BIOLOGICAL ACTIVITY OF THE GREEN ALGA *CHLORELLA ELLIPSOIDEA*[1] AGAINST IMMATURE STAGES OF MOSQUITOES

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ABSTRACT. Green algae, *Chlorella ellipsoidea* when tested against 1st-stage larvae of *Culex quinquefasciatus* caused mortality of larvae up to 73% after 3 days. Very low mortality in the pupal stages was observed. Both field collected and laboratory cultured algae induced mortality of the same magnitude. Algal suspension caused the highest mortality followed by supernatant; little or none was caused by residues. The toxin(s) produced by the algae was lethal and also delayed the development of mosquito larvae. Mortality increased with the increase in cell density of both algal suspension and the supernatant to some degree. The highest cell density tested against 4th-stage larvae and pupae induced little if any mortality in the former and no significant mortality in the latter. The alga was maintained successfully in the laboratory without addition of CO_2 and EDTA compounds.

Poisoning of fish, birds, cattle and humans by blooms of some toxic green and blue green algae has occurred in various parts of the world (Bradenburg and Shigley 1947, Ingram and Prescott 1954, Gentile 1971, Carmichael et al. 1975). Some of these algae have also been shown to be toxic to some species of invertebrates (Ryther 1954, Amonkar 1969, Angerilli and Beirne 1974).

During the course of studies on insecticidal control of mosquitoes in an urban cemetery (Mulla et al. 1977), a free floating, unicellular algal species, *Chlorella ellipsoidea* Gerneck was noticed in some of the flower vases. Subsequent field observations revealed that vases with high densities of this alga supported few if any immature stages of mosquitoes. Based on these observations it was concluded that this species of alga develops substance(s) which are lethal to immature, especially younger stages of mosquitoes or which alter their development. To lend support to this hypothesis, detailed studies in the laboratory were initiated to determine the biological activity of field collected and laboratory cultured *C. ellipsoidea* on the growth and survival of immature stages of *Culex quinquefasciatus* Say.

[1] (Chlorophyta:Oocystaceae).

METHODS AND MATERIALS

FIELD CULTURES. Suspensions of *C. ellipsoidea* were collected from flower vases of different lawns in Rose Hills Memorial Park, Whittier, Calif. The suspension was separated from solid contents of the vase by gradually pouring it into 1-gal. Mason glass jars. It was then strained through 80 mesh screen to obtain a uniform stock suspension. To determine the cell density, an aliquot of the stock suspension was diluted 100 times by using a graduated blood diluting pipette. Cell density was determined on a bright line hemocytometer under a compound microscope (40X).

To determine the activity of various fractions, 100 ml of the stock suspension was transferred to each of 3 china foam bowls. Another 300 ml of suspension was spun in a centrifuge (1EC) at 3500 rpm for 10 min. The supernatant was then gently poured into a glass beaker and divided into 3 equal parts. Each part was transferred to a bowl. The residue in the centrifuge tubes was collected by rinsing and reconstituting it to 300 ml with tap water. The residue suspension thus obtained was shaken thoroughly and divided into 3 equal parts, 100 ml/each bowl.

The various fractions of algal suspension, supernatant and residue were tested

against mosquito larvae and pupae in the laboratory. Twenty 1st-stage larvae of *Cx. quinquefasciatus* were placed in 100 ml of each fraction in each china foam bowl. Each fraction was replicated 3 times and the test conducted on 2 different occasions. Similar procedures were followed for pupae and 4th-stage larvae. The cumulative mortality and total emergence were assessed according to the procedure of Mulla et al. (1974). Water collected from vases without algae, and processed into 3 fractions in the manner described above, was also tested for biological activity against 1st-stage larvae. Three check replicates each containing 100 ml of tap water were run along with each test.

LABORATORY CULTURE. The alga was cultured at 85±2°F in 1-gal Mason jars maintained constantly under two 40 watt (cool-white) fluorescent lights at a distance of 9 in from the cultures. Six jars were set, each containing sufficient nutrients as used by Chimiklis and Karlander (1973); however, no EDTA (ethylenediaminetetraacetic acid) was added to the media as its addition did not result in higher cell density (unpublished data). A continuous flow of air was forced through all the jars, but a mixture of 5% CO_2-in-air was passed through only 3 jars at the rate of 55–60 bubbles/min. Each jar was inoculated with 25 ml of pure culture of *C. ellipsoidea* sufficient to provide an initial population of 1850 cells/ml. After the culture was established cell counts were periodically made. Equal amounts of nutrients, as used before, were added to each jar after a period of 17 days. The algal culture was discontinued after 30 days. Cultures obtained with or without CO_2 showed the same biological activity (unpublished data). Cultures were pooled together and assayed against the 1st-stage larvae as described above. Nutrient solution used for algal growth was also tested against the test insect. Checks with tap water were also run simultaneously.

RESULTS AND DISCUSSION

The biological activity of field collected *C. ellipsoidea* against 1st-stage larvae is shown in Table 1. The larval mortality seemed to be the highest in the suspension and the supernatant. This indicated that this alga released some water soluble substance(s) which were toxic to young larvae. Amonkar (1969) and Angerilli and Beirne (1974) also reported that blue green and green algae produced substances inhibiting larval development. The mortality caused by the whole suspension in general was similar to that produced by the supernatant, while mortality in the residue fraction was slight.

In these tests, only slight mortality was noted in the first 3 days posttreatment. However, during this period, it was observed that the majority of the larvae in the algal suspension and the supernatant were still in the 1st stage, while those in

Table 1. Evaluation of field collected green alga *C. ellipsoidea* at different densities against 1st-stage larvae of *Cx. p. quinquefasciatus* in laboratory.

	Mean % mortality in larvae (L), pupae (P), and % emergence (E) in algal cell density (million cells/ml)[a]											
	30			72			91			105		
Fraction	L	P	E	L	P	E	L	P	E	L	P	E
Algal suspension	27	3	70d	33	38	29a	66	7	27a	70	2	28a
Supernatant	55	2	43b	22	9	69d	36	7	57c	72	0	28a
Residue	13	3	84ef	13	4	83ef	25	6	69d	5	5	90g
Check	5	0	95gh	3	2	95gh	1	1	98h	11	0	89efg

[a] ±4-5 × 10^6 cells/ml.

Values followed by different letters are significantly different from each other at 1% level based on Duncan's multiple range test.

the check were mostly in the 3rd stage. The rate of larval development in the residue suspension was the same as that in the check; this indicated that the active principles were released in the media with a lapse of time. Emergence of adults in the check and residue was completed within 10 days, whereas in the algal suspension and the supernatant it took 14–16 days, thus showing that the transspecific chemicals also delayed development.

The mortality caused by the algal suspension and the supernatant fractions increased to some degree as the cell density increased; however, no definite relationship was found between cell density and mortality. The mortality was independent of the cell density in the residue suspension (Table 1). A significant difference of emergence was noticed between the lowest and higher rates of algal suspension. Ryther (1954) reported that the mortality of *Daphnia magna* also depended upon the growing stage of algae.

The highest cell density (105×10^6 cells/ml) of *C. ellipsoidea* was also assayed against 4th-stage larvae and pupae, and the activity shown by the alga is presented in Table 2. Results showed that emergence from 4th stage larvae exposed to the supernatant and residue was significantly lower than emergence from check. However, emergence from 4th-stage larvae exposed to algal suspension was not significantly different from emergence from the check. Emergence was insignificant when pupae were exposed to any of the fraction. These stages seemed to be more tolerant to these algal toxins than the younger stages. In this regard, the algal toxins followed the activity pattern of other auto- and transspecific ecological chemicals (Ikeshoji and Mulla 1970). The poor activity of this algal species against 4th-stage larvae and pupae could also be due to the short exposure of these stages, thus diminishing the intensity of the effects of the elaborated toxin(s).

Water collected from vases without algae and assayed against 1st-stage larvae did not induce any mortality higher than that in the check (data not presented). Therefore, the possibility of mortality being caused due to cemetery water and other contents of vases was eliminated.

The laboratory culture showed higher or similar biological activity in inhibiting larval development as shown by the same algal concentration of field collected algae (Table 3). Emergence of mosquitoes from the algal suspension and the supernatant was found to be highly significant as compared to the residue and check. Thus the evidence clearly indicated that this algal species produced certain toxin(s)

Table 2. Evaluation of field collected green alga *C. ellipsoidea* against 4th-stage larvae and pupae of *Cx. quinquefasciatus* in the laboratory.

	Mean % mortality in larvae (L), pupae (P) and % emergence (E)				
	Larvae exposed			Pupae exposed	
Fraction	L	P	E[b]	P	E
Algal suspension[a]	2	8	90ab	10	90 NS
Supernatant	2	10	88a	13	87 NS
Residue	3	8	89a	8	92 NS
Check	2	3	95b	13	87 NS

[a] 105×10^6 cells/ml.

[b] Values followed by the same letters are not significantly different from each other at the 5% level.

Table 3. Evaluation of laboratory cultured *C. ellipsoidea* against 1st-stage larvae of *Cx. p. quinquefasciatus*.

	Mean % mortality in larvae (L), pupae (P), and % emergence (E)		
Fraction	L	P	E
Algal suspension[a]	81	2	17a
Supernatant	78	2	20a
Residue	4	3	93b
Check	7	0	93b

[a] 99×10^6 cells/ml.

Values followed by different letters are significantly different from each other at the 0.01 level based on Duncan's Multiple range test.

which inhibited development of early stages of mosquito larvae. The nutrient media used for algal cultures did not show any activity. Another species of *Chlorella, C. sorokiniana* assayed at much higher density (2.45×10^9 cells/ml) did not show any activity against mosquito larvae (unpublished data).

MASS CULTURE. It was observed that a continuous flow of air was necessary in the laboratory culture to keep the cells in suspension. The effect of 5% CO_2-in-air mixture was also compared with air alone. The flow of CO_2 was reported necessary for *Chlorella sorokiniana* (Chimiklis and Karlander 1973) and *C. pyrenoidosa* (Spoehr et al. 1949). In this study the flow of CO_2, however, was not found to result in very marked increase in cell density, although the cell densities were always higher in the CO_2-air mixture culture than in the air culture alone, during the first 22 days (Fig. 1). Nevertheless, the possibility of obtaining better cultures of *C. ellipsoidea* at the higher rate of CO_2 could not be ruled out. Two prominent peaks of algal growth were noted during the study period. After 10 days of culturing, the algal cell density showed a downward trend. More nutrients were added on the 17th day assuming that all the nutrients had been assimilated by the growing algal cells. The maximum cell density occurred on the 22nd day and then suddenly decreased in CO_2-air mixture. In the air culture (W/O CO_2) the density of the cells increased, peaking on the 29th day, after which it declined abruptly.

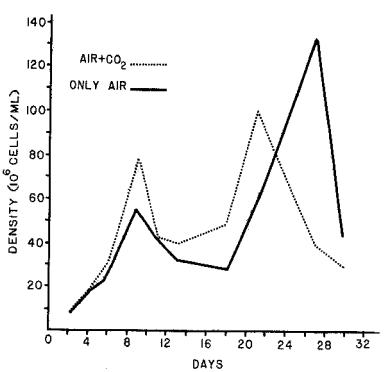

Fig. 1. Effect of 5% CO_2-in air mixture on the growth of *Chlorella ellipsoidea* in a laboratory culture.

Various other media are being tested currently for obtaining better production of this alga and for further investigations regarding the chemical nature and characterization of these toxin(s).

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