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UTILISATION OF BLUE-GREEN ALGAE AS BIOFERTILISERS

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KEY WORDS

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ABSTRACT

Biologically active compounds may be liberated from blue-green algae growing on the surface of moist soils. Such compounds may also be released as exudates from algae grown in liquid culture.

This report describes inoculation of soils in pots, containing radish or tomato plants, with algal suspensions or exudates, which resulted in increased growth rates of both plants and increased their overall yield. Autoclaved exudates were generally as effective as fresh exudates. Interaction of effects between the various active substances depends on the algal species and method by which the soils are amended.

INTRODUCTION

Blue-green algae (Cyanobacteria) are a diverse assemblage of prokaryotic photosynthetic microorganisms. The morphology and, to some extent, metabolism of the various genera varies greatly and due to an increasing awareness of the ecological importance of these algae much information on these aspects is now available[3,9].

Blue-green algae can grow in many different habitats ranging from sub-zero Arctic ice to geothermal springs above 50°C. Many genera can grow on the surface of temperate soils, where they are often visible as a thin greenish crust on the soil. Such terrestrial blue-green algae are ecologically important insofar as many species can fix nitrogen *i.e.* reduce molecular atmospheric nitrogen to ammonium which can then be utilized for amino acid and protein biosynthesis. Nitrogenous compounds which can be assimilated by other plants are ultimately

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released to the soil after death of the algal cells and subsequent mineralisation and nitrification. Algal nitrogen fixation, release of the fixed nitrogen and it's assimilation by higher plants may be rapid[15], especially in specialised symbioses between blue-green algae and fungi, liverworts, pteridophytes or angiosperms where the algae are in intimate contact with their hosts. Biological nitrogen fixation by free-living or symbiotic blue-green algae can greatly contribute to the pool of combined nitrogen in a soil when compared to other nitrogen input sources, especially in areas where inorganic nitrogen fertilisers are not used. Over 20 kg N/ha/yr may be fixed by blue-green algae in temperate soils[7,10].

Although nitrogen fixation by blue-green algae may often be an important factor in maintaining soil fertility, the algae may directly or indirectly benefit higher plants in other ways. The algae can bind soil particles together, due to the adhesive properties of the copious mucilage excreted by many algal species. Thus they may reduce soil erosion and also evaporative water loss from soils[9] as desiccated algal crusts form a relatively impermeable cover over the soil surface. Some reports indicate that blue-green algae may release biologically active compounds into the soil and that these compounds may then be assimilated by higher plants, significantly enhancing their growth[6,13,16]. At present few data are available concerning this aspect of blue-green algal physiology.

The aim of our work has been to determine the nature and extent of any effects on the growth of higher plants due to inoculation of soils with filamentous blue-green algae. In particular the ability of the algae to supply plants with compounds other than sources of combined nitrogen is considered.

MATERIAL AND METHODS

The experiments were performed in the springs of 1976 and 1977 in a greenhouse at the Institute of Physiological Botany, Uppsala University. Plants grown and tested were radish plants (Weibull's Wonder) and tomato plants (Stella and Potentat II). The Blue-green algae used as biofertilisers were the nitrogen-fixing species (*Anabaena variabilis* Kütz. and *Nostoc muscorum* Ag. both obtained from the Culture Collection of Algae and Protozoa, Cambridge U.K. For comparison of the efficacy of different algal species, the symbiotic *Nostoc* sp., isolated from the lichen Collema, and the non-nitrogen-fixing blue-green algae *Nodularia* sp., *Plectonema* sp., and *Oscillatoria* sp. were also tested.

The soil in the experiments was artificial, prepared from milled peat which was fertilized with N, P, K, Ca, Mg and microelements (Hammenhög's Grön Kronmull and Plantjord). The soil cultures were performed in expanded polystyrene pots of 1 or 5 l capacity. The algae were cultivated separately in magnetically stirred and aerated 5 l batch cultures using the medium of Allen and Arnon[1]: the nitrogen-fixing species without inorganic N in the medium. Algal suspensions contained cells equivalent to approximately 500 mg dry weight/l when used in the experiments.

The algal fertilisers were of two kinds; a) Algal cells suspended in water, 'Suspension', and b) Filtrates of culture medium containing any exudates released by live or lysing algal cells, 'Exudates'. It was unavoidable however, that the latter also contained the nutrient salts of the algal growth medium. They were present in very low concentrations compared to concentrations in the soils used.

Application of the algal fertilisers was made either at the start of the experiments only or, additionally, at regular intervals.

Total nitrogen analyses were performed by Statens Lantbrukskemiska Laboratorium, Uppsala.

RESULTS

Algal inoculation or addition of nitrate had no significant effect on the yield of radish plants, two weeks after sowing, as shown in Table 1. After 4 weeks algal inoculation caused a significant increase in yield of whole radish plants compared to uninoculated controls. In soils amended with nitrate only, there was no increase in yield compared to plants grown without nitrate. Similarly addition of nitrate only, gave a slight increase in root fresh weight, but algal inoculation with or without added nitrate caused a considerable increase in root fresh weight over uninoculated control plants. Total nitrogen contents of radish roots were increased by addition of nitrate only, but inoculation with algae with or without supplementary nitrate caused a decrease in total nitrogen contents of the roots. Inoculation with algae alone caused a decrease in the total nitrogen of leaves. Addition of nitrate only caused an increase in leaf total nitrogen. But, unlike the roots, the highest total nitrogen levels were found in leaves from plants treated with algae and nitrate.

Table 1. Effect of inoculation with Nostoc suspension on the yield of radish plants. Radish seeds sown in pots 36 cm long × 12 cm deep. 3 kg soil/pot × algae = Nostoc suspension, equivalent to 56 mg dry wt algae/pot. + NO_3 = $NaNO_3$, 165 mg N/pot. Results are the means ± standard errors of 30 plants/treatment. Harvested 2 and 4 weeks after sowing

Treatments	2 weeks after sowing	4 weeks after sowing			
	Fresh wts whole plants (g)	Fresh wts whole plants (g)	Fresh wts roots (g)	% total N leaves	% total N roots
+ Algae + NO_3	0.303 ± 0.014	6.96 ± 0.33	2.00 ± 0.23	6.22	3.20
− Algae + NO_3	0.300 ± 0.016	5.87 ± 0.32	1.65 ± 0.16	6.07	3.44
+ Algae − NO_3	0.337 ± 0.019	7.22 ± 0.44	2.28 ± 0.29	5.85	3.12
− Algae − NO_3	0.302 ± 0.019	5.87 ± 0.30	1.51 ± 0.16	6.01	3.35

Table 2. Effect of different Nostoc and Anabaena treatments on the yield of radish plants. Radish seeds sown in round pots of 22 cm diam. and 18 cm deep. 4 kg soil/pot. The amount of biofertiliser are shown in the table. Radishes harvested 5 weeks after sowing. Results are the means ± standard errors of 14 plants/treatment

Alga	Treatment	Dry wt algae (mg)	Roots fresh wts (g)	Stems and leaves fresh wts (g)	% increase over control	
					Roots	Stems and leaves
None	Control		4.2 ± 0.9	7.3 ± 0.7	–	–
Anabaena	Autoclaved exudate	exuded from 458	5.5 ± 1.0	9.4 ± 0.8	31	29
Anabaena	Fresh exudate	exuded from 558	7.0 ± 1.2	9.4 ± 0.7	6	29
Anabaena	Suspension	44	5.8 ± 0.8	8.9 ± 0.5	38	22
Anabaena	Suspension	438	5.3 ± 0.7	8.9 ± 0.7	26	22
Nostoc	Fresh exudate	exuded from 90	6.3 ± 0.7	8.7 ± 0.8	50	19
Nostoc	Suspension	147	5.3 ± 0.8	9.3 ± 1.1	26	27

The effects of algal suspension and exudates of two genera are given in Table 2. All algal treatments augmented leaf and root growth of radish plants. Anabaena and Nostoc exudates had the greatest effect on roots, whereas Anabaena exudates and Nostoc suspensions were most effective on leaf fresh weights. Autoclaved exudates of Anabaena were less effective than non-autoclaved exudates for increasing root growth, but autoclaving exudates did not affect their ability to increase leaf growth. Effects of all treatments were comparatively greater for roots than for leaves. Inoculation with suspensions of other filamentous blue-green algae also enhanced growth of tomato plants, as shown in Plate 1. However, only Nostoc and Anabaena were selected for further study as they had the greatest effect on growth.

Growth of tomato plants was augmented by inoculation with exudates or suspensions of Anabaena or Nostoc, as shown in Fig. 1. Exudates were more effective than suspensions in promoting growth. Autoclaved Anabaena exudates were as effective as non-autoclaved exudates. Similarly, fresh weights of tomato fruits were significantly greater when soils were amended with Anabaena exudates or suspensions, as shown in Table 3. At harvest no fruit were present on untreated control plants from the experiment with Nostoc. Fruit were present on plants treated with exudates or suspensions of Nostoc. Greater yields were

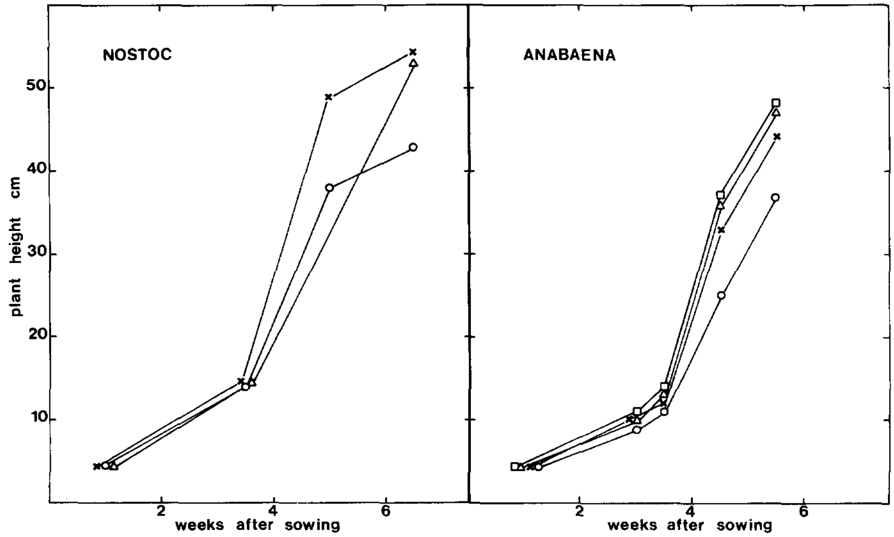

Fig. 1. Effect of different Nostoc and Anabaena treatments on the growth of tomato plants. Plants of 4–5 cm height each planted in 1 kg soil/pot. The standard error after 32 days of growth was ± 3.2 cm of 8 plants/treatment. Nostoc: o—o Control, watered with distilled water; △—△ Fresh exudate (from 53 mg algae/pot); ×—× Suspension (53 mg dry wt algae/pot). Anabaena: o—o Control; △—△ Fresh exudate; □—□ Autoclaved exudate (both from 271 mg dry wt algae/pot); and ×—× Suspension (271 mg dry wt algae/pot).

Table 3. Effect of different Nostoc and Anabaena treatments on the yield of tomato plants. The plants and treatments are those presented in Fig. 1. Suspension 1 was diluted 10 × compared to suspension 2 presented in the Anabaena series. Plants harvested after 9 weeks growth

Alga	Treatment	Fruit fresh wt (g)	Number of plants harvested
None	Control	5.2 ± 2.1	7
Anabaena	Autoclaved exudate	24.6 ± 2.1	6
Anabaena	Fresh exudate	27.6 ± 4.5	6
Anabaena	Suspension 1	21.3 ± 2.3	6
Anabaena	Suspension 2	19.3 ± 1.6	6
Nostoc	Fresh exudate	17.1 ± 1.1	4
Nostoc	Suspension	18.9 ± 1.7	3

Plate 1. Effect of inoculating soil with different blue-green algae on the growth of tomato plants. Photographed 4 weeks after sowing.

obtained from plants treated with exudates compared to plants treated with suspensions.

DISCUSSION

Inoculation of soil by Nostoc was beneficial to radish plants. The increasing fresh weights of roots and leaves was due to compounds other than those acting simply as sources of combined inorganic nitrogen, because additions of high levels of nitrate-nitrogen only had little effect on yields, whereas addition of algae had a marked effect independent of whether nitrate was added or not.

Experiments with tomato plants further indicate that the algae produce growth-stimulating compounds. Again the effect is unlikely to be due to fixation and transfer of combined nitrogen from the algae, because even the non-fixing algae, Plectonema and Oscillatoria enhanced plant growth. Biologically active compounds are released by algae growing in liquid culture as demonstrated by treatments with algal exudates. Such compounds are also released by algae growing on the soil surface following addition of algal suspensions. Growth-stimulating compounds may be released either actively by living algal cells or following cell death and lysis. The life cycle of filamentous blue-green algae is often rapid, with cells having a life span of only a few hours[11,12].

We have not established the nature of any active compounds released by Nostoc and Anabaeba. However because exudates retain most of their potency after autoclaving, cytokinins may be present rather than the heat-labile auxins and gibberellins. But as the effects vary with algal species, and whether suspensions or exudates were added, other biologically-active compounds are almost certainly produced and released by the algae. Venkataraman and Neelakantan[16] demonstrated the presence of auxins, vitamin B12 and amino acids produced by *Cylindrospermum muscicola*, a filamentous blue-green alga, and all the compounds could be assimilated by rice plants. Our results suggest an interaction between biologically-active compounds; the size and nature of the overall effect being dependent on algal species and whether suspensions or exudates are added.

Reasons for the general reduction in total nitrogen contents of plants grown on inoculated soils are unknown. Although this phenomenon may be partly explained by the fact that the algae, despite their capacity for fixing atmospheric nitrogen, wil preferentially assimilate combined inorganic nitrogen. Thus they may successfully compete with radish or tomato plants for this available nitrogen.

Our results demonstrate the beneficial effects of algal inoculation on two different cash crop plants. At present, use of blue-green algae as an alternative nitrogen source, at least in temperate soils, is uneconomic compared to inorganic nitrogen fertilisers[5]. The major limiting factor being the requirement for a moist soil surface to prevent desiccation of the algal cells. Nevertheless algae can rapidly recover from desiccation[14] and in many locations moist surfaces would be maintained; *e.g.* irrigated fields, glasshouses and domestic situations. Therefore if algae could be cultured cheaply (perhaps by harvesting unwanted algal blooms in amenity waters?) they could prove to be a remarkably cost-effective fertiliser complement; benefits from such treatments being due to growth-stimulating compounds released by the algae rather than nitrogen fixation. Storage and transport of the algae would present no difficulties as algae mixed with sand and then dried can remain viable for many years.

Two major areas worthy of further research are suggested by our results. First, investigations into the exact nature of substances released by blue-green algae are required. Second, the effectiveness of algal treatments on other plants, besides the two tested by us, should be ascertained.

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