EFFECT OF TEN CHLOROPHYTES ON LARVAL SURVIVAL, DEVELOPMENT AND ADULT BODY SIZE OF THE MOSQUITO *AEDES AEGYPTI*

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Abstract. The effect of ten microalgal chlorophytes isolated from mosquito breeding containers on the survival, larval development and adult body size of the mosquito *Aedes aegypti* was investigated. All larvae fed with six of the microalgal isolates died after 7 days. These isolates were found to be resistant to digestion by mosquito larvae. Delayed pupation and body size reduction of the mosquitos fed with *Chlorococcum* UMACC 218 and *Scenedesmus* UMACC 220 were observed. In contrast, larvae fed with *Ankistrodesmus convolutus* UMACC 101 and *Chlorococcum* UMACC 213 were bigger in size than those fed with normal insectory feed. The present study showed that microalgal chlorophytes have the potential to be used as larvicidal agents for mosquitos.

INTRODUCTION

Control of disease-bearing vectors relies heavily on the extensive and intensive use of chemical insecticides. These chemicals are to certain extent quite successful in curbing the diseases concerned. However, in view of some of the side effects of chemical agents used in vector control, interest in environmentally friendly approaches and the use of biological control agents, have been revived. Use of certain strains of *Bacillus thuringiensis* and *B. sphaericus* has been successful in mosquito control. However, rapid settling of the mosquitocidal preparations of these bacteria prevents sustained contact of their toxins with the target population and necessitates repeated treatment to effect continuous control. In the case of mosquitos, microalgae deserve particular attention since microalgae are the principal food for larvae of many species and have been known to exert some inhibitory effects upon certain components of the aquatic fauna.

Phytoplankton (microalgae) are the primary food for many species of mosquito larvae. Some species of these microalgae provide healthy, nutritious food for mosquito larvae whereas other species are harmful to the larvae. It is common in nature for mosquito larvae to die before completing their development because they are poisoned by toxic algae or they starve to death while feeding on algae that are indigestible. The detrimental effects of algal growth are not only harmful to larval development but also prevent mosquito oviposition. The deleterious effects of some species of algae on mosquito populations were demonstrated by several authors. For instance, Angerilli and Beirne (1974) and Mulla *et al* (1987) observed that a free floating unicellular *Chlorella ellipsoidea* produces certain substances that are lethal to the immature stages of the mosquitos as they alter the development. Rashed and El-Ayouty (1992) showed that *Chlorella vulgaris* has some mosquito regulating effects and it is not a sufficient food source for larval development when tested against *Culex pipiens*. Larval pupation was delayed and no pupation was noticed until 21 days.

Mosquito-indigestible phytoplankton have good field characteristics as a biological control agent against mosquitos because they are naturally present in the habitats of mosquito larvae and are able to multiply and persist in these habitats. Another major advantage of phytoplankton for mosquito control is the expectation that mosquitos will not evolve resistance to their use.

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The significance of mosquito-indigestible algae has not generally been recognized because, even when these algae are abundant, their occurrence in nature is usually in combination with other kinds of algae that provide sufficient nutrition. However, they sometimes do have an impact in nature. For example, Marten (1984) reported that *Ae. albopictus* larvae in Hawaii were dying of starvation in container-breeding habitats where *Kirchneriella irregularis* had taken over as the dominant phytoplankton. When a small quantity of *K. irregularis* is introduced to a container habitat where it was not already present, it often dominated and rendered the water unsuitable for *Ae. albopictus* larvae. Marten (1986) showed that mosquito-indigestible algae can take over other phytoplankton when introduced even in very small quantities, provided the algae are local strains, as they are highly competitive within the particular habitat to which they have been introduced.

Use of indigestible microalgae is, therefore, a good alternative for mosquito control. The first important step is identification of a suitable algae present in the natural mosquito breeding habitat. The objective of this study was to investigate the larvicidal properties of the indigenous microalgae of Malaysia. At present, the University Malaya Algal Collection Center (UMACC) has 43 isolates associated with mosquito breeding grounds in Malaysia. We report here on the larvicidal properties of ten chlorophytes from this collection.

MATERIALS AND METHODS

Evaluation of selected algal species as larvicides against *Aedes aegypti* larvae

Ten unicellular species of green algae isolated from mosquito breeding containers such as empty coconut shells, discarded tires and metal containers, were selected for the study. The axenic cultures were identified by reference to the strain numbers used in the University Malaya Algae Culture Collection (UMACC) catalogue (Phang and Chu, 1999). They were *Scenedesmus* UMACC 010, *Ankistrodesmus convolutus* UMACC 101, *Chlorella* UMACC 184, *Chlorella* UMACC 185, *Chlorella* UMACC 187 *Chlorella* UMACC 193, *Chloroccocum* UMACC 213, *Chlorella* UMACC 217, *Chloroccocum* UMACC 218 and *Scenedesmus quadricauda* UMACC 220.

Each isolate was cultured using Bold's Basal Medium (Phang and Chu, 1999) in 1 liter conical flasks with a 10% inoculum (OD_{620nm} = 0.2). At stationary growth, the cultures were centrifuged at 150*g* for 10 minutes to harvest the algal cells. The harvested cells were diluted with distilled water to obtain a concentration of OD_{620nm} = 0.6. Larvicidal activity of the algal suspensions against mosquito larvae was determined by transferring 200 ml of the algal suspension to a glass beaker containing 25 second instar larvae of *Ae. aegypti*. Four replicates were used each time and the test was repeated three times. The mosquito larvae used in this study were obtained from the insectary of the Institute for Medical Research, Kuala Lumpur. The control consisted of larvae in distilled water fed with finely ground partially cooked liver, which is the normal insectory feed (Cheong, 1965). Larvae mortality was assessed every 24 hours with dead organisms removed each time. Lethal time (LT) values were calculated using probit analysis (Finney, 1989). The percentage mortality for each test was calculated. Daily observations on larval and pupal mortality were continued through adult emergence or until termination of the test after a maximum of 21 days.

Adult body size was determined by measuring the wing length (distance from axial incision to the apical margin, excluding fringe of scales) of each individual. Wing length was chosen as an indicator of body size because it is directly proportional to dry body weight (McCombs, 1980). For statistical analysis, mean wing lengths of 30 to 50 adults of both sexes in each test were chosen randomly and were subjected to analysis of variance and compared by one way analysis of variance.

Feeding tests using *Aedes aegypti* to determine digestibility of the microalgae

Axenic algal cultures were grown in Bold Basal Medium until the inocula reached exponential phase cultures. The inoculum was centrifuged at 150*g* for 10 minutes and washed with distilled water. The process were repeated twice. The supernatant was discarded and the residue was resuspended in distilled water to obtain OD_{620} of 0.2 at a 250 ml volume. The algal suspension were trans-

ferred to a beaker for the digestion experiment.

Ae. aegypti larvae reared in the insectary were starved for 12 hours before the experiment. Some 25 larvae were placed in the algal suspension. The larvae were allowed to feed for one hour before being removed from the suspension. The attached algae were removed by washing with distilled water and the larvae were placed in distilled water to allow further digestion. After every hour, five larvae were picked and then rinsed with distilled water.

The food bolus was removed with forceps and washed three times in distilled water while it was still packaged by the peritrophic membrane. The gut contents were then teased from the membrane with very fine pin into a vial containing 1 ml sterile distilled water. The times required for ingested cells to pass through the larval digestive tracts were determined by examination under microscope. Cell counts were carried out to determine the percentage of digestion of the algal species.

Biochemical composition of algae

Proteins and carbohydrates were extracted using NaOH and HCl and the content was determined by spectrophotometry (Bradford, 1976; Kochert, 1978). Lipids were extracted in chloroform: methanol ratio of (1:2) and determined by gravimetry (Bligh and Dyer, 1979).

Digestive enzymes studies

Digestive enzymes of the *Ae. aegypti* larvae were investigated by feeding the larvae with the ten algae separately, for four days. For positive controls, guts of the larvae maintained in the insectary and fed with partially cooked liver were used. For negative controls, guts from unfed larvae were used. The gut from each larva was homogenized in doubled distilled water and the extract was separated on SDS-polyacrylamide slab gel using the discontinuous system consisting of 4% acrylamide stacking gel and 12% acrylamide separating gel. Approximately 10 µl of the gut samples were boiled at 100ºC for five minutes before loading onto the gel. The separated protein bands were visualized by staining with Coomassie brilliant blue. The selected digestive enzymes, such as α-amylase, lipase and cellulase were loaded separately onto the gel with the test sample. The enzymes α-amylase from *Bacillus amyloquefaciens*, lipase from *Rhizopus arrhizus* and cellulase from *Trichoderma viride* were used as standards. Enzymes were considered present in the gut of the larvae if the appropriate bands appeared in the sample's lane and the studied enzymes had the same molecular weight.

Cell walls characterization of microalgal isolates

Exponentially growing cells of the ten species were harvested and preserved in 2.5% glutraldehyde in cacodylate buffer (pH 7.2) for 4 hours at 4ºC. The specimens were washed in cacodylate buffer and transferred to 1.0% osmium tetroxide in cacodylate buffer for 1 hour. Subsequently they were washed in cacodylate buffer and stained with 2.0% uranyl acetate in water for 30 minutes each. The specimens were infiltrated with epoxy resin and polymerized in an oven at 60ºC overnight and sectioned using the ultrathin microtoem. The sections were then stained using 1.0% uranyl acetate and lead citrate and viewed under a transmission electron microscope (TEM-Model Hitachi S-430).

RESULTS

Evaluation of selected algal species as larvicides against *Aedes aegypti* larvae

The percentages mortality of larvae fed with the ten chlorophytes for 7 days is shown in Table 1. The maximum mortality was observed in the cases of *Scenedesmus* UMACC 010, *Chlorella* UMACC 184, *Chlorella* UMACC 185, *Chlorella* UMACC 187, *Chlorella* UMACC 193, and *Chlorella* UMACC 217 isolates, which caused almost 100% mortality to the larvae. Only 10.0% and 13.0% of the larvae fed with *Chloroccocum* UMACC 218 and *Scenedesmus quadricauda* UMACC 220 survived after 21 days. The development of these larvae was delayed with pupation occurring after 11.0 and 10.5 days respectively; only 7.0% of the larvae emerged to adults (Table 2).

Larvae fed on *Ankistrodesmus convolutus* UMACC 101 and *Chloroccocum* UMACC 213, had high survival rates of 99.0% and almost all larvae pupated by 3 or 4 days. All adults emerging from the treatments were further analyzed for

Table 1
Percentage LT_{50} mortality (%), and % of undigested cells of *Aedes aegypti* larvae fed with ten cell density of microalga.

Algal isolates	% mortality after 7 days	LT_{50} (day) 95% CL	% of undigested cells
Scenedesmus UMACC 010	100.0	2.58 (2.15 - 2.99)	60.53
A. convolutus UMACC 101	0.0	9.95 (9.43 - 10.54)	3.81
Chlorella UMACC 184	100.0	1.26 (0.95 - 1.53)	88.66
Chlorella UMACC 185	98.9	1.66 (1.36 - 1.94)	85.80
Chlorella UMACC 187	97.6	1.35 (1.13 - 1.54)	72.10
Chlorella UMACC 193	99.1	2.81 (2.44 - 3.25)	64.58
Chloroccocum UMACC 213	3.0	7.27 (6.68 - 8.11)	3.56
Chlorella UMACC 217	100.0	2.68 (2.37 - 2.97)	85.8
Chloroccocum UMACC 218	81.2	4.51 (3.87 - 6.05)	59.12
S. quadricauda UMACC 220	60.0	5.34 (4.73 - 9.10)	34.58

Table 2
Effect of four microalgal isolates on larval survival, development and adult size of *Aedes aegypti*.

Effect	Microalgal isolates				
	Scenedesmus UMACC 220	*Chlorococcum* UMACC 218	*Chlorococcum* UMACC 213	*A. convolutus* UMACC 101	**Normal Food
% mortality after 3 days	53.00	68.00	0.0	0.0	0.0
% mortality after 6 days	88.00	84.00	0.0	0.0	0.0
% mortality after 12 days	90.00	87.00	0.0	0.0	0.0
% mortality after 21 days	90.00	87.00	0.0	0.0	0.0
No. of days to first pupation	11.00	10.50	4.0	3.50	4.00
*Female wing length (mm)	2.79^{a}	2.86^{a}	3.17^{b}	3.21^{b}	3.10^{c}
*Male wing length (mm)	2.37^{a}	2.43^{a}	2.79^{b}	2.86^{b}	2.52^{b}

*Means followed by same letter in a column are not significantly different (Duncan's multiple range test, $p>0.05$).
**Normal food consisted of partially cooked liver.

their growth rate and body size by measuring the wing length. The wing length of the adults emerged from the treatments with *Chloroccocum* UMACC 218 and *Scenedesmus quadricauda* UMACC 220 were shorter for both males and females (Table 2) than other treatments, whilst those of adults from *Ankistrodesmus convolutus* UMACC 101 and *Chloroccocum* UMACC 213 treatments were longer than controls for both males and females.

The larvicidal property of the algal isolates was determined by calculating the lethal time (LT_{50}), which is the time in days taken to kill 50% of the larvae. Among the ten isolates tested, *Chlorella* UMACC 184, *Chlorella* UMACC 187, *Scenedesmus* UMACC 010 and *Chlorella* UMACC 185 were found to be most effective having LT_{50} of 1.35, 1.66, 2.58 and 2.60 days respectively at the concentration of OD_{620} 0.2. *Scenedesmus* UMACC 220 and *Chloroccocum* UMACC 218 exhibited moderate larvicidal effect with the same cell density (Table 1).

Feeding tests using *Aedes aegypti* to determine digestibility of the microalgae

Table 1 also shows the percentage of undigested cells for the various isolates. The highest percentages of undigested cells were observed in

Table 3
Biochemical composition (% DW) of ten microalgal isolates (mean, standard deviation, n=4).

Isolates	Protein	Lipids	Carbohydrate
Scenedesmus UMACC 010	19.07 ± 12.61	10.63 ± 3.23	15.13 ± 2.39
Ankistrodesmus convolutus UMACC 101	19.33 ± 4.68	19.55 ± 4.03	21.68 ± 9.36
Chlorella UMACC 184	24.45 ± 18.24	16.75 ± 10.68	17.53 ± 12.93
Chlorella UMACC 185	20.45 ± 5.97	11.28 ± 5.28	9.93 ± 2.35
Chlorella UMACC 187	24.83 ± 11.09	9.43 ± 5.39	14.78 ± 4.46
Chlorella UMACC 193	27.40 ± 18.13	11.23 ± 2.67	17.85 ± 7.64
Chloroccocum UMACC 213	21.48 ± 13.83	13.88 ± 2.82	15.89 ± 2.26
Chlorella UMACC 217	23.30 ± 12.89	11.08 ± 8.23	14.23 ± 4.49
Chloroccocum UMACC 218	23.85 ± 10.88	12.35 ± 4.50	19.88 ± 7.78

Chlorella UMACC 184, *Chlorella* UMACC 185, and *Chlorella* UMACC 217, which were 80.66, 75.80 and 65.64% respectively. The feeding of larvae with *Ankistrodesmus convolutus* UMACC 101 and *Chlorococcum* UMACC 213 showed lower percentages of undigested cells.

Biochemical composition of algae

All the cultures were harvested for biochemical analysis during the stationary growth phase. The protein, carbohydrate and lipid contents for all the ten isolates ranged from 19 to 25%, 15 to 22% and 9 to 12% of the dry weight respectively (Table 3). On the basis of the biochemical composition, the nutritional values in terms of the protein, carbohydrate and lipid contents of the ten algae were similar.

Digestive enzymes

α-amylase was present in the gut of larvae fed with *Ankistrodesmus convolutus* UMACC 101, *Chlorella* UMACC 184, *Chlorella* UMACC 185, *Chlorella* UMACC 193, *Chloroccocum* UMACC 218, *Scenedesmus quadricauda* UMACC 220 and partially cooked liver. No amylase was observed from the guts of larvae fed with *Scenedesmus* UMACC 010, *Chlorella* UMACC 187, *Chloroccocum* UMACC 213, *Chlorella* UMACC 217 and non-fed larvae. Lipase was only present in the gut of larvae fed with *Chlorella* UMACC 184, *Chlorella* UMACC 185, *Chlorella* UMACC 193, *Chlorella* UMACC 217 and partially cooked liver. Cellulase was present in larvae fed with all ten isolates and partially cooked liver (Table 4).

Table 4
Enzyme activity in the gut of *Aedes aegypti* of non-fed larvae and larvae fed with the ten chlorophytes and partially cooked liver.

Algal isolates	α-Amylase	Lipase	Cellulase
Scenedesmus UMACC 010	-	-	+
A. convolutus UMACC 101	+	-	+
Chlorella UMACC 184	+	+	+
Chlorella UMACC 185	+	+	+
Chlorella UMACC 187	-	-	+
Chlorella UMACC 193	+	+	+
Chloroccocum UMACC 213	-	-	+
Chlorella UMACC 217	-	+	+
Chloroccocum UMACC 218	-	-	+
S. quadricauda UMACC 220	+	-	+
Normal food	+	+	+
No food	-	-	+

Morphological characterization of microalgal isolates

Transmission electron micrographs of *Scenedesmus* UMACC 010 showed that the cell wall consist of a thick inner cellulose layer, a very thin middle layer (which is referred to as the trilaminar zone and bounded by membranes on either side) and the outer pectic layer (Fig 1).

The electron-microscopy studies of *Chlorella* UMACC 184 showed that the cell wall is composed of an inner microfibrillar zone and an outer trilaminar zone (Fig 2).

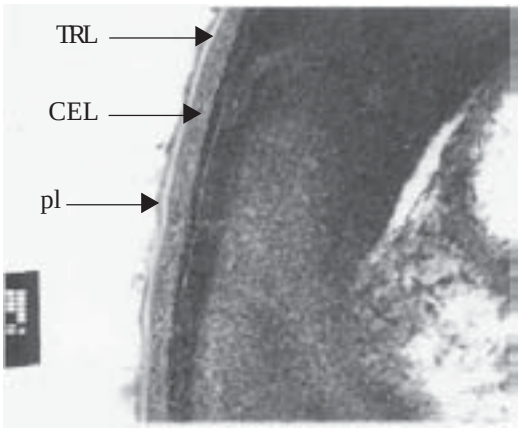

Fig 1–Longitudinal section of *Scenedesmus quadricauda* UMACC 010 showing cellulosic layer (CEL), trilaminar layer (TRL) and pectic layer (pl). Magnification 120,000x.

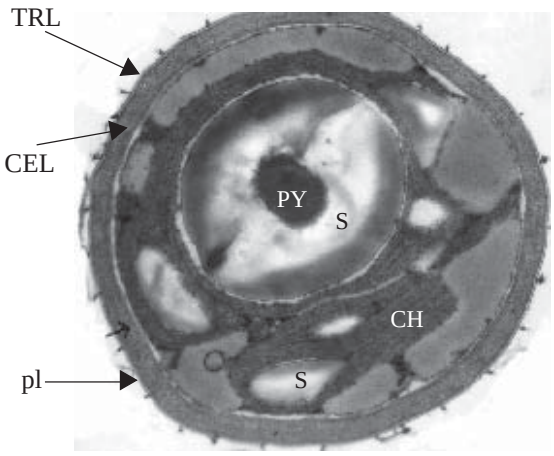

Fig 2–Longitudinal section of *Chlorella* UMACC 184 showing cellulosic layer (CEL), trilaminar layer (TRL) and pectic layer (pl). Magnification 35,000x.

Electron micrographs of the cross-section of *Chlorococcum* UMACC 213 showed that the cell walls are relatively thin with a thin inner layer and an outer gelatinous layer (Fig 3).

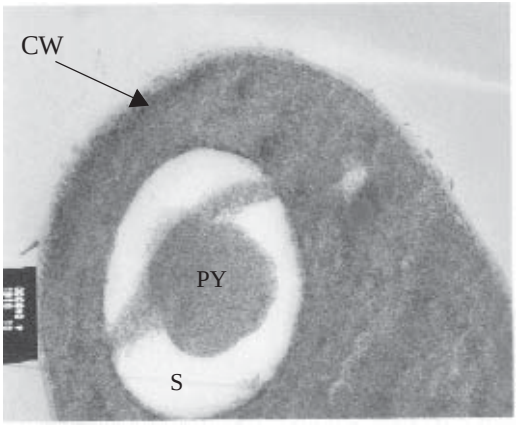

Fig 3–Electron micrograph showing thin sections of the cell wall (CW), storage materials (S) and pyrenoids (py) of *Chlorococcum* UMACC 205. Magnification 48,000x.

DISCUSSION

Chlorella UMACC 185, *Chlorella* UMACC 187, *Scenedesmus* UMACC 010 and *Chlorella* UMACC 191 were effective larvicides against the mosquito larvae, with most dying with their guts full of algal cells. The larvae showed no growth and died within a few days during the second or third instar of development, and those which occasionally reached the fourth instar were usually in an emaciated condition. Pre-pupal mortality may be due to failure of proper sclerotization (Zebitz, 1986) suggesting that the algae might interfere with the hormonal control of moulting (Sagar and Sehgal, 1997). The algae also induced some morphological abnormalities, as observed by the shrunken appearance of the treated larvae. Dhillon and Mulla (1982) reported similar observations when *C. ellipsoidea* were inoculated in the breeding containers of the first stage larvae of *Ae. aegypti* and *Cx. quinquefasciatus*. The potential deleterious effects of some species of algae on mosquito populations as demonstrated by several authors (Dhillon and Mulla, 1981; 1982; Rashed and El-Ayaouty, 1992) were supported in the present study.

Growth was slowest (more than two weeks to reach pupal stage) for larvae fed on *Scenedesmus* UMACC 220 and *Chloroccocum* UMACC 218 resulting in adults with significantly smaller body size than controls. The larvae were able to reach adult stages when fed with these isolates but growth was slow, probably due to interference with the endocrine mechanism (Benerjee and Rembold, 1993). Some green algae produce substances that inhibit larval development and delay the develop-

ment of the surviving larvae to the adult stage (Rashed and Al-Youty, 1992).

Conversely, larvae fed on *Ankistrodesmus convolutus* UMACC 101 and *Chloroccocum* UMACC 213 showed enhanced development. The larvae of *Ae. aegypti* all survived and developed normally to the adult stage. Growth was rapid (approximately one week to reach the pupal stage) with larvae and adults attaining larger sizes than the controls.

The emergence of larvae fed on *Scenedesmus* UMACC 220 and *Chloroccocum* UMACC 218 were significantly different from the controls. Emergence of larvae exposed to *Ankistrodesmus convolutus* UMACC 101 and *Chloroccocum* UMACC 213 were not, however, significantly different from the controls. Emergence of adults from the larvae of controls and those fed on *Scenedesmus* UMACC 220 and *Chloroccocum* UMACC 218 were completed within 10 days, whereas larvae treated with *Scenedesmus* UMACC 220 and *Chloroccocum* UMACC 218 took 16-24 days, showing that the development of mosquito larvae fed with these chlorophytes was delayed (Dhillon and Mulla, 1981).

Larvae fed with *Scenedesmus* UMACC 220 and *Chloroccocum* UMACC 218 had significantly shorter wing lengths than controls whereas those fed with *Ankistrodesmus convolutus* UMACC 101 and *Chloroccocum* UMACC 213 had longer wing lengths. Since an optimal larval diet increases both wing length (McCombs, 1980) and survival rates (Harmis, 1983), the present data suggest that most of the algae tested have larvicidal effects. Survival rates were high using *Ankistodesmus convolutus* UMACC 101 and *Chlorococcum* UMACC 213 suggesting that these are an adequate source of food for the development of *Ae. aegypti* larvae.

Larvae of *Ae. aegypti* placed in the suspensions containing the algal *Chlorococcum* UMACC 213 or *Ankistrodesmus convolutus* UMACC 101 lost their contents quickly (broken or dissolved) when ingested by *Ae. aegypti* larvae. The cells underwent considerable changes or were broken up. The cell walls are plasmolysed or dissolved. Many cells that contained full chloroplasts when ingested, showed reduced/fragmented chloroplasts when the gut contents were examined. This could be due to the partial digestion of cells.

The chlorophytes *Scenedesmus* UMACC 010, *Chlorella* UMACC 185, *Chlorella* UMACC 187, *Chlorella* UMACC 193 and *Chloroccocum* UMACC 218 were found to be resistant to digestion. None of the isolates examined lost their contents rapidly and the cells remained intact; even after being ingested by *Ae. aegypti*. Howland (1930) and Laird (1988) reported that 75% of the ingested algae may be unaffected and a high proportion of the resistant species belong to the chlorococcales. Howland (1930) further stated that *Scenedesmus quadricauda* cells are not easily plasmolysed even after several days starvation of the larvae *Cx. molestus*. The digestibility of microorganisms in larval food is determined by the resistance of their outer wall and the duration of exposure in the gut (Clements, 1992).

The present study further confirms the potential of the algae as biological control agents against mosquitos. Algae have many advantages as biological control agents when compared with other mosquitocidal bacteria because they are naturally present in mosquito habitats and are suitable food for the mosquito larvae (Kiviranta and Abdel-Hameed, 1994; Saario *et al*, 1994). In mosquito breeding grounds, there are zooplankton, such as copepods, which also feed on microalgae (Marten, 1984). The grazing of such phytoplankton results in the indigestible algae becoming dominant populations (Porter, 1973; Steinman, 1996). This is an advantage in the control of mosquito larvae.

The lipid, carbohydrate and protein contents of the ten chlorophytes were lower than those reported for other chlorophytes such as *Chlorella* sp and *Scenedesmus* sp (Renauld *et al*, 1994; Chu *et al*, 1995) although the nutritional content did not vary among the ten isolates, indicating that the differential mortality of the larvae was not due to malnourishment.

The enzyme α-amylase was present in the gut of larvae fed with *Ankistrodesmus convolutus* UMACC 101, *Chlorella* UMACC 184, *Chlorella* UMACC 185, *Chlorella* UMACC 193, *Chloroccocum* UMACC 218 and *Scenedesmus quadricauda* UMACC 220 and partially cooked liver, showing that it was involved in the digestion pro-

cess of the mosquito larvae (Yang and Davies,1971; Dadd,1975). Lipase was only present in the gut of larvae fed with *Chlorella* species. The presence and function of intestinal lipase in insects is only sparsely documented, especially in the larval stage (Clements, 1963). Cellulase was present in larvae fed with all the ten isolates and partially cooked liver. Cellulose digestion in insects is rare but occurs in several insects that have nutritionally poor diets (Martin, 1991).

Larvae, therefore, appear to possess sufficient normal enzymes to digest the algae when exposed to them and the enzymes of the larvae fed with the ten isolates tested were not significantly different. Digestibility of the algae depends on other factors as well, such as the shape, size and cell wall properties of the algae (Atkinson *et al*, 1972). These other factors may therefore be more important in accounting for the effects observed in this study.

In a survey of a number of these algae, Atkinson *et al* (1972) found that about half the species of C*hlorella* and *Scenedesmus* examined had a thick trilaminar layer outside the cell proper. This layer is extremely resistant and is believed to consist of polymerized carotenoid material like sporopollenin. Northcote *et al* (1958) and Soeder (1964), also reported that the sporopollenin of the *Chlorella* cell wall is located in the trilaminar outer component and perhaps at its outer surface.

The digestibility of the microalgae in larval food is determined by the resistant properties of their outer wall and the duration of exposure in the gut. Thick-walled organisms are relatively indigestible. When *Chlorella* UMACC 185 and *Scenedesmus* UMACC 010 were examined under a transmission electron microscope, the cell walls of these isolates showed a thin trilaminar layer thought to consist of sporopollenin, and the low digestibility of these isolates seems to be due to sporopollenin, a carotenoid polymer impervious to all digestive enzymes. In contrast, *Chlorococcum* UMACC 213 is rapidly digested and this may be due to their thin cell walls as observed under a transmission electron microscope in this study.

The limited survey of *Scenedesmus quadricauda* UMACC 010 and *Chlorella* UMACC 184 examined in the present work showed that sporopollenin might be present in *Chlorella* UMACC 184 as well as in S*cenedesmus quadricauda* UMACC 010, in all probability in the outer component or trilaminar zone.

The electron micrograph showed that *Chlorococcum* UMACC 213 had a thin cell wall compared with *Scenedesmus quadricauda* UMACC 010 and *Chlorella* UMACC 184. The cell wall has no trilaminar layer, as shown in both the *Scenedesmus quadricauda* UMACC 010 and *Chlorella* UMACC 184.

Investigations on the ultra-structural and biochemical properties of the cell wall may help to explain why *Chlorella* UMACC 185, *Chlorella* UMACC 187, *Scenedesmus* UMACC 010 and *Chlorella* UMACC 191 are larvicidal to *Ae. aegypti* but *Ankistodesmus convolutus* UMACC 101 and *Chlorococcum* UMACC 213 are not. The resistance of algal isolates in the alimentary canals of mosquito larvae also deserve a detailed investigation. The most significant outcome of this study is the demonstration that phytoplankton can have a decisive impact on the success of mosquito larvae.

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