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Gibberellic Acid, A Plant Growth Regulator, Have Multitude of Effects on Invertebrates and *Saccharomyces Cerevisiae*

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Introduction

Gibberellic acids are key hormones in plant kingdom. It has major effects on the plant growth, seed growth, stem lenghth, flower development etc [1]. Gibberellic acids are also produced by some microbes [2]. These microbes produce gibberellic acids to innterfere with the growth of plant cells. However, detailed effects of this molecule on animals and yeast cell is not known.

Metabolic agents are chemical substances that have the ability for emancipating an effect on the sum total of physical and chemical changes taking place in the tissues of an organism. They bring about anabolism as well as catabolism reactions. Metabolic agents have either positive or negative effects on organisms, but some of them may have no effect at all.

Drosophila, commonly known as “fruit flies” having size around 3 mm. *Drosophila* head consists of eyes and antennae. *Drosophila* males are smaller than females, and have greater amount of black pigments at the tail end [3]. The genus contains more than 1700 species based on morphology and anatomy. *Drosophila* is commonly used in genetics research. There are several advantages with *Drosophila* such as small size, low costs of lab set up, ease of availability and culture, higher reproduction with short life cycle, and

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availability of mutant strains [3]. Studies done on embryonic development of *Drosophila* have led to the understanding of various biological processes including the basics of many genetic birth defects in higher organisms like mammals.

The genome of *Drosophila* contains more than 14000 genes. *Drosophila* eggs contain respiratory filaments at front. The larvae feed in decaying environment. The development depends upon many factors like temperature, food and numbers. At temperature higher than the required, they wil die within a short time.

MALE *DROSOPHILA* FEMALE *DROSOPHILA*

Figure 1. Physical apprearance of *Drosophila* adults.

Saccharomyces cerevisiae are eukaryotic microorganism which usually measures about 3-4 microns in diameter although some measures over 40 microns [4]. Their sizes vary according to the species. The mode of reproduction is asexual by mitosis, sexual and also by simple asymmetric division called budding. *Saccharomyces cerevisiae* are usually unicellular, even though some species of *Saccharomyces cerevisiae* may develop into multicellular form called pseudo hyphae, which is seen in most molds. *Saccharomyces cerevisiae* divides asexually as long as enough nutrients are available [4]. In sexual reproduction, two different forms of cell a haploid cell and alpha haploid cell conjugate with each other.

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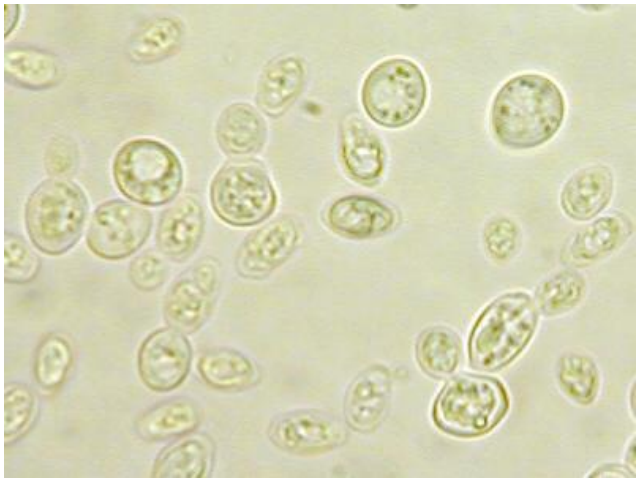

Figure 2. *Saccharomyces cerevisiae* as seen under microscope.

Saccharomyces cerevisiae is one of the eukaryotic microorganisms that have been explored thoroughly as model organism. Researchers use the information to dig into the biology of eukaryotic cells and also human system. The species of *Saccharomyces cerevisiae* is commonly used as baker's yeasts as well as in fermentation industry because of its ability to convert carbohydrates to carbon dioxide and alcohols. But there are many other species of *Saccharomyces cerevisiae* that are known to cause harmful infectious diseases in human beings e.g. *Candida albicans*. *Saccharomyces cerevisiae* is a eukaryotic microorganism that are widely and abundantly available. This microorganism has been explored by many researchers as model organism. With the basis of all these information, further researches have been done and still continuing. The current study will provide information about the effect of this hormone on *Saccharomyces cerevisiae*.

Daphnia, also known as water fleas, are zooplanktons that thrives in freshwater all over the world [5]. Their size ranges from 0.5 mm to 1cm according to species. They are usually seen by naked eyes and under microscope their internal organs as well as heart beat can be visualized because their body cover or carapace is transparent. *Daphnia* bears compound eye and a pair of antennae. Males are smaller compared to females and females have brood chamber for carrying eggs. *Daphnia* grows and matures fast and its life span is about eight weeks. *Daphnia* reproduces both by asexual and sexual methods. *Daphnia* are extremely sensitive water invertebrates. So they are often used as an indicator of water toxicity. They do not have lungs or red blood cells and thus, respiration

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is by diffusion. Also haemoglobin is extracellular and under traumatic environmental conditions like low oxygen, haemoglobin production increases. Thus they appear red due to their transparent carapace.

Drosophila is an important model organism, in which many aspects of physiology and development are similar to humans. It is a simple insect to understand the concepts that can be applied to mammals.

A study has been performed to check progressive developmental variations in *Drosophila* by the treatment of halogenated thymidine analogs. Various morphogenic lesions have been found to develop in the hatching *Drosophila* adult when 5-bromodeoxyuridine is provided to the larvae. The experiment has been done to see if 5-bromodeoxyuridine has been integrated in the DNA of *Drosophila* which would lead to induction of visible morphological modifications. In order to examine the circulation of 5-bromodeoxyuridine, radio autographic technique was used. Label was found to be distributed in the nuclei of larval tissues, imaginal disc cells and gonads. [6]

The larvae of *Drosophila hydei* was treated with gibberellic acid by injecting it and the changes brought about by the plant hormone at genetic level was observed [8]. Distinctive patterns of puffs present in giant chromosome could be seen. The giant chromosomes are present in the salivary glands. From this study it became clear that the function of genes have been altered in a specific manner and let to the formation of two different new puffs, 72B and 21B. These two new chromosomal puffs were not normal. Out of these two new puffs, either one was present in the treated larvae on the basis of the stages of larvae. Some of the normal puffs which are responsible for pupa formation was also affected. The larvae which were about to develop into pupa were injected with gibberellic acid. As a result of it, there were major decrease in the number of flies formed. [8]

Daphnia is a crustacean that is very commonly used as a model organism in the discipline of ecology, ecotoxicology as well as in evolution of aquatic organisms [5]. The study of these organisms is very crucial as *Daphnia* is susceptible to various environmental stress and this may help us understand the possible harm that might cause to other organisms due to altered environment. Moreover *Daphnia* holds a key position in the food web of aquatic habitat and any deleterious changes in *Daphnia* will directly or

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indirectly affect other life forms. Also there are many favourable factors like easy to culture, short generation time, parthenogenetic reproduction, that make *Daphnia* particularly popular for practical study.

Materials and Methods

Media Preparation for *Saccharomyces cerevisiae*:

Potato Dextrose Broth (PDB) was used as media for culturing *Saccharomyces cerevisiae*. Standard 100 ml conical flasks were used for preparing media. Media at different concentrations were prepared: 3X, 2X, 1.2X and 0.4X. At first the highest concentration media was prepared and subsequent dilutions to obtain the required concentrations were prepared using autoclaved distilled water under sterile condition.

Preparation for Plate Reading:

Gibberellic acid was dissolved in Tris buffer pH 7.5. Control was 100 µl diluted *Saccharomyces cerevisiae* culture (1ml *Saccharomyces cerevisiae* culture + 9ml media) and 100 µl Tris buffer. The dilution of *Saccharomyces cerevisiae* culture was done with respective concentrations of media (3X, 2X, 1.2X, 0.4X).

Results and Discussion:

We tested the effect of gibberellic acid on *Saccharomyces cerevisiae*, *Drosophila* and *Daphnia*.

TABLE 1: The effects of Gibberellic acid on *Drosophila* (20 mg/ml).

	Observation	3rd day	8th day	10th day	13th day	15th day	20th day	23rd day	24th day	26th day	28th day
Control	No. of larva	33	70	100	110	177	130	131	80	15	10
	No. of pupa		40	135	150	180	205	120	125	110	110
	No. of flies	5	5	50	75	90	110	50	8	6	7
Gibberellic acid	No of larva	-	8	12	7	8	-	-	-	-	-
	No. of pupa	-	-	15	18	18	18	17	16	16	16
	No. of flies	5	4	3	5	15	18	16	2	0	0

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TABLE 2: Effect of Gibberellic acid on *Daphnia* (The table shows percentage of *Daphnia* that remained alive after treatment).

	2nd day	3rd day	5th day	6th day
Control	100%	100%	100%	100%
Gibberellic acid (0.2 mg/ml)	10%	5%	0%	-

Gibberellic acid is a plant growth regulator that has deteriorating effect on *Daphnia*. In a research study it showed teratogenic effects leading to abnormality of second antenna, loss of function in the tail spine, deformities in the rostrum, malpighian tubes as well as sensory bristles. This led to the death of *Daphnia*.

These effects were generally more at lower concentrations of media, in case of yeast. Gibberellic acid has been previously reported to enhance spore formation (9). Thymidine is a nucleoside which is made up of thymine. It is commonly used in cell proliferation and the amount of thymidine integrated is directly proportional to the amount of cell multiplication.

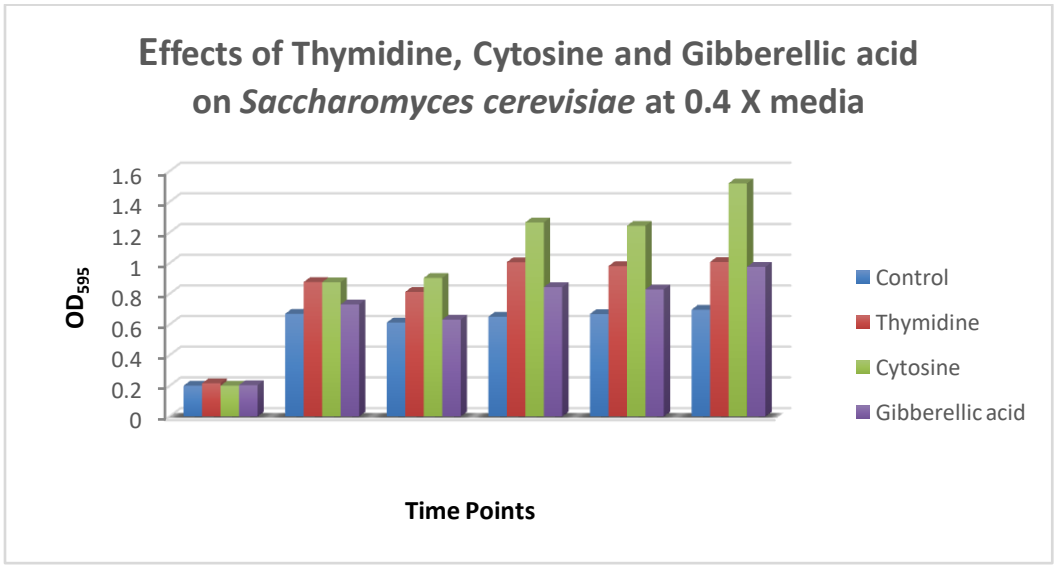

Figure 3. Effects of Thymidine, Cytosine and Gibberellic acid on *Saccharomyces cerevisiae* at 0.4 X media

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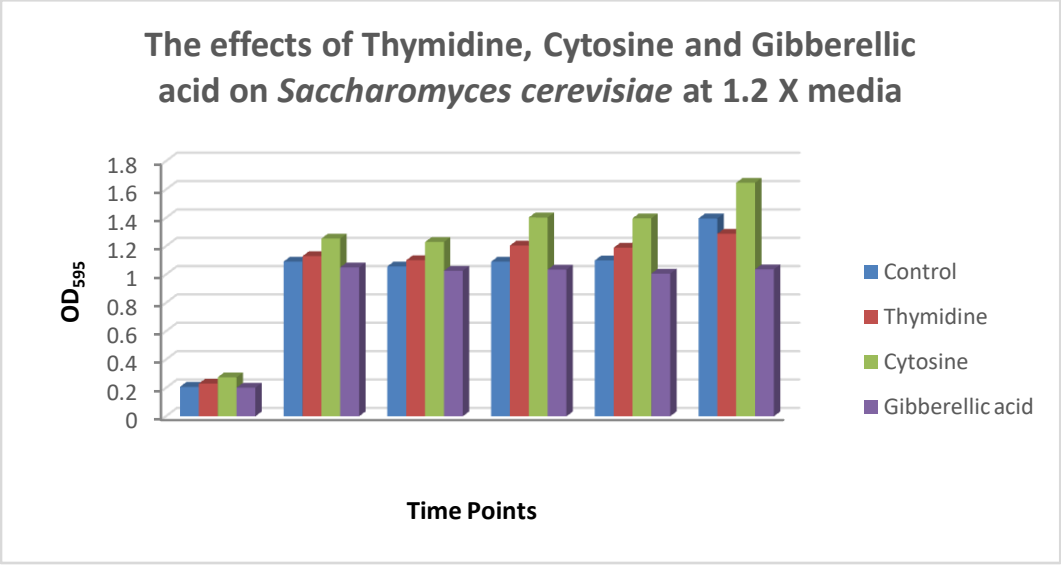

Figure 4. Effects of Thymidine, Cytosine and Gibberellic acid on *Saccharomyces cerevisiae* at 1.2 X media

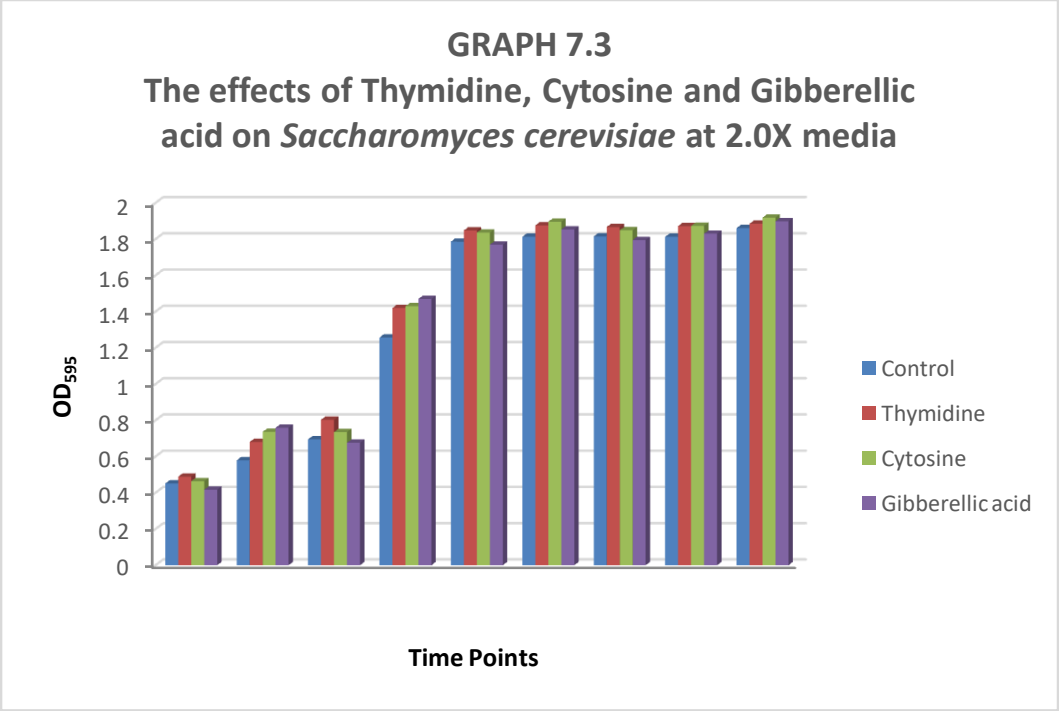

Figure 5. Effects of Thymidine, Cytosine and Gibberellic acid on *Saccharomyces cerevisiae* at 2 X media

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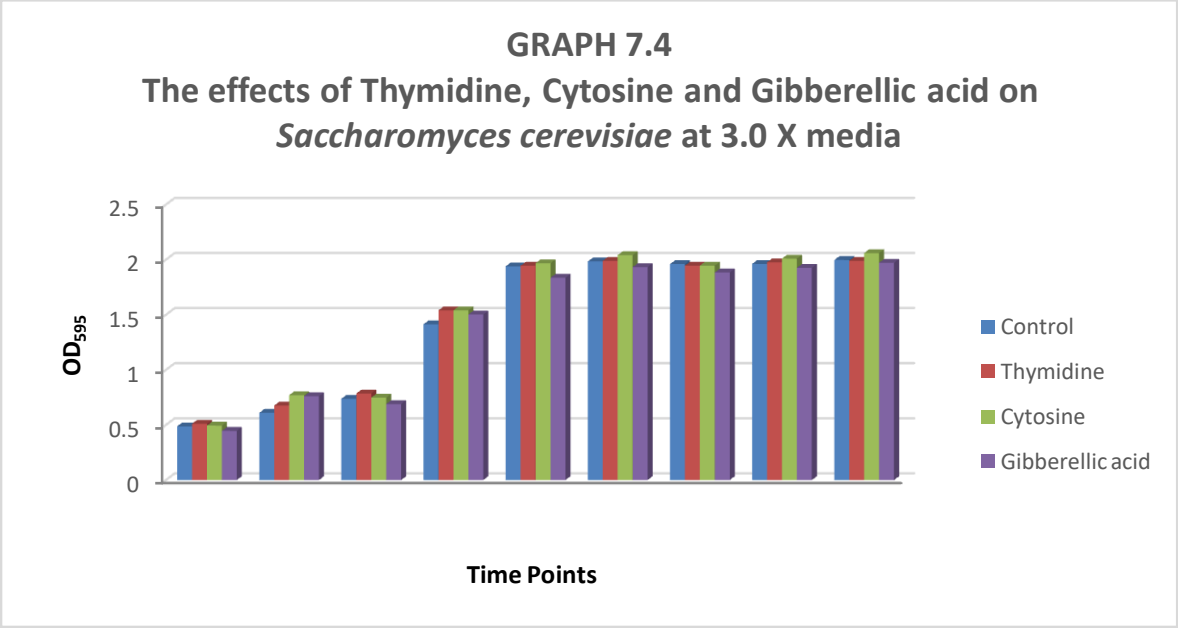

Figure 6. Effects of Thymidine, Cytosine and Gibberellic acid on *Saccharomyces cerevisiae* at 3 X media

At 0.4X media concentration, we observed significantly positive effects of thymidine, cytosine and gibberellic acid on the growth of *Saccharomyces cerevisiae* (**Figure 3**). However, higher the concentration of media, there is little or no effects of it on the growth of *Saccharomyces cerevisiae*. (**Figure 4,5,6**).

Conclusion:

The morphogenetic, developmental as well as toxicity effects of certain metabolic compounds on non-mammalians like *Saccharomyces cerevisiae, Drosophila and Daphnia* were analysed. Several distinctive features were visible upon treatment. From this study we could grasp the idea about the level of toxicity of certain metabolic compounds and correlate these findings with its effect in mammals. We observed developmental changes that were induced by some metabolic compounds in the case of *Drosophila* and *Daphnia.* The rate of reproduction, formation of larva or pupa, mortality rate in *Drosophila* were all changed in the presence of these agents.

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There are many genes which are analogous to each other between different species. So, considering lower forms of organisms as model, will give us an insight about detailed effects of these compounds. However, further investigation is needed to determine the specific effects of these metabolic compounds on mammals.

Author Contributions

DKC did all the experimental work and data compilation. GS prepared the paper.

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