Women's Coll. Ann. Rev.
Vol. 15 (1990).

SENSITIVITY OF ANABAENA ARYZAE AND TOLYPOTHRIX TENUIS (CYANOBACTERIA) TO POLYTRIN INSECTICIDE

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Summary

The effect of increasing concentration of the insecticide Polytrin (0.063 ------1.5 recommended field rate) on the growth of cyanobacteria Anabaena oryzae and Tolypothrix tenuis under asceptic conditions was studied. Percentage dry weight compared to control, total carbohydrates and intracellular nutrient mineral content have been estimated.

addition of Polytrin to growing cultures of A.oryzae and T.Tenuis resulted in a slight reduction of growth at low concentrations, but rapid decrease in growth was observed at higher rates for both strains. The growth of Tolypothrix tenuis was inhibited completely at 1.5 field rate.

Nitogen content of both strains were gradually reduced by increasing the insecticide concentration, while carbohydrate content of algal cells increased.

The intracellular K content of A. oryzae showed slight rise at low insecticide concentrations but a marked reduction at higher concentrations while a marked reduction in K content of T.tenuis was noticed even at the least used concentration.

Slight rise in P and Mg content was observed at low rate followed by decrease at higher applcations in both strains. Na did not show wide change by the insecticide. Zn, Cu and Mn content may show some little rise with Polytrin concentration in both organisms.

Introduction

The successful use of algal inoculation as in India and Japan demonstrates that blue-green (BGA) can serve as an alternative or supplemental source of nitrogen for rice cultivation, (Agrwal, 1979; Alyer et al, 1972; Watanabe, 1967 and Roger & Kuhsooriya, 1980). Blue-green algae may give some advantages not necessarily associated with nitrogen-fixation, such as through the production of growth-promotive substances (Roger and Kulasooriya, 1980).

From the various agronomic practice adopted along the cultivation cycles which may directly influence the growth of blue green algae is the application of pesticides. The resistance to high levels of pesticides seems to be more characterisitc of BGA than of the eukaryotic algae (Watanabe, 1967, venkataraman & Kajyalakshm, 1971, 1972;Singh, 1978). But some pestisides like chloropicrin may affect all algae without discrimination(Ishizawa and Musuguchi, 1966). Laboratory experiments showed that, metabolic products of the insecticides Aldrin, Dieldrin and endrin are inhibitory to blue-green algae. (Battino-Viterbo et al, 1973). Also, Wright et al, (1977) showed that, 5 ppm of the herbicide propanil prevented the growth of Anabaena cylindrica, Tolypcthrix tenuis and Nostoc endophytum in flask cultures.

Although a number of studies have investigated the growth responses of cyanobacteria to pesticides, available literature shows that, there are only few attempts to define systems for the study of the effect of pesticide on the intracellular mineral constituents of cyanobacteria.

The purpose, of the present study was to assess the changes in growth, intracellular carbohydrates, macro-and microelement of the cyanobacteria Anabaena oryzae and Tolypothrix tenuis when subjected to different concentrations of the foliar pyrethroid insecticide, Polytrin, in batch culture grown photoautotrophically in continuous illumination.

Material and Methods

Cyanobacteria:

Anabaena aryzae, Egyptian strain and Tolypothrix tenuis, Japanese strain were provided from the Agriculture Research Centre Giza, Egypt.

Insecticide:

Plytrin, common same Cypermethrin CGA 55' 186, 200 Emulsifiable concentrate (200 EC) was provided from Ciba-Geigy Limited, Cairo, Egypt. It is a foliar pyrethroid insecticide, its imperical formula is $C_{22}H_{19}Cl_2NO_3$.

Experimental procedure:

Cyanobacteria were grown in batch cultures under sterile conditions. Inocula were grown to mid-exponential phase. Aliquots of (5 ml) cell suspensions were inoculated into 500 ml Erlenmeyer flasks containing 200 ml of (Watanabe et al, 1959) medium. Cultures were grown under conditions of continuous illumination at an incident light intensity of 20 W/m^2 provided by white fluoresent lamps. The insecticide Polytrin was added at time-zero to cyanobacterial cultures, the concentrations used were 0.063(1/16), 0.125 (1/8), 0.25 (1/4) 0.5(1/2), 1.0 and 1.5 recommended field rate (0.5 ml 200 EC/L). Experiments were repeated at least twice.

Cells were harvested by centrifugation at the end of the exponential growth phase, the harvested cells were washed twice with distilled water.

Growth was determined in terms of (mg dry wt/100 ml culture) following drying for 24 hours at 70 °C.

Analytical methods:

Total carbohydrates were estimated by the phenol sulphuric acid reagent and calibrated against glucose standard (Dubois et al, 1956).

Intracellular ion concentrations were analyzed from the dried algal cells which were powedered and digested in nitric-perchloric-sulphuric acid. Phosphorus was analyzed using the vanadatomolybdate colorimetric method, (Chapman and Prott, 1978).

Mg, Fe, Mn, Zn, and Cu were determined by atomic absorption spectrophotometry. K and Na were estimated by flame photometry (Jackson, 1967).

Nitrogen was measured by kjeldahl method (Jackson, 1967).

Results

Effect of Polytrin on the growth of algae:

Growth of *Anabaena oryzae* and *Tolypothrix tenuis* in the presence of different insecticide concentrations in the medium (Table 1) showed that, growth was slightly reduced at low insecticide concentratoins (0.063 & 0.125 of the recommended field rate) 80.4% - 72.2% of control culture, but rapid decrease in growth was observed at higher concentrations. Minimal growth of 20.3% and 28.5% in dry weight was obtained at 1.5 and 1.0 field rate for A.oryzae, while the corresponding values for T.tenuis amounted only to 0% and 9.5%. (Table 1). The insecticide caused also yellowing of some Anabaena cells after growth at 1.5 field rate.

Effect of Polytrin on cell composition of carbohydrates and nutrient elements:

Polytrin had a detectable effect on the growth of A.oryzae and T. tenuis at concentrations greater than 0.25 field rate. Analysis of organisms exposed to all used concentration of insecticide (Tables 2,3 & 4) revealed marked differences in the content of carbohydrate, macro and micro-elements as compared to untreated organisms in the exponential phase of growth.

Table 2. shows that Polytrin caused inhibition of nitrogen fixation. The nitrogen content of both strains were gradually reduced by concentration and amounted to 56.7% and 0% of the control for A. oryzae and T. tenuis respectively at 1.5 recommended field rate.

Total carbohydrate content of algal cells increased with increasing the insecticide concentrations (Table2) reaching about twice the control value at 1.5 field rate in A.oryzae, while growth of T.tenuis was inhibited completely at that concentration.

Analysis of the intracellular macroelements of A.oryzae (Table 3) showed a slight rise in K content at low concentration and a marked reduction at higher concentrations from 0.25 recommended field rate upwards, while the marked reduction in K content with T.tenuis began from the least concentration 0.063 field rate.

Slight rise in P and Mg content was observed at low concentration followed by decrease at higher application in both strains.
Na did not show clear dependence on the insecticide concentration. Zn, Cu and Mn content showed some rise with Polytrin concentrations in both studied organisms.

Discussion

A comparison of the effect of used concentrations of Polytrin on cell growth and adaptation in A.oryzae and T.tenuis reveals that Anabaena oryzae is more tolerant to the used concentrations than Tolypothrix tenuis. Inhibition was observed at concentrations greater than

0.25 field rate. This result is similar to some previous observations on ther pesticides. Wright et al, (1977) showed that 5 ppm. of the herbicide propanil prevented the growth of Anabaena cylindrica, Tolypothrix tenuis and Nostoc endophytum in flask cultures. Venkatoraman & Rajyalakshmi (1972) observed that, among 10 Anabaena strains tested for their resistance to Ceresan, 9 could tolerate 100 ppm but 1 was inhibited by concentrations higher than 1 ppm. Cylindrospermum sp. was observed to be less resistant to insecticides than aulosira fertillissima and Plectonema boryanum Singh, (1973).

Under Egyptian conditions (El-Nawawy et al, 1962; Hamdi et al, 1970; Ibrahim, 1972, and Shalan et al, 1984) showed that most Anabaena strains and Tolypothrix tenuis were inhibited by high concentrations of various pesticides not including Polytrin.

The diminution of nitrogen content of A.oryzae and T.tenuis with Polytrin indicates a decrease in nitrogenase activity of these organisms. This loss of activity could reflect a high turn over for some protein (s) involved in N_2 fixation (Romos et al, 1985). Pesticides generally appear to limit N_2-fixing capacities of blue-green algae, thereby affecting the overall nitrogen economy of soils (DA Silva et al, 1975; Eid et al, 1962;

El-Nawawy and Hamdi, 1975; Huang, 1978; Ibrahim, 1972 and Inger, 1970).

Total carbohydrate content of A.oryzae and T.tenuis rose with increasing Polytrin concentration in the growth media. This means that, the cells did not probably completely lose the capacity for photosynthesis in the presence of the insecticide. Hamdi et al (1970),stated that, chlorophyll synthesis was stimulated by low levels of some herbicides. Increased levels of carbohydrates were typically found in nitrogen starved cyanobacteria (Allen & Smith, 1969 and Romos, 1985). Accumulation of carbohydrates could somehow counteract the negative effect of the active nitrogenous metabolites, as it seems to be the case for the control of nitrate utilization in the unicellular cyanobacterium Anacysts (Flores et al, 1983; Espin et al, 1982; Merrick, 1982). El-Mokadem et ál (1988) in a previous work have found more accumulation of carbohydrate ossociated with loss of nitrogen content in Anabaena oryzae grown under more saline conditions.

The elemental analysis of the dried cells revealed that, the algae exhibited changes in intracullular elements with increasing the insecticide. Lyzell et al, (1985), had stated that N:P ratio affects the growth rate of cyanobacteria.

It is recommended that for application of Polytrin insecticide in near by cotton or other crop fields, the rate would be adjusted in a manner not to harm the advantageous cyanobacteria added to rice as biofertilizer for nitrogen fixation.

Acknowledgement: The authors sincerely thank Prof. Dr. M.A. Hammouda, Women's College for his encourgement during this study.

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Table 1:Growth of Anabaena oryzae and Tolypothrix tenuis cultures at different concentrations of Polytrin insectcide.

Polytrin Concentration	Anabaena oryzae		Tolypothrix tenuis	
	mg.drywt 100/ml.cul.	Growth percent	mg.dry.wt/100ml. culture	Growth percent
Control	158 ± 19	100%	138 ± 17	100%
0.063 R.f.r.	127 ± 14	80.4%	109 ± 8	79%
0.0125 " "	114 ± 18	72.2%	78 ± 9	56.6%
0.25 " "	88 ± 10	55.7%	57 ± 4	41.3%
0.5 " "	74 ± 9	41.5%	40 ± 7	29%
1.0 " "	45 ± 7	28.5%	13 ± 3	9.5%
1.5 " "	32 ± 5	20.3%	Dead	0

Growth percent: calculated with reference to control growth as 100%; values based on mg. dry wt./100 ml culture the values are the mean of three independent observations.

R.f.r. : Recommended field rate.

Table 2: Effect of Polytrin insecticide on the intracullular carbohydrate and nitrogen content of <u>Anabaena oryzae</u> and <u>Tolypothrix tenuis</u>. (Cultures grown with and without Polytrin untill rate logarthmic phase).

Polytrin concentration	Anabaena oryzae		Tolypothrix tenuis	
	Carbohydrates	Total nitrogen	Carbohydrates	Total nitrogen
Control	13.4	5.82	21.2	7.38
0.063 R.f.r	17.8	5.31	26.3	6.39
0.125 " "	15.3	5.3	28.3	5.42
0.25 " "	19.5	5.3	28.1	5.28
0.5 " "	22.7	4.91	30.4	5.52
1.0 " "	25.0	4.41	29.8	4.16
1.5 " "	26.3	3.35	---	---

R.f.r Recomended field rate

Table 3: Effect of Polytrin insecticide on the intra-cellular macro & micro elements of *Anabaena oryzae*.

Treatment	P	K	Na	Mg	Fe	Mn	Zn	Cu
	mg./g .dry weight				μg./g. dry weight			
Control	11.6	14.8	0.14	6.9	192.2	83.95	82.0	24.0
0.063 R.f.r	13.9	17.7	0.14	7.5	128.1	62.1	57.6	25.3
0.125 " "	12.6	16.5	0.28	7.2	138.1	73.0	57.0	20.0
0.25 " "	7.2	0.7	0.14	5.2	73.0	87.9	161.0	32.0
0.5 " "	6.6	0.8	0.14	3.8	64.4	120.5	116.2	34.7
1.0 " "	5.5	0.1	0.14	4.0	61.9	151.5	140.6	61.3
1½ " "	4.9	0.2	0.24	4.2	48.3	93.5	116.0	49.3

R.f.r : Recommended field rate.

Table 4: Effect of Polytrin insecticide on the intracellular macro and micro elements of Tolypothrix tenuis

Treatment	P	K	Na	Mg	Fe	Mn	Zn	Cu
	mg./g .dry wt.				μg./g. dry wt.			
Control	11.1	3.5	0.21	4.5	222.	58.4	51	50
0.063 R.f.r	14.7	0.4	0.28	5.8	291.	98.1	131	62
0.125 " "	14.2	0.5	0.56	6.4	277	98.1	127	64
0.250 " "	4.7	0.5	0.56	1.9	96.1	65.4	117	50
0.500 " "	5.1	0.3	0.38	2.1	117	62.5	164	80
1.00 " "	N. D.				N. D.			
1.5 " "	--	--	--	--	--	--	--	--

R.F. r: Recommended field rate