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Response of *Westiellopsis prolifica* Janet. and *Scytonema cincinnatum* Thuret ex Born et Flah. (Cyanobacteria) to the effect of 2,4-D on growth and nitrogen fixation

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Abstract
Effect of different concentrations (0.5–100 mg.ml^{-1}) of 2,4 - Dichlorophenoxy acetic acid on the growth of the cyanobacteria *Westiellopsis prolifica* Janet and *Scytonema cincinnatum* Thuret ex Born. et Flah. has been studied. 2,4-D stimulated the growth up to 5 mg.ml^{-1} in *W. prolifica* and up to 1 mg.ml^{-1} in *S. cincinnatum* respectively over the control. Higher concentration of 2,4-D (above 10 mg.ml^{-1}) proved lethal for both *W. prolifica* and *S. cincinnatum*, even though it could tolerate up to 100 mg.ml^{-1}.

Keywords: 2,4 D, Cyanobacteria, *Scytonema*, *Westiellopsis nitrogen fixation*

Cyanobacteria are oxygenic photosynthetic prokaryotes which can fix atmospheric nitrogen and are abundant in rice field ecosystem. It is known that cyanobacteria contribute 25-30 kg N/ha to rice besides increasing the yield to the tune of 10 -15 percent. However, the cyanobacteria in rice fields are subjected to various field problems such as salinity, acidity, herbicide application, etc. which affect their growth and function (Gopalaswamy, 2001). In the present day agriculture, weed control using herbicides is a common practice. Use of many high yielding varieties of paddy has necessitated routine application of variety of agrochemicals including insecticides and herbicides. A considerable amount of these herbicides get access to terrestrial ecosystems and affect the non target organisms. Many of them are potential biofertilizers and help in improving crop yields (Likhitkar and Tarar, 1995). Herbicides impose chemical stress which influences the biological activity of cyanobacteria (Goyal *et al.*, 1991). Herbicide 2,4-D a synthetic growth hormone analogue is likely to affect the growth of cyanobacteria. Hence the present study was undertaken to know the relative tolerance of the cyanobacteria, *W. prolifica* and *Scytonema cincinnatum* , to 2,4-D.

Materials and Methods

W. prolifica Janet (CU 45286) and *S. cincinnatum* Thuret.ex Born. et Flah. (CU 45294) were isolated from the paddy field soil of Malappuram District, Kerala state (pH 6.5) and axenic cultures of the organisms were obtained by streak plate method. The cultures were grown under continuous light at 25 ±1^{0} C in BG- 11 N-free medium (Rippka *et al.*, 1979). An equal volume of exponentially growing homogenized cyanobacteria were inoculated to culture media containing 2,4-D having 0.5,1, 5 ,10, 50,100 mg ml^{-1} concentration and the experiment was set up to 35 days. The morphology was studied by observing the cyanobacteria through a stereo microscope. The growth was estimated by measuring the optical density in a spectrophotometer set at a wavelength of 760 nm against a reference blank containing a sterile medium. The total chlorophyll, protein content and ammonia excretion were also estimated as an indicator of growth. The chlorophyll content was assayed after extracting the cyanobacteria in 80% acetone and measured at a wavelength of 665 nm, 645 nm, and 630 nm (Parsons and Strickland, 1965). The total protein content was measured according to Lowry *et al.* (1951) and Price (1965). The ammonia excretion was estimated by phenol-hypochlorite method (Solorzano, 1969).

Results

The effect of different concentration of 2,4-D on growth, pigmentation, protein content and ammonia excretion of *W. prolifica* and *S. cincinnatum* were examined. The extent of sensitivity was slightly different in both the organisms. Results indicated that growth was stimulated in medium with 2,4-D up to 5 mg.ml^{-1} in *W. prolifica* and up to 1 mg ml^{-1} in *S. cincinnatum* respectively over the control. Relatively higher concentrations (above 10 mg.ml^{-1}) proved injurious for both *W. prolifica* and *S. cincinnatum*, eventhough it could tolerate up to 100 mg ml^{-1} (Figure 1 & 2). In *S. cincinnatum* there was only a marginal decrease in biomass content at 5 mg.ml^{-1} 2,4-D. Thus *W. prolifica* showed better growth than *S. cincinnatum*. The chlorophyll and proteins decreased with increasing concentration of herbicide which showed a trend similar to that of biomass production (Figure 3-6). A decrease in ammonia production was observed with increasing concentration of herbicide. The release of ammonia was more from *W. prolifica* than in *S. cincinnatum* (Figure 7).

Discussion

The increasing use of herbicides in rice fields, particularly the 2,4-Dichlorophenoxy acetic acid (2,4-

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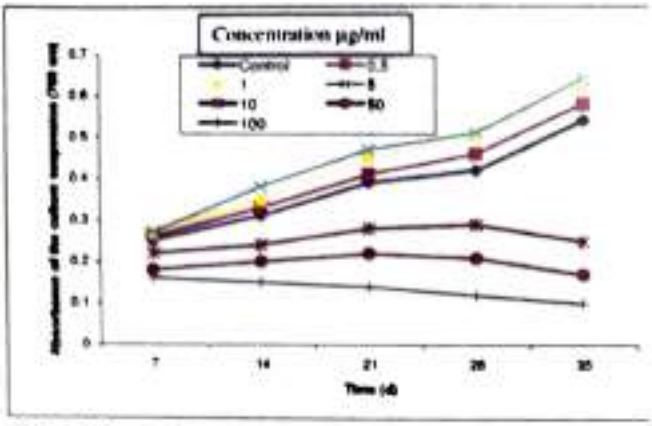

Fig.1: Effect of 2,4-D on the growth (absorbance of the culture suspension at 760 nm) of *Westiellopsis prolifica* up to 42 days of incubation.

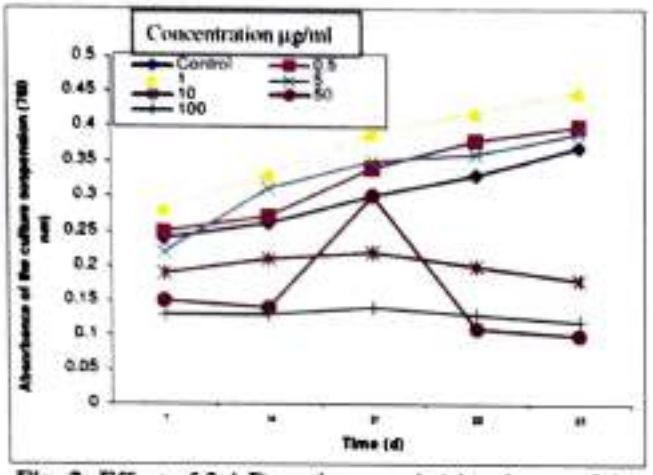

Fig. 2: Effect of 2,4-D on the growth (absorbance of the culture suspension at 760 nm) of *Scytonema cincinnatum* up to 35 days of incubation.

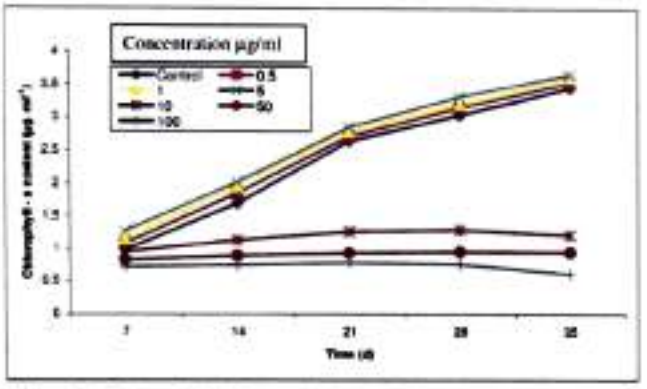

Fig. 3: Effect of 2,4-D on chlorophyll-a content of *W prolifica* up to 35 days of incubation

D), a synthetic growth hormone analogue is likely to affect the growth of cyanobacteria. In the present investigation, 2,4-D has been observed to be toxic to *W. prolifica* and *S. cincinnatum* above 10 $mg.ml^{-1}$. The results indicated that the level of herbicide used strongly affected the cyanobacterial growth and biochemical metabolites. Both cyanobacteria exhibited a differential degree of sensitivity to 2,4-D. The possible reason for such behaviour of the cyanobacteria may be due to the differential permeability of this herbicide across the cell membrane. It was found that up to 5 $mg.ml^{-1}$, the herbicide was stimulatory for *W. prolifica*. However, more than 5 $mg.ml^{-1}$ is inhibitory to biomass production, chlorophyll synthesis and nitrogen fixation. The effect of herbicide on pigment synthesis in turn reduces the rate of photosynthesis, cell division and finally the growth rate. In *S. cincinnatum* 1 $mg.ml^{-1}$ 2,4-D is stimulatory while above 5 $mg.ml^{-1}$ shows an inhibitory effect. However, a complete inhibition was not observed even up to 50 $mg.ml^{-1}$ during the present investigation and a minimum growth seen even at 100 $mg.ml^{-1}$, suggest the variations in tolerance level at generic level. There are reports that *W. prolifica* can tolerate up to 150 $mg.ml^{-1}$ 2,4-D (Nanda and Padhi, 1992). A stimulatory effect on the cyanobacteria at low concentration of herbicide was attributed to the direct effect exerted by the utilization of either the chemical itself or its degradory products (Goyal *et al.*, 1991).

According to Khalil *et al.* (1980), *Chlamydomonas* and *Anabaena* can grow well in 25 $mg.ml^{-1}$ 2,4-D. A gradual reduction in total nitrogen fixed by cyanobacteria was observed with increase in the concentration of various herbicide. It agrees with the findings of Goyal *et al.* (1991) and Likhitkar and Tarar (1996). Tiwari *et al.* (1981) reported that 2,4-D induces growth and heterocyst formation in cyanobacteria but higher concentration affect the growth. Thus it was expected that 2,4-D at higher concentration may block carbondioxide fixation either by reducing photolysis of water or by interfering at the level of electron transport chain (Moreland, 1980).

The inhibitory effect on cyanobacterial growth has been reported for many herbicides (Padhy, 1985). This inhibition may be caused due to the primary effect of these herbicides at the photosynthesis level which then leads to several secondary effects. The site of action of herbicides inhibiting electron transport is closely associated with PSII. Therefore reactions coupled with PSII such as noncyclic electron transport with water as electron donor and various electron acceptors get inhibited. The severe depletion of phycobilins on addition of 2,4-D can also force the cyanobacterial cells to have only cyclic photophosphorylation resulting the cessation of carbondioxide fixation. This can limit the supply of carbon compounds which serve as the main source of energy and raw material for the synthesis of other cellular constituents.

There are reports that the physiological damage generated at higher doses of 2,4-D may be partially reduced by glucose or amino acids (Nanda and Padhi, 1992). Since the heterocyst development depends on the cyanobacterial growth, the herbicide concentration inhibiting growth also inhibits the heterocyst formation (Ahluwalia and Dahuja, 1997).

The depletion of cyanobacterial pigments seems to result from photooxidation induced by the inability of chlorophyll to dissipate its absorbed excitation energy due to inhibition of electron transport. The inhibition limits the availability of NADPH (Moreland, 1980). Since energy and reducing potential provided by NADPH play a vital role in many biosynthetic pathways, the changes in other parameters are also expected.

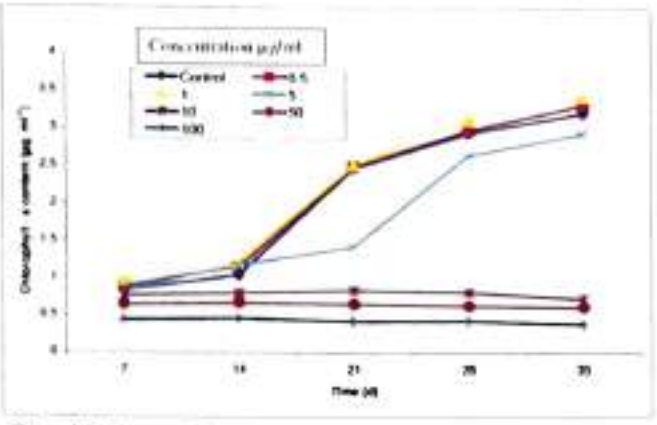

Fig. 4: Effect of 2,4-D on chlorophyll-a content of *Scytonema cincinnatum* up to 35 days of incubation.

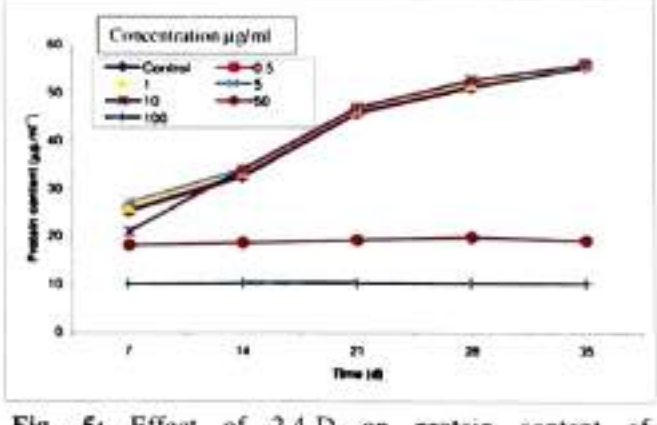

Fig. 5: Effect of 2,4-D on protein content of *Westiellopsis prolifica* up to 35 days of incubation.

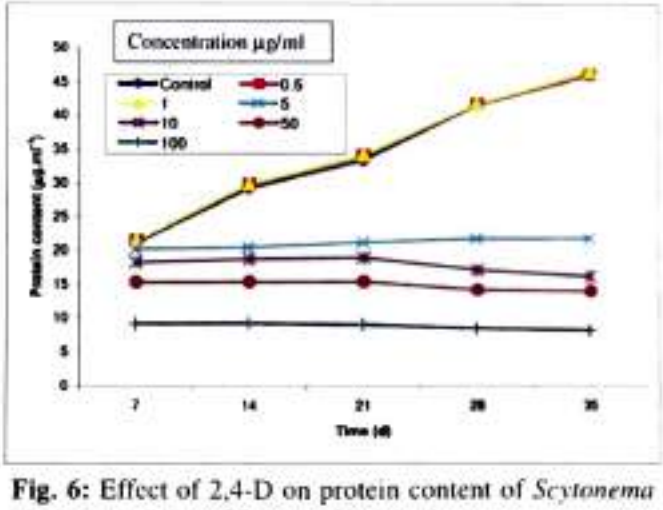

Fig. 6: Effect of 2,4-D on protein content of *Scytonema cincinnatum* up to 35 days of incubation.

The possible utilization of phycobilin pigments by cyanobacterial cells under nitrogen stress caused by the 2,4-D results in reduction of protein level. These phytotoxic chemicals move into the cell affecting the electron transport and enzymatic activities resulting in the destruction of metabolic process (Lal and Saxena, 1980). According to Ahluwalia and Dahuja (1997), the release of ammonia at higher concentration of herbicide may be partly due to the death of cyanobacterial cells.

There are several findings on the toxic effect of herbicides to a large number of cyanobacteria, thus affecting the total productivity (Kolte and Goyal, 1990; Venkataraman *et al.*, 1994). Herbicides reduce the growth, heterocyst differentiation and nitrogen fixation

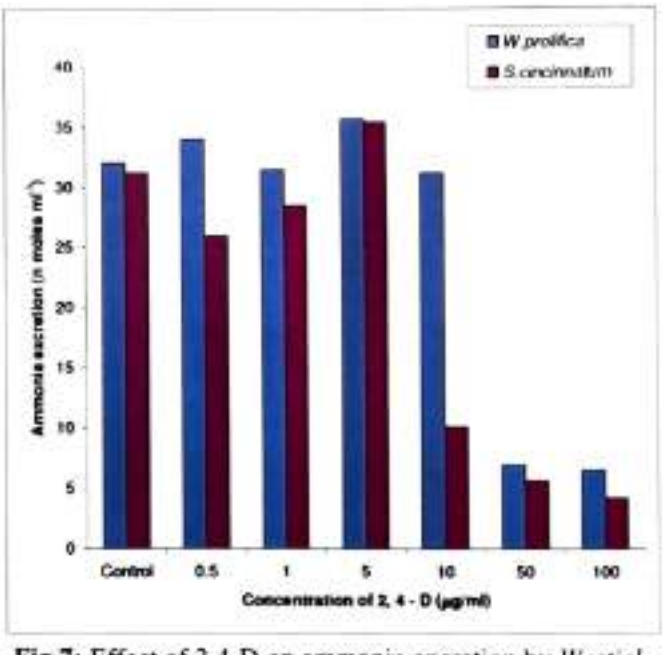

Fig 7: Effect of 2,4-D on ammonia excretion by *Westiellopsis prolifica* and *Scytonema cincinnatum* on 35th day of incubation

of cyanobacteria (Ahluwalia, 1988). It is evident from the present study that *W. prolifica* and *S. cincinnatum* remain unaffected at low concentration of 2,4-D and thus this biological system can be effectively employed for nitrogen build up in paddy field soils.

Acknowledgements

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