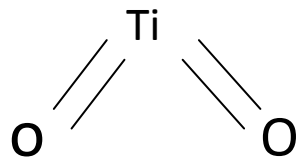


Fig2: Chemical structure of TiO_2

The function and use of this titania is very large i.e. from paint to sunscreen to food colouring. When applied as a food colouring, it has E number that is E171.Traditionally,

TiO_2 FPs was considered as weak soluble and low toxicity particles. That is why, they were conventionally treated as a "negative control" in various in vitro and in vivo particle for their toxicological studies (Zhao *et al* 2009). Nevertheless, this aspect was objected after lung tumours generated in rats after two years of liability to high concentrations of fine TiO_2 particles (Lee *et al* 1985). The International Agency for Research on Cancer (IARC), due to this reason, has categorized TiO_2 as a Group 2B carcinogen which are probably carcinogenic to humans (IARC). But, the tumourogenic effect of fine TiO_2 has been interrogating and applied to lung overwhelm rather than particular carcinogenicity of fine TiO_2 .

Various animal models are making use of multiple exposure routes of authorization, that includes inhalation, dermal exposure, intratracheal instillation, oral gavage, intragastric, intraperitoneal or the intravenous injection have been completely second handed in these studies. Studies have affirmed that the TiO_2 NPs can be more toxic than FPs. Oberdorster *et al.* advocated that TiO_2 NPs (21 nm) creates a higher pulmonary inflammatory feedback than TiO_2 corresponds to mass burden, with higher quantities of TiO_2 NPs indulging the alveolar interstitium in the lungs. Sager *et al*. have stated the same results after intra-tracheal instillation of well-dispersed condensation of TiO_2 NPs. Broad application of TiO_2 NPs deliberates significant impeding for human contact and environmental discharge, which unavoidably permits for a probable health hazard to humans, domestic animal, and the eco-system (Long *et al*). Even though the nanoparticle(NP) size has currently been defined as <100 nm, we have also incorporated some studies that have distinct particle sizes that are >100 nm as NPs. The molecular mechanisms and role of carcinogenesis will also be reviewed, to deal with the health burdens concerning carcinogenesis due to particle exposure. Titania nanoparticles have in warded a good attention for applications such as optical devices, sensors, and photocatalysis (Harizanov *et al* 2000*;Li et al* 2002).There are several factors in determining important properties in the performance of TiO_2 for applications such as particle size, crystallinity and the morphology. So in this study the toxicity of TiO_2, *Drosophila* as a model organism was selected and it was experimented and some remarkable result were observed.

2. Review of Literature:

The development of industry was immensely augmented the generation and build up of waste byproducts. In common, the manufacture of functional products has been centered on and the production of waste byproducts has been largely unnoticed. This has created harsh environmental troubles that have become a chief apprehension. Researchers all around the world have been operating on different approaches to direct this matter. Photo induced processes have been considered and many applications have been improved. One major technique for removing industrial waste is the use of light energy (electromagnetic radiation) and particles susceptible to this energy to mineralize waste which aids in its removal from solution. Titanium dioxide (TiO_2) is regarded very close to an ideal semiconductor for photocatalysis because of its high stability, low cost and safety towards both humans and the environment.

Titanium oxide (TiO_2) is obtainable in the form of nanocrystals or nanodots having a high surface area. They demonstrate magnetic properties. Titanium belongs to Block D, Period 4 while oxygen belongs to Block P, Period 2 of the periodic table. Titanium oxide is also known as flamenco, rutile, titanium dioxide and dioxotitanium. These nanoparticles are acknowledged for their ability to slow down bacterial growth and avert further configuration of cell structures. It is extensively used as UV-resistant material and in the field of producing chemical fibre, plastics, printing ink, coating, self-cleaning glass, self-cleaning ceramics, antibacterial material, air purification, sewage treatment, chemical industry, cosmetics, sunscreen cream, natural white moisture protection cream, beauty and whitening cream, morning and night cream, moistening refresher, vanishing cream, skin protecting cream, face washing milk, skin milk, powder make-up, foods packing material, coating for paper-making industry and used for improving the impressionability and opacity of the paper and used for producing titanium, ferrotitanium alloy, carbide alloy etc in the metallurgical industry, astronautics industry, conducting material, gas sensor, and moisture sensor.

As one of the assuring nanomaterial TiO_2 has have stimulated a large attention in catalysis ,coating, chemical sensing and a lot of other scientific field due to unusual properties beneficial microsized TiO_2 particulates **(**Zhang *et al;* Mallakpour *et al;*Lu *et*

al;Wu et al 2011). Unfortunately TiO_2 with a high ratio of surface area to volume be inclined to agglomerate which rigorously obstruct their application.

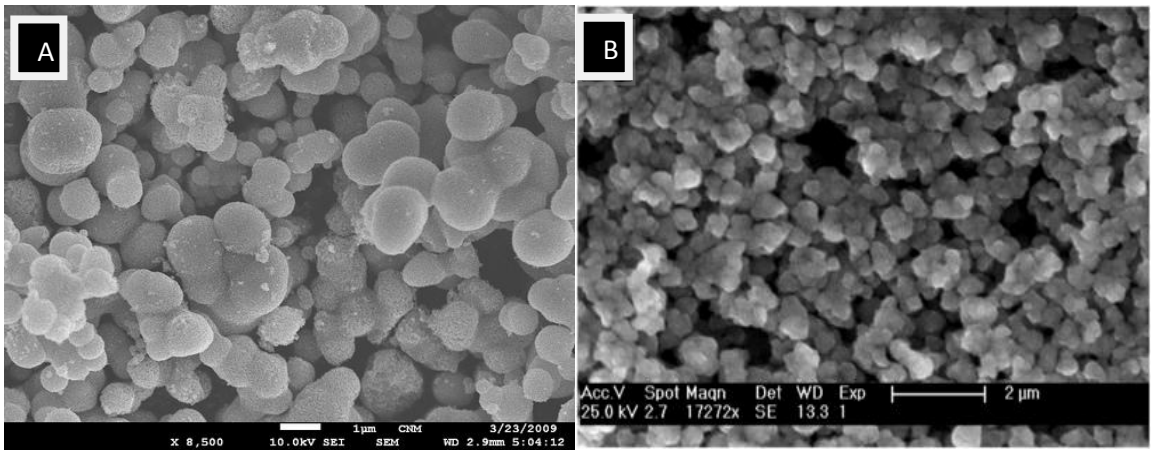


Fig3: SEM structure of TiO_2 NPs(Picture taken by A.Christopher Johnson and Vilas B. G. Pol G Ouyang, K Wang and X Y Chen)

To avoid inorganic nanoparticle with a high surface activity, researcher have prepared various methods to alleviate nanoparticle, where Langmuir-boldgett films, vesicles, and surface active support have been comprehensively determined on (Wu & Yang2000). Of numerous method for stabilizing inorganic nanoparticles, surface chemical conversion with many agents like organic molecules is particular significance , because it can be significantly used to efficiently put off agglomeration and improve the dispensability and constancy of nanoparticles in various organic solvents.TiO_2 that is the pigmentary titanium dioxide, are used by the paper industry, usually consists of semi-spherical particles having size all very close to the range 0.25 to 0.4 micrometers. Most of this is transported to paper mills in the form of aqueous slurries having solids levels near to 70%. This may seem to be an impractically high number until one considers the fact that the mineral is much denser than water, so the volume fraction is much lower than the mass fraction. Dispersants used in stabilizing the aqueous slurries can contribute a very high negative colloidal charge to the system. In addition, the surface or the bulk phase of TiO_2 products is often treated so that it contains a minor amount of such materials as aluminium, silicon, or phosphorous and such treatments also affect the colloidal charge. To enhance the opacity of paper products, the brightness of white-top linerboard and similar products, and brightness of some paper products.

2.1 Strategies for Use:

The major thing to keep in mind about titanium dioxide particles is that they intensify opacity by scattering light. Their capability to spread light relies on their being present in the sheet as detach particles, not clumps. This means that the TiO_2 product should be well disseminated before it is added. Premature mixing of titanium dioxide slurry with alum or other cationic materials should be prevented in order to minimize self-agglomeration of the pigment. Good optical competence typically can be achieved by first adding the pigment at a place where it becomes well mixed with the supply, and then adding a preservation aid. Even better optical competence can be accomplished in some cases if adequate highly charged cationic polymer is supplemented before the TiO_2 to create cationic sites on the surfaces of fibres and fines. The amount has to be optimized, since an excess of cationic polymer in the solution merely will agglomerate the TiO_2 to itself. Some of the negative features of titanium dioxide are (a) relatively high abrasiveness, and (b) absorption of ultraviolet light, reducing the effectiveness of fluorescent whitening agents. Though TiO_2 is often used to meet brightness goals, this approach tends to be expensive relative to other options such as increased bleaching and use of calcium carbonate filler, *etc.*

2.2Uses:

TiO_2 is a white pigment and due to its brightness and very high refractive index it is most extensively used. Approximately four million tons of this pigments are inspiredper annum worldwide. Also, TiO_2 accounts for 70% of the total production volume of pigments worldwide, and is in the top five NPs used in consumer products. TiO_2 can be used in paints, coatings, plastics, papers, inks, medicines, pharmaceuticals, food products, cosmetics, and toothpaste. It can also be used as a pigment to whiten skim milk.TiO_2 NPs are also used in sunscreens.

- TiO_2 has long been used as a constituent for expressing prosthetic implants, particularly for the hip and knee. These inserts sporadically fail due to deprivation of the materials in the insert or an unrelieved inflammatory response to the insert material.
- Used as an UV-resistant material, chemical fiber, plastics, printing ink, coating.

- Photocatalyst that is it exhibits as a good photo catalytic properties self-cleaning glass, self-cleaning ceramics, antiseptic compositions, antibacterial material, air purification, sewage treatment, chemical industry.
- Cosmetics, sunscreen cream, natural white moisture protection cream, beauty and whitening cream, morning and night cream, moistening refresher, vanishing cream, skin protecting cream, face washing milk, skin milk, powder make-up;
- Coating, printing ink, plastics, foods packing material like Coating for paper-making industry, used for improving the impressionability and opacity of the paper and used for producing titanium, ferrotitanium alloy, carbide alloy etc. in the metallurgical industry and also in the astronautics industry.
- Degrading organic contaminants and germs.

2.3 Applications:

TiO_2 has various applications in worldwide, some of those major applications are discussed below:

2.3.1 *TiO_2 nanopowder as special paper coating pigment*:

The progressing technologies of printing and packaging have located higher hassle on the surface of the paper sheet. To assemble the more rigid necessities, many papers are covered with appropriate pigment-rich formulation to give gloss, smoothness, color, print detail, and brilliance by filling in the void area on the surface of the paper sheet and covering the highest sitting fibers on the base paper surface (Smook 1997). The covering mixtures are highly intended water-based deformation containing, among other additives, inorganic pigments, binder thickeners and other additives. Pigment is the most important component in the coating, so pigment is commonly the most major factor affecting the properties of the coating materials (Gullichsen*et al*. 2000). Pigments are utilized as a merge of different sizes and shapes of different pigment materials. Various domain pigments with greater cost are often commenced in small quantities to optimize the coating properties (Ninness*et al*. 2003).

2.3.2 *In photo induced hydrophilic coatings and self-cleaning devices:*

The deposition of mud or dust, soot, vehicular tire out and other particulates results in

the requirement of cleaning the surfaces of buildings. The development of organisms, such as bacteria, algae and fungi mutilates the frontage of buildings and results in mechanical deteriorating and ultimate annihilation. To avoid this, buildings can be covered with a sheet of photocatalyst. Photocatalysis occurs in the occurrence of light with the energy equivalent to the band gap energy of the photocatalyst and affects the coating to chemically breakdown the organic particles adsorbed on the surface of the photocatalyst. Also, contact angle of water is enhanced, making the surface super hydrophobic which would permit muds or dusts to be washed away easily (Parkin*et al* 2005).

The common idea of the self-cleaning capacity of TiO_2 is well known and the application of TiO_2 coatings to housing for self-cleaning purposes is of considerable interest (Mills *et al 2005*). If a TiO_2 film is primed with a certain percentage of SiO_2, it obtains super hydrophilic properties after UV elucidation. In this case, electrons and holes are still formed but they react in a diverse way than normal photocatalysts. The electrons tend to decrease the Ti(IV) cations to Ti(III), and the holes oxidize the O_2- anions. In this process, oxygen atoms are evicted, causing oxygen vacancies. Water molecules can then dwell in these oxygen vacancies, creating adsorbed OH groups that tend to make the surface hydrophilic. The longer the surface is irradiated with UV light, the smaller the acquaintance angle of water becomes. After about 30 min under a UV light source of moderate intensity, the interaction angle approaches zero, meaning that water has a tendency to spread perfectly across the surface.

2.3.3 *Wastewater treatment*:

Over the last two decades, photocatalysis with TiO_2 nanoparticles has been exposed which is helpful for the deprivation of wastewater pollutants. This process has numerous advantages including total mineralization of organic pollutants like aliphatics, aromatics, polymers, dyes, surfactants, pesticides and herbicides to CO_2, water and mineral acids, no waste solids to dispose of and mild temperature and pressure conditions. Photocatalysis with TiO_2 nanoparticles uses two kinds of reaction systems, namely suspension and immobilized systems (Mahmoodi and Aram 2009.). TiO_2 powders have higher photocatalytic efficiency than a coating because of their greater precise surface area.

though, the separation of powder from the liquid state used in water management and recycling processes is upsetting because of the arrangement of aggregates, and also the deepness of diffusion of UV light is restricted due to strong absorption by both catalyst particles and dissolved organic species. These difficulties can be conquer and the application of TiO_2 nanoparticles can also be extended by utilizing various materials as substrates. Glass beads or fiberglass mesh structures may enhance the surface area available for catalysis.

2.3.4 *Degradation of pesticides*:

Pesticides (herbicides, insecticides, and fungicides) have a huge assortment of structures and have been generated to display an projected effectiveness to specific pests, fungal diseases and weeds. Pesticides are generally applied as formulations to such objectives in the field, and are related to cumulative and toxic. Their existence as contaminants in aquatic environments may create grave problems for human beings and other organisms. The use of TiO_2 for environmental cleanup of organic pollutants through photo oxidation has acknowledged a great deal interest in the past decade.

2.3.5 *Production of hydrogen fuel:*

H_2 has high prospect as a source of energy from the characteristics of environmental conservation and energy sanctuary to understand a sustainable society in the future. For this purpose, H_2 should be created from renewable resources and natural energy sources. Photocatalysis using solar energy has been extensively studied as a possible system to produce hydrogen from water ever since the Honda-Fujishima effect was first reported in 1972. The possibility of solar photo electrolysis was demonstrated for the first time with a system in which an n-type TiO_2 semiconductor electrode was associated through an electrical load to a platinum black counter electrode. When the surface of the TiO_2 electrode was irradiated with near-UV light, photocurrent flooded from the platinum counter electrode to the TiO_2 electrode through the external circuit, presenting that water can be decayed into oxygen and hydrogen without the application of an external voltage.

2.4 Model organism: The fruit fly

The fruit fly *Drosophila Melanogaster* is the well known sightseer and an organism of preference in genetics laboratories. As a link between genetics and developmental biology

are both built and traveled upon, it becomes crucial that developmental biologists study *Drosophila* to assist in the coalition of these two disciplines. Some of the important questions in developmental biology can only be responded with genetics. So we must learn about the geneticists' organisms and make them ours as well. An embryologist well-schooled in *Drosophila* development is both rare and valuable. Study of this laboratory work out as well and it could formulate some bucks in the future.

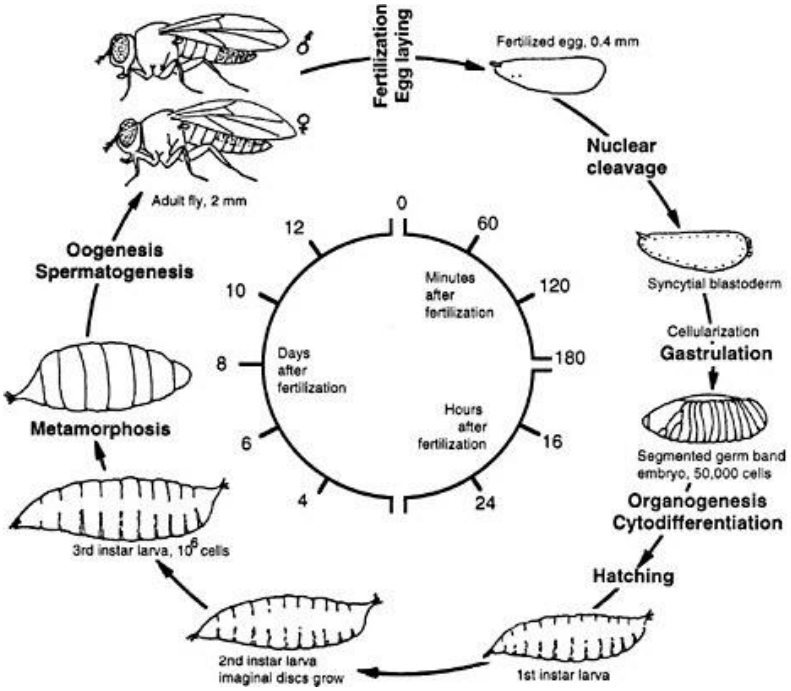


Fig4: Life cycle of *Drosophila Melanogaster*

The eggs are just about one-half mm in length, white, oval, and slightly flattened in lateral view that look much like a kernel of rice. The ovum is surrounded by an inner, very thin vitelline envelope and an outer, tough extracellular coat called a chorion. At its anterior end two small filaments, extensions of the chorion, enlarge from the dorsal surface. These are respiratory filaments and provide for gas exchange, as their name implies. Eggs are laid half-buried in rotten fruit or the medium in your culture jars, and the filaments protrude into the air. Eggs hatch in 22–24 hours at 25°C.

Eggs hatch in 22–24 hours at 25°C. The larva that emerges looks like a tiny worm and is called the first instar larva. It feeds on the substrate that the eggs were laid in and, after another 25 hours, molts into a larger wormlike form, the second instar larva. This feeds as well and, after about 24 hours, molts into the third instar larva. This is the largest of the larval forms. It feeds, but it also starts to climb upward out of its food, so that it will be in

a relatively clean and dry area to undergo pupation. The third instar molts into a pupa after 30 hours. The pupa is stationary, and in its early stages is yellowish-white. As it advances the pupa becomes gradually darker. During the pupal stage, the larva is metamorphosing into the adult fly, also called the imago. In doing so, it degrades most of the larval structures, although some larval organs are preserved. The larval nervous system, for example, is not degraded, but still it endures major restructuring; the malpighian tubules (excretory structures), fat bodies, and gonads are kept as well. Most of the adult structures, though, developed into anew from two sets of cells that have been carried as undifferentiated, mitotic cells within the larva all over its instar stages: these are the imaginal discs (imaginal since they are for the imago) and the histoblasts. Development of the Fruit Fly 8-2 Imaginal discs These are small, almost teardrop-shaped packets of epithelial cells that will form the epidermal structures of the adult, such as the wings, legs, eyes, mouthparts, and genital ducts. Imaginal discs are carried around within the larva, growing in size but not differentiating. During the pupal stage, they evert and differentiate into their adult structures. Histoblasts These cells are found in small groups (nests) within the larva. They form the abdominal epidermis and the internal organs of the adult. They, too, grow by mitosis during the larval stages and then differentiate during the pupal stage. They are recognizable within the larva as clumps of small cells nestled among the huge differentiated polytene larval cells. The pupal stage lasts for 3–4 days, after which the adult fly, or imago, emerges from the pupal case (eclosion). Adult male flies are sexually active within hours of emerging, females don't have ripe eggs until two days after eclosion, and the cycle begins again. Pause for a minute. Think about what you've just read. It's weird! What does the Drosophila do during its life cycle? It has a larval form: a fully differentiated, feeding organism, that carries around, as extra baggage, cells that will replace it—cells that will become another fully differentiated, feeding organism. The larva is only a vessel, a nurturing culture environment for these cells that become the adult. At the appointed time, the larva self-destructs as these "passenger cells" differentiate. It is an astonishing way to make an adult.

2.4.1 Drosophila gut region :

The gut is one of the largest organs in the body cavity. Aside from its central role in digesting and absorbing nutrients, the inner lining of the digestive tract also serve as the

first line of defence against a wide variety of pathogens. The gut is also a major source of neuronal and endocrine signals able to modulate nutrient storage or food intake by regulating the activity of other organs, such as the pancreas and the brain in mammals. Hence, far from being a passive tube exclusively concerned with digestion, the gut is emerging as a major regulator of multiple biological processes. As a result, what had

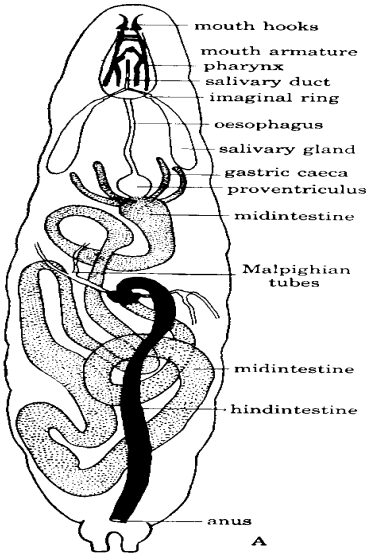


Fig5: Gut structure of *Drosophila*

historically been a relatively obscure organ is now coming to the forefront of research in areas as diverse as stem cell biology, neurobiology, metabolism, and immunity. The gut has also been a relatively understudied organ in *Drosophila Melanogaster*, given that, since the advent of the genetic revolution, this model system has played such a central role in the investigation of developmental processes. Consequently, physiological studies have typically been relegated to less genetically amenable insects, possibly because of their larger size. The potential to combine genetic and functional approaches in *Drosophila* has only been realized in recent years, and concurrent with this, there has been a surge of interest in the fly gut and its functions. In this review, we summarize our knowledge at times fragmented or sketchy of the *Drosophila* digestive tract, with an emphasis on the adult mid gut and its functional underpinnings.

The fruit fly *Drosophila Melanogaster* has the longest history of any model organism and has been widely used to study genetics and developmental biology**.** The fruit fly (*Drosophila Melanogaster*) is a small insect that feeds and breeds on spoiled fruit. It has been used as a model organism for over 100 years and thousands of scientists around the

world work on it. Part of the reason for this is historical. Scientists today choose to study the fruit fly because so many others have done so before them. There are established methods for handling flies in the laboratory and an immense volume of data has accumulated about fly biology.

As with most of the long-established model organisms, the initial choice was for practical reasons. The fruit fly is small and has a simple diet. Therefore, large numbers of flies can be maintained inexpensively in the laboratory. The life cycle is also very short, taking about two weeks, so large-scale crosses can be set up and followed through several generations in a matter of months. Fruit flies also have large polytene chromosomes, whose barcode patterns of light and dark bands allow genes to be mapped accurately.

Due to these advantages, fruit flies were extensively used in the early 20th century to work out the principles of genetics. Indeed, they are still used in this capacity to teach genetics in schools. Mutants are available for a large number of genes and new mutations can be induced very easily by exposing flies to radiation or adding mutagenic chemicals to their food. This ability to recover mutants means that flies can be used to investigate the genetic basis of any conceivable biological process. The fruit fly (*Drosophila Melanogaster*) has been used as a model organism for nearly a cent.

3. Material and Method:

3.1 *Nanoparticles* TiO_2 *used in this method*:

50mg of TiO_2 powder was weighed and taken. This powder was dissolved in 50ml of distilled water to prepared 1000ppm of solution. Sonication was done in 100% amplitude with pulse 0.6 for 10mins to maintain a homogenize solution.

3.2 *Fly husbandry:*

Canton-S(CS) is one of the most commonly used wild type strains among *Drosophila Melanogaster*. The CS stock was established by C.B.Bridges and it was chosen because of its low mutation rate. This strain of *Drosophilas* have also strong phototaxis response. Canton-S flies were obtained from the university of Calcutta, Kolkata, and are reared on standard cornmeal–yeast media .They were kept in their incubator after that the food were prepared for their amplification.Culturing *Drosophila Melanogaster*as we have undoubtedly noticed from the fruit basket that sat too long, *Drosophila* thrive on

fermenting soft fruits. A very suitable culture medium, therefore, is crushed banana. It provides all the necessary nutrients for both the larval and adult stages. The banana can be kept along with the flies in sterile pint jars with cotton or foam rubber plugs. Another standard medium, commonly used by laboratories that raise *Drosophila*, is a cornmeal yeast-agar mixture. Cornmeal-yeast-agar culture for *Drosophila*.

3.1PREPARATION OF FOOD:

3.1.1 *Steps for making the food:*

- 100 ml. of distilled water was taken and it was divided equally that is in 50:50 ratio in two beaker. One of the 50ml. distilled water was boiled slightly in a container, where 0.8gm. of agar and 4gm. Of sucrose was added and it was stirred completely.
- In another container 5gm.of corn meal, 2.5gm. of yeast and the rest 50ml.distilled water was mixed together until all lumps were removed.

Then the cornmeal-yeast mixture was added to agar-sucrose mixture and was boiled for 5 minutes. Composition of the food are given in the table below; and then it

Components	Amounts
Corn Meal	5gm
Yeast	2.5gm
Agar	0.8gm
Sucrose	4 gm
Propionic Acid	0.3µl
Nipagin	0.05gm
Ethanol	0.25ml

Table 1: Composition of FLY FOOD media (per 100ml D/W.)

was stirred vigorously..

- The mixture was cooled to 60°c. and after waiting 15 minutes. nipagin was added which was taken with ethanol followed by propionic acid.

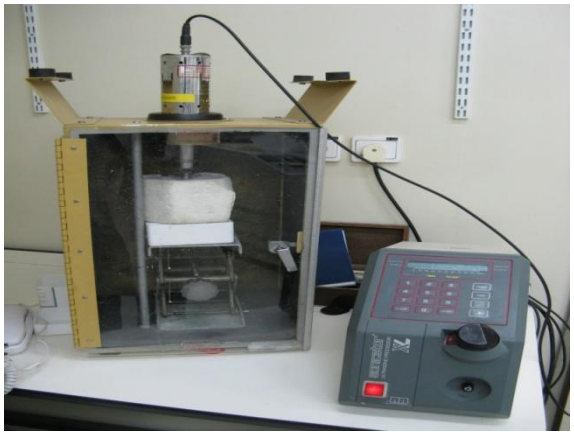


Fig6: sonication of TiO_2 using Sonicator

- 50mg. of TiO_2 NPs were taken and were mixed in 50ml. of distilled water and then was sonicated to agitate the particle and to maintain a homogenize condition. A picture(fig) of the sonicator was given that works on the basis of ultrasonic frequencies.
- 10 numbers of sterile test tubes were taken i.e. two test tubes for each concentration.TheTiO_2concentration which were taken are 50ppm ,100ppm 200ppm, 250ppm and control. In each test tube 10ml.of the prepared food was poured with the respective nanoparticle but except the control one.
- The test tubes were plugged with sterile cotton.

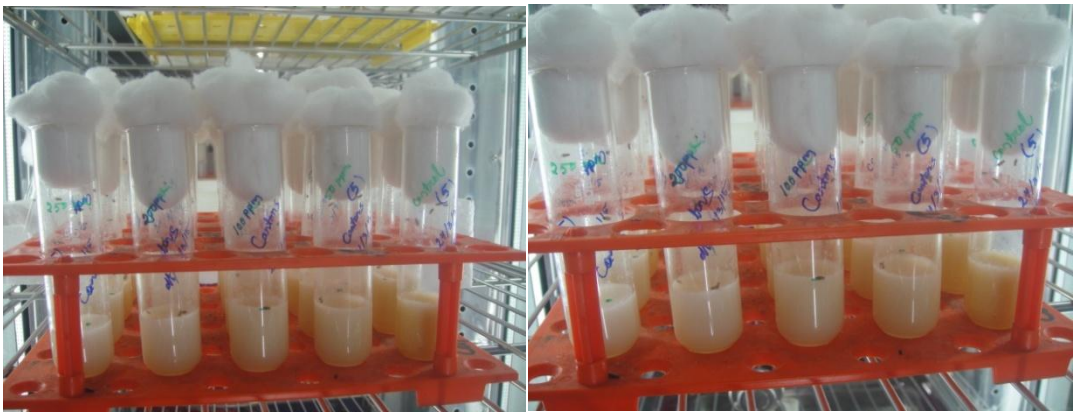


Fig7: Fly Culture in different concentration

3.1.2 *Survivorship assay*:

From the sample culture, male and female were separated. For the separation first the flies were given anasthesia by the use of diethyl ether. Then they were kept in a glass slide of a

microscope and by using the microscope they were separated .The male has a clear black spot in the posterior part while the female has not. After that the separated virgin male and female were taken for the treatment. *Drosophila* embryos were laid over 2hr time period on control medium, 50 of which were collected, from which 3 female and 2 male were separated. Separation was done by seeing them under microscope, a picture was shown below put them in each test tube starting from 50 to 250ppm respectively

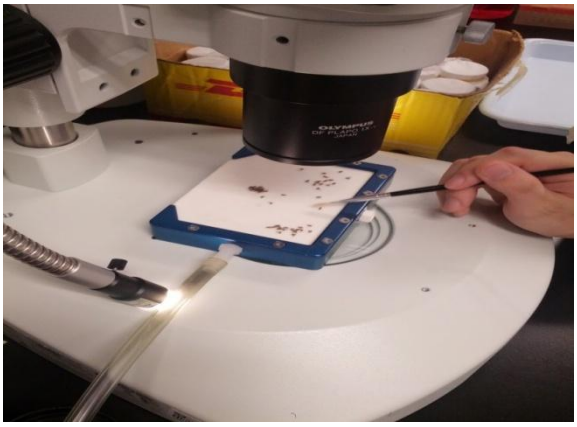


Fig8: picture showing separation of male and female flies under microscope

and a control one. The time from first larval instar hatch was also recorded. Virgin male and female flies were taken for each test tube to grow in 3:2 ratio i.e. three female and two male and then were mated. The number of successful mating (those resulting in offspring) and number of progeny produced were recorded and compared to control lines (mated pairs not exposed to TiO_2 toxicity during larval development). Then the lifecycle was checked every day for the change.

3.1.2 *Drosophila crawling behaviour*:

Larval crawling ability is used for identifying the early stage changes in the neuronal activity of *Drosophila* larvae. Such experiment is used for examining the effect of drugs on certain neurons. The larval crawling behaviour assay becomes more useful if expression or abolition of a gene causes lethality in pupal or adult stages, as these flies do not survive to adulthood where they otherwise could be assessed. This assay is also be used in conjunction with bright light or stress to examine additional behavioural responses in *Drosophila* larvae. In the locomotors Assay total distance travelled or body wall contractions was measured with time. In this experiment to check their crawling

behaviour after growing in the TiO2 media was checked. briefly, a brush was used to transport the individual larva (approximately 2-3 was taken) to a 1. 15 cm Petri dish containing 2% agarose (previously poured and allowed to harden) over graph paper with a 0.2 cm grid. it was cut in a circular manner as like as the petri plate's shape.

Then the number of grid lines were counted and the squares crossed by the flies in 2 minute and their starting point and ending point were noted**.** The crawling behaviour was often seen at the stage of third instar of the fly.

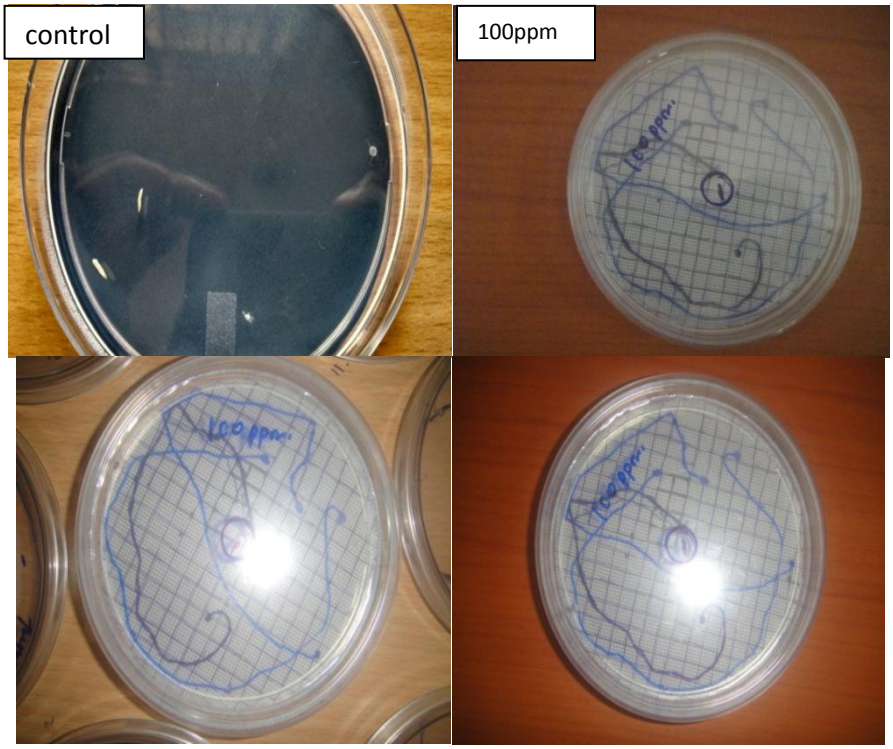


Fig9: The crawling behaviour assay of *Drosophila*(CS) in control and 100ppm

By seeing the larva's footprint which were fall in the agar plate, the marking were done to know how many squares one larvae crossed.

3.1.3 Larva fixation step:

First the larva were put into an eppendorf tube which contain the mixture of formal dehyde and PBST(PBS+Tween-20).The PBST taken was of 14ml. and 1ml.of formaldehyde to make 15ml.of solution.

- The larva were dipped in PBST for 10mins.and for three times.
- Then in distilled water for two times and for 10mins.

- After that the larva were dipped in 50% followed by 70%,90%, and 100% ethanol respectively for 10mins. each.
- Then dipped in xylene for 2mins.
- After that paraffin wax was heated and in the melting state, they were mixed with xylene in 3:1 ratio. for approximately 30mins.and were kept in thermostat.
- Then again with xylene and wax in a 1:1 ratio for 2hrs.
- Then only in wax for three times and each was given approximately 1hr,
- All those processes were done very quickly because of the paraffin wax which has the property to became solidify suddenly in room temperature.

After this the prepared paraffin was poured in a paper block which was used for giving a defined shape to the embedding medium. In these mixture 4 no. of larva were dipped keeping all their head regions into one edge of the paper block that makes easy for the dissection .Then they were kept in room temperature to let the paper block become solid. At the time of washing when the larva were kept on the eppendorf tube containing formaldehyde and PBST solution, it was observed that in 250ppm two of the larva's total body was turned to black colour. That can also caused due to some effect of the TiO_2

3.1.4 *Slide preparation for Histological analysis*:

For each conc. 10 slides were taken. They were coated by egg white that is albumin protein. Albumin has a good adhesive property .Then one by one the slides were shown on the spirit lamp for heating, by heating the slides a fume was appeared. After that the slides were ready. For the sectioning i.e. tranverse section, microtome was used. A picture of microtome was shown below. The size of the T.S. was set to 5µm. manually.

- The slides were deparaffinised using xylene for 2-3minutes..It was checked under microscope.
- Then the slides were dipped in distilled water for two times,10minutes. each.
- After that the slides were kept in haematoxylin for approximately 25minutes. Then poured in water for 10mins.for three times. If the blue stain was there then it was washed thoroughly one or more times till no blue stain was seen in the water.
- Then the slides were taken away from the water and poured in 30% ethanol and then 50% ethanol,70% ethanol, 90% ethanol and 95% ethanol respectively.

- After that they were kept in absolute ethanol for 10mins., two times. Then the slides were kept in eosine for 2mins.(if the stain is more than two months old then or less than a week then dip it for around 10mins.).

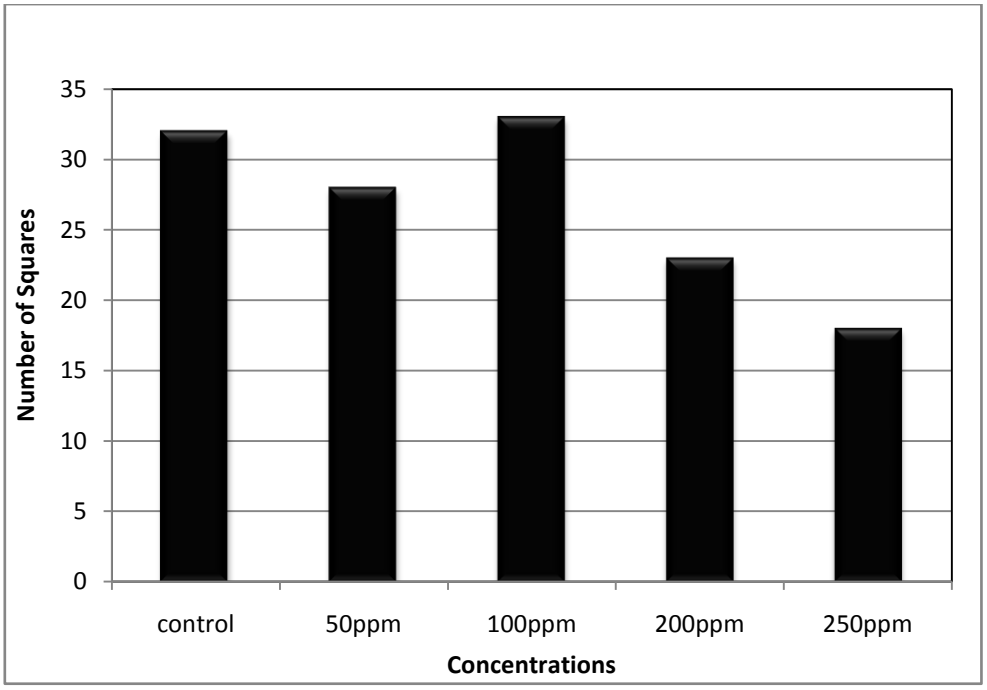
Fig.10:Structure of the Microtome

- After that the slides were washed by xylene for 1mins.for two times. Then they were kept for mounting in DPX. Lastly cover slip was put over it and it was seen under inverted microscope.

4. Result and Discussion:

4.1 *Larva crawling behaviour*:

Normal wild type larva will wander ~32 squares/2minute, and exhibit ~40-50 body wall contractions in two minutes. As we targeted the expression of wild type and mutant As shown below, wild type larvae crawl up to 32squares. In 50 ppm it was seen that the crawling is quite slow i.e. ~28 squares but in 100ppm it was very fast ~33 which is higher than the wild type. This shows that there must be a change in its neuronal activity that may affects to its behaviour and in 200 and 250ppm, the crawling is again slow.



Graph 1: Developmental event (at 25^{o}C)

4.2 *Growth and Development:*

After keeping the Canton-S in the TiO_2media,it was observed that the growth and development was influenced highly and it was finely seen during the larval stage. The delaying in the larval stage which occur after the hatching of egg to the pupal stage. In the 50 ppm the egg hatched just after one day of the control one. In 100 ppm there was delay in the beginning of 1st instar and also 2nd instar development was quite slow. but most abnormalities were seen in case of 200ppm and 250ppm. The delay was often observed at every stages of the larva. Generally it takes 1to 5 days or a maximum 6 days to grow a larva from 1st instar to the pupal stage, but in case of 200ppm and 250ppm it takes around 9-10 days for the completion of the developmental cycle. A table was shown below that describes the days of their growth and development.

Conc./ no. of days	control	50ppm	100ppm	200ppm	250ppm
Day1	Egg hatches	No egg	No egg	No egg	No egg
Day2	1st instar	Egg hatches	delay	delay	Delay
Day3	2nd instar	1st instar	Egg hatches	Egg hatches	Egg hatches
Day4	3rd instar	2nd instar	1st instar	1st instar	1st instar
Day5	Pupa forms	3rd instar	2ndinstar(very small no.)	1st instar remains	2nd instar appear(very small no. of)
Day 6	Pupa entering to adult	Pupa forms	2nd instar	2ndinstar(very small no.)	2nd instar remains
Day 7	Ready to emerge from pupa case	Pupa entering to adult	3rd instar	3rd instar appear	3rd instars appear
Day 8	adult	Ready to emerge from pupa case	Pupa forms	Pupa forms	Pupa forms

Table 2: Growth and Development of flies according to days

4.4 *Histological Analysis*:

The slides were seen on the inverted microscope and pictures were captured .The magnification of the microscope was set to 40x and pictures were taken.

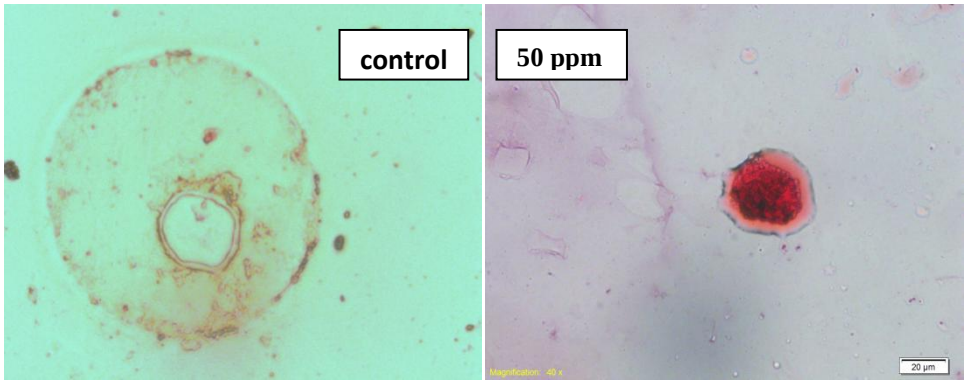


Fig11: Histology result of control and 50ppm

5. Conclusion:

As we get to know that Titanium dioxide (TiO_2) nanoparticles (NPs) are synthesized worldwide in huge quantities for accomplishing in a wide range of applications. TiO_2 NPs have many physicochemical properties on the contrary of their fine particle (FP) analogs, which might affect their bioactivity. So by taking this NPs Larval crawling ability is used for identifying the early stage changes in the neuronal activity of *Drosophila* larvae. So from this study we observed that in the higher concentration if TiO_2, there are certain changes occur at the gut of *Drosophila* which might be also responsible as a disease causing agent in the human body. So human being should also avoid those foods that contain TiO_2 in a large amount.

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