African Journal of Microbiology Research Vol. 5(18), pp. 2760-2764, 16 September, 2011
Available online http://www.academicjournals.org/ajmr
DOI: 10.5897/AJMR11.560
ISSN 1996-0808 ©2011 Academic Journals

Full Length Research Paper

Influence of bispyribac sodium on nitrogenase activity and growth of cyanobacteria isolated from paddy fields

Gulten Okmen* and Aysel Ugur

Department of Biology, Faculty of Science, Mugla University, Mugla 48127, Turkey.

Accepted 30 July, 2011

The goal of this study was to determine the effect of bispyribac-sodium on the nitrogenase activities and growth of cyanobacteria isolated from paddy fields. Ten cyanobacterial species were used in this study. Cyanobacterial species were isolated from soil and water samples obtained from rice fields in Corum, Turkey. Among all *Anabaena* strains, the maximum activity was determined in *Anabaena* sp. O-22 (2.26 µl ethylene/mg.h) whereas; the lowest activity was shown in *Gloeothece* sp. O-Y (0.04 µl ethylene/mg.h). The maximum inhibition was seen in *Anabaena* sp. O-22, *Synechocystis* sp. O-X and *Anabaena* sp. O-16 in 100 µg/ml bispyribac-sodium concentration. Although low bispyribac-sodium concentrations somewhat stimulated growths of *Anabaena* sp. O-X2, O-Ç, O-4, O-16, O-8 and *Synechocystis sp.* O-X, the biomass of all cultures were severely inhibited at higher concentrations. The growths of *Anabaena* sp. O-22 and *Synechocystis* sp. O-X completely repressed at 100 µg/mL and at higher bispyribac-sodium concentrations, whereas, *Anabaena* O-X2, O-6, O-4 and O-16 completely suppressed at 500 µg/mL bispyribac-sodium concentration. The end of the study *Anabaena* sp. O-22 has been proposed as biofertilizer. The results obtained may be useful for the production of rice.

Key words: Cyanobacteria, nitrogenase activity, herbicide.

INTRODUCTION

The utilization of nitrogen gas (N_2) as a source of nitrogen is called nitrogen fixation and it is a property of only certain prokaryotes (Manahan, 1997; Madigan et al., 1997). Nitrogen fixing cyanobacteria are important photosynthetic microorganisms because they contribute to soil fertility by fixing the atmospheric nitrogen.

Nitrogen fixing cyanobacteria are found in many different ecosystems. Certain photosynthetic bacteria fix N_2, but only under anaerobic conditions. Nitrate, phosphate, light intensity, metal, osmotic and herbicide stresses are important environmental conditions affecting algal growth and nitrogenase activity (Meeks et al., 1983; Castenholz, 1988; Lehtimaki et al., 1997; Liengen, 1999; Banerjee et al., 2004; Okmen and Donmez, 2007a; Okmen et al., 2007b; Okmen et al., 2007c). These effects depend on the type and nature of environmental conditions, the organisms present as well as the experimental conditions (Tozum and Sivaci, 1993).

The herbicide Nominee commonly know as bispyribac-sodium. Sangakkara et al. (2004) is reported to increase the rice yield by selectively eliminating weeds from paddy fields. Although, the use of the herbicide is aimed at eliminating weeds, a major portion is deposited on the surface of the soil and might adversely affect the nontarget soil microflora.

Bispyribac-sodium, sodium 2.6- bis [(4, 6- dimethoxy-2- pyrimidinyl) oxy] benzoate, which was first developed by Japan Kumiai Chemical, belongs to the pyrimidinyl oxybenzoic acid group (Wu and Mei, 2011). Bispyribac-sodium has been applied post emergence to control many weeds. Rice-field herbicides while protecting rice-seedlings selectively, destroy the weeds and indirectly bring an increase in grain yield (Still and Kuziriam, 1967; Park and Park, 1971; Fischer et al., 2000). This class of herbicides (penoxsulam, imazamox and bispyribac-sodium) act through inhibition of acetolactate synthase (specific to plants and microorganisms) and thereby block the biosynthesis of the branched-chain aminoacids which lead to decreased protein synthesis and cessation of growth (Osuna et al., 2002). However, bispyribac-sodium inhibits the synthesis of key aminoacids causing susceptible plants to stop growing and die within about two to three weeks (Slade et al., 2006).

*Corresponding author. E-mail: gultenokmen@gmail.com. Tel. +90252 211 1676. Fax. +90252 223 8656.

Many reports available indicate interaction between cyanobacteria and herbicides, including effects of herbicides on algal growth, photosynthesis, nitrogen fixation, biochemical composition and metabolic activities as well as degradation and removal of herbicides by algae and cyanobacteria (Lundqvist, 1970; Ibrahim, 1972; Singh, 1974; Dasilva et al., 1975; Tiwari et al., 1981; Maule and Wright, 1983; Stratton, 1984; Mattoo et al., 1984; El-Sawy et al., 1984; Singh et al., 1986; Goyal, 1986; Tandon and Lal, 1988; Singh and Tiwari, 1988; Mishra and Pandey, 1989a; Bhuniaa et al., 1991; Leganés and Fernández-Valiente, 1992; El Sheekh et al., 1994; Caux et al., 1996; Jeong-Dong and Choul-Gyun, 2006; Okmen, 2007c;).

Until now, very little work has been done on the effects of rice field herbicides on nitrogen fixation and the studies carried out provide a preliminary idea about the inhibitory or stimulatory effect of the herbicides on diazotrophic growth in cyanobacteria.

Previous studies have investigated the influence of selective pesticides on the growth of cyanobacteria (Pandey, 1985; Singh and Tiwari, 1988; Mishra et al., 1989b; Prosperi et al., 1993; Jin et al., 1996; Nystrom et al., 1999; Jianyi et al., 2002; Okmen et al., 2007c). Most reports demonstrated that the sensivity of cyanobacteria toward herbicides and their growth and nitrogen fixation behavior changed in the presence of herbicides. There are no reports on the effects of bispyribac-sodium on nitrogenase activity of cyanobacteria. In this paper, we report the experimental findings obtained on the effect of a rice herbicide bispyribac-sodium, on the nitrogenase activity, growth of ten diazotrophic cyanobacterial strains, namely *Anabaena*, *Synechocystis* and *Gloeothece* sp.

MATERIALS AND METHODS

Test organisms

Samples were collected from paddy fields in Osmancik, Corum-Turkey. The unicellular and filamentous, heterocystous cyanobacteria used in this study (*Anabaena*, *Synechocystis* and *Gloeothece* sp.) were isolated from soil and water samples obtained from rice fields in Osmancik, Corum, Turkey. Nitrogen-free BG-11 medium was used for isolation of nitrogen fixing cyanobacteria. Isolation and purification were performed by dilution and plating of soil and water samples. Stock cultures were grown in the N-free BG-11 medium as previously described (Castenholz, 1988). Temperature was maintained at 20°C and cultures were grown under a cool white light. Cells in the logarithmic phase of growth were collected from cultures and used as inocula for experiments. Experiments were conducted in batch cultures by using 10 ml of inoculated medium flasks in 25 ml. Culture media were adjusted accordingly pH 8 with 1 N NaOH and 1 N HCl. Illumination was supplied with 11 µmol/m^2 cool white light (Fogg et al., 1973; Rippka, 1988).

Determination of nitrogenase activity

Nitrogenase activity was measured by acetylene reduction technique (Burlage et al., 1998). Cultures (10 ml) were grown under the different concentrations of bispyribac-sodium and were enclosed by parafilm plastic. Then 1 ml of acetylene gase was injected into the serum bottles and cultures were incubated for 12 h under the experiment conditions. After the incubation periods, samples (1 ml) were taken from serum bottles with gas-tight syringes, injected into the gas chromatograph, and ethylene concentrations were determined using Agilent 6890 GC-FID.

Determination of dry weight

The pellets of centrifuged cultures were washed with distilled water three times, then dried to a constant weight at 70°C for 12 h and dry weights were measured (Castenholz, 1988; Cappuccino and Sherman, 2001).

Influence of bispyribac – sodium on nitrogenase activity and growth

The influence of different concentrations of bispyribac– sodium (5-500 µg/mL) on the nitrogenase activity were also tested on *Anabaena*, *Synechocystis* and *Gloeothece* sp. The experimental cultures were grown in 25 ml flasks containing 10 ml N-free BG-11 medium under the same conditions as described below. According to Rippka (1988), the cultures were grown in a liquid sterilized medium at 20 ± 2°C under cool white light for 35 days. At the end of 35 days, nitrogenase activity of the cultures were determined using the acetylene reduction technique.

Appropriate control systems containing no solvent and pesticide were included in each experiment. Control and treated cultures were grown under the same temperature and light intensity as mentioned above. All experiments were performed in triplicate and the average values were presented.

RESULTS

Cyanobacterial species were isolated from soil and water samples obtained from rice fields in Corum, Turkey. In this study, 10 cyanobacteria had studied and determined the effets of different concentrations of bispyribac-sodium on nitrogenase activities of cyanobacteria. These included 8 *Anabaena* sp., 1 *Synechocystis* sp. and 1 *Gloeothece* sp. strains.

When *Anabaena*, *Synechocystis* and *Gloeothece* sp. were cultured in the presence of various bispyribac-sodium concentrations, distinct effects were seen on nitrogenase activities and growths. The growths and nitrogenase activities of cyanobacteria treated with different concentrations of bispyribac-sodium under 11 µmol/m^2 light intensity are listed in Tables 1 and 2.

Among all *Anabaena* strains, the maximum activity was determined in *Anabaena* sp. O-22 (2.26 µl ethylene/mg.h) whereas, the lowest activity was shown in *Gloeothece* sp. O-Y (0.04 µl ethylene/ mg.h). The nitrogenase activities of *Anabaena* sp. O-X2, O-4 and O-8 were stimulated in initial period but, increasing concentrations repressed the nitrogenase activity. Bispyribac-sodium experiments have shown that the initial nitrogenase activity of *Gloeothece* sp. O-Y at low concentrations of bispyribac-sodium (5 to 10 µg/mL) did not change but, the activity repressed with increasing bispyribac-sodium concentrations in *Gloeothece* sp.O-Y (Table 1). The maximum inhibition was

Table 1. Effects of bispyribac- sodium on nitrogenase activity of cyanobacteria.

Microorganisms	Ethylene amount (µL/ mg.h)						
	Concentrations (µg/mL)						
	Control	5	10	25	50	100	500
Anabaena sp. O-X2	0.58±0.002	0.65±0.01	0.53±0	0.44±0	0.29±0	0.23±0.01	-
Anabaena sp. O-Ç	1.50±0.001	0.22±0.01	0.33±0.014	0.31±0.05	0.18±0	0.15±0.02	-
Anabaena sp. O-6	1.2±0.003	0.35±0.001	0.26±0.014	0.23±0.001	0.28±0	0.05±0	-
Anabaena sp. O-K	1.28±0.001	0.9±0.009	0.31±0.035	0.01±0.05	0.04±0.02	0.05±0.02	0.05±0.01
Anabaena sp. O-4	0.3±0.001	0.77±0.002	0.6±0.002	0.59±0.09	0.6±0.001	0.6±0.05	-
Anabaena sp. O-22	4.45±0.005	2.26±0.2	0.03±0.2	0.009±0	0.008±0.5	-	-
Anabaena sp. O-16	4.8±0.005	0.95±0.006	0.96±0.05	0.34±0.005	0.14±0.4	-	-
Anabaena sp. O-8	0.86±0.001	1.04±0.001	2.1±0.08	1.38±0.16	0.84±0.001	0.82±0.08	-
Synechocystis sp. O-X	2.62±0.002	1.18±0.008	1.40±0.001	1.45±0.003	0.9±0.04	-	-
Gloeothece sp. O-Y	0.30±0.004	0.25±0.001	0.27±0	0.20±0.007	0.08±0.02	0.04±0.07	0.04±0.01

(-): No effect Values are mean ± Standard deviation.

Table 2. Effects of bispyribac- sodium on growth of cyanobacteria.

Microorganisms	Dry weight (mg/mL)						
	Concentrations (µg/mL)						
	Control	5	10	25	50	100	500
Anabaena sp. O-X2	1.2±5	1.8±15	1.6±10	1.5±10	1.5±0	0.7±10	-
Anabaena sp. O-Ç	0.4±8	0.5±20	0.5±0	0.7±10	0.4±14.5	0.4±20	0.1±12
Anabaena sp. O-6	5±10	0.9±10	1.1±14	1.1±4	1.4±18	0.4±20	-
Anabaena sp. O-K	1.4±9	0.8±5	0.6±16	0.4±6.3	0.2±17	0.01±25	0.01±20
Anabaena sp. O-4	0.3±10	1.2±7	1.5±0	0.8±5	0.9±15	1±1.8	-
Anabaena sp. O-22	0.1±15	0.1±7	0.004±10	0.004±20	0.004±20	-	-
Anabaena sp. O-16	0.3±2	0.8±10	0.3±10.5	0.3±24	0.1±16	0.1±20	-
Anabaena sp. O-8	0.7±2.5	1.1±0	1.1±0	0.9±10	0.8±20	0.7±15	0.5±20
Synechocystis sp. O-X	0.2±0	0.3±10	0.4±7	0.5±14	0.2±20	-	-
Gloeothece sp. O-Y	0.7±0	0.6±7.8	0.7±5	0.4±18	0.3±0	0.4±10	0.4±18

(-): No effect Values are mean ± Standard deviation.

seen in *Anabaena* sp. O-22, *Synechocystis* sp. O-X and *Anabaena* sp. O-16 in 100 µg/ml bispyribac-sodium concentration. With the exception of *Anabaena* sp. O-K and *Anabaena* sp. O-Y, the nitrogenase activities of all other strains were inhibited at 500 µg/mL bispyribac-sodium concentration.

The growths of *Anabaena* sp. O-X2, O-Ç, O-4, O-16, O-8 and *Synechocystis* sp. O-X were stimulated in low bispyribac-sodium concentrations, but the biomass of all cultures were severely inhibited in higher concentrations. Bispyribac-sodium experiments have shown that the initial biomass of *Anabaena* sp. O-22 at low concentrations of bispyribac-sodium (5 µg/ ml) did not change but, the growth repressed with increasing bispyribac-sodium concentrations. The negative impacts of high bispyribac-sodium on the biomass of *Anabaena* sp. O-6 and O-K cultures were also demonstrated. The growths of *Anabaena* sp. O-22 and *Synechocystis* sp. O-X completely repressed at 100 µg/ mL and at higher bispyribac-sodium concentrations, whereas, *Anabaena* O-X2, O-6, O-4 and O-16 completely suppressed at 500 µg/mL bispyribac-sodium concentration (Table 2).

DISCUSSION

Variation in growth conditions influenced the growths and nitrogenase activities of all genera. Although, the use of the herbicide is aimed at eliminating weeds, a major portion is deposited on the surface of the soil and might adversely affect the nontarget soil microflora. The nitrogen- fixing cyanobacteria are known to dominate the water-logged paddy fields and help in the nitrogen economy of rice agriculture (Singh, 1961; Stewart, 1967;

Henriksson et al., 1975). Information on resistance to herbicides, and for bispyribac in particular, is lacking. In Turkey, bispyribac-sodium is mostly used for eliminating weeds in paddy fields in the Corum- Osmancik region (THOA, 2002). For this reason, the herbicide was chosen for this study.

In this study, bispyribac–sodium stimulated nitrogenase activity of *Anabaena* sp.O-X2, O-4 and O-8 at 5 µg/ml but not in higher concentrations. It was demonstrated that *Anabaena* sp. was capable of growing both photoautotrophically and photoheterotrophically like bacteria to a great extent (Jin et al., 1996; Yan et al., 1997).

In the other cyanobacteria tested the nitrogenase activities and growths were inhibited during the initial concentration (5 µg/ml) (Table 1). Gonzalez-Barreiro et al. (2006) showed that the serious effects on growth for microalgae by herbicide added to culture medium. The main characteristic of cell death or decrease of cell viability, whether from senescence, acute stress, or aging, seems to be the loss of the ability of cells to maintain homeostasis (Gahan, 1984). Most reports have demonstrated that the inhibitory effect of herbicide became greater with an increase in herbicide concentration and suggested that the reduction in the dry matter of algae may be due to a decrease in algal photosynthesis caused by the inhibition of synthesis of chlorophyll, which is the most important pigment in algal cells for collecting solar energy for photosynthesis (Caux et al., 1996; Prosperi et al., 1993).

The nitrogenase activities of *Anabaena* sp. O-K, O-6 and O-16 were more inhibited than growths. The nitrogenase activity of *Anabaena* sp. O-22 was repressed about 50% by 5 µg/ml bispyribac-sodium but growth was unaffected. In the case of *Synechocystis* sp. O-X, the herbicide also inhibited nitrogenase activity by about 50% but the dry matter increased. Powell et al. (1991) reported that nitrogenase activity was more sensitive to the isopropylamine salt of glyphosate than was photosynthetic O_2 evolution.

In *Anabaena* sp. strains O-X2, O-4 and O-8 it were found that very low concentrations of bispyribac-sodium (5 µg/ml) stimulated both nitrogenase activity and growth but increased concentrations repressed both nitrogenase activity and growth. The observed effects of bispyribac-sodium on cyanobacterial growth in this study are similar to those reported by Shen et al. (2005) for butachlor and acetochlor on several *Anabaena* species. Butachlor and acetochlor stimulated growth of these cyanobacteria under low concentrations (1 to 8 mgL^{-1}), but showed high toxicity at concentrations above 16 mgL^{-1}. Hammouda (1999), on the other hand, demonstrated the utilization of carbofuran at low concentrations by a nitrogen-fixing cyanobacterium, *Anabaena doliolum*. The 2.4D, a synthetic growth hormone analog, is reported to stimulate growth and heterocyst formation in the cyanobacterium at lower concentrations (Mishra and Tiwari, 1986). Growth studies showed that the strains used in the present study were capable of growing both photoautotrophically and photoheterotrophically (Yan et al., 1997; Guoan et al., 1997).

The data obtained in this study provide information about the inhibitory effect of the bispyribac- sodium on growths and nitrogenase activities of cyanobacteria, which exhibits different sensitivity to the herbicide. These findings suggest a limit or avoidance of the use of bispyribac- sodium in paddy fields, due to its inhibitory effect on biological nitrogen fixation and hence a possible reduction in rice crop yields.

In this study, we have shown a clear physiologic distinction betweeen *Anabaena* sp O-22 sp. and the other strains. Generally *Anabaena* sp O-22 had the best optimal performance of nitrogenase activity in all environmental conditions, so it is thought that it is a suitable genus for biofertilizer. A better understanding of the mechanism of action of the herbicide on biological nitrogen fixation requires further study of the biochemical targets of the herbicide in cyanobacteria.

REFERENCES

Banerjee M, Mishra S, Chatterjee J (2004). Scavenging of nickel and chromium toxicity in *Aulosira fertilissima* by immobilization: Effect on nitrogen assimilating enzymes. Electron. J. Biotechnol., 7(3): 13-14.

Bhuniaa K, Basun K, Roy D, Chakrabarat I, Banerjees K (1991). Growth, chlorophyll- a content, nitrogen-fixing ability and certain metabolic activity of *N. muscorum:* Effect of methylparathion and benthiocarb. Bull. Environ. Contam. Toxicol., 47: 43-50.

Burlage RS, Atlas R, Stahl D, Geesey G, Sayler G (1998). Techniques in Microbial Ecology. Oxford University Press, America, pp. 8-14.

Cappuccino JG, Sherman N (2001). Microbiology A Laboratory Manual. Sixth Edition, Benjamin Cummings, S. Francisco, p. 119.

Castenholz RW (1988). Culturing methods for cyanobacteria. Methods Enzymol., 167: 68-93.

Caux PY, Menard L, Kent R (1996). Comparative study of the effects of MCPA, butylate, atrazine and cyanazine on *Selenastrum capricornutum*. Environ. Pollut., 92(2): 219-225.

Dasilva EJ, Henriksson LE, Henriksson E (1975). Effects of pesticides on blue- gren algae and nitrogen fixation. Arch. Environ. Contam. Toxicol., 3: 193.

El 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*. Ecotoxicol. Environ. Saf., 29(3): 349-358.

El-Sawy M, Mahmoud SAZ, El-Haddad ME, Mashour WA, Salem KG (1984). Effect of different herbicides on nitrogen fixation by blue-green algae in paddy soil. In: J. Szegi (Ed.), Soil Biology and Conservation of the Biosphere, Akademiai Kiado, Budapest, pp. 297-306.

Fischer AJ, Bayer DE, Carriere MD, Ateh CM, Yim KO (2000). Mechanisms of resistance to bispyribac-sodium in an *Echinochloa phyllopogon* accession. Pestic. Biochem. Physiol., 68: 156-165.

Fogg GE, Stewart WDP, Fay P, Walsby AE (1973). Culture, nutrition and growth. In: The Blue Green Algae, Academic Press, London, NewYork, pp. 129-142.

Gahan PB (1984). Reversibl and irreversibl damage in plant cells of different ages, In: Davies, D.C. Sigee (Eds.), Cell Ageing and Cell Death. Cambridge Univ. Press, London, pp. 155-169.

González-Barreiro O, Rioboo C, Herrero C, Cid A (2006). Removal of triazine herbicides from freshwater systems using photosynthetic microorganisms. Environ. Pollut., 144(1): 266-271.

Goyal SK (1986). Interaction between pesticides and cyanobacteria. Proceedings, National Symposium on Current Status of BNF, Haryana Agricultural University, Hissar (1986), pp. 93-96.

Guoan AY, Xue Y, Wei W (1997). Effects of the herbicide molinate on mixotrophic growth, photosynthetic pigments, and protein content of *Anabaena sphaerica* under different light conditions. Ecotoxicol. Environ. Saf., 38:144-149.

Hammouda O (1999). Response of the paddy field cyanobacterium *Anabaena doliolum* to carbofuran. Ecotoxicol. Environ. Saf., 44:215–219

Henriksson E, Henriksson LE, DaSilva EJ (1975). A comparison of nitrogen fixation by algae of temperate and tropical soils. In: W.D.P. Stewart (eds.), Nitrogen fixation by free- living microorganisms. Cambridge Univ. Press, 6: 36-49.

Ibrahim AN (1972). Effect of certain herbicides on growth of nitrogen fixing algae and rice fields. Symp. Biol. Hung., 11: 445.

Jeong-Dong K, Choul-Gyun L (2006). Differential responses of two freshwater cyanobacteria, *Anabaena variabillis* and *Nostoc commune*, to sulfonylurea herbicide bensulfuron-methyl. J. Microbiol. Biotechnol., 16(1): 52-56.

Jianyi M, Ligen X, Shufeng W, Rongquan Z, Shuihu J, Songqi H, Youjun H (2002). Toxicity of 40 herbicides to the green alga *Chlorella vulgaris*. Ecotoxicol. Environ. Saf., 51:128-132.

Jin CY, Song LR, Li SH (1996). The mixotrophic growth of *Anabaena* sp.. Acta Hydrobiol. Sin., 2: 134-137.

Leganés F, Fernández-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.

Lehtimaki J, Moisander P, Sivonen K, Kononen K (1997). Growth, nitrogen fixation and nodularin production by two Baltic Sea cyanobacteria. Appl. Environ. Microbiol., 63(5): 1647-1656.

Liengen T (1999). Environmental factors influencing the nitrogen fixation activity of free- living terrestrial cyanobacteria from a hight arctic area. Can. J. Microbiol., 45(7):573-581.

Lundqvist I (1970). Effect of two herbicides on nitrogen-fixation by blue-green algae. Svensk. Bot. Tidskr., 64: 460-461.

Madigan MT, Martinko JM, and Parker J (1997). Brock Biology of Microorganisms. Prentice-Hall Int. Ltd. 8th edition. In: Chapter 4, London, pp. 13-15.

Manahan SE (1997). The Nitrogen Cycle. In: Environmental Science and Technology, Lewis Publishers, NewYork, pp. 466-468.

Mattoo AK, St. John JB, Wergin WP (1984). Adaptive reorganization of protein and lipid components in chloroplast membranes as associated with herbicide binding. J. Cell. Biochem., 24: 163-175.

Maule A, Wright SJL (1983). Physiological effects of chloropropham and 3- chloroaniline on some cyanobacteria and a green algae. Pestic. Biochem. Physiol., 19: 196.

Meeks JC, Wycoff KL, Chapman JS, Enderlin CS (1983). Regulation of expression of nitrate and dinitrogen assimilation by *Anabaena* species. Appl. Environ. Microbiol., 45(4): 1351-1359.

Mishra AK, Pandey AB (1989a). Toxicity of three herbicides to some nitrogen-fixing cyanobacteria. Ecotoxicol. Environ. Saf., 17(2): 236-246.

Mishra AK, Pandey AB, Kumar HD (1989b). Effects of three pesticides on MSX- induced ammonia photoproduction by the cyanobacterium *Nostoc linckia*. Ecotoxicol. Environ. Saf., 18: 145-148.

Mishra AK, Tiwari DN (1986). Effect of tryptophan on 2,4-dichlorophenoxyacetic acid toxicity in the nitrogen-fixing-cyanobacterium *Nostoc linckia*. J. Basic Microbiol., 26(1): 49-53.

Nystrom B, Bjornsater B, Blanck H (1999). Effects of sulfonylurea herbicides on non-target aquatic microorganisms. Aquatic Toxicol., 47: 9-22.

Okmen (Kurucuoglu) G, Donmez G, Donmez S (2007b). Influence of osmotic and metal stresses on nitrogenase activity of cyanobacteria isolated from paddy fields. Afr. J. Biotechnol., 6(15): 1828-1832.

Okmen G, Donmez G, Donmez S (2007c). Influence of nitrate, phosphate and herbicide stresses on nitrogenase activity and growth of cyanobacteria isolated from paddy fields. J. Appl. Biol. Sci., 1(1): 57-62.

Okmen G, Donmez G, Donmez S (2007c). Influence of Nitrate, Phosphate and Herbicide Stresses on Nitrogenase Activity and Growth of Cyanobacteria Isolated from Paddy Fields. J. Appl. Biol. Sci., 1(1): 57-62.

Osuna MD, Vidotto F, Fischer AJ, Bayer DE, Prado RD, Ferrero A (2002). Cross- resistance to bispyribac-sodium and bensulfuron-methyl in *Echinochloa phyllopogon* and *Cyperus difformis*. Pestic. Biochem. Physiol., 73: 9–17.

Pandey AK (1985). Effects of propanil on growth and cell constituents of *Nostoc calciola*. Pestic. Biochem. Physiol., 23: 157-162.

Park JK, Park RK (1971). Chemical weed control in rice culture in Korea. Asian Pacific Weed. Sci. Soc., 3: 13-17.

Powell HA, Kerby NW, Rowell P (1991). Natural tolerance of cyanobacteria to the herbicide glyphosate. New Phytol., 119: 421-426.

Prosperi C, Luna C, Valiente EF (1993). Influence of pH light intensity and oxygen on the short-term effect of ammonium on nitrogenase activity of cyanobacteria from rice fields. Environ. Exper. Bot., 33(4): 545-552.

Rippka R (1988). Isolation and purification of cyanobacteria. Methods Enzymol., 167: 3-27.

Sangakkara UR, Nissanka SP, Marambe B, Hurle K, Rubin B (2004). Weeds, herbicide use and resistance in rice fields of Sri Lanka, 4th International Crop Science Congress, Brisbane, Australia. 26 September-1 October.

Shen JY, Lu Y, Cheng G (2005). Effects of chemical herbicides on toxicity of non-target nitrogen-fixing cyanobacteria in paddy fields. in: Vietnam Weed Science Society Asian-Pacific, Proceedings of the 20th Asian-Pacific Weed Science Conference, pp. 665–670.

Singh LJ, Tiwari DN (1988). Effects of selected rice field herbicides on photosynhesis, respiration and nitrogen assimilating enzyme systems of paddy soil diazotrophic cynanobacteria. Pestic. Biochem. Phys., 31: 120-128.

Singh LJ, Tiwari DN, Singh HN (1986). Evidence for genetic control of herbicide resistance in rice field isolate of *Gloeocapsa* sp. capable of aerobic diazotrophy. J. Gen. Appl. Microbiol., 32: 81-88.

Singh PK (1974). Algicidal effect of 2,4-dichlorophenoxy acetic acid on blue-green algae *Cylindrospermum sp.*. Arch. Microbiol., 97: 69-72.

Singh RN (1961). The role of blue-green algae in nitrogen economy of Indian Agriculture, Indian Coun. Agric. Res., New Delhi, P. 175.

Slade JG, Poovey AG, Getsinger KD (2006). Bispyribac-sodium: Emerging herbicide for aquatic plant management. MidSouth Aquatic Plant Management Society, 25th Annual Meeting, Alabama, October, pp. 24-26.

Stewart WDP (1967). Transfer of biologically fixed nitrogen in sand dune slack region, Nature, 214: 603-604.

Still CC, Kuziriam O (1967). Enzymatic detoxification of 3',4'dichloro-propionanilide in rice and barnyard grass, a factor in herbicide selectivity. Nature, 216: 799-800.

Stratton GW (1984). Effects of the herbicide atrazine and its degradation products, alone and in combination on phototrophic microorganisms. Arch. Environ. Contam. Toxicol., 13: 35-42.

Tandon RS, Lal R, Narayana Rao VV (1988). Interaction of endosulfan and malathion with blue-green algae *Anabaena* and *Aulosira fertilissima*. Environ. Pollut., 52(1): 1-9.

THOA (Turkish Head Office of Agriculture Report) (2002). Weed management in the cultured plants growing regions of Corum. Head-Office of Agriculture, Ankara, Turkey.

Tiwari DN, Pandey AK, Mishra AK (1981). Action of 2,4 dichlorophenoxy acetic acid on growth and heterocyst formation in the blue gren alga *Nostoc linckia*. J. Biosci., 3: 33-39.

Tozum- Calgan SRD, Sivaci Guner S (1993). Effects of 2,4D and methylparathion on growth and nitrogen fixation in cyanobacterium, *Gloeocapsa*. Intern. J. Environ. Stud., 43: 307-311.

Wu S, Mei J (2011). Analysis of the herbicide bispyribac-sodium in rice by solid phase extraction and high performance liquid chromatography. Bull. Environ. Contam. Toxicol., 86: 314-318.

Yan GA, Yan X, Wu W (1997). Effects of the herbicide molinate on mixotrophic growth, photosynthetic pigments and protein content of *Anabaena sphaerica* under different light conditions. Ecotoxicol. Environ. Saf., 38(2): 144-149.