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Evaluation of Phytotoxic potential of Cyanobacterial extracts in Crop plants (*Zea mays* & *Oryza sativa* L.) system

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ABSTRACT
Cyanobacteria from freshwater and also from marine sources produce a wide array of toxic chemicals and secondary or bioactive metabolites. These are mainly nitrogen-rich alkaloids and peptides and are now identified as to pose threats both to human and environmental health as well as on aquatic and terrestrial plants. For the evaluation of phytotoxic effect of Cyanobacteria (*Microcystis aeruginosa*) on crop plants, *Oryza sativa* L *and Zea mays* were selected as test system, Cyanobacterial crude extracts along with Endosulfan (an insecticide), Bavistin (Fungicide), Glyphosate (Herbicide), *Lantana camara* (Phytotoxin) and *Parthenium hysterophorus* (Phytotoxin) have been assessed for the analysis of physiological behavior of both the crop plants. The result confirmed that these plants were sensitive to cell-free extracts of a toxic *Microcystis aeruginosa* and that germination inhibition was dose dependent. Present investigation showed that exposure of Cyanotoxin inhibited the growth and development of both rice and maize seedlings, however more potential in maize than rice. This may suggest as a minimum threshold limit of cyanotoxins, phytotoxins and agrochemicals that can be tolerated by the plants which can be further applied as bio-control agent.

Keywords **-** *Cyanobacteria, Exo-toxins, Cyanotoxin, Agrochemicals, Phytotoxins*

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I. INTRODUCTION

Cyanobacteria are assemblage of gram-negative eubacteria. As autotrophic prokaryotes, Cyanobacteria are common inhabitants of water logged area through and are very significant due to nitrogen fixing ability of its heterocystous forms in nature. Cyanobacteria play spectrum of remarkable roles in the field of energy production, bio-fertilizer, human food, animal feed, polysaccharides, biochemical and pharmaceuticals and in cleaning up of the environment, etc. Cyanobacteria have both beneficial and detrimental properties when judged from a human prospective.

Most of the toxic Cyanobacteria have been recognized to produce a range of hepatotoxic toxins - microcystins. Among these toxic Cyanobacteria, microcystis is the most common & cosmopolitan genus from which 35 variants of microcystins have been isolated [1]. The main producers are: *Microcystis, Oscillatoria, Anabaena, Nostoc, Synechocystis, Scytonema, Gleotrichia, Aphanizomenon, Cylindrospermum, Hapalosiphon, Schizotrix* and *Nodularia.* Cyanobacteria in the genus *Microcystis* produce an array of secondary metabolites classified as microcystins, aeruginosins, microginins, anabaenopeptins, cyanopeptolins, microviridins and cyclamides [2]. The Cyanobacterial toxins are known to affect a number of processes in plant tissues, and their presence in water used for irrigation may have considerable impact on the growth and development of crop plant. Microcystins have been detected in the tissues of exposed plants, suggesting that the uptake of these toxins by edible plants may have significant implications for human health [3]. The mutagenic role of Cyanobacterial toxins, as well as the potential for development and application of these compounds as algaecides, herbicides and insecticides, and specifically present relevant results from investigations into toxins of Cyanobacteria from the Florida, Everglades and associated water ways. Cyanobacteria are potent producers of structurally and functionally diverse natural compounds that exhibit a wide range of biological activities [4]. These compounds are known to modify growth, development of plants, including germination and early seedling growth [5]. Although rare, phytotoxic effects of Cyanotoxins on terrestrial plants have got attention of researchers, showing physiological and morphological alterations by Cyanotoxins in a range of terrestrial plants [6] [7].

Chhattisgarh has very good irrigation systems, with dams and canals on various rivers.

Average rainfall in the state is around 1400 mm and the entire state falls under Rice-agro-climatic zone. Agricultural land is 53.76%, out of which 37% field area is under paddy (*Oryza Sativa* L.) cultivation (water logged field). Chhattisgarh is the main harbor for the faster growing and toxin producing Cyanobacterial flora which we can investigate for the study of cyanotoxic behavior of Cyanobacteria. The aim of this work was to contribute to the present knowledge by studying the effects of toxic Cyanobacteria on crop plants. Keeping in view the present work has been done for the evaluation of phytotoxic potential of *Microcystis aeruginosa* comparing with other agrochemicals and phytotoxins.

II. MATERIAL AND METHOD

Selection of test material

The During present investigation for evaluation of phytotoxic potential of Cyanobacteria, the most common, frequent and prevalent strain - *Microcystis aeruginosa* was selected and cross testing with other chemicals [Glyphosate (Herbicide), Endosulfan (an insecticide), Bavistin (Fungicide), *Lantana camara* (Phytotoxin) and *Parthenium hysterophorus* (Phytotoxin)] was done in crop plant system. As the common crops of Chhattisgarh state, *Oryza sativa* and *Zea mays* were selected for the analysis of toxic effect of different treatments on physiological parmeters.

Preparation of extracts

Microcystis aeruginosa was isolated from local water resorviors and the crude extract was extracted with the help of method followed by [8] [9], with slight modification and was used directly for assessment of physiological activity of crop plants. Leaf extracts of *Lantana camara* and *Parthenium hysterophorus* were prepared with the help of method followed by the [10].

Physiological study

Healthy and uniform seeds of *Oryza sativa* L *and Zea mays* were taken and before use in experiment the surface sterilization of seeds were done with 0.1% $HgCl_2$ for 2-3 mins followed by washing four times with sterile distilled water to remove traces of $HgCl_2$. There after seeds were subjected to imbibitions to various concentration of Cyanobacterial crude extracts (50% & 100%), Glyphosate (50% & 100%), Endosulfan (50% & 100%), Bavistin (50% & 100%), *Lantana camara l*eaf extract (50% & 100%) and *Parthenium hysterophorus* leaf extract (50% & 100%), for different time duration (i.e. 24hr., 48hr. & 72hr.). The gradations of pesticides were made from their mother solution which was on the basis of the concentration used in agro-fields. The mother stock solution for Endosulfan was 2.5ml / 500ml, and for Bavistin and Glyphoste 50g / 100ml was taken. 20 seeds per treatment were taken. After the treatment, seeds of each treatment were rinsed with distilled water and allow germinating and grow in petriplates filled with moistened sand in laboratory condition. The emergence of radical was considered for germination of seeds. The seedling growth studied after 5 days of plating. Fresh and dry weights of total seedlings were measured. Root length/shoot length, Chlorophyll a-b were estimated. The data was average of three replicates and have been analyzed statistically. The following parameters have been employed and assessed.

Germination and seedling growth

Healthy seeds of *Oryza sativa* L *and Zea mays* were collected from previous season's harvest. The germinated seeds up to 5^{th} day after crude extract treatment were counted at the interval of 24 hours and daily progress in germination was recorded. Double distilled water was used in experimental practices.

Shoot length and root length

The mean shoot and root length (cm) after 10 days was observed and measurements were recorded. Each mean value of 3 observations were taken in table for analysis. For the purpose of measuring root and shoot length, outline of root and shoot after straightening them on the centimeter graph paper was drawn and the length of shoot and root was readied from the graph paper.

Fresh weight and dry weight of 10 days old seedling:

Ten plants (10 days old) from each treatment were randomly selected to evaluate the effect of treatments on the biomass production. Fresh weight of treated as well as control plants were taken and the plants were dried in an incubator at 60^{o} C for 48 hours, so that dry weight was recorded. Each plant weight was taken by digital electronic balance.

Estimation of chlorophyll content

A known amount of selected flora, leaf tissue 100 mg was suspended in 10 ml of 80% acetone mixed well and kept at 4°C over night in dark. Supernatant was withdrawn after centrifugation (5000 rpm) and absorbance was recorded at 663 and 645 nm in spectrophotometer, then amount of chlorophyll content was calculated, according to [11].

III. RESULTS

Observed results revealed that the growth of both crop plants was inhibited to some extent. All

the different treatments showed milder effect on higher plants at 50% concentration but 100% concentration showed the complete inhibitory effect and as the time duration was extended the inhibition effect was more pronounced.

Germination and seedling growth

The seedling growth studied after 5 days of plating (Table- 1) (Fig. 1) showing effect of *Microcystis aeruginosa* on *Oryza sativa* L *and Zea mays*. Crude extracts had more powerful inhibitory effect on maize then on rice as the time duration was increased. At lower concentration crude extract treatment showed enhancement in the percentage of germination whereas at higher concentration levels seed germination was adversely effected and comparatively absent at 100% concentration, as in case of *Lantana camara* leaf extract & *Parthenium hysterophorus* leaf extract.

Shoot length, Root length R/S ratio

Due to the effect of crude extract of *Microcystis aeruginosa* the root length and shoot length was decreased as the concentration was increased, in case of both the crop plants as compared to control. The root and shoot length were adversely affected by higher concentrations of Cyanobacterial crude extracts, Agrochemicals and phytochemicals. It was dose dependent. A gradual increase in the length was observed in case of Endosulfan. (Table-2) (Fig. 2). Lateral roots were not observed in any of the treated sample whereas lateral roots were observed in all the samples treated with Endosulfan. No root length and shoot length observed in case of *Lantana camara,,* as the germination was totally absent due to lethal nature of phytotoxins.

Fresh weight and dry weight

With increasing the concentration of various treatments the FW and DW was decreasing gradually as compare to control. The crude extract of *Microcystis aeruginosa* showed the complete inhibition at 100% concentration (Table-3) (Fig. 3). Exception was observed in case of Endosulfan as the Endosulfan stimulates the abnormal growth of root length and shoot. In case of Bavistin and Glyphosate the fresh weight and dry weight decreased as the concentration was increased, which showed that the effects of both the agrochemicals are dose dependent. *Lantana camara* and *P. hysterophorus* showed the inhibitory effect at both the concentrations.

Estimation of chlorophyll content

Significant dose dependent decrease in chlorophyll content was also observed, when the concentration of crude extract was raised from 50%-100%. Total chlorophyll and chlorophyll-a content decreased significantly with an increase in various treatments but chlorophyll-b was increased with increase in Endosulfan concentration. It enhances the growth of shoot with increasing in time duration from 24h to 72h. *Zea mays* treated with Cyanobacterial crude extracts of *Microcystis aeruginosa* showed the less chlorophyll-a content at 50% concentration as compare to *Oryza sativa* L. (Table-4) (Fig.4).

IV. DISCUSSION AND CONCLUSION

After 5 days of plating and incubation in the presence of Cyanobacterial extracts of *Microcystis aeruginosa*, *Zea mays* species recorded the germination rate of 0-30% and *Oryza sativa* L. showed the germination rate of 0-50% as compare to control (98-100%); simultaneously a gradual decrease in their growth parameters was observed. Crude extracts had more powerful inhibitory effect on *Zea mays* then the *Oryza sativa* L. Treatment at lower concentration of crude extracts percentage of germination showed enhancement whereas at higher concentration levels seed germination was adversely effected and comparatively absent at 100% concentration. At 50% concentration of crude extracts the germination was 30% in 24h and 10% in 48h while it was 0% in 72h treatment and at 100% concentration, 24h and 72h treatment responded 0% germination, whereas 48h treatment showed 10% germination in *Zea mays.* Effect of Bavistin showed the more inhibitory action as compare to Glyphosate, in *Zea mays*. An increase in germination value compared to control may be specific response of *Zea mays* to the treatment of Endosulfan [12]. At 50% concentration for 24h Imbibition, germination was 43% as compare to 48h (25%) and 72 h (30%) in seeds treated with Endosulfan in case of *Zea mays*. Phytotoxic *Lantana camara* showed the 0% germination in both the concentrations (50% & 100%) for both the crop plant. Such findings may suggest as a minimum threshold limit of crude extracts and pesticides that can be tolerated by the crop plants. In case of *Oryza sativa* L. the germination rate in treatment of Glyphosate and Endosulfan were found 8-30% & 0-36.5% respectively and Bavistin showed similar response like Glyphosate, whereas 0-15% germination was recorded in *Parthenium hysterophorus.*

It may be due to the disturbance of the osmotic relations of the seed and water, thus reducing the amount of absorbed water and retarding seed germination by enhanced salinity and conductivity of the solutes. Exposure of Cyanobacterial aqueous extracts may affect the metabolic activities of seeds during germination process [13]. Seeds exposed to cyanotoxins in scientific trials have been found to exhibit a lower

TABLE -1: Effect of Crude extract of (*Microcystis aeruginosa)*, Agrochemicals and Phytochemicals on Seed germination of Crops (Zea *mays & Oryza sativa* L.) by Seed treatment at different concentration (50% & 100%) at different Period (24, 48 & 72 hours), after 10 days of treatment (Mean ± SD).

Different conc. (%) of Crude Extract, Agro-chemicals & Phytochemicals.		Conc. (%)	% Germination of Crop's seed					
			Zea mays			*Oryza sativa L.*		
			24	48	72	24	48	72
Control		0	98.0 ±0.04	96.5 ±0.05	100 ±0.00	98.0 ±0.04	98.5 ±0.05	100 ±0.00
Extract	*Microcystis aeruginosa*	50	30.0 ±0.21	10.0 ±0.17	xx	50.0 ±0.22	20.0 ±0.08	13.0 ±0.01
		100	xx	10.0 ±0.61	xx	26.5 ±0.14	xx	xx
Agrochemicals	Glyphosate	50	30.0 ±0.04	20.0 ±0.09	33.0 ±0.22	25.0 ±0.12	16.5 ±0.41	30.0 ±0.63
		100	13.0 ±0.10	xx	23.0 ±0.31	8.00 ±0.50	16.5 ±0.19	32.5 ±0.21
	Endosulfan	50	43.0 ±0.11	25.0 ±0.04	30.0 ±0.51	36.5 ±0.03	25.0 ±0.52	xx
		100	20.0 ±0.21	20.0 ±0.21	xx	25.0 ±0.08	25.0 ±0.61	25.0 ±0.11
	Bavistin	50	43.0 ±0.05	30.0 ±0.51	1.5 ±0.05	38.0 ±0.08	xx	4.50 ±0.73
		100	20.0 ±0.30	23.0 ±0.11	16.5 ±0.09	15.0 ±0.21	18.0 ±0.35	8.50 ±0.37
Phytochemicals	*Lantana camara*	50	xx	xx	xx	xx	xx	Xx
		100	xx	xx	xx	xx	xx	Xx
	Parthanium hysterophorous	50	20.0 ±0.12	6.5 ±0.26	xx	15.0 ±0.61	12.0 ±0.61	Xx
		100	xx	3.00 ±0.03	xx	3.00 ±0.34	6.50 ±0.07	Xx

TABLE –2: Effect of Crude extract of (*Microcystis aeruginosa)*, Agrochemicals and Phytochemicals on Root length, Shoot length and Root/Shoot ratio of Crops (*Zea mays & Oryza sativa* L.) by Seed treatment at different period (24, 48 & 72 hours), after 10 days of treatment (Mean ± SD).

Crude Extract, Agrochemicals and Phytochemical at different hrs.			Root & Shoot length of Crop Plants (in cm.)					
			Zea mays			*Oryza sativa* L.		
			Root length	Shoot length	R / S ratio	Root length	Shoot length	R / S ratio
0 (Control)			11.36 ± 0.06	14.76 ± 0.12	0.81 ± 0.07	12.64 ± 0.09	16.56 ± 0.06	0.76 ± 0.11
Extract	*Microcystis aeruginosa*	24	7.54 ± 0.06	10.82 ± 0.06	0.70 ± 0.10	10.18 ± 0.04	13.44 ± 0.08	0.75 ± 0.16
		48	4.96 ± 0.02	6.28 ± 0.09	0.79 ± 0.20	7.08 ± 0.31	9.52 ± 0.22	0.74 ± 0.06
		72	XX	XX	XX	5.06 ± 0.02	6.64 ± 0.06	0.76 ± 0.02
Agrochemicals	Glyphosate	24	7.08 ± 0.08	11.17 ± 0.03	0.63 ± 0.07	10.6 ± 0.11	13.14 ± 0.05	0.81 ± 0.13
		48	4.10 ± 0.09	5.78 ± 0.06	0.71 ± 0.01	6..92 ± 0.06	8.82 ± 0.01	0.78 ± 0.11
		72	2.86 ± 0.07	3.78 ± 0.06	0.75± 0.05	4.94 ± 0.02	6.74 ± 0.11	0.73 ± 0.03
	Endosulfan	24	11.90 ± 0.03	14.83 ± 0.02	0.80 ± 0.25	12.72 ± 0.04	16.90 ± 0.01	0.75 ± 0.09
		48	8.96 ± 0.05	10.2 ± 0.31	0.87 ± 0.02	7.68 ± 0.06	9.52 ± 0.04	0.81 ± 0.06
		72	5.86 ± 0.03	6.72 ± 0.05	0.86 ± 0.40	5.26 ± 0.01	6.64 ± 0.06	0.79 ± 0.01
	Bavistin	24	7.96 ± 0.08	11.54 ± 0.01	0.69 ± 0.04	9.45 ± 0.19	13.18 ± 0.05	0.72 ± 0.05
		48	4.90 ± 0.06	6.82 ± 0.15	0.72 ±0.06	6.92 ± 0.11	9.52 ± 0.03	0.73 ± 0.09
		72	3.05 ± 0.04	3.86 ± 0.08	0.79 ± 0.06	5.26 ± 0.09	6.64 ± 0.04	0.64 ± 0.09
Phyto-chemicals	*Lantana camara*	24	XX	XX	XX	XX	XX	XX
		48	XX	XX	XX	XX	XX	XX
		72	XX	XX	XX	XX	XX	XX
	Parthenium hysterophorus	24	7.18 ± 0.11	9.87 ± 0.05	0.73 ± 0.08	9.48 ± 0.33	13.08 ± 0.08	0.72 ± 0.16
		48	4.22 ± 0.17	6.82 ± 0.04	0.69 ± 0.08	5..92 ± 0.07	9.42 ± 0.22	0.63 ± 0.41
		72	XX	XX	XX	3.58 ± 0.05	5.16 ± 0.05	0.69 ± 0.11

TABLE -3: Effect of different concentration of Crude extract of (*Microcystis aeruginosa)*, Agrochemicals and Phytochemicals on Fresh wt., Dry wt., FW/DW Ratio, Dry Wt.% & Moisture content of Crops - *Zea mays* & *Oryza sativa* L. (Mean ± SD).

Different conc. of Crude Extract (%), Agro-chemicals and Phytochemical.		Fresh & Dry weight of Crop Plants (in gm.)										
		Zea mays					*Oryza sativa* L.					
		Fresh Wt.	Dry Wt.	FW/DW Ratio	Dry Wt. %	Moist. Cont.	Fresh Wt.	Dry Wt.	FW/ DW Ratio	Dry Wt. %	Moist. Cont.	
0 (Control)		5.35 ±0.08	1.08 ±0.12	6.43 ±0.09	20.18 ±0.08	79.81 ±0.05	0.246 ±0.11	0.057 ±0.04	4.31 ±0.01	23.17 ±0.01	76.82 ±0.06	
Extract	*Microcystis aeruginosa*	50	3.94 ±0.08	0.83 ±0.09	4.74 ±0.02	21.06 ±0.01	78.93 ±0.01	0.157 ±0.08	0.057 ±0.09	2.75 ±0.08	36.30 ±0.06	63.69 ±0.05
		100	XX	XX	XX	XX	XX	0.127 ±0.03	0.041 ±0.02	3.09 ±0.01	32.28 ±0.02	67.72 ±0.04
Agrochemicals	Glyphosate	50	4.05 ±0.08	0.86 ±0.05	4.70 ±0.09	21.23 ±0.05	78.76 ±0.11	0.174 ±0.31	0.067 ±0.03	2.90 ±0.06	38.50 ±0.01	61.49 ±0.01
		100	2.28 ±0.08	0.57 ±0.08	4.00 ±0.06	25.00 ±0.09	75.00 ±0.04	0.119 ±0.09	0.034 ±0.01	3.50 ±0.04	28.57 ±0.04	71.42 ±0.10
	Endosulfan	50	5.19 ±0.10	1.06 ±0.07	4.89 ±0.06	20.42 ±0.01	79.57 ±0.01	0.155 ±0.09	0.042 ±0.05	3.69 ±0.04	27.09 ±0.12	72.9 ±0.04
		100	4.10 ±0.09	0.78 ±0.06	5.14 ±0.06	19.45 ±0.05	80.54 ±0.12	0.136 ±0.05	0.04 ±0.08	3.40 ±0.03	29.41 ±0.11	70.58 ±0.07
	Bavistin	50	3.99 ±0.03	0.72 ±0.22	5.54 ±0.07	18.04 ±0.05	81.95 ±0.02	0.14 ±0.11	0.04 ±0.21	3.50 ±0.04	28.57 ±0.02	71.42 ±0.01
		100	3.66 ±0.05	0.73 ±0.06	5.22 ±0.04	19.94 ±0.02	80.05 ±0.04	0.126 ±0.04	0.037 ±0.01	3.40 ±0.01	29.36 ±0.05	70.63 ±0.04
Phytochemicals	*Lantana camara*	50	XX	XX	XX	XX	XX	XX	XX	XX	XX	XX
		100	XX	XX	XX	XX	XX	XX	XX	XX	XX	XX
	Parthenium hysterophorus	50	3.79 ±0.02	0.62 ±0.04	6.06 ±0.01	16.48 ±0.05	83.51 ±0.04	0.14 ±0.05	0.047 ±0.04	3.00 ±0.09	33.33 ±0.07	66.66 ±0.05
		100	XX	XX	XX	XX	XX	0.127 ±0.08	0.037 ±0.05	3.40 ±0.05	29.13 ±0.07	70.86 ±0.01

TABLE – 4: Effect of different concentration of Crude extract of (*Microcystis aeruginosa)*, Agrochemicals and Phytochemicals (Mean ± SD) on Chlorophyll-a, Chlorophyll-b & total Chlorophyll content of Crops (*Zea mays & Oryza sativa* L.).

Different concentration (%) of Crude Extract, Agrochemicals and Phytochemical			Chlorophyll of Crop Plants (in mg.)					
			Zea mays			*Oryza sativa* L.		
			Chl. a	Chl. b	Total Chl.	Chl. a	Chl. b	Total Chl.
0 (Control)			1.286 ±0.112	0.963 ±0.068	2.243 ±0.076	0.813 ±0.068	0.796 ±0.062	1.613 ±0.086
Extract	*Microcystis aeruginosa*	50	0.887 ±0.092	0.654 ±0.048	1.602 ±0.068	0.616 ±0.121	0.465 ±0.076	1.075 ±0.076
		100	xx	xx	xx	0.472 ±0.102	0.338 ±0.049	0.812 ±0.047
Agrochemicals	Glyphosate	50	0.879 ±0.069	0.713 ±0.078	1.596 ±0.097	0.786 ±0.092	0.642 ±0.113	1.431 ±0.092
		100	0.613 ±0.067	0.546 ±0.095	1.161 ±0.089	0.689 ±0.087	0.595 ±0.124	1.279 ±0.088
	Endosulfan	50	1.293 ±0.086	0.978 ±0.074	2.271 ±0.065	0.826 ±0.087	0.792 ±0.091	1.818 ±0.066
		100	1.189 ±0.127	0.857 ±0.088	2.048 ±0.068	0.723 ±0.078	0.711 ±0.098	1.442 ±0.052
	Bavistin	50	0.868 ±0.068	0.664 ±0.088	1.532 ±0.078	0.731 ±0.090	0.612 ±0.094	1.341 ±0.086
		100	0.526 ±0.086	0.446 ±0.096	0.976 ±0.091	0.635 ±0.076	0.528 ±0.121	1.063 ±0.074
Phytochemicals	*Lantana camara*	50	xx	xx	xx	xx	xx	xx
		100	xx	xx	xx	xx	xx	xx
	Parthenium hysterophorus	50	0.568 ±0.091	0.394 ±0.082	0.966 ±0.069	0.556 ±0.087	0.387 ±0.083	0.943 ±0.065
		100	xx	xx	xx	0.362 ±0.068	0.275 ±0.098	0.637 ±0.086

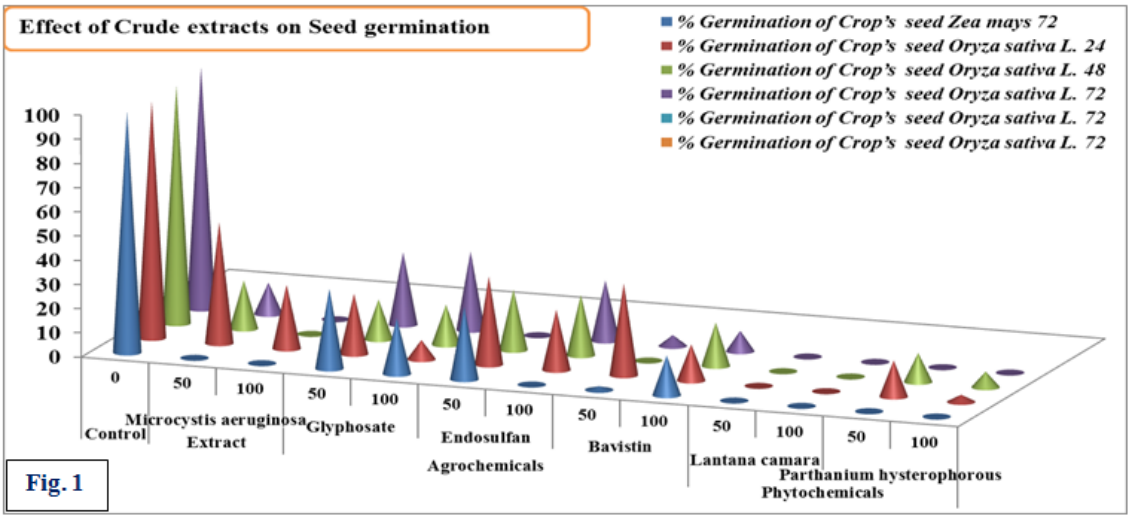


Fig. 1

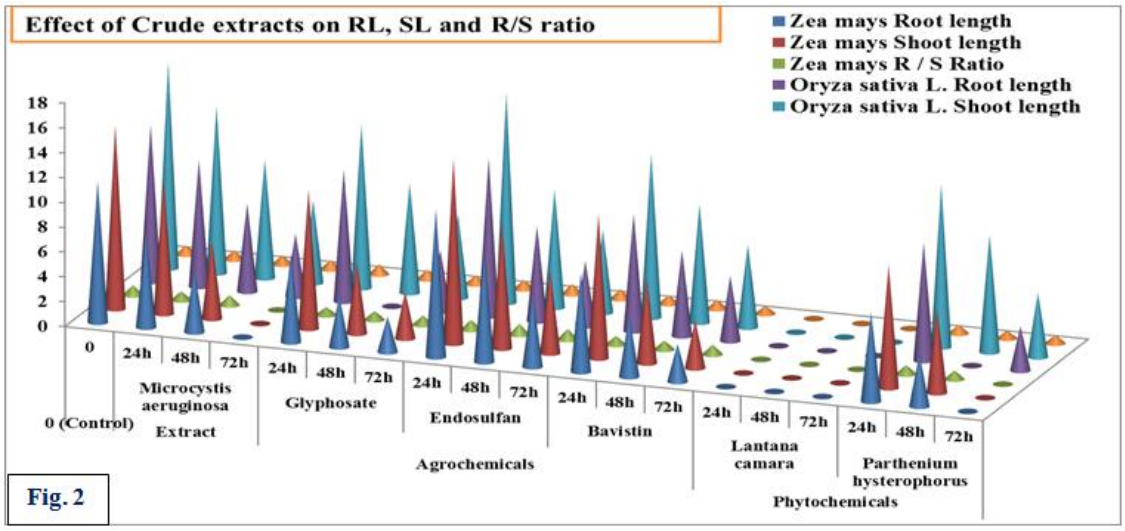


Fig. 2

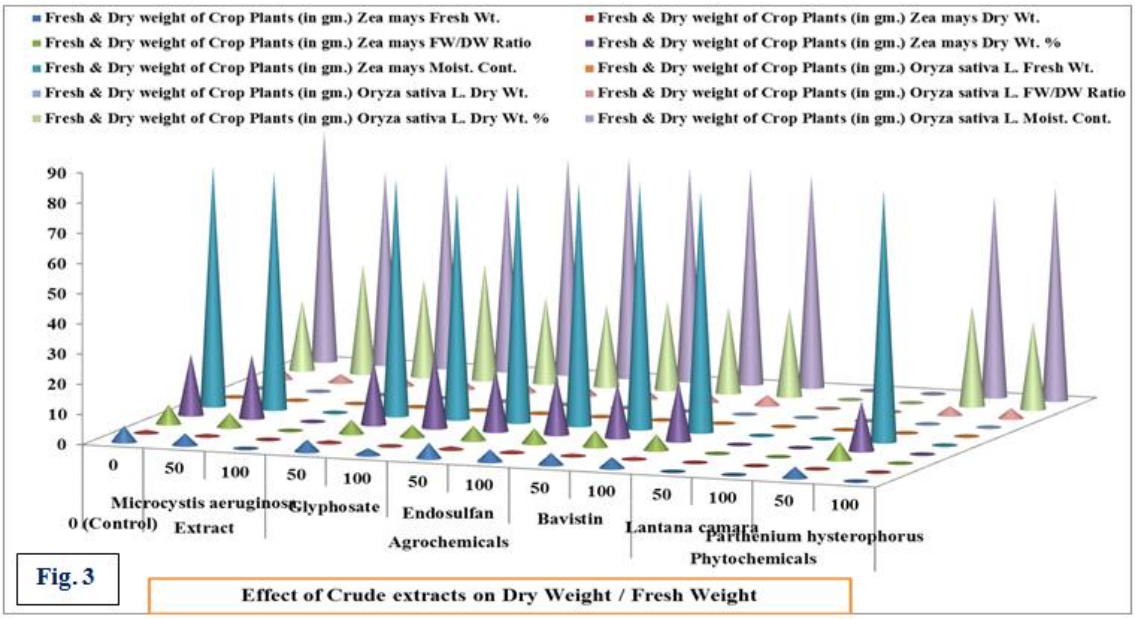
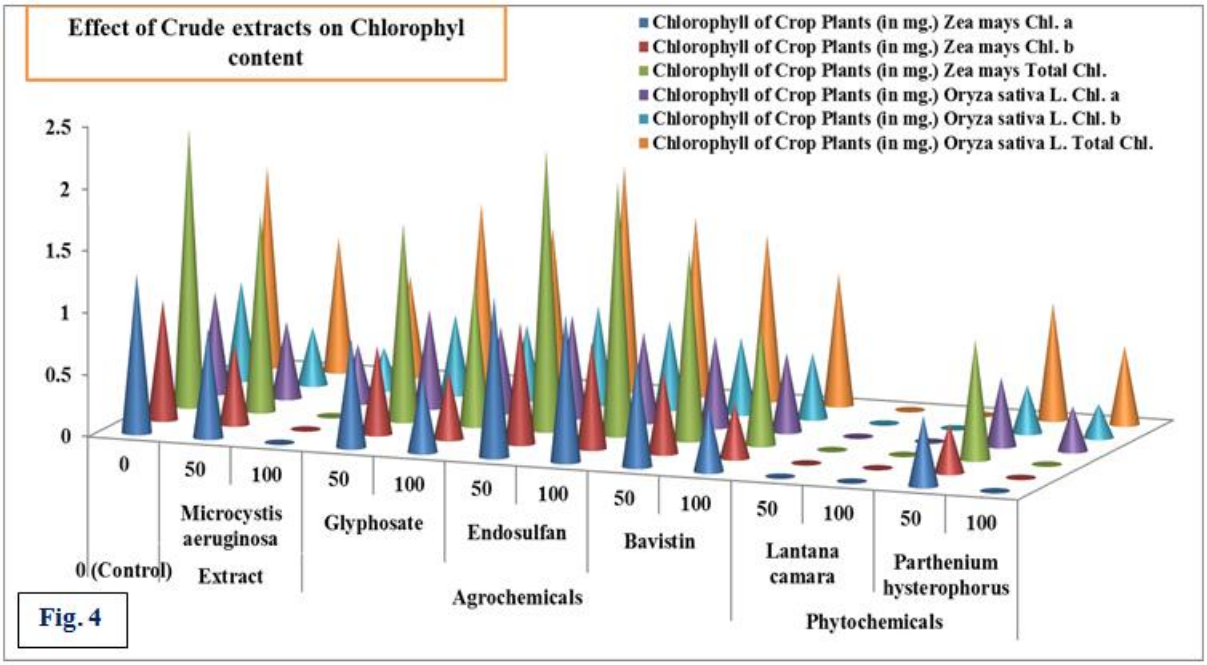


Fig. 3

Effect of Crude extracts on Dry Weight / Fresh Weight



germination rate than control units however; resistance to cyanotoxins varies with different plants, for example - rice seeds have been found to be more resistant than rape seeds [6]. *Medicago sativa* showed inhibition of germination when exposed to Cyanobacterial toxins (Microcystins and Anatoxin-a) and Cyanobacterial cell-free crude extracts [14]. Reduction of germination rate was also observed in *Lens esculenta, Zea mays, Triticum durum* and *Pisum sativum* when exposed to MC-LR [15]. Seedling growth is usually inhibited at micro molar microcystin concentrations which are seldom found in nature. The effects of Cyanobacteria aqueous extracts containing Microcystin-LR (MC-LR) on the seed germination and growth of *Pisum sativum, Lens esculenta, Zea mays* and *Triticum durum* were investigated [15].

Results of the present analysis revealed that seeds treated with extract of *Microcystis aeruginosa* showed decrease of root length and shoot length, as the concentration was increased in *Zea mays* and *Oryza sativa* L., as compared to control. The shoot and root lengths of maize and rice were measured at different stages of the growth and the higher shoot and root lengths were recorded at 50% concentration of all treatments. Root length and shoot length of the seedlings were decreased as concentration increased at different time durations for all the pesticides except Endosulfan that showed increase in root length and shoot length as the concentration increases, as compare to control. The dose dependent phenomenon was absent in case of Endosulfan and treatment of Endosulfan for 48h showed the more prominent growth. Its toxicity is much higher after 48h exposure than 24h. Shoot and root lengths of the seedling decreased as increasing concentrations of both pesticide treatments in different time durations. However, exceptionally in an individual treatment with 200 & 500 ppm Endosulfan for 6h & 12h duration showed increase in root and shoot lengths when compared to controls [16]. Mustard seedlings when subjected to crude extracts of *C. raciborskii*, the growth of roots and hypocotyls were inhibited [17]. However, in Bavistin treated seeds the RL and SL were decreased with increasing concentration at different time duration.

In case of *Lantana camara* (Phytotoxin) treatment, the germination was not observed, which indicates that the *L. camara* crude extract was more prominent or inhibitory as compare to Cyanobacterial crude extracts. Root and shoot length in *Oryza sativa* L. was found higher as compare to *Zea mays*, because the paddy fields occupied by cyanotoxin releasing Cyanobacteria. Lower optimum concentration of Cyanobacterial crude extracts can be used as biocidal agent in place of chemical based pesticides that will be harmless for crop plants. Treatment of *Parthenium hysterophorus* showed unsatisfactory result at all the three time durations as compare to control. Reduction in growth was correlated with the concentration in case of all the treatments, perhaps plants suffer from chemical stress in chemical based pesticides. Treatment of Endosulfan and Bavistin showed the abnormal increase in growth or stimulated growth with increase in concentration from 50% -100% along with time duration. Investigations showed that exposure of microcystins inhibited the growth and development of both rice and rape seedlings,

however, microcystins had more powerful inhibition effect on rape than rice in germination of seeds and seedling height. [6]. The behavior of some pesticides particularly organophosphate insecticides, herbicides and systemic fungicides in soil shows that higher concentrations require more time to degrade and there are reports to show that higher concentrations of pesticides have harmful effects on various growth parameters of plants.

Fresh weight and dry weight of total seedlings for all the treated samples showed the correlation with the growth of seedlings. Both weights were decreasing gradually with increasing the concentration of various treatments and FW/DW ratio was also decreasing when the concentration was increasing from 50% to 100% for all the treatments. Use of benlate fungicide has also been found to cause an increase in fresh and dry weights of *Sesbania sesban* at 0.25g/l concentration [12]. [18] have reported that systemic fungicides which are based on SBI (sterol biosynthesis inhibitor) are closely related to plant growth regulators the use of which at higher than labeled rates shorten the internodes which may lead to slow shoot growth. Use of high rates of systemic fungicides as seed treatments may also reduce the growth of small grains such as barley and wheat. Increase in total phenols at higher concentrations of systemic fungicide provides further insight to the reduction in growth parameters [19]. On the other hand phytotoxin in the form of phenols have been found to have an adverse affect on germination and growth parameters [12].

In-vivo efficacy of Cyanobacterial crude extracts has been performed by estimation of total chlorophyll content, during the present research work. Chlorophyll-a, a measure of phytoplankton in routine water monitoring, can be useful as a first estimation of maximum intracellular microcystin concentration [20]. Microcystins can either enter the plants through roots or remain in the leaves after spray irrigation with toxin containing water. Translocation of toxin within the plant is possible [21]. MC-LR inhibited plant growth and photosynthetic oxygen production, and bleached chlorophyll pigments [22]. The loss of chlorophyll content in treatments may be due to the interfearence in fat metabolism inhibiting root and shoot growth and root growth, photosynthesis, nutrient uptake, leaf area, biomass etc. [23]. Recently the crude extracts of five freshwater Cyanobacteria were found to have auxin-like activity on potato tissue cultures [24]. Plants are generally not killed by cyanotoxins but plant growth may be inhibited [25] and result in a yield reduction [26].

The total chlorophyll contents of the treated plants decreased significantly as the carbaryl concentration was increased. Chlorophyll-a & b were estimated that was significant dose dependent and decrease in chlorophyll content was observed as increase in concentration from 50%-100%. Total chlorophyll and chlorophyll-a content decreased significantly with an increase in various treatments but chlorophyll-b was increased with increase in Endosulfan concentration. *Zea mays* treated with Cyanobacterial crude extracts of *Microcystis aeruginosa* showed the less chlorophyll-a content at 50% concentration (0.887) as compare to control (1.28). In case of Glyphosate the chlorophyll-a (0.932 @50% and 0.768@100%) and chlorophyll-b (0.613 @50% and 0.546@100%) were decreased with increasing concentration and in case of Endosulfan chlorophyll-a content was increased with increasing concentration while Chlorophyll –b was decreased with increasing concentration. Bavistin showed the same response as showed by Glyphosate as compare to control in *Zea mays.* In all the treatments the chlorophyll-a and chlorophyll-b was reduced with increasing concentration. Crude extracts of 50% and 100% showed the decreased chlorophyll content - a and b. Glyphosate (0.786 @ 50% and 0.689 @ 100%) for chlorophyll-a, similar results were recorded for chlorophyll-b; Endosulfan (0.826 @ 50% and 0.723@ 100%) and Bavistin (0.731 @ 50% and 0.635@ 100%) were observed similar response in case of chlorophyll-a. *P. hysterophorus* showed the unsatisfactory result with all the different concentrations for Chlorophyll-a. At higher concentration all the pesticides and phytotoxins showed the inhibitory effect.

Obviously the Cyanobacteria are used, for a long time, in many parts of the world in agricultural practices, especially for Paddy fields [27]. The increase in crop yields as a result of algal inoculation can not only be attributed to nitrogen-fixing property of Cyanobacteria, but may be largely due to the growth regulating substances endogenously produced by these organisms [28] [29] [30]. This suggestion is greatly supported by the fact that non-nitrogen fixing species as *Phormidium* sp. and *Oscillatoria* sp. [31] [32] stimulated the growth of rice. It is expected that unicellular green algae or other autotrophic/diazotrophic microorganism would provide solutions for the toxic and stimulatory mechanisms involved in such effects of Cyanobacteria on crop plants.

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