# Effect of herbicides on the composition of cyanobacteria in transplanted rice

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## Summary

**The effect of butachlor (1.5 kg ha$^{-1}$ a.i.), thiobencarb (1.5 kg ha$^{-1}$ a.i.), propanil (1.5 kg ha$^{-1}$ a.i.) and 2,4-D (1.5 kg ha$^{-1}$ a.i.) on the presence of nitrogen-fixing photosynthetic procaryotic cyanobacteria was studied in rice bays during the summer monsoon at Varanasi, India. Herbicides at the recommended rate had little effect on the percentage of *Phormidium*, *Microcystis* and *Microcoleus* spp. present in the algal biomass. All herbicides increased the proportion of the blue-green algae such as *Nostoc*, while only propanil reduced the proportion of *Anabaena* in the algae population. In general, the recommended rates of herbicide did not result in major changes in the composition of the algal population.**

## Introduction

The soil microflora, especially nitrogen-fixing photosynthetic procaryotic cyanobacteria, make a valuable contribution to soil fertility by fixing atmospheric nitrogen (Singh 1961). Although cyanobacteria are found in all environmental situations, their luxuriant growth is most common in waterlogged paddy fields, where, however, herbicides are commonly used to suppress weeds. The use of algae as biofertilizers in paddy fields is thus subject to toxic chemicals which often strongly inhibit normal physiological processes by impairing regulation of the growth cycle and ultimately disrupting growth and development of such flora (Wright 1978).

Despite the importance of the microalgae in maintaining soil fertility, comparatively few studies have been undertaken on their reaction to pesticides (Greave 1982). Studies of modification of the composition of cyanobacterial species in herbicide-treated paddy soils are of greater significance to agriculture than assessments of herbicide toxicity to the individual species in pure culture. In the present study an attempt has been made to examine the effect of field doses of common herbicides to paddy fields on the composition of the cyanobacteria.

## Materials and methods

Rice seedlings were transplanted in experimental plots (0.5 m$^2$) on the Agricultural Farm, Banaras Hindu University. The plots were separated by earthen bunds (10 cm high). The rice herbicides 2,4-dichlorophenoxyacetic acid (2,4-D), thiobencarb and butachlor were applied at the rate of 1.5 kg ha$^{-1}$ a.i. 6 days after transplanting and propanil at the same rate 15 days after transplanting. The treatments were replicated thrice. Fertilizer at the recommended rates (120 kg nitrogen, 26.4 kg phosphorus and 26.4 kg potassium ha$^{-1}$) was applied at transplanting. The central plots were free from herbicides. Ten days after each application of herbicide samples were collected from 10 different sites in each plot. They were washed of soil particles, teased carefully and mounted on the slides to count the number of different algal filaments/cells under the microscope. Fifty microscopic fields were considered for each sample. For accurate analysis of populations, diluted samples (10–15 fold) were plated on agar nutrient so that colony-forming units of individual species could be distinguished.

## Results and discussion

After 2 weeks of flooding a lush green mat of algae, mostly comprising the cyanobacteria (90%), were found growing uniformly on the soil surface beneath the water. The percentage composition of both non-heterocystous and heterocystous forms, as affected by herbicides are presented in Table 1. Clearly, normal doses of the herbicides tested have no effect on the growth of non-heterocystous cyanobacteria. In all herbicide-treated plots *Phormidium* and *Microcystis* species dominated (Table 1*a*), the less abundant being *Cylindrospermum*, *Anabaena variabilis* and *Fischerella* (Table 1*b*).

**Table 1** Effect of various herbicides on the percentage of each algal species in total cyanobacterial population

| Algal species | Herbicide used (1.5 kg ha$^{-1}$) | | | | |
|---|---|---|---|---|---|
| | control | 2,4-D | benthicarb | butachlor | propanil |
| (*a*) Non-heterocystous species | | | | | |
| *Microcystis* | 15.22 | 20.08 | 15.67 | 14.47 | 6.09 |
| *Aphanothece* | 4.26 | 6.21 | 6.13 | 3.61 | 5.70 |
| *Anacystis* | 8.25 | 3.72 | – | 4.26 | – |
| *Aphanocapsa* | 4.06 | 2.07 | 5.33 | 2.97 | 2.85 |
| *Synechococus* | 1.52 | 0.41 | – | 7.62 | 4.40 |
| *Gloeocapsa* | 3.65 | 1.24 | 1.29 | 5.03 | 5.57 |
| *Oscillatoria* | 5.78 | 15.11 | 15.50 | 10.85 | 13.22 |
| *Phormidium* | 15.53 | 14.07 | 19.54 | 19.25 | 17.38 |
| *Lyngbya* | 2.03 | 1.96 | 1.93 | 2.84 | 3.76 |
| *Microcoleus* | 9.14 | 10.97 | 16.80 | 14.08 | 17.50 |
| (*b*) Heterocystous species | | | | | |
| *Cylindrospermum* | 2.84 | 8.07 | – | – | – |
| *N. punctiforme* | 1.31 | 2.88 | 0.96 | 1.29 | 1.94 |
| *N. linckia* | 0.70 | 2.00 | 1.29 | 0.70 | 0.80 |
| *N. piscinale* | 0.87 | – | 1.00 | 0.60 | 0.99 |
| *N. elliposporum* | 0.97 | 2.70 | 1.58 | 0.94 | 1.79 |
| *A. variabilis* | 0.50 | – | – | 0.60 | – |
| *A. doliolum* | 0.50 | – | 1.09 | 0.40 | – |
| *Anabaena* | 0.50 | 1.34 | 1.49 | 0.65 | – |
| *Aulosira fertilissma* | 4.67 | 5.48 | – | – | 6.09 |
| *Calothrix* | 1.52 | 1.86 | 4.20 | 3.10 | 3.63 |
| *Fischerella* | – | – | – | 3.61 | 0.55 |
| Blue-green algal spore | 15.73 | 3.62 | 6.13 | 5.94 | 7.26 |

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The frequency distribution pattern of the non-heterocystous form in the herbicide-treated plots was invariably higher than for the heterocystous form compared to the control.

However, herbicide-induced growth and toxicity varied from species to species because of the increase or decrease in the frequency distribution of cyanobacteria with reference to one herbicide or the other. An increase in the frequency of *Microcoleus, Phromidium Oscillatoria, Microcystis* and *Aphanothece* species was observed in all the herbicide-treated fields compared to the control. The same was true for certain species of *Nostoc* and *Calotherix* which are known to fix molecular nitrogen.

Numerous spores of cyanobacteria were found in the samples collected from both control (untreated) and herbicide-treated fields. Interestingly, the total frequency of spores in the control sample was much higher than in any one of the treated samples. Apparently, herbicides either inhibit the development of spores or induce their germination. Most of the spores when allowed to germinate on agar plates resembled those of *Nostoc* or *Anabaena* spp. Certain spores, resembling those of *Cylindrospermum* in size and shape, failed to germinate, however. Our studies reveal the dominance of filamentous nitrogen-fixing forms over the unicellular forms. *Anabaena* species was present occasionally.

Thiobencarb and propanil reduced the numbers of nitrogen-fixing heterocystous forms, although 2,4-D also affected certain species similarly. Rice yield after herbicide treatment was higher than in the control plots. Thus the application of herbicides to paddy crops seems to have little or no effect on the blue-green algae but, by inhibiting weeds, increased rice grain yield (Table 2).

Our present observations demonstrate that the herbicides commonly used in rice paddies do not significantly detract from microbial growth, particularly that of the photosynthetic blue-green algae which play such an important role in the fertility of these ecosystems. This observation contradicts laboratory observations in which these herbicides have been shown to inhibit the growth of blue-green algae (Tiwari *et al.* 1984; Pandey *et al.*, 1984). Almost all the species of algae recorded in the control samples were found growing in the herbicide-treated plots, except for a few herbicide-sensitive strains. Reports of herbicide toxicity to these algae under laboratory conditions indicate that the field doses should be quite inhibitory. Possibly the interactions of several factors in the field might reduce herbicide toxicity to these algae. Tiwari *et al.* (1981) have shown that the presence of organic carbon or nitrogen sources prevent or interfere with the toxicity of herbicides like butachlor, 2,4-D and thiobencarb by forming complexes that could hinder the availability of herbicides to algal cells. Other factors such as pH, presence of combined nitrogen sources and certain chelating agents are believed to reduce the toxicity of herbicides to blue-green algae (Pandey 1981). Water-logged fields being slightly alkaline and micro-anaerobic naturally promote the growth of these algae. These factors, together with the secretion of much mucilage and other extra-cellular products by most of the blue-green genera, could interfere with herbicide toxicity.

**Table 2** Effect of herbicides on grain yield (kg $ha^{-1}$) of rice

| Treatment | Yield |
|---|---|
| thiobencarb[A] | 2975.00 |
| butachlor[A] | 2573.00 |
| 2,4-D[A] | 2270.00 |
| propanil[B] | 1923.00 |
| l.s.d. ($P = 0.05$) | 190.00 |

[A]Granular; [B]liquid.

It is difficult for us to arrive at any conclusion whether herbicides actually caused the inhibition of nitrogen fixing potential of blue-green algae, because the experimental plots received nitrogenous fertilizers which are known to inhibit nitrogen fixation, it is not possible to say that the herbicides used do not reduce the nitrogen-fixing potential of blue-green algae. However, since the fertilizers used in this experiment did not appear to inhibit the growth of the microorganisms, the results suggest that herbicides, at the recommended rates, have relatively small adverse effects on blue-green algae.

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