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Penicillin, Streptomycin and Other Antimicrobial Substances of Cynobacterial Hepatotoxins by Plant Tests

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ABSTRACT

The world-wide occurrence of harmful cyanobacterial blooms CyanoHAB in fresh and brackish waters create problems for all life forms. During CyanoHAB events, toxic cyanobacteria produce cyanotoxins at high level under stress conditions that can cause chronic and sub-chronic toxicities to animals, plants and humans. Cyanotoxicity in eukaryotes has been mainly focused on animals, but during recent years, data, related to cyanotoxin impact on both aquatic and terrestrial crop plants irrigated by water containing these toxins have become more and more available. This last cited fact is gaining importance since plants could in a direct or indirect manner contribute to cyanotoxin transfer through the food chain, and thus constitute a potent health risk source. The use of this contaminated irrigation water can also have an economical impact which appears by a reduction of the germination rate of seeds, and alteration of the quality and productivity of crop plants. The main objective of present investigations is to discuss the eventual phytotoxicity of cyanotoxin as the major agricultural impacts induced by the use of contaminated water for plant irrigation. Thus cyanobacterial phytotoxicity strongly suggests a need for the surveillance of CyanoHAB and the monitoring of water irrigation quality. Therefore it is advisable to farmers that they should not use such water bodies for irrigation without prior examination.

Keywords— *Microcystis aeruginosa*, *Vigna radiata*, Cyanobacterium, CyanoHAB, cyanotoxin, water irrigation

I. INTRODUCTION

Cyanobacteria are one of the most diverse groups of Gram-negative photosynthetic prokaryotes which frequently form cyanobacterial harmful blooms (CyanoHAB) in eutrophic water bodies. Some species of cyanobacteria are able to produce toxins which may be divided in to three main groups; hepatotoxin, neurotoxin and cyanotoxin (Codd, 2000). These toxins can cause water quality problems for fisheries, aquaculture, farming and sanitary hazards for human and animals. Therefore, survey of CaynoHAB and cyanotoxin have been carried out in several countries, avoiding sanitary risks by a safe use of cyanotoxin-contaminated water (Chorus and Bartram, 1999). However, in water irrigation, cyanotoxin surveys have not yet been considered within any water quality official monitoring programme, in spite of the possible contamination of edible plants constituting a significant indirect route of human exposure to cyanobacterial toxins (Codd, 2000). Accordingly, many studies clearly indicate that irrigation with water containing cyanotoxin can be a threat for both the quality and yield of crop plants (Crush et al. 2008, Saqrane and Odura, 2009). Ko's et al. (1995) reported for the first time that cyanotoxin inhibited the growth of mustard seedlings. Recently, the effects of cyanotoxins on seed germination and contaminated with cyanotoxin in irrigation leads to lower field productivity. In this way several terrestrial plants were investigated using both cyanobacterial standard toxin and cyanobacterial cell-free crude extract by several workers like Saqrane et al. (2008) and Crush et al. (2008). In the present investigation the attempt has been made to study the effect of cell free extract of a toxin producing cyanobacterium *Microcystis aeruginosa* on seed germination of *Vigna radiata* because it is one the most medicinally used pulse crop of this region

II. MATERIALS AND METHODS

The toxic property of *Microcystis aeruginosa* was first observed in laboratory cultures while attempting to grow seeds of *Vigna radiata*. *Microcystis aeruginosa* has been isolated in unialgal form following Bold (1942) and then purified by following Watanabe and Kiyohara (1963) method. Then following methodology have used in the present investigation:

Author's formulated culture medium

Filtered and sterilized natural water 990 ml

Soil extract-w/v 10 ml/l This *M. aeruginosa* was cultured in the culture roomat 30 C temperature with light exposure of 3200 lux for 16 hours of 24 hours cycle

Extraction of toxic substances

Author's modified medium was taken for the culture of this cyanobacterium as it was growing luxuriantly under natural conditions and the toxin extraction was done from 30 days old culture, because on this day the cyanobacterial growth was maximum followed by senescence. The investigations for the extraction of toxic substances were carried out by adopting two methods:

A. Extraction from algal cells (Ramamurthy and Krishnamurthy, 1967)

The cyanobacterium was thoroughly washed in sterilized glass distilled water and 1 ml of the packed cell volume of the selected species of cyanobacterium from fresh sub-cultures was then crushed with 2 ml of sterilized glass distilled water. A bluish green viscous fluid was there by obtained. The toxic property was tested by following Florey et al (1949) and Waksman (1952).

B. Extraction from culture filtrate

The cyanobacterial suspension along with whole culture medium was filtered through Watman's No.1 filter paper. The filtrate was collected in beaker. This filtrate was dried in oven at 30^0 C ± 5 C.The dried filtrate was mixed with 1 ml distilled water and collected in glass vial. The toxic property of this extract was also tested by the methods being described in the ensuing paragraph.

Assessment of cyanobacterial toxin

Since *Microcystis aeruginosa* was predominant cyanobacterial form in many aquatic reservoirs of Agra, hence it was selected for the study. This canobacterium was grown in culture flask in author's modified medium for 30 days and culture extract was obtained by filtration. This extract was used for the treatment of the seeds. The extract was used as such in various dilutions and proceeded as below:

1. The moong (*Vigna radiata*) seeds were soaked for 10 hours in extract of this cyanobacterium in the test tubes
2. Then the treated seeds were transferred on the blotting paper in the petridishes.
3.The observations for the seed germination were taken after every 12 hours up to 48 hours.
4. Length of radicle of the germinated seeds was measured (Allen, 1973)

III. RESULTS AND DISCUSSION

To study the effect of bloom toxicity upon seed germination the seeds of a pulse *Vigna radiata* has been selected, it was because, in case of water scarcity, farmers used pond water to irrigate their agricultural fields. After going through the observation as shown in table no-1 it became clear that with the increase of cyanobacterial cell free extract concentration the growth of radicle was found decreasing. However in higher concentration, few seeds could not germinate. These results showed the inhibitory effect of cyanotoxin upon seed germination (plate 1-2). The cell free cyanobacterial extract in different concentrations affected the germination of seed. During the course of study it was found that in the presence of cyanobacterial toxin seed germination was found delayed and further the length of radicle decreased as the concentration of cyanobacterial extract increased. Thus the effect of cyanotoxin on seed germination was dose dependent. As the cell free extract concentration increased beyond 6ml in 4ml distil water, the percentage of germination started decreasing. Further, it was noted that with the increase of cell-free extract concentration the length of radicle of germinated seed was affected. These results clearly showed the inhibitory role of cyanotoxin. It appeared that exposure to cyanobacterial.

TABLE 1:EFFECT OF CYANOBACTERIAL EXTRACT ON SEED GERMINATION

PD NO	CONCENTRATIONS	N0. OF SEEDS GERMINATED	LENGTH OF RADICLE OF GERMINATED SEEDS *(cm.)										AVE-RAGE
			1	2	3	4	5	6	7	8	9	10	
1	10 ml Distil Water(Control)	10	4.7	3.4	2.8	3.6	3.3	2.0	1.6	1.4	1.3	1.7	2.6
2	9 ml Distil Water + 1ml. cyanobacterial extract	10	3.1	3.1	3.6	4.2	2.5	1.4	1.9	2.8	1.4	1.1	2.5

3	8 ml Distil Water + 2 ml cyanobacterial extract	10	2.9	1.8	3.3	2.3	2.0	2.2	2.3	2.1	1.9	1.8	2.3
4	7 ml Distil Water + 3ml. cyanobacterial extract	10	3.4	3.2	2.3	2.8	2.1	2.0	1.3	1.7	1.4	1.7	2.2
5	6 ml Distil Water + 4ml. cyanobacterial extract	10	2.8	3.5	2.8	2.0	1.8	2.0	2.6	1.7	1.3	0.6	2.1
6	5 ml Distil Water + 5ml. cyanobacterial extract	10	2.8	2.2	2.0	1.8	2.0	1.5	2.5	2.0	0.8	1.4	1.9
7	4 ml Distil Water + 6ml. cyanobacterial extract	9	2.6	2.2	1.3	2.3	1.5	1.6	1.6	1.7	1.8	0.0	1.7
8	3 ml Distil Water + 7ml. cyanobacterial extract	9	2.5	2.4	1.3	1.0	1.6	1.3	0.8	2.5	2.5	0.0	1.6
9	2 ml Distil Water + 8ml. cyanobacterial extract	8	2.4	1.5	2.0	0.0	2.1	2.2	1.8	1.9	0.0	1.3	1.5
10	1 ml Distil Water + 9 ml cyanobacterial extract	7	2.0	0.0	2.0	1.5	1.8	2.0	0.0	1.9	1.5	0.0	1.3

Pd No. Petridish Number

*The Seeds Of *Vigna radiata*

**Test Organism *(Microcystis aeruginosa)*

aqueous extract may affect the metabolic activities of seeds during the germination process. Similar results were obtained by Chen et al. (2004) using rice and rape seeds. The inhibition of seed germination was also observed after the exposure of Alfalfa (Medicago sativa) seedling to cyanobacterial cell-free crude extract, by Pflumacher et al. (2006). All these investigations suggested that exposure to cyanotoxin via irrigation contaminated with toxins of cyanobacteria could pose a threat to the quality and yield of crop plants in the agricultural environment Therefore, it is suggested that farmers should not use the CyanoHAB containing water to irrigate their land without prior examination.

Fig1 - Photograph showing effect of cyanotoxin on seed germination

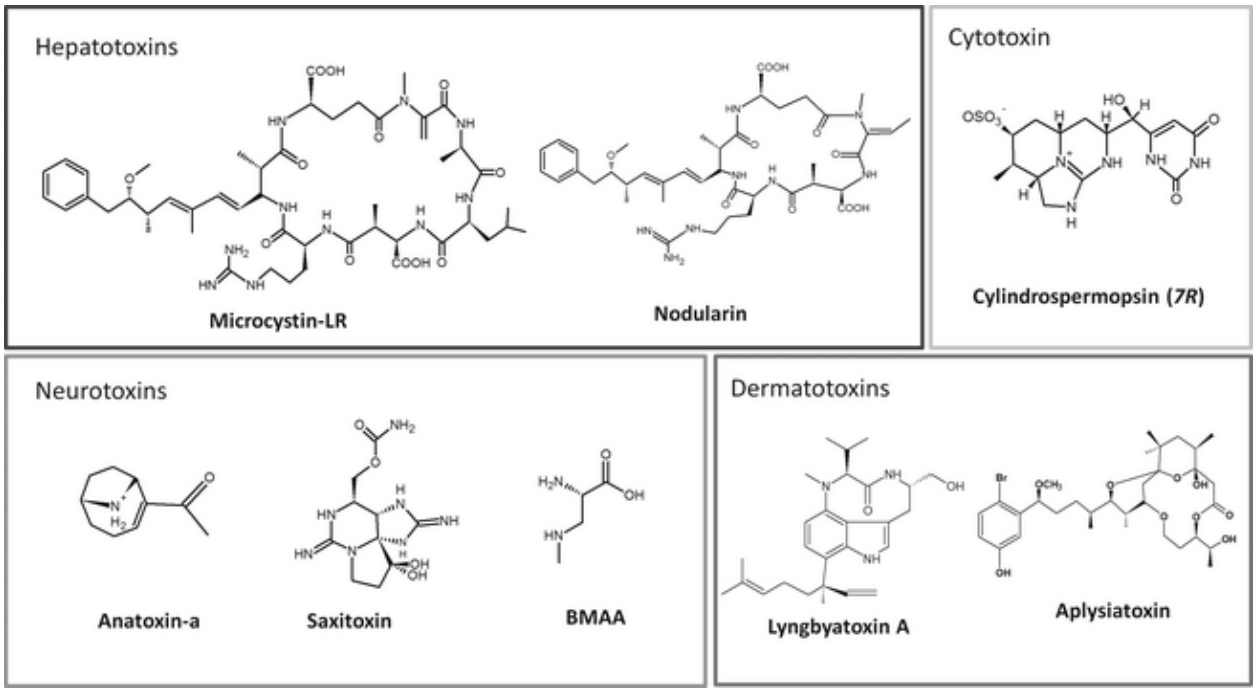
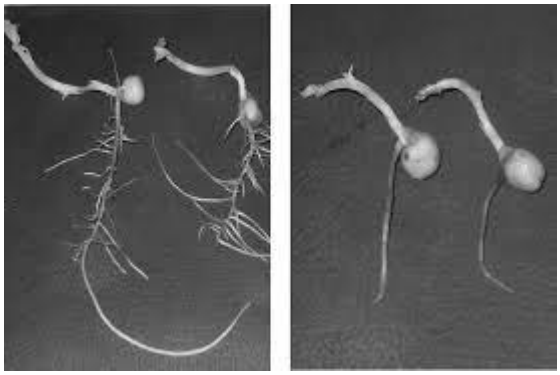


Fig2 - Photograph showing effect of cyanotoxin on seed germination



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