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EFFECT OF FUNGICIDES ON PROLINE CONTENT OF *NOSTOC SP*

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ABSTRACT

This study describes the toxic effects and most widely used fungicides on the intracellular proline content of cyanobacterium *Nostoc sp*. In spite of chlorophyll reduction the intracellular proline content increased in the presence of plant protecting chemicals. The intracellular proline content accumulation was more in the presence of salt stress (300mM) and slightly less with fungicides. Mancozeb at 3.0µg/ml other fungicides also increased the proline content but with gradual increase in fungicide (3.5 - 5.0 µg/ml) concentration led to a significant change, maximum yellowing was observed resulting in proline content degradation. The order of toxicity was Mancozeb> Difeconazole> Ediphenphos> Hexaconazole.

Key words : Fungicides, Proline, degradation, accumulation

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INTRODUCTION

The aquatic ecosystem is at stake due to the presence of pesticides, heavy metals, salts in agricultural, urban and industrial wastes. The levels of environment pollution can be screened with the presence of cyanobacteria the primary producers in fresh water ecosystems. Cyanobacteria are the oldest microorganisms belonging to Precambrian period. They have developed a protective mechanism to several stress factors. Stress on plants, bacteria, protozoa, algae and other resulted in accumulation of proline content[1]. Proline accumulation has been reported to be an important index for stress tolerance capacity in plants[2]. It functions as stabilizer[3]. A metal chelator[4]. An inhibitor of lipid peroxidation[5]. And a singlet scavenger[6].Most of the work done in India and abroad was on higher plants, and the work done with algae was using synthetic pyrethroids so there is a need to study the effect of fungicides on proline content hence an attempt was made to exploit the proline differentiation.

MATERIALS AND METHODS

Pesticide: For evaluating the pesticide toxicity Carbendazim 50% WP, Mancozeb 75% WP, Hexaconazole 5% SC and Ediphenphos 50% EC was separately added to the fresh medium in calculated amounts to obtain a final concentration of 0.5, 1.0 – 4.0µg/mL. For control sets microorganism was grown without adding the pesticide. Commercial grade Fungicides were produced by Rasayan chemicals and Crystal phosphates ltd (India). Nacl (AR) used for the study was obtained from Himedia lab, Mumbai

Test strain: Cyanobacterial strain was isolated from the rice field of Vadrapalli, Visakhapatnam, AndhraPradesh, India. The test strains were raised in BG-11medium (pH 7.3) without nitrogen source[7] . The flask and the medium were sterilized in an autoclave (Kemi Model KAUC-S4 India) maintaining 15Lb/in^2 pressure for 15minutes. 50mL of the inoculum were suspended in 500mL sterile medium taken in 1000mL Erlenmeyer flasks (Triplicates) maintaining the culture O.D at (0.4 ± 0.1) at 560nm. Cultures were allowed to grow for 15-20days under 2000 ± 100 lux provided by 20W fluorescent tubes following 16:8 light/dark regimes at a temperature of 30˚C ± 2˚C. The biomass obtained was washed to remove the pesticide.

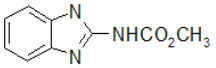
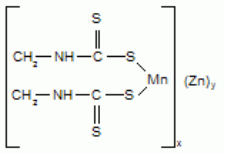
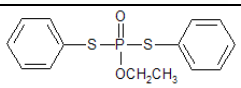
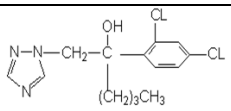
Growth Measurement: The test cyanobacterial strain growth was determined for a period of 20days by recording the optical density values of the 5mL of suspension at 560nm on a U.V-Visible Spectrophotometer (SHIMAZU Spectrometer UV-1800 ENG 240V) at 4days interval.

Biochemical analysis: Biochemical analysis of 20 day's old harvested biomass of the test organism under stress and control conditions was carried out in triplicate for evaluating chlorophyll, proline.

Chlorophyll: Extraction was made using 5mg dry weight in 10mL 95% methanol in the test tube that was placed in water bath at 65˚C for 30minutes. The pellet was discarded and the absorbance at 650nm and 665 against 95% methanol as blank[8].

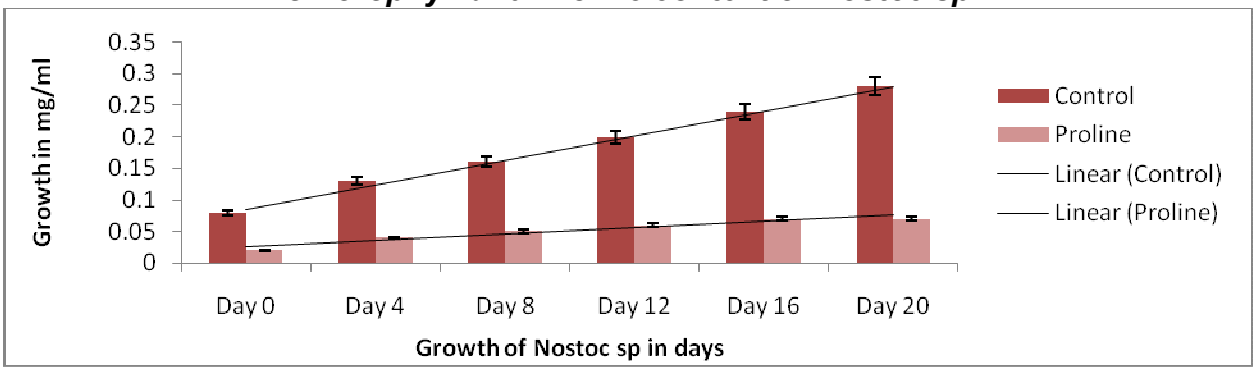
Proline: Cells were suspended in 10 ml of 3% sulphosalicylic acid and centrifuged at 5000g for 10min to remove cell debris. To 2ml of supernatant, 2ml of ninhydrin was added, followed by addition of 2ml glacial acetic acid and incubated at boiling temperature for one hour. The mixture was extracted with toluene. Proline was quantified spectrophotometrically 520nm from organic phase[9].

Table 1
Physico-chemical properties of selected Fungicides

Pesticides	Emperical formula	Chemical name and number (Chemical abstract Service)	Chemical structure	Molecular weight ($g.mol^{-1}$)	Melting point (°C)
Carbendazim (Fungicide)	$C_9H_9N_3O_2$ CAS No:10605-21-7	Methyl-benzimidazol-2-ylcarbamate		191.2	307°C
Mancozeb (Fungicide)	$(C_4H_6MnN_2S_4)_x(Zn)_y$ CAS No:8018-01-7	Mangaese ethylenebis (dithocarbamate) (polymeric) coplex with zinc salt		541.0	138°C
Edifenphos (Fungicide)	$C_{14}H_{15}O_2PS_2$ CAS No:17109-49-8	O-ethyl S,S-diphenyl phosphorodithioate		310.4	25 °C
Hexaconazole (Fungicide)	$C_{14}H_{17}CL_2N_3O$ CAS No: 79983-71-4	2-(2,4-dichlorophenyl)-1-(1H-1,2,4-triazol-1-yl)hexan-2-ol		314.2	111°C

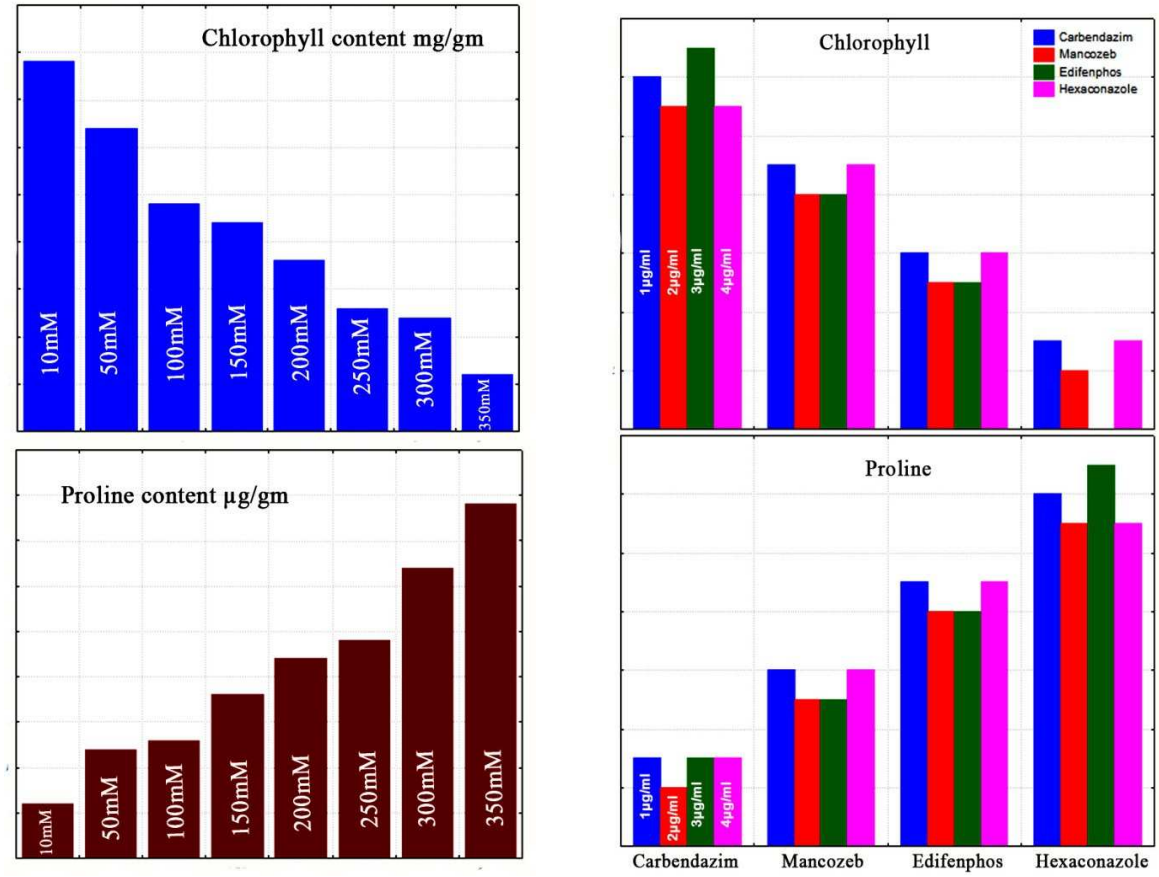
RESULTS AND DISCUSSION

Figure 1
Chlorophyll and Proline content of Nostoc sp



The main concentration was laid on the proline content of the cyanobacteria in stress conditions. The cyanobacteria were led to death by proline structural proteins. Transgenic micro algal cell was developed to study the molecular mechanism of proline mediated tolerance to toxic metals[10]. Literature on higher plants, algae and cyanobacteria in relation to proline accumulation was not seen. Algal growth photosynthesis and biochemical composition were affected by pesticides[11]. Antioxidant enzyme activity was checked in *Nostoc muscorum*[12].

Figure 2
(A) Effect of Salt stress on Nostoc sp
(B) Effect of Fungicides on Nostoc sp



(A) **(B)**

The effect of organophosphate pesticide chlorpyriphos on biochemical composition was noted in *Nostoc sp*[13]. Proline content enhancement under salt stress was reported[14]. Folded protein structures are stabilized by proline[15]. And by interacting with phospholipids the membranes are regulated[16]. Cytosol harbors proline accumulation and maintains cellular osmotic adjustment[17]. Higher chlrophyllase enzyme, Mg^{2+} ion replacement, photochlorophyll and pigment degradation are the reasons for reduced growth in algae and cyanobacteria[18]. Photosynthetic activity and growth are reduced by salt stress[19]. Oxidative stress by free radicals is reduced by proline[20]. In the present study intracellular proline content was enhanced by salt and fungicide stress in cyanobacteria *Nostoc sp*. The maximum proline content was seen in Mancozeb at 3.0µg/ml concentration and least proline content was recorded with Ediphenphos at 3.0µg/ml. After 3.0µg/ml concentration the blue green algae started to die by degrading chlorophyll content. The order of toxicity was Mancozeb> Difeconazole> Ediphenphos> Hexaconazole. At 300mM salt concentration high proline content was observed.

From the above experimental findings proline seems to be stress induced substance in *Nostoc sp* and it helps in sustaining environmental severities. Hence a genetically engineered pesticide resistant *Nostoc sp* can be a better biofertilizer.

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