Volume : 2 | Issue : 8 | August 2013 • ISSN No 2277 - 8179

Research Paper

Toxicity of Agricultural Herbicide 2, 4-Dichlorophenoxyacetic acid on growth, Photosynthesis and Respiration of Rice Field Cyanobacterium

Botany

KEYWORDS : Cyanobacteria: Synechococcus aeruginosus; herbicides; 2,4-D; photosynthesis; respiration ; rice field.

Bhagya Lakshmi Jyothi. K.

Department of Biotechnology,Dr.Lankapalli Bullayya College,Visakhapatnam, Andhra Pradesh,India.

ABSTRACT

One of the most commonly used paddy field herbicide in India is 2, 4-Dichlorophenoxy acetic acid. A study was conducted to investigate the toxic effect of 2, 4-D on the growth, cellular metabolites, rate of photosynthesis, (O2 evolution) and rate of respiration (O2 consumption) in a Synechococcus aeruginosus collected from rice field soils of Krishna district, Andhra Pradesh, India and grown in Hughe's medium (1958) as modified by Allen (1968). An experiment was designed to find out the toxic effect of 2, 4-D on the growth of Cyanobacterium when exposed to different concentrations and time intervals incubated at 500, 800 and 1000 mg per ml concentration of herbicide for 0, 24 and 48 hrs time intervals. The increasing dose of 2,4-D and treated / incubation time (immediately on the addition of chemical 0,24and48 hours)gradually declined the rate of photosynthesis and respiration. The herbicide reduced the cellular metabolites gradually with the increase of 2,4-D concentration and exposure time.

Introduction

In tropical countries such as India, the blue–green algae inhabitated mainly in the paddy fields (Singh, 1961; Venkataraman, 1972) and play a considerable leading role in the ecosystem of rice agriculture (Watanabe and Brotonegoro, 1981). The most important functional characteristics of blue green algae growing in the soils of paddy – fields are the atmospheric carbon and dinitrogen fixation (Stewart W.D.P.1970) and reclamination of the soil by enriching with nitrogenous substances and metabolites to obtain more crop yield and reduce application of nitrogenous and chemical fertilizers.

To obtain a high yield of grains formers employ synthetic chemical fertilizers in the rice – fields, which enrich the nitrogen content and phosphate content. Because of the abundance of nitrogen and phosphates in the water and soil, growth of unwanted angiospermic weeds is promoted viz. Cyperus rotundes Linn(Cyperaceae), Ischaemum pilosum hack (Graninaceae) etc. (Tadulingam & Narayana, 1955). Weeds compete rice plants for CO_2, space, moisture, sunlight and nutrient, results in low agricultural output. It has been observed that grain yield in paddy is drastically reduced if it is not deweeded at early stage of growth (Dangwal et al, 2012).

Nowadays, to avoid high labour costs, farmers have been heavily employing pesticides in crop-fields and rice-fields (Brian, 1964) to eradicate weeds (Thakur *et al., 1995; Amaral-As-Dos and Dos-S-Amaral, 1997; Street and Kurtz, 1998) and pest organisms (Battah et al., 2001) in agricultural practices and these chemicals affect secondarily the metabolism of non-target organisms such as blue-green algae (Cyanobacteria) widely distributed in rice-fields (Battah et al.,2001; Nirmal Kumar et al, 2012) and occupy an important position within soil and water microflora.*

Among the metabolic processes, photosynthesis and respiration are the important metabolic processes of photoautotrophic organisms such as algae. Majority of the herbicides inactivates photosynthesis and respiration by interfering photophosphorylation, pigments, nitrogen fixation, lipid metabolism and cell division and finally retard the growth (Mishra and Pandey, 1989; Victor Galhang et al, 2009; Okmen et al, 2013). Generally it appears that herbicides affect more than one metabolic reaction in the cell.

The herbicides DCMU (3-(3, 4 –dichlorophenyl), 1, 1-dimethyl urea), Diuron, 2 4 –D and maleic hydrazide inhibited the growth and oxidation of water in photosynthetic algae (Hamdi et al., 1970; Corbett, 1974; Dodge, 1975) and stam F-34 (Propanil) inhibited respiration (Hofstra and Switzer, 1968) and reduced the cytochrome of photosystem II of photosynthesizing isolated chloroplasts (Nishimura and Takamiya, 1966). All the urea herbicides (DCMU) were known to inhibit the hill reaction in photosynthesis (Corbett, 1974) and competed with plastoquinone (PQ) binding proteins and interrupted the electron transport system (Sherman et al., 1987). In Chlorella pyrenoidosa Wedding et al., (1954) reported in inhibition of photosynthesis and respiration by the herbicide 2, 4-D which also inhibited the growth, protein and chlorophyll-a content of Chlorella vulgaris and Spirulina platensis cells (Saadet and Ozlem, 2008). In Synechococus aeruginosus and Scenedesmus incrassatules 2, 4-D inhibited the activity of amylase (Reddy et al., 1987).

Tiwari et al. (1982) observed the inhibition of growth, photosynthetic pigments, nitrogen fixation and macromolecules by 2, 4-D in Anabaena cylindrica. In Chlorella pyrenoidosa Bertagnolli and Nadakavukaren (1974) reported the inhibition of photosynthesis and respiration in terms of 02 consumption and liberation respectively.

Because of the wide applicability of 2, 4-D in rice-fields, the deleterious effects of 2, 4-D on nitrogen fixation (Inger, 1970; Hamdi et al., 1970; Tiwari et al., 1982) and growth of blue-green algae (Singh, 1974; Tiwari and Pandey 1981; Das and Singh, 1978) were reported but the literature is meager and scanty on 02 evolution and consumption of blue-green algae. Keeping this in view, the present study was undertaken to observe the effects of 2, 4-D on photosynthesis and respiration in terms of 02 liberation and consumption respectively and cellular metabolites of Synechococcus aeruginosus.

Synechoeoccus aeruginosus cyanobacterium found abundantly in rice – fields and 2 4-D is a strong herbicide having a wide practical applications in the fields of rice crops and cereals used to arrest a number of broad leaf weeds.

MATERIALS AND METHODS

Isolation of Test Organism

Synechococcus aeruginosus a prokaryotic unicellular, blue-green algae is very commonly found in rice-fields, chosen for the present study was isolated from rice fields of Krishna District (Andhra Pradesh). Isolation and purification were performed by the usual protocols of dilution and plating.

Raising of clonal population:

Single individual cells are pipetted out on agarised (1.5%) basal medium plates and incubated in wooden culture chambers fitted with cool fluorescent lamp (600 lux). Visible colonies were picked up singly by fine glass micropipettes under a binocular, dissecting microscope and inoculated into basal liquid medium. The process was repeated two to three times in order to get uni algal clonal population.

Axenic culture:

The obtained unialgal cultures were made bacteria free by ultra violet irradiation following the method described by Venkataraman (1969) and the axenic cultures are maintained in the Hughe's medium as stock cultures in the laboratory.

Volume : 2 | Issue : 8 | August 2013 • ISSN No 2277 - 8179

Culture conditions:
The axenic populations of the alga are maintained in hughe's medium and experiments are also conducted in a Hughe's medium and experiments are also conducted in the same medium. Wooden cabinets fixed with fluorescent lamps emitting a light intensity of 600 lux are used for incubation of the experimental and stock cultures which are maintained at 280 + 20C. The light and dark cycles was adjusted to 16h/82h and cultures were shaken by hand two to three times daily to prevent clumping of algal cells.

Culture medium:
Synechococcus aeruginosus was grown in Hughe's medium (1958) as modified by Allen (1968) which in composed of distilled water and the following chemical ingredients (mg/L); Na No3 1500; Na2 Co3 20; Mg So4. 7 H20 75; K2 H P04 30; Ca Cl2 27; Na2 Si O3: 9 H20 59; EDTA 1; Citric acid 6 6; Ferric citrate: 6 and trace elements liquid from stock solutions 1 ml/L. (Ingredients of trace elements are H3 BO3 2860; Mn Cl2 1810; Zn SO4 220; Mo Co3 390; Cu So4 80; Co (No3)2 495). The pH of the medium was adjusted to 8.5 either with 0.1 N Na OH/ 0.1 N Hcl.

The medium was sterilized at 1210C, 1.05 kg/ cm2 for 30 min.

Treatment with herbicide:
2, 4-D was used to treat the alga and it was purchased from commercial source and the effective doses 500,800,1000 mg/ml of 2,4-D were selected for the present study. The fresh stock solutions of 2, 4-D are prepared by dissolving the chemical in sterilized basal medium mixed with 0.1 N sodium hydroxide. The desired concentration of 2, 4-D (500, 800, 1000 mg/ml) are prepared by making proper dilution of the stock solution. Freshly prepared herbicide solution was used to perform the experiments. All experimental cultures including control are set up in triplicate. Three replicates are made for every herbicide concentration and control.

Methodology
Short-term experiments are performed to study the effect of 2, 4-D (herbicide) on photosynthesis (02 evolution) and respiration (02 consumption). In these experiments varied concentrations of 2, 4-D are added to 10 days old algal culture in the basal medium adjusted to 8.5 pH and buffered with Tris (0.001 mg per ml). After the addition of different concentrations of 2, 4-D immediately (0 hour), 24 and 48 hours incubation, the rate of 02 evolution and 02 consumption are determined by photowarburg's monometric method (Umbreit et al., 1959). Control experiments without herbicide are also performed. After introduction of 5 ml of algal culture into reaction vessel, 02 evolution and consumption are measured at regular intervals of 10 minutes upto 30 minutes. The temperature was always kept at 28 + 20C and a light intensity of 1,000 Lux was provided for 02 evolution experiments. The rate of 02 evolution was expressed as micromoles (μM) of oxygen for 100 mg fresh weight of algae. Immediately after the measurement of photosynthetic rate, 0.2 ml of 10 percent potassium hydroxide (KOH) absorbed paper was introduced carefully into the central well of the reaction vessel to absorb the liberated carbon dioxide (CO2) and to increase the exposed area of the alkali. The rate of respiration was measured in the dark at 28 + 20C and expressed as 'μ' moles of 02 consumption per 100 mg fresh weight of algae. Besides the effect of 2, 4-D on 02 evolution and consumption, its effects are also observed on growth in terms of chlorophyll-a and on changes in cellular metabolites after 48 hours of incubation period.

Growth estimations:
The growth of the *Synechococcus aeruginosus* was measured in terms of proteins as well as optical density of 80 percent acetone soluble pigment viz. chlorophyll-a at 0, 2 4 and 48h.

Determination of chlorophyll-a:
Algal cultures are centrifuged at 5000 rpm for five minutes and the supernatant was drained off. The algal pellet was suspended in 5 ml of 80 percent acetone and kept overnight in refrigerator at 10^{0} centigrade and then centrifuged.

Absorbance of acetone extracted chlorophyll-a was measured at 660nm in a spectrophotometer and estimated by using the formula of Mac Lachlan and Zalik (1963) as mentioned by Holden (1976) on fresh weight basis (1963).

Cell metabolite contents i.e. carotenoids (Jensen, 1978), biliproteins (Siegelman & Kycie, 1978), proteins (Lowry et al, 1951), carbohydrates (Spiro, 1966), α-amylase enzyme (Oser, 1965 & Bernfeld,1955); intracellular acid phosphatase (Patni &Aaronson, 1978), RNA (Elliot & Wald ,1954), DNA (Fiske & Subba Rao ,1925) was determined at regular intervals.

RESULTS
The results in Table 1 and 2 indicate the adverse effect of 2, 4-D on growth in terms of optical density of chlorophyll-a and the rate of photosynthesis and respiration at '0' hour time and significant effects have been noticed after 24 and 48 hours period. In control cultures of Synechococcus aeruginosus the growth and rate of 02 evolution and consumption were augmented with the incubation period, whereas a reverse trend was noticed in 2, 4-D treated culture. The rate of photosynthesis and respiration was increased during the observed intervals (10, 20 and 30 minutes) irrespective of concentration of 2, 4-D at 0, 24 and 48 hours incubation. Cellular metabolites (chlorophyll-a, carotenoids, carbohydrates, proteins, nucleic acids, biliproteins and the activity of α-amylase and acid phosphatase) are also estimated in 2, 4-D containing algal cultures after 48 hours to know the immediate toxic effects of herbicide on the synthesis of various algal cell components.

The contents of cellular metabolites of algae are gradually decreased with the increase of 2, 4-D concentration than control. At 1,000 μg per ml concentration of 2, 4-D (sub-lethal dose) the percent inhibition of cellular metabolites (chlorophyll-a, carotenoids, carbohydrates, proteins, RNA, DNA, C-phycocyanin and allophycocyanin) and the activity of α-amylase and acid phosphatase were 52.29, 86.84, 88.23, 53.12, 57, 79.83, 80.67, 88.67, 85.17 and 82.60 percent respectively. Among the cellular metabolites, C-phycocyanin, allophycocyanin, carbohydrates and DNA are significantly inhibited at 1000 μg per ml 2, 4-D dose more than 50 percent as compared to other cellular metabolites. The percent inhibition of algal cellular metabolites in short-term experiments indicates that the toxicity of 2,4-D is dose-dependent as well as time dependent .

Table 1: Short-term effect of 2, 4-D on photosynthesis and respiration in *Synechococcus aeruginosus*

Time (hr)	Concentration of 2, 4-D (μg/ml) in the BM	O.D.	Photosynthesis			Respiration		
			μ moles 02 evolved /100 mg f.w.			μ moles 02 Consumed/100 mg f.w.		
			10 min	20 min	30 min	10 min	20 min	30 min
0	0 (Control)	0.052	76.95	94.95	116.10	72.97	63.18	76.50
	500	0.053	68.59	87.07	105.68	54.66	60.78	68.58
	800	0.050	65.16	78.21	85.86	46.16	52.46	55.25
	1000	0.048	60.15	62.63	68.64	38.48	40.35	45.28
		(7.69)	(21..83)	(34.03)	(40.87)	(47.26)	(36.13)	(40.81)

Time (hr)	Concentration of 2, 4-D (µg/ml) in the BM	O.D.	Photosynthesis			Respiration		
			µ moles 02 evolved /100 mg f.w.			µ moles 02 Consumed/100 mg f.w.		
			10 min	20 min	30 min	10 min	20 min	30 min
24	0 (Control)	0.090	82.76	104.40	110.64	74.65	75.96	80.95
	500	0.034	60.42	75.98	93.37	69.25	72.36	79.50
	800	0.030	46.75	58.43	75.96	55.25	60.50	65.15
	1000	0.25	28.71	40.75	66.94	50.29	52.25	53.46
		(72.22)	(65.30)	(60.96)	(39.49)	(32.63)	(31.21)	(33.95)
48	0 (Control)	0.110	99.93	112.50	115.12	78.76	86.25	89.76
	500	0.030	50.78	56.44	61.00	65.15	67.25	72.35
	800	0.020	33.90	40.31	56.50	48.25	50.15	52.25
	1000	0.15	25.80	38.40	44.81	(40.36)	42.18	45.65
		(86.36)	(74.18)	(65.86)	(61.07)	(48.75)	(51.09)	(49.14)

2, 4-D =2, 4-Dichlorophenoxyacetic acid; hr = hours; min = minutes; f.w. = fresh weight; BM = Basal medium; O.D. = Growth in terms of optical density at 663 nm.

Table 2: Effect of 2, 4-D on cellular metabolites of *Synechococcus aeruginosus* during O2 evolution (photosynthesis) and consumption (respiration) experiments

Time (hr) (48h)	Concentration of 2, 4-D (µg.ml) in BM	Chlorophyll-a (mg/g f.w.)	Carotenoids (mg/ml)	Carbohydrates (µg/100 mg f.w.)	Proteins (µg/100 mg f.w.)	RNA (µg/100 mg f.w.)	DNA (µg phosphate/ 100 mg f.w.)	C-phyco-cyanine (mg/ml)	Alloph-yco-cyanine (mg/ml)	A-amylase (µg maltose released/ 15 min/100 mg protein)	Acid phosphatase µg phosphate released /30 min/100 mg protein)
	0 (Control)	0.143	0.013	100.32	113.39	37.60	20.12	0.022	0.017	181.92	43.43
	500	0.125	0.0101	97.33	100.25	33.41	19.68	0.021	0.013	171.45	38.42
	800	0.114	0.009	93.42	98.43	29.84	17.91	0.016	0.009	169.86	32.61
	1000	0.102	0.009	88.35	95.42	25.79	14.33	0.014	0.005	158.65	27.58
		(28.67)	(30.75)	(11.91)	(15.84)	(31.40)	(28.77)	(36.36)	(70.58)	(12.79)	(38.49)
	0 (Control)	0.163	0.021	121.43	137.52	49.77	30.72	0.038	0.022	209.98	57.76
	500	1.116	0.008	90.25	91.13	26.71	13.34	0.016	0.012	151.32	30.13
	800	0.111	0.007	81.59	81.32	19.85	10.21	0.013	0.008	142.29	26.96
	1000	0.101	0.004	75.32	77.32	17.82	8.25	0.010	0.004	121.35	23.20
		(38.03)	(80.95)	(37.97)	(43.77)	(64.19)	(73.14	(73.68)	(81.81)	(42.20)	(59.83)
	0 (Control)	0.192	0.038	132.26	157.49	64.61	42.43	0.053	0.034	275.11	71.45
	500	0.108	0.007	85.16	80.24	20.90	10.91	0.012	0.009	121.02	21.49
	800	0.099	0.006	70.45	73.71	17.84	9.08	0.009	0.006	101.90	18.64
	1000	0.082	0.005	62.00	67.72	13.03	6.21	0.006	0.004	80.30	12.43
		(57.29)	(86.84)	(53.12)	(57.00)	(79.83)	(85.17	(88.67)	(88.23)	(70.81)	(82.60)

2, 4-D = 2, Dichorophenoxyacetic acid; hr = hours; min = minutes; f.w. = fresh weight; BM = Basal medium; Pnp = p-nitrophenol; ( ) = Figures in bracket indicate the percent inhibition at 1000 µg per ml 2, 4-D over the control.

DISCUSSION

The blue-green algae are an important segment of soil and water logged paddy fields of tropical countries and their significance as bio-fertilizers contributing to the nitrogen fertility was well documented (Venkataraman, 1972; Watanabe and Furusaka, 1980). Much of the information on the effects of numerous herbicides on algal growth has been reviewed ((Chinnaswamy and Patel, 1984). Among the herbicides, 2, 4-D, a cheapest herbicide, has been commonly employed in the rice fields to control weeds and to enhance the crop yield (Street and Kurtz, 1998) and also for its effect on nitrogen fixation of blue-green algae (Inger, 1970).

The data obtained on *Synechococcus aeruginosus* indicated that 2, 4-D inhibited the O2 evolution (photosynthesis) and O2 consumption (respiration) gradually, evidencing the interference and reduction of photosynthesis and respiration and the cellular metabolites with increase in the concentration and time. (Tables 1 and 2). Wedding *et al.* (1954) reported that inhibitory effect of

Volume : 2 | Issue : 8 | August 2013 • ISSN No 2277 - 8179

2, 4-D on photosynthesis and respiration of *Chlorella pyrenoidosa* which are decreased depending upon the pH and concentration of undissociated 2, 4-D molecule in the growth medium. Anderson and Thomson (1973) reported that 2, 4-D caused the destruction of chloroplast thereby affecting the photosynthesis in plants and microorganisms. But in blue-green algae, photosynthetic pigments are attached on thylakoids (Fogg *et al.,* 1973; Vanden Hoek *et al.,* 1995) and arranged in arrays (Sherman *et al.,* 1987). Bertagnolli and Nadakavukaren (1974) carried out the experiments on the effect of 2, 4-D in *Chlorella pyrenoidosa* and postulated that the herbicide 2, 4-D damaged the chloroplast and also interfered in photosynthesis and respiratory process of algal cells. Wedding and Black (1962) studied the effect of 2, 4-D on *Chlorella pyrenoidosa* cells and reported that 2, 4-D enhanced, the inhibition of the incorporation of phosphate (P-32) into ATP which is a product formed in photophosphorylation and oxidative phosphorylation. Pfister and Urbach (1983) reported that the herbicides generally interfered with respiratory process and inhibited the action of the electron transport system and the related enzymes resulting in affecting the oxidative phosphorylation. The herbicides interfere with the photochemical activity of photosynthetic pigments which cause limiting of the availability of chemical energies as ATP and the reducing power as NADPH, resulting non-availability of energies and reducing power must be leading to the production of phytotoxicity.

The inhibition of photosynthetic electron transport system was caused due to binding up of parathion and pesticide to certain proteinaceous molecules located on the surface of photosynthetic membranes (Bose and Saroja-Subbaraj, 1984). It is well known from literatures that 2,4-D herbicide cause inhibition of thylakoid electron transport chain (Boger and Sandmann,1998; Rutherford and Krieger –Lisz-kay,2001; Jones,2005;Moreland 1980;Campbell et al., 1998;Kobbia et al .,1991; Xia,2005;Galhano et al.,2009), therefore we can assume that the function of PS II oxygen evolving complex is affected by 2,4-D herbicide. The inhibition of respiratory O2 consumption by 2,4-D could be related with the inhibition of photosynthesis as both processes share common intermediates in the cyanobacteria electron transport chain (Schmetterer,1994).

The detrimental effects of 2, 4-D in *Synechococcus aeruginosus are* expressed by the percentage reduction of the quantities of cellular metabolites which may attribute and give clue to the site of action of 2, 4-D in *Synechococcus aeruginosus.* The percentage of inhibition of chlorophyll-a, carotenoids, carbohydrates, proteins, RNA, DNA, C-phycocyanin, allophycocyanin and the activities of α amylase and the acid phosphatase at 1000 mg per ml 2, 4-D after 48 hours incubation were 57.29, 86.84, 53.12, 57, 79.83, 85.17, 88.67, 88.23, 70.81 and 82.10 respectively. Among the cellular metabolites the percent inhibition of C-phycocyanin, allophycocyanin, carbohydrates and RNA were high as compared to other cellular metabolites. Probably it appears that 2, 4-D got absorbed primarily by the cell wall of blue-green alga and bio-accumulated on the thylakoids membranes and it affected the pigment synthesis of photosynthesis of blue-green alga *Synechococcus aeruginosus* (Biliproteins, Chlorophyll) as reported by Tiwari *et* al (1982) followed by inactivating the photosynthesis and respiration process (Tables 1 and 2) or by impairing the pigments and uncoupling the phosphorylation as suggested by Wedding and Black (1962) and Pfister and Urbach (1983) which in turn might have led to affect the other metabolic pathways thereby reducing the cellular metabolites (Tables 1 and 2) and the growth of *Synechococcus aeruginosus.*

The inhibition of respiration by these chemicals (herbicides) can be attributed to affect the permeability of cell wall and bio-accumulation on the thylakoids as mentioned in *Chlorella* sp. and damage the thylakoids membranes caused by binding of 2, 4-D to certain proteinaceous molecules located on the surface of these membranes (Bose and Saroja-Subbaraj, 1984). Moreover, blue-green algal respiration takes place mostly from thylakoid membranes where pigments are arranged in arrays and thereby the herbicide interferes with the common components of electron transport system of respiration and photosynthesis (Peschek, 1987; Kumar, 1999) thereby affecting the uncoupling of phosphorylation and inhibition of carbondioxide production as suggested in microorganisms by Bartha *et al.* (1967).

Conclusion

Experiments are conducted with a view to determining the deleterious and differential effects of herbicide on the photosynthesis and respiration as well as cellular metabolites of Cyanobacteria. Results suggested that S*ynechococcus aeruginosus* was the most susceptible organism to 2,4-D .The herbicide highly affected the photosynthesis and respiration in this alga with the increased dose of the herbicide and duration of incubation time. Besides the above results, further suggests that the growth of the alga was reduced followed by decrease of the quantities of cellular metabolites. In conclusion , although the 2,4-D is useful agent to control the growth of weeds, their application should be considered because of its toxicity to the rice-field Cyanobacteria.

Acknowledgements

The author is thankful to the Department of Botany, Andhra University, Visakhapatnam ,Andhra Pradesh, India for providing facilities required to carry out this work.

REFERENCE

1.Amaral-AS-dos and Dos-S-Amaral-A. (1997). Herbicide management for weedy rice control. Lavoura – Arrozeira 50: 13-15. | | 2.Anderson, J.L. and Thomson, W.T. (1973). Residue Rev. 47: 167-189. | | 3.Baratha, R. Lanzilotta, R.P. and Pramer, D. (1967). Stability and effects of some pesticides in soil. Applied Microbiol. 15: 67-75 | | 4.Battah, M.G., Shabana, E.F., Kobbie, I.A. and Eladel, H.M. (2001). Differential effects of thiobencarb toxicity on growth and photosynthesis of Anabaena variabilis with changes in phosphate level. Ecotoxicol Environ. Saf. 49: 235-239. | | 5.Belma Aslim and Sahlan Ozten K(2009). "Toxicity of herbicides to cyanobacterial isolates"-Journal of Environmental Biology, 30(3) 381-384 . | | 6.Bertagnolli, B.L. and Nadakavukaren, M.J. (1970). Effect of 2, 4-dichlorophenoxyacetic acid on the fine structure of Chlorella pyrenoidosa. Chick. J. Phycol. 6: 98. | | 7.Bertagnolli, B.L. and Nadakavukaren, M.J. (1974). Some physiological responses of Chlorella pyrenoidosa to 2, 4-Dichlorophenoxyacetic acid. J. Expl. Bot. 25: 180-188. | | 8.Boger P,Sandman G.(1998). Action of modern herbicides. In: Raghavendra AS, editor, Photosynthesis A Comprehensive Treatise. Cambridge: Cambridge University Press. pp337-351. | | 9.Bose, S. and Saroja – Subbaraj, G. (1984). Photosynthetic behaviour of cells of Chlorella protothecoides adapted to methyl parathion. Ind. J. Exptl. Biol. 22: 374-378. | | 10.Brian, R.C. (1964). Weed Res. 4: 105-117. | 11.Butler, G.I. Dear son, T.R. and O'Kelly, J.C. (1975). The effect of Atrazine, 2, 4-D Methoxychlor, Carboryl and Diazinon on the growth of planktonic algae. Br. Phycol. J. 10: 371-376. | | 12.Campbell D. Hurry V. Clarke AK, Gustafsson P. Oquist G.(1998). Chlorophyll fluorescence analysis of cyanobacterial photosynthesis and acclimation. Microbial Mol Biol Rev 62:667-683. | | 13.Chinnaswami, R. and Patel, R.J. (1984). Blue-Green algae and pesticides. Pesticides. pp. 5-9. | | 14.Corbett, J.R. (1974). The Biochemical Mode of Action of Pesticides. Academic Press, New York, London and San Francisco. Pp.330. | | 15.Dangwal, L.R. Amandeep Singh, Tajinder Singh and Antima Sharma(2012). "Major weeds of Paddy fields in district Rajouri (J&K) India". APPN Journal of Agricultural and Biological Science, Vol.7 No.7 . | | 16.Dodge, A.D. (1975). Some mechanism of herbicide action. Sci. Prog. 62: 447-466. | | 17.EI.Sheekh MM, Kotkat HM, Hammouda OHE, 1994. Effect of atrazine herbicide on growth, photosynthesis, protein synthesis, and fatty acid composition in the unicellular green alga Chlorella kesleri. Ecotoxical Environ Saf. 29(3): 349-358. | | 18.Fogg, G.E. Stewart, W.D.P. Fay, P. and Walsby, A.E. (1973), Photosynthesis and Chemosynthesis. In "The blue-green algae". Academic Press, London – New York. pp.143-160. | | 19.G. Okmen, O. Turkcan and P. Erdal.(2013) "Effect of Herbicides on chlorophyll-a, β-carotene, phycocyanin and allophycoganin content of Anabaeno sp. Journal of Applied Biological sciences 7 (2) 20-27. | | 20.Hamdi, Y.A., Nawawy, A.S.E. and Tewfik, M.S. (1970). Effect of herbicides on growth and nitrogen fixation of alga, Tolypothrix tennis. Acta. Microbiol. Pol. B. 2: 53-56. | | 21.Hofstra, G. and Switzer, C.M. (1968). The phytotoxicity of propanil. Weed Sci. 16: 23-28. | | 22.Inger, L. (1970). Effect of two herbicides on nitrogen fixation by blue-green algae. Svensk.Bot. Tidskr. 64: 460-461. | | 23.Israel, G. (1983). Toxicity of pesticides on the rice field algae. Ph.D. Thesis in Botany, Andhra University, Waltair. pp.32-88. | | 24.Jones, R. (2005). The ecotoxicological effects of Photosystem II herbicides on corals. Mar Pollut Bull 51:495-506. | | 25.Kobbia IA. Shabana EP. Khalil Z. Zaki FT. (1991). Growth criteria of two common cyanobacteria isolated from Egyptian flooded soil, as influenced by some pesticides. Water, Air, and Soil Pollut 60: 1573-29 | | 26.Kumar, H.D. (1999). Cynophyta and prochlorophyta. In "Introductory phycology". Affiliated East-West press private limited, New Delhi. pp.87-141.s | | 27.Leganes F, Fernandez-Valiente E. (1992). Effects of phenoxy acetic herbicides on growth, photosynthesis, and nitrogenase activity in cyanobacteria from rice fields. Arch Environ Contam Toxicol. 22: 130-134. | | 28.Mishra AK, Pandey AB. (1989). Toxicity of three herbicides to some nitrogen-fixing cyanobacteria. Ecotoxicol/ Environ Saf. 17 (2): 236 | | 29.Moreland DE. (1980). Mechanisms of action of herbicides. Annu Rev. Platn Physiol. 31: 597-638. | | 30.Nishimura, M. and Takamiya, A. (1966). Energy and electron transfer system in algal photosynthesis-1. Action of two photochemical systems in oxidation reduction reactions of cytochrome in Porphyra sp. Biochem. Biophya. Acta. 120: 45-56. | | 31.Nirmal Kumar, Anubhuti Bora, Rita Kumar and Manmeet Kaur Amb. (2012)."Differential Effects of Agrndosulfan and Tebuconazole on photosynthetic pigments, metabolism and assimilating enzymes of three heterotrophic, cyanobaceia." – J. Biol. Environ. Sci., 6 (16), 67-75. | | 32.Pescheck, G.A. (1987). Respiratory electron transport. In "The Cyanobacteria" Ed.p. Fay and C. Van Baalen Elesevier, New York. pp.119-161. | | 33.Pfister, K. and Urbach, W. (1983). Effects of Biocides and growth regulatory: Physiological basis. Encyclopedia of plant physiology New Series. 12: 329-391. | | | 34.Rutherford, AW, Krieger-Liszkay A. (2001). Herbicides induced oxidative stress in photosystem. II Trends Biochem Sci 26: 648-653. | | 35.Saadet Demirors Saygideger and Ozlem Okkay.(2008). "Effect of 2, 4-Dichlorophenoxyacetic acid on growth, protein and chlorophyll-a content of chlorella vulgaris and spirulina platensis cells." Journal of Environmental Biology, 29 (2) 175-178. | | | 36.Saroja-Subbaraj, G. and Bose, S. (1983). Binding characteristics of methyl parathion to photosynthetic membranes of Chlorella. Pestic. Biochem. Physiol. 20: 188-193. | | 37.Sherman, L. Bricker, T., Guikema, J. and Pakrasi, H. (1987) The protein composition of the photosynthetic complexes from the cyanobacterial thylakoid membrane. In "The cyanobacteria". (Ed) P. Fay and C. Van Baalen Elesevier, Amensterdan, New York. pp. 1-33. | | 38.Singh, P.K. (1974). Algicidal effect of 2, 4-D Dichlorophenoxyacetic acid on blue-green alga, Cylindrospermum sp. Arch. Microbiol. 97: 69-72. | | 39.Singh, R.N. (1961). The role of blue-green algae in nitrogen economy of Indian Agriculture, Indian Council of Agricultural Research, New Delhi. India. pp.1-175. | | 40.Stewart W.D.P.(1970).Algal fixation of atmospheric nitrogen. Plant and Soil.32:555-558. | | 41.Street, J.E. and Kurtz, M.E. (1998). The preplant use of 2, 4-D in Rice. In "Research report of Mississippi – Agricultural and Forestry experimentation station". 22: 4-26. | | 42.Thadulingam,C and Venkatanarayana,G.(1955). A hand book of some south Indian weeds. Revised and enlarged by C.Rajashekhar Mudiliar and J.Sankaram. Printed by Superintendent, Govt.,press,Madras.pp.60-67. | | 43.Thakur, R.B., Jha, G. and Choudhary, S.K. (1995). Economics of weed control in transplanted rice. J. Applied. Biology. 5: 75-76. | 44.Tiwari, D.N., Pandey, A.K. and Mishra, A.K. (1981). Action of 2, 4-dichlorophenoxyacetic acid and rifampicin on heterocyst differentiation in the blue-green alga, Nostoc linckia. J. Biosci. 3: 33-39. | | 45.Tiwari, D.N., Pandey, A.K., Mishra, A.K. and Vibha Srivastava (1982). Role of 2, 4-D on growth and macromolecular synthesis in Anabaena cylindrical, Ind. J. Botany 5: 111-114. | | 46.Umbreit, W.W., Burris, R.H. and Stanffer, J.F. (1959). Monometric techniques 4th ed. Burgess Publishing Co. Minneopolis. Minnesote pp.4 | | 47.Victor Galhano, Francisco Peixoto, jose games – Laranjo, Eduardo Fernandez valiente(2009). "Comparative Toxicity of Bentazon and mohinate on growth, Photosynthetic pigment, photosynthesis, and respiration of the Portuguese Ricefield cyanobacterium Nostoc muscorum". Environmental toxicology 147–156. Available on line at Wiley InterScience (www.interscience.wiley.com) Do1 10.1002/ tox.20486. (accessed: 24th Mar, 2009). | | 48.Ware, G.W. (1980). Effects of pesticides on non-target organisms. Residue. Rev. 76: 173-201. | | 49.Watanabe, I. and Furusaka, C.(1980). Microbial ecology of flooded rice soils. In "Advances in Microbial Ecology."Vol.4.(Ed.) M. Alexander ,plenum publishing corporation.pp.125-167. | | 50.Wedding, R.T. and Black, M.K. (1962). Response of oxidation and coupled phosphorylation in plant mitochondria to 2, 4-Dichlorophenoxyacetic acid. Pl. Physiol. 62: 364-370. | | 51.Wedding, R.T., Erickson, L.C. and Brannaman, B.L. (1954). Effect of 2, 4-Dichlorophenoxyacetic acid on photosynthesis and respiration. Pl.Physiol. 29: 64-69. | | 52.Xia J. (2005). Response of growth, photosynthesis and photoinhibition of the edible cyanobacterium Nostoc sphaeroides colonies to thiobencarb herbicide. Chemosphere, 59-561-566.