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IMPACT OF HERBICIDES ON CHLOROPHYLL-A, B-CAROTENE, PHYCOCYANIN AND ALLOPHYCOCYANIN SUBSTANCE OF *NOSTOC MUSCORUM* L.

***Komala E.**

Department of Botany, Govt. Degree College Husnabad Dist; Siddipet– 505467.

***Corresponding Author: Komala E.**
Department of Botany, Govt. Degree College Husnabad Dist; Siddipet- 505467.

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ABSTRACT
Continual increases within the human population and growing concerns associated with the energy crisis, food security, disease outbreaks, global warming, and other environmental issues require a sustainable solution from nature. One among the promising resources is cyanobacteria, also called blue green algae. They require simple ingredients to grow and possess a comparatively simple genome. Cyanobacteria are known to supply a large style of bioactive compounds. Pigments are frequently employed in medicine, food, pharmacology, cosmetics, ink, and textile preparations. Cyanobacteria have an excellent potential to be used as pigment producers because they're easy to grow with simple nutrient requirements, unlike other microbial systems, and additionally, have an outsized number of pigments. Cyanobacteria produce some pigments like chlorophyll-a, carotenoids, phycobiliproteins. Additionally, cyanobacteria can fix free atmospheric nitrogen. Environmental conditions affect cyanobacterial growth and pigment production. Herbicides use commonly in paddy fields. Because of this, herbicides can affect to non-target microorganisms such as cyanobacteria. In this study, production of some pigments by *Nostoc muscorum* were studied using different herbicides in BG-11 medium. As herbicide, cyhalofop- butyl and fenoxaprop-p-ethyl used in this study. In this work, different herbicide concentrations were applied on pigment production. Initial fenoxaprop-p-ethyl concentrations (10.0, 20.0, and 30.0 mg/L) stimulated chlorophyll-a, β-caroten, phycocyanin and allophycocyanin contents. But increasing herbicide concentrations suppressed to the all of pigment contents. The all of pigment contents of *Nostoc muscorum* completely suppressed by 500 mg/L fenoxaprop-p-ethyl concentration. The other herbicide is cyhalofop-butyl. Initial cyhalofop-butyl concentrations partly stimulated the pigment contents. But, the pigment contents of *Nostoc muscorum* increased sharply by 30 mg/L cyhalofop-butyl concentrations. The all of pigment contents of *Nostoc muscorum* completely repressed by 500 mg/L cyhalofop-butyl concentrations.

KEYWORDS: *Nostoc muscorum*, chlorophyll-a, β- caroten, phycocyanin, allophycocyanin, herbicid, and cyhalofop- butyl and fenoxaprop-p-ethyl.

INTRODUCTION
Cyanobacteria are the prokaryote, Gram-negative and oxygenic phototrophs (Wilmotte, 1994). They have a wide distribution and are ubiquitous in occurrence (Henson *et al.,* 2004) including extreme habitats of the world (Schulz and Scherer, 1999). Role of cyanobacteria in soil fertility is well established. Paddy fields are one of the most favorable natural niches for the growth and proliferation of cyanobacteria (Whitton, 2000) where they play a major role in primary productivity as well as the nitrogen economy of that ecosystem. Cyanobacterial growth and diversity are mainly governed by soil physicochemical properties. They prefer a natural to alkaline pH for optimum growth. Many cyanobacteria fix nitrogen and comprise one of the largest global suppliers of fixed nitrogen in flooded/irrigated rice fields (Singh, 1961; Roger, 1996). Many nitrogen-fixing strains of cyanobacteria have been isolated and used in biofertilizer consortia in south East Asian countries. Potentiality of cyanobacteria as biofertilizers, soil conditioners, plant growth regulators and soil health ameliorators has been well recognized (Vaishampayan et al., 2001, Whitton, 2000). Members of the order Nostocales and stigonematales are widespread having particular significance in these environments (Desikachary, 1959).

Cyanobacteria improve the soil fertility by increasing organic content, water holding capacity, nitrogen content, phosphate solubilization, and secretion of polysaccharides (Tiwari *et al.,* 1991; Whitton and Potts, 2000). These properties of cyanobacteria prove it suitable and eco-friendly bio fertilizer. Tolerant strains of cyanobacteria to regularly used pesticides and potential to degrade them are desirable qualities for cyanobacterial bio fertilizer. Paddy fields favor the luxuriant growth of cyanobacteria and most of the biological nitrogen

fixation of this ecosystem is done by N2-fixing cyanobacteria (Irisarri *et al.*, 2001). Many nitrogen-fixing strains of cyanobacteria have been isolated and used in bio fertilizer consortia in Southeast Asian countries.

Herbicides, and fungicides, and insecticides are common pesticides used in agricultural fields. Pesticides help in improving agricultural productivity. Fungal diseases are most common on plants throughout the world. Substantial amount of fungicides are being poured in fields regularly. Fungicides besides controlling fungi also cause adverse effects on non-target organisms including cyanobacteria. Modern sustainable paddy cultivation worldwide involves extensive use of agrochemicals such as insecticides, fungicides but especially herbicides. Herbicide demand has unique characteristics compared with other common productive inputs in rice culture systems such as land, labour, seeds and chemical fertilizers (Yamamoto H, Nakamura K. 2003). The goal of herbicide use is to kill or stunt weed infestation allowing the rice to grow and gain a competitive advantage. The use of rice herbicides has been expanding enormously worldwide over the past 20- 40 years (*Monaco et.al*, 2002).

Cyhalofop butyl is intended to provide post emergent control of selected grassy weeds in rice. It is to be used for the control of barnyard grass (*Echinochloa* spp) and silver top (*Lepthochloa fusca)* in rice. This herbicide will be applied at 0.5-1.0 L/ha (142-285 g ac/ha) as a foliar spray together with spraying oil at 1 L/ha. The main effects associated with repeat dose toxicity of cyhalofop-butyl in animals were hepatocellular proliferation, inflammation and gross enlargement of liver and bile duct hyperplasia. Macroscopic and microscopic abnormalities in liver and kidneys were also consistently observed (Gajanayake 2005).

Fenoxaprop- P-ethyl is an aryloxy phenoxy propionate post emergence herbicide inhibiting fatty acid synthesis in grasses through inhibition of acetyl CoA carboxylase (Pornprom *et.al.* 2006). The herbicide can be used on several crops: Gelmini *et al.* (Gelmini *et.al.* 2001). Reported the use of fenoxaprop-P-ethyl (FPE) on onion, Nisha and Chopra (Nisha C, Chopra NK. 2005) on wheat and (McMullan. 1994). Described its use on barley. The use of fenoxaprop-P-ethyl against annual and perennial grasses in rice is well documented by many authors (Bhattacharya *et.al.* 2001 and Bhattacharya *et.al*. 2004. Traditionally, paddy fields are home-ecosystems to many species (Min *et.al.* 2001). The nitrogen-fixing cyanobacteria form a prominent component of microbial population in rice paddy fields, since they significantly contribute to fertility as natural biofertilizers (Fernández *et.al*. 2000). Their contribution to the maintenance of soil fertility, by fixing atmospheric N2 (diazotrophy), is particularly important in rice field soils (Whitton 2000). Otherwise, cyanobacteria are characterized by the production of various pigments on natural or synthetic media. These pigments are usually described in terms of various shades of blue, violet, red, yellow, and green.

Pigment producing microorganisms are yeast, fungi, bacteria, micro algae and are quite common in nature. Carotenoids are yellow to orange-red pigments present in a wide variety of bacteria, algae, fungi and plants (Goodwin and Briton 1980) having the functions of food colorants, absorbers of light energy, oxygen transporters, provitamin A, scavengers of active oxygen, antitumor and enhancers of in vitro antibody production (Krinsky 1979) (Mathews. 1979). Besides this, carotenoids protect the pigment–protein complexes and the chloroplast against photo oxidation (Demmig-Adams 1990). A number of natural carotenoids pigments produced by plants also contribute to enhanced immune system and reduced risk of degenerative diseases, such as cancer, cardiovascular diseases, macular degeneration and cataract by scavenging reactive oxygen radicals and acting as anti-aging agent (Mayne 1996).

The influence of herbicides on cyanobacteria has been extensively reviewed in many studies (Padhy 1985). Generally, cyanobacteria are quite sensitive to herbicides, because they share many of the physiological features of higher plants, which form the site of herbicide action (Whitton 2000.). 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 (Gelmini.2001). Furthermore, an ideal bio fertilizer strain of cyanobacteria must have the ability to tolerate or even resist to toxic actions of herbicides (Singh *et.al.* 2003). High bensulfuron-methyl concentrations (8- 10 ppm) inhibited the growth and photosynthesis of over 50% in *A. variabilis* and *Nostoc commune* rice field isolated; nitrogenase activity decreased by 94-98% in *A. variabilis* and by 85-86% in *N. commune* after 24 hours' incubation with 10 ppm and 20 ppm of the herbicide, respectively (Kim and Lee 2006). (Ahluwalia Kaur and Dahuja. 2002) proved that the incorporation of relatively higher doses (> 5 µg.ml-1) of diquat into *N. muscorum* and Cylindrospermum sp. cultures could be highly toxic, thereby reducing their chlorophyll a content and contributing to a progressive decrease in growth which culminates in complete lysis of the cells with the increasing level of the herbicide. (Okmen and Ugur 2011) reported that bispyribac-sodium (100 µg.ml-1) partly suppressed the growths and nitrogenase activities of ten cyanobacteria.

Production of pigments by cyanobacteria have been utilized as an important cultural characteristic in describing the organisms. Nevertheless, very little is known about the effect of herbicides on pigment production, because the formation of pigment is influenced by the pH of the medium, aeration, temperature of the growth and carbon and nitrogen

sources. Most reports demonstrated that the sensivity of cyanobacteria toward herbicides and their metabolic activities behavior changed in the presence of herbicides. Until now, a work has not been done on the effects of cyhalofop butyl, and fenoxaprop-P-ethyl on pigment contents of cyanobacteria. In this work, we report the experimental findings obtained on the effect of a rice herbicides cyhalofop butyl, and fenoxaprop-P-ethyl on the chlorophyll- a, β-caroten, phycocyanin and allophycocyanin contents of *N. muscorum*. These parameters may be of great relevance to determine the toxicity of cyhalofop butyl, and fenoxaprop-P-ethyl on *N. muscorum* and, the studies carried out provide a preliminary idea about the inhibitory or stimulatory effect of cyhalofop butyl, and fenoxaprop-P-ethyl on photosynthetic activities in cyanobacteria.

MATERIALS AND METHODS

Test organisms and cultivation Cyanobacterial culture obtained from Department of Botany Kakatiya University Warangal. This including *N. muscorum*. Stock cultures were grown within the N-free BG- 11 medium as previously described (Castenholz 1988). Temperature was maintained at (25 ± 2°C) and cultures were grown under a cool white light. Cells within the logarithmic phase of growth were collected from cultures and used as inoculate for experiments. Experiments were conducted in batch cultures by using 10 ml of inoculated medium flasks in 25ml. Culture media were adjusted accordingly pH 8 with 1N NaOH and 1N HCl. Illumination was provided with 600 lux cool white light (Fogg *et.al.*,197).

Influence of fenoxaprop-P-ethyl and cyhalofop butyl on pigment contents

The influence of various concentrations of fenoxaprop-P-ethyl (10.0- 500mg/L) and cyhalofop butyl, (10.0-500mg/L) on the chlorophyll- a, β-caroten, phycocyanin and allophycocyanin contents were also tested on *N. muscorum*. The experimental cultures were grown in 25ml flasks containing 10ml N-free BG-11 medium under the identical conditions as described below. per (Rippka 1988), the cultures were grown during a liquid sterilized medium at (25 ± 2°C) under cool white light (600 lux) for 30 days. At the top of 30 days, chlorophyl, β- caroten, phycocyanin and allophycocyanin contents of the cultures were determined as described below techniques.

Appropriate control systems containing no solvent and herbicide 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.

ANALYTIC METHODS

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 12h and dry weights were measured (Fogg *et.al.*, (1973).

Determination of chlorophyll a content

The spectrophotometric method (Shimadzu, UV-1201V, Japan) recommended by Porra *et al.*, (32) was used for determination. Chlorophyll *a* content was calculated on wet weights. All pigment extractions were subsequently repeated until no more pigment was extracted.

Determination of β-caroten content

The β- caroten contents was determined spectrophotometrically at 436 nm against a heptane blank (Anonymous, 2002). The quantities of β- caroten in the extracts were calculated from the measurement of absorbance at 436 nm using the equations. β- Caroten contents were calculated on dry weights. All pigment extractions were subsequently repeated until no more pigment was extracted (Anonymous, 2002).

Determination of phycocyanin and allophycocyanin contents

The spectrophotometric method recommended by Boussiba and Richmond (1979) was used for determination. Samples were centrifuged, ultrasonicated and the pigment contents were estimated in the supernatant according to Boussiba and Richmond (1979). Pigment contents were determined at 615nm and 652nm. The quantities of phycocyanin and allophycocyanin in the extracts were calculated from the measurement of absorbance at 615 and 652nm using the equations. Phycocyanin and allophycocyanin contents were calculated on dry weights. All pigment extractions were subsequently repeated until no more pigment was extracted (Boussiba and Richmond. 1979).

RESULT AND DISCUSSION

In this study, we had been determined the results of various concentrations of fenoxaprop-P-ethyl and cyhalofop butyl on pigment contents of *N. muscorum*. When *N. muscorum* was cultured within the presence of varied fenoxaprop-P-ethyl and cyhalofop butyl concentrations, distinct effects were seen on pigment contents. The pigment contents of cyanobacterium treated with different concentrations of fenoxaprop-P-ethyl under 600 lux candlepower.

The nitrogen- fixing cyanobacteria are known to dominate the water- logged paddy fields and help within the nitrogen economy of rice agriculture (Singh 1961). Although the utilization of the herbicide is geared toward eliminating weeds, a serious portion is deposited on the surface of the soil and might adversely affect the non-target soil microflora. Information on resistance to herbicides, and for these herbicides, are lacking. In Turkey, fenoxaprop-P-ethyl and cyhalofop butyl are mostly used for eliminating weeds in paddy fields. For this reason, the herbicides were chosen for this study.

The maximum chlorophyll *a* content was determined in *N. muscorum* (0.003 mg/ml) at 6.25 mg/L fenoxaprop-P-ethyl concentration whereas, the lowest chlorophyll *a* content of *N. muscorum* was shown at 50 mg/L fenoxaprop-P-ethyl concentration. The chlorophyll-a contents of this strain were completely repressed during the 100 mg/L fenoxaprop-P-ethyl concentrations. 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 1996).

Similarly, the highest β- caroten content (0.26 mg/L) was determined by 6.25 mg/L fenoxaprop-P-ethyl concentration in *N. muscorum*. The β- caroten contents of this strain were partly repressed during the 50 mg/L fenoxaprop-P-ethyl concentrations, whereas β- caroten contents of this strain were completely repressed during the 100 mg/L fenoxaprop-P-ethyl concentrations.

Otherwise, the highest phycocyanin content was determined in *N. muscorum* (0.006 mg/ml) at 6.25 mg/L fenoxaprop-P-ethyl concentration. The phycocyanin contents of this strain were partly repressed during the 50 mg/L fenoxaprop-P-ethyl concentrations, whereas phycocyanin contents of this strain were completely repressed during the 100 mg/L fenoxaprop-P-ethyl concentrations (Figure 3). Allophycocyanin contents of *N. muscorum* were partly inhibited up to (25 mg/L) fenoxaprop-P-ethyl concentration whereas allophycocyanin contents of strain were completely inhibited up to 100 mg/L fenoxaprop-P-ethyl concentration. It is similar to the previous report by Marco *et. al.*, (1990), who found that the organophosphorus insecticide trichlorfon (at concentrations ranging from 20 to 300 µgml-1) decreased biliprotein content in *N. muscorum*.

In this study, the maximum chlorophyll *a* content was determined in *N. muscorum* (0.004 mg/ml) at 25 mg/L cyhalofop butyl concentration whereas, the lowest chlorophyll *a* content of this strain was shown at 200 mg/L cyhalofop butyl concentration. Growth studies showed that the cyanobacterial strains could grow both photoautotrophically and photoheterotrophically (Guoan *et.al.*, 1997). In *N. muscorum*, a concentration of 25 ppm carbofuran was observed to be stimulatory under most of the experimental conditions established by Kar and Singh (1978).

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