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Toxicity of Carbofuran to Blue-green Alga *Nostoc muscorum*

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The pesticides and herbicides are being used extensively in Agriculture but their effects on widely distributed blue-green algae are not yet explored. It is now well recognised that the blue-green algae occupy an important position among the soil and water microflora and therefore, any adverse effects caused by indiscriminative use of the pesticides and herbicides on these organisms might influence the total productivity. There are few reports on the tolerance of blue-green algae towards these chemicals under defined conditions (SHILO 1965, BATTERTON et al., 1971, SINGH 1973, 1974, DA SILVA et al., 1974, MAKI et al., 1975, DAS and SINGH 1977 a,b)but there is no published records on the effects of pesticide carbofuran on these organisms. The present study deals with the effects of commonly used pesticide furadan (carbofuran) on the growth and nitrogen fixing ability of blue-green alga Nostoc muscorum.

MATERIALS and METHODS

The nitrogen fixing blue-green alga Nostoc muscorum ISU was used as the experimental material. The alga formed fine homogenous suspension in culture media on shaking and heterocyst differentiation in filaments occurred at regular intervals in the nitrogen free media.

The commercial grade furadan containing 3% active ingradient of carbofuran was used as the pesticide. Its stock solution was prepared freshly for experiments in sterilized media and its various concentrations (µg/ml) were added to the culture media contained in culture tubes (18x55 mm) and conical flasks of 100 ml capacity. The tubes and flasks containing media free from the pesticides were treated as control.

The alga was grown in modified Chu-10 medium (SAFFERMAN and MORIS 1964) with trace elements (ALLEN and ARNON 1955) in nitrogen free and nitrate containing (0.232 gm/l) media. The nitrogen free and nitrate containing media were referred as C-N and C+N respectively throughout the text. Cultures were grown in cotton stoppered conical flasks maintained in a culture room at temperature of 24±2°C with light intensity of 3000 lux provided by means of a bank of cool white flourescent tubes for 10 hrs per day. Corning glass vessels and autoclaved media were used in all experiments. Weekly transfer of cultures into fresh media were ensured to keep the alga in its exponential phase.

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Cultures were hand shaken twice daily and growth was measured in terms of Klett's units with the help of a Systronics double cell colorimeter using red filter of spectral range 625 to 700 nm. The Klett's units were converted to optical density by multiplying with a factor 0.002. Growth was also measured by taking acetone soluble pigments with the help of a Systronics spectro colorimeter at 655 nm after extracting pigments in 10 ml of 80% acetone. For gravimetric determination, 10 days old cultures were filtered through whatman No.42 filter paper. These filters were oven dried at 80°C for 24 hrs, cooled and weighed. Algal biomass was expressed in mg dry weight/50 ml of culture. Specific growth rate constant values were calculated by counting cell numbers using Fein-Optik haemocytometer counting chamber with the help of formula $Kt = \log_{10} (Nt/No)$ where = specific growth rate constant, t = 10 days, Nt = cell number at time t and No = cell number at time 0 (KRATZ and MYERS 1955). Heterocyst frequency of the alga grown in C-N media for 10 days in presence and absence of different concentrations of pesticides was calculated from the number of vegetative cells present in between two successive heterocysts.

Survivality of the alga was studied in C+N agar plates incorporated with varying concentrations (10 to 1000 µg/ml) of pesticide. Aliquot of 0.1 ml of diluted algal suspension was spread aseptically on to each of the agar plates and incubated under the light in the culture room. After a week of growth colonies were counted under binocular microscope and survival data was ploted taking the survival on control plates as 100 per cent.

Toxicity test for alga was performed using higher concentrations (1000, 1200 and 1500 µg/ml) of the pesticide. Exponentially growing cultures were used as inoculum. After 10 days of growth the treated cultures were thoroughly washed by repeated centrifugations to ensure complete removal of pesticide and the algal pellet with due dilutions was plated on C+N agar plates which were incubated under light.

Detoxification test was done taking three higher concentrations (250, 500 and 1000 µg/ml) of pesticide in 50 ml of medium. For replacement of alga, each time the cultures were centrifuged aseptically at 3,000 r.p.m. for 10 minutes and fresh alga with an optical density equal to that of first inoculum was added to the supernatant containing varying concentrations of pesticide.

Total nitrogen fixed by the alga in presence of different concentrations of pesticide (25-1000 µg/ml) was studied by inoculating 0.5 ml exponentially growing culture in 100 ml conical flasks containing 50 ml of C-N media along with the pesticide. The flasks without the pesticide served as control. After 10,15 and 20 days of incubation, growth was stopped by adding 2 ml of concentrated H_2SO_4. Samples were subjected for Kjeldahl digestion and the total nitrogen was estimated by micro-Kjeldahl method. The nitrogen present

in the pesticide was also analysed separately and the amount was deducted from the total nitrogen to know the actual amount of nitrogen fixed during the incubation period.

RESULTS and DISCUSSION

The growth response of the alga towards the furadan was determined in both C+N and C-N media (Fig.1). The alga tolerated a concentration of 1,000 µg/ml and higher concentrations did not support growth in both media. The growth remained almost unaffected at the level of 10 µg/ml while at the concentration of 25 µg/ml growth was stimulated. At the concentrations above 25 µg/ml a progressive decline in the growth was observed as evidenced by low optical density of the cultures, low absorbance of acetone soluble photosynthetic pigments (chlorophyll a) and also water soluble (phycocyanin) pigments. The specific growth rate constant values (K) gradually decreased with increase of pesticide level from 50 to 1000 µg/ml (Table 1). Gravimetric determination also followed the similar trend (Table 1).

TABLE 1

Effect of different concentrations of furadan on growth of *Nostoc muscorum*

Furadan (µg/ml)	Mean cell No.per ml on 10th day [a]	Specific growth rate constant (K)	Dry Wt.of the culture in gm on 10th day [b]
Control	3.4×10^6	0.074	0.035
10	3.6×10^6	0.077	0.036
25	3.9×10^6	0.081	0.052
50	3.5×10^6	0.072	0.028
100	3.2×10^6	0.068	0.020
250	1.9×10^6	0.044	0.014
500	1.3×10^6	0.027	0.004
1000	0.8×10^6	0.007	0.001

[a] Initial cell No/ml = 6.6×10^5, [b] Initial dry weight = 0.002 gm

Toxicity test revealed that the concentration of 1200 µg/ml was algistatic where as the higher concentrations were algicidal. The alga survived to a concentration of 1,000 µg/ml on agar plates. The survival curve was sigmoidal type indicating enhancement of survival at lower concentrations but there was gradual fall in the survival from 50 to 1000 µg/ml of pesticide (Fig.2A). Detoxification test showed interesting results (Table 2). After 10 days of incubation the growth was found to be very slow but the fresh alga which was incubated to the supernatent showed much better growth on the 5 days of incubated period. The growth of the alga determi-

TABLE 2

The loss of toxicity due to removal and reinoculation of alga (mean initial O.D. of cultures were 0.012 and 0.0125 in Ist and 2nd experiment respectively)

Optical Density of cultures after Ist incubation (10 days)			Optical Density of cultures after 2nd incubation(5 days)			Optical Density of cultures after 3rd incubation(5 days)		
250 µg/ml	500 µg/ml	1000 µg/ml	250 µg/ml	500 µg/ml	1000 µg/ml	250 µg/ml	500 µg/ml	1000 µg/ml
Expt.1 0.018	0.017	0.012	0.029	0.023	0.021	0.032	0.024	0.022
Expt.2 0.015	0.014	0.012	0.032	0.025	0.021	0.040	0.028	0.023

ned in third transfer after 5 days incubation was also slightly better than that of first transfer but the rate of the growth was comparatively slow than the second one. The percentage of heterocysts was also found to increase from 2-3% in 25 µg/ml and after which it gradually decreased. Only 3% of heterocysts were present in 1000 µg/ml (Fig.2A). There was distinct decrease of nitrogen fixing activity from 50 to 1000 µg/ml on prolonging incubation periods. However, a slight increase in the fixation rate was observed in the concentration of 25 µg/ml (Fig.2B).

The enhancement of growth, heterocyst frequency and nitrogen fixation at lower dozes of furadan (0.75 µg/ml of carbofuran) is very interesting. DA SILVA et al. (1975) reported slight stimulating activity of nitrogen fixation with few pesticides. Lower concentrations of herbicide 2,4-D also encouraged growth and nitrogen

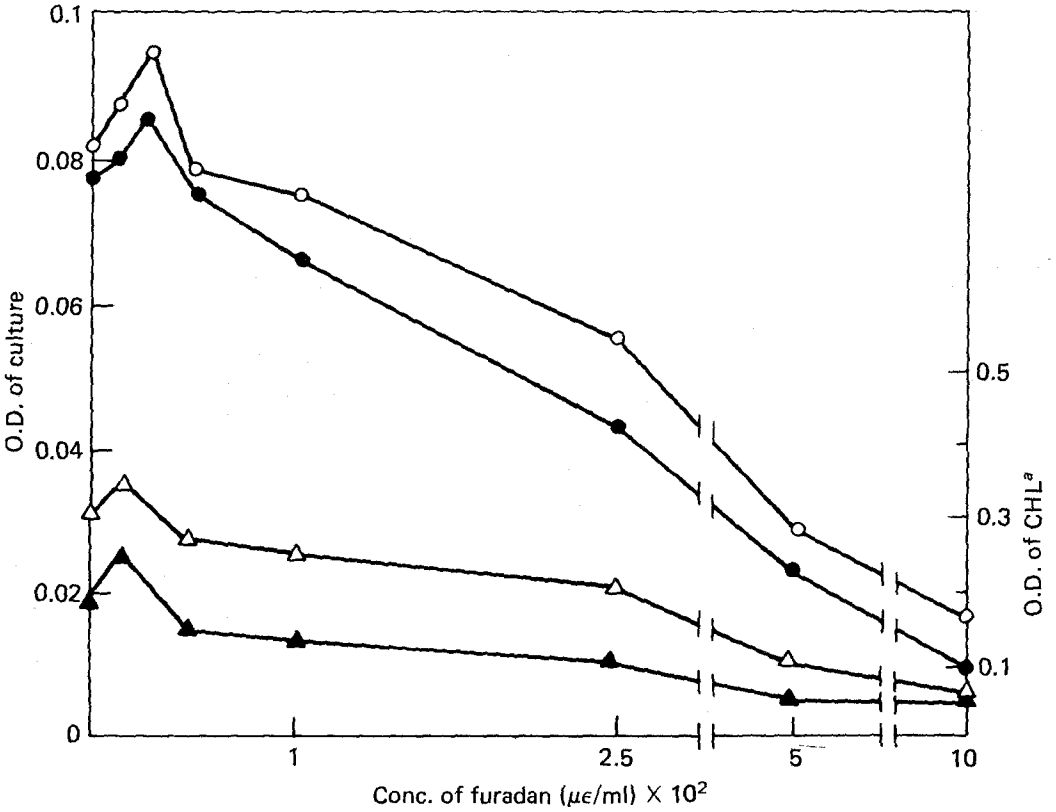

Fig. 1. Growth of *N. muscorum* in presence of various concentrations of furadan. Optical density (O.D) of culture in C+N (0–0) and C-N (●–●) medium; O.D. of acetone soluble pigments of C+N (Δ-Δ) and C-N (▲-▲) grown cultures.

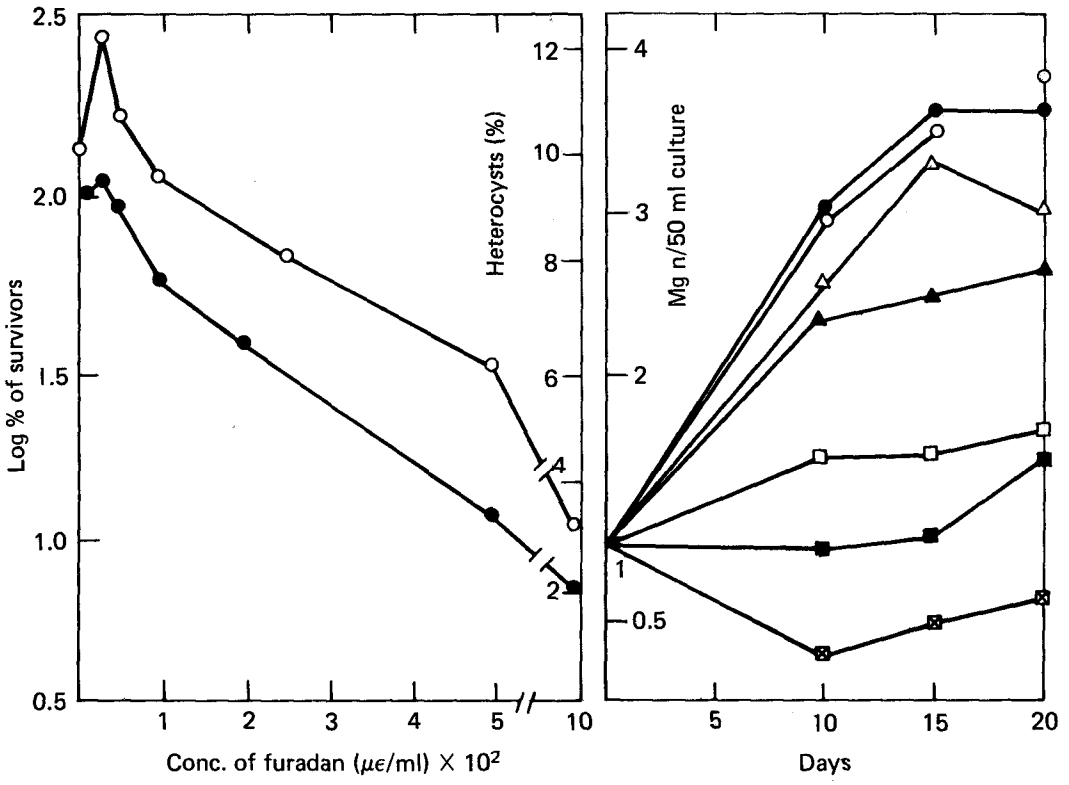

Fig. 2A Survival (0–0) and heterocyst frequency (●–●) of alga in presence of furadan.

Fig. 2B Effect of furadan on nitrogen fixation. Control (0–0) 25 ug/ml (●–●); 50 ug/ml (Δ-Δ); 100 ug/ml (▲-▲); 250 ug/ml (□-□); 500 ug/ml (■-■) and 100 ug/ml (⊠-⊠)

fixation of alga Anabaenopsis raciborskii (DAS and SINGH 1977a). The suppression of heterocyst frequency from 50-1000 µg/ml in 10 days incubation period revealed that pesticide is utilized as N source by the alga. The concentrations of 200 and 300 µg/ml could kill 50% of the population on the agar plates and liquid cultures respectively, the concentrations of 1000-1200 µg/ml were algistatic and more than 1200 µg/ml was algicidal. However the inoculum had also significant influence on the toxicity (KAR and SINGH, unpublished). The application of 17 kg of furadan (0.5 kg carbofuran/ha) is recommended to control rice pests which will be around 2-4 ppm in fields. It is likely that this concentration may not affect the survival, growth and nitrogen fixation of blue-green algae. However, the rate of nitrogen fixation gradually decreased from 50-1000 µg/ml and therefore excessive use of pesticide might effect the over all nitrogen economy of soils. Comparative studies of several blue-green algae both in field and laboratory conditions are required to reach at a definite conclusion.

The detoxifying experiment revealed interesting observations that pesticide is metabolized or biodegraded by the alga and therefore, frequent inoculation and removal of algae might help in detoxifying polluted waters. Similar effects were also observed with BHC using Anabaena aphanizomenoides and Anabaenopsis raciborskii (DAS and SINGH 1977c), FITZERALD (1975) reported detoxifying effect of algicides with bloom forming blue-green algae.

SUMMARY

Effect of commercial grade pesticide furadan (3% a.i. as carbofuran) was studied on the survival, growth and nitrogen fixation of blue-green alga Nostoc muscorum. The lower concentration of furadan i.e. 25 µg/ml enhanced survival, growth and nitrogen fixation in the alga whereas these were gradually inhibited in higher concentrations (50-1000 µg/ml) and the presence of more than 1200 µg/ml was algicidal. The preliminary observations revealed that pesticide is biodegraded by the alga.

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