



Impact of herbicide Glyphosate on metabolic activities of Cyanobacterial species

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Abstract

Nitrogen fixing cyanobacteria widely used as biofertilizers make a valuable contribution to soil fertility by fixing atmospheric nitrogen. Application of agricultural pesticides for improving crop productivity has resulted in either stimulatory or inhibitory effects on the soil microflora. This study was carried out to determine the impact of herbicide Glyphosate on the growth and nitrogen fixation by six cyanobacterial species found abundantly in the soil of farmland of Nagpur. Lower concentrations of Glyphosate showed increase in chlorophyll content as well as nitrogen fixation whereas higher concentration was inhibitory. The results showed that *Cylindrospermum indicum* was more sensitive to Glyphosate but *Aulosira fertilissima* and *Calothrix marchica* were most tolerant among the test organism.

Keywords- Cyanobacteria, Glyphosate, Chlorophyll content, Nitrogen fixation

Introduction

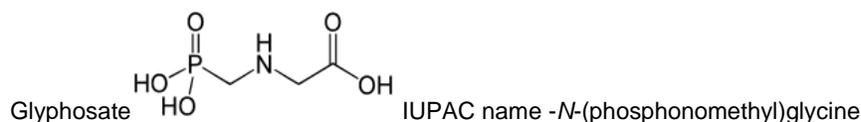
Application of chemicals for crop protection plays an integral part in maintaining sufficient food production and sustainable agriculture development. In modern agriculture weed control by herbicides is a common practice to increase crop productivity. Herbicides are frequently applied to the soil before or after plant emergence. Herbicide may adversely affect soil fertility by upsetting the components of the populations of micro-organisms that inhabit the soil. Continuous applications of herbicides are not only detrimental to undesired weeds, but also to the beneficial microbial flora of soil including cyanobacteria. Herbicides influence the density and composition of microbial populations in soil as well as the growth of individual species in pure culture. Successful exploitation of diazotrophic cyanobacteria as biofertilizer requires them to have capability to either tolerate or resist toxic actions of various herbicides.

Effect of herbicidal chemicals on nitrogen fixing blue-green algae is of great importance, as far as their survival in soil is concerned because many of them are used as a biofertilizer. Various herbicides have been known to be toxic to a large number of useful cyanobacteria thus influencing the total productivity (Singh, 1973; Kolte and Goyal, 1990; Venkataraman *et al.*, 1994). Herbicides reduce growth, heterocyst differentiation and nitrogen fixation by cyanobacteria (Kapoor and Sharma, 1980; Ahluwalia, 1988; Kaur, *et al.* 1997). Suresh Babu, *et al.* 2001, studied the effect of lindane on growth and metabolic activity of cyanobacteria. Response of glyphosate toxicity on photoautotrophic cyanobacterium *A. doliolum* and its mutant strain was investigated by Shikha, *et al.* 2004. The effects of three herbicides trifluralin, 2,4-D, linuron on the growth of 10 threatened aquatic cyanobacterial isolates were tested by Aslim and Ozturk, 2009. Two strains of paddy field isolates of free-living, diazotrophic filamentous forms of cyanobacteria (*Nostoc* sp. L. ACN 101 and *Westiellopsis* sp. L. ACW 101) were tested for their responses to glyphosate treatment under culture conditions (Balakumar and Ravi, 2001). Effect of glyphosate on *Chlorella pyrenoidosa* was studied by Hernando, *et al.* 1989. Present study is aimed to elucidate the effect of herbicide Glyphosate on six cyanobacterial species with different concentration and observed for changes in their chlorophyll content and nitrogen fixing capacity.

Material and method

Herbicide used

Glyphosate (*N*-(phosphonomethyl)glycine) is a broad-spectrum systemic herbicide used to kill weeds, especially annual broadleaf weeds and grasses known to compete with commercial crops grown around the globe.



Organism and Growth conditions

The starter cultures of cyanobacteria, *Cylindrospermum indicum*, *Nostoc commune*, *N. linckia*, *Anabaena variabilis*, *Aulosira fertilissima* and *Calothrix marchica* were obtained from the National Facility for Blue Green Algae Collection Centre, IARI, New Delhi. The unialgal cultures of nitrogen fixing organisms were routinely grown in BG-11 medium (Rippaka, *et.al.* 1979). Cultures were incubated for the growth in an air-conditioned culture room maintained at 25±2° C fitted with cool day fluorescent light with alternate light and dark (16: 8) duration. Experiments were carried out in 250 ml Erlenmeyer flasks containing 100ml culture medium inoculated with 5 ml of homogenous suspension of all species.

Determination of Growth and Nitrogen fixation

At the end of 30 days, chlorophyll and nitrogen content of the cultures were determined. Growth was measured in terms of chlorophyll content (McKinney, 1941) and total nitrogen fixation by conventional microkjeldhal method (Jackson, 1973). Appropriate control systems containing no pesticides were included in each experiment. Control and treated cultures were grown under the same temperature and light intensity as mentioned above. All experiments were performed in triplicate and the average values were presented.

Statistical Analysis

All experiments were performed in triplicates. Data presented in this study are presented in means ± standard deviation (SD).

Result and Discussion

Effect of Glyphosate on Chlorophyll content of test organism

The nitrogen fixing cultures of various species exhibited a various reaction with increase in the concentration of Glyphosate. The growth of various species treated with different concentration of Glyphosate is given in Table 1. *Cylindrospermum indicum* shows 45.62% and 20.26% increase in chlorophyll content at 100 ppm and 200 ppm concentration over control respectively whereas at 400 ppm concentration it declined by 57.46%. Glyphosate treatment stimulated chlorophyll content of *Nostoc commune* by 39.5% and 7.22% at 100ppm and 200 ppm respectively and markedly inhibited the chlorophyll by 29.58 % at 400ppm. *Nostoc linckia* shows similar effect of Glyphosate as 23.00% and 8.33% increase in chlorophyll content at 100 ppm and 200ppm respectively while 23.93% decrease at 400 ppm of Glyphosate. *Anabaena variabilis* on treatment at 100ppm concentration shows 22.92 % increase over control while at 200ppm and 400ppm the growth was inhibited by 6.51% and 32.86% respectively. Glyphosate treatment at various concentration caused stimulation in chlorophyll content of *Aulosira fertilissima* and *Calothrix marchica* after 30 days of treatment. 28.48%, 26.93% and 9.62% increase in growth was observed in *Aulosira fertilissima* at 100ppm, 200ppm and 400ppm concentration respectively. In *Calothrix marchica* 41.21%, 35.52% and 11.82% increase over control was observed at 100 ppm, 200 ppm and 400 ppm concentration respectively.

Effect of Glyphosate on Nitrogen fixation of test organism

At lower concentration of Glyphosate i.e. 100 ppm all the test organisms show stimulatory effect for nitrogen fixation. *Cylindrospermum indicum* on treatment at 100 ppm concentration shows 52.44% increase in nitrogen over control, whereas at 200ppm and 400 ppm concentration it drastically decreased and resulted into 4.44% and 33.33% decrease over control respectively. Nitrogen fixation was increased in *Nostoc commune* at 100ppm concentration by

54.39% and further increase in concentration inhibited nitrogen fixation by 1.67% and 55.23 % at 200 ppm and 400ppm respectively. Similarly in *Nostoc linckia* at 100 ppm increase of 54.34 % was observed and at 200 ppm and 400 ppm it drastically decreased by 7.9% and 52.89% respectively. Glyphosate treatment stimulated nitrogen at 100 ppm and 200 ppm in *Anabaena variabilis* by 65.58% and 4.6% and at 400 ppm it inhibited by 51.16%. Stimulatory effect was observed in *Aulosira fertilissima* at 100ppm and 200 ppm by 44.10% and 33.14% and at 400 ppm inhibited nitration fixation by 7.58% over control. In *Calothrix marchica* the Glyphosate at 100ppm and 200ppm shows stimulatory effect on nitrogen fixation by 49.36 % and 37.02 % over control respectively and at 400 ppm it decreased by 4.43%. From the result in Table 1 and 2 it shows that growth and nitrogen fixation efficiency appears to be independent of each other in these algae with reference to the external stress of Glyphosate. Similar observation were made by Pande and Goyal, 1982; Kaushik and Venkataraman, 1983; Goyal *et.al.*1991. Glyphosate was better tolerated by test algae at lower concentration. Glyphosate proved to be significant in *N.commune*, *N.linckia*, *Aulosira fertilissima* and *Calothrix marchica*. While higher concentration of Glyphosate disturbed the biological activity of test organism. The stimulatory effect at the low concentration of Glyphosate attributed to the direct effect exerted by utilization of either chemical itself or its degradatory products (Goyal, *et.al.*, 1991). Okmen *et.al.* 2013 similarly concluded that initial concentration stimulated chl-a, β -carotene, phycocyanin and allophycocyanin content, but increasing herbicide concentration suppressed all of the pigment content in *Anabeana* sp.

Table 1. Effect of Glyphosate on chlorophyll content of different cyanobacterial species. (mg/l)

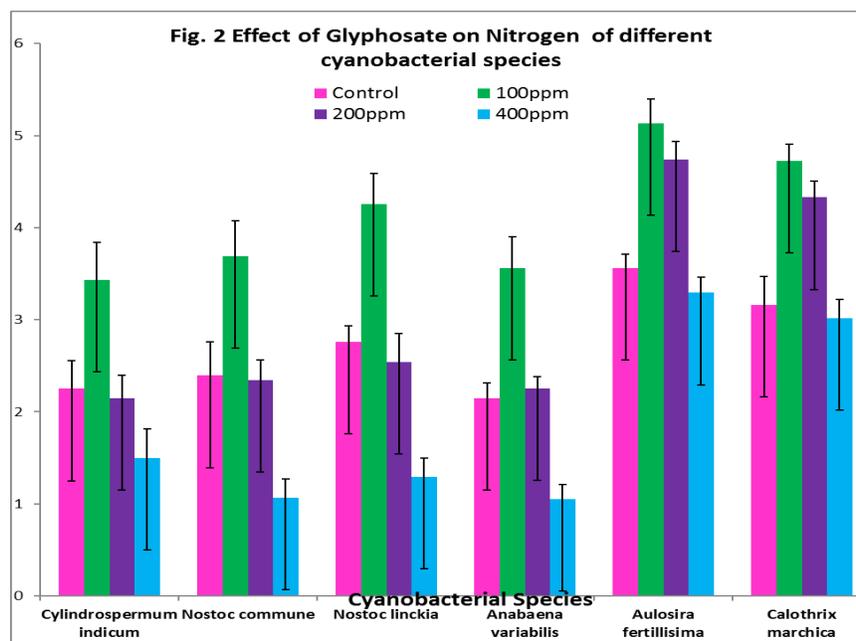
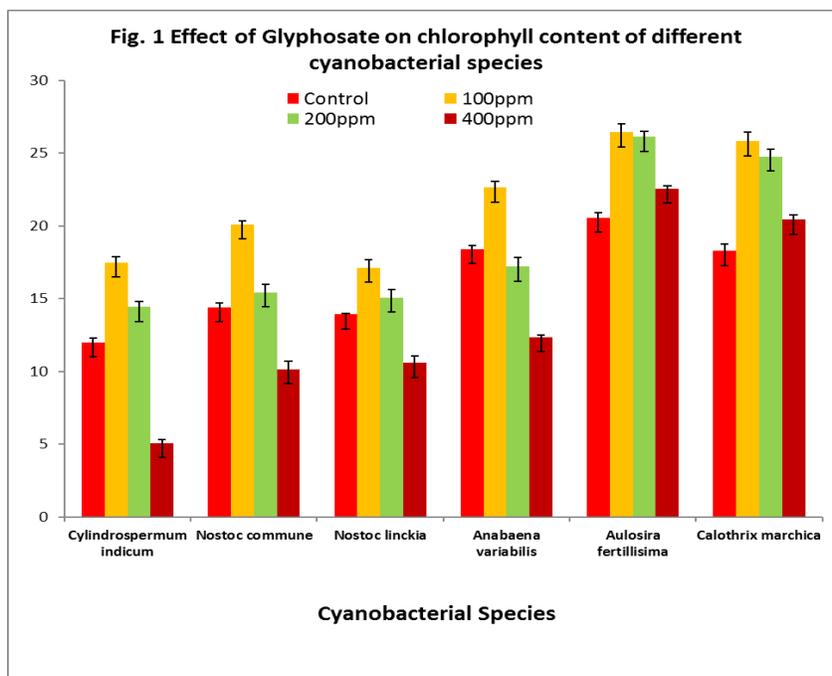
	Cyanobacterial Species	Control	100ppm	200ppm	400ppm
1.	<i>Cylindrospermum indicum</i>	11.99±0.30	17.46±0.43 (+45.62)	14.42±0.41 (+20.26)	5.1±0.24 (-57.46)
2.	<i>Nostoc commune</i>	14.40±0.29	20.09±0.25(+39.5)	15.44±0.54 (+7.22)	10.14±0.54 (-29.58)
3.	<i>Nostoc linckia</i>	13.91±0.08	17.11±0.58 (+23.00)	15.07±0.57 (+8.33)	10.58±0.49 (-23.93)
4.	<i>Anabaena variabilis</i>	18.41±0.24	22.63±0.40 (+22.92)	17.21±0.64 (-6.51)	12.36±0.14 (-32.86)
5.	<i>Aulosira fertilissima</i>	20.57±0.35	26.43±0.59 (+28.48)	26.11±0.38 (+26.93)	22.55±0.21 (+9.62)
6.	<i>Calothrix marchica</i>	18.27±0.50	25.80±0.62 (+41.21)	24.76±0.48 (+35.52)	20.43±0.32 (+11.82)

Values in parenthesis is percentage increase or decrease over control

Table 2. Effect of Glyphosate on Nitrogen of different cyanobacterial species.

	Cyanobacterial Species	Control	100ppm	200ppm	400ppm
1.	<i>Cylindrospermum indicum</i>	2.25±0.30	3.43±0.41 (+52.44)	2.15±0.25 (-4.44)	1.5±0.32 (-33.33)
2.	<i>Nostoc commune</i>	2.39±0.36	3.69±0.38 (54.39)	2.35±0.21 (-1.67)	1.07±0.20 (-55.23)
3.	<i>Nostoc linckia</i>	2.76±0.18	4.26±0.33 (+54.34)	2.54±0.31 (-7.9)	1.30±0.20 (-52.89)
4.	<i>Anabaena variabilis</i>	2.15±0.17	3.56±0.34 (+65.58)	2.25±0.13 (+4.6)	1.05±0.16 (-51.16)
5.	<i>Aulosira fertilissima</i>	3.56±0.15	5.13±0.27 (+44.10)	4.74±0.20 (+33.14)	3.29±0.17 (-7.58)
6.	<i>Calothrix marchica</i>	3.16±0.31	4.72±0.18 (+49.36)	4.33±0.18 (+37.02)	3.02±0.20 (-4.43)

Values in parantheses is percentage increase or decrease over control



A gradual reduction in the total nitrogen fixed by algae was observed with the increase in concentration of various herbicides (Roychaudhary and Kaushik, 1986 and Goyal, *et. al* 1991). The toxic effects of DDT and its metabolites are dose related and lower concentration could not measurable affect soil algae (Megharaj *et.al*, 1999). Venkateswarlu, 1993 in his studies on the influence of individual herbicides, fungicides and insecticides on different cyanobacteria showed that herbicides influenced morphology and also photosynthesis by affecting electron transport. Issa, 1999 reported that Glyphosate and its formulation inhibited both growth and oxygen evolution in two soil algae *Oscillatoria angustissima* and *Calothrix parietina* and concluded that the target site for Glyphosate is in the pathway

of aromatic amino acid biosynthesis. The variation in the relative sensitivity of the strain to the growth toxic effects of the herbicides tested under laboratory conditions seems to result from interactions between mode of herbicidal actions with morphological, physiological, biochemical and genetic properties of cyanobacteria. Herbicides have differential effects on various metabolic processes and the sensitivity of the strain varies depending upon the species, kind of herbicides and chemical formulation (Powell, *et.al*, 1991, Anand and Subramanian, 1997, Fairchild, *et.al*, 1998). Surendra Singh 1990 suggested that herbicide mainly inhibits the nitrogen fixation by inhibiting CO₂ assimilation, photosynthetic electron flow, ATP and reductant supply to heterocysts in cyanobacteria.

Chlorophyll a content of both the wild type and mutant strain in the presence of glyphosate (N-phosphonomethyl glycine) initially showed an increasing trend when supplemented with Pi and a declining tendency under the Pi-starved condition in cyanobacterium *Anabaena doliolum* (Shikha and Singh, 2004). Aslim and Ozturk, 2009 study indicated that as the concentrations of the herbicides were increased, significant changes were recorded in cyanobacterial growth rates. Glyphosate had two different effects upon photosynthetic pigments: inhibition of chlorophyll synthesis and a decrease in carotenoids. Oxygen uptake was not affected, but oxygen evolution was strongly inhibited. Hernando, *et.al.*, 1989 suggest that glyphosate acts as an electron transport inhibitor, acting on both photosystems, but its effect was greater on PS II than PS I. Nirmal Kumar, *et.al.*, 2012 observed that *Aulosira fertilissima* was the most susceptible organism to insecticide and fungicides than *Westiellopsis prolifica* and *Anabaena fertilissima*. Balakumar and Ravi, 2001 concluded from their studies that between the two cyanobacteria, *Westiellopsis* has a more efficient machinery to degrade glyphosate compared to *Nostoc*. It can be concluded from the results observed in the present investigation that among the six cyanobacterial species tested *Aulosira fertilissima* and *Calothrix marchica* exhibited better response to Glyphosate whereas *Cylindrospermum indicum* was sensitive.

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