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Experimental Production and Chemical Composition of *Culex* Mosquito Larvae and Pupae Grown in Agro-industrial Effluent

M.A.B.[1] HABIB, F.M. YUSOFF[2], S.M. PHANG[3] and S. MOHAMED[4]

[1]*Department of Aquaculture*
Bangladesh Agricultural University
Mymensingh 2202
Bangladesh

[2]*Department of Biology, Faculty of Science and Environmental Studies*
Universiti Putra Malaysia
43400 UPM Serdang, Selangor D.E.
Malaysia

[3]*Institute of Postgraduate Studies and Research, Studies*
Universiti Malaya
50603 Kuala Lumpur
Malaysia

[4]*Faculty of Food Science and Biotechnology*
Universiti Putra Malaysia
43400 UPM Serdang, Selangor D.E.
Malaysia

Abstract

An experiment was conducted to grow larvae and pupae of *Culex* mosquito in palm oil mill effluent (POME), latex concentrate rubber effluent and microalgae *Selenastrum gracile* (isolate no. 166) as cultured in 70 l tanks. No larval growth was observed in rubber effluent and very few larvae were found in microalgal tanks. Sampled *Culex* larvae and pupae grew in POME tanks, and were considered for biochemical analyses. The crude protein, real protein, lipid and ash content of *Culex* pupae were significantly higher ($p < 0.05$) than the larvae of the third and the fourth instars. All the essential amino acids of pupae except threonine, valine, isoleucine and

lysine were significantly higher ($p < 0.05$) than those of the fourth instar followed by the third instar. The polyunsaturated fatty acids (PUFAs) of pupae such as linolenic acid (18: 3n-3), ϒ-linolenic acid (18: 3n-6), arachidonic acid (20: 4n-6), eicosapentaenoic acid (22: 5n-3) and docosahexaenoic acid (22: 6n-3) were significantly higher ($p < 0.05$) than those of the third and the fourth instars. The pupae bioaccumulated significantly higher ($p < 0.05$) amount of essential minerals than larvae of the fourth instar followed by the third instar. The production of *Culex* pupae per 20 l of POME was 670 g, which is equivalent to 33.50 $kg \cdot ton^{-1}$. Due to presence of high micronutrients the larvae and pupae may be used as food for the rearing of post-larvae of 6-7 days old and fry of catfish, and shrimp and prawn. The presence of chitin as body covering of larvae and pupae helps to develop the chitinuous body covering of shrimp and prawn.

Introduction

Mosquito larvae and pupae grow in the aquatic habitat and contribute as a good natural live food for larvivorous as well as carnivorous fish (Blaustein 1992, Espinoza et al. 1997, Singaravelu et al. 1997) and other aquatic predators (Marian et al. 1983). Further, Degani and Yehuda (1996) reported that mosquito larvae are nutritious due to their high protein content (50%) and that they could be used to formulate artificial diet for fish (Degani and Yehuda 1996), at least to fulfill to some extent the increasing demand of protein (Habib et al. 1992).

The *Culex* mosquito, unlike *Anopheles* and *Aedes*, usually lays its eggs in polluted water containing decomposed organic matter (Pennak 1978, Blaustein 1992) with high organic loads. The larvae of *Culex* can easily grow even without the presence of dissolved oxygen (Pennak 1978, Sasa et al. 1988), due to the presence of air sacs in which larvae can store air containing oxygen for long period and use the oxygen for respiration (Ward and Whipple 1959, Pennak 1978). Richards (1938) found a large population of *Culex pipiens* larvae in water containing decaying plants on Long Island, New York, USA. Horsfall (1972) stated that the occurence of this mosquito is hardly noticeable in quiet water and larvae usually disappear. Researchers reported that tannery wastes were usually heavily populated with *Culex fatigens* larvae and almost 50% of septic tanks produce this mosquito as well (Horsfall 1972).

Agroindustrial effluents of the rubber industry and palm oil mills are present in substantial quantities in Malaysia (Phang and Ong 1988; Isa 1993, Habib et al. 1998). These wastes contain high levels of organic matter (Phang 1990, Habib et al. 1998) as well as reasonable amounts of protein, carbohydrate, nitrogenous compounds, amino acids, lipids, fatty acids and minerals (Phang 1990, Kekwick 1997, Habib et al. 1998). Some animal wastes like pig manure, poultry manure etc. also contain the above mentioned nutrients (Shaw and Mark 1980, Nuov 1995). The nutrients may be utilized by *Culex* mosquito larvae for their growth and development (Habib et al. 1992) like other insect larvae such as chironomid

larvae (Kugler and Chen 1968, Shaw and Mark 1980, de Smet 1982, Marian and Pandian 1985; Habib et al. 1992; Berg 1995; Habib et al. 1997) and house fly (*Lucilia sericata*) larvae as live maggots (Nuov 1995) that enrich themselves with essential nutrients. The larvae and pupae of these flies can then be utilized as consumable live food by shrimp, larvivorous and carnivorous fish, aquarium fish and catfish (Shaw and Mark 1980, Habib et al. 1992, Nuov 1995, Yusoff et al. 1996, Espinoza et al. 1997). In this way nutrients from wastes can be recycled to consumable live food for aquatic organisms (Habib et al. 1998). Few research work, except those of Phang (1990), Anton (1994), and Yusoff et al. (1996) have been conducted to recover nutrients as the consumable form for fish from effluents by microalgae and chironomid larvae (Habib et al. 1997). Therefore, the present work was undertaken to grow *Culex* mosquito larvae and pupae in rubber effluents, POME and algae, and to evaluate their nutritional status.

Materials and Methods

The experiment was conducted in 70 l aerated tanks with three replications at the hatchery complex of Universiti Putra Malaysia, and the experimental design are shown in table 1. The microalga, *Ankistrodesmus falcatus* (UMACC 166) was inoculated from pure culture at an optical density of 0.20 at 620 nm in aerated tanks containing commercial fertilizer (N:P:K=1.0:1.0:0.70) dissolved with tap water. As the sources of nitrogen, phosphorus and potash, 260 $mg \cdot l^{-1}$ urea, 1.05 $g \cdot l^{-1}$ triple super phosphate and 216.50 $mg \cdot l^{-1}$ murate of potash, were used respectively with slight modification of the formulation of Geldenhuys et al. (1988) and Habib et al. (1997). The algal tanks were exposed to *Culex* mosquito adults for eight days after inoculation to facilitate oviposition (egg laying). The palm oil mill effluent (POME) and latex concentrate rubber effluent (LCRE) were collected from nearby rubber mills and 20 l of effluent was kept in every 70 l plastic tank. Adequate aeration was provided in all the tanks. The depth of media was maintained at 15 cm throughout the experiment. The parameters such as pH, chemical oxygen demand (COD), total suspended solids (TSS), total dissolved solids (TDS), total nitrogen, ammoniacal nitrogen (NH_3-N) and

Table 1. Treatment conditions for the experiment

Tank	Duration (Day)	Replication	Depth of media (cm)	Substrate (20 l)
1	20	3	15	Palm oil mill effluent
2	20	3	15	Rubber effluent
3	20	3	15	Algal culture

ortho-PO_4 of POME, LCRE and algal tanks before and at the end of the experiment were analyzed following the methods of Clesceri et al. (1989). During the study, pH, dissolved oxygen and NH_3-N were recorded daily. The cell and optical density (OD) of samples of algal tanks were done by sampling 10 ml samples every alternate day by using an improved Neubaur haemocytometer under microscope and an UV-Spectrophotometer at 620 nm, respectively. It was done just to monitor the growth of algal cells.

The *Culex* larvae of third and fourth instars, and pupae, grown in tanks, were collected using 0.50 mm (mesh size) net. All the larvae were then cleaned by repeated washings in tap water followed by distilled water finally. Some of the larvae were preserved in 10% buffered formalin (solution of 4.0 g NaH_2PO_4 and 12.0 g Na_2HPO_4. $7H_2O$ in 10 ml 40% formalin and the volume adjusted to 100 ml with distilled water) for subsequent identification with a microscope, using the keys of Chernovskii (1949), Ward and Whipple (1959), Needham and Needham (1966); Karunakaran (1969), Pennak (1978) and Sasa et al. (1988). The samples of *Culex* larvae and pupae were stored at 0°C for two days and then kept at -80°C for three days prior to freeze drying. The samples were freeze dried at -50°C under 150 millitorr vacuum pressure for two days. After freeze drying, the samples were ground, sieved through 250 mm mesh, packed and preserved at -80°C for chemical analyses.

The proximate composition of stored samples was determined following the methods of Horwitz (1984). Crude protein by Kjeltec Auto 1030 Analyser and crude lipid by solvent extraction were determined. Real (true) protein of larvae and pupae was determined following the extraction method using 5 ml 0.5 M NaOH and Bio Rad reagent protein reagent to develop colour (Kochart 1978). Then the readings of supernatant of samples after centrifugation through 1030 UV Spectrophotometer at 595 nm. Amino acids of preserved samples were determined by Waters HPLC 5100 (Millipore 1990). Two mg of deproteinized samples were hydrolyzed with 0.50 ml 4.0 M methanesulfonic acid containing 0.20% tryptamine at 115°C for 24 hrs and cooled samples were diluted with 10 ml of 2.50 $mmol \cdot ml^{-1}$ L-a-amino-n-butyric acid as an internal standard and then filtered through 0.45 mm Whatman filter paper. Amino acids were then detected after injection of 10 ml sample at 37°C onto a single column Waters HPLC 501 unit with double pump (Millipore 1990) with 30 minutes retention time comparing with peaks of known standard as described by Habib et al. (1997). The lipids were collected from frozen samples after homogenized with chloroform:methanol:water (1.0:2.0:0.80) following Folch et al. (1957). The fatty acid methyl esters (FAME) were obtained after hydrolysis of 10 mg lipid sample at 100°C for 30 minutes with 3.0 ml 14% boron trifluride-methanol following the methods of

Benitez (1989) and modified by Habib et al. (1998). Then the fatty acids were determined after injection of 1.0 ml FAME at 230°C onto a Thermon 3000 capillary column in Shimadzu GC-14A with 37 minutes retention time and as described by Habib et al. (1997). The peaks of amino and fatty acids were identified comparing with the peaks of known standards of amino and fatty acids from Sigma Chemical Co. (Benitaz 1989).

The mineral content of samples was detected first by energy dispersive analysis of X-ray (EDAX) through scanning electron microscope (SEM) (Habib et al. 1997), and then detected and quantified by inductively coupled plasma Mass Spectrophotometer (ICP-MS) after digestion of 10 mg dry sample with 10 ml 6.0 N conc. nitric acid and 1.0 ml hydrogen peroxide except Sulphur (Habib et al. 1997, Habib 1998). Sulphur was analysed by Elemental Analyser after burning the sample at 670°C for 2.0 minutes and then detected and quantified by S-column (Perkin Elmer 1994). To compare treatment means of specific growth rates, total carotene, total biomass, protein, lipid, carbohydrate, essential amino acids, unsaturated fatty acids and essential minerals of *C. vulgaris* and *M. micrura*, one way ANOVA was performed using SAS computer package followed by Tukey test (Zar 1984).

To compare the mean values of crude protein, crude lipids, ash, true protein, amino acids, unsaturated fatty acids and essential minerals in the larvae of the third and the fourth instars, and pupae of *Culex* mosquito, data were analysed by one way ANOVA using statistical analysis system (SAS) computer package followed by Tukey's test (Zar 1984).

Results and Discussion

The characteristics of palm oil mills effluent (POME), latex concentrate rubber effluent (LCRE) before and at the end of the experiment, and content of algal tank on the 8th day (peak of growth of *Ankistrodesmus falcatus*), and at the end of experiment were analyzed (Table 2). The raw POME and LCRE were acidic when collected and slighty acidic at the end of the experiment. The pH of algal tanks was above 8.50 and alkaline in nature. There was no dissolved oxygen up to the 10th day of experiment in the POME tanks but the levels increased from 0, in the first day, to 2.1 ppm at the end of the experiment (16th day) though the tanks were well aerated. The COD, TSS, TDS, total N and ortho-PO_4 of POME levels declined at the end of the experiment due to nutrient removal by mosquito larvae and pupae, and the degradation into inorganic nutrients by bacteria under aerobic conditions.

Culex larvae grew in POME and algal tanks but not in LCRE tanks (Table 3). On the 2nd day, plenty of adult mosquitoes were observed resting on the surface of all POME tanks and *Culex* larvae were found near the surface of POME in the 5th day. A layer of froth formed on the surface of POME tanks and continued up to the 15th day of the experiment. *Culex* larvae and pupae were collected using net (mesh size 500 µm) from POME tanks. Similar observations were previously recorded when egg yolk was used as a medium (Habib 1984).

Culex larvae and pupae were collected for the 2nd time from POME tanks after 12 days of first collection. For the 3rd time after 25 days of first collection, very few larvae were found in tanks. Usually these adult mosquitoes were attracted by the odour of decomposed POME. Habib et al. (1992 and 1997) reported that decomposed matter usually attracted the adult mosquitoes to lay eggs. Similar observation was stated by Yashouv and Allhones (1962). Culex larvae and pupae have air sacs to store air that assist for long period of respiration (Ward and Whipple 1959, Pennak 1978, Habib and Ahmed 1987). When comparing the different

Table 2. Characteristics* of palm oil mill effluent (POME), latex concentrate rubber effluent (LCRE) and algal water before and at the end of experiment

Parameters	Raw POME (before expt.)	POME (after expt.)	LCRE (before expt.)	LCRE (after expt.)	Algae tank (betore expt.)	Algae tank (after expt.)
pH	3.9±0.03	6.4±0.03	4.35±0.05	7.3±0.04	9.2±0.03	8.6±0.04
Dissolved Oxygen	0	2.1±0.02	0	4.2±0.03	6.5±0.04	5.8± 0.03
COD	35836.4±180.5	1912.4±33.3	7856.45± 82.5	2215.50±25.3	425.6±3.5	76.4± 1.88
TSS	67932.7±98.7	128.8±2.6	6475.15± 63.1	135.25±3.1	43.5±1.1	12.5± 0.06
TDS	39233.6±75.2	36.5±1.1	5847.36± 51.2	33.15±0.8	6.7± 0.05	10.8± 0.04
Total N	1194.5±40.2	109.3±2.2	1175.05± 20.6	145.70±2.3	246.4± 2.8	42.6± 0.8
NH_3-N	50.6±1.2	88.4±1.3	480.25±10.4	65.25±1.2	3.3±0.04	5.2± 0.04
Ortho-PO_4	180.8±2.7	135.2±1.9	182.45±2.1	155.05±2.1	14.5± 0.07	8.7± 0.07

*Unit in $mg \cdot l^{-1}$ except pH

Table 3. Proximate Composition ($g \cdot 100^{-1}$g dry sample) of *Culex* larvae of 3rd and 4th instars, and pupae grown in palm oil mill effluent

Proximate composition	3rd instar	4th instar	Pupae
Moisture	6.91±0.03^a	6.94±0.03^a	6.93±0.03^a
Crude protein	48.72±0.35^c	54.75±0.37^b	58.40±0.38^a
Crude lipid	13.55±0.13^b	15.44±0.15^a	16.15±0.12^a
Ash	11.85±0.11^c	14.60±0.12^b	17.20±0.14^a
NFE	18.94±0.04^a	8.23±0.03^b	1.28±0.03^c
Total	99.97	99.96	99.96
Real Protein	40.55±0.31^c	46.85±0.33^b	50.75±0.32^a

Means of (± SE, n = 9) with different superscripts in each row indicate significant differences ($p<0.05$, df. 23, 3)

species of only the *Culex* laid eggs, larvae grew in POME in the absence of dissolved oxygen. Since the *Culex* mosquito bites and some species are disease carriers (Pennak 1978, Gullan and Cranston 1995) all the pupae were carefully collected.

The levels of protein, lipid, ash, nitrogen free extract (NFE) and moisture of POME, and *Culex* larvae of 3rd and 4th instars, and pupae are presented in table 3. The protein, lipid and ash contents of pupae were significantly ($p < 0.05$) higher than those of larvae of 4th instar followed by 3rd instar. The percentage of ash was higher in larvae of the fourth instar than the others indicating the bioaccummulation of minerals in high quantity in their tissue from the cultured media. This was consistent with the report of Habib et al. (1997) on the growth of *Chironomus* fly larvae. Some other reports on the excellent growth of Chironomid larvae in poultry manure (Shaw and Mark 1980), polluted water (de Smet 1982), insect larvae in artificial medium (Habib 1984), oraganic matter (Marian and Pandian 1985), egg yolk (Habib and Sultan 1987), artificial medium as mustard oil cake, wheat flour and cowdung (Habib et al. 1992) and fly larvae in pig manure (Nuov 1995). Due to the presence of high crude protein, the larvae and pupae may be used as live food or processed (frozen) food for catfish, aquarium fish and climbing perch. Nuov (1995) reported very excellent growth of African catfish fry in cage after feeding the high protein and lipid content house fly larvae as live maggot.

Though the crude protein of pupae is higher than the larvae of 3rd and 4th instars, pupae contain chitinuous body covering which is not digestible in the early stages of post-larvae of fish and shrimp due to lack of chitinase enzyme in the stomach (Steffens 1989). Mass mortality of post-larvae usually happens due to not digesting the chitin as well as some toxic effects of chemicals of chitin (Steffens 1989). The chitinuous body covering remain very soft in 3rd instar larvae which is easily digestible, but it is a little bit hard in 4th instar and very hard in pupal stage . The chitinase enzyme develops fry of 15-18 days old which helps to digest chitin. Usually post-larvae of 7-10 days old can not produce this enzyme. The *Culex* larvae should be given as food to the post-larvae of early stage of fisheries organisms, and pupae will be given as food to the fry. Otherwise, mass mortality of post-larvae of 4-8 days old may happen due to lack of chitinase in post-larvae. Habib et al. (1993) reported that mass mortality happened when *Chironomus* larvae of 4th instar and pupae were fed to 4-5 days old *C. batrachus* post-larvae. They found intact body coverings of *Chironomus* larvae and pupae in the intestine of *C. batrachus* post-larvae which was not digested due to absence of chitinase.

All the essential amino acids (EAAs) were present in *Culex* larvae and pupae (Table 4). The levels of EAAs such as histidine, arginine,

methionine, leucine, phenylalanine and lysine were significantly ($p < 0.05$) higher in pupae (De la Noüe and Choubert 1985) followed by larvae of 4th instar and then 3rd instar. But, tyrosine and leucine in pupae were significantly ($p < 0.05$) higher than others. Biosynthesis of methionine, a S-contained essential amino acid was recorded in considerable amount in all the stages which was probably due to high quantity of sulfur in POME. The high level of total essential amino acids in pupae demonstrate their high biological value to that of fish protein (Keembiyehetty and Delbert 1993) like silkworm pupae (Habib et al. 2001) and make them a rich source of food for many organisms (Armitage 1995).

The polyunsaturated fatty acids (PUFAs) such as linoleic acid (18:2n-6), linolenic acid (18:3n-3) and ϒ-linolenic acid (18:3n-6) were present in higher and considerable amount in pupae followed by larvae of 4th and 3rd instars (Table 5). Aquatic animals can biosynthesize both unsaturated fatty acids and PUFAs of 18 20 and 22 carbon from saturated fatty acids and lower carbon unsaturated fatty acids (Halver 1980). The 20 carbon chained PUFAs such as eicosatrienoic acid (20:3n-6), arachidonic acid (20:4n-6), eicosapentanoic acid (20:5n-3), docosapentaenoic acid (22: 5n-3) and docosahexaenoic acid (22: 6n-3) were biosynthesized in higher amounts in *Culex* pupae than larvae of 4th instar, followed by 3rd instar. These are essential for the proper development of marine fish and shimp and prawn larvae and fry (Armitage 1995; Degani and Yehuda 1996).

Table 4. Amino Acids ($g \cdot 100^{-1}$g protein) of *Culex* larvae of 3rd and 4th instars, and pupae grown in palm oil mill effluent

Amino acids	3rd instar	4th instar	Pupae
Aspartic acid	9.15±0.10	6.12±0.07	8.45±0.16
Glutamic acid	8.05±0.07	7.55±0.05	6.85±0.13
Serine	6.60±0.07	8.77±0.07	5.02±0.09
Glycine	7.95±0.06	7.25±0.06	6.88±0.06
Histidine*	$1.90±0.02^{b}$	$1.76±0.03^{b}$	$2.51±0.03^{a}$
Arginine*	$3.25±0.03^{c}$	$3.75±0.05^{b}$	$4.86±0.08^{a}$
Threonine*	$2.25±0.03^{c}$	$3.56±0.04^{a}$	$2.88±0.06^{b}$
Alanine	10.45±0.10	8.05±0.06	7.15±0.07
Proline	6.60±0.05	6.70±0.05	5.44±0.11
Tyrosine*	$4.20±0.04^{b}$	$4.66±0.04^{b}$	$6.21±0.13^{a}$
Phenylalanine*	$3.12±0.04^{c}$	$4.55±0.04^{b}$	$4.82±0.10^{a}$
Valine*	$6.05±0.06^{a}$	$5.95±0.05^{a}$	$6.12±0.14^{a}$
Methionine*	$6.65± 0.05^{b}$	$6.80±0.06^{b}$	$7.45±0.12^{a}$
Cystine	1.05±0.03	0.75±0.02	1.10±0.07
Isoleucine*	$5.18±0.04^{b}$	$3.72±0.03^{c}$	$4.65±0.08^{b}$
Leucine*	$4.35±0.05^{c}$	$4.70±0.04^{b}$	$5.19±0.06^{a}$
Lysine*	$5.60±0.03^{b}$	$5.95±0.06^{a}$	$6.15±0.07^{a}$
Tryptophan*	$2.30±0.03^{a}$	$1.90±0.04^{b}$	$2.45±0.03^{a}$
Total	93.45	92.49	94.18

Means (± SE, n = 9) of *essential amino acid with different superscripts in each row indicate significant differences ($p<0.05$, df. 23, 3). The recovery of total amino acids varied from 92.49 to 94.18% based on total protein

Twenty two essential minerals were quantified by inductively coupled plasma- Mass Spectrophotometer (ICP-MS) present in POME and *Culex* larvae and pupae (Table 6). The accumulatation of most of the minerals in larvae and pupae was directly related with those of POME. Some of the very important minerals such as Na, P, K, Fe, Mg, Mn, I, S, Si, Se, Zn, Ca, Cl, Co, Cu, Cr, Al, Mo, B, Ge, As and V were available in *Culex* larvae and pupae which may help for proper physiological processes of aquatic animals after consumption of larvae and pupae (Kamen 1987, Lovell 1989, McDowell 1992). The aquatic animals require minerals for their body development, like terrestrial animals. They have an opportunty to absorb dissolved minerals in water through the gills and intestinal wall after drinking water (Lovell 1989). However, minerals may be supplemented in fish, shrimp and prawn by feeding them with these larvae and pupae. The amount of mineral bioaccumulation in larvae and pupae increased 3rd instar (1.85%) to pupae (2.60%). Total amount of minerals in animal body is approximately 3.50% (McDowell 1992). Therefore, biominerals may be supplemented to the aquaculture organisms through feeding of larvae and pupae of *Culex* mosquito. It might be better than use inorganic minerals in the diet of fisheries organisms.

The production of *Culex* pupae·20l^{-1} of POME was 670 g which is equivalent to 33.50 kg·ton^{-1} (Table 7). Therefore, considering the high

Table 5. Fatty acids (g·100^{-1}g lipid) of *Culex* larvae of 3rd and 4th instars, and pupae grown in palm oil mill effluent

Fatty acids	3rd instar	4th instar	Pupae
Lauric acid (12:0)	3.22±0.06	5.15±0.13	4.88±0.06
Myristic acid (14:0)	12.66±0.12	9.35±0.16	8.15±0.08
Palmitic acid (16:0)	15.45±0.22	13.33±0.19	14.47±1.66
Heptadecanoic acid (17:0)	2.21±0.04	2.39±0.03	2.63±1.03
10-heptadecanoic acid (17:01)*	1.39±0.03	1.25±0.02^{b}	1.45±0.03
Stearic acid (18:0)	10.41±0.54	12.20±0.14	11.42±0.06
Oleic acid (18:1n-9)*	13.54±1.25^{c}	15.50±0.17^{b}	17.78±1.02^{a}
Linoleic acid (18:2n-6)*	9.53±0.62^{a}	7.15±0.05^{b}	6.54±0.34^{c}
Linolenic acid (18:3n-3)**	3.72±0.03^{c}	4.33±0.04^{b}	5.66±0.04^{a}
¡-Linolenic acid (18: 3n-6)**	0.75±0.03^{c}	1.27±0.02^{b}	2.05±0.03^{a}
Arachidic acid (20:0)	3.56±0.03	5.18±0.04	4.35±0.04
Eicosatrienoic acid (20:3n-6)**	2.85±0.03^{c}	4.66±0.03^{a}	3.75±0.03^{b}
Arachidonic acid (20:4n-6)**	4.35±0.04^{b}	2.85±0.03^{c}	4.96±0.04^{a}
Eicosapentaenoic acid (20:5n-3)**	2.76±0.02^{b}	0.79±0.02^{c}	3.85±0.03^{a}
Behenic acid (22: 0)	3.15±0.03	3.25±0.03	3.22±0.03
Docosapentaenoic acid (22: 5n-3)**	2.20±0.03^{a}	2.15±0.03^{a}	2.26±0.03^{a}
Docosahexaenoic acid (22: 6n-3)**	1.05±0.02^{b}	1.02±0.02^{b}	1.35±0.02^{a}
Total	92.80	92.82	93.55

Means (± SE, n = 9) of *unsaturated and **polyunsaturated fatty acids with different superscripts in each row indicate significant differences ($p<0.05$, df. 23, 3)

production of *Culex* pupae and their good nutritional qualities (presence of EAA, PUFAs and EMs) for feeding of aquarium fish as well as post-larvae of 6-7 days old and fry of catfish and snakeheads, and shrimp and prawn, *Culex* pupae may be produced using POME as culture medium. The presence of chitin as body covering of larvae and pupae of this mosquito helps in the proper development of the chitinuous body covering of shrimp and prawn.

Table 6. Mineral content ($mg \cdot g^{-1}$ sample on dry weight basis) of *Culex* larvae of 3rd and 4th instars, and pupae grown in palm oil mill effluent

Minerals	3rd instar	4th instar	Pupae
P*	11345.60±75.25^{c}	14675.35±125.20^{b}	16334.25±134.25^{a}
Ca*	2632.15±38.15^{c}	3210.60±57.50^{b}	3536.12±50.15^{a}
Na*	145.35±2.14^{c}	160.25±3.12^{b}	170.05±3.20^{a}
K*	3855.20±82.15^{c}	4525.15±145.15^{b}	5235.45±155.75^{a}
Mg*	340.30±12.20^{c}	455.60±15.50^{b}	515.20±14105^{a}
Fe*	13.50±0.25^{c}	16.80±0.38^{b}	18.55±0.33^{a}
Zn*	15.75±0.21^{c}	17.10±0.25^{b}	17.35±0.24^{a}
Mn*	32.30±1.30^{c}	36.50±1.35^{b}	42.30±1.48^{a}
Mo*	9.25±0.30^{c}	12.15±0.42^{b}	14.35±0.53^{a}
Co*	1.98±0.02^{c}	2.42±0.03^{b}	2.85±0.03^{a}
Cr*	3.25±0.03^{c}	4.35±0.03^{b}	5.02±0.04^{a}
Cu*	8.12±0.12^{c}	9.75±0.15^{b}	11.15±0.16^{a}
S*	15.60±0.65^{c}	18.30±0.73^{b}	23.50±0.85^{a}
Se*	10.85±0.35^{c}	13.25±0.37^{b}	16.33±0.38^{a}
Si*	15.40±0.43^{c}	19.72±0.45^{b}	23.25±0.53^{a}
B*	5.30±0.04^{c}	7.45±0.06^{b}	8.85±0.07^{a}
Al*	13.15±0.45^{c}	15.05±0.55^{b}	17.05±0.65^{a}
Ni*	1.85±0.02^{b}	1.96±0.02^{b}	2.66±0.02^{a}
Sn*	1.75±0.02^{b}	2.35±0.02^{a}	2.45±0.02^{a}
V*	0.22±0.02^{b}	0.25±0.02^{b}	0.35±0.03^{a}
As*	7.75±0.06^{c}	9.55±0.06^{b}	11.05±0.07^{a}
Li*	2.30±0.02^{c}	2.75±0.02^{b}	3.17±0.03^{a}
Pb*	4.55±0.04^{c}	6.22±0.05^{b}	6.85±0.05^{a}
Cd	0.42±0.03	0.55±0.04	0.67±0.03
Total	18481.39	23223.42	26018.82
% of body weight	1.85	2.32	2.60

Means of (± SE, n = 9) *essential mineral with different superscripts in each row indicate significant differences ($p<0.05$, df. 23, 3)

Table 7. Production and estimated income from *Culex* pupae grown in palm oil mill effluent

Sub-cycle	Yield of pupae			Estimated total income (@ of RM $10 \cdot Kg^{-1}$)	
	$g \cdot 20^{-1}$l	$Kg \cdot ton^{-1}$	m $kg \cdot 15^{-1}$m ton	(b RM)	(b $US)
1	480	24	360	3.60	0.95
2	190	9.50	142.50	1.43	0.37
3	-	-	-	-	-
Total	670	33.50	502.50	5.03	1.32

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