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Effect of herbicide Butachlor on the frequency of the Heterocyst in *Nostoc punctiforme* (Kutz) Har.

Arun Kumar*

University Department of Botany, Ranchi University, Ranchi, Jharkhand, India

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Abstract- Cyanobacteria are structurally diverse assemblage of gram-negative eubacteria, a link between bacteria and green plants. *Nostoc punctiforme* (Kutz) Har., is a heterocyst containing cyanobacteria. They perform oxygenic photosynthesis and fix nitrogen and abundant in the algal ecosystem of the western part of Orissa. In modern agricultural practices herbicides occupy an important place to kill or reduce growth of unwanted weeds. These are chemical compounds which selectively interfere with the growth of unwanted weeds. Butachlor is an amide herbicide. It is a pre-emergent herbicide and used as controller for all small broad-leaved weeds. Butachlor at recommended doses (100 to 200 ppm) proved to be toxic to cyanobacteria while lower doses favored growth and nitrogen fixation. In tropical Paddy fields the use of butachlor is common in summer and in winter crops of Paddy. Heterocyst frequency in the cyanobacterial filament is an important attribute to view the function of the species with respect to nitrogen fixation. With the increase of butachlor concentration in the culture of nostoc punctiforme, the frequency of heterocyst gradually decreased. Lower concentration of this herbicide is recommendable.

Key words: Butachlor, herbicide, cyanobacteria, heterocyst, *Nostoc punctiforme*

INTRODUCTION

Cyanobacteria are structurally diverse assemblage of gram-negative eubacteria. In evolutionary terms they represent a link between bacteria and green plants. *Nostoc punctiforme* (Kutz) Har. is a heterocyst containing cyanobacteria. They perform oxygenic photosynthesis and fix nitrogen. They are most abundant in the algal ecosystem of the western part of Orissa. Due to their nitrogen fixing ability, they maintain the fertility status of the Paddy fields. This stimulated me to take the *Nostoc punctiforme* as study material.

Nostoc punctiforme (Kutz) Har. is an ensheathed heterocystous uniseriate, unbraced filamentous form of cyanobacteria. Typical cell measures about 2-5 μ in diameters. In *Nostoc* innumerable usually contorted, threads are aggregated within mucilage with a firm boundary. At first colonies are mostly spherical and this general shape may be retained in the globular all ellipsoidal colonies of *Nostoc punctiforme*. The sheath of individual trichomes is usually yellow or brown but are only recognizable in the peripheral parts. Production of hormogonia or of akinetes generally takes place almost simultaneously throughout a colony. Akinesis formation usually commences midway between two heterocysts, are some specialized cells with thick hyaline walls, which are derived from ordinary fields. In position heterocyst may be terminal all intercalary.

In modern agricultural practices herbicides occupy an important place. These are chemical compounds which

*Corresponding author :
Phone : 9631088409
E-mail : drarunkumar21@gmail.com

selectively interfere with the growth of unwanted weeds. These weeds compete with the cultivated plant for growth and nutrition and therefore should be removed from the crop fields to maintain and enhance crop yield. Weeds can be handpicked but it is time consuming and expensive. Therefore, modern agronomics use the selective herbicides which kill or retard the growth of the weeds and improve the growth and yield of the cultivated plants. Butachlor is an amide herbicide. It is a pre-emergent herbicide and used as controller for all small broad-leaved weeds.[1] According to Singh (1973)[2], butachlor at recommended doses (100 to 200 ppm) proved to be toxic to cyanobacteria while lower doses favored growth and nitrogen fixation. In tropical Paddy fields the use of butachlor is common in summer and in winter crops of Paddy.

Heterocyst frequency in the cyanobacterial filament is an important attribute to view the function of the species with respect to nitrogen fixation.[3] Various workers have tried to evaluate herbicidal toxicity through the assessment of heterocyst frequency and differentiation.[4]

MATERIAL & METHODS

The pure culture of *Nostoc punctiforme* (Kutz)Har. was obtained from the Regional Agricultural Research Institute Chip ilima, Odisha. The *Nostoc punctiforme* was cultured in BGII medium.[5] Before the inoculation of Nostoc punctiforme the cultural medium was subjected to autoclaving for sterilization. The *Nostoc punctiforme* culture was maintained under continuous white light (of 1950 lux approx.) at the temperature 25°C ± 2°C. Besides the culture flasks were subjected to handshaking intermittently.

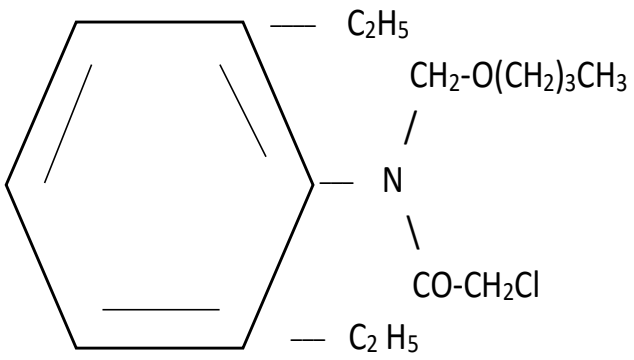
Conical flasks of size 250 ml were used for the experimental setup. Four sets of flasks, each with four replicates containing 50 ml of nutritional media were taken. To each flask 2 ml of *Nostoc punctiforme* (Kutz)Har culture was introduced. One set of inoculated flasks were kept as control. To the other three sets 0.1, 0.5 and 1.0 ppm of butachlor were introduced. All the conical flasks were maintained at the temperature 25°C ± 2°C under continuous white light.

A drop of *Nostoc punctiforme* culture was taken out of the culture flask and was put on a clear glass slide. After using the cover slip, the slide was subjected to microscopic examination. Each focus number of vegetative cells and number of heterocyst were counted. Ten such random observations were made. The heterocyst frequency was calculated as:

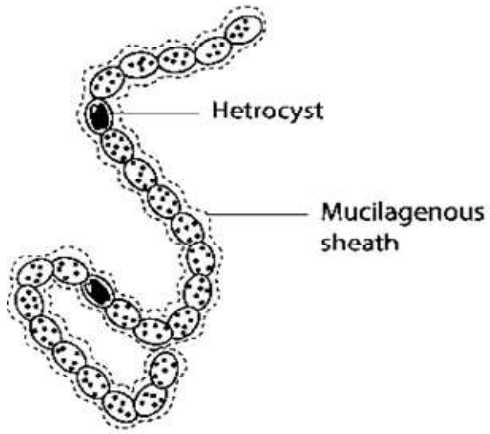
$$\text{Heterocyst frequency (\%)} = \frac{\text{Number of heterocyst}}{\text{Total no of vegetative cells + heterocyst}} \times 100$$

RESULT & DISCUSSION

The heterocyst frequency *Nostoc punctiforme* with respect to control and butachlor treatment during 5th, 10th and 15th days of incubation. On treated with butachlor (Figure-1A) the *Nostoc* heterocyst (Figure-1B) frequency significantly declined from the control value i.e. from 4.317% on 15th day to 3.49 % in 0.1 ppm, 2.11% in 0.5 ppm and 1.312% in 1.0 ppm. The significance of data between the treatment and the days were subjected to the analysis of variance. The analysis revealed the variation of heterocyst frequency with respect to different treatment and different days were statically significant.



BUTACHLORE HERBICIDE

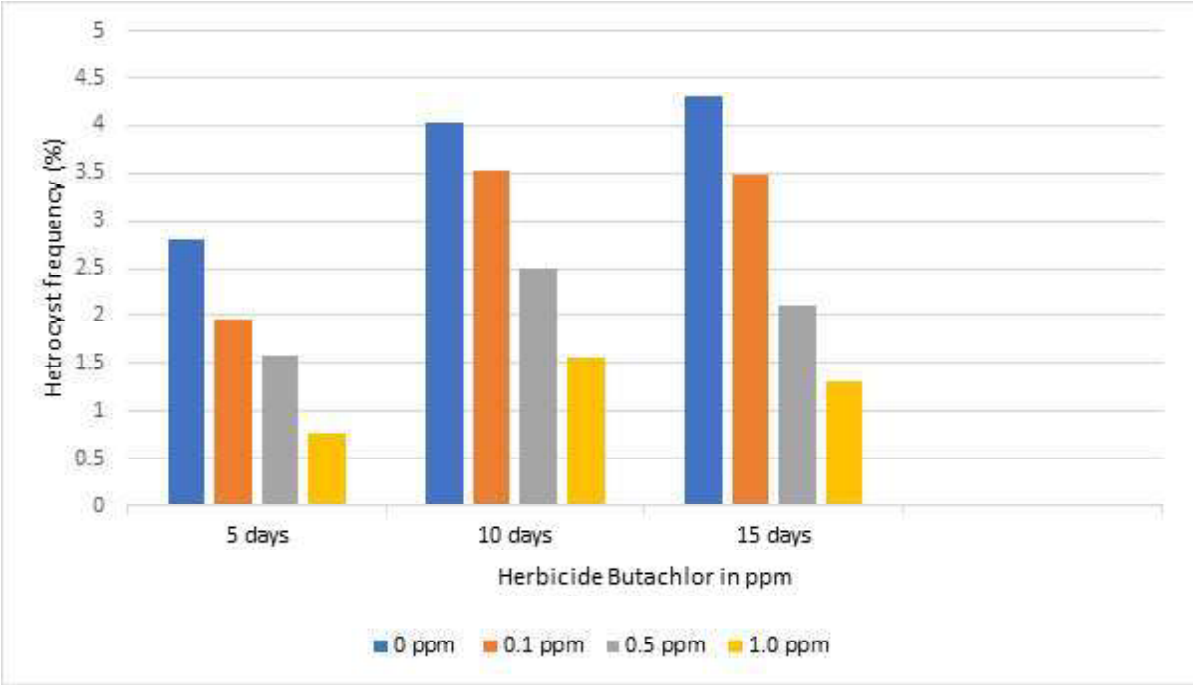


NOSTOC WITH HETEROCYST

Table 1- Effect of different concentration of Butachlor on heterocyst frequency (%) in *Nostoc punctiforme* at the interval of 5 days up to 15 days

Days	Herbicide Butachlor (ppm)			
	0	0.1	0.5	1.0
5 d	2.87	1.95	1.58	0.76
10 d	4.04	3.53	2.50	1.56
15 d	4.31	3.49	2.11	1.31

Graph 1- Effect of different concentration of Butachlor on heterocyst frequency (%) in *Nostoc punctiforme* at the interval of 5 days up to 15 days.



As revealed in the present study, frequency of heterocyst of Nostoc punctiforme declined with the increase of concentration of herbicide butachlor in comparison to the controlled one. The impact of different herbicides on heterocyst frequency have been investigated.[3,4,6] All of them have reported lowered heterocyst frequency because of the different herbicide treatments. Since heterocyst is considered as the site for nitrogen fixation, the butachlor treatment, thus impairs the nitrogen fixation process in *Nostoc punctiforme* by not allowing the differentiation of the heterocyst. Impact of different pesticides on the heterocyst differentiation in the cyanobacterial species have also been reviewed by Singh (1973)[2].

The experimental evidence clearly showed the impact of herbicide butachlor on the frequency of heterocyst. With the increase of butachlor concentration in the culture of nostoc punctiforme, the frequency of heterocyst is gradually decreased. Lower concentration of this herbicide is recommendable.

The heterocyst frequency, as revealed in the present study did exhibit a decline because of butachlor treatment[3,4,6] have investigated the impact of different herbicides on heterocyst frequency of cyanobacteria.

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