Differential Effect of Endosulfan and Lasso on Growth and Nitrogen Fixation of Two Species of *Nostoc*.

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Abstract

Cyanobacteria present abundantly in soil are important in maintaining field fertility through nitrogen fixation. In present day agricultural practices, for improving crop productivity application of agricultural pesticides has become necessary. Cyanobacteria are also affected by pesticides as non-target organism. For successful exploitation of nitrogen fixing cyanobacteria as biofertilizer, it is evident to select a tolerant strain along with understanding of their tolerance. The response of two nitrogen fixing cyanobacterial species of *Nostoc* to Endosulfan and Lasso was hence examined in present study. Photosynthetic pigment and nitrogen fixing capacity of two species of *Nostoc, N. commune and N. linckia* were recorded after 15 days of treatment with various concentration of Endosulfan and Lasso. Stimulatory and inhibitory type of responses were recorded. 10 ppm and 20 ppm concentration of Endosulfan enhanced growth in *N. commune* while 40ppm inhibits growth. In *N. linckia* all the concentration reduced growth. While all the conc. of Endosulfan inhibits nitrogen fixing capacity of both the species of *Nostoc*. In *N. commune* as well as *N. linckia* 10 ppm and 20 ppm of Lasso stimulated growth and at 40 ppm conc. the growth was inhibited. Nitrogen fixation was stimulated in *N. commune* at 10ppm concentration. In *N. linckia* it was stimulated at 10ppm and 20 ppm concentration. Differential response towards tolerance level of Endosulfan and Lasso was observed among species of the same genus.

Keywords: Cyanobacteria, Pesticides, Endosulfan, Lasso, *Nostoc.*

Introduction:

Cyanobacteria are nitrogen fixing oxygenic prototroph's with potential as source of nitrogenous biofertilizer. Many species are capable of not only surviving, but thriving in conditions previously thought to be inhabitable, tolerating desiccation, high temperature, extreme pH, high salinity and pesticides illustrating their capacity to acclimate to extreme environment. Nitrogen fixing cyanobacteria widely used as biofertilisers make a valuable contribution to soil fertility by fixing atmospheric nitrogen.

Modern sustainable agriculture worldwide involves extensive use of agrochemicals such as insecticides, fungicides, herbicides and pesticides. Pesticides appear to be very effective in controlling a wide range of pests that infect crop including rice, pepper, etc. The effects of few pesticides have been studied with respect to growth, photosynthesis, nitrogenase activity and carbon fixation etc. Pesticides affect cyanobacteria under laboratory conditions differently (Kannaiyan,1985) and their effect with reference to the growth of these organisms has been classified as stimulatory, inhibitory and ineffective (Metting, 1985).

Several workers have reported the toxicity of a variety of pesticides, herbicides and fungicides to pure culture of blue-green algae (Ahmad & Venkataram,1973, Adhikary, 1989, Singh, 1990). The results of their investigations indicate that blue-green algae show variable resistance to different pesticides (Nirmal Kumar *et.al.* 2012, Giriyappanwar, 2014). Therefore a deeper understanding of the ecology and physiology of cyanobacteria becomes essential for the successful development of the algalization technology. An attempt has,

therefore been made in the present work to determine the effect of the most commonly used pesticide Lasso and Endosulfan on the growth and nitrogen fixation of two nitrogen fixing species of cyanobacteria *Nostoc commune* and *N. linckia*.

Material and Methods:

Organism and Growth conditions

The starter cultures of cyanobacteria, *Nostoc commune* and *N. linckia* were obtained from the National Facility for Blue Green Algae Collection Centre, IARI, New Delhi. The unialgal cultures of nitrogen fixing organisms was 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 two species of *Nostoc*.

Determination of Growth and Nitrogen fixation

At the end of 30 days, chlorophyll and nitrogen content of the cultures were determined as described below.

Growth was measured in terms of chlorophyll content (McKinney, 1941) and total nitrogen fixation by conventional microkjeldhal method (Jackson, 1973). Appropriate control system 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.

Pesticides used

Lasso- Alachlor is an herbicide from the chloroacetanilide family. It is used to control the growth of broad-leafed weeds and grasses in corn and many other crops.

Endosulfan- Endosulfan is an off-patent organochlorine insecticide. Endosulfan has been used in agriculture around the world to control insect pests including whiteflies, aphids, leafhoppers, and cabbage worms.

Statistical Analysis

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

Result and Discussion:

The present results indicate gradual but substantial inhibition in growth with increasing concentration of herbicides. The growth of Nostoc commune and N. linckia treated with different concentration of Endosulfan are listed in Table 2. It can be seen that the endosulfan applied at 10 ppm and 20 ppm concentration stimulated the algal growth of *N. commune* by 10.8% and 1.45% over control respectively, but then markedly inhibited the growth at 40 ppm by 27%. The

Chlorophyll content reduced by 27.44% exposed to 40 ppm concentration in *N.commune* after incubation of 30 days. Nitrogen fixation was reduced in *N.commune* with the treatment of endosulfan at all the concentration by 4.31%, 36.2% and 62.06% respectively.

Endosulfan treatment reduced chlorophyll content of *N.linckia* by 7.96%, 7.53 % and 20.49 % respectively. The results were highly indicative of their inhibitory effects on photosynthetic activities of the cells. Similar results were observed by Kumar *et.al.*. 2008 with endosulfan on cyanobacteria.

In *N. linckia* 6.42%, 34.64% and 56.42% reduction was recorded in nitrogen fixation with treatment of endosulfan after 30 days of incubation. *N. linckia* was more sensitive to endosulfan treatment than *N. commune*.

Lasso treatment at various concentration caused stimulation and inhibition in chlorophyll content of *N. commune* and *N. linckia* after 30 days of treatment. It is shown in Table 3 that initial concentration *i.e* 10 ppm and 20 ppm stimulated the growth by 37.69% and 9.00% respectively in *N. commune* while at 40 ppm concentration the alga shows drastic decrease in chlorophyll content 31.87%. Nitrogen fixation was also increased in *N. commune* at 10 ppm concentration by 19.39% while further increase in concentration of Lasso inhibited nitrogen fixation by 6.03% and 65.51%. Lasso treatment stimulated chlorophyll content by 34% and 3.62% in *N. linckia* at 10 ppm and 20 ppm concentration respectively. While at 40 ppm concentration it was reduced by 31.49%. Corresponding to Chlorophyll content nitrogen fixation increased with 10 ppm concentration in *N. linckia* by 23.92% while at 20 ppm and 40 ppm of Lasso declined nitrogen fixation by 22.83% and 65% after 30 days of incubation.

From the results shown in Table 2 and 3 it is concluded that nitrogen fixation was affected drastically in presence of both the pesticides. Adhikary *et.al.*,1984 studied the effect of carbamate insecticides Sevin on the growth survival and nitrogen fixation of *Anabaena* spp. and *Westiellopsis prolifica* and quoted similar result. From the results it was noted that growth and nitrogen fixation efficiency appears to be independent of each other in these organisms with reference to the external stress of agrochemicals. Similar results were observed by Gangwane, 1979 and Goyal, *et.al.* 1991. Kumar *et.al.* 2012 also reported 77% to 93% reduced activity of nitrate reductase after treatment with endosulfan and Tebuconazole.

Growth and nitrogen fixation of Nostoc species were inhibited at higher concentration of pesticides. The stimulatory effect at the low concentration of Endosulfan and Lasso was attributed to the direct effect exerted by utilization of either chemical itself or its degradatory products (Goyal, *et. al.* 1991). The toxic effects of DDT and its metobolities are dose related and lower concentration could not measurably affect soil algae(Megharaj, *et.al.* 1999). The results were highly indicative of inhibitory effects of Endosulfan and Tebuconazole on photosynthetic activities of the cells(Nirmal Kumar *et.al.*2012). Mohapatra and Schiewer (2000) have demonstrated with organophosphorus insecticides that toxicant-membrane interaction is responsible for changes in fluorescence behavior and pigment content

of *Synechocystis* PCC 6803. . Okmen *et.al.*, 2013 similarly concluded that initial concentration 6.25 mg/L stimulated Chl-a, β-carotene, phycocyanin and allophycocyanin content, but increasing herbicide concentration suppressed all of pigment content in *Anabaena* sp. Singh, 1990 reported that the herbicide Stam F-34 inhibited the heterocyst differentiation and nitrogenase activity even at a low concentration (0.5 ug ml^{-1}). Further he suggested that herbicide mainly inhibits the nitrogen fixation by inhibiting CO_2 assimilation, photosynthetic electron flow, ATP and reductant supply to heterocyst in cyanobacteria. Islam *et. al.* 2007 observed that the effect of Furadan on the growth and nitrogen fixation by BGA isolated was stimulatory under autotrophic conditions.

Effect of Endosulfan and Lasso on the two species of Nostoc shows that both the species respond differently though they belong to same genera. Similar results were reported by Kaushik and Venkataraman, 1983. They showed that insecticides, such as BHC, Carbofuran and Phorate effect differently on pigment synthesis and nitrogenase activity and different genera respond differently and there is an inter and intra-specific strain variation. Kaur, *et.al.*, 1997 reported that test species exhibited different degree of sensitivity to Machete. The possible reason for such behavior of the test algae is attributed to the differential permeability of chemical across the cell membrane. Das and Adhikary, 1996 reported variation in the tolerance level to a specific pesticide existed among species of the same genus and also between genera.

Table. 1- Properties of the pesticides used for the study

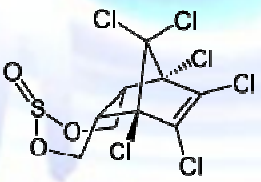
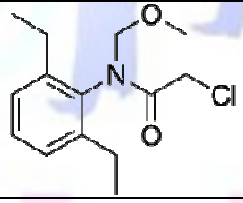
Properties	**Endosulfan**	**Lasso**
IUPAC name	6,7,8,9,10,10-Hexachloro-1,5,5a,6,9,9a-hexahydro-6,9-methano-2,4,3-benzodioxathiepine-3-oxide	Alachlor 2-Chloro-*N*-(2,6-diethylphenyl)-*N*-(methoxymethyl) acetamide
Chemical structure		

Table.2 Effect of Endosulfan on growth(Chlorophyll content mg/ml) and Nitrogen of *N. commune* and *N. linckia*

N. commune	Control	Concentration of Endosulfan		
		10 ppm	20ppm	40ppm
Chlorophyll content	14.43±0.65	15.99±0.71 (+10.81)	14.64±0.30 (+1.45)	10.47±0.22 (-27.44)
Nitrogen	2.32±0.25	2.22±0.12 (-4.31)	1.48±0.18 (-36.2)	0.88±0.12 (-62.06)
N. linckia	Control	Concentration of Endosulfan		
		10 ppm	20ppm	40ppm
Chlorophyll content	13.81±0.25	12.71±0.07 (-7.96)	12.77±0.22 (-7.53)	10.98±0.43 (-20.49)
Nitrogen	2.80±0.16	2.62±0.12 (-6.42)	1.83±0.12 (-34.64)	1.22±0.15 (-56.42)

The values are mean of three readings. ± Standard deviation. Values in parantheses represent percent increase(+) or decrease (-) over control.

Table. 3- Effect of Lasso on growth(Chlorophyll content mg/L) and Nitrogen of *N.commune* and *N.linckia*

N. commune	Control	Concentration of Lasso		
		10 ppm	20ppm	40ppm
Chlorophyll content	14.43±0.65	19.87±0.90 (+37.69)	15.73±0.15 (+9.00)	9.83±0.21 (-31.87)
Nitrogen	2.32±0.25	2.77±0.24 (+19.39)	2.18±0.21 (-6.03)	0.80±0.04 (-65.51)
N. linckia	Control	Concentration of Lasso		
		10 ppm	20ppm	40ppm
Chlorophyll content	13.81±0.25	18.52±0.49 (+34.10)	14.31±0.07 (+3.62)	9.46±0.22 (-31.49)
Nitrogen	2.80±0.16	3.47±0.10 (+23.92)	2.16±0.13 (-22.85)	0.98±0.08 (-65.00)

The values are mean of three readings. ± Standard deviation. Values in parantheses represent percent increase(+) or decrease (-) over control.

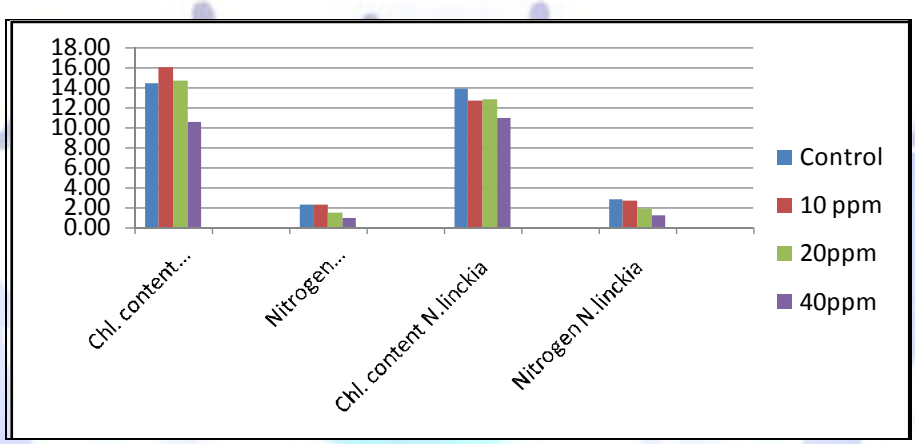


Figure. 1- Effect of various concentration of Endosulfan on chlorophyll content and nitrogen of *N.commune* and *N. linckia*

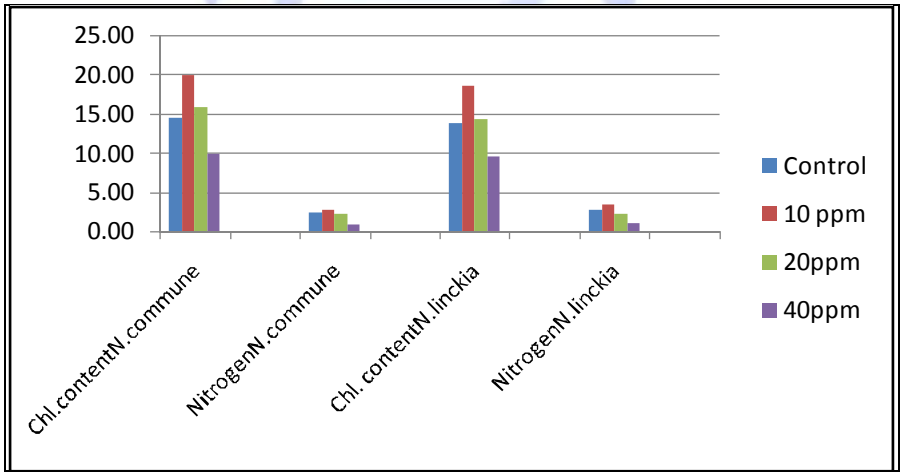


Figure. 2- Effect of various concentration of Lasso on chlorophyll content and nitrogen of *N.commune* and *N. linckia*

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