Natural tolerance of some rice field diazotrophic cyanobacteria on exposure to herbicide Bispyribac-sodium

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Abstract: As cyanobacteria are beneficial for rice crops as as biofertilizer,it becomes necessary to study the effect of different herbicides commonly used on the growth and survivability of these microorganisms.we studied effect of herbicide bispyribac sodium(commonly known as Nomnigold) on four selected strains namely Anabaena circinalis, Scytonema hofmanni, Aulosira prolifica and Tolypothrix fragilis isolated from soil samples obtained from rice fields of four sites of Kuthilia farm,Rewa.Maximum deleterious effect was found at 300ppm of herbicide Nomnigold but at 75ppm decrement rate of survived colonies of Anabaena and Scytonema reduced and showed regeneration in Aulosira and Tolypothrix which also affected their chlorophyll and Nitrogen contents were obtained maximum (0.475mg/l and 0.3984mg/l Chl'a') and(10.27mg/l and 11.74 mg/l N2) at 75 ppm which is minimum dose of Nomnigold (22.5-30g/Ha) found suitable for crops as well as for the cyanobacteria present.

Keywords: Biofertilizer,Nomnigold or Bis pyribac sodium,cyanobacteria, Aulosira prolifica and Tolypothrix fragilis,Herbicide,Regeneration

Introduction

Cyanobacteria has dual capacity of nitrogen fixation and carbon assimilation which makes them useful as biofertilizer which help in promotion of plant growth and enhanced soil fertility.

Worldwide modern suistainable rice cultivation involves extensive use of agrochemicals such as insecticides,fungicides but especially herbicides which are used to kill or stunt weeds growth providing the desired crop a competitive advantage but their harmful effect on useful microorganisms like cyanobacteria is often ignored.

Thus before recommending a herbicide for a particular weed it is generally ensured that the prescribed dose does not have any negative effect on the desired crop and the microorganisms.

The herbicide Nomniee commonly known as bispyribac sodium is a post emergence herbicide has been reported to increase rice yield by selectively eliminating weeds from rice fields,[3],[14],[15].

Bispyribac-sodium, sodium 2.6- bis [(4, 6-dimethoxy-2- pyrimidinyl) oxy] benzoate, which was first developed by Japan Kumiai Chemical, belongs to the pyrimidinyl oxybenzoic acid group [22].This herbicide is ALS(acetolactate synthase)inhibitor and thus block synthesis of branched chain aminoacids which lead to decrease in protein synthesis and cessation of growth.Interaction between cyanobacteria and herbicides with different modes of action on physiological and cellular traits of several cyanobacteria has been indicated in reports of [2],[5],[6],[10],[11],[12],[16],[17],[19].Generally ,cyanobacteria are quite sensitive to herbicidesbecause they store many physiological features of higher plants,which form the site of herbicide action[21].Thus a comparative study has been designed to observe natural tolerance of selective cyanobacteria strains on exposure to different doses of herbicide Nomnigold by examining their nitrogen and chlorophyll content after 30 days treatment.

Material and Methods

The filamentous ,heterocystous cyanobacteria used in this study were ***Anabaena circinalis, Scytonema hofmanni, Aulosira prolifica*** **and** ***Tolypothrix fragilis*** were isolated from soil samples obtained from four sites of Kuthilia farm,Rewa.Chu 10 media was used for isolation of nitrogen fixing bacteria.Isolation and purification were performed by dilution and plating of soil samples .temperature was maintained at 25-28ºC under white light of 800 lux.cells in the logarithmic phase of growth were

collected from stock cultures and used as inocula in the experiment.

Four replicates of four petriplates were prepared. One set of petriplates was used as control and the other three sets of petriplates used for three different concentrations of herbicide Nomnigold at 75 ppm, 150 ppm and 300 ppm, for each strain of cyanobacteria stated above.After treatment with herbicide Nomnigold,the cultures were again incubated up to30 days. after which decrease in number of colonies by the effect of herbicide is observed each day ,until all the colonies are killed or start regenerating.The decrement rate of survived colonies of the isolates were calculated by the following method:Number of the colonies on the herbicide treated plates/number of colonies on the untreated plates*100. The difference in number of colonies survived at different concentrations of herbicide is determined stastically by **Bartellets method ANOVA (Analysis of variance)** and represented graphically by plotting graph of no. of colonies survived against herbicide concentration.After which Chlorophyll'a' and nitrogen content of herbicide treated strains of cyanobacteria also estimated after 30 days of incubation according to chlorophyll 'a' estimation methods suggested by ISO [8], Chorus and Bartram[7] and nitrogen estimation by Ci Xueyan[20] and ISO[9].

Results

In this study four cyanobacteria isolates viz, *Anabaena circinalis*, *Scytonema hofmanni*, *Aulosira prolifica* and *Tolypothrix fragilis* have been studied and the effect of different concentrations of bispyribac sodium on cholorophyll and nitrogen activity of cyanobacteria after 30 days.cyanobacterial isolates showed gradual inhibition in the growth with the increasing concentration of herbicide up to 30 days at 75ppm,150ppm and 300 ppm respectively.

Deleterious effect of Nomnigold has been observed on on *A. circinalis*(fig.2.1) , population decreased significantly as shown by Anova analysis [Fcalculated(30,8.5) > Fcritical(30,1.5), P value (30,2.93E-15) < .05] with the increasing concentration of herbicide where means(shown in table 1:1) after 30 days readings were 606 ±99, 398 ±79, 296 ±71 at 75,150 and 300 ppm respectively as compared to control 1974.. During the end of month from 25^{th} to 30^{th} day the number of survived colonies decreases from 70 to 6 CFU (3.5% to 3%) at 75-300ppm respectively and rest of the colonies turn yellowish white in color. Chlorophyll content (Chl'a') (as in table 3.1) of survived colonies of *A.circinalis* on treatment with Nomnigold was .2958mg/l after 30 days incubation with herbicide at 75ppm and Nitrogen content(table 3.2) reaches to 8.80 mg/l after same time period.On *S. hofmanni* relatively lesser decrement rate was found as compared to *Anabaena circinalis a*nd the maximum mean obtained was 317.63±41.1 at 75ppm as compared to control of mean value 829±1.73 but the difference in means was variant as per Anova analysis [Fcalulated (30,(9.09)>Fcritical (30,1.5), p (1.84 E-16)<.05]. The number of survived colonies from 25^{th} to 30^{th} day the population stops decreasing and its number remains constant (fig.2b)and only 15%colonies survived at 75ppm, and 7%-2% at 150 ppm and 300ppm respectively .the chlorophyll content reaches (0.37 and0 .356 mg/l Chl'a') at 75 and 150 ppm respectively and N_2content to 8.80mg/l .

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Table 1:1:-Mean values of survived colonies of four species of cyanobacteria at different concentration of herbicide Nomnigold

Species	*Anabaena circinalis*				*Scytonema hofmanni*				*Aulosira prolifica*				*Tolypothrix fragilis*			
Herbicide concentration	Control	75ppm	150ppm	300ppm	Control	75ppm	150ppm	300ppm	Control	75ppm	150ppm	300ppm	Control	75ppm	150ppm	300ppm
Mean	1974.03	606.28	398.97	296.83	829	317.63	239.76	163.50	1830.16	1128.17	770.86	521.09	1136.03	894.76	812.4	566.82
SE	± 4.17	±99.02	±79.97	±71.61	±1.73	±41.11	±42.35	±36.56	±4.01	±61.81	±49.88	±36.13	±1.36	±16.01	±17.25	33.42

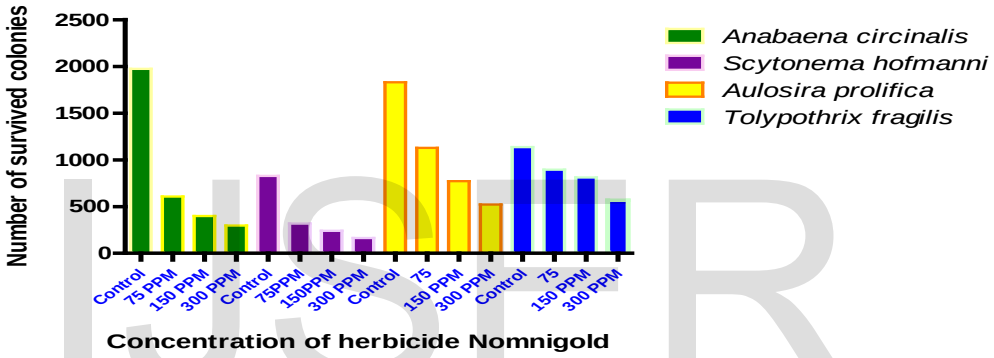

fig.1:1Graph representing effect of herbicide Nomnigold on the four strains of cyanobacteria

The decrease in number of colonies of *A.prolifica* further slows down on Nomnigold treatment and the difference in colonies at different concentrations is also not very significant, as per Anova analysis [Fcalulated (30,(3.6) > Fcritical (30,1.5), p (1.84, E-06)<.05] and mean values of 1128.17±61.8, 770.86±49.8, 521.09±36.1 at 75, 150 and 300ppm as compared to control (1830±4.01) as in table 1.1. From 20th day at 75 and 150ppm the decrease in number of colonies becomes slow and then constant and from 25th day starts showing regeneration as in (fig.2c) The percentage of remaining survived colonies was 42%, 25%, 11% at 75,150 and 300ppm respectively and the colonies appear brownish-green in color.

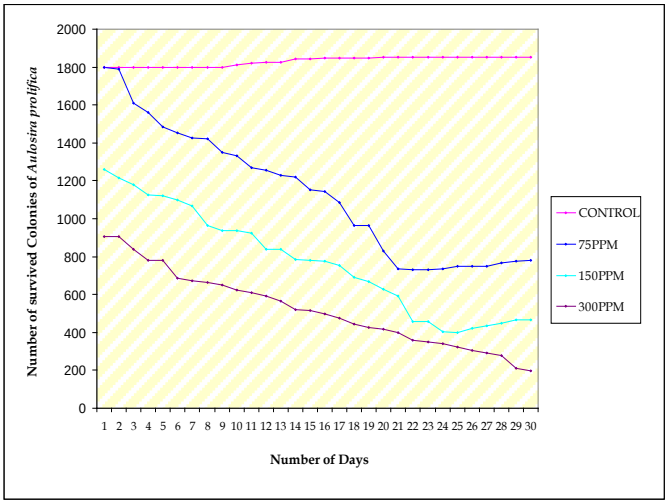

Fig.2a

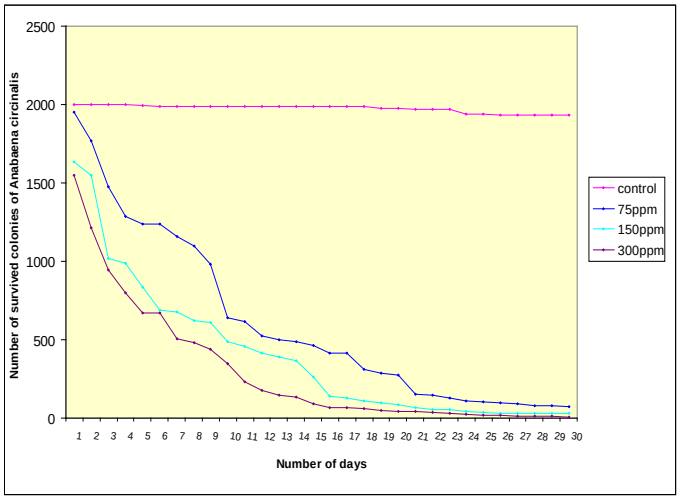

fig.2b

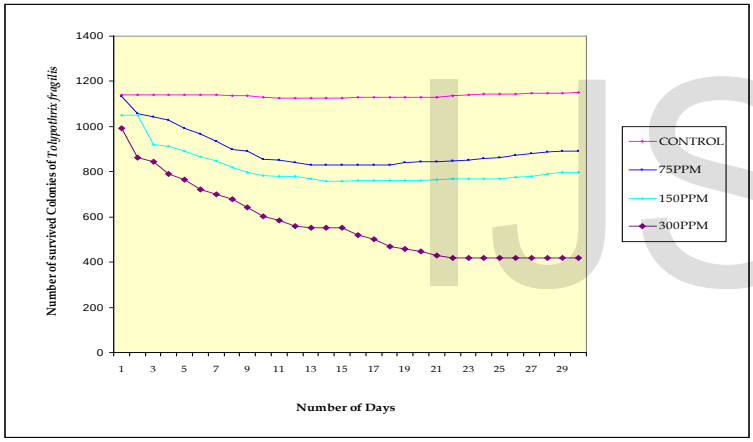

fig.2c

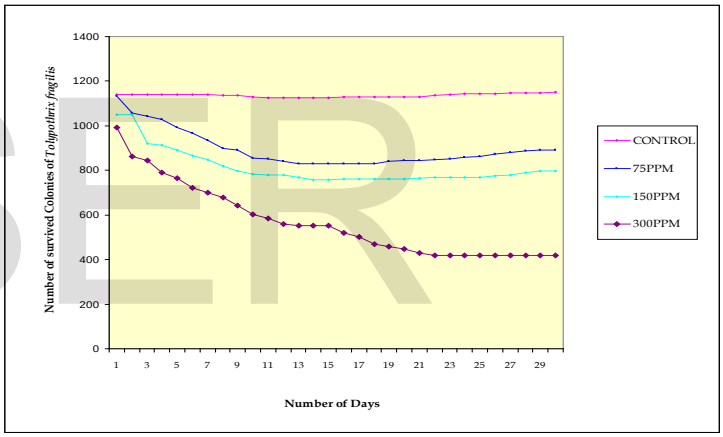

fig.2d

Figure 2:Graph representing effect of herbicide Nomnigold on four cyanobacteria strains a) *Anabaena circinalis,b) Scytonema hofmanni,c) Aulosira prolifica and d) Tolypothrix fragilis*

On treatment of *Tolypothrix* colonies with Nomnigold the decrement rate becomes extremely slow and gradually becomes constant,this could be from 20th day onwards and the difference in colonies at differen concentrations is also not very significant, Anova analysis shows [Fcalulated (30, (3.6)>Fcritical (30,1.5), p (1.84 E-o6)<.05] was less significant and mean values were 894.76±16, 812.4 ±17, 566.8 ± 33.4 at 75, 150 and 300ppm as compared to control (1136±1.3) after 30 days incubation period.From 25th day the survived colonies of *Tolypothrix* starts showing regeneration, the decrement rate decreases from 24.7 to 22% and 32.7 to 30.6 at 150ppm and at 300ppm 36.4% survival is seen as shown in fig.(2d) colonies appear distinct and brownish green in color.

Table 3.1: Effect of Nomnigold at different concentrations on Chlorophyll 'a' content(in mg/l) of four species of *Cyanobacteria*

Cyanobacteria species	***Anabaena circinalis***	***Scytonema hofmanni***	***Aulosira prolifica***	***Tolypothrix fragilis***
Herbicides Concentration				
Control	0.4708	0.4782	0.5202	0.4198
75ppm	0.2958 ±.12	0.37 ±.15	0.475 ± .19	0.3984 ± .16
150ppm	0.2398 ±.09	0.356 ± 14	0. 459 ± .18	0.3908 ± .15
300ppm	0.1562± .06	0.3034 ± .12	0.455 ± .18	0.3574 ± .14

Table 3.2.: Effect of Nomnigold at different concentrations on Nitrogen content(in mg/l) of four species of cyanobacteria

Cyanobacteria species	***Anabaena circinalis***	***Scytonema hofmanni***	***Aulosira prolifica***	***Tolypothrix fragilis***
Herbicides Concentration				
Control	11.0	11.88	10.47	11.97
75ppm	8.80 ± 0.19	8.80 ± 0.20	10.27 ± 0.18	11.74 ± 0.17
150ppm	7.7 ± .23	5.28 ± 0.21	9.76 ± 0.19	11.5 ± 0.17
300ppm	5.28 ± 0.25	6.51± 0.16	8.73 ± 0.25	11.26 ± 0.12

Where maximum Chl'a' content as in table 3.1 on Nomnigold treatment was 0.3984 mg/l for *T.fragilis* and for *A.prolifica* it was 0.475 mg/l. And the Nitrogen content as in table 3.2 reaches to 11.74 mg/l and 10.27 mg/l respectively for the two strains. Thus *T.fragilis* and *A.prolifica* showed better results than rest of the species and proved to be tolerant against high doses of the herbicide.

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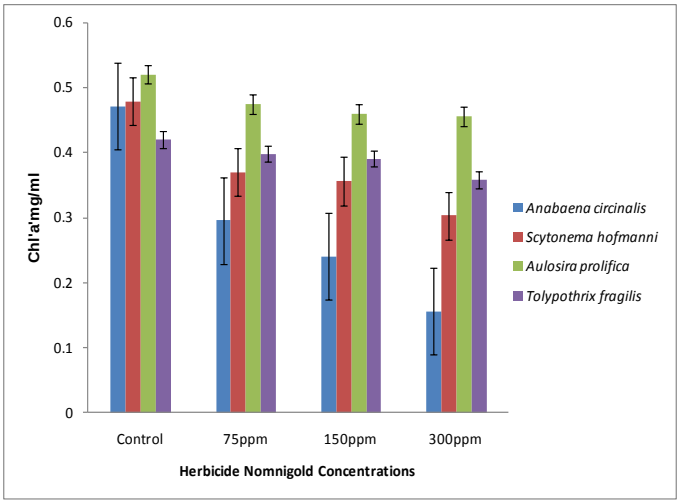

Fig.3a

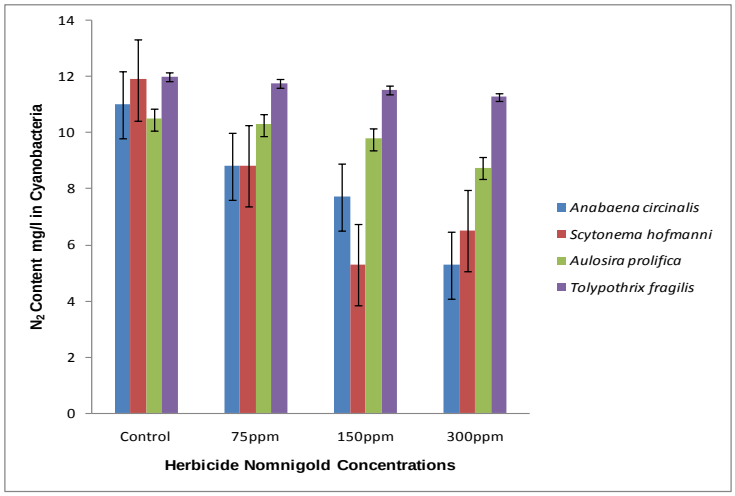

fig.3b

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Figure3.Graphs representing Chlorophyll 'a' content (3a) and Nitrogen content(3b) respectively in four strains of cyanobacteria.

Discussion

Bispyribac sodium was found to be deleterious at its recommended dose and double of the recommended dose to most of the cyanobacteria found in the rice fields as it inhibits the acetolactate synthase enzyme of the photosynthetic pathway which is also found in higher plants but it was found less harmful at low doses. In the study of Okmen and ugur[12] showed that bispyribac sodium stimulated activity of Anabaena species at 5µg/l but not in higher concentrations,in some cyanobacteria growth is inhibited at the initial concentration of 5µg/l. Gonzlaez –Barreiro [4] demonstrated harmful effects of herbicide on microalgae Generally w qhen added to the culture media.Caux [1] and Prosperi [13] demonstrated inhibitory effect of herbicide become greater on increased herbicide concentration and suggested that the reduction in the dry matter of algae may be due to decrease in algal photosynthesis caused by inhibition of synthesis of chlorophyll. Inderjit and kaushik[6] demonstrated in their experiment that higher dose of pretilachlor (40 ai mg/L), inhibited Chl aconcentrations of A. fertilissima.

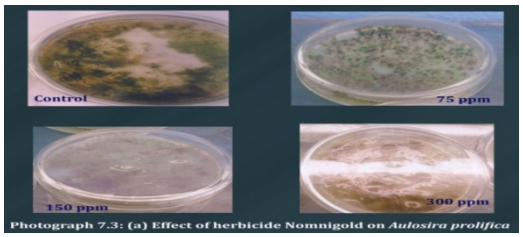

Fig.4a

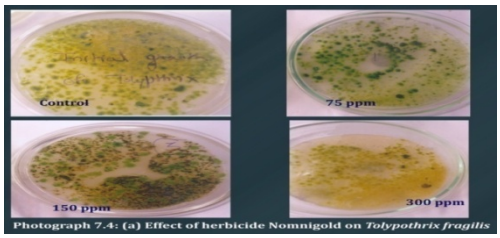

fig.4b

Figure.4.Photograph representing effect of herbicide Nomnigold at different concentrations on a) *Aulosira prolifica and b) Tolypothrix fragilis* showing minimum decrement rate.

In our study maximum deleterious effect of herbicidewas seen on Anabeana and Scytonema at 300ppm due to which chlorophyll reduced to 66% and 37% and Nitrogen reduced to 51% and 45% respectively from control followed by Aulosira and Tolypothrix where chlorophyll content reduced to 12% and 14.7% and nitrogen reduced to 16% and 5% from control but at 75 ppm which is half the recommended dose the 37 % and 22% for Anabaena and Scytonema least (8.6%,5%)for Aulosira and Tolypothrix.,likewise nitrogen content reached 20%,28%,1.9% and 1.9% for the four above strains respectively which is far less than that obtained at 300ppm ,this is because decrement rate in number of colonies of Aulosira and Tolypothrix as in figure 4,after 30 days was very less and after 20 to 25 days of incubation started showing regeneration.

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Conclusion

This increased growth observed at very low doses are result of a phenomenon 'hormesis'where herbicides can result in stimulatory effect..and at higher concentration result in inhibitory effect on plant as cyanobacteria has many metabolic mechanisms similar to that of higher plants,they also show regeneration .also in sensitive plants bispyribac sodium is adsorped by leaf surface and translocated through the plant,but in rice plants it is rapidly metabolized to nonherbicidal products so its extremity is reduced to large extent and becomes less harmful to microorganisms and may act as growth hormones and induce certain genes which increase heterocyst frequency in regenerated colonies.

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