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EFFECT OF FURADAN ON THE GROWTH AND NITROGEN FIXATION BY BLUE GREEN ALGAE

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Abstract

The study was carried out to determine the effects of pesticide on the growth and nitrogen fixation by blue green algae (BGA) that isolated from three different soils of Bangladesh *viz*., saline soil, calcareous soil and red soil. Furadan 5G, one of the most commonly used pesticides was selected for the study of eighteen taxa of blue green algae (BGA) which brought under the unialgal culture in the selected soil types. Variations observed in growth and nitrogen fixation among the isolates of a particular type of soil. The culture resulted as BGA grow slow under heterotrophically grown cultures than that of autotrophically grown cultures. On the other hand, nitrogen fixation in heterotrophically grown cultures was observed at higher rate. However satisfactory result was observed in both cases when field dose of Furadan was applied practically. In this case, heterotrophically grown cultures were more tolerant to pesticides with respect to growth and nitrogen fixation.

Key words: Blue green algae, Furadan, nitrogen fixation, heterotrophic growth, autotrophic growth.

Introduction

Molecular nitrogen is biologically unavailable unless fixed by some prokaryotic organisms such as bacteria and blue-green algae containing nitrogenous enzyme and chemically in the industries Although nitrogen constitutes nearly 78% by volume of the air and this inert gas approximately amounts to 36 thousand tons over each acre of the earth's surface (Young and Johnson 1982) but the living organisms of the world surface from nitrogen unavailability. Nevertheless microorganisms are known to fix an estimated 175 million tons of nitrogen annually (Burns and Hardy 1975) or 75% of our total supply. The remainder is produced as chemical fertilizer in the factories. With the alarmingly rising world population and the declining supply of fossil fuels required to manufacture chemical nitrogen fertilizer, it is becoming increasingly necessary to rely more and more on microorganisms to satisfy plant needs for nitrogen.

In other words, the use of chemical fertilizers as a major input for crop production will not only create fertility problem but the resultant toxicity will also seriously affect the population of natural flora and fauna, causing an ecological imbalance (Haque 1993). Reports are also available that continuous use of chemical fertilizers in rice cultivation degrades the soil, which becomes unfit for further cultivation. Besides this, chemical fertilizers are responsible for environmental polluting.

Although it is not possible to ignore the dependency on chemical nitrogen fertilizers especially for rice production yet, at least, it is essential for the alternatives, which would add fertility to the soil, improve the soil hygiene and be friendly to the environment. Biological nitrogen fixation can occupy that position.

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Some bacteria and blue-green algae are able to reduce atmospheric nitrogen to ammonia. Several of them do even live in symbiosis or in association with green plants. The nodule bacteria (e.g. *Rhizobium*) of legumiosae are best known. They are host-specific. *Rhizobium japonicum* lives in symbiosis with soy beans, *Rhizobium trifolii* with clover, *Rhizobium meliloti* with lucerne. *Anabaena azollae* (a blue-green alga) co-operates with an aquatic fern, *Nostoc muscorum* (another blue-green alga) with the tropic plant *Gunnera macrophylla*. The leguminous genus *Pisum* contains species living in continuous symbiosis with nodule bacteria, others that develop non-functional nodules, and finally such species that do not grow nodules at all, being consequently unable to form symbioses. A number of free-living soil bacteria, e.g. bacteria of the genera *Azobacter* (aerobic), *Closterium* (strictly anaerobic), *Klebsiella* (optionally aerobic), and *Rhodospirillum* (anaerobic, photo synthetically active) belong to the nitrogen reducing species. Nitrogen fixation has been thoroughly covered during the last years, since genetic engineering fosters the hope for techniques improving the nitrogen supply of plants. The production of synthetic nitrogen fertilizer is expensive and extraordinarily costly in terms of energy. Bacteria, too, are not able to produce ammonia at low energy costs, since the triple bond of nitrogen belongs to the strongest covalent bonds occurring in biologically important molecules. The conversion of 1 mole nitrogen to 2 mole ammonia requires 25 moles ATP, i.e. the fixation of 1 gram nitrogen costs 10 g glucose - under favourable conditions. *Azotobacter's* reaction is especially pricey: it needs 100 g glucose for the fixation of 1 g nitrogen. The interactions of *Anabenae-Azolla* symbioses differ from that of leguminosae-nodule bacteria interactions. Little is known about the way in which *Anabena* and *Azolla* recognize each other. *Anabaena* enters the fern's tissue at tip of growing shoots. Nitrogen fixation takes place in specialized cells, the heterocysts that alternate with vegetative, photo synthetically active cells in the alga's filaments. Roughly every tenth cell is a heterocyst. In the case of the *Anabaena-Azolla* interactions, the penetrating *Anabaena* cells are small, and heterocysts are lacking. Heterocyst development starts not before *Anabaena* has colonized the fern tissue and has settled within the intracellular cisterns (Hill 1977). Azolla is common in the rice fields of Eastern Asia, where a considerable amount of the nitrogen bound by this fern is of benefit to the rice plants. Previously mentioned Rhizobium, *Azotobacter*, blue-green algae*, Azospirillum* etc. which are capable of fixing atmospheric nitrogen, which enriches the soil, but in the context of wet land rice culture, free-living blue-green algae (BGA) are the important one to consider as their potential contribution could be 37-150 kg N/ha/cropping season with an average of 27 kg N/ha/crop equaling to the application of 25-30 kg N/ha (Mian 1993).

Blue-green algae (Prokaryotic autotrophy) are common in tropical and sub-tropical regions of the world. They are particularly common in rice field niche. Some blue-green algae can fix atmospheric nitrogen and maintain rice field fertility (De 1939). These organisms are now being used as biofertilizers for rice crop in many countries of the world such as India, Burma, Thailand, Philippines, Vietnam etc (Venkataraman 1981).

The application of nitrogen fixing blue-green algae in rice cropping as biofertilizers has gained much significance in rice cultivation in many rice growing countries of the world (Venkataraman 1981). Reduction of chemical fertilizer input up to 30% by supplementing with blue-green algae is a significant finding when the conservation of energy is contemplated (Venkataraman 1981). One of the important problems that have been noticed in mass multiplications of blue-greens under field conditions is the destruction of algal populations by pesticides applications intended to control the insects and pests of the rice crop (Venkataraman 1973). These chemicals also damaged a wide variety of beneficial microorganisms because of their persistence in the environment (Subramanian 1988). Therefore, pesticides used in routine applications in rice fields have important ecological effects in addition to those usually intended. Several workers have reported the toxicity of a variety of pesticides, herbicides and fungicides to pure cultures of blue-green algae (Ahmad and Venkataraman 1973, Adhikary 1989). The results of their investigations indicate that blue-green algae show variable resistance to different pesticides. Apart from a few published records the effects of pesticides on blue-green algae has not been studied in details. An attempt has, therefore, been made in the present work to determine the effect of the most commonly used pesticide-Furadan on the growth and nitrogen fixation of blue-green algae isolated from different types of soils.

Materials and Methods

The experiment was carried out in the laboratory designed for research on "Azolla and blue-green algae". *Azolla pinnata* R. Br. mosquito Fern, water velvet (English), it is an aquatic plant can spread rapidly, and has the ability to survive on moist soil in and around rivers, ditches, and ponds. It forms dense surface mats, which degrade water quality by reducing oxygen levels, and can interfere with boating, fishing and recreational activities. Azolla is useful as a "soybean plant in rice field", because it can assimilate atmospheric nitrogen gas owing to the nitrogen fixation by cyanobacteria (blue green algae) living in the cavities located at the lower side of upper (dorsal) lobes of leaf. Blue-green algae (BGA), technically known as cyanobacteria, are microscopic organisms that are naturally present in lakes and streams. They usually are present in low numbers. Blue-green algae can become very abundant in warm, shallow, undisturbed surface water that receives a lot of sunlight. All BGA are prokaryotic, i.e. they lack true nucleus, nucleolus and nuclear membrane in their cells. They also lack plastids, flagella, and the sexual mode of reproduction. But somatic fusion of nuclear materials, a kind of genetic recombination known as parasexuality is present in some species. The symbiotic association of the algae aids in the creation of a huge amount of biomass on the surface of the water. It is then harvested, dried and used as biofertilizer to supplement the needs of nitrogen in coffee farms. Some experimental studies with the BGA in Bangladesh have many characteristic features similar to true bacteria and for this reason these are now classified by many with the bacteria and are called cyanobacteria. One of the bacterial features is fixation of the atmospheric nitrogen to soil and water, particularly in tropical countries. This N_2-fixation ability of the BGA was first proved experimentally and conclusively by Dr PK De of the University of Dhaka in 1939 with the soil BGA collected from Faridpur rice-fields. Agriculturally, this information is very important and now a day various BGA are used as biofertilisers to improve soil-fertility in tropical Asia, Africa and Latin American countries. The most commonly used pesticide Furadan 5G was used for the study. For the experiment blue-green algae were isolated from saline soil, calcareous soil and red soil of Bangladesh. For presentation this chapter may be divided into the followings sections:

Soils and selection of sites

Three different types of paddy soils *viz*. saline soil, calcareous soil and red soil were selected for the study. For each type of soil three locations were considered and there were nine locations in total under study. The selected areas were located in three administrative divisions namely Khulna, Rajshahi and Dhaka comprising four Agro-ecological Zones (AEZ) and four general soil types of Bangladesh. Locations, AEZ and General Soil Types under study have been presented in Table 1.

Table 1. Soils, location, AEZ and general soil types under study.

Soils	Locations	AEZ	General Soil types
Saline 1 (S1)	Khulna	G T F	Non-calcareous Dark Grey F Soil
Saline 2 (S2)	Bagerhat	G T F	Non-calcareous Grey F Soil
Saline 3 (S3)	Satkhira	G T F	Calcareous Grey F Soil
Calcareous 1 (C1)	Rajshahi	Low G R F	Calcareous Dark Grey F Soil
Calcareous 2 (C2)	Ishurdi	High G R F	Calcareous Dark Grey F Soil
Calcareous 3 (C3)	ATI, Ishurdi	High G R F	Calcareous Dark Grey F Soil
Red 1 (R1)	M (forest)	M Tract	Deep Red Brown Terrace Soil
Red 2 (R2)	M (Crop field)	M Tract	Deep Red Brown Terrace Soil
Red 3 (R3)	Gazipur Sadar	M Tract	Deep Red Brown Terrace Soil

G=Ganges; T=Tidal; R=River; F= Floodplain; M= Madhupur.

Collection and preparation of soil samples: Soil samples were collected from the rice fields of the selected areas from 0-15 cm depth. After collection the soils samples were divided into equal halves, one for physicochemical analyses and other for Microbiological studies. The soil samples were made free form roots and gravels.

Physicochemical analyses of soil

For physicochemical analyses of soils, composite samples were air dried ground and passed through 10 mesh sieve. Different parameters such as Soil texture, pH, Organic matter content, Total nitrogen, Available Phosphorus, Available Sulfur, Electrical Conductivity (EC), Exchangeable potassium, calcium and sodium, Cation exchange capacity (CEC) etc. were analyzed from the soil samples using standard methods like., Hydrometer method for soil texture, wet oxidation methods for organic matter contents, Kjeldahl digestion method for total nitrogen contents etc. (Table 2).

Table 2. Name of different parameters and its methods used for the study.

Parameters	Methods
Soil texture	Hydrometer method (Piper 1950) and the textural classes were determined following Marshall's "Triangular Co-ordinates" using USDA system.
Soil pH	The pH of soils was measured with the help of glass electrode pH meter using soil water ratio 1:2.5 as described by Jackson (1962).
Organic matter content	The percentage of organic carbon in soil was determined by wet oxidation methods as out lined by Walkley and Black (1935). The percent organic matter content was then calculated by multiplying the percent organic carbon with the Vanbemmelen Factor, 1.73.
Total nitrogen	Total nitrogen content of soils was determined by the Kjeldahl digestion method. Catalyst mixture (K_2SO_4: $CuSO_4$, $5H_2O$: Se = 10: 1: 0.1), 30% H_2O_2 and concentration H_2SO_4 were used for digesting the soil samples. Nitrogen was estimated by distillation with 40% NaOH followed by titration of distillate trapped in H_3BO_3 with 0.01 N H_2SO_4 (Page *et al.* 1989).
Available Phosphorus	Available Phosphorus in soil was determined by extracting the soil samples with 0.5 M $NaHCO_3$ solution of pH 8.5 (Olsen *et al.* 1954). The P in the extract was then determined by developing blue-color with $SnCl_2$ reduction of Phospho-molybdate complex and measuring the color by spectrophotometer at 660 nm wavelength.
Available Sulphur	Available Sulphur in soil was determined by extracting the soil samples with CaCl2 (0.15%) solution. The S content in the extract was determined turbid metrically and the turbid was measured by spectrophotometer at 420 nm wavelength (Black 1965).
Electrical Conductivity (EC)	Electrical conductivity (EC) of aqueous soil extracts, obtained from 1.5 soils to water mixture is by vacuum filtration and measured by filling and EC electrode with soil extract using 0.01N KCl solution to calibrate the meter (Biswas and Mukherijee, 1987).
Exchangeable potassium, calcium and sodium	Exchangeable Potassium, Calcium and Sodium content of soil were determined by Flame photometer after extraction with 1N NH_4OAC of pH 7.0 (Black 1965).
Cation exchange capacity (CEC)	Cation exchange capacity of soil was determined by saturating the soil samples with NaOAC and replacing Na+ from the saturated sample by 1N NH_4OAC (pH 7.0). Sodium in the solution was then determined by Flame Photometer at 589 nm wavelength (Jackson 1962).

Microbiological Study

For microbial study different standard methods like; blue green algae broth and agar media, and soil-water media were followed such as preparation of media, preparation of dilution series, inoculation, isolation and identification of BGA, preparation of agar plates, preparation of unialgal cultures, measurement of dry weight, heterotrophic growth, measurement of nitrogen fixation, tolerance of blue green algae to pesticides,etc.

The composition of BGA broth and agar media:

The following were dissolved in 850ml of distilled water (for BGA broth media):

1.5g $NaNO_3$, 0.04 g K_2HPO_4, 0.075 g $MgSO_4$-$7H_2O$, 0.036 g $CaCl_2$-$2H_2O$, 0.006g Citric acid, 0.006 g Ferric ammonium citrate, 0.001 g EDTA, 0.02 g Na_2CO_3, 1.0 ml Trace metal mix

The pH of the media was adjusted to 7.1 and volume was made to 1000ml with distilled water and was autoclaved or filter sterilized.

Trace metal mix contained: 2.86 g H_3BO_3, 1.81 g $MnCl_2$-$4H_2O$, 0.222g $ZnSO_4$-$7H_2O$, 0.39 g Na_2MoO_4-$2H_2O$

0.079 g $CuSO_4$-$5H_2O$, 0.0494 g $Co(NO_3)_2$-$6H_2O$, (to add 10gm agar for BGA agar media).

Finally the cultures of BGA isolates were treated with Basudin 10g concentrations of 2.5, 7.5, 50, 100, 200 and 500 ppm and were incubated under growth conditions after which dry weight was measured.

Results and Discussion

This chapter presents the results of experiments followed by discussion on different aspects of blue-green algae (BGA) isolated from three different types of soils *viz*. saline soil, calcareous soil and red soil as affected by the used pesticide namely Furadan. The physical and chemical properties of soil were identified to determine the effect of Furadan more precisely. Table 4 shows the physicochemical properties of soils of the study area.

Table 4. Physicochemical properties of soils.

Locations	pH	OM (%)	Textural class	Total N (%)	Av. P (ppm)	Av.S (ppm)	CEC (me/ 100g)	Exchangeable me/100g			EC (ds/m)
								K	Ca	Na	
Khulna (S1)	5.4	1.62	SC	0.15	8.4	18.5	16.5	0.23	14.75	0.08	14.7
Bagerhat (S2)	7.0	1.75	SC	0.13	17.2	22.0	18.0	0.29	15.5	0.30	16.1
Satkhira (S3)	5.7	1.25	SCL	0.08	16.0	13.3	18.0	0.30	16.4	0.52	17.5
Rajshahi C1)	8.2	1.73	Sl	0.12	15.8	21.0	16.8	0.29	13.75	0.33	3.6
Ishurdi (C2)	8.3	1.67	SCL	0.10	12.9	19.0	18.8	0.25	10.75	0.38	2.8
ATI, Ishurdi (C3)	8.4	1.65	CL	0.10	16.8	25.0	15.9	0.27	11.25	0.34	3.7
Madhupr (forest) (R1)	5.2	0.95	CL	0.07	6.3	11.0	14.8	0.14	10.37	0.32	2.8
Madhupr (Crop field) (R2)	5.0	1.23	C	0.08	10.0	12.1	13.3	0.13	11.87	0.33	2.3
Gazipur Sadar (R3)	5.4	1.34	C	0.09	12.5	12.5	16.3	0.12	11.68	0.33	2.9

S= Silty, C= Clay, L= Loam

Heterotrophic growth of BGA

Eighteen isolates of BGA were tested for heterotrophic growth on glucose, sucrose and fructose separately in darkness. The cultures were incubated in the dark for one month with the substrates. All the isolates under study were able to grow under heterotrophic conditions (Table 3) but differences on their substrate preference were observed. All the isolates from saline soils preferred both glucose and fructose. On the other hand, all the isolates from red soils preferred both sucrose and fructose and the isolates from calcareous soils preferred only glucose. Among eighteen isolates four can just survive on sucrose and glucose, two were dead on glucose, eight on sucrose and six on fructose.

Similar pattern was noticed to the substrate preference among the isolates of saline soils and red soils. The isolates from both these types of soils were also able to grow under heterotrophic conditions on fructose. The isolates from both saline soils and calcareous soils were not successful to grow on sucrose.

Various sugars are being continuously produced due to decomposition of organic matter under wetting and drying of paddy fields. The blue-green algal isolates which are able to use sugars will be of additional advantage in rice fields ecosystem because rice fields are frequently distributed due to intercultural operations for which BGA are mixed up with soils below the photic zone. Therefore it is very essential to know the heterotrophic potency of BGA isolates from the Agricultural point of view.

Blue-green algae were long been considered to be exclusively autotrophic. However, certain filamentous and unicellular forms can grow heterotrophically in the dark (Khoja and Whitton 1971, Rippka 1972). Blue-green algae have been reported to grow heterotrophically which is supported only by sugars, the kind of which varies from organisms to organisms (Khoja and Whitton 1975, Rippka 1972). This result also supports the findings of Gallon *et al*. (1991) and Hashem *et al*. (1987) who reported that *Oscillatoria* sp. UCSB-8 can grow heterotrophically on glucose and *Oscillatoria* sp. UCSB-25 can also grow heterotrophically but on fructose.

The frequent occurrence of BGA in soils and in habitats rich in organic matter and also in highly eutrophic environments such as mud flats, drainage ditches, sewage, oxidation ponds and fish culture ponds suggest that they might grow in the dark at the expense of organic nutrients, which may be ecologically important.

Table 3. Heterotrophic growth of BGA isolates on glucose, sucrose and fructose and its dry weight in both heterotrophic and autotrophic condition.

BGA isolates	Substrate			Dry weight (mg/50 ml)	
	Glucose	Sucrose	Fructose	Autotrophic	Heterotrophic
Anabaena S1	+	-	+	4.3	2.9
Nostoc S1	+	-	+	3.5	1.8
Anabaena S2	+	-	+	4.9	3.1
Nostoc S2	+	0	+	3.7	2.6
Anabaena S3	+	-	+	5.4	3.9
Nostoc S3	+	0	+	2.3	1.2
Anabaena C1	+	-	-	4.8	4.5
Nostoc C1	+	-	-	5.5	2.8
Anabaena C2	+	0	-	7.2	4.8
Nostoc C2	+	-	-	6.5	3.6
Anabaena C3	+	0	-	6.9	3.9
Nostoc C3	+	-	-	7.3	4.5
Anabaena R1	-	+	+	5.5	3.8
Nostoc R1	0	+	+	2.8	1.2
Anabaena R2	-	+	+	6.1	3.2
Nostoc R2	0	+	+	2.7	1.9
Anabaena R3	0	+	+	3.1	1.7
Nostoc R3	0	+	+	2.6	1.7

+ = Positive growth, - = dead and 0 = Just survived

Growth performance (dry weight) of BGA

Maximum growth was observed in the isolates of calcareous soils ranging from 5.5 to 7.3 mg/50 ml cultures. The growth in the isolates of saline soils was similar to those in red soils. The growth in the isolates of saline soils ranged from 2.3 to 5.4 mg/50 ml cultures where as in the isolates of red soils ranged from 2.6 to 6.1 mg/50 ml cultures. Variations were also observed among the isolates of a particular type of soil. Results presented in Table 3 show that in case of saline soil growth was maximum in *Anabaena* from Satkhira and was minimum in *Nostoc* which was also from Satkhira in case of calcareous soil maximum growth was observed in *Nostoc* from Agriculture Training Institute (ATI), Ishurdi and the minimum was observed in *Nostoc* also but from Rajshahi. In case of red soil the growth was the maximum in *Anabaena* from Madhupur (crop field) and the lowest observed in *Nostoc* from Gazipur Sadar.

Among the isolates the highest growth of 7.3 mg/50 ml cultures was observed in *Nostoc* from calcareous soil and the lowest was observed also in *Nostoc* but from saline soil, which was a 2.3 mg/50 ml culture. The pattern of growth in heterotrophic cultures of the BGA isolates was different from those in autotrophic cultures. Furthermore, the values for growth (dry weight) obtained under heterotrophic conditions where less than those obtained under autotrophic conditions (Table 3).

The growth of BGA isolates from calcareous soils was faster than those from saline and red soil, might be due to that the pH of calcareous soil is neutral to slightly alkaline and also due to accumulation of $CaCO_3$ in calcareous soils which favour the growth of BGA. In contrast, red soils and saline soils have low pH values. Furthermore, nutritional problems in saline soils and red soils might be the reason for slower growth in these two soils.

The values for growth obtained under heterotrophic conditions were, however, found to be less than those attained under autotrophic conditions. The decreased growth rates observed in BGA under heterotrophic conditions have been variously ascribed by Stewart (1977) to permeability problem in the uptake of organic substances; by Carr (1973) to lack of transcriptional control, to inability to use the sugars (Rippka 1972) and to inefficient oxidative phosphorylation.

Nitrogen fixation by BGA isolates under autotrophic conditions

Marked variations in pattern of nitrogen fixation were displayed by the isolates. Nitrogen fixation was the maximum in the isolates of BGA obtained from calcareous soil followed by those from saline soils. The minimum nitrogen fixation was recorded in the isolates of red soils. Nitrogen fixing capacity by the isolates of a particular type of soil was also varied. In saline soil nitrogen fixation was recorded slightly higher in *Anabaena* than that in *Nostoc* but in calcareous soils nitrogen fixation in *Anabaena* and *Nostoc* was almost the same. *Anabaena* from red soils fixed nitrogen higher than the value that was done by *Nostoc*. Nitrogen fixation by the isolates was at an increasing rate up to 50 days in all types of soils studied.

Nitrogen fixation by BGA isolates heterotrophic conditions

The pattern of nitrogen fixation in heterotrophically grown cultures was similar to that in autotrophically grown cultures but the rate of nitrogen fixation was higher in heterotrophically grown cultures compared to that in autotrophically grown cultures (Figs. 1 to 3).

The higher nitrogen fixation in heterotrophically grown cultures may be explained by the availability of exogenous sugars. Reports are available that the rate of respiration is higher under heterotrophic condition than under autotrophic conditions (Hashem *et al.* 1990) Respiration is needed to support nitrogenous activity and the higher nitrogen fixation observed in heterotrophically grown cultures may be related to the higher rate of respiration. Furthermore, a higher rate of respiration in heterotrophically grown cultures will lead to lower intracellular conc. of O_2 as a result; the rate of O_2 inactivation of nitrogenous would be decreased. It is, therefore, important to choose BGA that fix nitrogen under autotrophic and heterotrophic conditions. This is essential because of diverse condition under which BGA may occur and fix nitrogen. Also because the paddy field ecosystem is constantly changing physically, chemically and biologically. The nitrogen fixation in BGA likewise will also change.

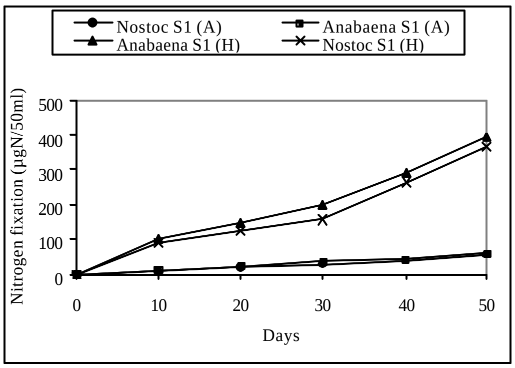

Fig. 1. Nitrogen fixation by BGA isolates of saline soils under autotrophic (A) and heterotrophic (H) conditions.

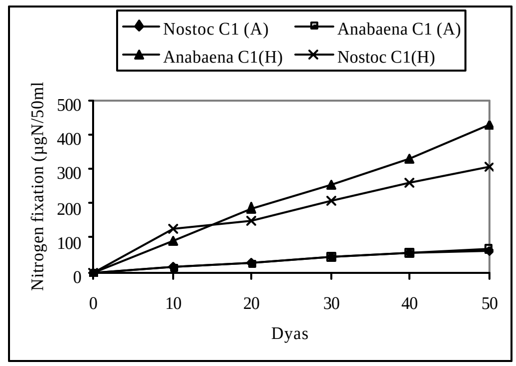

Fig. 2. Nitrogen fixation by BGA isolates of calcareoussoils under autotrophic (A) and heterotrophic (H) conditions.

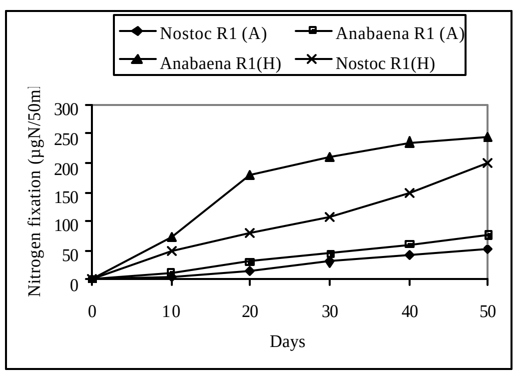

Fig. 3. Nitrogen fixation by BGA isolates of red soils under autotrophic (A) and heterotrophic (H) conditions.

Effect of Furadan on the growth of BGA

The results on the effect of Furadan 5G on the growth of BGA isolates of the selected soils have been presented in Figs. 7 to 9. Furadan did not affect results show that the growth of BGA isolates under autotrophic conditions up to concentration of 50 ppm, though the recommended dose of this pesticide for field application is 4.4 ppm (Figs. 4 to 6). An increase in growth in heterotrophically grown cultures occurred after the application of Furadan at concentration up to 500 ppm. Furthermore, compared to autotrophically grown cultures was slower with respect to growth at low concentration.

The BGA isolates were observed more tolerant to Furadan in comparison to Basudin. In autotrophically grown cultures growth was stimulated up to 50 ppm of Furadan and in heterotrophically grown cultures up to 500 ppm. The results of the study indicate that the effects of Basudin and Furadan are stimulatory at field dose on the growth of BGA under autotrophic conditions. The stimulatory effect of pesticides on BGA has been reported by Gangawane and Saler (1979). Under heterotrophic conditions BGA can tolerate high concentration of Pesticides for their growth. However, to have clear conclusion further study is required.

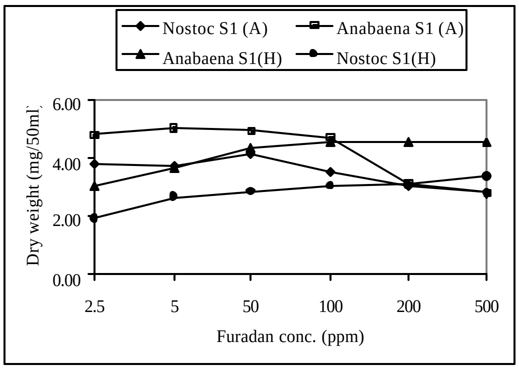

Fig .4. Effect of Furadan on growth of BGA isolates of saline soils under autotrophic (A) and heterotrophic (H) conditions.

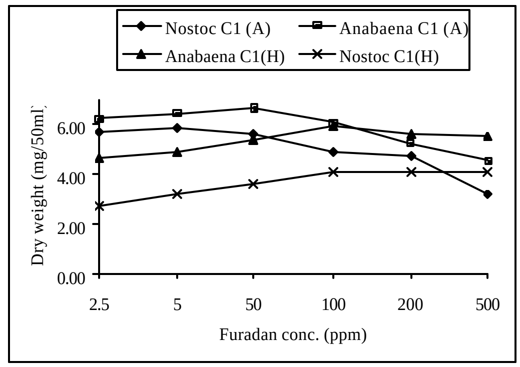

Fig. 5. Effect of Furadan on growth of BGA isolates of calcareous soils under autotrophic (A) and heterotrophic (H) conditions.

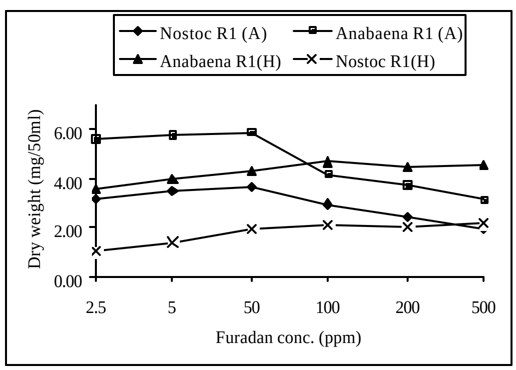

Fig. 6. Effect of Furadan on growth of BGA isolates of red soils under autotrophic (A) and heterotrophic (H) conditions.

Effect of Furadan on nitrogen fixation by BGA

Nitrogen fixation in autotrophically grown isolates increased after the application of Furadan at concentration up to 50 ppm (Figs. 7 to 9). In heterotrophically grown cultures nitrogen fixation increased by Furadan up to highest concentration used. Heterotrophically grown cultures fixed nitrogen at rates higher than that did by types of BGA isolates showed the similar pattern of nitrogen fixation after the application of Furadan. The BGA isolates were observed more tolerant to Furadan in comparison to Basudin. In autotrophically grown cultures nitrogen fixation was stimulated up to 50 ppm of Furadan and in heterotrophically up to 500 ppm.

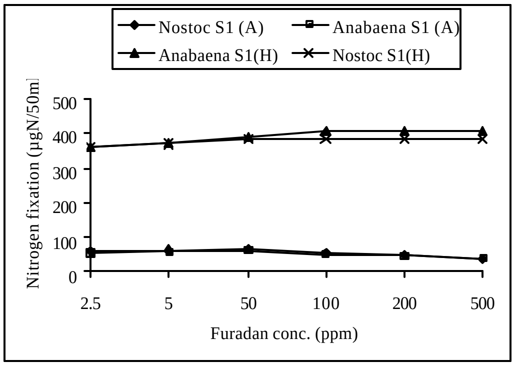

Fig. 7. Effect of Furadan on nitrogen fixation by BGA isolates of saline soils under autotrophic (A) and heterotrophic (H) conditions.

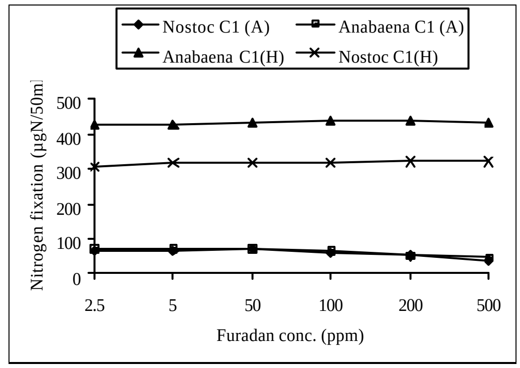

Fig. 8. Effect of Furadan on nitrogen fixation by BGA isolates of calcareous soils under autotrophic (A) and heterotrophic (H) conditions.

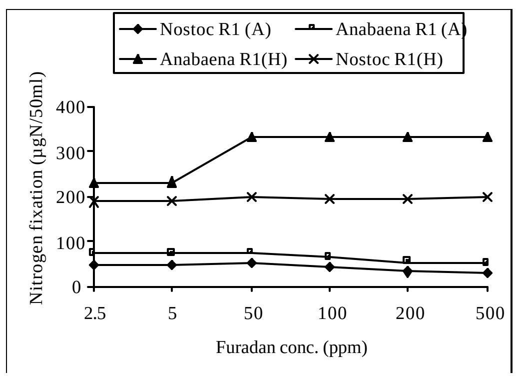

Fig. 9. Effect of Furadan on nitrogen fixation by BGA isolates of red soils under autotrophic (A) and heterotrophic (H) conditions.

The results of the study indicate that the effects of Furadan are stimulatory at field dose on the growth and nitrogen fixation by BGA under autotrophic conditions. Heterotrophically grown cultures were more tolerant to pesticides with respect to growth and nitrogen fixation.

Conclusion

Experiments were conducted with a view to determining the effect of pesticides on the growth and nitrogen fixation by blue-green algae (BGA) isolated from different types of paddy soils. All the isolates under study were tested for their ability to grow heterotrophically on glucose, sucrose and fructose separately in complete darkness. The isolates under study were capable of growing heterotrophically but they preferred different substrates. The isolates from saline soil were able to grow on both glucose and fructose. In contrast, isolates from red soil were successful to grow on both sucrose and fructose and the isolates from calcareous soil preferred only glucose. The growth rate was the maximum in the isolates of calcareous soil. The growth rate in the isolates of saline soil was similar to those in red soil. The values for growth (dry weight) observed in heterotrophically grown cultures were less than those observed in autotrophically grown cultures.

Marked variations in pattern of nitrogen fixation were displayed by the isolates of BGA. The maximum nitrogen fixation was observed in the isolates in the calcareous soil followed by those from saline soil. The minimum nitrogen fixation was observed in the isolates of red soil. Nitrogen fixation capacity among the isolates of particular type of soil was also varied. The nitrogen fixation capacity in the isolates of BGA under autotrophic conditions was similar to those under heterotrophic conditions except that the rate of nitrogen fixation under heterotrophic condition was higher compared to that under autotrophic condition.

The recommended doses of Furadan for field application are 7.5 and 4.4 ppm respectively. The growth under autotrophic condition was not affected up to 50 ppm, though the recommended dose is 4.4 ppm. In heterotrophically grown cultures an increase in growth was recorded at concentration up to 500 ppm. Under heterotrophic conditions BGA can tolerate high concentrations of pesticides for their growth.

The effect of Furadan was also studied on nitrogen fixation by BGA isolates where nitrogen fixation in autotrophically grown cultures was also increased up to 50 ppm but in heterotrophically grown cultures nitrogen fixation was increased up to 500 ppm. Heterotrophically grown cultures fixed nitrogen at rates higher than that did by autotrophically grown cultures at all the concentrations used.

From the present study it seems clear that the effect of Furadan on the growth and nitrogen fixation by BGA isolates was stimulatory under autotrophic conditions and more tolerant with respect to growth and nitrogen fixation.

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