International Journal of Advanced Research in Biological Sciences
ISSN: 2348-8069
www.ijarbs.com
(A Peer Reviewed, Referred, Indexed and Open Access Journal)
DOI: 10.22192/ijarbs Coden: IJARQG (USA) Volume 9, Issue 2 -2022

Research Article

DOI: http://dx.doi.org/10.22192/ijarbs.2022.09.02.013

Effect of Pesticides on Growth (Chlorophyll-a) and Nitrogenase Activity of the Two Fast Growing Strains of *Scytonema chiastum* Geitle and *Scytonema bewsii* Fritsch et Rich of the Family *Scytonemataceae* (Cyanobacteria)

Nikhil Chandra Halder
Department of Botany, Uluberia College,
Uluberia, Howrah, West Bengal, Pin–711315, India
*Corresponding author: *nchalder.algae@gmail.com*

Abstract

Present paper deals with the two fast-growing filamentous and heterocystous Cyanobacterial strains Viz. *Scytonema chiastum* Geitler and *Scytonema bewsii* Fritsch et rich of the family, Scytonemataceae were studied for growth (Chlorophyll-a) and nitrogenase activity in 6 different concentrations of (0, 5, 10, 20, 30,50 ppm) of pesticides Butachlor (50% EC). Results indicate that *Scytonema chiastum* showed the stimulatory effect on growth maximum in lower concentrations up to 5 ppm and 10 ppm on 10^{th} day and medium upto 20^{th} day and 30^{th} day, respectively. *Scytonema bewsii* showed upto 20 ppm concentrations were stimulatory on the 10th, 20th, and 30th, respectively. However, between them, *Scytonema bewsii* showed more tolerance up to 30 ppm rapidly while *Scytonema chiastum* are unable to easily grow up to the same concentration. Results of nitrogenase activity of both the strains decreasing trend in higher concentration i.e., up to 5ppm to 20 ppm. But gradually increasing concentration, i.e., 30 ppm and 50 ppm, successively showed totally lethal and absent on the 10th, 20^{th} and 30^{th} days.

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

1. Introduction

The growth of Scytonematacean form is also very common, particularly in moist rice fields throughout the corp period, which contribute in many ways to the crop and the soil. Simultaneously, a provided mixture of BGA bio-fertilizer cultures also contains the growth forming propagula provided to farmers for algalization in their fields. So, it is essential to find out which dose of these pesticides has no adverse effect on their growth and nitrogen fixation efficiency. Every year about one-third of the total yield crops were damaging and losses India due to the development of rice-borer, leaf and plant grasshoppers, termites, paddy root weevil, hoppers, Gandhi-bugs, hairy caterpillars, mites, snails, shrimps and crabs in fields. Therefore, frequent application of parathion phosrate, malathion, Butachlor, diazinon, monocrotophos, endrin, quinophos, BHC, and aldrin are benign made by farmers to control these pests and diseases and these are also more effective. Among them malathion, dimecron, butachlor and BHC are commonly used to

control the pests. Pests and diseases cause losses in crop yields that are about thirty percent of the total yield in India. In rice-field soils of topical countries, abundance occurrence of various nitrogen fixing Cyanobacteria (BGA) also have much significance in supplying nitrogen for the crop and enriching the soil products, i.e., organic matter, growth promoting substances, amino acids peptides etc. But the application of these pesticides is also common in rice fields, affecting the growth of these Cyanobacteria.

2. Materials and Methods

2.1. Isolation and Maintain of the strains

In total 21 strains belonging to the genera *Scytonema* of the family Scytonemataceae have been taken for the present study and which are isolated from their natural materials and from enrichment cultures of the soil samples collected from various fields of rice growing localities of several districts of Uttar Pradesh and West Bengal in India. All the selected strains were found different in their morphological and physiological behaviours. Finally, a few strains were selected as fast-growing strains after comparing their growth (chlorophyll-a and dry weight Nitrogenase activity) with other strains of the order Scytonemataceae and Nostocaceae. A similar quantity of the inoculum was taken from their exponential growth phase of unialgal cultures grown in BG11 nitrogen-free liquid medium (Stanier et. al., 1971). The streaking method carried out the isolation of these filamentous forms (Kaushik, 1987). Cyanobacterial samples were inoculated upto 30 days at 32 ± 2°C and 4000-5000 Lux light intensity under 14/10 LD. The final pH was adjusted at 7.5. Required concentrations of Butachlor (50% EC) were prepared aseptically in 10 ml containing sterilized medium after making their stock solutions.

2.2. Morphological observation and identifications

Morphological observations were recorded with the help of Nikon and MOTIC microscopes with attaching photosystems.

2.3. Identification of these filamentous cyanobacteria

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

2.4. Physiological studies

(a) Determination of chlorophyll-a (µg/ml): The replicates were in the test tubes containing 10 ml of liquid medium. The growth of strains was measured after a definite period i.e., 10th day, 20th day and 30th day, respectfully of inoculation by an increase in chlorophyll-a µg/ml.

(b) Estimation of Nitrogenase activity: Nitrogen as activity was analysed in the cultures grown in nitrogen-deficient medium .at the exponential stage of growtg. The activity was measured in terms of Acetylene Reduction Assay (ARA) (Kaushik and Venkataraman-1983 using Gas Chromatograph (Amil- Nucon model-5700) with para pack N and T columns (Stewart et al., 1967).

Acetylene equivalent to 10% of the total air space was injected into glass vial of 15 ml capacity. The vials were stopped with sub seals and incubated for 120 minutes at 28 ± 2^{0}C under 4000 – 5000 lux light intensity. The reaction was stopped by injecting 0.1 ml of 50 % TCA (Trichloro acetic acid) and the gas phase was analyzed for ethylene and the activity was expressed as n mole C_2H_4 / µg chl / h and it is also presented as n mole C_2H_4 /vial /h. Experiments were performed in three replicates.

3. Results

3.1. *Scytonema chiastum* Geitler

I. Growth (chlorophyll a µg/ml):

The present alga has also increased in growth upto 10 ppm but the maximum percentage increase (72.47%, 91.17% and 100%) was in 5 ppm concentration. It is also observed that the percentage increase over the control was higher in younger growth. From 20 ppm concentration, it was interesting that the percentage of inhibition in growth was more pronounced during early days than later days. The growth was completely absent in 50 ppm concentration **(**Table 1, Fig.1).

3.2. Nitrogenase activity:

In terms of n mole C_2H_4/ µg chl.a/h, it was gradually poor than the control in all treated replicates and maximum inhibition was found in the highest concentration and later days also and it was absent in 30 and 50 ppm concentration **(Table 2, Fig.2)**.

However, an increase in nitrogenase activity in terms of n mole C_2H_4/ vial/h was observed in 5 ppm concentration on all days (10th to 30th days) and in 10 ppm only on 10th day. In remaining concentrations (10 and 20 ppm), it was always lower than the control. Further, in 30 ppm and 50 ppm, it was absent due to poor or absence of growth **(**Table 3**)**.

Table 1: Butachlor effect on Growth (chl.a a µg/ml) of *Scytonema chiastum*

	10th Day	20th Day	30th Day
Cont.	0.139	0.204	0.327
5 ppm	0.278(+100%)	0.390(+91.17%)	0.564(+72.47%)
10 ppm	0.226(-62.58%)	0.296(+45.09%)	0.471(-44.03%)
20 ppm	0.069(-50.35%)	0.183(-10.29%)	0.306(-06.42%)
30 ppm	0.023(-83.45%)	0.079(-61.27%)	0.145(-55.65%)
50 ppm	----	-----	-----
(---) Growth absent.			

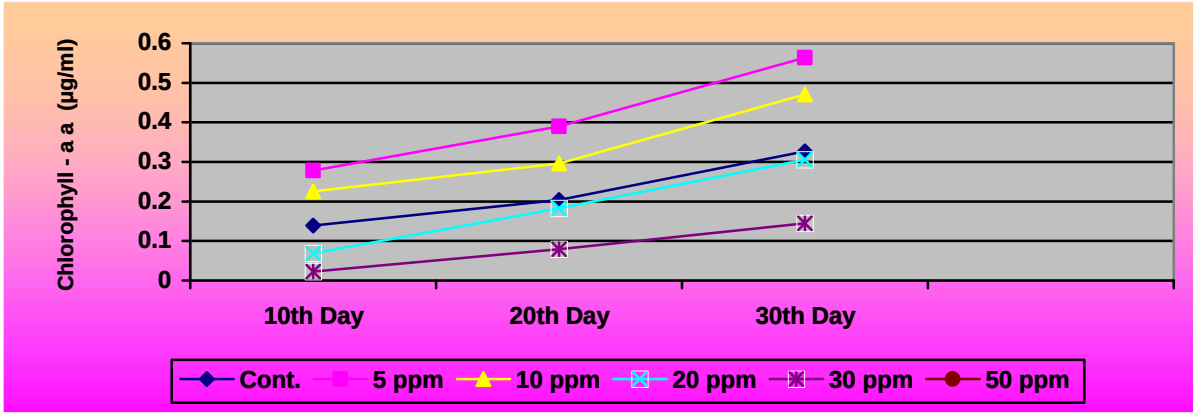

Figure-1: Butachlor effect on Growth (chl.a a µg/ml) of *Scytonema chiastum*

Table 2: Butachlor effect on Nitrogenase activity (n mole C_2h_4/µg/chl.a/h) of *Scytonema Chiastum*

	10th Day	20th Day	30th Day
Cont.	2.44	2.25	1.72
5 ppm	2.38(-2.45%)	1.38(-38.66%)	1.33(-22.67%)
10 ppm	1.92(-21.31%)	1.02(-54.66%)	0.91(-47.09%)
20 ppm	1.60(-34.47%)	0.80(-64.44%)	0.57(-66.86%)
30 ppm	----	----	-----
50 ppm	----	----	----
(---) Nitrogenase activity absent.			

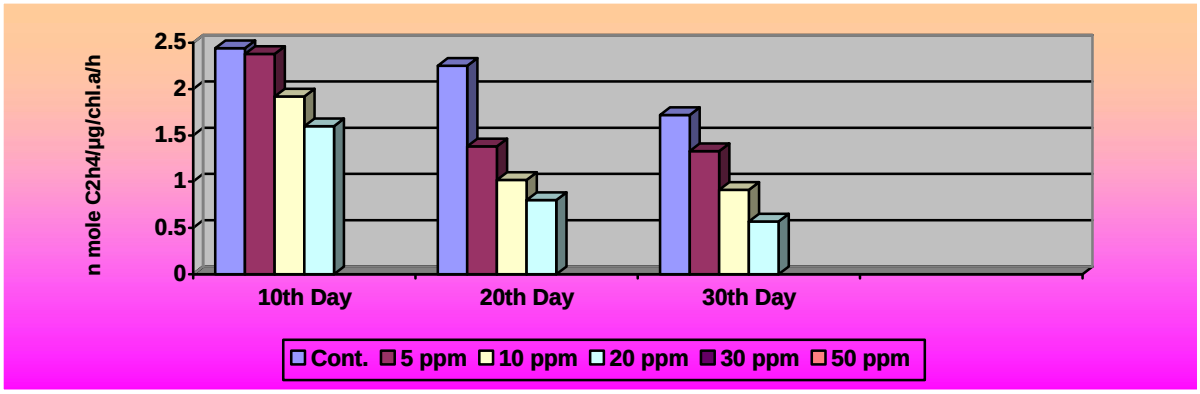

Figure-2. Butachlor effect on Nitrogenase activity (n mole C_2h_4/µg/chl.a/h) of *Scytonema Chiastum*

Table 3. Butachlor effect on Nitrogenase activity (n mole C_2h_4/vial/h) of *Scytonema Chiastum*

	10th Day	20th Day	30th Day
Cont.	3.39	4.59	5.62
5 ppm	6.61(+94.98%)	5.38(+17.21%)	7.50(+33.45%)
10 ppm	4.33(+27.72%)	3.01(-34.42%)	4.28(-23.84%)
20 ppm	1.10(-67.55%)	1.46(-68.19%)	1.74(-69.03%)
30 ppm	----	----	----
50 ppm	----	----	----
(---) Nitrogenase activity absent.			

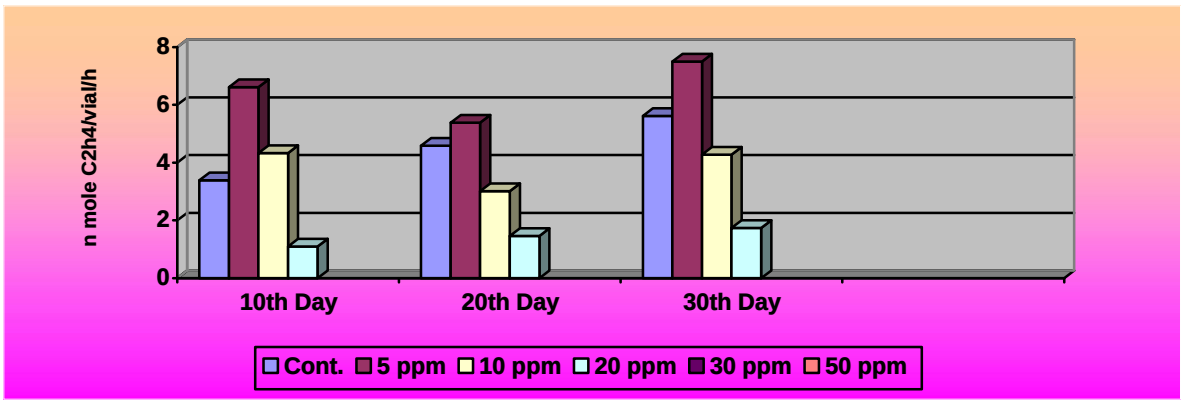

Figure-3. Butachlor effect on Nitrogenase activity (n mole C_2h_4/vial/h) of *Scytonema Chiastum*

2. *Scytonema bewsii* Fritsch et Rich

I. Growth (Chlorophyll-a µg/ml): The growth of alga increased upto 30 ppm while it was absent in 50 ppm concentration except on 20th day when inhibition in growth was present in 30 ppm. The increasing percentage was highest in each concentration on 10th day in comparison to other days. However, compared to used concentrations, 20 ppm has the highest increase in growth **(**Table-4, Fig.4**)**.

II. Nitrogenase activity: In terms of n mole C_2H_4/ µg chl. a/h, it was showing lower values in all treated replicates compared to the control and the highest inhibition (88.66%) was present on 20th day in 20 ppm concentration all treated replicates. Interestingly, in 30 ppm concentration, nitrogenase activity was completely absent on all estimation days **(**Table-5, Fig.5**)**.

In terms of n mole C_2H_4/vial/h, the nitrogenase activity increased upto 10 ppm only on 10th day while on remaining days it was gradually poor than the control upto 20 ppm concentration, otherwise trend of the results is similar to the above results **(**Table 3**)**.

Table 4: Butachlor effect on Growth (chl.-a µg/ml) of *Scytonema biwsii*

	10th Day	20th Day	30th Day
Cont.	0.039	0.082	0.116
5 ppm	0.125(+220.51%)	0.161(+96.34%)	0.225(+93.96%)
10 ppm	0.146(+274.35%)	0.177(+115.85%)	0.253(+118.10%)
20 ppm	0.170(+335.89%)	0.196(+139.02%)	0.311(+168.10%)
30 ppm	0.040(+02.56%)	0.060(-26.82%)	0.117(+00.86%)
50 ppm	----	-----	----
(---) Growth absent.			

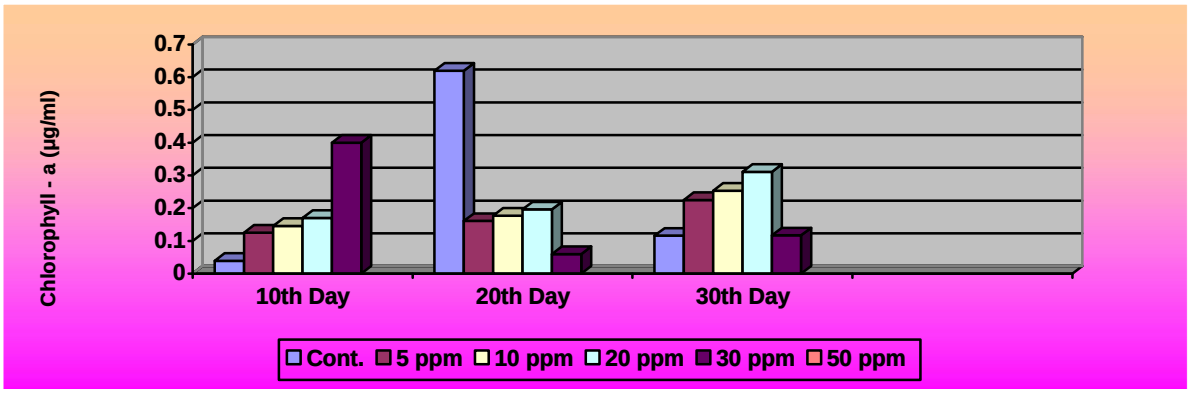

Figure – 4. Butachlor effect on Growth (chl.-a µg/ml) of *Scytonema biwsii*.

Table 5: Butachlor effect on Nitrogenase activity (n mole C_2h_4/µg/chl.a/h) of *Scytonema biwsii*

	10th Day	20th Day	30th Day
Cont.	1.56	5.56	2.63
5 ppm	1.04(-33.33%)	1.32(-76.25%)	0.98(-62.73%)
10 ppm	0.76(-51.28%)	1.17(-78.95%)	0.77(-70.72%)
20 ppm	0.28(-82.05%)	0.63(-88.66%)	0.45(-82.88%)
30 ppm	-----	-----	-----
50 ppm	-----	-----	-----
(---) nitrogenase activity absent.			

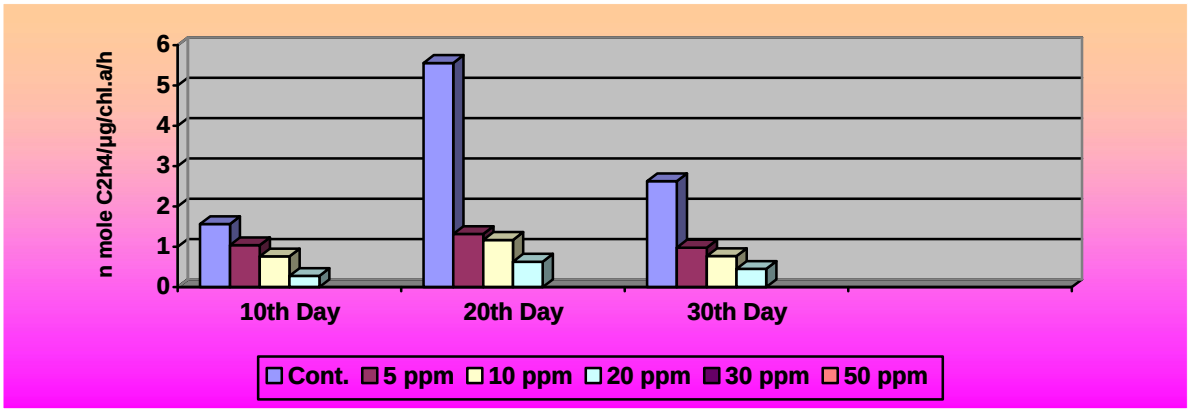

Figure-5. Butachlor effect on Nitrogenase activity (n mole C_2h_4/µg/chl.a/h) of *Scytonema biwsii*

Table 6: Butachlor effect on Nitrogenase activity (n mole / vial/h) of *Scytonema biwsii*

	10th Day	20th Day	30th Day
Cont.	0.60	4.5	2.05
5 ppm	1.3(+116.66%)	2.1(-53.33%)	2.20(-27.86%)
10 ppm	1.10(+83.33%)	2.07(-54.00%)	1.94(-36.39%)
20 ppm	0.47(-21.66%)	1.2(-73.33%)	1.39(-54.42%)
30 ppm	-----	-----	-----
50 ppm	-----	------	-----
(---) nitrogenase activity absent.			

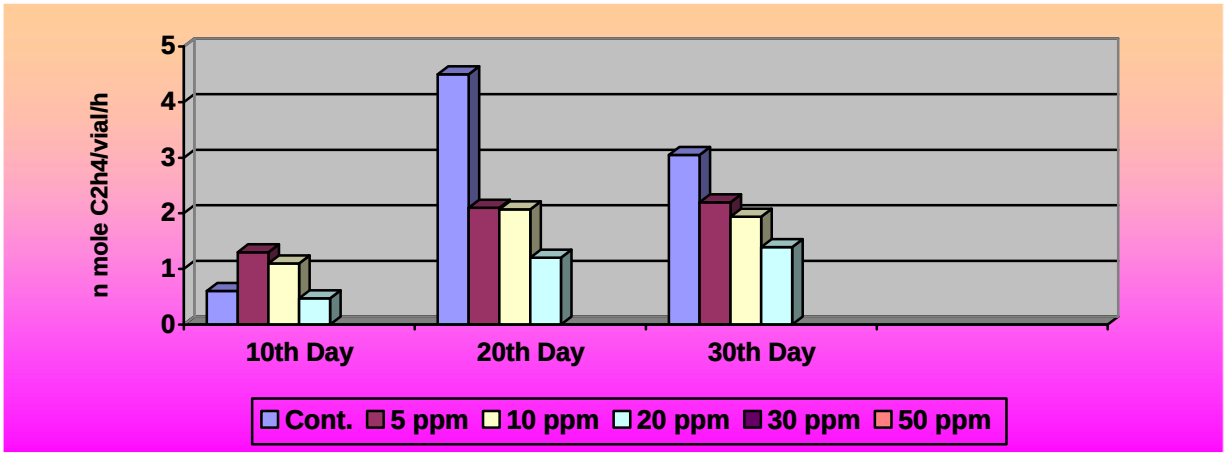

Figure-6. Butachlor effect on Nitrogenase activity (n mole C_2h_4/vial/h) of *Scytonema biwsii*

4. Discussion

Previously, Anand and Veerappan (1980) recorded the survivability of *Anabaena vaginicola*, *Cylindrospermum muscicola* and *Nostoc entophytum* upto 250 ppm concentration of dimecron. However, *Anabaena* sp. and *Nostoc* sp. are more resistant than *Cylindrospermum* sp. Reddy (1976) did not noted any significant effect of 25 ppm concentration of dimecron on *Tolypothrix tenuis* and *Anabaena* sp. Halder. N.C. et.al. (2010), studied on *Scytonema chiastum*, *Scytonema bewsii*, *Tolypothrix distorta* and *Camptylonemopsis lahorensis* for different concentrations of Dimecron (50% EC) on the growth (Chlor0phyll-a) and nitrogenase activity. Results indicate that *Scytonema chiastum* shows the

stimulatory effect on growth maximum in 5 ppm and10 ppm on 20th day and 30th day, respectively. *Scytonema bewsii* showed upto 5 ppm concentration were stimulatory on 20th day and 30th, respectively. Whereas *Tolypothrix distorta* and *Camptylonemopsis lahorensis* were stimulatory up to 10 ppm concentration on 20th day and 30th day, respectively. However, among them *Scytonema chiastum* and *Camptylonemopsis lahorensis* showed more tolerance upto 40 ppm while others were unable to grow up to in same concentration. The nitrogenase activity of all the strains increases in low concentration, i.e., upto 5ppm to 10 ppm. But gradually increasing concentration i.e., 20 ppm and 30 ppm, successively showed and decreasing trend and up to 40 ppm and 50 ppm concentration, their growth and nitrogenase activity was lethal and absent on 10th, 2oth and 30th days successively. And also, Chandra Bhan (1993) recorded stimulation in growth and nitrogen fixation rate of taken strains in presence of upto 20 ppm concentrations of dimecron. According to him, *Nostoc*, *Scytonema* and *Anabaena* have more tolerance of dimecron (upto 60 ppm) than *Calothrix and Aulosira*.

5. Conclusion

Although, the present results are not comparable because mostly all previously taken strains are not the same species taken for the present study. But all the present strains also have tolerance upto 40 ppm concentrations of dimecron except *S. bewsii* which has only upto 30 ppm concentrations. Therefore, the present results are more comparable to the results of Chandra Bhan (1993) and Reddy (1976). But the present results indicate that usually, all strains of the genera *Scytonema chiastum*, *Scytonema bewsii* behave distinctly.

Scytonema bewsii showed more tolerance upto 30 ppm rapidly while *Scytonema chiastum* cannot easily grow up to in same concentration and nitrogenase activity of both the strains decreasing trend in higher concentration i.e., up to 5ppm to 20 ppm. But gradually increasing concentration, i.e., 30 ppm and 50 ppm, successively showed totally lethal and absence on the 10th, 20th and 30th days. Therefore, the present results are comparable in individual parameters with mostly all the previous worker's results above. There is no similar trend in the same pesticides on different species.

Conflict of Interest

The author of this paper have no conflict of interest.

References

Agardh, C.A. 1848 Species, genera et ordines algarum.

Anand, N. and Veerappan B. 1980 Effect of pesticides and fungicides on blue-green algae. Phykos, 19: 210-212.

Chandra Bhan 1993 Studies on the effect of dimecron on *Anabaena*, *Nostoc* and *Scytonema*; D. Phil thesis pp, 59-68. Allahabad Univesity, Allahabad.U.P.

Desikachary, T.V. 1948 On *Camptylonema indicum* Schmidle and *Camptylonemopsis* gen. nov. Proc. Indian Acad. Sci. B 28:35-50.

Desikachary, T.V. 1959. Cyanophyta. I.C.A.R., New Delhi, 686.

Halder,N.C.,V.Kwivedi and Rashmi Singh (2010)Effect of Dimecron 50% EC on growth and Nitrogenase activity of the four fast growing strains of Scytonemataceae. National Journal of Life Sciences, Vol 7(1)2010:163-168.

Kaushik.B.D.& Venkataraman,G.S.(1983) Response of Cyanobacterial nitrogen fixation To insecticides.Curr.Sce. 52:321-323

Komarek, J. and Anagnnostidis, K.1986 Modern approach to the classification system of Cyanophytes, 2- Chroococcales Arch. Hydrobiol Suppl. 73, Algological Studies 43:157-226.

Komarek, J. and K. Anagnostidis, 1988 Modern approach of the classification system of cyanophytes 4-Nostocales. Arch. Hydrobiol Suppl. 82.3, Algological studies 3rd part. 247-345.

Raghav Reddy, H.R. 1976 Studies on enfluence of pH. phosphate and pesticides on nitrogen fixation and radio carbon assimlation by two blue-green algae. M.Sc. Thesis subm., Univ. Agri. Sci. Banglore.

Stewart. W.D.P. 1972 Heterocysts of blue-green algae. In:Desikachary. T.V. ed. Taxonomy and biology of blue-green algae., Madras p.227-235.

Stewart, W.D.P., Fitzerald, C.P. and Burris, R.H. 1967 In situ studies on nitrogen fixation using the acetylene reduction technique. Proc. Nat. Acad. Sci. Wash., 58:2071-2078.

Stewart. W.D.P. 1980 Some aspects of structure and function on nitrogen fixing cyanobacteria. Ann. Rev. Microbiol. 34:497-536.

Tiwari, G.L. 1972 a A study of the Blue-green algae of paddy field soils of India. Hydrobiologia 393:335-350.

Tiwari, G.L. 1975 A study of the Blue-green algae from paddy field soils of India. Taxonomic consideration of non-heterocytous blue-green algae. Nova Hedwiegia, 26:765-798.

Tiwari, G.L. 1979 A study of the Blue-green algae from paddy field soils of India. IV-Taxonomic consideration of Nostocales and Stignematales. Nova Hedwiegia, 63:133-159.

Access this Article in Online	
	Website: www.ijarbs.com
Quick Response Code	Subject: Phycology
DOI:10.22192/ijarbs.2022.09.02.013	

How to cite this article:
Nikhil Chandra Halder. (2022). Effect of Pesticides on Growth (Chlorophyll-a) and Nitrogenase Activity of the Two Fast Growing Strains of *Scytonema chiastum* Geitle and *Scytonema bewsii* Fritsch et Rich of the Family *Scytonemataceae* (Cyanobacteria). Int. J. Adv. Res. Biol. Sci. 9(2): 109-116.
DOI: http://dx.doi.org/10.22192/ijarbs.2022.09.02.013