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The effects of *Cyanobacterium Chroococcus Minor* and *Oscillatoria amonea* on Seed Germination of Plant *Vigna radiate*

Abstract- Cyanobacteria are prokaryotic photosynthesis algae able to produce toxins and that cause problems in water quality, agriculture and aquatic organisms As well as concerns about the public health for humans. Among the cyanobacterial toxins, microcystins (MC) are the most common. In this study, Identification and Isolation of blue green algae *Chroococcus minor* and *Oscillatoria amonea* Belonging to the cyanobacteria division, BG-11 media were used to cultivation. In suitable laboratory conditions ($25 \pm 2^\circ\text{C}$, $260\mu\text{E}/\text{m}^2/\text{sec}$) for 16:8 hrs. Light: dark. The culture was harvested after 30 days and determine the effects of the extracellular of both algae on seed germination for *Vigna radiate*. The results showed reduction in root and shoot length of the exposed seedlings. Observed throughout the study that with increased concentration of cyanobacterial cell extract been found on the growth radicle and shoots decreasing. It was found the begin decomposition of roots and is not configured to lateral root at treatment (5 ml D.W + 5ml extract) recorded 4.9 cm. the highest inhibitor in seed germination it is recorded 4.7 cm at treatment 10 ml extract (100% extract), compare with control treatment as it recorded 18.8 cm (100% D.W).

Keywords- *Cyanobacterium* , *Microcystins* , *Vigna radiata* , , cyanotoxin

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1. Introduction

Cyanobacteria are photosynthetic prokaryotes that capture sunlight for energy using chlorophyll a and various accessory pigments that are common in all freshwater systems, are known to produce various kinds of secondary metabolites that can affect many biochemical processes in cells. These secondary metabolites are sometimes called allelochemicals and toxins because they can influence the growth of surrounding organisms such as bacteria, microalgae, fungi, invertebrates, and some plants [1,2]. Irrigation of edible plants with Cyanobacteria-containing water may pose threat of indirect exposure of human health to cyanotoxins via bioaccumulation of these toxins in plant tissues. Moreover, Cyanotoxins have been shown to inhibit plant growth and development [3,4,5] The use of this contaminating water changing the quality and productivity of crop plants such as onion (*Allium cepa*), lentils (*Lens esculenta* Moench), wheat (*Triticum durum* L.) *Lemna minor*, (*Mimosa pigra* L.), peas (*Pisum sativum* L.) and maize (*Zea mays* L.) [6,7] moreover, cyanotoxin transfer through the food chain through accumulates in their edible tissues [6,9]. The most common cyanobacterial toxins are microcystin-LR are dangerous hepatotoxins, which can be produced

by some strains of Cyanobacteria generally those such as *Nostoc*, *Nodularia*, *Anabaena*, *Oscillatoria*, *Aphanizomenon*, *Microcystis*, *Anabeaopsis*, {e.g. microcystin-LR} [2,10]. Its impact on the metabolism plant development and physiology of plant such as effect on plant biomass accumulation, plant growth, photosynthesis, nutrient absorption and seeds germination [5,7]. The current research focuses on microcystin compound because it is widespread, produced by many species of cyanobacteria and is hydrophobic under certain conditions [11,12]. The toxins are found during the growth stage of the bloom usually released into water (extracellular toxins) when the cells rupture or die. Extracellular toxins may be absorbed by clays, and organic materials dissolve in the water column, which is difficult to remove than the intracellular toxins [13,14]. In most cases, cyanobacterial toxins are found intracellularly (approximately 95%) in the cytoplasm and are retained within the cell [15]. The study aims effect of toxin produced by microalgae *Chroococcus minor* and *Oscillatoria amonea* on the seeds germination (radical and shoot) *vigna radiate*.

2. Material and Methods

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Chroococcus minor and *Oscillatoria amonea* was collected and isolated from gardens Mustansiriyah University. Using the method of Streak plating [16]. The algae was identified by using an optical microscope (Olympus compound) according to [17].

I. Growth condition

According to method [18] Cultures had been cultivated in 1000 ml conical flasks with 500 ml BG-11 medium, the content of the medium consist of the following composition (g/l): 150 NaNO₃, 30 K₂HPO₄, 75 MgSO₄.7H₂O, 27.81 CaCl₂.H₂O, 6 Citric acid, 6 Ferric ammonium Citrate, 1 EDTA.Na₂, 20 Na₂CO₃, 0.222 ZnSO₄.7H₂O, 11.8 MnCl₂.4H₂O, 0.0124 (NH₄)Mo₇O₂₄.4H₂O, 2.86 H₃BO₃, 0.072 CuSO₄.5H₂O, 0.048 CO (NO₃)₂.6H₂O. Culture was kept at a constant room temperature (batch culture) incubated in 25±2C° with 200μE / m² /Sec for 8:16 h dark: light. Shaken by hand two or three times a day to avoid sticking. All the media and glassware always were sterilized prior to inoculation.

II. Extraction of toxic substances

Extraction from culture filtrate was determined according to Sengar *et al.* [19]. It was growing culture under normal conditions and then screening for extracting toxins after thirty days (an old culture) means the rapid increase in growth when Reach aging.

III. Assessment of cyanobacterial toxin

Table 1: Effects of extract of *Chroococcus minor* and *Oscillatoria amonea* on germination of *Vigna radiata* seeds

Treatment	Concentrations	Total length(cm)	length of radical(cm)	length of shoot(cm)	The number lateral branches
1	10 ml Distil water (control)	18.7	4.2	14.5	12
2	9 ml D.W +1ml extract	10.8	3.9	6.9	9
3	8 ml D.W+ 2 ml xtract	9.6	5.3	4.3	7
4	7 ml D.W+ 3 ml extract	8	2.8	6.2	4
5	6 ml D.W+ 4ml extract	7.2	3.5	3.7	3
6	5ml D.W+5ml extract	4.9	2.9	2	2
7	4 ml D.W+ 6ml. extract	3	2.3	1	0.00
8	3 ml D.W+ 7ml extract	3.1	2	0.8	0.00
9	2 ml D.W+ 8ml extract	2.8	1.8	1	0.00
10	1 ml D.W+ 9 ml extract	2	1.5	1	0.00
11	10 ml extract	2	1	1	0.00

Cyanobacterium *Chroococcus minor* and *Oscillatoria amonea* was filtration after growing 30 days in BG-11 culture media. This extract (various dilutions) was used for the treatment of the seeds.

3. Material and Methods

This study revealed that extracellular of cyanobacteria *Chroococcus minor* and *Oscillatoria amonea* have toxic effects on the seed germination includes the growth, biomass accumulation, root hair formation, root tip formation and root cortical cells of *Vigna radiata* seedling cultured *in vitro* when compare with control. The studies also showed that root part is more sensitive to MCs that did shoot part this can be explained by the fact that the roots are the organ where nodulation take place, and it is the first organ to deal with this MCs stress [1]. The Highest inhibitor it is recorded 2 cm at treatment 10 ml extract (100% extract), compare with control treatment as it recorded 18.7 cm (100% D.W) (Table 1 and Figure 1). The extent of germination inhibition depends on treated concentration. It was observed that the seeds germination inhibited with increase of the extract concentration It was found through the study that the concentrations at treatment (5 ml D.W + 5ml extract) recorded 4.9 cm It was found the decomposition of roots and is not configured to lateral root (Figure 1).

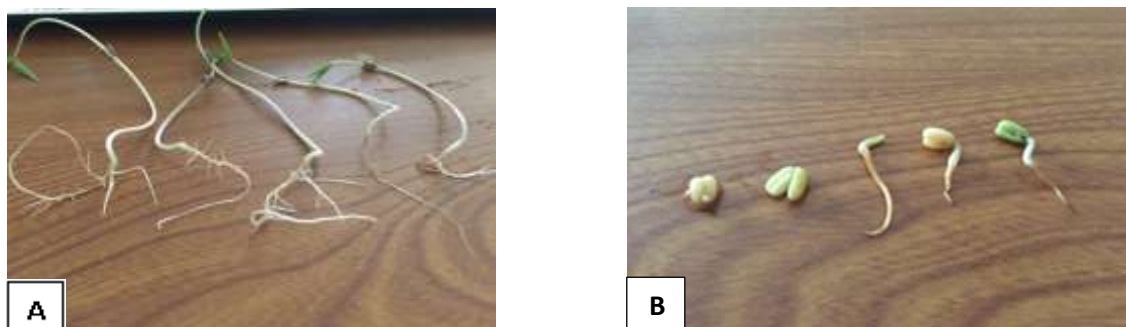


Figure 1: Effect of extract of *Chroococcus minor* and *Oscillatoria amonea* on seed germination *Vigna radiata* A)100% Distil water (control) B) 100% extract



Figure 2: Effect of extract of *Chroococcus minor* and *Oscillatoria amonea* on seed germination *vigna radiata* at treatment (5 m D.W+ 5 ml extracts)

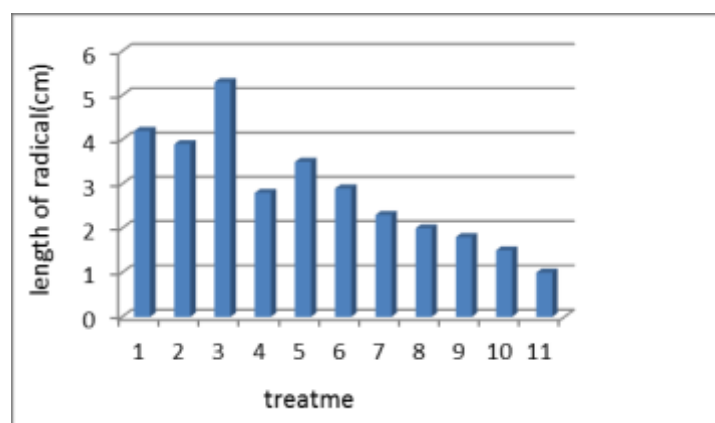


Figure 3: Effect of different concentrations of microcystins on the growth of radical of *Vigna radiate* seedling cultured *in vitro* for 48hr.

Microcystins affecting the physiology and the growth of the whole plant [21,22,7]. The authors reported the inhibition of the growth of shoot and root parts, histological alterations, brownish aspect, necrosis and tissue lysis [2]. Microcystin is a potent inhibitor of protein phosphatases in both animals and plants. Protein phosphatases are key

regulatory enzymes that catalyze de-phosphorylation of serine/threonine residues in phosphoproteins. In plants, these enzymes regulate important processes such as ion channel activity, carbon and nitrogen metabolism, tissue development and photosynthesis [22,23].

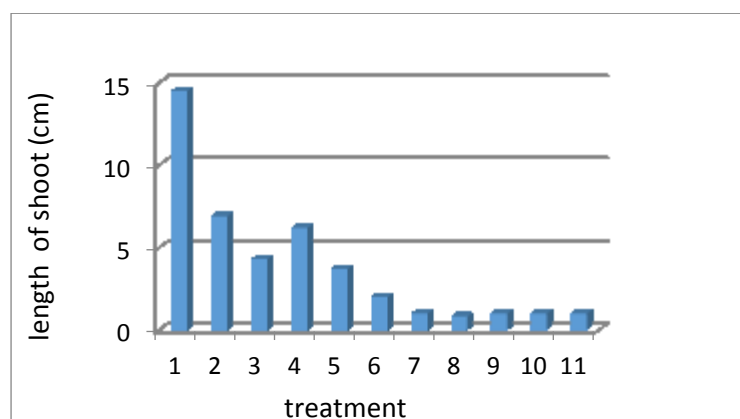


Figure 4: Effect of different concentrations of microcystins on the growth of shoot of *Vigna radiate* seedling cultured *in vitro* for 48 hr.

Seedlings can take up microcystin, resulting in inhibitory effects on development, root growth; Necrotic lesions on root are also observed and likely due to microcystin induced oxidative stress [23]. Modifying histological tissue basic root of *pisum sativum* seedlings when exposure to cyanobacteria extract containing MC-LR such as a delay in differentiating root system and inhibition of lateral root primordial formation. Most of the physiological effects associate with inhibition of protein phosphatase [24]. Percentage of seed germination was found to be 100% with distilled water control and combined treatment of *Lyngbya* sp. with groundnut shell at concentration of 12.5% and 25% respectively. While at higher concentration of combined treatment, 40-60% reduction in germination percentage was exhibited [24]. This selective growth could be ascertained to growth promoting effect of low concentration of mineral elements present in the diluted effluent [4]. In fact, toxicity the bloom depend on different factors, toxin-producing strains, and vary the intensity and diversity of species of cyanobacteria on the length of time and depends on growth rate, and the exponential phase is commonly increased production cyanotoxins [25]. MCs are inhibitors of protein phosphatase and it is affirmed by [26] that the inhibitors of protein phosphatase such as okadaic acid and calyculin-A inhibit root hair formation and alter the shape of root cortical cells of *Arabidopsis*. Similar results were found by [27] that showed histological changes of roots of *P. sativum* after exposure to 11.6 $\mu\text{g/mL}$ equiv. MC-LR. El-Sheekh *et al.* [26] observed that the compounds produced by extra *oscillatoria* sp. such as phenolic and alkaloids the effect of an inhibitor of seed germination on many processes such as photosynthesis, respiration, mineral uptake, chlorophyll and protein synthesis. Described by

Nakano *et al.* [29] the alkaloids that affect the inhibition of the growth of both mono- and dicotyledonous plants. This study concludes that the toxins posed by blue-greens local isolated an effect on seed germination and thus going to affect production of agricultural crops must be established mechanism to get rid of such toxins.

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