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EFFECT OF MALEIC HYDRAZIDE ON GROWTH AND DEVELOPMENT OF *NOSTOC SPONGIAEFORME*

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Maleic hydrazide (MH) inhibited the growth of *Nostoc spongiaeforme* at 10,000μg/ml concomitant with quantitative reduction of cellular metabolites. MH at lower concentration (1000μg/ml) stimulated the growth and cellular metabolites of *Nostoc spongiaeforme* when compared to control. The heterocyst frequency and precent sporulation decreased with increasing concentrations of MH.

Keywords : Maleic hydrazide (MH), *Nostoc spongiaeforme*

Introduction

Maleic hydrazide (MH) is a potential growth inhibitor to various algae (Kim and Greulach, 1962; Tamiya *et al.* 1962) by inhibiting their metabolic p ocesses and acts as a mutagen in green algae (Sarma and Tripathi, 1973) and blue-green algae (Gupta and Kumar 1970). Pachpande and David (1980) reported that MH inhibited the growth in terms of dry weight in *Chlorococcum infusionum Meneghini* (Schrank) even at low concentrations. MH specifically inhibited DNA replication (Scott, 1968) and to cause severe depression in the rate of DNA synthesis (Evans and Scott, 1964). Tyagi (1973) observed the ineffectiveness of MH on heterocyst differentiation in *Anabaena doliolum.* The present paper deals with the effect of MH on growth and development of blue-green alga, *Nostoc spongiaeforme.*

Material and Methods

The heterocystous, sporulating blue-green alga, *Nostoc spongiaeforme* (IARI, New Delhi culture collection) was used in the experiment. The alga was grown in Chu No. 10 medium as modified by Gerloff *et al.* (1950). Both the experimental and stock cultures were incubated in fluorescent light (600 lux) and maintained at 28$\pm$2°C. The source of inoculum was a homogeneous spore suspension obtained by crushing the sporulated axenic clonal populated alga with glass beads in sterilized distilled water. The number of spores per ml was estimated with Neubauers

haemocytometer. Growth was measured interms of optical density of 80 per cent acetone extracted cholorophyll-a pigment. The techniques for Quantitative estimation of cellular metabolites are same as mentioned earlier (Sarada, 1990). Heterocyst frequency was expressed interms of average number of heterocysts present in hundred vegetative cells in twentyfive actively growing vegetative filaments. The percentage of sporulation was calculated as the number of spores per hundred vegetative cells in a filament. The percentage of sporulation is based on an average of ten sporulating filaments. MH (BDH, England) was used during the present investigation.

Results and Discussion

The growth response of *Nostoc spongiaeforme* to MH is shown in Fig. 1. Four days lag phase, twenty days exponential phase and more than seven days declining phase were observed on the growth *N. spongiaeforme*. As in control, MH grown *N. spongiaeforme* cultures expressed 4 days lag period except in 7500 µg/ml culture where the lag period was ten days. The lowest concentration of MH (1000 µg/ml) promoted the growth which was higher than control. The next higher concentrations of MH, decreased the growth inversely proportional to the respective concentrations. The growth was completely inhibited at 10,000 µg/ml MH grown cultures.

The data shown in Table 1 revealed the levels of various cellular metabolites such as pigments (Chlorophylls and carotenes) proteins, carbohydrates and nucleic acids (DNA and RNA) in various concentrations of MH grown cultures. These cellular metabolites decreased with the increased doses of MH except at 1000 µg/ml dose, in which the quantity of cellular metabolites was higher than control. The results obtained, suggested that MH affects not only the photosynthetic pigments, but also the cell at multiple sites as evidenced by the reduction of proteins, carbohydrates and nucleic acids.

It is evident from Table 2 that the heterocyst frequency was reduced with the increasing levels of MH. Heterocysts were developed on 6th day in control and 1000 µg/ml treated MH culture, followed by reduction in frequency upto 10th day and increased further in the latter period. The experimental cultures showed the reduction of percent heterocysts with the age and concentration of MH as compared to control. Heterocyst development was completely suppressed in 7500 µg/ml concentration. Though 1000 µg/ml concentration promoted the growth and cellular metabolites it did not show

Table 1 : Effect of maleic hydrazide on cellular metabolites in *Nostoc spongiaeforme*

Concentrations	Chloro-phyll-a (mg/g)	Carotenoids (mg/ml)	Proteins (μg/100 mg) fresh weight	Carbohydrates (μg/100 mg) fresh weight	RNA (μg/100mg) fresh weight	DNA (μg phosphate/ (100 mg) fresh weight
Basal medium (control)	0.1156	0.0029	39.28	361.25	44.05	6 08
Basal medium+1000 μg/ml maleic hydrazide	0.1493	0.0032	41 11	440.00	49.02	6.92
Basal medium+5000 μg/ml maleic hydrazide	0.0953	0.0025	20.00	260 00	32.50	1.30
Basal medium+7500 μg/ml maleic hydrazide	0.0856	0.0021	18.38	238.50	25.60	0.95
Basal medium+10000 μg/ml maleic hydrazide	—	—	—	—	—	—

Table 2 : Effect of maleic hydrazide on heterocyst frequency in *N. spongiaeforme*

Concentrations	% Heterocyst frequency						
	Days						
	2	4	6	8	10	12	14
Basal medium (control)	—	—	3.45	2.91	2.70	3.00	3.28
Basal medium+1000 μg/ml maleic hydrazide	—	—	3.12	2.76	2.61	2.82	3.05
Basal medium+5000 μg/ml maleic hydrazide	—	—	—	2.32	1.98	1.73	2.06
Basal medium+7500 μg/ml maleic hydrazide	—	—	—	—	—	—	—
Basal medium+10,000 μg/ml maleic hydrazide	—	—	—	—	—	—	—

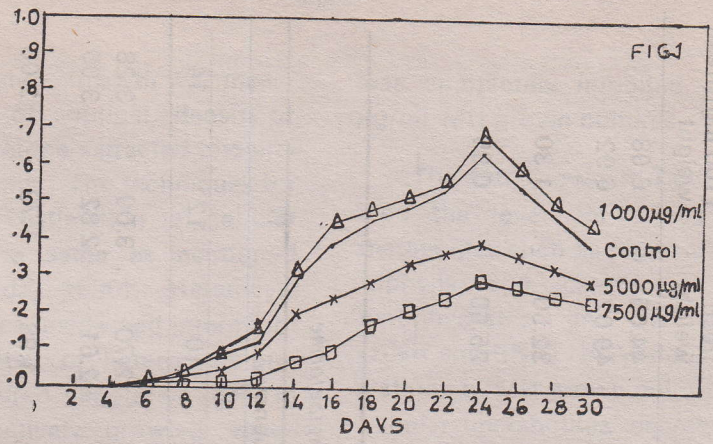

Fig. 1 Growth of *Nostoc spongiaforme* in the basal medium containing varied doses of Maleic hydrazide

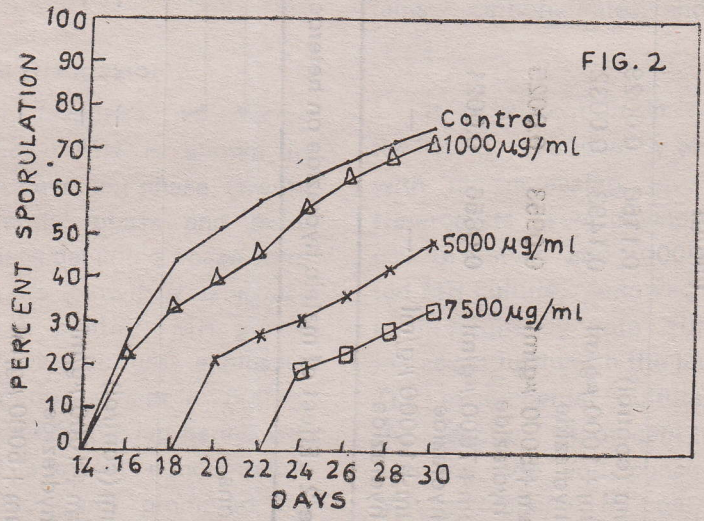

Fig. 2 Effect of maleic hydrazide on sporulation in *Nostoc spongiaeferme*

any such promotory effect on heterocyst frequency In maleic hydrazide treated culture (5000 μg/ml) the heterocysts were observed on 8th day.

The spore differentiation was observed on the 16th day of life cycle in control and 1000 μg/ml MH grown cultures (Fig. 2). In 5000 and 7,500 μg/ml concentration the spore formation was observed on 20th and 24th day, respectively. The percent sporulation was reduced with increasing doses of MH.

From the above results, MH inhibited the growth and cellular metabolites. The inhibitory action of MH on the growth and cellular metabolites might be due to cell wall permeability or disruption by physical interaction, which depends upon the concentration of MH, thereby affecting photosynthesis (Ware 1980, Rivera and Penner 1979, Lal and Saxena, 1980). The detrimental effect of MH on chloroplast indicated the direct or indirect effect on photosynthesis and other related metabolic path ways involving the replacement of Uracil in nucleic acids (Butenko and Baskakov, 1960, Kim and Greulach, 1963) because MH is an isomer of uracil (Rakittin *et al.* 1971). Hughes and Spragg (1958) postulated that MH reacted with—SH group and inhibited the reduction of proteins—SS by GSH during mitosis.

Apart from growth inhibition and reduction of cellular metabolites, MH inhibited and delayed the developmental stages such as heterocysts and spores in *N. spongiaeforme*. The differentitation of heterocyst was affected due to MH by inhibiting the activity of photosynthetic pigments involved in photochemical activity which limited the availability of chemical energy such as ATP, reducing power of NADPH to the alga. The unavailability of ATP and NADPH and reduction of required proteins and nucleic acids might have inhibited the difterentiation/development of heterocyst. Tyagi (1973) observed the ineffectiveness of MH (1 to $10\times10^{-4}\mu M$) on heterocyst differentiation in *A. doliolum*. But in the present study MH showed inhibitory effect on heterocyst differentiation in *N. spongiaeforme*.

In blue-green algae the transformation of vegetative cell into spore is a morphogenetic process during which cell size increased and several metabolic changes occurred in the cell such as reduction of pigments, polyphosphate granules and increase of cyanophycin granules (Fogg *et al.* 1973; Singh and Srivastava, 1968; Pandey and Talpasayi 1980; Wolk 1965, Tyagi 1974; Simon 1977). The delay of spore inception may be due to the delay of formation of metabolites required for spore

formation. This suggests that MH exerts its action on spore formation and percent sporutation.

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