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Effects Of 1-Naphthaleneacetic Acid, A Plant Hormone, On Invertebrates And Saccharomyces Cerevisiae

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ABSTRACT
In this short communication, 1-naphthaleneacetic acid (NAA), which is used as a common plant growth regulator, was tested for its effects on Drosophila, Daphnia and Saccharomyces cerevisiae. Both Drosophila and Daphnia are invertebrates, but importantly, Daphnia is aquatic. All three organisms used here are established model organisms. This plant hormone was clearly toxic for both types of invertebrate model organisms, and had growth inhibitory effect on yeast cells. In Drosophila, NAA not only caused reduction in number of larvae or pupae, but complete development was affected. Number of new Drosophila produced in next generation were very low compared to controls. Similarly, when Daphnia were cultured in presence of NAA, most of the Daphnia were dead within a week. The effects of NAA was recorded at different time points in all three organisms, and the effect was more severe at later time points, or at higher concentrations of NAA.

Key words : 1-naphthaleneacetic acid, Saccharomyces cerevisiae, Daphnia

1. INTRODUCTION

1-naphthaleneacetic acid (NAA) is a white amorphous synthetic organic compound which is commonly used as plant growth regulator [1]. NAA, which comes under auxin family of plant hormones, plays important role as rooting agent [2]. Like all other chemical compounds of auxin family, NAA too is toxic at high concentrations to plants [3]. Even at low concentrations, it is slightly toxic however, at high concentrations, it is toxic to rats as well [4]. So, there is possibility that it might be toxic to invertebrates like *Drosophila*, *Daphnia* and *Saccharomyces cerevisiae*.

Drosophila, also known as fruit flies, is around three mm in size. The genus contains more than 1500 species and each of them has differences in morphology and anatomy [5]. The genome of *Drosophila* contains more than 14000 genes [6]. *Drosophila* is an important model organism, in which many aspects of physiology and development are similar to humans [6]. It is a simple insect to understand the concepts that can be applied to higher organisms including mammals.

Daphnia, also known as water fleas, are zooplanktons that thrive in freshwater all over the world [5, 6]. Their size ranges from 0.5 mm to 1 cm 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* 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 stresses 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 indirectly affect other life forms.

Saccharomyces cerevisiae are eukaryotic microorganism which usually measures about 3-4 microns in diameter although some measures over 40 microns [7]. 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 is* one of the eukaryotic microorganisms that have been explored thoroughly as model organism [8]. Researchers use the information to dig into the biology of eukaryotic cells and also human system.

2. MATERIALS AND METHODS

Culturing Saccharomyces cerevisiae:

Potato Dextrose Broth (PDB) was used as media for culturing *Saccharomyces cerevisiae*. Media at two different concentrations 1.2 X and 0.4 X were prepared. Standard 100 ml conical flasks were used for routine culture at 27 degree C.

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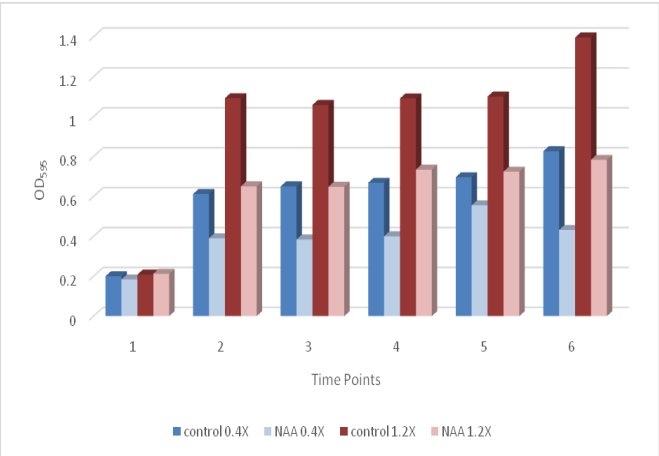
Preparation of NAA Solution for plate reading:

4 mg NAA was dissolved in 1ml tris buffer (pH 7.5). 100 µl of the prepared NAA solution was taken and mixed with 100 µl of diluted *Saccharomyces cerevisiae (*1 ml *Saccharomyces cerevisiae* culture + 9 ml PDB media) in a microplate. Thus, the final concentration of NAA was 2 mg/ml. A control was also prepared by mixing 100 µl of diluted *Saccharomyces cerevisiae* (1 ml *Saccharomyces cerevisiae* culture + 9 ml PDB media) with 100 µl tris buffer pH 7.5. The cultures were allowed to grow at 27 degree C, and optical density (at 595 nm) was recorded at different time intervals.

Culture of Drosophila:

Standard 100 ml culture tubes were used for culture and 600 ml bottles were used for raising stocks at large scales. The tubes and bottles were closed by cotton plugs, and incubated at 25°C-28°C. For maintaining as well as raising stocks, both male and female flies in the ratio of 7:3 were transferred in the tubes containing culture media. Cultures were routinely monitored for growth and development. Propionic acid was added in the media to avoid possible fungal contamination. Propionic acid acts as fungicide. Male and female flies have visible differences; the end of the abdomen is pointed in females and round in males. The abdomen of female *Drosophila* have seven segments, but male flies have only five. Similarly, males have visible sex combs, and a fringe black bristles on the distal side of basal tarsal joint of the first legs. Taking into considerations those distinctive features, male and female flies were sorted out and transferred in the culture tubes and bottles.



Handling Drosophila:
Before transferring flies in culture tubes, they were immobilized either by keeping them at -20°C for 2-5 minutes or by anaesthetising with ether. However precautions were taken not to overdo the freezing or ether treatment, as this can kill *Drosophila*.

Treating Drosophila with NAA:
To see the effect of NAA on *Drosophila*, NAA was mixed with media at different concentrations (12 mg/ml and 20 mg/ml) in different culture vial. In each vial, *Drosophila* was added in the ratio of 1 male: 4 female. Different stages of the life cycle of *Drosophila* namely were analysed.

Culturing Daphnia:
Daphnia were cultured in an aquarium or 300 ml bottles. Aged tap water was used for culture. Yeast culture or green algae were used as feed for *Daphnia*.

Results and Discussion

The effect of Naphthaleneacetic acid (NAA) on three eukaryotic model organisms, namely *Drosophila, Daphnia* and *Saccharomyces cerevisiae,* was investigated in this project. NAA clearly inhibited the growth of *Saccharomyces cerevisiae* [Figure 1]. Naphthaleneacetic acid is usually considered to be slightly toxic but at high concentrations, it is very toxic to animals. In an experiment on rats, NAA, as low as 1 mg/gm, was found to be toxic [3].

Figure 1. Effects of Naphthaleneacetic acid on *Saccharomyces cerevisiae*'s growth at 0.4 X and 1.2 X media concentrations. Naphthaleneacetic acid inhibited the growth of Saccharomyces cerevisiae.

Drosophila treated with Naphthaleneacetic acid had delayed pupa formation at both the concentrations used [Table. 1, 2]. Many new flies were emerged on the 10^{th} day in control culture tubes of both concentrations i.e. 20 mg/ml and 12 mg/ml. However NAA-treated culture tubes had 5-8 times less number of new flies. Thus, it was clear that NAA had inhibitory effect on the growth of *Drosophila.*

Table 1: Effect of Naphthaleneacetic acid (20 mg/ml) on *Drosophila*

	Observations	0th day	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	5	50	75	90	110	50	8	6	7
NAA	No. of larva	-	-	20	27	18	22	5	-	-	-	-
	No. of pupa	-	-	-	-	17	25	33	30	33	33	33
	No. of flies	5	5	5	5	2	1	30	30	5	1	-

Table 2: Effect of Naphthaleneacetic acid (12 mg/ml) on *Drosophila*

	Observations	0th day	3rd day	6th day	9th day	10th day	14th day	15th day
Control	No. of larva	-	-	22	16	5	8	10
	No. of pupa	-	-	15	18	47	50	110
	No. of flies	5	5	5	10	12	40	65
NAA	No. of larva	-	-	3	3	3	-	-
	No. of pupa	-	-	0	0	1	1	1
	No. of flies	5	2	2	2	2	3	4

Control **NAA-treated**

Figure 2. NAA-treated *Drosophila* and control.

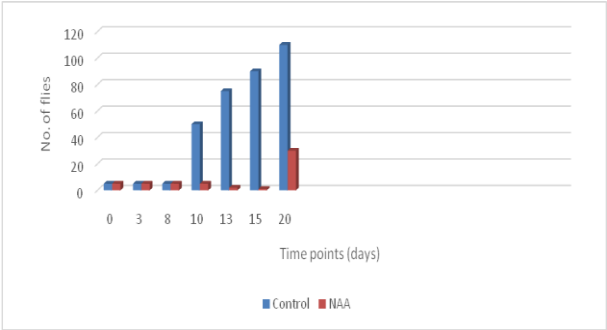


Figure 3. Comparison of number of flies between Control and NAA-treated (20 mg/ml) culture tubes.

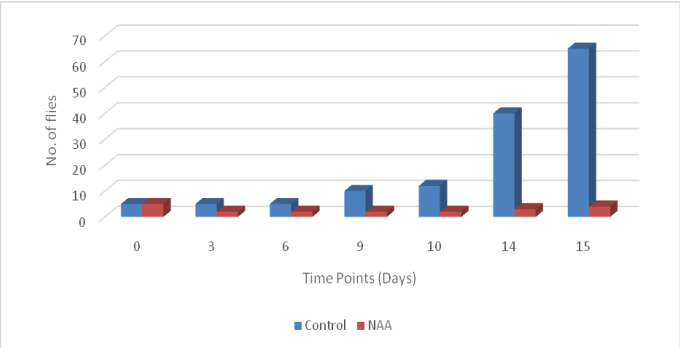


Figure 4. Comparison of numbers of flies between Control and NAA-treated (12 mg/ml) culture tubes.

TABLE 3: Effect of Naphthaleneacetic acid (NAA) on *Daphnia* (The table shows percentage of *Daphnia* that remained alive after treatment).

	0th day	2nd day	3rd day	5th day	6th day	7th day	12th day	15th day	18th day
Control	100%	100%	100%	100%	100%	Several	Several	Several	Several
Naphthaleneacetic acid (0.2 mg/ml)	100%	95%	75%	35%	5%	0%	0%	0%	0%

Naphthaleneacetic acid was also found to be toxic in *Daphnia*. Developmental as well as toxicity effects of Naphthaleneacetic acid on *Drosophila, Daphnia,* and *Saccharomyces cerevisiae* were analysed in this paper. Several distinctive features were visible upon treatment. From this study, we could grasp the idea about the level of toxicity of Naphthaleneacetic acid and correlate these findings with its effect in higher organisms including mammals.

There are many genes which are analogous to each other between different species. So, considering lower forms of organisms as model, it will provide an insight about detailed effects of this compound. However, further investigation is required to find out the specific effects of NAA on mammals.

Effects of various phytohormones are under study and it is thoroughly reviewed about the effect of phytohormones on regulation of osmolyte accumulation under abiotic stress condition [12]. Various other studies have been done in this field with successful results [13-20].

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