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Research Article

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Herbicidal Effect on *Tolypothrix distorta* Kuetz. ex Bron. et Flah and *Camptylonemopsis lahorensis* (Ghose) under the family Scytonemataceae and their Comparative Studies on Growth (Chlorophyll-a.µg/ml) and Nitrogenase Activity (n mole C_2H_4/µgchl.a/h) under culture in three different days

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Abstract

This experimental work effect on herbicides (Butachlor 50% EC) of two different fast-growing strains viz., *Tolypothrix distorta* Kuetz. ex Bron. et Flah and*Camptylonemopsis lahorensis* (Ghose) Desikachary under the family Scytonemataceae(Cyanobacteria) have been taken for their Comparative Studies on the herbicidal effect on Growth (Chl-a.µg/ml) and Nitrogenase activity in six different concentrations each of the culture medium in three different days .

Results indicated that *Camptylonemopsis lahorensis* showed more growth in nitrogen free culture medium and gradually increased more growth on 10th to 20th and 30th day respectively. But in lower concentration of herbicidal effect showed *Camptylonemopsis lahorensis* and *Tolypothrix distorta*, both the genera showed maximum stimulatory effect on maximum days and gradually increased more growth on different days i.e. on 20th day and 30th day. And both different genera showed maximum stimulatory effect upto in 5 ppm concentrations and the growth was i.e. Chl.-a. 0.963 µg/ml on 30th day .*Tolypothrix distorta* and *Camptylonemopsis lahorensis* showed talerancy upto 40 ppm concentration and*Camptylonemopsis lahorensis* is able to grow up to 50 ppm concentration.

In case of nitrogenase activity in 5ppm concentration, result is maximum. But gradually increasing concentration showed the decreasing trend successively.In 5 ppm concentration *of Camptylonemopsis lahorensis* maximum nitrogenase activityshowed 1.97 mole C_2H_4/µgchl.a/h as compare to *Tolypothrix distorta.* But gradually moe concentration showed the decreasing trend and up to 50 ppm concentration in both the strains. But in *Tolypothrix distorta* nitrogesase activity was totally absent in three different days.

Keywords: Cyanobacteria, Scytonemataceae, Herbicides, Butachlor ,Growth, Chlorophyll-a, Nitrogenase activity.

1. Introduction

Scytonemataceae (Cyanobacteria) strains are very much common and for nitrogen fixing capacity present in moist rice fields. They contribute the nitrogen to development of soil health and ecology in many ways. About one third of the total yield herbicides and different diseases cause losses in crop yields losses in every year due to grass hoppers, gandhi- bugs and application of monocrotophos, diazinon, malathion,butachlor phosrate, quinophos, BHC, endrin, herbicides and diseases and these are also more effective. Among them herbicides of butachlor ,malathion and dimecron are generally used to control the pests. Indiscriminate application of agrochemicals (chemical fertilizers: N, P, K, Zn etc.), weedicides and pesticides by farmers in their agriculture fields to enhance the yield and to earn *more* money has created an alarming situations for agriculture sector. Unbalanced application of agrochemicals has greatly influenced the growth and population of microorganisms beneficial to agriculture. It is necessary to conserve these organisms by application of nutrient substances in balanced amount and also by using bio fertilizer in agriculture fields for Sustainable development of agriculture sector.

Therefore, our present study to select two fast growing and efficient nitrogen fixing strains have been taken for checking the herbicides or pesticides viz. Butachlor, to find out the precise effect on their growth and nitrogen fixation. They are *Tolypothrix distorta* and *Camptylonemopsis lahorensis* are very common and generally dominant in upland and moist lowland reice-field ecosystems.

2. Materials and Methods

2.1. Isolation and Maintain of the strains

Our present study, more than 20 strains of the family Scytonemataceae, have been collected from the different natural soil samples of various fields of rice growing localities and out of which two different fast-growing genera and species of *Tolypothrix distorta* Kuetz. ex Bron. et Flah and *Camptylonemopsis lahorensis* (Ghose) Desikacharyhave been taken for the present work. At last, two different genera were selected as fast-growing strains after comparing their chl-a µg/ml i.e., growth and Nitrogenase activity. A similar 10 ml required concentrations of Butachlor (50% EC) of the particular inoculum was taken and pH was adjusted at 7.5 and grown in BG-11 medium (Stanier et. al., 1971). The streaking method was applied for isolation (Kaushik, 1987). Selected two samples were prepared and aseptically inoculated in 28 ± 2°C and in 3000-4000 Lux light intensity under ^{14}/10 LD upto 30 days.

2.2. Morphological observation and identifications

We were used by the Nikon and MOTIC microscopes with attaching photosystemsfor recording the morphological observations.

2.3. Identification of these filamentous Cyanobacteria

The isolated strains and taxonomic identifications were made by our present observations, Desikachary (1959), Komarek & Anagnostidis (1986,1988), and Tiwari et.al. and Tiwari (1972,1975 and 1979).

2.4. Physiological studies

(a) Estimation chlorophyll-a (µg/ml): Each of thethree replicates the inoculation of the two different strains were measured of chlo-a µg/ml on 10th day, 20th day and 30th day respectfully.

(b) Determination of Nitrogenase activity: According to Assay (Kaushik and Venkataraman-1983 using Gas Chromatograph (Amil- Nucon model-5700) with para-pack N and T columns (Stewart et al., 1967) the Nitrogenase activities were measured in terms of Acetylene Reduction assay value .In the laboratory, in 3 replicates in each in nitrogen-deficient liquid medium Nitrogenase activities were analysed both the strains grown at the exponential stage of growth.

In total 15 ml capacity of Acetylene equivalent to 10% of the total air space was injected into glass vial . The vials reactions were stopped with sub-seals and incubated at 28 ± 2^{0}C under 3000 – 4000 lux light intensity for 120 minutes. The reaction was stopped with the injecting 0.1 ml of 50 % TCA (Trichloro acetic acid) . The gas phase was analyzed for ethylene and the activity was expressed as n mole C_2H_4/µg chl-a / h and it is also presented as n mole C_2H_4 / µg vial /h. Experiments were performed by three replicates.

3. Results

3.1. *Tolypothrix distorta* Kuetz. ex Bom. et Flah.

I. Estimation of Growth (Chlo-a µg/ml):

Estimation of growth only upto 10 ppm concentration increasing but the maximum increase over the control was in 5 ppm on all the day. In 20 and 30 ppm, gradual inhibition was present and growth was quite absent in 50 ppm concentration. Further, it was observed that pesticidal effect was more on 10th day than 20th and 30thday **(Table-1. Fig.1)**.

3.2. Estimation of Nitrogenase activity:

The nitrogenase activity gradually inhibited in terms of n mole C_2H_4/µg chl-a/h in increasing concentrations on all days but in 5 and 10 ppm concentration. It has some increasing values from 20th and 30th day respectively. In 50 ppm, it was completely absent **(Table-2, Fig.2)**.

However, in terms of n mole C_2H_4/vial/h the increase in nitrogenase was present only in 5 ppm from 10th to 30th day and in 10 ppm concentration show the activity on 20th to 30thday. In remaining concentration, this pesticide causes gradually inhibitory effect and it was quite absent in 50 ppm concentration due to absent of growth **(Table-3)**.

Table-1. Herbicidal effect on Growth (chl.-a. µg/ml) of *Totypothrix distorta*

	10th Day	20th Day	30th Day
Cont.	0.195	0.243	0.417
5 ppm	0.243(+24.61%)	0.678(+179.01%)	0.963(+130.93%)
10 ppm	0.221(+13.33%)	0.529(+117.67%)	0.872(+109.11%)
20 ppm	0.104(-46.66%)	0.218(-10.28%)	0.409(-41.91%)
30 ppm	0.083(-57.43%)	0.196(-19.34%)	0.251(-24.61%)
50 ppm	-----	-----	----

(---) Growth absent.

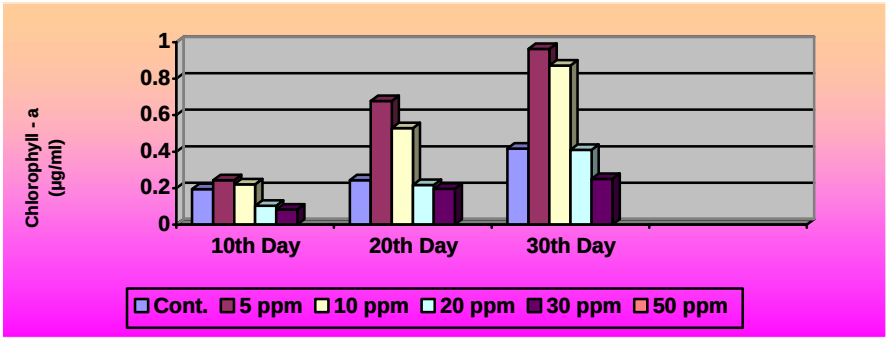

Fig.-1:Herbicidal effect on Growth (chl.-a. µg/ml) of *Totypothrix distorta*

Table-2. Herbicidal effect on Nitrogenase activity (n mole C_2H_4/µg/chl.a/h) of*Tolypothrix distorta*

	10th Day	20th Day	30th Day
Cont.	1.48	0.93	0.82
5 ppm	1.30(-12.61%)	1.13(+21.50%)	1.06(+29.26%)
10 ppm	1.22(-17.56%)	0.93(-3.22%)	0.86(+4.87%)
20 ppm	1.07(-27.70%)	0.74(-20.43%)	0.51(-37.80%)
30 ppm	0.82(-44.59%)	0.67(-27.95%)	-----
50 ppm	----	----	-----

(---) nitrogenase activity absent.

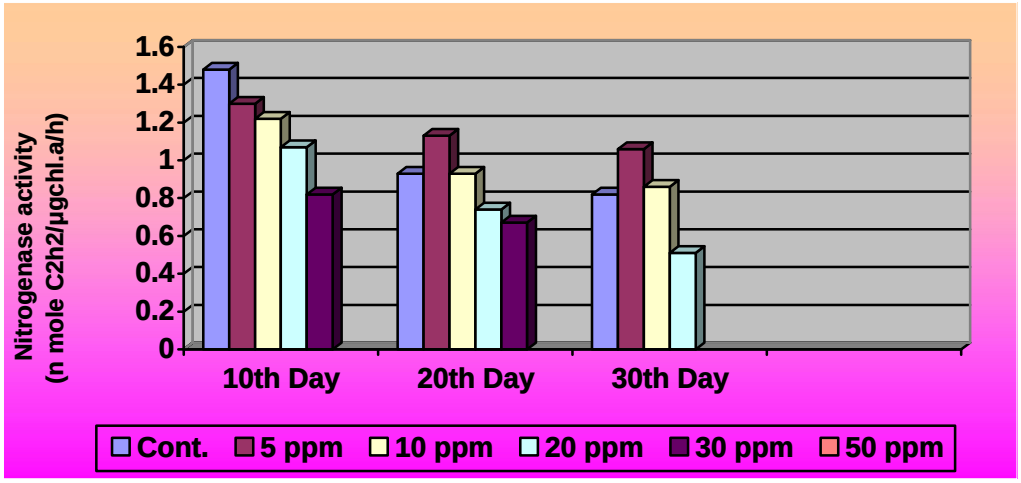

Fig.-2: Herbicidal effect on Nitrogenase activity (n mole C_2h_2/µgchl.a/h) of *Tolypothrix distorta*

Table-3. Herbicidal effect on Nitrogenase activity (n mole C_2H_4/vial/h) of *Tolypothrix distorta*

	10th Day	20th Day	30th Day
Cont.	2.88	2.25	03.41
5 ppm	3.15(+09.37%)	7.66(+240.44%)	10.20(+199.12%)
10 ppm	2.69(+06.59%)	4.76(+111.55%)	07.49(+119.64%)
20 ppm	1.11(-61.45%)	1.61(-28.44%)	02.08(-39.00%)
30 ppm	0.68(-76.38%)	1.31(-41.77%)	01.00(-70.67%)
50 ppm	-----	-----	-----

(---) nitrogenase activity absent.

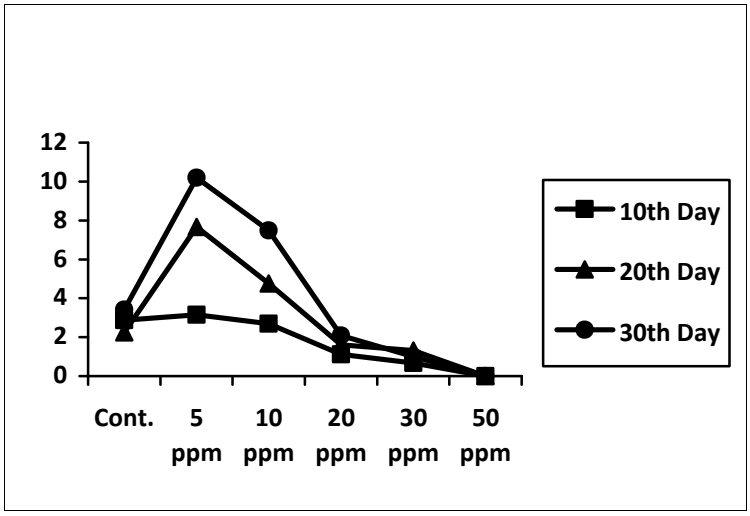

Fig.-3: Herbicidal effect on Nitrogenase activity (n mole C_2H_4/vial/h) of*Tolypothrix distorta*

3.3. *Camptylonemopsis lahorensis* (Ghose) Desikachary

I. Growth (Chlo-a µg/ml): The present **Growth** showed increasing tendency only upto in 10 ppm concentration on 10th, 20th and 30th day. But on 30thday, 20 ppm concentration also has some increase (01.57%) over the control. Otherwise, from 20 ppm, it was gradually inhibited in higher concentration. However, alga was showing negative result in 50 ppm concentration of butachlor **(Table-4, Fig.3)**.

II. Nitrogenase activity: The result in terms of n mole C_2H_4/**µ**g chl.-a/h throughout inhibition was present upto 20 ppm concentration and completely absent in 30 and 50 ppm concentration. However, it was also observed that trend inhibition was gradually lower on 30th day in each concentration as compared to 10th and 20th day **(Table-5, Fig.4)**.

In terms of n mole C_2H_4/vial/h was also showing similar trend of results in all concentrations except on 20th & 30th day result in 5 ppm concentration. **(Table-6)**.

Table- 4. Herbicidaleffect on Growth (chl-.a µg/ml) of *Camptylonemopsis lahorensis*

	10th Day	20th Day	30th Day
Cont.	0.261	0.452	0.572
5 ppm	0.390(+49.41%)	0.549(+21.46%)	0.690(+20.62%)
10 ppm	0.345(+32.18%)	0.482(+06.63%)	0.653(+14.16%)
20 ppm	0.184(-29.50%)	0.347(-23.23%)	0.581(+01.57%)
30 ppm	0.162(-37.93%)	0.319(-29.42%)	0.479(-16.25%)
50 ppm	0.114(-56.32%)	0.216(-52.21%)	0.270(-51.54%)
(---) Growth absent.			

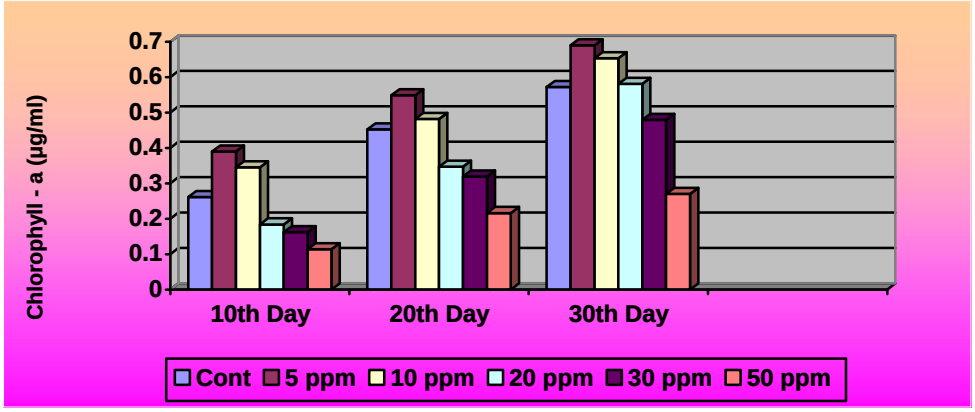

Fig.-4: Herbicidaleffect on Growth (chl-.a µg/ml) of *Camptylonemopsis lahorensis*

Table-5. Herbicidal effect on Nitrogenase activity (n mole C_2H_4/µg chl. a/h) of *Camptylonemopsis lahorensis*

	10th Day	20th Day	30th Day
Cont.	1.89	1.47	1.21
5 ppm	0.82(-56.6%)	1.40(-4.76%)	1.19(-1.65%)
10 ppm	0.68(-64.02%)	0.73(-50.34%)	0.93(-23.14%)
20 ppm	0.62(-67.19%)	0.62(-57.82%)	0.76(-37.19%)
30 ppm	-----	-----	-----
50 ppm	------	-----	-----
(---) nitrogenase activity absent			

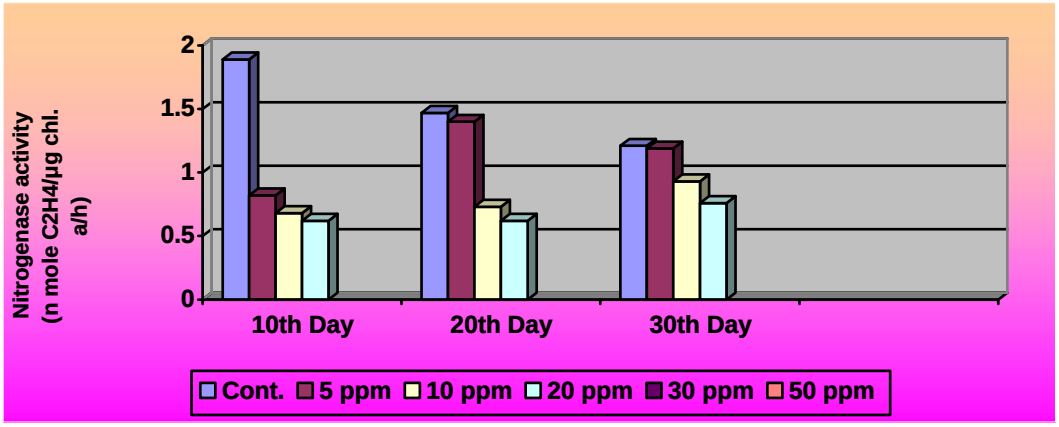

Fig-.5: Herbicidaleffect on Nitrogenase activity (n mole C_2H_4/µg chl a/h) of *Camptylonemopsis lahorensis*

Table-6. Herbicidal effect on Nitrogenase activity (n mole C_2H_4/Vial/h) of *Camptylonemopsis lahorensis*

	10th Day	20th Day	30th Day
Cont.	4.93	6.6	6.92
5 ppm	3.19(-35.29%)	7.6(+15.15%)	8.21(-18.64%)
10 ppm	2.34(-52.53%)	3.5(-46.96%)	6.07(-12.28%)
20 ppm	1.14(-76.87%)	2.1(-68.18%)	4.41(-36.27%)
30 ppm	-----	-----	-----
50 ppm	-----	-----	-----

(---) nitrogenase activity absent

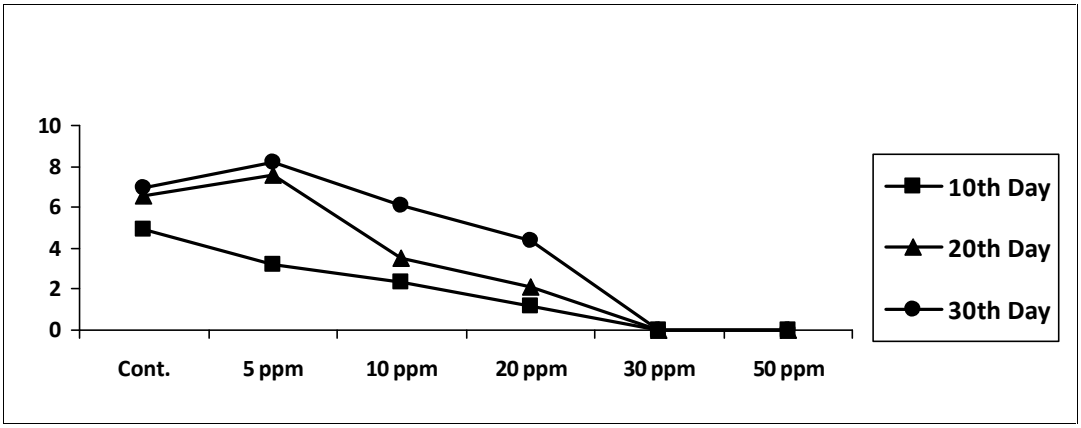

Fig.-6: Herbicidal effect on Nitrogenase activity (n mole C_2H_4 / Vial/h) of *Camptylonemopsis lahorensis*

4. Discussion

Anand and Veerappand (1980) have observed the effect of different concentration of dimecron on *Anabaena vaginicola, Cylindrospermum muscicola* and *Nostoc entophytum* and only one strain of *Scytonema.* Previously Tiwari and Pandey (1981) observed that the stimulatory effect of 5 ppm concentration of butachlor on growth of *Nostoc calcicola* and inhibitory at 7 ppm concentration. Roy Choudhury and Kaushik (1986) had not observed that any effect on growth and Chlorophyll-a synthesis of *Tolypothrix ceylonica and Scytonema cinnatum* at 10 ppm concentration treatment, but its higher concentration than that developed marked inhibition.

Dikshit and Tiwari (1990) noted inhibition in chlorophyll-a of *Anabaena variabilis, Aulosira implexa* var. *tenuis, Aulosira fertilissima var. tenuis, Scytonema chiastum* and *S. stuposum* in presence of 100 ppm concentration of cyathion (malathion) and their growth was completely absent in it 200 ppm concentration except *S. chiastum.*

Kolte and Goyal (1990) also did not found any adverse effects on the nitrogenase activity of *Calothrix marchica* in presence of subnormal, normal and supernormal concentration of the present pesticide. Nagpal and Goyal (1992) found more comparability of butachlor with *Anabaena oryzae, A. anomala, N. paludosum and N. calcicola* except *A. anomala* which did not grow beyond 10 ppm concentration.

Zargar and Dar (1990) again reported that 70 ppm concentration of butachlor was not effective for nitrogen content dry biomass, chlorophyll-a content and heterocyte differentiation but its higher concentration (90 and 110 ppm) created marked reduction in all the parameters.

Halder. N.C. et.al. (2010) studied on*Scytonema chiastum, Scytonema bewsii, Tolypothrix distorta* and*Camptylonemopsis lahorensis*for different effect onDimecron their growth and nitrogenase activity.The results of this pesticidal effect of Dimecron (50% EC) *Tolypothrix distorta* have more tolerance of higher concentrationin 30ppm upto 30 days. Whereas other strain i.e. *Camptylonemopsi lahorensis* has increased growth only upto 20 ppm concentration upto 30th day.

The present results of Butachlor (50% EC) indicates that it develops stimulation in higher concentration their tolerancy is more in *Camptylonemopsis lahorensis* which is upto 50 ppm concentration. Thus according to tolerance *Camptylonemopsis lahornsis* is the better strain but for stimulatory effect than others.

Nitrogenase activity in terms of per n mole C_2H_4µg chl a/h *Tolypothrix distorta* is the better strain which shows the ARA value even upto 30 ppm concentration of butachlor because other strains performe activity only upto 20th day. Although these values are lower than the control's values. *T. distorta* has higher values than the control in 10 ppm on 30th day and in 5 ppm on 10th to 30th day. While others have no stimulation in their nitrogenase activity in treated replicates. Further its values in terms of n mole C_2H_4/ vial/h are also in higher concentrations (upto 30 ppm) only in *Tolypothrix distorta* which has higher values than the control in 10 ppm on 20th and 30th day and in 5 ppm on 10th to 30th day. The maximum increase over the control was in 5 ppm concentration.

Thus the our present study will be more useful in this respect.

5. Conclusion

The present results indicate that *Camptylonemopsis lahorensis* showed more growth in nitrogen free culture medium and gradually increased more growth on 10th to 20th and 30th day respectively. But in lower concentration of herbicidal effect showed *Camptylonemopsis lahorensis and Tolypothrix distorta,* both the genera showed maximum stimulatory effect on maximum days and gradually increased more growth on different days i.e. on 20th day and 30th day respectively. *Tolypothrix distorta* maximum stimulatory effect in 5 ppm concentrations showed maximum growth i.e. Chl.-a. 0.963 µg/ml on 30th day.

But gradually increasing concentration showed the decreasing trend up to in 50 ppm concentration in both the strains. But in *Tolypothrix distorta* nitrogesase activity was totally absent on 10th, 20th and 30th days respectively.

Results of Herbicides or butachlor indicated that it develops stimulation in higher concentration and tolerancy is more in *Camptylonemopsis lahorensis,* which is maximum and upto 50 ppm concentration. Thus according to tolerance *Camptylonemopsis lahornsis* is the better strain than *Tolypothrix distorta.*

Conflict of Interest

The authors of this paper have no conflict of interest.

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