ISSN:1511-3701

Pertanika Journal of

TROPICAL Agricultural Science

VOLUME 27 NO.2
SEPTEMBER 2004

A scientific journal published by Universiti Putra Malaysia Press

Pertanika Journal of Tropical Agricultural Science

About the Journal

Pertanika a leading agricultural journal in Malaysia began publication in 1978. After 15 years as a multidisciplinary journal, the revamped *Pertanika Journal of Tropical Agricultural Science* now focuses on tropical agricultural research. The journal is current and regular, bringing the latest information related to plant and animal sicences, fisheries, food sciences and forestry to the attention of researchers and scientists. It is published two times a year i.e. in March and September.

Aims and Scope

The journal will accept contributions from managers, researchers and scientists in the fields of biochemistry, ecology, genetics, physiology, pathology and management and production of plants and animals of economic importance. *Pertanika Journal of Tropical Agricultural Science* will consider for publication articles both in English and Bahasa Melayu. Articles must be original reports of research, not previously or simultaneously published in any other scientific or technical journal.

Submission of Manuscript

Three complete clear copies of the manuscript are to be submitted to

The Chief Editor
Pertanika Journal of Tropical Agricultural Science
Universiti Putra Malaysia
43400 UPM, Serdang, Selangor Darul Ehsan
MALAYSIA
Tel: 03-89468854 Ext: 8854; Fax: 03-89416172

Proofs and Offprints

Page proofs, illustration proofs and the copy-edited manuscript will be sent to the author. Proofs must be checked very carefully within the specified time as they will not be proofread by the Press editors.

Authors will receive 20 offprints of each article and a copy of the journal. Additional copies can be ordered from the Secretary of the Editorial Board.

Published by Universiti Putra Malaysia Press
ISSN No.: 1511-3701

DR. NAYAN DEEP S. KANWAL
EXECUTIVE EDITOR
Pertanika Journals
Research Management Centre (RMC)
Universiti Putra Malaysia
43400 Serdang, Selangor, Malaysia
Tel: +603 8947 1622, +6016 217 4050
E-mail: ndeeps@admin.upm.edu.my

Pertanika Journal of Tropical Agricultural Science

Volume 27 Number 2 (September) 2004

Contents

Pertanika J. Trop. Agric. Sci. 27(2): 79 - 89 (2004) ISSN: 1511-3701

Growth and Nutritional Value of a Tropical Green Alga, *Ankistrodesmus convolutus* Corda, in Agro-industrial Effluents

[1, *]M.A.B. HABIB, [2]F.M. YUSOFF, [3]S.M. PHANG, [4]S. MOHAMED & [5]M.S. KAMARUDIN

[1] *Department of Aquaculture, Faculty of Fisheries*
Bangladesh Agricultural University, Mymensigh 2202, Bangladesh
[2] *Department of Biology, Faculty of Science and Environmental Studies*
Universiti Putra Malaysia, 43400 UPM Serdang, Selangor, Malaysia
[3] *Institute of Postgraduate Studies and Research*
Universiti Malaya, 50603 Kuala Lumpur, Malaysia
[4]*Faculty of Food Science and Biotechnology*
Universiti Putra Malaysia, 43400 UPM Serdang, Selangor, Malaysia
[5]*Department of Agronomy, Universiti Putra Malaysia,*
43400 UPM Serdang, Selangor, Malaysia

Keywords: Growth, *A. convolutus*, essential amino acids, polyunsaturated fatty acids

ABSTRAK

Penggunaan efluen agro-industri bagi pengkulturan mikroalga telah dikaji dengan menggunakan mikroalga air tawar tropikal, Ankistrodesmus convolutus *yang dikultur di dalam pelbagai cairan efluen getah lateks pekat (LCRE), getah piawaian Malaysia (SMRE) dan kelapa sawit tercerna (POMED).* Ankistrodesmus convolutus *yang dikultur di dalam 40% dan 60% LCRE, 60% SMRE dan 10% POMED telah meningkatkan kadar pertumbuhan spesifik (dari segi bilangan sel dan klorofil a) yang jauh lebih tinggi ($P < 0.05$) dibandingkan dengan alga yang tumbuh di dalam efluen lain dan baja tak organik (N:P:K = 1:1:0.5) sebagai kawalan. Jumlah biojisim yang dikultur dalam 60% LCRE, 60% SMRE dan 10% POMED adalah lebih tinggi ($P < 0.05$) dibandingkan alga yang tumbuh di dalam efluen lain dan kawalan.* Ankistrodesmus convolutus *yang dikultur di dalam 40% dan 60% LCRE, 60% SMRE dan 10% POMED mempunyai protein mentah dan lipid yang lebih tinggi ($P < 0.05$) dibandingkan dengan mikroalga yang tumbuh dalam medium lain. Kebanyakan asid amino perlu (EAA) adalah lebih tinggi dalam alga yang tumbuh di dalam 60% LCRE, 60% SMRE, dibandingkan dengan efluen lain.* Ankistrodesmus convolutus *yang dikultur di dalam 10% POMED mengandungi jumlah EAA yang tinggi kecuali treonin dan tirosin dibandingkan dengan alga yang dikultur dalam medium POMED lain dan kawalan.* Ankistrodesmus convolutus *yang dikultur di dalam 40% dan 60% LCRE mengandungi jumlah asid lemak poli-tak-tepu (PUFA), C18 dan C20, yang lebih tinggi ($P < 0.05$) daripada alga dalam medium SMRE lain dan kawalan, kecuali asid eikosadienoik (20:2n-11). Corak PUFA yang sama direkodkan dalam alga yang dikultur dalam 60% SMRE kecuali asid eikosatrienoik (20:3n-6) dan asid arakidonik (20:4n-6).* Ankistrodesmus convolutus *yang dikultur di dalam 10% POMED mengandungi PUFA seperti asid linoleik (18:2n-6), asid linolenik (18:3n-3) dan asid arakidonik yang lebih tinggi dibandingkan dengan alga yang tumbuh dalam medium POMED lain dan kawalan. Kajian ini menunjukkan* A. convolutus *yang dikultur dalam 40% - 60% getah dan 10% POMED mempunyai nilai pemakanan yang lebih tinggi dibandingkan dengan alga yang dikultur dalam medium lain.*

ABSTRACT

Use of agro-industrial effluents for microalgal culture was investigated using a tropical freshwater green alga, Ankistrodesmus convolutus *cultured in various dilutions of latex concentrate effluent (LCRE), standard Malaysian rubber effluent (SMRE) and digested palm oil mill effluent (POMED).* Ankistrodesmus convolutus *grown in 40% and 60% LCRE, 60% SMRE and 10% POMED showed significantly higher ($P < 0.05$) specific*

* Corresponding Author:
E-mail: *ahsan@royalten.net*

growth rate in terms of cell number and chlorophyll a than that grown in other effluent media and inorganic fertiliser (N:P:K = 1:1:0.5) as control. Total biomass of this microalgae grown in 60% LCRE, 60% SMRE and 10% POMED was significantly higher (P < 0.05) than that cultured in other effluent media and the control. Ankistrodesmus convolutus *cultured in 40% and 60% LCRE, 60% SMRE and 10% POMED showed significantly (P < 0.05) higher amount of crude protein and lipid than that grown in other effluent media and the control. Significantly higher (P < 0.05) amount of most of the essential amino acids (EAAs) except a few were found in* A. convolutus *cultured in 60% LCRE and 60% SMRE than that grown in other effluent media and control.* Ankistrodesmus convolutus *cultured in 10% POMED resulted in significantly higher (P < 0.05) amount of all the EAAs except threonine and tyrosine that were grown in other POMED media and control.* Ankistrodesmus convolutus *grown in 40% and 60% LCRE, contained significantly higher (P < 0.05) amounts of all the C18 and C20 polyunsaturated fatty acids (PUFAs) than that cultured in other SMRE media and control, except eicosadienoic acid (20: 2n-11). A similar trend of PUFAs was recorded in* A. convolutus *cultured in 60% SMRE except eicosatrienoic acid (20:3n-6) and arachidonic acid (20:4n-6). It was found that* A. convolutus *contained significantly (P < 0.05) higher amount of PUFAs such as linoleic acid (18:2n-6), linolenic acid (18:3n-3) and arachidonic acid (20:4n-6) when grown in 10% POMED than that cultured in other POMED media and control. This study showed that* A. convolutus *grown in 40-60% rubber and 10% POMED has higher nutritional value that that cultured in other effluent media and inogranic fertilizer.*

INTRODUCTION

Microalgae biosynthesize essential nutrients like proteins, lipids, essential amino acids (EAAs), polyunsaturated fatty acids (PUFAs) and essential minerals (Chu *et al.* 1991; 1995; Habib *et al.* 1997; Vazhappilly and Chen 1998) when grown in appropriate media. Most of the freshwater microalgae contain high amount of C1'8 PUFAs and low amount of C20 PUFAs, but C22 PUFAs are very rarely found (Ben-Amotz *et al.* 1985; Geldenhuys *et al.* 1988; Chu *et al.* 1994) when grown in media without manipulation of nutrients. On the other hand, microalgae can biosynthesize high amount of carotenoids, some EAAs, C18, C20 and C22 PUFAs if the culture media contain one or more electrolytes and nutrients such as peptides, hydrocarbons, sucrose, glucose, short carbon chain compounds, minerals (Tan and Johns 1991; Laliberte and de la Noüe 1993; Barclay *et al.* 1994; Combres *et al.* 1994; Vazhappilly and Chen 1998).

The agroindustrial effluents such as rubber and palm oil mill effluents contain high organic matter, as indicated by high chemical oxygen demand (COD) (Phang 1987; Isa *et al.* 1988; Habib *et al.* 1998). They are also rich in nutrients including protein, lipid, carbohydrate and micronutrients as amino acids, fatty acids and minerals (Hwang 1978; Okiy 1987; Isa *et al.* 1993; Kekwick 1997; Habib *et al.* 1998). In addition, these effluents can be important sources of inorganic nutrients and short carbon chain compounds like those found in heterotrophic culture media (Isa *et al.* 1988; Phang 1990). The aim of this work was to illustrate the recovery of valuable inorganic and organic nutrients in agro-industrial waste water through biosynthesis by microalgae.

MATERIALS AND METHODS

The raw latex concentrate rubber effluent (LCRE) from Atherton Estate factory, Atherton, Negeri Sembilan, standard Malaysian rubber effluent (SMRE) from Kilang Getah MARDEC Berhad, Selangor D.E. and palm oil mill effluent from Golden Hope Mill, Pulau Carey Island, Selangor D. E. were collected and carried to the laboratory. All the effluents were collected at the discharge point from mills to the drain. The raw LCRE and SMRE were aerated in tanks for 5 days to break down the sulphides overnight, and to increase pH value to approximately 7.0. On the sixth day, the effluents were diluted into four different concentrations i.e. 20%, 40%, 60% and 80% of 100% effluent. The raw POME was digested and organic nutrients were broken into simpler forms by bacteria in aerobic conditions using continuous air flow. After 16 days, the brownish coloured liquid portion was formed in the upper layers. The upper layer of digested POME (POMED) was then diluted into four concentrations i.e. 5%, 10%, 20%, 30% of 100% POMED.

Ankistrodesmus convolutus (isolate no. 101) was innoculated in 5 different concentrations of LCRE and SMRE including 100% LCRE and SMRE, four different concentrations of digested Palm Oil Mill Effluents (POMED) and in inorganic fertilizer, N:P:K (1.0: 1.0: 0.50) as control in 70 1 tanks in the hatchery (Table 1). Each treatment had three replicates. The pH,

optical density, chlorophyll *a*, and specific growth rates in terms of cell numbers and chlorophyll *a* were recorded (Clesceri *et al.* 1989). Total carotene, observed yield, proximate composition, amino acids and fatty acids of the microalga were analyzed from samples collected before reaching the stationary phase of the growth. The microalge were cleaned with deionized water and centrifuged repeatedly. The cleaned precipitate of alga was freeze dried at – 50°C under 150 millitorr pressure for 1.5 days. The freeze-dried samples were preserved at –80°C for biochemical analyses.

Proximate composition of the samples was determined according to the methods of Horwits (1984). The deproteinized samples were hydrolyzed with 2.0 ml 4.0 M methanesulfonic acid containing 0.20% tryptamine. The amino acids were determined from the peaks which appeared after injection of 10μl filtered hydrolyzed samples onto a single column Waters HPLC 501 (Millipore 1990) and as described by Habib *et al.* (1997b). The fatty acid methyl esters (FAME) were obtained form lipid hydrolyses of samples by refluxing at 100°C with 14% boron trifluoride-methanol according to the methods of Folch *et al.* (1957) as modified by Benitez (1989). The FAME of 1.0 μl were injected at 130°C onto a single column Shimadzu GC-14A gas chromatography unit and fatty acids were analyzed following Habib *et al.* (1997b). To compare treatment means of proteins, lipids, carbohydrates, ash, essential amino acids (EAAs) and unsaturated fatty acids (UFAs) of *A. convolutus* grown in different media, one way ANOVA was performed using SAS computer package followed by the Tukey Test (Zar 1984).

RESULTS AND DISCUSSION

Among the growth parameters, specific growth rate (SGR) of cell and chlorophyll *a*, and total biomass of *A. convolutus* cultured in 40% & 60% LCRE, 60% SMRE and 10% POMED were significantly ($p < 0.05$) higher than those of *A convolutus* grown in other effluent media and the control N:P:K fertilizer (Table 2). All the growth parameters of *A. convolutus* grown in 10% POMED were significantly ($P < 0.05$) higher than that cultured in other POMED media and the control (Table 2). High growth performances in the above mentioned media might be due to adequate micro nutrients available in the media, appropriate colour of media which permitted sufficient light to penetrate in the media and sufficient supply of filtered air to overcome carbon dioxide (Phang 1991a; Anton 1994; Johns 1994; Yusoff *et al.* 1997). However, the C:N:P ratios of different dilutions of raw LCRE and SMRE (Table 3) showed almost three times less than the recommended ratio (56.30: 8.60: 1.20 for microalgae) (Edwards *et al.* 1980) which agrees with the findings of Phang and Ong (1988).

Rubber effluent is deficient in carbon content which may be partially solved through adequate filtered air supply and light penetration (Johns 1994) as well as flow of carbon dioxide gas (Phang and Ong 1988; Geetha *et al.* 1994) into the effluent media. Values of pH of all the media at the exponential phase of *A. convolutus* growth were above 10, indicating high cell growth and production during the study. Phang (1990; 1991) also reported high growth of microalgae in agro-industrial wastes. Anton *et al.* (1994) reported that *Scenedesmus quadricauda* showed high growth rate for concentrations of palm oil mill effluent (POME) below 14% collected at a point of discharge into river three km away from the mill. In addition, Phang (1987) and Yusoff and Chan (1997) found that diluted and digested palm oil mill effluent enhanced higher growth of a green alga, *Selenastrum capricornutum*. The effluent media contained high chemical oxygen

TABLE 1
Different concentrations (%) of raw latex concentrate rubber effluent (LCRE) standard Malaysian rubber effluent (SMRE) and digested palm oil mill effluent (POMED)

Treatment	Raw LCRE (%)	Raw SMRE (%)	POMED (%)
T1	20.0	20.0	5.0
T2	40.0	40.0	10.0
T3	60.0	60.0	20.0
T4	80.0	80.0	30.0
T5	100.0	100.0	-

TABLE 2
Specific growth are (μ/day) of cell and chlorophyll *a* (Chl-*a*), total carotene and total biomass of *Ankistrodesmus convolutus* grown in different dilutions of latex concentrate rubber effluent (LCRE), standard Malaysian rubber effluent (SMRE), digested palm oil effluent (POMED), and fertilizer. Means (± SE) with different superscripts in each row indicate significant differences (P<0.005), *mg/g. **mg/l

Parameters	20%LCRE	40%LCRE	60%LCRE	80%LCRE	100%LCRE	Fertilizer
SGR of Cell	0.32±0.03^{b}	0.38±0.03^{a}	0.40±0.03^{a}	0.30±0.03^{bc}	0.27±0.02^{c}	0.33±0.02^{b}
SGR of Chl-*a*	0.31±0.02^{b}	0.36±0.03^{a}	0.38±0.03^{a}	0.28±0.02^{c}	0.27±0.02^{c}	0.33±0.02^{b}
Total carotene*	6.29±0.05^{b}	6.45±0.05^{a}	6.65±0.05^{a}	6.46±0.05^{a}	6.37±0.05^{a}	5.53±0.04^{b}
Total biomass (Chl-*a* x 67)**	509.20 ±8.43^{f}	791.94 ±11.75^{b}	925.94 ±13.95^{a}	616.40 ±9.45^{c}	519.25 ±8.77^{e}	559.45 ±9.35
	20%SMRE	40%SMRE	60%SMRE	80%SMRE	100%SMRE	N:P:K
SGR of Cell	0.30±0.02^{b}	0.32±0.03^{b}	0.39±0.03^{a}	0.30±0.03^{b}	0.26±0.02^{c}	0.33±0.02^{b}
SGR of Chl-*a*	0.31±0.02^{b}	0.33±0.03^{b}	0.37±0.03^{a}	0.27±0.02^{b}	0.26±0.02^{b}	0.33±0.02^{a}
Total carotene*	6.35±0.05^{a}	6.55±0.05^{a}	6.73±0.05^{a}	6.53±0.05^{a}	6.45±0.05^{a}	5.55±0.04^{b}
Total biomass (Chl-*a* x 67)**	475.70 ±6.15^{f}	787.25 ±10.75^{b}	907.85 ±13.92^{a}	600.32 ±9.21^{c}	506.52 ±7.55^{e}	562.13 ±9.15^{d}
	5% POMED	10% POMED	20% POMED	30% POMED		Fertilizer
SGR of Cell	0.29±0.02^{bc}	0.36±0.03^{a}	0.26±0.02^{c}	0.22±0.02^{d}	-	0.32±0.02^{b}
SGR of Chl-*a*	0.33±0.03^{b}	0.38±0.03^{a}	0.27±0.02^{c}	0.21±0.02^{d}	-	0.33±0.02^{b}
Total carotene*	6.45±0.05^{b}	6.95±0.06^{a}	6.34±0.06^{b}	6.25±0.05^{b}	-	5.57±0.04^{c}
Total biomass (Chl-*a* x 67)**	509.20 ±8.66^{c}	924.60 ±12.96^{a}	800.65 ±11.82^{b}	769.83 ±9.62^{c}	-	564.81 ±9.22^{d}

demand, total solids, total suspended solids, total N, ortho-phosphate (Table 3) which proved its potential as a source of inorganic nutrients for good growth of microalgae and other aquatic microplants. Phang (1991), Geetha *et al.* (1994) and Habib *et al.* (1998) reported that rubber and palm oil effluents media were rich in inogranic nutrients, COD, total N, broken protein, lipids, carbohydrates and minerals.

Both protein and lipids were significantly ($P < 0.05$) higher in *A. convolutus* cultured in 40% and 60% LCRE, 60% SMRE and 10% POMED than other media (Table 4). Carbohydrate content of *A. convolutus* grown in N:P:K, and 20% LCRE, 20% and 40% SMRE, and 5% POMED were significantly ($P < 0.05$) higher than that of *A. convolutus* cultured in other effluent media. It was observed that both protein and carbohydrates of *A. convolutus* were higher than the lipid contents except in 10% POMED. The high growth and nutritional contents of the alga were probably due to the availability of essential nutrients in the effluents (Combres *et al.* 1994; Yusoff *et al.* 1996).

Ankistrodesmus convolutus biosynthesized all the 11 essential amino acids (EAAs) when cultured in effluent media. It contained significantly higher ($P < 0.05$) amount of EAAs viz. Histidine, threonine, tyrosine, phenylalaniene, valine, methionie, lysine and tryptophan cultured in 60% LCRE than that grown in other LCRE media and control (Table 5). Significantly ($P < 0.05$) higher amounts of arginie, methionine, leucine, lysine and tryptophan were biosynthesized when grown in 60% SMRE than grown in other SMRE media and control (Table 6). Furthermore, *A. convolutus* contained significantly ($P < 0.05$) higher amount of all the EAAs except threonine and tyrosine when grown in 10% POMED than that grown in other POMED media and control (Table 7). Combres *et al.* (1964) and Laliberte and de la Noüe (1993) reported that micronutrients in culture media including nitrogenous compounds, phosphorus compounds, minor nutrients are channeled into protein, carbon compounds and essential nutrients like amino acids. It was found that the nonessential amino acids such as aspartic acid, glutamic acid, serine, glycine, alanine and proline had considerable contribution to total amino acids of *A. convolutus* cultured in all the media including the control.

Ankistrodesmus convolutus contained most of the polyunsaturated fatty acids (PUFAs) of C18 and C20 in considerable amounts when grown in all the effluent media compared to that

TABLE 3
Average chemical contents (mg/L except pH) of different dilutions of raw latex concentrate rubber effluent (LCRE), standard Malaysian rubber effluent (SMRE) and digested palm oil mill effluent (POMED)

Parameters	20%LCRE	40%LCRE	60%LCRE	80%LCRE	100%LCRE
pH	7.10 ± 0.10	6.90 ± 0.10	6.80 ± 0.10	6.60 ± 0.15	4.45 ± 0.09
Dis. O_2	3.90 ± 0.10	3.75 ± 0.09	3.60 ± 0.10	3.50 ± 0.15	0
COD	1505.24 ± 2.67	3062.49 ± 3.66	4605.73 ± 5.54	6269 ± 8.12	7906.22 ± 12.44
TS	1256.40 ± 2.87	2556.80 ± 3.66	3622.20 ± 5.15	4842.60 ± 7.22	6562.72 ± 10.35
TSS	1175.08 ± 1.12	2306.12 ± 2.08	3588.17 ± 3.11	4774.23 ± 4.15	5945.29 ± 6.23
Total N	231.03 ± 1.08	472.06 ± 2.05	668.09 ± 2.88	934.12 ± 3.67	1205.15 ± 5.28
NH_3-N	92.33 ± 0.52	179.64 ± 0.86	282.45 ± 1.15	377.33 ± 1.90	496.66 ± 2.44
PO_4-P	32.95 ± 0.44	70.89 ± 0.62	102.84 ± 0.79	142.78 ± 0.79	189.73 ± 1.68
C:N:P ratio	15.70:9.0:1.0	16.20:9.33:1.0	16.80:9.24:1.0	16.47:9.19:1.0	15.63:8.97:1.0
Parameters	20%SMRE	40% SMRE	60% SMRE	80% SMRE	100% SMRE
pH	6.90 ± 0.10	6.70 ± 0.10	6.50 ± 0.10	5.60 ± 0.15	4.35 ± 0.12
Dis.O_2	2.85 ± 0.10	2.75 ± 0.09	2.60 ± 0.10	2.50 ± 0.15	0
COD	695.55± 2.56	1266.52 ± 4.54	1985.33 ± 4.54	2724.12 ± 5.66	3506.08 ± 8.66
TS	505.47 ± 1.04	1033.45 ± 2.55	1617.41 ± 3.25	2165.78 ± 6.88	2862.35 ± 7.55
TSS	466.05 ± 0.83	916.09 ± 1.14	1464.14 ± 2.07	2036.17 ± 3.55	2550.22 ± 5.38
Total N	176.08 ± 1.22	345.15 ± 1.68	542.22 ± 2.15	716.30 ± 2.74	985.37 ± 3.66
NH_3-N	55.20 ± 0.45	97.40 ± 0.66	155.60 ± 0.75	218.92 ± 0.90	296.15 ± 1.45
PO_4-P	22.02 ± 0.28	45.03 ± 0.46	68.05 ± 0.67	87.07 ± 0.83	125.08 ± 1.07
C:N:P ratio	11.85:10.50:1.0	10.55:9.83:1.0	10.94:10.26:1.0	11.73:10.74:1.0	10.51:10.25:1.0
Parameters	5% POMED	10% POMED	20% POMED	30% POMED	100%POMED
pH	7.10 ± 0.10	6.90 ± 0.10	6.80 ± 0.10	6.60 ± 0.10	6.40 ± 0.10
Dis.O_2	3.90 ± 0.10	3.75 ± 0.09	3.60 ± 0.10	3.50 ± 0. 10	2.10 ± 0.10
COD	1042.65. ± 12.75	2179.50 ± 25.55	4245.45 ± 75.66	6458.85 ± 65.12	21842.40 ± 30.42
TS	443.15 ± 5.04	975.72± 8.05	1926.02 ± 11.25	2844.32 ± 15.07	10128.80 ± 85.50
TSS	252.25 ± 3.03	524.52 ± 4.04	959.65 ± 6.07	1645.25 ± 8.12	5443.50 ± 25.90
Total N	58.52 ± 2.18	118.55 ± 3.28	228.03 ± 4.36	334.16 ± 5.42	1093.30 ± 48.60
NH_3-N	4.55 ± 0.18	8.94 ± 0.41	17.14 ± 0.82	29.55 ± 1.90	88.40 ± 3.50
PO_4-P	8.35 ± 0.26	17.89 ± 0.46	34.20 ± 1.29	51.65 ± 2.33	74.20 ± 6.10
C:N:P ratio	46.83:7.01:1.0	46.70:6.77:1.0	46.55:6.69:1.0	46.89:6.47:1.0	47.02:6.28:1.0

cultured in control. *A. convolutus* grown in 40% and 60% LCRE showed significantly higher ($P < 0.05$) amounts of all the C18 and C20 PUFAs, except eicosadienoic acid (20:2n-11), than that cultured in other LCRE media and control (Table 8). In addition, *A. convolutus* grown in 60% SMRE and 10% POMED biosynthesized higher amounts of C18 and C20 PUFAs except eicosatrienoic acid (20:3n-6) and arachidonic acid (20: 4n-6) than that cultured in other SMRE and POMED media, and control (Tables 9 and 10). The micronutirents of the culture media were probably channelled into essential nutrients like unsaturated fatty acids through enzymatic activities (Laliberte and de la Noüe 1993; Johns 1994; Che *et al.* 1995; Habib *et al.* 1997 a, b).

This study showed that valuable micronutirents available in rubber and palm oil effluents may be bioaccumulated by microalgae in the form of proteins, lipids, carotene, amino acids, unsaturated fatty acids and PUFAs which can then be channelled to other organisms located higher in the food chain.

ACKNOWLEDGEMENTS

The authors are grateful to the Ministry of Science and Environmental Malaysia, for providing funds through Intensification of Research Priority Areas (IRPA) 1/027/05/078 and Research and Development (R&D) 01/026 projects to carry out the research work. Thanks also to the laboratory technicians of Ecology and Food Science Departments, Electron Microscope Unit, and Institute of Post-graduate Studies and Research, University of Malaya.

TABLE 4

Proximate composition (g/100 g dry sample) of *Ankistrodesmus convolutus* grown in different dilutions of latex concentrate rubber effluent (LCRE), standard Malaysian rubber effluent (SMRE), digested palm oil effluent (POMED) and fertiliser. Mean(±SE) with different superscripts in each row indicate significant difference (P<0.05)

Proximate Composition	20%LCRE	40%LCRE	60%LCRE	80%LCRE	100%LCRE	Fertilizer
Moisture	5.66 ± 0.02^{a}	5.60 ± 0.02^{a}	5.63 ± 0.01^{a}	5.62 ± 0.02 ^{a}	5.64 ± 0.02^{a}	5.61 ± 0.01^{a}
Crude protein	39.63 ± 0.82^{c}	42.62 ± 1.08^{a}	43.45 ± 1.18^{a}	40.05 ± 0.92^{c}	41.42 ± 0.98^{b}	38.15 ± 0.94^{d}
Crude lipid	14.05 ± 0.09^{c}	15.75 ± 0.12^{a}	15.72 ± 0.11^{a}	15.12 ± 0.11^{b}	14.32 ± 0.11^{c}	10.46 ± 0.09^{d}
Ash	10.45 ± 0.09^{e}	12.92 ± 0.16^{d}	14.29 ± 0.18^{c}	17.42 ± 0.10^{b}	18.68 ± 0.14^{a}	8.06 ± 0.08^{f}
Cardohydrate	21.98 ± 0.18^{b}	18.21 ± 0.12^{b}	16.33 ± 0.11^{c}	16.61 ± 0.16^{c}	16.24 ± 0.14^{d}	21.62 ± 0.22^{a}
NFE	8.20± 0.06^{b}	4.86 ± 0.03^{c}	3.54 ± 0.03^{d}	5.14 ± 0.05^{c}	3.65 ± 0.04^{d}	16.06 ± 0.07^{a}
Prox. Comp.	20%SMRE	40%SMRE	60%SMRE	80%SMRE	100%SMRE	N:P:K
Moisture	5.55 ± 0.02^{a}	5.53 ± 0.01^{a}	5.55 ± 0.01^{a}	5.57 ± 0.02^{a}	5.57 ± 0.02^{a}	5.59 ± 0.01^{a}
Crude Protein	38.68 ± 0.55^{c}	39.12 ± 0.62^{c}	42.02 ± 0.95^{a}	40.88 ± 0.75^{b}	40.52 ± 0.71^{b}	38.02 ± 0.67^{c}
Crude lipid	14.15 ± 0.07^{b}	14.05 ± 0.07^{b}	15.15 ± 0.13^{a}	14.36 ± 0.11^{b}	14.24 ± 0.09^{b}	10.51 ± 0.06^{c}
Ash	8.25 ± 0.05^{e}	10.94 ± 0.07^{d}	13.25 ± 0.09^{c}	16.09 ± 0.09^{b}	18.32 ± 0.08^{a}	8.11 ± 0.07^{e}
Cardohydrate	21.88 ± 0.13^{a}	21.38 ± 0.12^{a}	19.41 ± 0.08^{b}	17.66 ± 0.10^{c}	17.28 ± 0.11^{c}	21.46 ± 0.13^{a}
NFE	11.44 ± 0.06^{b}	8.94 ± 0.06^{c}	4.58 ± 0.04^{d}	5.40 = 0.04^{d}	4.03± 0.05^{e}	16.27 ± 0.06^{a}
Prox. Comp.	5%POMED	10% POMED	20% POMED		30% POMED	Fertilizer
Moisture	5.56 ± 0.01^{a}	5.53 ± 0.01^{a}	5.57 ± 0.01^{a}		5.55 ± 0.02^{a}	5.59 ±0.02^{a}
Crude Protein	42.16 ± 1.06^{b}	43.66 ± 1.14^{a}	41.88 ± 1.23^{b}		40.22 ± 1.14^{c}	37.94 ± 0.89^{d}
Crude lipid	16.24 ± 0.13^{b}	17.68 ± 0.15^{a}	14.55 ± 0.12^{c}		13.39 ± 0.12^{d}	10.38 ± 0.09^{e}
Ash	10.66 ± 0.11^{d}	13.72 ± 0.16^{c}	15.96 ± 0.18^{b}		18.25 ± 0.21^{a}	8.15 ± 0.06^{e}
Cardohydrate	20.48 ± 0.52^{a}	15.59 ± 0.34^{c}	17.23 ± 0.35^{b}		16.90 ± 0.32^{b}	21.54 ± 0.45^{a}
NFE	4.85 ± 0.11^{b}	3.78 ± 0.04^{c}	4.77 ± 0.04^{b}		4.66 ± 0.05^{b}	16.36 ± 0.14^{a}

TABLE 5

Amino acids (g/100g protein) of grown in *Ankistrodesmus convolutus* 20%, 40%, 60%, 80% and 100% LCRE, and fertilizer. Mean (±SE) of *essential amino acid with different superscripts in each row indicate significant differences (P<0.05)

Amino Acids	20% LCRE	40% LCRE	60% LCRE	80% LCRE	100% LCRE	N:P:K
Aspartic	13.86 ± 0.28	9.42 ± 0.17	7.46 ± 0.11	12.26 ± 0.27	11.45 ± 0.22	9.50 ± 0.17
Glutamic acid	9.55 ± 0.17	5.66 ± 0.11	5.35 ± 0.07	8.66 ± 0.21	7.25 ± 0.13	10.91 ± 0.24
Serine	5.85± 0.12	6.15 ± 0.13	6.63 ± 0.08	6.45 ± 0.16	9.25 ± 0.10	7.87 ± 0.11
Glycine	7.95 ± 0.19	7.66± 0.10	3.37 ± 0.04	7.86 ± 0.18	6.90 ± 0.09	8.55 ± 0.15
Histidine*	0.99 ± 0.02^{d}	2.25 ± 0.02^{b}	2.94 ± 0.03^{a}	1.15 ± 0.02^{d}	1.55 ± 0.02^{c}	2.22 ± 0.03^{b}
Arginine*	2.52 ± 0.03^{c}	2.75 ± 0.03^{b}	2.83 ± 0.03^{b}	2.77 ± 0.03^{b}	2.88 ± 0.03^{b}	3.55 ± 0.04^{a}
Threonine*	1.16 ± 0.02^{d}	2.74 ± 0.03^{b}	3.06 ± 0.04^{a}	1.189 ± 0.02^{c}	1.96 ± 0.02^{c}	1.14 ± 0.02^{e}
Alanine	14.26 ± 0.33	10.68 ± 0.22	8.17 ± 0.08	13.35 ± 0.30	12.75 ± 0.25	12.43 ± 0.19
Proline	8.15 ± 0.16	7.65 ± 0.13	6.32 ± 0.05	9.52 ± 0.22	9.15 ± 0.15	9.15 ± 0.12
Tyrosine*	2.48 ± 0.02^{d}	3.15 ± 0.04^{c}	5.33 ± 0.04^{a}	2.42 ± 0.02^{d}	2.66 ± 0.03^{d}	4.01 ± 0.06^{b}
Phenylalanine	1.25 ± 0.02^{d}	3.45 ± 0.05^{b}	4.61 ± 0.05^{a}	1.35 ± 0.02^{d}	1.42 ± 0.02^{d}	3.14 ± 0.03^{c}
Valine*	1.05 ± 0.02^{e}	3.92 ± 0.04^{b}	4.55 ± 0.04^{a}	1.16 ± 0.02^{e}	1.66 ± 0.02^{d}	2.02 ± 0.02^{c}
Methionine*	3.66 ± 0.06^{d}	6.96 ± 0.13^{b}	7.82 ± 0.12^{a}	5.98 ± 0.06^{c}	5.88 ± 0.10^{c}	2.33 ± 0.03^{e}
Cystine	2.95 ± 0.05	2.04 ± 0.04	2.93. ± 0.03	2.75 ± 0.05	2.45 ± 0.05	0.76 ± 0.02
Isoleucine*	3.05 ± 0.04^{a}	2.45 ± 0.03^{b}	2.47 ± 0.03^{b}	2.30 ± 0.03^{b}	2.35 ± 0.03^{c}	2.47 ± 0.02^{b}
Leucine*	2.16 ± 0.02^{e}	4.75 ± 0.06^{a}	4.48 ± 0.05^{b}	2.05 ± 0.02^{e}	2.52 ± 0.03^{d}	3.35 ± 0.04^{c}
Lysine*	5.88 ± 0.04^{e}	8.85 ± 0.15^{b}	10.15 ± 0.19^{a}	6.25 ± 0.11^{d}	7.58 ± 0.13^{c}	4.22 ± 0.06^{f}
Tryptophan*	0.75 ± 0.02^{d}	1.48 ± 0.02^{b}	1.66 ± 0.02^{d}	0.80 ± 0.02^{d}	1.05 ± 0.03^{c}	0.70 ± 0.02^{d}

TABLE 6
Amino acids (g/100g protein) of *Ankistrodesmus convolutus* grown in 20%, 40%, 60%, 80% and 100% SMRE, and fertilizer. Means (± SE) of *essential amino acid with different superscripts in each row indicate significant differences (P<0.05)

Amino Acids	20% SMRE	40% SMRE	60% SMRE	80% SMRE	100% SMRE	N:P:K
Aspartic	12.44 ± 0.27	12.66 ± 0.26	9.21 ± 0.19	10.55 ± 0.18	11.12 ± 0.19	9.60 ± 0.17
Glutamic acid	13.66 ± 0.32	13.75 ± 0.30	10.37 ± 0.18	11.05 ± 0.17	12.66 ± 0.21	10.85 ± 0.24
Serine	8.12 ± 0.15	6.44 ± 0.11	4.85 ± 0.04	6.12 ± 0.10	7.66 ± 0.11	7.82 ± 0.11
Glycine	6.88 ± 0.10	7.32 ± 0.13	5.78 ± 0.05	6.56 ± 0.11	5.75 ± 0.10	8.62 ± 0.15
Histidine*	1.96 ± 0.02^{d}	2.05 ± 0.02^{d}	2.45 ± 0.03^{b}	3.15 ± 0.03^{a}	2.23± 0.03^{c}	2.04 ± 0.03^{d}
Arginine*	2.35 ± 0.03^{d}	2.38 ± 0.03^{d}	4.98 ± 0.05^{a}	4.57 ± 0.08^{b}	2.46 ± 0.03^{d}	3.46 ± 0.04^{c}
Threonine*	3.01 ± 0.04^{c}	3.33 ± 0.03^{b}	3.97 ± 0.04^{a}	3.88 ± 0.04^{b}	3.25 ± 0.04^{b}	1.22 ± 0.02^{d}
Alanine	7.38 ± 0.15	7.45 ± 0.13	6.10 ± 0.06	7.12 ± 0.13	6.86 ± 0.13	12.45 ± 0.19
Proline	6.16 ± 0.11	5.45 ± 0.09	5.55 ± 0.05	5.16 ± 0.08	5.62 ± 0.11	9.10 ± 0.12
Tyrosine*	3.55 ± 0.04^{d}	3.85 ± 0.05^{c}	4.35 ± 0.04^{a}	3.82 ± 0.04^{c}	4.42 ± 0.07^{a}	4.06 ± 0.04^{b}
Phenylalanine	4.76 ± 0.05^{a}	4.72 ± 0.06^{a}	4.62 ± 0.04^{a}	4.15 ± 0.06^{b}	3.66 ± 0.04^{c}	3.21 ± 0.03^{d}
Valine*	4.02 ± 0.03^{b}	3.98 ± 0.04^{b}	4.13 ± 0.03^{b}	4.52 ± 0.07^{a}	4.05 ± 0.03^{b}	2.06 ± 0.02^{c}
Methionine*	2.55 ± 0.02^{d}	2.62 ± 0.03^{d}	6.22 ± 0.11^{a}	4.95 ± 0.06^{b}	3.75 ± 0.03^{c}	2.50 ± 0.03^{d}
Cystine	2.96 ± 0.03	2.96 ± 0.03	2.02 ± 0.02	3.66 ± 0.04	4.22 ± 0.04	0.85 ± 0.02
Isoleucine*	2.72 ± 0.03^{d}	2.66 ± 0.02^{d}	2.94 ± 0.03^{c}	3.88 ± 0.03^{a}	3.45 ± 0.03^{b}	2.62. ± 0.02^{d}
Leucine*	2.96 ±0.03^{d}	3.05 ± 0.03^{d}	5.36 ± 0.12^{a}	4.45 ± 0.07^{b}	3.36 ± 0.03^{c}	3.31 ± 0.04^{c}
Lysine*	3.36 ± 0.04^{c}	4.16 ± 0.04^{d}	9.88 ± 0.18^{a}	7.18 ± 0.18^{b}	5.05 ± 0.10^{c}	4.29 ± 0.06^{d}
Tryptophan*	1.23 ± 0.02^{c}	1.32 ± 0.02^{c}	1.88 ± 0.02^{a}	1.72 ± 0.02^{b}	1.66 ± 0.02^{b}	0.68 ± 0.02^{d}

TABLE 7
Amino acids (g/100 protein) of *Ankistrodesmus convolutus* grown in 5%, 10%, 20% and 30% digested palm oil effluent (POMED) and fertilizer. Means (± SE) of *essential amino acid with different superscripts in each row indicate significant differences (P<0.05)

Amino Acids	5% POMED	10% POMED	20% POMED	30% POMED	N:P:K
Aspartic	12.22 ± 0.26	10.60 ± 0.18	11.85 ± 0.25	12.85 ± 0.25	9.55 ± 0.17
Glutamic acid	10.86 ± 0.22	7.57 ± 0.14	8.66 ± 0.15	9.45 ± 0.17	10.88 ± 0.24
Serine	7.55 ± 0.13	5.24 ± 0.05	6.75 ± 0.12	7.64 ± 0.13	7.82 ± 0.11
Glycine	6.15 ± 0.11	4.13 ± 0.04	5.56 ± 0.09	5.86 ± 0.10	8.44 ± 0.15
Histidine*	2.05 ± 0.02^{c}	2.98 ± 0.03^{a}	2.40 ± 0.03^{b}	1.75 ± 0.02^{d}	2.26 ± 0.03^{b}
Arginine*	2.86 ± 0.03^{c}	4.97 ± 0.05^{a}	3.41 ± 0.04^{b}	1.66 ± 0.02^{d}	3.50 ± 0.04^{b}
Threonine*	3.45 ± 0.05^{a}	3.50 ± 0.03^{a}	3.60 ± 0.03^{a}	1.24 ± 0.02b	1.12 ± 0.02^{b}
Alanine	7.56 ± 0.14	5.18 ± 0.10	6.27 ± 0.13	5.88 ± 0.10	11.78 ± 0.19
Proline	8.16 ± 0.08	4.37 ± 0.08	5.86 ± 0.11	6.45 ± 0.12	9.22 ± 0.12
Tyrosine*	3.69 ± 0.05^{b}	3.72 ± 0.05^{b}	3.78 ± 0.04^{b}	3.78 ± 0.03^{b}	4.12 ± 0.06^{a}
Phenylalanine	3.48 ± 0.04^{b}	4.51 ± 0.06^{a}	3.32 ± 0.03^{b}	2.98 ± 0.02^{c}	3.10 ± 0.03^{c}
Valine*	2.55 ± 0.03^{b}	4.63 ± 0.04^{a}	2.40 ± 0.02^{c}	2.35 ± 0.02^{c}	2.09 ± 0.02^{d}
Methionine*	4.26 ± 0.05^{c}	7.22 ± 0.16^{a}	5.10 ± 0.10^{b}	3.05 ± 0.04^{d}	2.22 ± 0.03^{e}
Cystine	0.88 ± 0.02	0.63 ± 0.02	2.86 ± 0.03	4.46 ± 0.05	0.88 ± 0.02
Isoleucine*	2.15 ± 0.02^{c}	3.43 ± 0.04^{a}	1.15 ± 0.03^{d}	1.05 ± 0.02^{d}	2.36 ± 0.02^{b}
Leucine*	4.25 ± 0.04^{c}	6.59 ± 0.13^{a}	5.46 ± 0.11^{b}	3.15 ± 0.03^{d}	3.28 ± 0.04^{d}
Lysine*	5.09 ± 0.09^{c}	7.66 ± 0.17^{a}	6.16 ± 0.13^{b}	5.86 ± 0.06^{b}	4.16 ± 0.06^{d}
Tryptophan*	1.55 ± 0.02^{c}	1.86 ± 0.02^{a}	1.70 ± 0.02^{b}	1.72 ± 0.02^{b}	0.71 ± 0.02^{d}

TABLE 8
Fatty acids (g/100g lipid) of *Ankistrodesmus convolutus* grown in 20%, 40%, 60%, 80% & 100% latex concentrate rubber effluent (LCRE) and fertilizer. Means (± SE) of *unsaturated and **polyunsaturated fatty acids with different superscripts in each row indicate significant differences (P<0.05)

Fatty Acids	20% LCRE	40% LCRE	60% LCRE	80% LCRE	100% LCRE	N:P:K
Capric acid (10:0)	7.42 ± 0.07	5.72 ± 0.05	6.90 ± 0.07	7.75 ± 0.07	7.22 ± 0.07	8. 72 ± 0.09
Lauric acid (12:0)	8.57 ± 0.07	4.55 ± 0.04	4.15 ± 0.04	8.12 ± 0.04	5.28 ± 0.04	7.85 ± 0.07
Myristic acid (14:0)	9.08 ± 0.08	7.66 ± 0.06	7.12 ± 0.07	9.16 ± 0.08	9.55 ± 0.10	10.66 ± 0.10
Palmitic acid (16:0)	12.66 ± 0.11	8.38 ± 0.07	11.34 ± 0.11	12.82 ± 0.10	10.47 ± 0.09	14.05 ± 0.12
Heptadecanoic acid (17:0)	3.14 ± 0.03	3.06 ± 0.03	2.15 ± 0.02	3.25 ± 0.03	3.32 ± 0.03	3.82 ± 0.04
10-Heptadecanoic acid (17:01)*	2.04 ± 0.02^{b}	1.55 ± 0.02^{d}	1.24 ± 0.01^{e}	1.76 ± 0.01^{c}	1.47 ± 0.02^{d}	2.96 ± 0.03^{a}
Stearic acid (18:0)	11.27 ± 0.09	12.71 ± 0.12	10.55 ± 0.09	11.25 ± 0.11	11.82 ± 0.12	12.16 ± 0.11
Oleic acid (18:1n-9)*	5.15 ± 0.05^{b}	5.62 ± 0.06^{a}	5.66 ± 0.05^{a}	5.25 ± 0.05^{b}	5.20 ± 0.06^{b}	5.05 ± 0.04^{b}
Linoleic acid (18:2n-6)*	4.56 ± 0.04^{c}	6.70 ± 0.06^{a}	6.88 ± 0.06^{a}	4.72 ± 0.04^{c}	5.66 ± 0.05^{b}	4.62 ± 0.08^{c}
Linoleic acid (18:3n-3)**	9.47 ± 0.08^{c}	13.88 ± 0.11^{a}	14.06 ± 0.12^{a}	9.55 ± 0.08^{c}	11.44 ± 0.10^{b}	9.87 ± 0.10^{c}
γ-Linoleic acid (18:3n-3)**	1.25± 0.02^{b}	1.58 ± 0.02^{a}	1.66 ± 0.02^{a}	1.22 ± 0.02^{b}	1.27 ± 0.02^{b}	1.13 ± 0.02^{c}
Arachidic acid (20:0)	2.62 ± 0.02	4.46 ± 0.05	3.15 ± 0.03	4.18 ± 0.05	5.12 ± 0.04	2.82 ± 0.02
Eicosadienoic acid (20:2n-11)*	1.71 ± 0.02^{a}	1.62 ± 0.02^{a}	1.70 ± 0.02^{a}	1.66 ± 0.02^{a}	1.72 ± 0.03^{a}	0.83 ± 0.02^{b}
Eicosadienoic acid (20:3n-6)**	1.76 ± 0.03^{b}	2.72 ± 0.03^{a}	2.82 ± 0.03^{a}	1.78 ± 0.03^{b}	1.86 ± 0.03^{b}	1.20 ± 0.02^{c}
Arachidonic acid (20:4n-6)**	2.42 ± 0.03^{c}	3.37 ± 0.03^{a}	3.48 ± 0.03^{a}	2.55 ± 0.03^{c}	3.13 ± 0.03^{c}	0.51 ± 0.02^{d}
Eicosapentaenoic acid (20:5n-3)**	1.45 ± 0.02^{c}	2.48 ± 0.03^{a}	2.59 ± 0.03^{a}	1.35 ± 0.02^{c}	2.34 ± 0.03^{b}	1.09 ± 0.02^{d}

TABLE 9
Fatty acids (g/100g lipid) of *Ankistrodesmus convolutus* grown in 20%, 40%, 60%, 80% & 100% standard Malaysian rubber effluent (SMRE) and fertilizer. Means (± SE) of *unsaturated and **polyunsaturated fatty acids with different superscripts in each row indicate significant differences (P<0.05)

Fatty Acids	20% SMRE	40% SMRE	60% SMRE	80% SMRE	100% SMRE	N:P:K
Capric acid (10:0)	8.82 ± 0.16	7.33 ± 0.14	6.30 ± 0.15	6.76 ± 0.13	7.44 ± 0.15	8.75 ± 0.09
Lauric acid (12:0)	6.95 ± 0.07	5.77 ± 0.06	4.48 ± 0.07	5.50 ± 0.07	5.60 ± 0.08	7.80 ± 0.07
Myristic acid (14:0)	9.33 ± 0.17	8.25 ± 0.17	5.88 ± 0.16	6.96 ± 0.14	7.15 ± 0.16	10.61 ± 0.10
Palmitic acid (16:0)	12.55 ± 0.09	13.75 ± 0.10	10.58 ± 0.09	10.22 ± 0.09	11.38 ± 0.09	14.09 ± 0.12
Heptadecanoic acid (17:0)	2.03 ± 0.02	2.15 ± 0.02	1.92 ± 0.02	1.88 ± 0.02	1.69 ± 0.02	3.77 ± 0.04
10-Heptadecanoic acid (17:01)*	1.04 ± 0.02^{b}	0.89 ± 0.02^{c}	0.86 ± 0.02^{c}	0.91 ± 0.02^{c}	0.88 ± 0.02^{c}	2.92 ± 0.03^{a}
Stearic acid (18:0)	14.32 ± 0.12	12.55 ± 0.13	11.15 ± 0.12	12.58 ± 0.13	12.73 ± 0.15	13.13 ± 0.11
Oleic acid (18:1n-9)*	3.55 ± 0.04^{d}	3.66 ± 0.04^{d}	4.95 ± 0.03^{a}	4.72 ± 0.04^{b}	4.67 ± 0.03^{b}	4.48 ± 0.04^{c}
Linoleic acid (18:2n-6)*	5.34 ± 0.04^{c}	5.55 ± 0.05^{c}	7.75 ± 0.04^{a}	6.48 ± 0.03^{b}	6.62 ± 0.04^{b}	5.27 ± 0.08
Linoleic acid (18:3n-3)**	9.65 ± 0.10^{d}	11.66 ± 0.11^{c}	15.79 ± 0.12^{a}	13.49 ± 0.11^{b}	1152. ± 0.11^{c}	9.86 ± 0.10d
γ-Linoleic acid (18:3n-3)**	1.66 ± 0.02^{b}	1.52 ± 0.02^{c}	1.88 ± 0.02^{c}	1.72 ± 0.02^{b}	1.69 ± 0.02^{b}	1.05 ± 0.03^{d}
Arachidic acid (20:0)	5.25 ± 0.12	5.33 ± 0.12	3.69 ± 0.11	4.39 ± 0.11	3.45 ± 0.12	2.86 ± 0.03
Eicosadienoic acid (20:2n-11)*	0.79 ± 0.02^{d}	0.88 ± 0.02^{d}	2.55 ± 0.02^{b}	2.12 ± 0.12^{b}	1.52 ± 0.02^{c}	0.89 ± 0.02^{d}
Eicosadienoic acid (20:3n-6)**	1.85 ± 0.06^{c}	1.92 ± 0.06^{c}	3.10 ± 0.05^{a}	2.99 ± 0.05^{a}	2.68 ± 0.05^{b}	1.27 ± 0.03^{d}
Arachidonic acid (20:4n-6)**	2.26 ± 0.03^{c}	2.33 ± 0.03^{c}	2.77 ± 0.03^{b}	2.66 ± 0.03^{b}	2.94 ± 0.04^{a}	0.44 ± 0.02^{d}
Eicosapentaenoic acid (20:5n-3)**	1.95 ± 0.03^{d}	1.95 ± 0.03^{c}	2.59 ± 0.03^{a}	2.48 ± 0.03^{a}	2.28 ± 0.03^{b}	1.02± 0.03^{e}

TABLE 10
Fatty acids (g/100g lipid) of *Ankistrodesmus convolutus* grown in 5%, 10%, 20% and 30% POMED and N:P:K. Means (± SE) of *unsaturated and **polyunsaturated fatty acids with different superscripts in each row indicate significant differences ($P < 0.05$, df, 39, 5)

Fatty Acids	5% POMED	10% POMED	20% POMED	30% POMED	N:P:K
Capric acid (10:0)	5.02 ± 0.06	5.15 ± 0.08	5.99 ± 0.05	7.30 ± 0.04	8.71 ± 0.09
Lauric acid (12:0)	6.45 ± 0.11	5.95 ± 0.12	6.34 ± 0.10	7.48 ± 0.11	7.83 ± 0.07
Myristic acid (14:0)	8.91 ± 0.30	8.55 ± 0.11	7.26 ± 0.12	6.33 ± 0.10	10.64 ± 0.10
Palmitic acid (16:0)	12.33 ± 0.21	12.32 ± 0.20	14.02 ± 0.17	13.69 ± 0.19	14.05 ± 0.12
Heptadecanoic acid (17:0)	1.75 ± 0.02	1.88 ± 0.02	2.15 ± 0.03	1.55 ± 0.04	3.74 ± 0.04
10-Heptadecanoic acid (17:01)*	1.06 ± 0.02^{c}	1.25 ± 0.02^{c}	2.08 ± 0.03^{b}	2.02 ± 0.03^{b}	2.88 ± 0.03^{a}
Stearic acid (18:0)	14.92 ± 0.18	11.88 ± 0.21	13.25 ± 0.16	13.66 ± 0.11	13.90 ± 0.15
Oleic acid (18:1n-9)*	5.76 ± 0.11^{b}	6.05 ± 0.14^{a}	5.12 ± 0.08^{c}	4.32 ± 0.07^{d}	4.43 ± 0.04^{d}
Linoleic acid (18:2n-6)*	6.02 ± 0.04^{c}	8.44 ± 0.04^{a}	6.26 ± 0.05^{c}	5.40 ± 0.03^{d}	5.17 ± 0.08^{d}
Linoleic acid (18:3n-3)**	10.02 ± 0.18^{b}	15.88 ± 0.22^{a}	10.12 ± 0.14^{b}	8.44 ± 0.11^{c}	8.20 ± 0.10^{c}
γ-Linoleic acid (18:3n-3)**	2.35 ± 0.02^{a}	2.42 ± 0.02^{a}	1.75 ± 0.02^{b}	1.52 ± 0.02^{c}	1.04 ± 0.01^{d}
Arachidic acid (20:0)	3.66 ± 0.12	2.16 ± 0.11	5.32 ± 0.14	4.55 ± 0.14	1.82 ± 0.02
Eicosadienoic acid (20:2n-11)*	0.59 ± 0.03^{d}	0.65 ± 0.03^{d}	1.28 ± 0.03^{b}	1.45 ± 0.03^{a}	0.87 ± 0.02^{c}
Eicosadienoic acid (20:3n-6)**	1.45 ± 0.03^{c}	1.79 ± 0.04^{b}	1.55 ± 0.03^{c}	2.37 ± 0.03^{a}	1.22 ± 0.02^{d}
Arachidonic acid (20:4n-6)**	2.60 ± 0.03^{b}	2.80 ± 0.03^{a}	1.34 ± 0.02^{c}	1.38 ± 0.02^{c}	0.49 ± 0.02^{c}
Eicosapentaenoic acid (20:5n-3)**	1.82 ± 0.03^{a}	1.86 ± 0.04^{a}	1.64 ± 0.02^{b}	1.60 ± 0.02^{b}	1.07 ± 0.02^{c}

REFERENCES

ANTON, A., KUSNAN and A.R.M. HUSSIN. 1994. Effects of palm oil mill effluent on algae: a laboratory bioassay. In *Proceedings of the 1st Asia-Pacific Conference on Algal Biotechnology*, ed. S.M. Phang, L.Y. Kun, M.A. Borowitzka and B.A. Whitton, p. 320-323. Kuala Lumpur, Malaysia: University of Malaya.

BARCLAY, W.R., K.M. MEAGER and J.R. ABRIL. 1994. Heterotrophic production of long chain omega-3 fatty acids utilizing algae and algae-like microorganisms. *Journal of Applied Phycology* **6:** 123-129.

BENITEZ, L.V. 1989. Amino acid and fatty acid profiles in aquaculture nutrition studies. In *Fish Nutrition Research in Asia, Proceedings of the 3rd Asian Fish Nutrition Network Meeting*, ed. S.S. De Silva, p. 23-35. Metro Manila, Philippines: Asian Fisheries Society.

BLIGH, E.G. and W.J. DYER. 1959. A rapid method of total lipid extraction and purification. *Canadian Journal of Biochemistry and Physiology* **37:** 911-917.

CHU, W.L., S.M. PHANG, S.H GOH and B. NORMAN. 1994. Environmental effects on growth and biochemical composition of *Ankistrodesmus convolutus*. In *Proceedings of the 1st Asia-Pacific Conference on Algal Biotechnology*, ed. S.M. Phang, L.Y. Kun, M.A. Borowitzka and B.A. Whitton, p. 16-27. Kuala Lumpur, Malaysia: University of Malaya.

CLESCERI, L.S., A.E. GREENBERG and R.R. TRUSSELL. 1989. *Standard Methods for the Examination of Water and Wastewater*. 17th edition. Washington D.C.: USA: American Public Health Association, American Water Works Association and Water Pollution Control Federation.

EDWARDS, P., O.A. SINCHUMPASAK and E.A.O. OUANO. 1980. A Study of a sewage fed high-rate stabilization pond in Thailand. Wastewater Treatment and Resources Recovery (IDRC – 154e). Ottawa, Canada: International Development Research Centre.

FOLCH, J., M. LEES and G.H. STANDLEY. 1957. A Simple method for the isolation and purification of total lipids from animal tissues. *Journal of Biological Chemistry* **226:** 497-509.

GEETHA, P.K., S.M. PHANG, M.A HASHIM and N. BLAKEBROUGH. 1994. Rubber effluent treatment in a high rate algal pond system. In *Proceedings of the 1st Asia-Pacific Conference on Algal Biotechnology*, ed. S.M. Phang, L.Y. Kun, M.A Borowitzka and B.A. Whitton, p.

306-312. Kuala Lumpur, Malaysia: University of Malaya.

GELDENHUYS, D.J., R.D. WALMSLEY and D.F. TOFRIEN. 1987. An investigation of a fertilizer tap water medium for mass algal production in outdoor plastic-enclosed systems. *Biotechnology and Bioengineering* **30:** 153-156.

HABIB, M.A.B., F.M. YUSOFF, S.M. PHANG, K.J ANG and S. MOHAMED. 1997a. Nutritional values of chironomid larvae grown in plam oil mill effluent and algal culture. *Aquaculture* **158:** 95-10.

HABIB, M.A.B., F.M. YUSOFF, S.M. PHANG, M.S. KAMARUDIN and S. MOHAMED. 1997b. Chemical characteristics and essential nutrients of agroindustrial effluents in Malaysia. *Asian Fisheries Sciences* **11:** 279-286.

HORWITZ, W. 1984. *Offical Methods of Analysis of the Association of Official Analytical Chemists.* 14th edition. Washington D.C., U.S.A: Association of Official Analytical Chemists.

HWANG, T.K., S.M. ONG, C.C. SEOW and H.K. TAN. 1978. Chemical composition of palm oil mill effluents. *Planter, Kuala Lumpur* **54:** 749-756.

ISA, Z. 1993. Wastes from rubber processing industries. In *Waste Management in Malaysia: Current Status and Prospects for Bioremediation,* ed. B.G. Yeih, K.S. Chee, S.M. Phang, Z. Isa, A. Idris and M. Mohamed, p. 137-151. Kuala Lumpur, Malaysia: Ministry of Science and the Environment.

ISA, Z., A. IBRAHIM, N.A.K. BAKTI and M.Z.A KARIM. 1988. Treatment of rubber factory effluent – a survey. In *Proceedings of a Seminar on Waste Treatment by Algal Cultivation,* ed. M.A Borowitzka and K. Mathew, p. 52-60. Murdoch, Australia: Murdoch Australia.

JOHNS, M.R. 1994. Heterotphic culture of microalgae. In *Proceedings of the 1st Asia-Pacific Conference on Algal Biotechnology,* ed. S.M. Phang, L.Y. Kun, M.A. Borowitzka and B.A. Whitton, p. 150-154. Kuala Lumpur, Malaysia: University of Malaya.

LALIBERTE, G. and J. de la NOÜE. 1993. Auto-, hetero- and mixotrophic growth of *Chlamydomonas humicola* (Chlorophyceae) on acetate. *Journal of Phycology* **29:** 612-620.

MILLIPORE. 1990. Waters chromatography division. Millipore Corporation, Massachusetts, USA, 3-22pp.

MIZUNO, T. 1978. *Illustration of the Freshwater Plankton of Japan.* Revised edn. 341 pp. Osaka, Japan: Hoikusha Publishing Co., Ltd.

OKIY, D.A. 1987. Chemical and biological characterization of the by products of Nifor palm oil mill. In *Proceedings of the International Oil Palm/Palm Oil Conference Technology,* ed. I.M.A. Ngan, J. H. Maycock, S.L.M. Ling and M.A. Augustine, p. 434-437. Kuala Lumpur, Malaysia: Palm Oil Research Instite of Malaysia.

PHANG, S.M. 1987. Agro-industrial wastewater reclamination in Peninsular Malaysia. *Archivfür HydrobiologieBeiheft Ergednisse der Limnologie* **28:** 77-94.

PHANG, S.M. and K.C. ONG. 1988. Algal biomass production in digested palm oil mill effluent. *Biological Wastes* **25:** 177-191.

PHANG, S.M. 1990. Algal production from agro-industrial and agricultural waste in Malaysia. *Ambio* **19(8):** 415-418.

PHANG, S.M. 1991. Performance of a laboratory-scale pond system for the secondary treatment of palm oil mill effluent. In *Proceedings of the Regional Seminar on Management and Utilization of Agricultural and Industrial Wastes,* ed. S.H. Goh, C.H Chuah, S.L. Tong, S.M. Phang and S. Vikineswary, p. 308-318. Kuala Lumpur, Malaysia: University of Malaya.

SUKENIK, A. and Y. CARNNELI. 1990. Lipid synthesis and fatty acid composition in *Nannochloropsis* sp. (Eustigmatophyceae) grown in a light dark cycle. *Journal of Phycology* **26:**463 – 469.

TAN, C.K. and M.R. JOHNS. 1991. Fatty acid production by heterotrophic *Chlorella saccharophilla. Hydrobiologia* **215:** 13-19.

VAZHAPPILLY, R. and F. CHEN. 1998. Eicosaphentaenoic acid and docosahexaenoic acid production potential of microalgae and their heterotrophic growth. *Journal of American Oil Chemistry Society* **75:** 393-397.

YUSOFF, F.M., A.D. OM and S.H. CHEAH. 1996. Use of agro-industrial effluent in

augementing microalgae production and fish fry growth in hatcery tanks. *Journal of Aquaculture in the Tropics* **11:** 119-126.

YUSOFF, F.M. and S.Y. CHAN. 1997. Nutrient status of palm oil, rubber and domestic effluents and their effects on algal growth. *Journal of Bioscience* **8:** 42-50.

ZAR, J.H. 1984. *Biostatistics.* Englewood Cliffs, USA: Prentice-Hall, Inc.

(Received: 18 October 2000)
(Accepted: 22 March 2004)

Pertanika J. Trop. Agric. Sci. 27(2): 91 - 99 (2004) ISSN: 1511-3701

Effects of Sole and Amended Agricultural by Products on Soil Fertility and the Growth and Chemical Composition of Budded Rubber

E.I MOYIN-JESU
Agronomy Department, Federal College of Agriculture
Akure Ondo State, Nigeria

Keywords: Sole and amended cocoa husk, soil fertility, budded rubber, Akure, rainforest zone

ABSTRAK

Satu eksperimen telah dijalankan untuk menentukan kesan sekam koko yang diperbaiki dan yang tidak menggunakan abu tandan kelapa sawit dengan najis ayam itik, ayam belanda, arnab dan sekam yang digunakan pada tangkai akar getah (tunas getah yang dicantumkan pada akar) di kawasan zon hutan hujan Akure, Nigeria. Ada tiga belas rawatan pembaikan organik dijalankan pada 6t/ha dengan baja 300 kg/ha sebagai rujukan pembaikan dan kawalan (tanpa baja, tanpa najis binatang). Rawatan disusun dalam aturan blok lengkap rawak (RBC) dan direplikakan sebanyak tiga kali. Rawatan pembaikan tanah organik juga dianalisis secara kimia dan parameter tunas getah direkodkan bagi ketinggian, jumlah dedaun, jumlah limfa, kawasan daun, nilai N, PL, K Ca, pH Mg tanah dan bahan organik (O.M) pada tanah dan daun. Keputusan menunjukkan ada peningkatan yang signifikan (P<0.05) pada kawasan dan jumlah daun, jumlah limfa, tanah dan nilai N, P, K, Ca, Mg dan pH tanah dan bahan organik (O.M) tunas getah di bawah rawatan pembaikan berbanding rawatan kawalan. Rawatan menggunakan tandan kelapa sawit dan najis ayam itik menghasilkan limfa daun yang tertinggi. Penggunaan baja NPK, sekam (betari sisa kilang) dan rawatan kawalan, mengurangkan jumlah limfa daun sebanyak 50%, 42% dan 92%. Pembaikan abu kelapa sawit + najis arnab juga menaikkan min ketinggian tunas pokok getah dengan 56% dan 46% berbanding najis ayam itik dan baja NPK. Abu tandan kelapa sawit dan hampas sekam koko meningkatkan K dan Mg pada tanah dan daun berbanding sekam sementara itu najis ayam belanda meningkatkan N, P, K, Ca, Mg pH tanah dan O.M pada tunas, tanah dan daun getah berbanding najis ayam itik dan arnab.

ABSTRACT

An experiment was out to determine the effectiveness of sole and amended cocoa husk, oil palm bunch ash with poultry, turkey, rabbit manure and spent grain on budded rubber root stock (grafted bud rubber on a root stock) in the field at Akure in the rainforest zone of Nigeria. There were thirteen organic amendment treatments applied at 6t/ha with 300 kg/ha fertilizer as a reference treatment and a control (no fertilizer; no manure). The treatments were arranged in a randomized complete block (RBC) design and replicated three times. The soil organic amendment treatments were also chemically analysed while the parameters recorded for the budded rubber were plant height, leaf number, number of nodes, leaf area, soil and leaf N, PL, K Ca, Mg Soil pH and Organic matter (O.M). The results showed that there were significant increases (P<0.05) in plant height, leaf area, leaf number, nodes number, soil and leaf N,P,K,Ca, Mg Soil pH and organic matter (O.M) of budded rubber under different organic amendment treatments compared to the control treatment. Oil palm bunch ash + poultry manure treatments had the greatest number of leaf nodes. Use of NPK fertilizer, spentgrain (sorghum based brewery waste) and control treatments reduced mean leaf node number by 50%, 42% and 92% respectively. The amendment oil palm ash + rabbit manure also increase the mean plant height of budded rubber by 56% and 46% compared to the poultry manure and NPK fertilizer respectively. Oil palm bunch ash and cocoa husk residues increased the soil and leaf K and Mg compared to the spent grain while the turkey manure increased growth of budded rubber, soil and leaf N, P, K, Ca, Mg Soil pH and O.M. more than poultry and rabbit.

INTRODUCTION

Rubber, (*Hevea brasiliensis*) is a member of the family Euphorbiaceae and is mainly cultivated for its latex production. The family also contains plants such as castor oil plant (*Ricinus comunis*), cassava (manihot esculentum) and many other

species of tropical importance.

The genus Heavea contains a number of species but *Hevea brasiliensis* is the only species with very high isoprene content in its latex. The latex produced by the tree in processed into commercial forms of rubber and has contributed enormously to the economies of Nigeria, Asian and other tropical countries in term or raw-materials for tire industries, foreign exchange earnings and provision of employment opportunities.

In spite of the utilization and importance of the crop, the rubber production in Nigeria is facing serious problems at present because the tree area aging and there is a scarcity of new rubber seedlings or budded rubber to replace over 532,364 hectares of aging and low production rubber trees in the field. This replacement is becoming difficult because ofa continuous decline in soil fertility.

Efforts to improve soil fertility using different types of inorganic fertilizers such as Urea, sulphate of ammonia and NPK tog row rubber seedlings or budded types, are limited by the cost of these materials, scarcity at the farmers' level and continued deterioration of soil properties (Folorunso *et al.* 1995).

Umoti (1990) reported that cocoa, rubber and oil palm removed large amounts of plant nutrients in the nursery and field. High productivity of these crops could be achieved and sustained by massive application of inorganic fertilizers. However, inorganic fertilizers are becoming very expensive (N1500.00 or US$15.00 per bag) which poses a constraint to low income farmers who produce the major percentiles of rubber, oil palm and cocoa in Nigeria.

Therefore, the complimentary use of organic fertilizers materials derived from oil palm, cocoa, rice, maize, cassava crops and animal wastes such as poultry and turkey manures could help to reduce the high cost of fertilizers. Due to their natural abundance, organic amendments such as oil bunch ash, wood ash spent grain (sorghum based brewery waste) and their amended forms with turkey and poultry manures as fertilizers have been used for growing maize, cassava and okra crops in Nigeria (Oladokun 1986; Folorunso 1999).

Based on an extensive literature review, it is concluded that there is scarcity of research information pertaining to the of oil palm bunch ash, wood ash, spent grain nad their amended forms with turkey and poultry manures for growing rubber either in the nursery or field. Therefore, there is a strong justification to investigate the use of these wastes to grow budded rubber in the field.

The objectives of the research were as follows:

i. To determine the effect of different organic amendments on the performance of budded rubber in the field
ii. To determine the effect of organic amendments on the soil and leaf chemical composition of rubber in the field.

MATERIALS AND METHODS

The experiment took place at Akure (Lat 7°N[1], 5° 10E[1]) in the rainforest zone of Nigeria. The annual rainfall is 1300mm and the mean termperature is 70°F while the soil is a sandy laom, skeletal, kaolinitic isohyperthermic oxic paleustalf (alfisol) or Ferric Luvisol (F.A.O).

Source and Preparation of the Organic Amendments Used

Oil palm bunch ash and wood were obtained from the oil palm and cassava processing units of Federal College of Agriculture, Akure respectively. Cocoa husk was collected form the college cocoa farms while the spent grains (sorghum based brewery waste) were collected from a nearby local brewery.

The turkey, poultry and rabbit manures were collected form the college livestock farms. The organic residues were processed to allow decomposition and reduction of C/N ratio. The dried cocoa husk were ground a hammer mill while the spent grain was chopped into pieces, and allowed to decompose. The turkey, poultry and rabbit manures were stored individually to allow for mineralisation and placed under shade.

Generally, all the organic wastes readily available, sustainable and inexpensive for growing comemrical quantities of budded rubber in the field.

Field Experiment

The budded rubber root stocks were bought form Rubber Research Institute of Nigeria (RRIN) Benin, Edo- State. The field site was cleared and the planting lines for the budded were marked with pegs.

There were thirteen organic amendment treatmnets viz:spent grain, rabbit manure, turkey manure, spent grain + turkey manure, oil palm bunch ash + rabbit manure, cocoa husk + turkey

manure with a field recommended chemical fertilizer NPK 15 – 15 – 15 treatment applied at 300kg/ha and a control (no fertilizer no manure).

The organic amendments were applied at 6t/ha for the ordinary forms of spent grain, rabbit manure, turkey and oil palm bunch ash while their amended froms were applied at a ratio of 50:50% by weight (3tha- 1 each).

Planting holes were dug 30cm deep and filled with top soil halfway. Then each organic amendment was added and allowed to decay for ten days before planting one budded rubber root stock per hole at a spacing of 6m x 3m. The 4th week after planting, sprouting of the scions occurred.

Weeding began 2 weeks after planting and every 3 weeks until experiment was terminated. 10ml a.i. of Karate/10L of water was sprayed on them (sprouted scions of rubber) to control termite attacks.

Five weeks after planting measurements or counts of plant height, nodes number, leaf area, stem girth and leaf number were made and these measurements or counts were representative leaves of the plant and this was used to calculate total leaf area (cm^2) per plant. (4.8 and 32 weeks after planting), also these measurements were done insitu.

Representative leaf samples were taken at 20 weeks after planting for each treatment, placed in labelled envelopes and oven dried at 70°C for 48 hours. Two grams of dried leaf samples were weighed into crucible and dry-ashed in a muffle furnace at 45°C for 6 hours.

The resulting ash was solubilized into solution and analyzed for phosphorus (P) using vanado-molybdate colouration and read on spectronic 20 at 442Um while the % K, Ca and Na were determined using a flame photometer. Magnesium content was determined with an atomic absorption spectrophotometer (Jackson 1958). The % N was determine using the micro-Kjeldahl method (Jackson 1964).

Soil Analysis Before Planting

30 core soil samples were collected from 0-15cm depth on the site, mixed thoroughly and the bulk samples were taken to the laboratory air-dried sieved with 2mm sieve and ready for routine analysis.

The soil pH (1:1 soil/water and 1:2 soil/ 0.01M $Cacl_2$) was determined using a glass/ calornel system (Crockford and Nowell 1956). Organic carbon determination was done using wet dichromate method (Walkley and Black 1934). The organic C ws multiplied by 1.723 to get organic matter (0.M).

The exchangeable cations were extracted using 1M NH_40 (ammonium acetate) solution and the amount of K, Ca and Na contents were determined on flame photometer using appropriate element filters while Mg content in the extract was read on atomic absorption spectrophotometer (Jackson 1958). The exchangeable acidity (H^+ and $A1^{3+}$) was measured from 0.01 M KCI extracts by titrating with 0.1 M HCI (Mclean 1965).

Total nitrogen was determined by micro-kjedahl method (Jackson 1964) and the soil available phosophorus was extracted using Bray Pi extractant (Murphy and Riley 1962) and the concentration measured on a spectronic 20 at 882Um.

Soil Analysis After Planting

Soil samples were taken from each treatment plot using soil auger, air-dried, sieved with 2mm sieve for routine analysis of total N, available P, exchangeable K, Ca, Mg and Na contents, soil pH and O.M as described earlier under soil analysis before planting.

Statistical Analysis

The data collected from the treatment effects of the organic amendments on the growth parameters such as plant height, leaf area, stem girth, leaf number, nodes number, leaf and soil N,P,K,Mg, Soil pH and O.M were analysed using an ANOVA F-test and their means were separated and compared along the treatment effects using Duncan Multiple Range Test (DMRT) at 5% level.

RESULT

Soil Analysis Before Planting of Budded Rubber

The physical and chemical properties of the soils used for growing rubber are presented in Table 1. Using the established soil critical levels in Southwest Nigeria for rubber production, the soils are low in organic matter when compared with the critical level of 3% O.M (Agbooda and Corey 1973). The total nitrogen was less than 0.14% considered as optimum for cocoa and rubber production (Obatolu 1989).

The available P was below the 10mg/kg P which is to be the optimum for crop production (Agboola and Corey 1973). The exchangeable

K, Ca, Mg and Na contents were below, 0.20, 0.25 and 0.18 mg/kg that are considered as adequate for cocoa, oil palm and rubber crops (Agboola 1974).

The low values of soil N, P, K, Ca, Mg and Na and O.M were indications of the low inherent soil fertility. Budded rubber root stocks planted on such would respond favourably to the application of the organic amendments. The soils were sandy loam in texture, skeletal, kaolinitic, isohyperthemic oxic paleustalf (Alfisol or Ferric Luvisol (FAO) or Akure series (local classification).

Chemical Composition of the Organic Fertilizer Materials Used

Table 2 presents the chemical properties of the organic materials used for growing budded rubber root stocks in the field. The poultry manure had the highest N, K and Mg levels when compared to turkey and rabbit manures while turkey manure had the highest P, Ca and Na levels.

The oil palm bunch ash had the highest %N, K, Ca Mg and Na levels when compared to cocoa husk and spent grain. Spent grain is fairly low in N, K, Ca, Mg and Na levels except in %P. The prior processing of the organic amendment treatments before application reduced their C/N ratio.

Leaf Number of Budded Root Stocks

The leaf number of budded rubber root stocks between 4 and 32 weeks after planting (WAP) under the different organic fertilizer treatments is presented in Table 3. There were significant increases ($p<0.05$) in leaf number of budded rubber under the different organic treatments compared to the control treatment.

The amended oil palm bunch ash+spent grain treatments increased the leaf number of budded rubber by 26% and 64% compared to the sole oil palm bunch ash and spent grain respectively. The amended spent grain + turkey manure and cocoa husk+spent grain treatments increased leaf number of budded rubber by 71% and 81% respectively when compared to the control treatment.

The NPK fertilizer treatment increased the leaf population of budded rubber by 48% compared to spent grain treatment. However, the amended cocoa husk+spent grain increased the leaf number of budded rubber by 20% compared to NPK fertilizer. Of all sole residue treatments, poultry manure resulted in the greatest increase in leaf number then spent grain and turkey manure.

Leaf Area of Budded Rubber

TABLE 1
Chemical analysis of the soil before the experiment

PH					Exhangeable Cations			
H_20	$Cacl_2$	Organic matter	N	P	K	Ca	Mg	Na
		%	mg/kg		mmol/kg soil			
5.80	5.30	0.45	0.08	7.62	0.09	0.11	0.17	0.16

TABLE 2
Chemical analysis of the organic fertilizers used for the experiment

Organic Material	N	Available P	Exchangeable Cations K	Ca	Mg	Na
	%	%		%		
Spent grain	0.78a	0.64c	0.12a	0.118a	0.20a	0.14b
Rabbit manure	2.67c	0.86e	0.29b	0.30b	0.18	0.16c
Turkey manure	4.08d	0.93f	0.42c	0.60c	0.17a	0.13b
Poultry manure	5.92e	0.75d	0.48c	0.54c	0.35b	0.09a
Oil palm bunch ash	1.54b	0.42b	2.40e	1.25e	0.78d	0.21d
Cocoa pod ash	1.44b	0.21a	1.26d	0.91d	0.48c	0.17c

TABLE 3
The leaf number of budded rubber at 4.8 and 32 WAP under different organic amendments

Treatments	Weels After Planting			
	4	8	32	Mean
Control (No fertilizer)	1	2	4	2.33k
NPK 15-15-15	6	10	15	10.3f
Poultry manure	6	12	18	12.00d
Rabbit manure	4	6	10	6.66h
Turkey manure	3	5	10	6.00i
Oil palm bunch ash	5	11	17	11.00f
Spent grain	3	4	9	5.30j
Spent grain + Cocoa husk	7	12	20	13.0b
Spent grain + Oil palm bunch ash	8	14	23	15.0a
Cocoa husk + turkey manure	5	11	17	11.00ef
Oil palm bunch ash + poultry manure	5	10	16	10.30f
Oil palm bunch ash + rabbit manure	6	10	16	10.66f
Spent grain + poultry manure	6	11	17	11.33c
Spent grain + rabbit manure	6	12	20	12.66c
Spent grain + turkey manure	4	8	12	8.00g

Treatment means followed by the same letters are not significantly different from each other using Duncan Multiple Range Test at 5% level.

TABLE 4
The leaf area (Cm^2/tree) of budded rubber at 4, 8 and 32 and WAP under different organic amendments

Treatments	Week After Planting			
	4	8	32	Mean (Cm^2)
Control	48	75	100	74.30m
NPK 15-15-15	118	236	590	314.90c
Poultry manure	81	160	398	213.00i
Rabbit manure	68	134	335	179.00k
Turkey manure	85	170	424	226.30h
Oil palm bunch ash	78	156	390	208.00j
Spent grain	61	123	308	163.90b
Spent grain + cocoa husk	97	194	484	258.40e
Spent grain + oil palm bunch ash	138	263	698	366.50a
Cocoa husk + turkey manure	128	255	638	340.60b
Oil palm bunch ash + poultry	91	181	453	241.50g
Oil palm bunch ash + rabbit manure	104	207	519	276.50c
Spent grain + poultry manure	85	170	426	227.00h
Spent grain + rabbit manrue	105	209	523	279.00e
Spent grain + turkey manure	114	227	568	3030.00d

Treatment means followed by the same letters are not significantly different from each other using Duncan Multiple Range Test at 5% level.

The leaf of budded rubber root stocks between 4 and 32 weeks after planting (WAP) increase significantly ($p<0.05$) under the different organic fertilizers as presented in Table 4. The amended oil palm bunch ash+spent treatment increased the mean leaf area of budded rubber in the field by 24% compared to the spent grain+rabbit manure. It also increased the leaf area of budded rubber by 43% compared to the sole form of oil palm bunch ash treatment.

The amended oil palm bunch ash+spent grain increased the leaf area of budded rubber by 14% compared to NPK fertilizer while the same parameter increased by 80% when

compared to the control treatment. Among the sole residues used, poultry manure increased the leaf most, when compared toe the spent grain and rabbit manure respectively.

Nodes Number of Budded Rubber

Table 5 shows the number of nodes of budded rubber increased significantly ($p<0.05$) between 4 and 32 weeks after planting (WAP) under the different organic fertilizer treatments. The amended oil palm bunch ash + poultry manure increased the mean nodes number of budded rubber by 50%, 42% and 92% compared to the NPK fertilizer, spent grain and control treatments respectively.

Oil palm bunch + rabbit manure increased the nodes number of budded rubber 29% and 53% compared to the oil palm bunch + poultry manure and oil palm bunch ash (sole form) respectively. Poultry manure treatment also increased the nodes number 27%, 45% and 55% compared to turkey manure, rabbit manure and spent grain treatments respectively.

Plant Height of Budded Rubber

Treatments had significant (<0.05) effect on plant height 32 weeks after planting (Table 6). The amended oil palm bunch ash + rabbit manure increased the plant height of budded rubber by 56% and 46% compared to the poultry manure and NPK fertilizer respectively. The same treatment increased the plant height of budded rubber by 85% compared to the control treatment.

The poultry manure treatment increased the plant height of budded rubber by 53% compared to the spent grain treatment.

DISCUSSION

Soil used for planting of budded rubber trees in the field were generally low in pH, O.M. N,P,K, Ca and Mg which could be responsible for the poor growth of budded rubber as shown in the control treatment. The observation was supported by Agboola (1982) who had reported that poor growth of rubber trees was noticeable in undertilized soils, hence, it is expected that the application of spent grain, oil palm bunch ash, turkey, poultry and rabbit manures and cocoa pod husk alone or in combination iwht manures to the soil would increase growth of budded rubber.

The increase in plant height, leaf area, nodes number, leaf number of budded rubber grown with the sole and amended organic fertilizers could be due to their rich chemical composition and this finding agreed with Umoti (1990) and Folorunso (1999) who reported that oil palm

TABLE 5
The increase in node number of budded rubber between 4 and 32 weeks after planting under different organic treatments

Treatments	Week After Planting			
	4	8	32	Mean (Cm^2)
Control	0	0	1	0.33h
NPK 15-15-15	1	2	3	2.00g
Poultry manure	1	4	6	3.66d
Rabbit manure	1	2	3	2.00g
Turkey manure	1	2	5	2.66e
Oil palm bunch ash	1	2	4	2.33f
Spent grain	0	1	4	1.66g
Spent grain + cocoa husk	1	3	6	3.33d
Spent grain + oil palm bunch ash	2	4	6	4.00c
Cocoa husk + turkey manure	2	4	8	4.66b
Oil palm bunch ash + poultry	2	4	6	4.00c
Oil palm bunch ash + rabbit manure	2	5	10	5.66a
Spent grain + poultry manure	1	2	5	2.66e
Spent grain + rabbit manrue	1	2	4	2.33f
Spent grain + turkey manure	1	2	5	2.67e

Treatment means followed by the same letters are not significantly different from each other using Duncan Multiple Range Test at 5% level.

TABLE 6
The increase in plant height (cm) of budded rubber between 4 and 32 WAP under different organic residues

Treatments	4	8 WAP	32	Mean
Control	4.30	6.00	9.00	6.42i
NPK 15-15-15	10.00	20.00	37.00	22.37d
Poultry manure	8.00	16.70	30.00	18.23f
Rabbit manure	9.00	17.00	32.00	19.34ef
Turkey manure	7.00	16.00	29.00	17.58f
Oil palm bunch ash	6.00	14.00	24.60	14.81g
Spent grain	5.60	13.00	22.00	13.54g
Spent grain + cocoa husk	10.00	18.90	33.00	20.64e
Spent grain + oil palm bunch ash	11.60	23.00	43.00	25.88c
Cocoa husk + turkey manure	6.10	11.00	19.00	12.04h
Oil palm bunch ash + poultry	12.00	28.00	41.20	27.06b
Oil palm bunch ash + rabbit manure	15.50	40.00	69.00	41.5a
Spent grain + poultry manure	10.00	23.80	39.00	24.28c
Spent grain + rabbit manure	10.00	19.30	35.00	21.46de
Spent grain + turkey manure	10.00	19.00	35.00	21.33d

TABLE 7
The leaf chemical composition of budded rubber at 20 weeks after planting under different organic residues

Treatments	N	P	K%	Ca	Mg	Na
Control (no fertilizer)	0.90k	0.141	0.20m	0.20k	0.15kl	0.10h
NPK 15-15-15	2.90b	0.61b	0.65b	0.20k	0.101	0.10h
Poultry manure	2.78c	0.35j	0.33j	0.41f	0.21j	0.15g
Rabbit manure	2.22g	0.35j	0.32j	0.30hi	0.25i	0.11h
Turkey manure	1.72h	0.27k	0.25L	0.28j	0.33h	0.14g
Oil palm bunch ash	1.35i	0.29k	0.28k	0.30hi	0.42de	0.39d
Spent grain	1.25j	0.27k	0.24L	0.36g	0.18k	0.10h
Spent grain + cocoa husk	2.35f	0.40hi	0.44ef	0.38g	0.32h	0.20f
Spent grain + oil palm bunch ash	2.46e	0.42h	0.45e	0.53e	0.48d	0.23f
Cocoa husk + turkey manure	2.40c	0.35j	0.36i	0.32h	0.37fg	0.41d
Oil palm bunch ash + poultry	2.85b	0.53c	0.50c	0.58b	0.53c	0.22f
Oil palm bunch ash + rabbit manure	3.20a	0.69a	0.95a	0.84a	0.86a	0.93a
Spent grain + poultry manure	2.70c	0.45f	0.42g	0.49d	0.64b	0.54b
Spent grain + rabbit manure	2.60d	0.45f	0.48d	0.44e	0.34h	0.50c
Spent grain + turkey manure	2.60d	0.43fg	0.40gh	0.48d	0.39f	0.26

Treatment means within each group followed by the same letters are not significantly different from each other using Duncan Multiple Range Test at 5% level.

bunch ash, cocoa husk and spent grain applied in sole form or amended poultry manure and pig manure at 6t/ha increased significantly the plant height, leaf area and leaf number of oil palm, rubber and coffee trees.

The nutrient contents in leaves of budded rubber in the control plots were below the critical of 0.25%P, 1.19%K, 0.8% Ca and 0.7% Mg as reported by Jones and Eck (1973), thus the leaves of the budded rubber exhibited deficiency symptoms of N (yellow colouration), P (purple colouration) and K (burnt leaf margin).

The application of turkey poultry and rabbit manure, oil palm bunch ash, spent grain and cocoa husk (either solely or amended forms) increased the leaf N,P,K, Ca and Mg contents of budded rubber compared to controls which may be related to their chemical composition (Table 2). This observation agreed with the views of Adu-Daaph *et al.* (1994) who reported that cocoa husk and oil palm bunch ash were good sources of P,K,Ca, Mg and Na when soil applied.

However, increased leaf and soil chemical

TABLE 8
The soil chemical composition of the field planted to budded rubber at 52 weeks after planting under different treatments

Treatments	PH (h_2O)	O.M %	N %	P Mg/kg soil	K	Ca	Mg Mmol/kg	Na
Control (no fertilizer)	5.40e	0.23g	0.03f	4.5j	0.03i	0.05i	0.04h	0.06g
NPK 15-15-15	5.10f	0.28g	0.32bc	34.2b	3.42bc	0.03i	0.02h	0.025g
Poultry manure	6.50c	2.30e	0.28cd	24.5g	2.49g	2.09g	1.32f	0.80d
Rabbit manure	6.45cd	2.26e	0.24d	26.5f	2.25h	1.97g	1.28f	0.69e
Turkey manure	6.60bc	2.35e	0.26d	22.7h	2.78de	2.07f	1.32f	0.74de
Oil palm bunch ash	6.90b	2.30e	0.23d	21.7hi	3.18c	1.89g	1.25f	0.64ef
Spent grain	6.20	1.76f	0.16e	20.5i	2.47g	1.04h	0.81g	0.62f
Spent grain + cocoa husk	6.48c	2.58d	0.29c	25.5f	2.48ef	2.74d	1.64e	0.88c
Spent grain + oil palm bunch ash	7.10a	3.31b	0.29c	33.2c	2.65e	2.63de	1.62e	0.85cd
Cocoa husk + turkey manure	6.53c	2.85c	0.30c	31.9d	2.91d	2.17f	2.46b	1.10b
Oil palm bunch ash + poultry	7.00ab	3.34b	0.35ab	35.2b	3.62b	3.89b	2.05c	1.09b
Oil palm bunch ash + rabbit manure	7.20a	3.80a	0.39a	37.2a	4.53a	4.29a	3.5a	1.25a
Spent grain + poultry manure	6.70b	2.93c	0.31bc	27.3ef	2.52f	3.62b	1.86d	1.20ab
Spent grain + rabbit manure	6.40cd	2.67d	0.28cd	28.6e	2.86d	3.40c	1.65c	0.92bc
Spent grain + turkey manure	6.80b	2.75cd	0.34b	26.3f	2.81de	2.60e	1.68e	0.93bc

Treatment means within each group followed by the same letters are not significantly different from each using Duncan Multiple Range Test at 5% level.

composition and growth parameters of budded rubber by the oil palm bunch ash and cocoa huks blended with turkey, rabbit and poultry manures compared with their sole forms could be explained in term of their better nutrient levels form organically-amended fertilizers than their unamended forms.

The effect of turkey and poultry manure on the growth, leaf and soil chemical composition of budded rubber compared to the spent grain and cocoa husk is consistent with the higher values of soil N, P,K,K, Ca, Mg and O.M of the former than that of the latter. The observation agreed with Folorunso (1999) who reported that the spent grain had lower C, N, Ca, Mg, P, K and Zn content and C/N ratio 1:14 which would make it more resistant to degradation and their nutrients might be made slowly available to plants compared to turkey, poultry and rabbit manures.

The fact that oil palm bunch ash and cococa husk pod increased the soil pH is consistent with the previous finding that the ash contained mainly K, Ca and Mg (Ojeniyi 1995). The NPK fertilizer also reduced the soil pH and this could be a result of acidication associated with continuous use of 300 kg NPK 15-15-15 fertilizer applied per hectare which was a standard practice used for budded rubber in the field. The above statement is consistent with the fact that application of high amount of NPK fertilizer would increase the NH_4^+ cation sorption on the soil surface leading to increased soil acidity (Barber 1962). The phenomenon is also responsible for the reduction in soil O.M. because the soil acidity reduced the activities of microbial organisms, especially aerobic bacteria (inotrosomonas and nitrobacter) which are N fixers cause poor organic matter build up.

CONCLUSION AND RECOMMENDATION

Oil palm bunch ash, cocoa pod husk, turkey, poultry manures, spent grain and rabbit manures are effective sources of nutrients because their additions to the soil have enhanced the leaf and soil N, P,K, Ca, Mg, soil pH and O.M. plant height, leaf area, leaf number and nodes number of budded rubber in the field.

Spent grain was the least effective in promoting budded rubber growth while the amended residues with turkey, poultry and rabbit manures performed well. Oil palm bunch ash + rabbit manure, oil palm bunch ash + poultry manure and cocoa husl + turkey applied at 6t/ha improved nutrient availability and would ensure sustainable cultivation of budded rubber on soils of low fertility in the humid tropics. Thus, they can be used in place of scarce and expensive inorganic fertilizers.

REFERENCES

ADU DAAPH H.K., J. COBBINA and E.O ASARE. 1994. Effect of cocoa pod ash on the the growth of maize. *J. of Agric. Cambridge* **132:** 31-33.

AGBOOLA, A.A. 1974. *Organic Material as Fertilization.* Pp147-152. F.A.O Bull 27, Rome Italy.

AGBOOLA, A.A. and R.B COREY. 1973. Soils testing N,P,K, for maize in the soils derived from metamorphic and igneous rocks of Western State of Nigeria. *Journal of West African Science Association* **17(2):** 93-100.

AGBOOLA, A.A. 1982. Organic manuring and green manuring in tropical agricultural system. In *Proceeding of 12th International Congress of Soil Science,* New Delhi India, p. 198-212, 8-16 February.

BARBER, S.A. 1962. Exchange equilbra, selectivity and effective diffusion coefficient of K in soils. *Soil Science Soc of Amer. J.* p. 39.

CROCKFORD, L. and R. NOWELL. 1956. *Laboratory Manual of Physical Chemistry. Experiments 31 and 32.* p. 58-59. New York: John Wiley and Sons.

FOLORUNOS, O.O, A.A. AGBOOLA and G.O. ADEOYE. 1995. Use of fractional recovery model to calculate potassium needs of some soil series in South Western Nigeria. *Journal of Technical Education, NBTE.* **1&2:** 57-67.

FOLORUNSO, O.O. 1999. Use of plant residues for improving soil fertility and yield of okra (Abelmoschus esculentum L). Ph.D. Thesis, Federal University of Technology, Akure.

JACKSON, M.L. 1958. *Soil Chemical Analysis.* p. 57-67. Englewood Cliffs N.J.: Prentice Hall.

JACKSON, M.L. 1964. *Soil Chemical Analysis.* pp. 86-92. Englewood Cliffs, N.J.: Prentice Hall Inc.

JONES, B.J. and H.V. ECK. 1973. Plant analysis as as aid in fertilizing corn and grain sorghum. In *Soil Testing and Plant Analyses Rev. ed Soil Sci. Soc. Amer,* ed. Walsh and J.D. Beaton, p. 349-364. Madison, Winconsin, USA.

MCLEAN, E.O. 1965. Aluminium. In *Methods of Soil Analyses Part 2,* ed. M.C.A. Black, MCA Black, p. 927-932.

MURPHY, J. and J.P. RILEY. 1962. A modified single solution method for determination of phosphate in natural waters. *Analytical Glumi – Acta.* **27**: 31-36.

OBATOLU, C.O. 1989. Complementary user of organic manures and inorganic fertiizers for cocoa and rubber production. In *1st National Conference on Organic Fertilizer Utilization,* p. 15-19, held I Kaduna, Nigeria.

OJENIYI, S.O. 1995. That our soil may not die. 10th inaugural lecture, p. 3-5, March 23, federal University of Technology, Akure.

OLADOKUN, M.A.O. 1986. Use of cocoa pod husk as fertilizer for maize production. *Nigeria Journal of Agronomy* **1(3)**: 103-109.

TITILAYO, E.O. 1982. The evaluation of various types of organic wastes as sources of nutrient for growth and yield maize. Ph.D Thesis, University of Ibadan, Ibadan Nigeria p. 70-80.

UMOTI V. 1990. Complimentary use of organic and inorganic fertilizers for tree and forest crop production in Nigeria. *3rd Annual Organic Fertilizer Conference,* p. 163-175, held in Durbar Hotel, Kaduna.

WALKEY, A. and I.A BLACK. 1934. An examination of degtajaroff method for determining soil organic acid filtration method. *Soil Sci.* **37:**29-38.

(Received: 19 March 2002)
(Accepted: 14 September 2004)

Pertanika J. Trop. Agric. Sci. 27(2): 101 - 105 (2004) ISSN: 1511-3701

Effects of Vaccination on the Prevalence of Peste Des Petits Ruminants (PPR) in Small Ruminants in Taraba State, Nigeria

*H.M MAI, [2]I. SAIDU, [3]O.L OBASI & [4]M.A. ILIYASU
[1]*Animal Production Programme, School of Agriculture,*
Abubakar Tafawa Balewa University, P.M.B. 0248 Bauchi, Bauchi State, Nigeria
[2]*Ministry of Agriculture and Rural Development,*
Veterinary Department, Bauchi, Nigeria
[3]*Faculty of Agriculture, University of Uyo Akwa-Ibom State, Nigeria*
[4]*Ministry of Agriculture and Rural Development,*
Veterinary Department, Taraba State, Nigeria

Keywords: Vaccination, Peste Des Petits Ruminants, small ruminants, Taraba State

ABSTRAK

Satu kajian telah dijalankan untuk menentukan taburan Peste Des Petits Ruminants (PPR) dan usaha pemvaksinan di Negeri Taraba, Nigeria menggunakan data yang dikumpulkan daripada Jabatan Perkhidmatan Veterinar di Kementerian Pertanian dan Pembangunan Luar Bandar Negeri antara tahun 1992 dan 1998. Keputusan menunjukkan bahawa penyakit tersebut adalah paling tersebar luas semasa bulan-bulan yang sejuk pada tahun tersebut (Hamattan) dan permulaan musim hujan. Begitu juga, tercetusnya penyakit tersebut yang meningkat kerana pengenduran program kempen pemvaksinan. Diperhatikan bahawa jumlah tercetusnya penyakit tersebut adalah rendah apabila pemvaksinan menggunakan Tissue-Culture-Rinderpest Vaccine (TCRV) yang diperkuatkan dan ia meningkat apabila pemvaksinan dikendurkan. Ia boleh disimpulkan daripada kajian ini bahawa kempen pemvaksinan intensif ruminan kecil terhadap PPR melalui peruntukan fasiliti secukupnya, vaksin TCRV, melatih pekerja luar dan kempen kesedaran massa di kampung-kampung adalah paling utama untuk mengawal ancaman penyakit ini di Nigeria.

ABSTRACT

An investigation was conducted in order to determine the distribution of Peste Des Petits Ruminants (PPR) and vaccination efforts in Taraba State of Nigeria using data collected from the Veterinary Services Department of the State's Ministry of Agriculture and Rural Development between 1992 and 1998. The results showed that the disease is most prevalent during the cold months of the year (Hamattan) and beginning of the rainy season. Similarly, outbreaks increased with the relaxation of vaccination campaign programmes. It was observed that the number of outbreak was low when a vaccination using Tissue-Culture-Rinderpest Vaccine (TCRV) was intensified and it increases when the vaccination was relaxed. It was concluded from this study that intensive vaccination campaign of small ruminants against the PPR through provision of adequate facilities, TCRV vaccines, training of field workers and mass enlightenment campaign in the villages are paramount to control menace of the disease in Nigeria.

INTRODUCTION

Peste Des Petits ruminants (PPR) is an acute and highly contagious rinderpest – like viral disease of small ruminants, especially sheep and goats. It causes higher morbidity and mortality in goats than in sheep. The disease is characterized by fever, pneumonia, occulonasal discharge, anorexia, necrotizing erosive stomatitis, enteritis and diarrhea (Nawathe and Taylor 1979).

The PPR is caused by a Morbillivirus which has been shown to be similar in ultrastructure to the rinderpest, canine distemper and measles viruses (Gibs *et al.* 1979). The virus has been classified as the 4th member of the Morbillivirus

* Corresponding Author
E-mail: *hassanmai@hotmail.com*

genus of the paramyxoviridae family (Abdulkadir 1989).

In Nigeria, Whitney *et al.* (1967) described the disease clinically in affected Nigerian goats under the name 'Kata". Since its recognition in Nigeria, PPR has continued to be a major disease of small ruminants causing sporadic outbreaks among susceptible sheep and goats. During the course of an epidemic, the villages are known to lose their entire populations of small ruminants. In Nigeria, over one hundred outbreaks are reported annually and many more go unreported (Nawathe 1984).

Hamdy *et al.* (1976) considered PPR to the responsible for losses to the tune of $1.5 million, and by now, with rising inflation, it could well be over $10.0 million annually. PPR therefore, is an economically significant disease considering the fact that it affects small ruminants, which are found practically in almost every household in Nigeria. These small ruminants are found to contribute significantly to be income of rural dwellers and the protein consumption in the country providing about 46% of the quantity of meat consumed in the country (Emmanuel 1980). This therefore, explains why control of PPR should be intensified by governments or voluntary organizations.

Attempts made so far in the control of the disease in Nigeria is by vaccination using the Tissue-Culture-Rinderpest-Vaccine (TCRV) which has been found to afford solid protection against PPR for over one year (Nawathe 1984).

The aim of the study therefore, was to determine the prevalence of PPR in Taraba State of Nigeria vis-á- vis the control measures using TCRV and make possible recommendations on how to control the disease.

MATERIALS AND METHODS

Data were collected from the Veterinary Department of the State's Ministry of Agriculture and Rural Development on PPR outbreaks and vaccination figures in Taraba State for a period of seven years, spanning from 1992 to 1998.

The vaccine used was Tissue-Culture-Rinderpest-Vaccine (TCRV), a heterelogous live attenuated viral vaccine prepared from the National Veterinary Research Institute (NVRI), Vom, Plateau State, Nigeria in which half the large ruminant dose was administered to the small ruminants which confers a solid immunity against PPR for at least one year. Therefore, the vaccination exercise was repeated annually.

The prevalence of the disease and specifically the relationships between vaccination campaign and outbreak of PPR in the State were determined.

Similarly, monthly variation for the reported outbreaks was determined using the Ratio-to-Moving average method or Time Series Analysis in which the data was recorded month by month for the entire period of the study i.e from January 1992 to December 1992, then January of the next year, 1993 to December, 1993 to so on up to 1998. A centered average was determined for all and each month, the averages were computed such that the data was represented using 12 months. The data was therefore summarized to be represented by 12 months and extraneous variations were eliminated, such that against each month is the seasonal index for that month. It was the seasonal index that was plotted against the months (x-axis) to obtain a plot that shows seasonal index of the PPR.

RESULTS

The result showed that the number of outbreaks increased only from 1993-1995. Thereafter, the outbreak number was on the decline (*Fig. 1*).

An increase in the number of animals vaccinated was also recorded in 1993, 1994 and 1997. The period from 1995 to 1998 was characterized by a fluctuation in the number of animals vaccinated. While the number of animals vaccinated was high in 1995, it was low in 1996 and increased again in 1997 with another decrease in 1998.

The relationship between the vaccination figures and the number of outbreak is shown in *Fig. 1.* When the vaccination campaign was intensified in 1994, the number of outbreaks was low. In 1995, the number of outbreaks increased as the vaccination figures went down. From 1995 to 1997, the outbreak was in continuous decline whereas 1998 witnessed a slight increase in the number of outbreaks.

A summary of monthly outbreaks of PPR from 1992 to 1998 revealed that most of the outbreaks occurred in January, March, April and May (*Fig. 2*). A similar result was observed using the seasonal index (*Fig. 3*).

DISCUSSION

Results of vaccination campaigns and the outbreak observed in this study further strengthened the assertion that intensified efforts towards effective

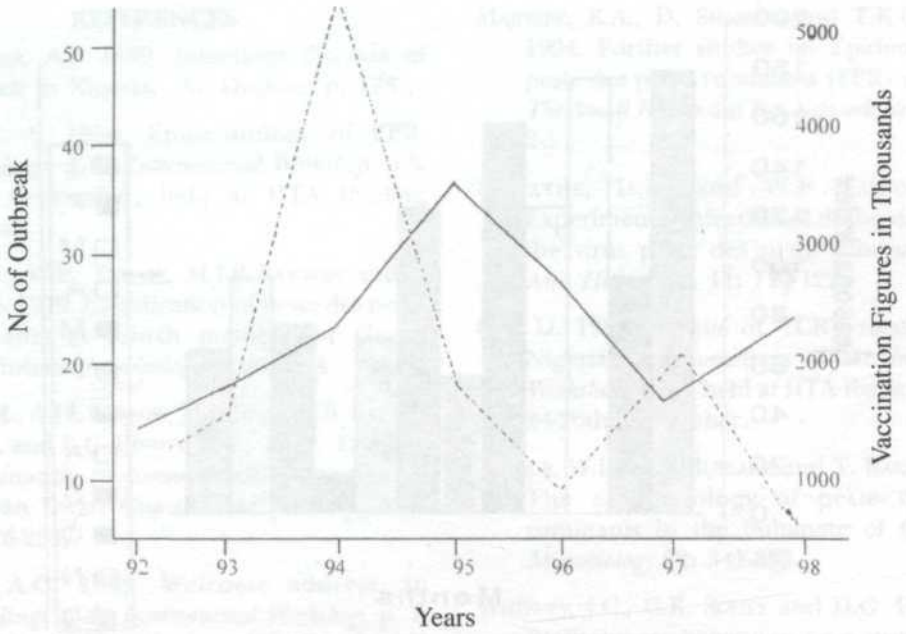

Fig. 1: Relationship between vaccination figures and outbreak of PPR in small ruminants in Taraba State, Nigeria

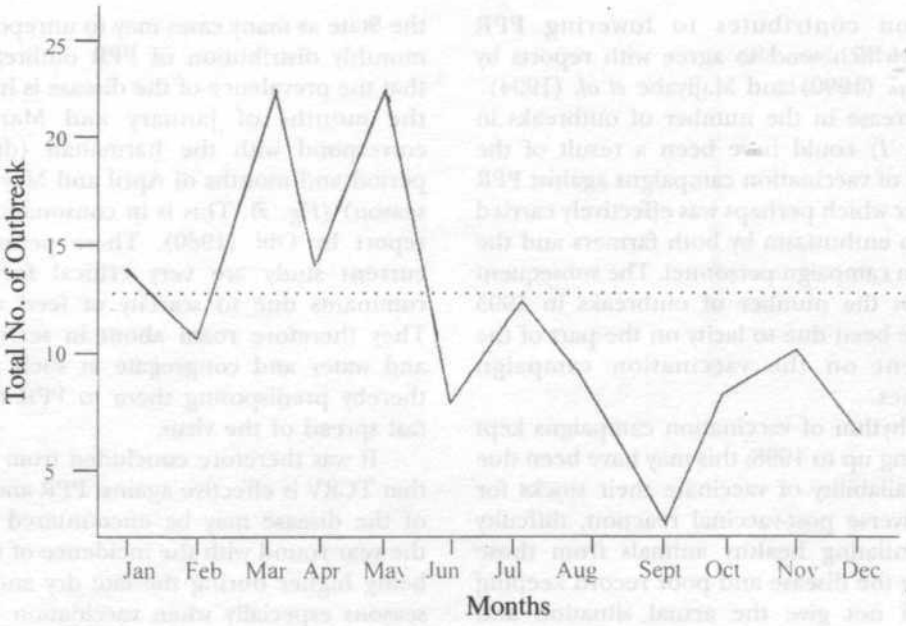

Fig. 2: Monthly distribution of PPR outbreaks in small ruminants in Taraba State of Nigeria (1992-1998)

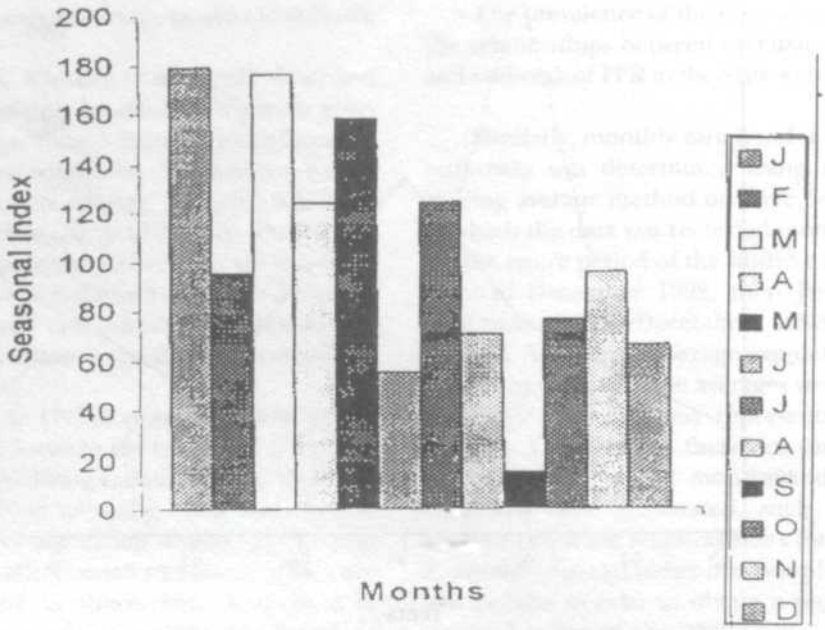

Fig. 3: Seasonal index of PPR in small ruminants in Taraba State of Nigeria from 1992 - 1998

vaccination contributes to lowering PPR outbreaks which tend to agree with reports by Taylor *et al.* (1990) and Majiyabe *et al.* (1994).

A decrease in the number of outbreaks in 1994 (*Fig. 1*) could have been a result of the launching of vaccination campaigns against PPR in that year which perhaps was effectively carried out due to enthusiasm by both farmers and the vaccination campaign personnel. The subsequent increase in the number of outbreaks in 1995 could have been due to lacity on the part of the government on the vaccination campaign programmes.

The rhythm of vaccination campaigns kept on declining up to 1998; this may have been due to non availability of vaccinate their stocks for fear of adverse post-vaccinal reaction, diffculty in differentiating healthy animals from those incubating the disease and poor record keeping which will not give the actual situation and information on the disease and vaccination campaign programme. Such outbreaks could result in economic losses in Nigeria to the tune of over one million naira (N1,000,000) annually (Lamorde 1980); with the current rise in inflation the losses could even exceed this figure.

It is possible that outbreaks recorded in the State not have reflected the actual situation of the State as many cases may to unreported. The monthly distribution of PPR outbreak showed that the prevalence of the disease is high during the months of January and March which correspond with the harmattan (dry season) period and months of April and May (pre-rainy season) (*Fig. 3*). This is in consonance with the report by Obi (1980). These periods in the current study are very critical for the mall ruminants due to scarcity of feed and water. They therefore roam about in search of feed and water and congregate at such few points thereby predisposing them to PPR due to the fast spread of the virus.

It was therefore concluded from this study, that TCRV is effective against PPR and outbreak of the disease may be encountered almost all the year round with the incidence of the disease being higher during the late dry and pre-rainy seasons especially when vaccination exercise is relaxed. Hence it is essential to employ effective and efficient control measures by seeking both national and international co-ordination and commencing the exercise prior to seasonal outbreak as it is evident that the vaccination is dwindling in Nigeria.

REFERENCES

ABDUL KADIR, A.I. 1989. Infectious diseases of livestock in Nigeria. An Outline. p. 173

EMMANUEL, A. 1980. Epidemiology of PPR. *Proceddings of the International Workshop*, p.3, 24-26 September, held at IITA Ibadan, Nigeria.

GIBS, E.P.J., W.P. TAYLOR, M.J.P LAWMAN and J. BRYANT. 1979. Classification of peste des petits ruminants as fourth member of Genus Morbilivirus. *Intevirology* **2:** 268-274.

HAMDY, F.M., A.H. DARDIRI, D.J. NDAAKA, S. BREESE, S.S Jr., and E.C. IHEMELANDU. 1976. Etiology of stomatitis pneumoenteritis complex in Nigerian Dwarf Goats. *Can. J. Comp. Med.* **40:** 276-284.

LAMORDE, A.G. 1980. Welcome address. In *Proceedings of the International Workshop*, p. 1-2. held at IITA Ibadan, Nigeria. 24-26th September.

MAJIYABE, K.A., D. SHAMAKI and T.K.C. UDEANI. 1994. Further studies on Epidemiology of peste des petits ruminants (PPR) in Nigeria. *The Small Ruminant Res Network Newsletter* **29**: 2-5.

NAWATHE, D.R. and W.P TAYLOR. 1979. Experimental infection of domestic pigs with the virus peste des petiti ruminants. *Trop. Ani. Hlth. Prod.* **11:** 120-122.

OBI, U. 1980. Results of TCRV vaccination in Nigeria. In *Proceedings of the International Workshop*, p. 82. held at IITA Ibadan, Nigeria. 24-26th September.

TAYLOR, W.P., S. AlBUSSAIDI and T. BARRET. 1990. The epidemiology of peste des petits ruminants in the Sultanate of Oman. *Vet Microbiology* **22:** 341-352.

WHITNEY, J.C., G.R. SCOTT and D.G. HILL. 1967. Preliminary observation on a stomatitis and enteritis of goats in southern Nigeria. *Bull Epizoot. Dis. Afr.* **15:** 31-41.

(Received: 25 March 2002)
(Accepted: 7 June 2004)

Pertanika J. Trop. Agric. Sci. 27(2): 107 - 112 (2004)
ISSN: 1511-3701

The Effect of Feeding Soyabeans Treated with Different Alkaline Salts on the Protein and Energy Utilisation by Starter Boilers

B.A AYANWALE
Department of Animal Production,
Federal University of Technology,
Mina. Nigeria

Keywords: Treated soyabeans, broilers, alkaline, salts, protein and energy

ABSTRAK

Satu eksperimen telah dijalankan untuk menguji kesan efisyen susu kacang soya beralkali terhadap protein kepada ayam pedaging. Benih soya direndam selama 24 jam dalam sebatian akueus sodium klorida (3% kelikatan), trona dan alum, dikeringkan, dikisar dan digunakan bagi melakukan rawatan pokok, kacang soya yang dirawat dengan sodium klorida (T2), trona (T3) dan alum (T4), dengan kacang soya yang dibakar sebagai kawalan (T1). Keputusan menunjukkan kacang soya yang direndam dalam sebatian garam alkali telah mengurangkan sedikit DM, CF, NFE and GE manakala EE dan abu mencatatkan kadar lebih rendah dalam kacang soya yang dibakar. Nilai PER untuk T1 dan T4 adalah sama dan lebih tinggi, manakala efisyen tanaga bertambah dalam T3 dan T4. Pada peringkat akhir fasa pertama, berat badan dan penambahan berat ayam pedaging yang mana ia dirawat dengan NaCI adalah lebih lemah berbanding dengan kumpulan lain, ini diakibatkan oleh jumlah pemakanan yang rendah ($p<0.05$) dan efisien pemakanan lemah ($p<0.05$). Ayam pedaging diberi makan kacang soya yang dirawat oleh trona dan alum mempunyai kadar efisyen tenaga yang lebih tinggi berbanding yang diberi dengan rawatan NaCI dan kawalan diet. Ia boleh disimpulkan bahawa mana-mana dari tiga garam alkali tersebut boleh digunakan untuk proses kacang soya untuk pemakanan ayam pedaging tetapi untuk tenaga yang lebih baik dan efisyen protein, penggunaan alum dan trona adalah lebih elok.

ABSTRACT

An experiment was conducted to evaluate the effect of feeding alkaline treated soyabeans to broilers on protein and energy efficiency of the starter boilers. Soyabean seeds were soaked in aqueous solution (3% concentration) of sodium chloride, trona, and alum respectively for 24 hours, air-dried, ground and used in compounding the three treatments, sodium chloride treated soyabeans (T2), trona (T3), and alum (T4), with roasted soyabeans as the control (T1). The results indicated that soaking soyabeans in alkaline salt solution slightly reduced DM, CF, NFE and GE while EE and Ash were lower in the roasted soyabean seeds. The PER values for T1 and T4 were similar and higher values while energy efficiency was improved in T3 and T4. At the end of the starter phase, the body weights and body weight gains of the broiler on diets in which soyabeans were treated with NaCI were poorer even compared to the other groups, which was partly attributed to the lower feed intake ($p<0.05$) and poor feed efficiency ($p<0.05$) of the group. The broilers fed trona and alum treated soyabeans had better energy efficiency ratio than the ones fed the NaCI treated soyabean and the control diets. It was concluded that any of the three alkaline salts could be used to process soyabeans for broiler consumption but for better energy and protein efficiency the utilization of trona and alum were preferable.

INTRODUCTION

In countries like Nigeria, the use of raw or whole soyabeans as a source of plant protein in animal diets was on the increase. However, raw soyabeans need to be processed before their incorporation into the animal diets in order to remove anti-nutritional factors including polyphenols and typsin inhibitors. It is also desirable to reduce the high levels of oligosaccharides, notably raffinose and stahyose, which cause flatulence and abdominal discomfort in animals. Soaking in water and boiling are the methods commonly in use (Fanimo 1996). Excessive soaking under tropical conditions can lead to serious microbial deterioration while

boiling uses firewood or other fuel, which may be scarce and expensive. This creates the obvious need for seeking alternative methods of processing soyabeans.

The addition of alkaline salts such as sodium bicarbonate has been shown to reduce soaking and cooking time (Singh *et al.* 1988). Polyphenols were removed by soaking legumes in water and sodium bicarbonate, which makes the process efficient (Laurena *et al.* 1986). Omueti *et al.* (1992) reported that soaking and blanching of soyabeans is an effective way of inactivating trypsin inhibitors and removing a significant proportion of polyphenols and oligosaccharies. Nelson *et al.* (1976) stated that removal of trypsin inhibitor by blanching was made more effective by addition of sodium bicarbonate. Ayanwale (1999) also reported that sodium sesquicarbonate (trona) can be used without detrimental effect on broiler performance and carcass quality. Consequently, this work was designed to evaluate the effect of sodium chloride, trona and alum, which are equally cheap and readily available, on the protein and energy utilization, by starter broilers.

MATERIALS AND METHODS

Processing of the Soyabeans

The different alkaline salt solutions, sodium chloride (NaCl), sodium sesquicarbonate (trona) ($Na_2CO_4NaHCo_3.H_2O$) and alum (Al_2 (So_4) $.24H_2O$) were prepared by adding 30g of each alkaline salts to 1,000 milliliters of water at room temperature. The raw soyabean seeds were then soaked in the prepared 3% solutions of the salts for 24 hrs, drained, air-dried, ground and used in preparing the four isocaloric and isonitrogenous diets (Table 1). The crude protein level of the diets is 24.16% while energy level is 3.18kcal/g.

Soaking was done in such a way that the soyabeans were always completely covered with the alkaline solution. This was ensured by checking at regular intervals. Four broiler starter diets were formulated and designated T1, T2, T3, and T4, respectively. Diet T1 was the control, which contained roasted soyabeans while diets T2, T3, and T4 contained sodium chloride, trona and alum-processed soyabeans respectively. (Table 1) Roasting of the soyabeans was done by autoclaving at 100 C for 30 minutes as described by Ewing ('963) since Kratzer (1990) reported that heating at 130 C for 60 minutes destroyeds or renders unavailable several essential amino acids.

Analytical Procedure

The proximate composition of the processed samples as shown in Table 2 was determined according to the Official Methods (A.O.A.C.,

TABLE 1
Composition of the starter diets T_1-T_4

Ingredients	(%)
Maize	50.23
Rice Offal	5.00
Fish Meal	5.00
Palm Oil	2.50
Bone Meal	2.50
Salt	0.30
Lysine	0.20
Methionine	0.20
Premix	0.25
Soyabeans	33.82
Total	100.00
Chemical composition	
Energy Kcal/g %	3.18
Crude protein %	24.16
Crude fibre %	3.95
Ether Extract %	4.40

a: to provide the following per kg diet: vitamin A 1500iμ; vitamin D3, 1600iμ; riboflavin 9.0mg; biotin 0.25mg; pantothenic acid 1.10mg; vitamin K 3.0mg; vitamin B_2 2.5mg; vitamin B_6 0.3mg; vitamin B_{12} 8.0mg, nicotinic acid 8.0mg; Fe 5.0mg; zn 4.5mg; Mn 10.0mg; Co 0.02mg and Se 0.01mg.b: T1-Roasted soyabeans; T2-soyabeans soaked in NaCl; T3-soyabeans soaked in trona; T4-soyabeans soaked in trona.

1990) and gross energy by Gallen Kamp oxygen calorimeter (Miller and Payne 1959).

Biological Evaluation

One hundred and eighty day-old Sussex broiler chicks of equal male to female ratio (1:1) were used for this work. They were randomly allocated in the diets at forty-five birds per diet and replicated in three groups of fifteen birds each. All experimental birds were given feed and water *ad-libitum*. Records of average growth rate and feed consumption were taken over a 28-d period from which values for average live body weight, gain, feed to gain ratio, protein efficiency ratio (PER) was calculated as energy intake per unit body weight gain (Ayanwale and Ogunmodede 1999) The birds were raised on a deep litter system. Heat was provided with 60 watts bulb supplemented with charcoal pot for brooding.

Determination of Nitrogen Utilization

At 3 weeks, two birds from each replicate were randomly selected and transferred to metabolic cages. They were allowed 7 days adjustment period. Weighed quantities of feed were supplied and droppings collected over a 72-hr period using total collection method (Longe 1980). Faecal samples were dried at 80°C, weighed and ground prior to chemical analysis. The faecal samples were collected daily, bulked for each replicate, weighed, dried and stored. From the data on nitrogen intake and excretion, the proportion of nitrogen was calculated.

Statistical Analysis

The experimental design was randomized complete block (RCB) design using the Replicates as the blocks. The statistical analysis was done according to Gomez and Gomez (1984) and mean separation by Duncan multiple range test (Duncan 1955).

RESULTS AND DISCUSSION

The proximate composition of the soyabean seeds processed by roasting and with sodium chloride, trona, and alum is shown in Table 2. The results indicated that processing of soyabeans by soaking in different alkaline solutions reduced the dry matter (DM), crude protein (CP) and crude fibre (CF) contents of the seeds. This is in agreement with the reports of Ku. *et al.* (1976) who attributed such reductions to the increased solubility of soyabean proteins at the alkaline pH of the salts resulting in increased leaching of the proteins into solution. However, the relatively lower ether extract content of the control (T1) could only be attributed to the higher temperature (100°C) at which the soyabean seeds were roasted compared to the soaked beans. Similarly, a decrease in fibre content due to (NaOH) alkali treatment of farm residue was ascribed almost entirely to a reduction in hemicellulose content of the residues (Moss *et al.* 1990). The nitrogen free extract (NFE) and gross energy (GE) were slightly higher in the roasted soyabeans than the treated ones due possibly to the solubility of parts of the nutrients in the alkaline salts. The lowest ash value was found in the control diet ($p<0.05$). This reflects the uptake of minerals from the solution. A similar observation on the uptake of inorganic minerals of cocoa pod husk resulting from alkali treatment (NaOH) was made by Sobamiwa and Longe (1994). Also the chelated minerals in the legume could be released into the solution leading to greater uptake as explained by Ayanwale (1999).

TABLE 2
Proximate composition of the soyabean seeds processed with different alkaline salts (%)

Parameters	T_1	T_2	T_3	T_4
Dry Matter	93.00	92.00	92.20	92.00
Crude Protein	43.82	43.7	43.45	43.65
Ether Extract	18.00	18.92	18.84	18.88
Crude Fibre	5.87	4.86	4.93	4.91
Ash	3.16	3.98	3.96	3.97
NFE	22.15	20.54	21.02	20.59
GE (Kcal/g)	6.00	5.68	5.80	5.69

NFE is Nitrogen Free Extract, GE is Gross Energy
T_1-Roasted soyabeans; T_2-soyabeans soaked in NaCl; T_3-soyabeans soaked in trona; T_4-soyabeans soked in alum.

Table 3 shows the performance characteristics of the broilers fed the alkali-treated soyabeans and the control soyabeans. The birds fed sodium chloride-treated soyabeans had significantly lower ($p>0.05$) body weight compared to the trona-fed diets. The weights of birds on the control diet were similar to the weights of those fed trona and alum diets. The weight gains of the broilers also followed the same pattern as the body weights. The observed reductions in the body weights and weight gains could be attributed to the reductions in the feed intake which is significantly lower ($p<0.05$) in the NaCl-treated soyabean-based diets. Although the feed consumption of NaCl-based diet and alum-based diets were similar, feed efficiency (feed/gain ratio) of the alum based diets were better. This was attributed to the differences in the composition of the two alkaline salts. Alum would have been more effective in the removal of the anti-nutritional factors of soyabeans than the sodium chloride since stronger alkaline salts are reported to be more effective in this aspect (Omueti *et al.* 1992).

The results presented in Table 4 show that nitrogen intake, nitrogen output and nitrogen retained in grams per day were not significantly ($p>0.05$) different for all the broiler groups. However, efficiency of protein utilization was better in trona and alum treated diets than in the control. This might be due to higher protein available to the broilers for utilization due to the destructive effects of the alkaline salts on the trypsin inhibitors of the soyabean seeds. The results of the in vitro trona treatment of soyabeans flour showed that trona destroyed the trypsin inhibitors present in raw soyabeans (Omueti *et al.* 1992).

The energy efficiency results shown in Table 5 indicate that the energy of the control diet (T1) was poorly utilized by the broilers as compared to those of the other diets. These observations could be due to the release of the inorganic mineral elements of the treated soyabeans. Some of the minerals when available serve as co-enzymes and co-factors of enzyme systems involved in both protein and energy metabolism (Lloyd *et al.* 1978; Church and Pond 1988) and their levels in the diets affect feed utilization. Although the formation of lysinoalanine has been reported for soyabeans soaked in aqueous NaOH (DeGroot and Slump 1969) the authors also remarked that feeding mildly treated soyabeans to broilers did not

TABLE 3
Performance characteristics of broilers fed alkali-treated soyabeans for 28 days

Parameters	T_1	T_2	T_3	T_4
Initial live weight (g/bird)	32.50	32.51	32.53	32.52
Final live weight (g/bird)	5400.00^{a}18.92	400.10^{a}23.90	484.53^{a}27.20	511.49^{a}29.92
Body weight gain (g/bird)	507.50^{a}4.11	367.59^{b}5.69	452.00^{b}3.57	478.97^{a}6.22
Feed intake (g/bird)	989.00^{a}3.60	921.66^{b}2.10	993.00^{a}2.40	952.97^{a}b2.40
Feed/gain ratio	1.95^{b}0.08	2.51^{a}0.26	2.20^{a}0.33	1.99^{a}0.07

Means denoted by the same letter in the same row are not significantly ($p>0.05$) different.
T1 is roasted soyabean diet; T2-NaCl treated soyabean diet; T3 trona treated soyabean diet; T4-alum-treated soyabean diet.

TABLE 4
Nitrogen utilization by broilers fed the experimental diets

Parameters	T_1	T_2	T_3	T_4	SEM
Nitrogen intake (g/bird/day)	3.41	3.36	3.39	3.40	0.06
Nitrogen output (g/bird/day)	1.15	1.12	1.13	1.14	0.20
Nitrogen retained (g/bird/day)	2.26	2.24	2.26	2.26	0.11
Nitrogen retention (%)	66.28	66.67	66.49	66.49	1.54
Protein Efficiency ratio	2.12a	1.83b	2.09ab	2.14a	0.12

T_1 is Roasted soyabean diet; T_2 NaCl-treated soyabean diet; T_3 trona treated soyabean diet; T_4 alum treated soyabean diet.

TABLE 5
Energy efficiency of broiler fed soyabeans treated with different alkaline salts

Parameters	T_1	T_2	T_3	T_4	SEM
Dry matter intake (g/day)	32.85	31.28	32.63	31.31	1.22
Energy intake (Kcal/day)	116.12	90.40	96.42	94.89	4.83
Live weight gain (g/bird	16.85	13.84	16.44	16.21	0.32
Energy efficiency	7.00a	6.46ab	5.86a	5.85b	0.17

Means detonated by the same letter in the same row are not significantly ($p>0.05$) different.
SEM is the standard error of mean
T1 is roasted soyabean diet; T2 is NaCl-treated soyabean diet; T3-trona-treated soyabean diet; T4-alum-treated soyabean diet.

produce any adverse effect in the birds, which is true of the diets used in this work. The results of the present work agree with the findings of Lauren *et al.* (1969) and Nelson *et al.* (1976) that soaking of legumes in alkaline salts removed polyphenols and destroyed trypsin inhibitors.

CONCLUSION

All the alkaline-treated soyabeans supported the growth of the broilers since all the birds were raised to the end of the starter phase. However, on the bases of body weight gain, feed consumption and PER similar optimal results were obtained in broiler fed roasted soyabeans and the ones fed alum-treated soyabeans more than those of the roasted ones. So, alum at 3% concentration could be recommended for treating soyabeans for starter broilers.

REFERENCES

Association Of Official Analytical Chemists (AOAC). 1990. *Official Methods of Analysis*, 15th edn. Washington D.C.

Ayanwale, B.A. 1999. Performance and carcass characteristics of broiler chickens fed sodium sesquicarbonate processed soyabean diets. *Tropical Journal of Animal Science* **2:** 85-89.

Ayanwale, B.A and B.K. Ogunmodede. 1999. Growth and energy efficiency of broilers fed different commercial premixes at two developmental stages. *Nigerian Journal of Animal Production* **26:** 43-47.

Church, D.C. and W.G. Pond. 1988. *Basic Animal Nutrition and Feeding.* 3rd edn. U.S.A: John Wiley and Sons.

Degroot, A.P. and P.I. Slump. 1969. Effects of severe alkali treatment of proteins on amino acid composition and nutritive value. *Journal of Nutrition* **98:** 45.

Duncan, D.B. 1955. Multiple range and multiple F-tests. *Biometrics* **11:** 1-42.

Ewing, W.R. 1963. *Poultry Nutrition.* 5th edition. p. 498-502. California: Ray Ewing Co. Div. Hoffman La Roche Inc.

Fanimo, A.O. 1996. Nutritional evaluation of full-fat soyabeans boiled for three time periods. *Pertanika Journal of Tropical Agricultural Science* **19:** 175-182.

Gomez, K.A. and A.A. Gomez. 1984. *Statistical Procedures for Agricultural Research.* 2nd edition. New York: John Wiley & Sons.

Kratzer, F.H., S.V.P. Bersh and R.E Ernst. 1990. Chemical and biological evaluation of soyabean flakes autoclaved at different temperatures for different durations. In *Proceedings of the Aquaculture Feed Processing and Nutrition Workshop*, ed. D.M. Akinyama and K.H. Ronnie (1991), p. 184-241. Thailand and Indonesia.

Ku, S.L.S. Weis, M.P. Steinberg, A.I. Nelson and T. Hymowitz. 1976. Extraction of oligosaccharides during cooking of whole soyabeans. *Journal of Food Science* **41:** 361-364.

Laurena, A.C.O., V.V. Gracia and E.M.T. Mendoza. 1969. Effect of soaking in aqueous, acid, and alkali solutions on removal of polyphenols and *in vitro* digestibility of cowpea. *Qual. Plant Foods and Human Nutrition* **36:** 107-118.

Longe, G.O. 1980. Effect of processing on the chemical composition and energy value of cassava. *Nutri. Rep.* **21:** 819-828.

LLOYD, L.E., M.C. DONALD and E.W. CRAMPTON. 1978. *Fundamentals of Nutrition.* 2nd edn. San Francisco: Freeman Coy.

MILLER, D.S. and P.R. PAYNE. 1959. A ballistic bomb calorimeter. *British Journal of Nutrition* **13:** 501-508.

NELSON, A.I., M.P. STEINBERG and L.S. WEI. 1976. Illinois process for preparation of soya milk. *Journal Food Science* **41:** 57-61.

MOSS, A.R., D.I. GIVENS and J.M. EVERYINGTOM. 1990. The effect of sodium hydroxide treatment on the chemical composition, digestibility and digestible energy content of wheat, barley and oat straws. *Animal Feed Science Technology* **29:** 73-87.

OMUETI, O.I.D. MORTON and P.W. EMERY. 1992. Nutrition characteristics of soyabean seed floor after processing with sodium bicarbonate or trona. *Journal Food Science and Technology* **43:** 121-180.

SINGH, K. B.W. BERSKINE, L.D. ROBERTSON, NAKKOUL and P.C. WILLIAMS. 1988. Influence of pre-treatment on cooking quality parameters of dry food legumes. *Journal of Feed and Agriculture* **44:** 135-142.

SOBMIWA, O. and O.G. LONGE. 1994. The nutritive value of alkali-treated cocoa husk meal in broiler chick diet. *Animal Feed Science Technology* **46:** 32-330.

STEEL, R.G.D. and J.H. TORRIE. 1981. *Principles and Procedures of Statistics-A Biometrical Approach.* 2nd edn. New York: McGraw-Hill.

DEGROOT, A.P. and P. SLUMP. 1969. Effects of severe alkali treatment of proteins on amino acid composition and nutritive value. *Journal of Nutrition* **98:** 45.

(Received: 26 June 2002)
(Accepted: 31 March 2004)

Pertanika J. Trop. Agric. Sci. 27(2): 113 - 120 (2004) ISSN: 1511-3701

A Study of Weed Populations and Their Buried Seeds in the Soil of MARDI Research Station and at Farmers' Rice Fields in Sungai Burung, Tanjung Karang, Selangor, Malaysia

ISMAIL B. S. & K. PHAIK-HONG
School of Environmental and Natural Resource Sciences,
Universiti Kebangsaan Malaysia
43600 UKM, Bangi, Selangor Darul Ehsan, Malaysia

Keywords: Weed flora composition, transplanted rice field, direct seeding, seedbank

ABSTRAK

Kajian populasi rumpai dan biji yang tertanam sebelum dan selepas penuaian telah dilakukan di kawasan sawah di Stesyen Penyelidikan MARDI dan Sungai Burung, Selangor. Hasil menunjukkan bahawa rumpai berdaun lebar sangat dominan di kawasan sawah di Stesyen MARDI dan Sungai Burung. Dua spesies iaitu Cyperus difformis (rusiga) dan Najas graminae *(daun lebar), masing-masing merupakan spesies dominan di kedua-dua kawasan tersebut. Populasi biji rumpai tidak berbeza di antara kedua-dua kawasan tersebut. Bagaimanapun, biji rumpai yang dikira sebelum dan selepas penuaian menunjukkan perbezaan bilangannya. Pada umumnya bilangan biji yang tertinggi di kedua-dua kawasan sawah tersebut dikesan pada kedalaman 0-5 cm.*

ABSTRACT

A study of weed populations and their buried seeds before and after harvesting was carried out at the MARDI Research Station and at Farmers' Rice Fields, Sungai Burung in Tanjung Karang, Selangor. The results showed that the broadleaf weeds were the most dominant in the MARDI and Sungai Burung rice fields. Two species, namely Cyperus difformis *(sedge) and* Najas graminae *(broadleaf) were the most dominant species in the MARDI and Sungai Burung rice fields respectively. The weed seed populations were similar in both rice fields. However, the weed seed counts before and after harvesting were different. In general, it was also found that the highest number of weed seeds in the soil was detected in the 0-5 cm layer of soil in both rice fields.*

INTRODUCTION

Weed infestation is one of the most problematic and troublesome scenarios in rice cultivation. Factors such as cultivation practice, land preparation, soil moisture content and management strategies greatly affect the presence and abundance of weeds in rice fields (De Datta 1981). Previous studies have shown that weeds found in direct seeded rice fields were more complicated than those in transplanted rice fields (Teerawatsakul 1976; Tengku Zunaidah 1996). As reported, weed composition and dominance were affected by water management and soil preparation. Rice field soils, which are flattened and submerged in water in the early stage of land preparation coupled with proper tillage, could reduce the growth of weeds (Khalid 1988; Yamada 1965). Noda (1977) reported that weeds in transplanted and direct seeded rice fields caused rice yield losses of 16 and 62%, respectively.

Weed problems begin with weed seeds in the soil and they continuously create problems even though attempts are made to prevent them from going to seed in the field (Wilson *et al.* 1985). Thus, knowledge of the total number and type of buried seeds is very useful in predicting which species are likely to emerge in a particular field (Lawson 1988). Reports on weeds from rice fields in Tanjung Karang are very limited. Recently, it was reported that *Limnophila erecta* and *Bacopa rotundifolia* were the newly detected dominant weeds in Sekinchan (Azmi and Baki 2003). Experiments were therefore conducted to determine the weed flora composition and seed population in wet-seeded rice fields in Tanjung Karang, Selangor.

MATERIALS AND METHODS

The Study Area

The study was conducted at the Research Station of MARDI and farmers' rice fields in Sungai Burung, Tanjung Karang, Selangor. The MARDI and Sungai Burung areas have 9.6 and 2,000 ha of rice fields respectively. Farmers in MARDI practise the wet direct seeding method in the monsoon season (Jun-Oct 2000) and the transplanting method in November-March (2000/01). Almost 98% of the rice fields in Sungai Burung are wet-direct seeded and the main sources of water supply are irrigation canals and rain.

Weed Population Determination

Two plots from each study area were selected randomly and used for the weed population assessment study. The paddy plants were approximately 2 to 2 $^1/_2$ months old in both sites. Weeds from each of thirty 1-m^2 quadrats (Kim and Moody 1983) in each plot were removed and counted by species. Summed dominance ratio (SDR) of each species was determined from the sum of the relative density, relative abundance and relative frequency.

Seed Bank Sampling

Soil samples were taken from the same locations as those used for weed population determination. Soil samples 7 cm in diameter were taken up to a depth of 15 cm from 30 quadrats in each study site. The soil depth of 15 cm was studied as reports have shown that the viability of the seed decreased at the depth of 10 cm and the buried seeds were skewed within the first 10 cm soil depth besides being well-correlated to the seedling emergence in fields (Baki *et al.* 1997; Graham and Hutchings 1988; Wilson *et al.* 1985; Zoner *et al.* 1984). The soil cores were divided into three different sections according to depth: 0-5 cm, 5-10 cm and 10-15 cm. Soil samples of the same depth for each particular site were pooled, mixed thoroughly and air-dried. The soil sampling before harvesting was conducted when the paddy plants were 2 to 2 $^1/_2$ months old whilst soil samples after harvesting were taken immediately after the crop was harvested. All sampling was done from the wet-direct seeded rice fields at both sites.

A modification of the seed separation methods described by Wilson *et al.* (1985), Sastroutomo and Yusron (1987) and Ball and Miller (1989) were used. For each soil, a 400 g soil sample was soaked in water and passed through a sieve with a screen size of 250 μm to collect weed seeds. Water was run through the screen to enhance sample movement. The contents collected in the screen were removed and air-dried. Seeds from the entire samples were sorted using a dissecting microscope and counted according to species. The total number of buried seeds found in the soil at different depths was expressed in numbers per m^2.

RESULTS

Weed Composition

A total of 17 weed species belonging to 13 families were found in these arable sites (Table 1). Among the families, Poaceae was the family with the highest number of species. Generally, broadleaf weeds were the most dominant (9 species) compared to grasses (4) and sedges (3). A species of aquatic ferns (*Marsilea crenata* Presl) from the family Marsileaceae was identified too. The number of weed species in the MARDI rice fields was slightly higher (14 species) than that in the Sungai Burung (13 species) area.

There were four species with SDR values of more than 5% in the MARDI rice fields. The most dominant weed was *Cyperus difformis* L. with a SDR value of 12.14%, followed by *Mimulus orbicularis* (Bl.), *Lemna minor* L. Griff and *Limnocharis flava* Buchenau in the respective order of 10.12, 7.78 and 5.39%. However in the Sungai Burung rice fields, the hierarchical order of the dominant weed species was *Najas graminea* (non Del.) Ridl, *L. minor*, *Sagittaria guayanensis* Kunth, *Hydrilla verticullata* (L.f) Royle and *Monochoria vaginalis* (Burm.f.) Presl with SDR values of 12.77, 8.92, 7.14, 5.42 and 5.19% respectively.

Based on the SDR value, the broadleaved weeds dominated the weed community in the MARDI and Sungai Burung rice fields accounting for *ca.* 32.48% and 39.60% respectively (*Fig. 1*).

Weed Seed Population in the Soil

Tables 2 and 3 illustrate the number of species collected from the study sites. Twenty-four species of weed seeds were recorded. The total weed seed populations in both rice fields were quite similar for both harvest times (369,720 and 526,042 seeds/m^2 in MARDI; 390,721 and 535,316 seeds/m^2 in Sungai Burung before and

TABLE 1
Summed dominance ratio values of weeds in transplanted rice fields of the Research Center MARDI and farmers' fields in Sungai Burung, Tanjung Karang, Selangor

Species	SDR (%)	
	MARDI	Sungai Burung
Alismataceae		
Sagittaria guayanensis	-	7.14
Limnocharitaceae		
Limnocharis flava	5.39	-
Cyperaceae		
Cyperus difformis	12.14	-
Cyperus iria	2.96	-
Hydrocharitaceae		
Hydrilla verticillata	1.57	5.42
Lemnaceae		
Lemna minor	7.78	8.92
Lentibulariaceae		
Utricularia aurea	0.88	1.03
Lythraceae		
Rotala indica	1.65	3.54
Marsileaceae		
Marsilea crenata	1.37	1.50
Najadaceae		
Najas graminea	3.52	12.77
Onagraceae		
Ludwigia adscendens	0.70	1.01
Poaceae		
Digitaria adscendens	-	0.56
Echinochloa colona	2.21	2.65
Echinochloa crus-galli	3.76	3.48
Leptochloa chinensis	-	1.79
Pontederiaceae		
Monochoria vaginalis	2.44	5.19
Scrophulariaceae		
Mimulus orbicularis	10.12	-

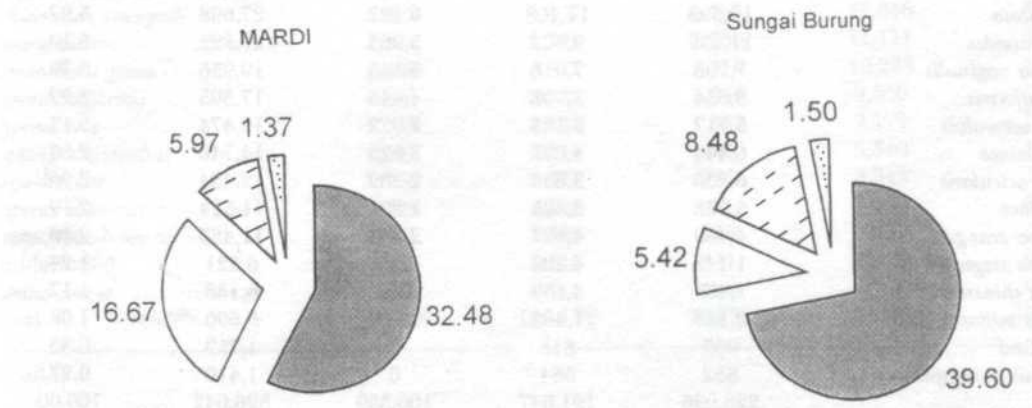

Fig. 1: SDR values of broadleaved weeds ( ), grasses( ), sedges ( ) and aquatic ferns ( ) in MARDI and Sungai Burung rice fields

TABLE 2
The distribution of weed seeds at the depth of 0-15 cm soil profile of arable rice fields in MARDI, Tanjung Karang, Selangor

Species	Soil depth (cm)				
	0-5	5-10	10-15	0-15	%
			seeds/m²		
Before harvesting					
Cyperus iria	33,351	25,958	16,955	76,264	20.63
Echinochloa colona	18,332	20,580	14,265	53,177	14.38
Limnocharis flava	17,117	11,459	10,056	38,632	10.45
Scirpus juncoides	12,700	12,161	9,121	33,981	9.19
Leguminosae	11,375	9,705	9,121	30,201	8.17
Eleocharis acicularis	10,160	6,665	3,157	19,982	5.41
Monochoria vaginalis	8,835	7,600	2,806	19,241	5.20
Utricularia aurea	5,963	9,939	1,988	17,890	4.84
Najas graminea	5,301	5,847	1,754	12,902	3.49
*Oryza sativa**	7,178	3,859	468	11,505	3.11
Cyperus difformis	7,841	2,105	1,052	10,998	2.97
Echinochloa crus-galli	4,417	4,560	1,637	10,614	2.87
Sagittaria guayanensis	2,761	6,314	818	9,893	2.68
Cleome viscosa	2,761	3,508	3,274	9,543	2.58
Lemna minor	2,430	4,209	0	6,639	1.80
Echinochloa stagnina	552	2,339	0	2,891	0.78
Leptochloa chinensis	994	1,403	0	2,397	0.65
Unidentified	1,215	0	585	1,800	0.48
Fimbristylis miliacea	0	1,169	0	1,169	0.32
Total	153,283	139,380	77,057	369,720	100.00
After harvesting					
Cyperus iria	53,008	35,313	16,838	105,159	20.00
Limnocharis flava	31,597	32,039	21,047	84,683	16.10
Echinochloa colona	20,683	18,709	10,758	50,150	9.53
Utricularia aurea	12,473	18,007	11,927	42,407	8.06
*Oryza sativa**	19,644	7,016	6,197	32,857	6.25
Leguminosae	16,110	13,213	1,006	30,329	5.77
Cleome viscosa	10,393	11,108	6,197	27,698	5.27
Scirpus juncoides	11,537	9,822	5,963	27,322	5.19
Monochoria vaginalis	7,795	7,016	5,145	19,956	3.79
Cyperus difformis	9,354	3,508	4,443	17,305	3.29
Ludwigia octovalvis	5,717	8,185	2,572	16,474	3.13
Najas graminea	6,444	4,677	3,625	14,746	2.80
Eleocharis acicularis	6,236	3,859	2,339	12,434	2.36
Lemna minor	6,028	3,625	1,871	11,524	2.19
Echinochloa crus-galli	4,469	4,677	2,339	11,485	2.18
Echinochloa stagnina	1,143	4,209	1,169	6,521	1.24
Leptochloa chinensis	1,455	3,859	819	6,133	1.17
Fimbristylis miliacea	2,183	1,403	2,104	5,690	1.08
Unidentified	935	818	0	1,753	0.33
Dactyloctenium aegyptium	832	584	0	1,416	0.27
Total	228,036	191,647	106,359	526,042	100.00

• Paddy

TABLE 3
The distribution of weed seeds at the depth of 0-15 cm soil profile of farmers' arable rice fields in Sungai Burung, Tanjung Karang, Selangor

Species	Soil depth (cm)				
	0-5	5-10	10-15	0-15	%
			seeds/m²		
Before harvesting					
Najas graminea	35,693	23,839	10,513	70,045	17.93
Sagittaria guayanensis	27,332	16,768	7,104	51,204	13.11
Scirpus juncoides	26,368	11,718	8,808	46,894	12.00
Monochoria vaginalis	16,507	15,657	12,786	44,950	11.50
Echinochloa colona	10,290	13,435	7,388	31,113	7.96
Utricularia aurea	12,970	6,364	5,115	24,449	6.26
Cleome viscosa	10,611	7,374	2,178	20,163	5.16
Eleocharis acicularis	7,396	8,990	1,988	18,374	4.70
*Oryza sativa**	11,898	4,445	1,705	18,048	4.62
Limnocharis flava	6,002	7,778	2,178	15,958	4.08
Cyperus iria	7,074	3,232	1,515	11,821	3.03
Cyperus difformis	2,358	6,465	947	9,770	2.50
Echinochloa crus-galli	4,716	2,121	852	7,689	1.97
Lemna minor	5,788	1,313	378	7,479	1.91
Unidentified	1,179	2,929	2,272	6,380	1.64
Leptochloa chinensis	2,359	1,212	0	3,571	0.91
Ludwigia octovalvis	1,822	707	284	2,813	0.72
Total	190,363	134,347	66,011	390,721	100.00
After harvesting					
Najas graminea	76,472	47,552	14,096	138,120	25.80
Scirpus juncoides	48,058	30,012	8,299	86,369	16.13
Echinochloa colona	27,245	20,138	11,141	58,524	10.93
Sagittaria guayanensis	12,979	27,414	9,436	49,829	9.31
Utricularia aurea	29,934	12,732	6,253	48,919	9.14
Monochoria vaginalis	11,225	15,980	1,819	29,024	5.42
*Oryza sativa**	15,084	8,055	3,638	26,777	5.00
Eleocharis acicularis	10,290	8,185	2,501	20,976	3.92
Echinochloa crus-galli	9,822	4,807	2,387	17,016	3.18
Cleome viscosa	7,133	2,728	1,250	11,111	2.08
Limnocharis flava	2,456	4,417	3,410	10,283	1.92
Cyperus difformis	5,145	2,339	2,046	9,530	1.78
Cyperus iria	2,339	3,248	1,592	7,179	1.34
Ludwigia octovalvis	1,637	3,248	455	5,340	1.00
Leguminosae	1,169	3,378	796	5,343	1.00
Cyperus compressus	2,339	1,560	0	3,899	0.73
Leptochloa chinensis	1,052	1,560	568	3,180	0.59
Unidentified	819	1,819	341	2,979	0.56
Lemna minor	350	0	568	918	0.17
Total	265,548	199,172	70,596	535,316	100.00

* paddy

TABLE 4
Estimation of weed seeds found in the arable soil at the Research Station MARDI and in farmers' fields Sungai Burung, Tanjung Karang, Selangor[a]

Soil depth (cm)	Estimated mean number of seed (seeds/m²)	
	Before harvesting	After harvesting
MARDI		
0-5	153,283 a	228,036 a
5-10	139,380 a	191,647 a
10-15	77,057 b	106,359 b
Total*	369,720	526,042
Sungai Burung		
0-5	190,363 a	265,548 a
5-10	134,347 b	199,172 b
10-15	66,011 c	70,596 c
Total*	390,721	535,316

a Means in a column followed by the same letters are not significantly different (P>0.05) according to the Tukey test.

* The total seed count between before and after harvesting is significantly different at the 5% level as determined by Tukey test.

after harvesting, respectively). Nevertheless, the estimated number of weed seeds after harvesting was higher than the number before harvesting (Table 4). Nineteen and twenty species of weed seeds were recorded in the MARDI rice fields before and after harvesting, respectively. Meanwhile, the corresponding number was 18 and 17 species in the Sungai Burung rice fields. The seeds of *Cyperus iria* L. were the most abundant in the MARDI rice fields while *N. graminea* had the highest percentage of total seeds found in the Sungai Burung rice fields irrespective of the harvest time. Most of the weed seeds were found in the top 5 cm of the soil at both sites. In general there was a significant difference in the seed count at the different depths of the Sungai Burung soil. The total number of weed seeds declined with increasing depth, i.e. they were lower at 10-15 cm than at 0-5 cm.

DISCUSSION

According to the findings of Saharan (1977), the most common broadleaved weeds found in rice fields were *M. vaginalis, L. flava, S. guayanensis* and *Jussiaea repens.* All these species except *J. repens* were found in this study. The abundance of broadleaf weeds in both study sites indicates that flooding has a major suppressive effect on the stand establishment and growth of grasses and sedges. Anon (1982) and Moody (1977) reported that almost all weeds cannot survive in water at the depth of more than 10 cm, except certain broadleaved weeds.

Seventeen out of the 55 weed species recorded by Itoh (1991) were identified in the MARDI and Sungai Burung rice fields. Only two species were in the group of common weeds, *viz. L. minor* and *Scirpus juncoides* meanwhile two other species (*H. verticullata* and *Rotala indica*) were rare species. Drost and Moody (1982) reported that moisture or saturated soil conditions after planting was the major factor affecting the composition of the weed flora and the dominance patterns of the major weed groups in particular populations. Water supplied to rice fields after seeding hinders the establishment of sedges and grasses. The SDR values reflect this, as the value for broadleaves is more than double those obtained for sedges and grasses. Thus, flooding favoured the growth of broadleaved weeds over sedges and grasses (Ho and Itoh 1991).

The total numbers of buried seeds (including viable and non-viable seeds) in the two sites were similar. This was due to the geographical factor. Both study sites were close to each other (5 km apart) and irrigated by canals from the same point. Kelly and Bruns (1975) have proven that water played a significant role in the process of weed dissemination. The same source of

irrigation might contribute to the similarity of weed seed counts at both sites.

On the other hand, seed counts before and after harvesting were significantly different in both areas. In most cases, the life cycle of weeds in rice fields is shorter compared to that of the rice plant. Within 3 months, weed seeds will ripen and fall, thus contributing to the seed reservoir in the soil. For instance, *E. crus-galli* has a growth duration of 90 days, which is shorter than the maturation period of the paddy plant (via. 120 days) (Azmi 1990). Thus, the weed seed population in the soil is higher after the harvest period. It is also reasonable to suggest that the species found possess characteristically efficient and strong vegetative growth coupled with the ability to produce a vast number of seeds.

Weed seeds of both rice fields were skewed mostly to the first 0-5 cm soil depth accounting for *ca.* 41.5 to 43.3% and 48.7 to 49.6% of the total seed counts in the MARDI and Sungai Burung rice fields, respectively. The large number of seeds may appear to be those seeds buried during previous years and the continuous seed shedding by the existing weeds onto the soil surface. Baki *et al.* (1997) have also proven that the total seed counts are high in the first 0-5 cm soil depth in arable peat of Selangor, Malaysia.

The depth from which soil samples are taken is an important factor to estimate the seedbank in the soil (Zhang *et al.* 1998). Studies have shown that burial depth significantly affects seed germination and seedling emergence in a variety of plant species under both greenhouse and field conditions (Fenner 1985; Maun and Lapierre 1986). In our study, soil samples were taken up to a depth of 15 cm to determine the density of the soil seedbank. This is because taking soil samples to ploughing depth may increase the chances of sampling the seeds of weed species in an area and thus enhance the degree of accuracy in the estimation of the existing soil seedbank.

Seedbanks can make eradication of weeds very difficult. It is possible to locate and destroy growing plants, but dormant viable seeds may remain undetected in the soil for many years and later on give rise to a new population of plants. Therefore, control of weed seed population in the soil is necessary for better weed management (Ismail and Kalithasan 1994) and may be more practicable if the seeds in the active soil seedbank are used (Zhang *et al.* 1998). Further studies on the viability of the weed seeds are required to achieve this goal.

ACKNOWLEDGEMENTS

This work was supported by research grant IRPA 09-02-02-0073-EA200 from the Ministry of Science, Technology and Environment of Malaysia.

REFERENCES

ANON. 1982. *Annual Report.* Rice Research Branch. MARDI: 131-151.

AZMI, M. 1990. Critical period for weed control in direct seeded rice. *Proc. 3rd Tropical Weed Science Conference.* p. 75-91. Malaysian Plant Protection Society, K.L.

AZMI, M. and B. B. BAKI. 2003. Weed species diversity and management practices in the Sekinchan Farm Block, Selangor South West Project – A highly productive rice area in Malaysia. *Proc. 19th Asian-Pacific Weed Science Society Conference,* p. 174-184. Weed Science Society of the Philippines, Manila.

BAKI, B.B., W.K. YONG and N.Y.F. WONG. 1997. Quantitative assessments and spatial pattern amalyses of weed seed banks of arable peat in Selangor, Malaysia. *The Korean J. of Weed Sci.* **17(3):** 269-280.

BALL, D.A. and S.D. MILLER. 1989. A comparison of techniques for estimation of arable soil seed banks and their relationship to weed flora. *Weed Res.* **29**: 365-373.

DE DATTA, S.K. 1981. *Principles and Practices of Rice Production.* New York: Wiley & Sons.

DROST, D.C. and K. MOODY. 1982. Effect of butachlor on *Echinochloa glabescens* in wet-seeded rice (*Oryza sativa*). *Philippines J. Weed Sci.* **9**: 57-64.

FENNER, M. 1985. *Seed Ecology.* New York: Chapman & Hall.

GRAHAM, D.J. and M.J. HUTCHINGS. 1988. Estimation of the seed bank of a chalk grassland ley established on former arable land. *J. Applied Ecol.* **25**: 241-252.

HO, N.K. and K. ITOH. 1991. Changes of weed flora and their distribution in the Muda area. In *8th MADA/TARC Quarterly Meeting.* Kedah: Alor Setar.

ITOH, K. 1991. *Life Cycles of Rice Fields Weeds and Their Management in Malaysia.* Japan: Tropical Agricultural Research Center.

ISMAIL, B.S. and K. KALITHASAN. 1994. Effects of glufosinate-ammonium and terbuthylazine on germination and growth of two weed species. *Plant Protection Q.* **9**: 15-19.

KHALID, S.C. 1988. Masalah rumpai dan perlaksanaan program pengurusan dalam kawasan KADA. *Pros. Seminar & Bengkel Pengurusan Rumpai Sawah Padi Kebangsaan*, 7-9 Jun, Pulau Pinang, Malaysia.

KELLY, A.P. and V.F. BRUNS. 1975. Dissemination of weed seeds by irrigation water. *Weed Sci.* **23**: 486.

KIM, S.C. and K. MOODY. 1983. Effect of a mixture of two rice cultivars on the competitive ability of rice against weeds and rice grain yields. *Philipp. J. Weed Sci.* **7**: 17-25.

LAWSON, H.M. 1988. The use of weed seedbank in the selection of herbicide recommendations. *Weed Res.* **28**: 486.

MAUN, M.A. and J. LAPIERRE. 1986. Effects of burial by sand on germination and seedling emergence of four dune species. *American J. of Botany* **73**: 450-455.

MOODY, K. 1977. Weed control in rice. In *5th BIOTROP Weed Science*, p. 374-424. K.L.: Rubber Research Institute.

NODA, K. 1977. Integrated weed control in rice. In *Integrated Control of Weeds*, ed. J.D. Fryer, and S. Matsunaka, p. 17-46. Tokyo: Univ. of Tokyo Press.

SAHARAN, H.A. 1977. Rice weed control in Malaysia. MARDI Report No. 66, Serdang.

SASTROUTOMO, S.S. and A. YUSRON. 1987. Buried weed seed population in arable soils. *11th the Asian-Pac. Weed Sci. Soc. Conf.*, pp. 46-60. Nov. 29-Dec. 5, Taipei, Rep. of China.

TEERAWATSAKUL, M. 1976. Weeds in paddy field and their control in Thailand. Weeds and Weed Control in Asia. Food and Fertilizer Technology Centre. China. Aug. 1981.

TENGKU ZUNAIDAH, T.J. 1996. Kajian populasi rumpai dan biji rumpai dan tanah di Tanjung Karang, Selangor. Tesis Sarjana Muda Sains. Universiti Kebangsaan Malaysia, Bangi.

WILSON, R.G., E.D. KERR and L.A. Nelson. 1985. Potential for using weed seed content in the soil to predict future weed problems. *Weed Sci.* **33:** 171-175.

YAMADA, N. 1965. Problems of irrigation and drainage practices in rice culture. *Int. Rice Com. Newsl.* **14(3):** 13-31.

ZHANG, J., A.S. HAMILL, I.O. GARDINER and S.E. WEAVER. 1998. Dependence of weed flora on the active soil seedbank. *Weed Res.* **38**: 143-152.

ZORNER, P.S., R.L. ZIMDAHL and E.E. SCHWEIZER. 1984. Effect of depth and duration of seed burial on kochia (*Kochia scoparia*). *Weed Sci.* **32:** 602-607.

(Received: 10 September 2003)
(Accepted: 20 April 2005)

Pertanika J. Trop. Agric. Sci. 27(2): 121 - 133 (2004) ISSN: 1511-3701

Physical and Chemical Properties of Coconut Coir Dust and Oil Palm Empty Fruit Bunch and the Growth of Hybrid Heat Tolerant Cauliflower Plant

[1]ASIAH A., [2]MOHD. RAZI, I., [3]MOHD. KHANIF Y.,
[4]MARZIAH M. & [5]SHAHARUDDIN M.
[1]Agrotech and BioSciences Division,
[2]Department of Crop Science,
[3]Department of Land Management,
[4]Department of Biochemistry, Universiti Putra Malaysia,
43400, UPM, Serdang, Selangor, Malaysia
[5]Malaysian Institute For Nuclear Technology Research,
Bangi, 43000, Kajang, Selangor, Malaysia

Keywords: Coconut coir dust, oil palm empty fruit bunch, growing medium, soilless culture, cauliflower

ABSTRAK

Penyelidikan yang dilaporkan dalam eksperimen ini memberi tumpuan terhadap ciri fizikal dan kimia habuk sabut kelapa (CD) dan tandan kosong kelapa sawit (EFB) berpotensi digunakan sebagai media pertumbuhan bagi kobis bunga hibrid di bawah rumah tanaman di tropika yang mempunyai kelembapan udara yang tinggi. EFB dan CD mempunyai ciri fizikal optimum untuk keperluan pertumbuhan tanaman pada awal penanaman. Walau bagaimanapun, kedapatan air tersedia bagi CD adalah 34% dibandingkan dengan 19% untuk EFB. Ruang rongga tersedia EFB , iaitu rongga yang melebihi 300 µm adalah tinggi dibandingkan dengan CD. Ciri kimia menunjukkan EFB adalah lebih alkali (pH 6.9) dibandingkan dengan CD(pH 5.3) dan rendah kekonduksian elektrik (EC) iaitu 1.3 dSm^{-1} dibandingkan dengan CD (1.9 dSm^{-1}). Keputusan juga menunjukkan CD mengandungi kepekatan nutrien yang tinggi dibandingkan dengan EFB. Walaupun EC asal CD lebih tinggi, jumlah bahan kering tanaman adalah tinggi dibandingkan dengan kobis bunga yang ditanam dalam EFB. Kedapatan fungi dan cendawan pada permukaan media EFB dan penguraian awal telah menyebabkan pertumbuhan tanaman yang kurang pada media EFB. Oleh itu, CD adalah media tanaman yang lebih sesuai dibandingkan dengan EFB untuk penanaman hibrid bunga kobis.

ABSTRACT

This research report is about the physical and chemical properties of coconut coir dust (CD) and oil palm empty fruit bunch (EFB), and their potential for use as growing media for hybrid cauliflower grown under humid tropical greenhouse conditions. The physical properties showed that both the EFB and CD provided optimum plant growth conditions at the start of the growing the period. However, the readily available water value for CD was 34% whereas that for EFB was 19%. The air filled porosity containing more pores larger than 300 µm were higher in EFB compared to CD. The chemical properties data suggested that EFB was more alkaline (pH of 6.9) than CD (pH of 5.3) and had lower electrical conductivity (EC) of 1.3 dSm^{-1} than CD (1.9 dSm^{-1}). The results also indicated higher nutrient contents in CD than in EFB. Despite a high initial EC value for CD than EFB, the hybrid cauliflower plant dry weights and total leaf area for CD grown plants were double those grown in EFB. The appearance of fungus and mushroom on the EFB medium surface and the early sign of decomposition may account for the lower plant growth in the EFB medium. Hence, CD is a more suitable growing medium for growing hybrid cauliflower compared to EFB.

INTRODUCTION

The principal difference in growing conditions between plants grown in other media and those raised in soil is the amount of space available for root development. In many cases the development of roots is restricted by the

container, pot or holding medium in which the plants are raised (Carlile 1997). The root of plants must obtain sufficient water, oxygen and nutrients from the surrounding environment. The life processes of the root system excrete CO_2 and other gases that must disappear within a certain time from the root environment. The structure of the growing medium must be soft and porous enough so that roots can easily penetrate widely into the material and it must also provide anchorage and support for the plants. The well being of a plant is strongly dependent on the correct functioning of the root system. The physical, chemical and biological properties of a growing medium can be used as a basis of classifying the suitability of a growing medium in relation to the needs of the roots.

Understanding the physical environment surrounding roots in containers (relative volume of air, water, and solid) is based on the relationship between energy status and water content of the medium (Roberts *et al.* 1989). This relationship is a reflection of the pore size distribution of the medium. A plot of this relationship, i.e., a plot of volumetric wetness (q) vs. water potential is called moisture characteristic curve or moisture retention curve (De Boodt and Verdonck 1972).

Ever since Bunt (1961) first reported moisture retention curves for pot-plant media, there has been considerable effort to determine the utility of these curves in explaining plant growth, and the best way to quantify these data for both descriptive and predictive purposes (Roberts *et al.* 1989). The phase distribution (air, water and solid) of a medium is important for horticulture particularly at matric potentials between –1 cm and –100 cm water column (pF 2.0 = 10 kPa) (De Boodt *et al.* 1974; Michiels *et al.* 1993). Lower matric potentials will give rise to severe loses in growth rate. Air filled porosity (AFP) of the medium is the ability of the medium to retain air, hence is important to ensure sufficient oxygen supply to the roots. As far as the chemical properties are concerned the growing medium must have a suitable pH value range of about 5.0-6.5, an electrical conductivity that may not exceed a certain level and free of harmful elements and chemicals.

The physical and chemical properties of coconut coir dust (CD) from numerous sources have been found to be within acceptable ranges and suitable for use as growing medium (Evans *et al.* 1996). However significant differences were observed among coconut coir dust sources with respect to physical and chemical properties (Evans *et al.* 1996), and these may explain the differences in results (good and negative) obtained from previous studies (Reynolds 1973; Meerow 1994; Radjaguguk *et al.* 1983) when using CD as growing media. Yahya and Mohd Razi (1996) suggested that the variation in chemical and physical properties of the media and differences in plant sensitivity to a defined root environmental condition might have contributed to the marked differences in some ornamental plant development observed in their studies. Therefore, the properties of the growing medium affect the plant growth.

Although CD and its mixture with other components have been widely and successfully used in different parts of the world as an environment friendly peat substitute for growing plants in containers (Meerow 1994, 1995), its basic properties and utilization in crop production in Malaysia is rather scarce (Yahya and Mohd Razi 1996). Another potential medium is oil palm empty fruit bunch (EFB). It is one of the solid wastes generated from the oil palm industry. Even though direct mulching using EFB on soil can improve soil structure, aeration and moisture capacity (Megat Johari *et al.* 1990) there has been little interest on utilisation of EFB as growing medium for containerized plants.

The objective of this study was to determine the differences in physical and chemical properties between the local CD and EFB, and their effect on the growth and nitrogen content in the hybrid cauliflower when used as growth media. In many cases the properties of CD were manipulated to prepare suitable coir based media for containerized plant (Handreck 1993; Meerow 1994, 1995; Yahya *et al.* 1997). However, in this study no manipulation was done to improve the basic properties of the medium by mixing with other organic and inorganic components.

MATERIALS AND METHODS

Experiment 1: Determination of Physical and Chemical Properties

Coconut coir dust (CD) obtained locally from Perak and freshly shredded oil palm empty fruit bunch fibres of mean gross length 9.10 ± 3.40 cm obtained from Kapar Klang were used in this study. Oil palm fibres were cut to a mean gross length of 3.46 ± 1.26 cm by an impact type cutter at a cutting speed of 2 kg/min. Short

fibres were then ground through 5.0-mm sieve and air-dried. The products are referred to as oil palm empty fruit bunch (EFB). Both CD and EFB were analysed for physical and chemical properties. Bulk density (g cm^{-3}) was determined using modified methods of De Boodt *et al.* (1974). The cylinder used was a brass ring, 4.0-cm in height and 7.60 cm (internal diameter), with internal volume of 181 ml. The bottom of the ring was fitted with iron gauze and rubber ring and the whole assembly were weighed. Another ring was then placed on top of the above-mentioned ring and stacked vertically. The rings were filled with a medium and gently tapped against a laboratory table at a height of ≈ 10.0 cm a sufficient number of times to obtain a certain bulk material in the bottom ring. The top ring was discarded. The bottom ring filled with medium was weighed and then seated on to the porous plate and saturated with water for 48 hours. The mass was determined after drying the medium in the oven at 105° C for 24 hours.

The particle density of the substrates was determined by using a pycnometer bottle (Blake and Hartge 1986). All determinations were replicated five times. The total pore space (volume %) and volumetric shrinkage (volume %) were calculated following the methods of Michiels *et al.* (1993).

The moisture retention curves of both coconut coir dust and oil palm empty fruit bunch were determined using the method of De Boodt *et al.* (1974). The moisture content was measured after equilibrating the samples on the pF equipment at matric potentials of –10, -50, –100, -300 cm water column and 15 bar (pF 1, 1.7, 2.0, 2.5 and 4.2). From this curve, the air volumes, readily available water, water buffer capacity, less readily available water and pore size distributions were calculated (Michiels *et al.* 1993).

The pH and electrical conductivity (EC) of the coconut coir dust and oil palm empty fruit bunch were determined using air-dried samples to water ratio of 1:2 (v/v). The samples were stirred with a glass rod and allowed to equilibrate for 4 hours. For pH measurement, the samples were stirred again immediately before measuring the pH using a calibrated pH meter. For EC measurement, the solution was then filtered through a Whatman No.41 filter paper and the extract was collected in a small beaker. The EC of the extract was measured using the EC probe with calibrated conductivity meter.

Total nitrogen was determined using Semi-Micro Kjedahl., potassium (K^+) contents using flame photometer and calcium (Ca^{2+}), magnesium (Mg^{2+}) and zinc (Zn^{2+}) contents using absorption following HNO_3-H_2O_2 digestion of the coconut coir dust and oil palm empty fruit bunch in the microwave. Phosphorus concentration (P) was determined colorimetrically by vanadium molybdate measured at 410 nm with a spectrophotometer.

Five replications were tested for each medium (coconut coir dust and oil palm empty fruit bunch). A t-test for two independent samples (two tailed test, a = 0.05) was done to determine if the medium significantly affected the measured physical and chemical properties.

Experiment 2: Growth Comparison of Hybrid Cauliflower Grown in Coconut Coir Dust and Oil Palm Empty Fruit Bunch

a. Plant Materials, Growth Conditions and Treatments

White polyethylene bags of 6 L volume were used as growing bags. A 10 cm circular opening was made at the top of each bag after filling with 6 L of growing medium (100% coconut coir dust or 100% empty fruit bunch) to plant the hybrid cauliflower seedling. Slits were made at the bottom of each bag to allow for drainage. The coconut coir dust or oil palm empty fruit bunch was then thoroughly wet with water and allowed to drain before seedling transplanting. Each bag was placed on a perforated square plastic basket fitted with 6 legs of 4 cm high from the greenhouse floor.

Seeds of hybrid heat tolerant cauliflower were germinated in germination trays filled with 100 % coconut coir dust or 100% oil palm empty fruit bunch and watered daily with tap water. Two weeks after germination, uniform sized seedlings were transplanted, one seedling per bag, into the 6 L growing bags filled with coconut coir dust or oil palm empty fruit bunch.

The growing bags were placed in a greenhouse under natural photoperiod and irradiance with day temperature ranging between 28°C and 38°C and minimum night temperature of 25°C. The seedlings were irrigated via drip emitter (2 L h^{-1}) with tap water for the first 3 days and a complete Cooper (1979) nutrient solution thereafter. Nitrogen was applied as KNO_3 at a concentration of 200 ppm N. The nutrient solution was adjusted to pH ranging from 5.5 to

5.8 with either dilute KOH or H_2SO_4. The electrical conductivity of the solution was 2.4 dS m^{-1}. Initially plants were irrigated twice daily for 15 minutes but as the environmental conditions promoting evapotranspiration increased and the plants grew larger fertigation was increased to three times/day. The experimental design was a randomized complete block design with the two growth medium as treatments each replicated five times.

b. Plant Samplings, Growth Parameters and Chemical Analysis

Plants were harvested at 21, 42 and 65 days after transplanting (DAP). The plants were cut just below the first leaf node, which was less than 1 cm from the growing medium surface. Five plants of each treatment were harvested at each harvesting time and separated into stem plus petioles, leaves and curd at final harvest. After weighing, the plant samples were dried in a forced air oven at 70° C for 96hr and weighed. The dried samples were ground through a 2 mm sieve for total N analysis.

RESULTS

The physical and chemical properties of coconut coir dust were evaluated as potential growing medium for containerized plants and compared to oil palm empty fruit bunch. The bulk density of oil palm empty fruit bunch (0.075 g cm^{-3}) was significantly higher than that of coconut coir dust (0.177 g cm^{-3}) as shown in Table 1. Oil palm empty fruit bunch also showed a significantly higher particle density (1.321 g cm^{-3}) compared to 0.758 g cm^{-3} for coconut coir dust. The total pore space for organic medium included the open, interconnecting and closed pores. Data in Table 1 show that coconut coir dust had significantly higher total pore space (96.26 %vol.) than oil palm empty fruit bunch (92.80% vol.). No significant difference was found in volumetric shrinkage values between coconut coir dust and oil palm empty fruit bunch. The volumetric shrinkage values for the coconut coir dust and oil palm empty fruit bunch were 11.21% and 10.13% respectively.

The moisture retention curves for coconut coir dust and oil palm empty fruit bunch are shown in *Fig. 1*. As suction was gradually increased more water was drawn out of the oil palm empty fruit bunch compared to the coconut coir dust, resulting in the low amount of water retained in oil palm empty fruit bunch as compared to coconut coir dust. Increasing suction will result in the progressive emptying of the pores of different sizes until at high suction values where only the narrow pores retain water. Hence, the shape of the moisture retention curve of a medium reflects its pore size distribution (Michiels *et al.* 1993).

From the moisture retention curves obtained for coconut coir dust and oil palm empty fruit bunch, its pore distributions were calculated. *Fig. 2* shows the distribution of pore size for oil palm empty fruit bunch and coconut coir dust. It is clear that oil palm empty fruit bunch contained relatively larger pores (> 300 μm diameter) than coconut coir dust, and once these large pores are emptied at a given suction, only small amounts of water remained.

This is reflected in the results as only 45% water was retained in oil palm empty fruit bunch at 1 kPa suction compared to 71% water retained in coconut coir dust as shown in *Fig. 1*. The air filled porosity (AFP) represented by relatively larger pores > 300 mm in oil palm empty fruit bunch was 47% whereas the AFP in coconut coir dust was only 25% (*Fig. 2*).

The coconut coir dust contained more pores of sizes between 60 mm and 300 mm than oil palm empty fruit bunch which resulted in a

TABLE 1
Physical properties of coconut coir dust and oil palm empty fruit bunch

Property	Coconut coir dust	Oil palm empty fruit bunch	t- value
Bulk density (g cm^{-3})	0.074	0.177	3.790**
Particle density (g cm^{-3})	0.758	1.321	2.659*
Total pore space (% vol)	96.264	92.796	2.932**
Shrinkage (% vol)	11.206	10.129	0.729^{NS}

The figures are means of 5 replications.
ns, *, ** Non significant or significant at $p < 0.05$ or 0.01, respectively.

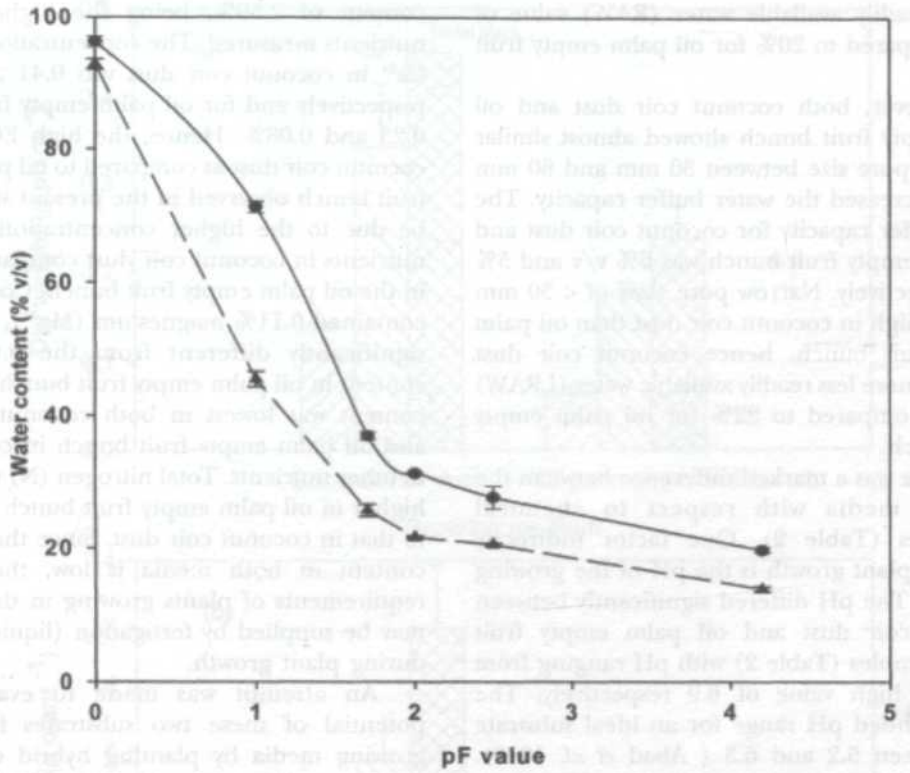

Fig. 1: Moisture characteristic curve for coconut coir dust ● and oil palm empty fruit bunch ▲

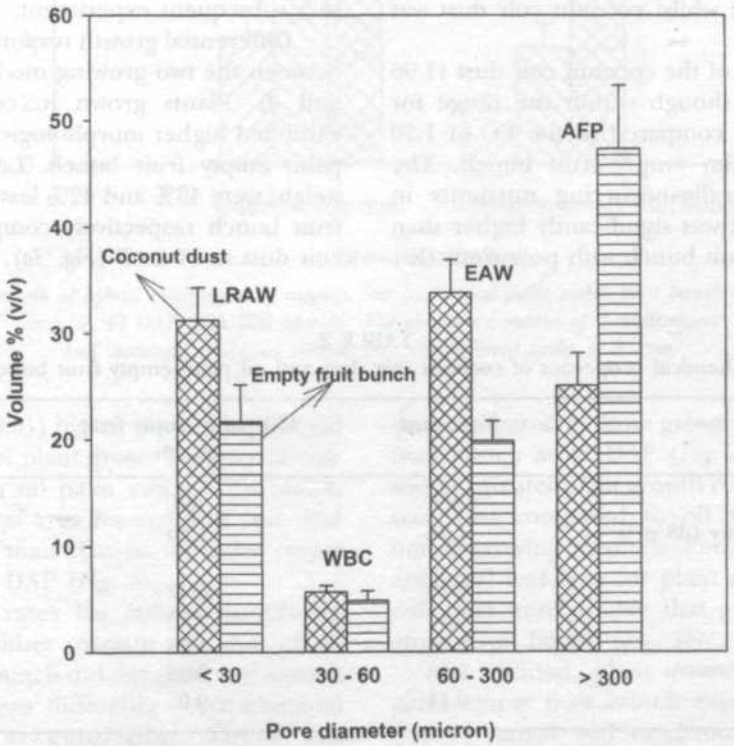

Fig. 2: Air filled porosity (AFP), Easily available water (EAW), Water buffer capacity (WBC) and Less readily available water (LRAW) for coconut coir dust and oil palm empty fruit bunch at different pore size distribution. The values are means of 5 replications and standard errors are shown

higher readily available water (RAW) value of 34% compared to 20% for oil palm empty fruit bunch.

However, both coconut coir dust and oil palm empty fruit bunch showed almost similar values of pore size between 30 mm and 60 mm which decreased the water buffer capacity. The water buffer capacity for coconut coir dust and oil palm empty fruit bunch was 6% v/v and 5% v/v, respectively. Narrow pore sizes of < 30 mm was also high in coconut coir dust than oil palm empty fruit bunch, hence coconut coir dust retained more less readily available water (LRAW) of 31% compared to 22% for oil palm empty fruit bunch.

There was a marked difference between the growing media with respect to chemical properties (Table 2). One factor indirectly affecting plant growth is the pH of the growing medium. The pH differed significantly between coconut coir dust and oil palm empty fruit bunch samples (Table 2) with pH ranging from 5.3 to a high value of 6.9 respectively. The recommended pH range for an ideal substrate was between 5.2 and 6.3 (Abad *et al.* 1989). Therefore, the pH value of oil palm empty fruit bunch was higher than the established limits for an ideal substrate whilst coconut coir dust was within the range.

The EC level of the coconut coir dust (1.96 dSm^{-1}) was high though within the range for ideal substrate as compared to the EC of 1.30 dSm^{-1} for oil palm empty fruit bunch. The amount of naturally–occurring nutrients in coconut coir dust was significantly higher than oil palm empty fruit bunch with potassium (K^+) content of 2.39%, being the highest for all nutrients measured. The concentration of P and Ca^{2+} in coconut coir dust was 0.41 and 0.18% respectively and for oil palm empty fruit bunch 0.23 and 0.08%. Hence, the high EC value of coconut coir dust as compared to oil palm empty fruit bunch observed in the present study could be due to the higher concentrations of these nutrients in coconut coir dust compared to that in the oil palm empty fruit bunch. Coconut dust contained 0.11% magnesium (Mg^{2+}), which was significantly different from the 0.05% Mg^{2+} content in oil palm empty fruit bunch. The Mg^{2+} content was lowest in both coconut coir dust and oil palm empty fruit bunch in comparison to other nutrients. Total nitrogen (N) was slightly higher in oil palm empty fruit bunch compared to that in coconut coir dust. Since the nutrients content in both media is low, the nutrient requirements of plants growing in these media may be supplied by fertigation (liquid feeding) during plant growth.

An attempt was made to evaluate the potential of these two substrates for use as growing media by planting hybrid cauliflower on both media and supplying both with complete nutrient solution including nitrogen at 200 ppm in a subsequent experiment.

Differential growth responses were observed between the two growing media studied (*Figs. 3* and *4*). Plants grown in coconut coir dust exhibited higher morphological growth than oil palm empty fruit bunch. Leaf and stem dry weight were 49% and 42% less in oil palm empty fruit bunch respectively compared to coconut coir dust at 21 DAP (*Fig. 3a*). These resulted in

TABLE 2
Chemical properties of coconut coir dust and oil palm empty fruit bunch

Property	Coir dust	Oil palm empty fruit bunch	t- value
pH	5.3	6.9	3.751**
Electrical conductivity (dS m^{-1})	1.96	1.30	3.973**
Total nutrient (%):			
Total nitrogen	0.39	0.42	2.870*
P	0.41	0.23	3.406**
K^+	2.39	1.56	2.507*
Ca^{2+}	0.18	0.08	2.499*
Mg^{2+}	0.11	0.05	2.859*

The figures are means of 5 replications.
*, **, Significant at P<0.05, or 0.01 respectively.

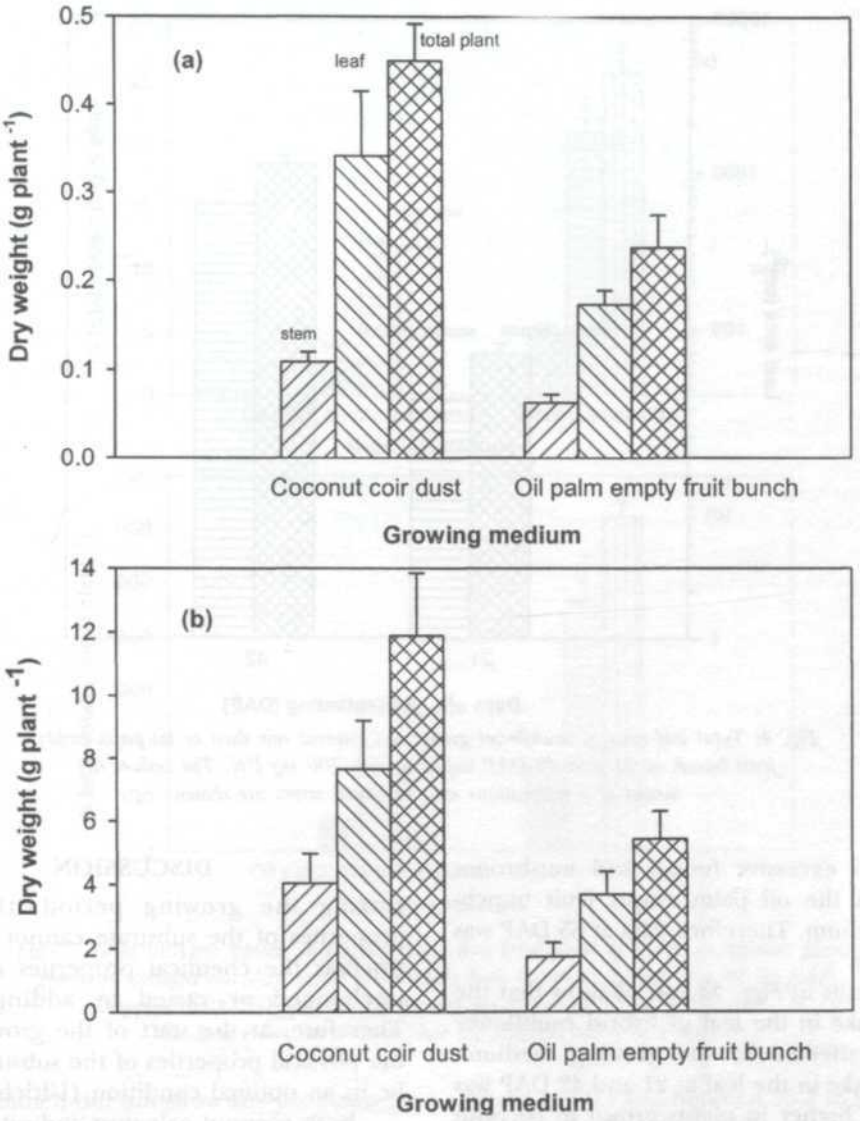

Fig. 3: Growth of hybrid cauliflower in coconut coir dust or oil palm empty fruit bunch at (a) 21 and (b) 42 DAP with 200 ppm N. The values are means of 5 replications and standard errors are shown. Note the different scales of Y axes

a significantly ($p<0.01$) higher above-ground total plant dry weight of plant grown in coconut coir dust compared to oil palm empty fruit bunch (*Fig. 3a*). Total leaf area for coconut coir dust plant was higher than that in oil palm empty fruit bunch at 21 DAP (*Fig. 4*).

The growth rates for hybrid cauliflower plants grown in either coconut coir dust or oil palm empty fruit bunch did not show any distinct dissimilarly. However differences were observed in dry weight accumulation. There was significantly higher dry weight accumulation in stem and leaves of plants grown in coconut coir dust compared to those grown in oil palm empty fruit bunch at 42 DAP (*Fig. 3b*). These results suggest greater plant growth response to coconut coir dust compared to oil palm empty fruit bunch growing medium. Total plant dry weight and total leaf area for plant grown in coconut coir dust were double that grown in oil palm empty fruit bunch (*Fig. 3b*).

In addition, plant growth in oil palm oil palm empty fruit bunch experienced damage due to fungal and mushroom growth in the medium at about 30 to 35 DAP (*Figs. 6a, 6b* and *6c*). The experiment was terminated after 42

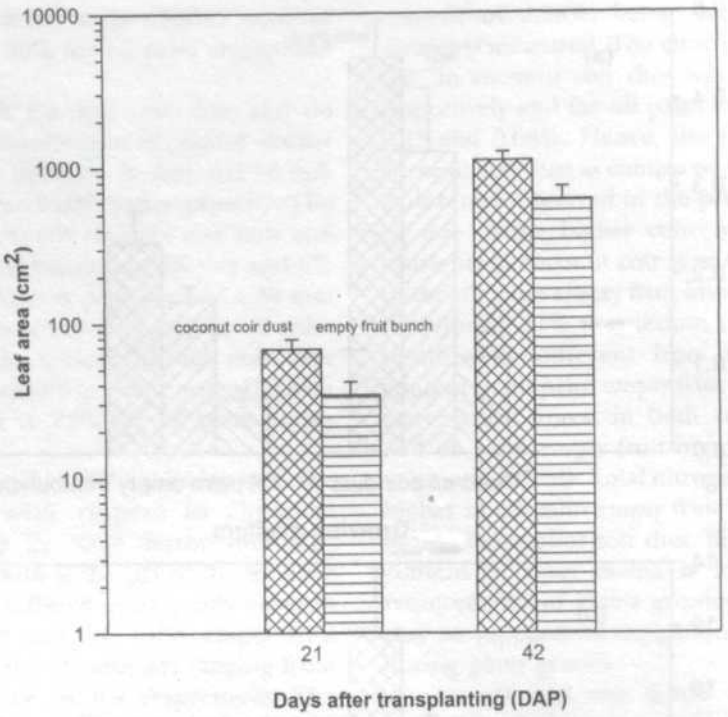

Fig. 4: Total leaf area of cauliflower grown in coconut coir dust or oil palm empty fruit bunch at 21 and 42 DAP supplied with 200 mg l^{-1}N. The values are means of 5 replications and standard errors are shown

DAP due to excessive fungal and mushroom problems in the oil palm empty fruit bunch-growing medium. Therefore data at 65 DAP was not available.

The results in *Figs. 5a* and *5b* show that the total N uptake in the leaf of hybrid cauliflower plants was affected by the growing medium. Total N uptake in the leaf at 21 and 42 DAP was significantly higher in plants grown in coconut coir dust than in plants grown in oil palm empty fruit bunch. Total N uptake in the leaf per plant ranged between 10.0 to 20.0 mg dry weight for plants grown in oil palm empty fruit bunch and coconut coir dust respectively. Total N uptake in the stem per plant was lower than in the leaf for all plants grown whether in coconut coir dust or oil palm empty fruit bunch. However, total N uptake was 30% higher in the stem of plants grown in coconut coir dust than that in oil palm empty fruit bunch.

The high dry weight accumulation in the plant grown in coconut coir dust (*Fig. 3a* and *3b*) resulted in double the total N uptake in the plants grown in coconut coir dust as com-pared to the plants grown in oil palm empty fruit bunch at both 21 and 42 DAP.

DISCUSSION

During the growing period, the physical properties of the substrate cannot be changed whereas the chemical properties can be kept unchanged or varied by adding nutrients. Therefore, at the start of the growing period, the physical properties of the substrates have to be in an optimal condition (Ulrich 1996).

Both coconut coir dust and oil palm empty fruit bunch were evaluated as lightweight materials with bulk density varying between 0.074 g cm^{-3} and 0.180 g cm^{-3} respectively (Table 1). The values were lower than the range for ideal substrates (Abad *et al.* 1989). However, the coconut coir dust bulk density was within the range (0.04 to 0.08g cm^{-3}) reported by Evans *et al.* 1996 for five coconut coir dust sources from the Philippines and by Patricia *et al.* (1997) for coconut coir dust from Mexico (0.075g cm^{-3}) and Sri Lanka (0.056g cm^{-3}). The advantage of the light weight material is that it allows ease of handling and transport, which is a very important commercial or economic consideration during handling and transport. Despite their low bulk density, both coconut coir dust and oil palm

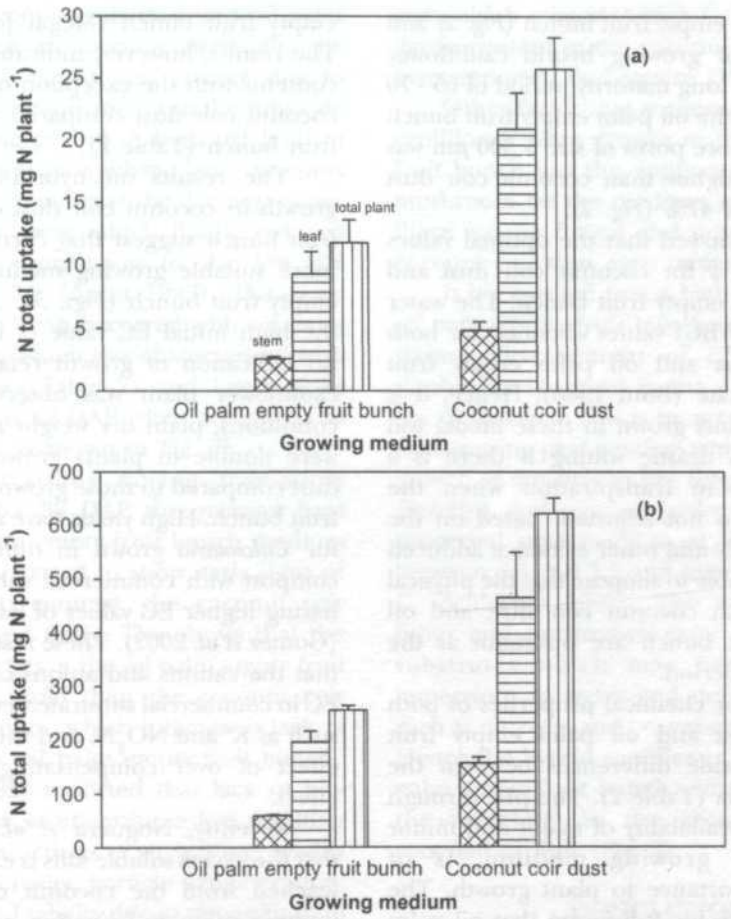

Fig. 5: Total nitrogen uptake in the leaf, stem and total plant of hybrid cauliflower plant grown in coconut coir dust or oil palm empty fruit bunch at 21 (a) and 42 (b) DAP supplied with 200 ppm N. The figures are means of 5 replications and standard errors are shown. Note the different scales of Y axes

empty fruit bunch did not show any problems to anchor of cauliflower plants.

Both the particle and bulk densities of oil palm empty fruit bunch were significantly higher than coconut coir dust (Table 1). These could be attributed to the different material composition of the two media. For most organic substrates, however the particle density is the parameter with the least quantum with respect to limitations on plant growth (Michiels *et al.* 1993).

The total pore space, which can be filled with water and air for both coconut coir dust and oil palm empty fruit bunch, was characteristically high (Table 1). Therefore both media should have sufficient amounts of air and available water for plant growth. The value obtained for coconut coir dust was 96 vol % which was close to the 95.3 vol % obtained by Prasad (1997) and between 95.1 to 96.3 vol % by Patricia *et al.* (1997) for coconut coir from Mexico and Sri Lanka. Furthermore, the total pore space has been reported to show small changes over time for black, white peat and sphagnum (Michiels *et al.* 1993) when using those substrates in an ebb/flood irrigation system.

Air filled porosity (AFP), readily available water (RAW), water buffer capacity (WBC) and less readily available water (LRAW) are the physical properties that have a direct effect on plant growth. It is usually recommended that air filled porosity (AFP) for growing medium in horticulture should be above 10-20% (De Boodt and Verdonck 1972; Bugbee and Frink 1986). The air content of the medium is particularly important when plants are grown for long periods of time in containers. In this study, the AFP was estimated to be above 25% in both coconut coir

dust and oil palm empty fruit bunch (*Fig. 2*) and thus sufficient for growing hybrid cauliflower plant which has a long maturity period of 65 - 70 days. The AFP of the oil palm empty fruit bunch which contains more pores of size > 300 μm was however, much higher than coconut coir dust with difference of 47% (*Fig. 2*).

The results showed that the optimal values of RAW were 34% for coconut coir dust and 19% for oil palm empty fruit bunch. The water buffer capacity (WBC) values obtained for both coconut coir dust and oil palm empty fruit bunch are adequate (Bunt 1988). Hence, it is expected that plants grown in these media will not be prone to drastic wilting if there is a sudden increase in transpiration when the watering regime is not adjusted. Based on the results of this study and other evidence adduced so far, it is reasonable to suppose that the physical properties of both coconut coir dust and oil palm empty fruit bunch are optimum at the start of growing period.

The results for chemical properties of both coconut coir dust and oil palm empty fruit bunch indicate wide differences between the two growing media (Table 2). The pH, through its effect on the availability of major and minor nutrients from growing medium, is of considerable importance to plant growth. The results shown in Table 2 indicate that oil palm empty fruit bunch is more alkaline (pH of 6.9) compared to coconut coir dust (pH of 5.3). This would favour coconut coir dust as a more suitable growing medium than oil palm empty fruit bunch since the optimal pH for plant growth in most substrates is 5.0–5.5 (Carlile 1997). On the other hand, high electrical conductivity (1.9 dS m^{-1}) for coconut coir dust may pose a problem by retarding plant growth. Fewer problems may occur with oil palm empty fruit bunch with its lower EC value of 1.3 dS m^{-1}.

Earlier (Evans *et al.* 1996; Meerow 1995) and recent studies (Abad *et al.* 2002) on coconut coir dust naturally occurring nutrients were reported to be low in mineral nitrogen (N), calcium (Ca^{2+}) and magnesium (Mg^{2+}) and can be improved by using fertilisers such as calcium nitrate, magnesium nitrate, magnesium sulphate or gypsum. These researchers also reported high levels of phosphorus (P) and potassium (K^+) content in coconut coir dust tested. Other researchers have also reported low levels of N, Mg^{2+} and P but high K^+ content in oil palm empty fruit bunch (Megat Johari *et al.* 1990). The results, however, indicated higher nutrient contents with the exception of total nitrogen in coconut coir dust compared to oil palm empty fruit bunch (Table 2).

The results on hybrid cauliflower plant growth in coconut coir dust or oil palm empty fruit bunch suggest that coconut coir dust is a more suitable growing medium than oil palm empty fruit bunch (*Figs. 3a, 3b* and 4). Despite the high initial EC value of coconut coir dust, no indication of growth retardation in hybrid cauliflower plant was observed. Under these conditions, plant dry weight and total leaf area were double in plants grown in coconut coir dust compared to those grown in oil palm empty fruit bunch. High yields have also been reported for *Calceolaria* grown in different mixtures of compost with commercial substrates (1:2 ratio) having higher EC values of 5.60 and 4.95 dS m^{-1} (Gomez *et al.* 2002). These researchers suggested that the cations and anions contributing to the EC in commercial substrate were mainly nutrients such as K^+ and NO_3^-N, and thus had a beneficial effect of over compensating for the osmotic effect.

Recently, Noguera *et al.* (2000) reported that the excess soluble salts is easily and effectively leached from the coconut coir dust material under customary irrigation regimes when used for ornamental plants in containers or 'growing bags' for tomatoes, flowers, etc., in garden greenhouses. In this study, it has been suggested that frequent fertigation applied to the medium may effectively leach out the excess soluble salt in coconut coir dust. Nevertheless, a high K^+ content in coconut coir dust as seen in Table 2 may contribute to a high EC but not to excess salinity, which may have caused an osmotic imbalance.

From the results of this study, it is postulated that the lower growth response of hybrid cauliflower plant to oil palm empty fruit bunch at 21 DAP could be due to high rates of immobilisation of soluble N by micro-organisms in the oil palm empty fruit bunch medium. This is reflected in lower total N uptake in the leaf and stem of plants grown in oil palm empty fruit bunch in comparison to those grown in coconut coir dust (*Figs. 5a* and *5b*). Thus, it was assumed that the liquid feed of 200 ppm N applied to the oil palm empty fruit bunch medium was not enough to compensate for a consistently high rate of N immobilisation.

Since both coconut coir dust and oil palm empty fruit bunch are organic materials, the properties of both media may change due to decomposition of organic matter in the substrate during the growing period. A high pH level of 5.9 to 6.9 resulting from applying large amounts of lime was shown to increase the decomposition rate of sphagnum peat, which finally reduced plant growth in comparison to the low pH between 4.4 and 5.2 (Anon 1997). The same phenomenon may have occurred with oil palm empty fruit bunch which was characterised with a high pH of 6.9 (Table 2) and lower plant growth response at 42 DAP when compared to coconut coir dust as shown in *Fig. 3b.*

Visual observations during this study indicated that after 30 DAP the growing bags containing oil palm empty fruit bunch medium flattened out and started to show early signs of decomposition. In contrast, the coconut coir dust medium stayed fluffy. This shows that the changes in properties of the oil palm empty fruit bunch are more rapid than the coconut coir dust growing medium, which indicates a lack of bio-stability in the oil palm empty fruit bunch.

Lemaire (1995) reported that lack of bio-stability may cause severe volume loss resulting in compaction, reductions in air volume, readily available water, porosity, particle size alteration, increases in pH and salinity due to mineralization and also change in the gaseous phase composition due to carbon dioxide production. These changes in properties may finally reduce the plant growth (Lemaire 1995). Thus the low hybrid cauliflower plant growth in the oil palm empty fruit bunch observed in this study may be due to the changes in the properties during the growing period.

High content of lignin, between 37% and 50% dry weight, was reported by Abad *et al.* (2002) in the coconut coir dust from different sources whilst lower values of 22% and 25% in the oil palm empty fruit bunch were reported by Zainon *et al.* 1998 and Megat Johari *et al.* 1990. Lignin is resistant to microbial degradation. Thus coconut coir dust is most likely to be more bio-stable than oil palm empty fruit bunch due to CD's higher lignin content. Evidence of a very high bio-stability index of 100 for coconut coir dust, which related to high C/N ratios, and high lignin content in the coconut coir dust has also been reported by Lemaire (1997).

Other factor that account for the low hybrid cauliflower plant growth in the oil palm empty fruit bunch was the appearance of fungus and mushroom on the medium surface. In contrast there was no fungal and mushroom growth in coconut coir dust even until 42 DAP.

It is expected that a high pH of 6.9 for the oil palm empty fruit bunch may be suitable for strong development of certain fungi and mushroom observed in the present study (*Figs. 6a, 6b* and *6c*). This is in agreement with other studies using peat medium which provide optimal growth of mushroom like fungi, cup fungi (*order Pezizales*) and agarics (*order Agaricales*) in the processed peat medium at high pH values of between 6.5 and 7.5 and temperature above 20° C (Schlechte 1997). The mycelium of these fungi and mushroom grow vigorously in the substrates which may become hardened, impervious to water and deficient in nutrients such as nitrogen and potassium (Schlechte 1997). Hence the hybrid cauliflower plant grown in oil palm empty fruit bunch with thick coverings of the mycelium in the growing bag showed retarded growth (*Fig. 3*).

CONCLUSIONS

The results presented show that the physical properties of both coconut coir dust and oil palm empty fruit bunch are optimum at the start of the growing periods. However, differences in chemical properties between the two media were more obvious compared to other properties. Under these conditions and with continuous liquid feed high in N content, the hybrid cauliflower grew better in 100 % coconut coir dust than 100% oil palm empty fruit bunch. Moreover, coconut coir dust was free from fungi and mushroom-like fungi during the growing period of cauliflower. It can be concluded that coconut coir dust can be used directly as growing medium whereas oil palm empty fruit bunch has to be composted, to achieve biostability. Hence, coconut coir dust is a more suitable growing medium for growing hybrid cauliflower than oil palm empty fruit bunch.

REFERENCES

ABAD, M., NOGUERA, M.D. MARTINEZ-HERRERO, F. FORNES and J.C. MARTINEZ. 1989. Physical and chemical properties of sedge peat-based media and their relation to plant growth. *Acta Hort.* **238:** 45-46.

ABAD, M., P. NOGUERA, R. PUCHADES, A. MAQUIEIRA and NOGUERA. 2002. Physico-chemical and chemical properties of some coconut coir dusts for use as a peat substitute for containerised ornamental plants. *Bioresource Tech.* **82:** 241-245.

ANON. 1997. Decomposition of peat substrates in relation to physical properties and growth of chamaecyparis. *Acta Hort.* **450:** 191-198.

BLAKE, G.R. and K.H. HARTAGE. 1986. Particle density. In *Methods of Soil Analysis, Part 1. Physical and Mineralogical Methods,* ed. A. Klute, p. 377- 382. USA: Madison, Winconsin.

BUGBEE, G.J. and C.R. FRINK. 1986. Aeration of potting media and plant growth. *Soil Sci.* **141(6):** 438-441.

BUNT, A.C. 1961. Some physical properties of pot-plant composts and their effect on plant growth. *Plant Soil.* **13:** 322-332.

BUNT, A.C. 1988. *Media and Mixes for Container-Grown Plants.* London: Unwin Hyman.

CARLILE, W.R. 1997. The requirements of growing media. In *Peat in Horticulture-its Use and Sustainability. Proc. International Peat Conference,* ed. G. Schmilewski, p. 17-23. Amsterdam.

COOPER, A. 1979. *The ABC of NFT (Nutrient Film Technique)*, London: Grower Books.

DE BOODT, M. and O. VERDONCK. 1972. The physical properties of the substrates in horticulture. *Acta Hort.* **26:** 37-44.

DE BOODT, M., O. VERDONCK and I. CAPPAERT. 1974. Method for measuring the water release curve of organic substrates. *Acta Hort.* **37:** 2054-2062.

EVANS, M.R., S. KONDURU and R.H. STAMPS. 1996. Source variation in physical and chemical properties of coconut coir dust. *Hort Sci.* **31:** 965-967.

GOMEZ, A.G., M.P. BERNAL and A. ROIG. 2002. Growth of ornamental plants in two composts prepared from agroindustrial wastes. *Bioresource Tech.* **83:** 81-87.

HANDRECK, K.A. 1993. Properties of coir dust, and its use in the formulation of soilless potting medium. *Comm. Soil Sci. Plant Anal.* **24:** 349-363.

LEMAIRE, F. 1995. Physical, chemical and biological properties of growing medium. *Acta Hort.* **396:** 273-284.

LEMAIRE, F. 1997. The problem of the biostability in organic substrates. *Acta Hort.* **450:** 63-69.

MEEROW, A.W. 1994. Growth of two subtropical ornamentals using coir dust (coconut mesocarp pith) as a peat substitute. *Hort Sci.* **29:** 1484-1486.

MEEROW, A.W. 1995. Growth of two tropical foliage plants using coir dust as a container medium amendment. *Hort. Tech.* **5:** 237-239.

MEGAT JOHARI, M.M., AHMAD JUSOH and G. ABDUL HALIM. 1990. Land application. In *Management and Utilization of Oil Palm Wastes. A Review,* p. 25-33 Selangor: Malindo Publisher.

MICHIELS, P., R. HARTMANN and C. CAUSSENS. 1993. Physical properties of peat substrates in an EBB/Flood irrigation system. *Acta Hort.* **342:** 205-219.

NOGUERA, P., M. ABAD, V. NOGUERA, R. PUCHADES and A. MAQUIEIRA. 2000. Coconut coir waste, a new and viable ecologically-friendly peat substitute. *Acta Hort.* **517:** 279-286.

PATRICIA, N., M. ABAD, R. PUCHADES, V. NOGUERA, J. MARTINEZ and A. MAQUIEIRA. 1997. Physical and chemical properties of coir waste and their relation to plant growth. *Acta Hort.* **450:** 365-373.

PRASAD, M. 1997. Physical, chemical and biological properties of coir dust. *Acta Hort.* **450:** 21-29.

RADJAGUGUK, B., SOEKOTJO, O.H. SOESENO and SANTOSO. 1983. A comparative study of peats and other media for containerized forest seedlings. *Acta Hort.* **150:** 449-458.

REYNOLDS, S.G. 1973. Preliminary studies in Western Samoa using various parts of the coconut palm (*Cocos nucifera* L.) as growing media. *Acta Hort.* **37:** 1983-1991.

ROBERTS, R.M., C.F. WILLIAM and R.A. LARSON. 1989. Hydrology of horticultural substrates: I. Mathematical models for moisture characteristics of horticultural container Media. *J. Am. Soc. Hort. Sci.* **114(1):** 48-52.

SCHLECHTE, G.B. 1997. Trouble creating fungi in peat of horticultural use. In Schmilewski, G. ed. In *Proc. Of the Int. Peat Conference: Peat in Horticulture-it's Use and Sustainability.* ed. G. Schmilewski, p. 89-93. 2-7 Nov. Amsterdam, Netherland. Paino Porrasoy Jyvaskyla, Finland.

ULRICH, B. 1996. Physical properties of different potting media and substrate mixtures-especially air and water capacity. *Acta Hort.* **450:** 263-270.

YAHYA, A. and I. MOHD RAZI. 1996. The growth and flowering of some annual ornamentals on coconut dust. *Acta Hort.* **450:** 31-38.

YAHYA, A., H. SAFIE and S. AB. KAHAR. 1997. Properties of cocopeat-based growing media and their effects on two annual ornamentals. *J. Trop. Agr. and Food. Sc.* **25(2):** 151-157.

ZAINON, O., A. MAT RASOL and H. HASSAN. 1998. Irradiation effect on chemical components of oil palm empty fruit bunch and palm press fibre. *Nuclear Sci. J. Malaysia* **16(1):** 21-30.

(Received: 21 October 2003)
(Accepted: 1 June 2005)

Pertanika J. Trop. Agric. Sci. 27(2): 135 - 142 (2004) ISSN: 1511-3701

Effect of Repeated Applications of Fipronil on Arthropod Populations in Experimental Plot Studies

ISMAIL B. S., NURAZIAH A., NOR-AINI D. & MAIMON A.
School of Environmental and Natural Resource Sciences
Universiti Kebangsaan Malaysia
43600 UKM, Bangi, Selangor, Malaysia

Keywords: Arthropod, fipronil, soil

ABSTRAK

Kesan dua kali semburan fipronil ke atas populasi Arthropod telah dikaji di plot kajian menggunakan Cuphea ignea *yang berusia 3 bulan. Lapan puluh satu famili daripada 12 order Arthropod telah diperangkap sebelum disembur. Empat order dominan telah dikenal pasti iaitu Hymenoptera (28.6%), Homoptera (19.1%), Collembola (17.8%) dan Diptera (16.2%). Beberapa order lain telah dikenal pasti wujud dalam bilangan yang rendah iaitu Hemiptera, Coleoptera, Orthoptera, Thysanoptera, Araneida, Acarina, Lepidoptera and Isopoda. Selepas semburan pertama dan kedua, jumlah famili dalam order Arthropod telah berkurang masing-masing kepada 44 dan 47 famili. Peratus populasi Collembola meningkat secara bererti selepas semburan pertama dan kedua berbanding bilangannya sebelum rawatan diberikan. Peratus populasi Homoptera (Aleyrodidae) pula meningkat selepas semburan pertama tetapi peratusannya menurun selepas semburan kedua. Famili Isotomidae (Collembola) meningkat secara signifikan selepas semburan pertama dan kedua. Beberapa order lain seperti Isopoda dan Lepidoptera telah tidak dapat dikesan selepas plot dirawat dengan fipronil.*

ABSTRACT

The effect of two applications of fipronil on arthropod populations were studied under experimental plot conditions using 3-month old Cuphea ignea. *Eighty-one families belonging to 12 orders of Arthropoda were trapped before spraying. The four dominant orders were Hymenoptera (28.6%), Homoptera (19.1%), Collembola (17.8%) and Diptera (16.2%). Other orders were present in small numbers i.e. Hemiptera, Coleoptera, Orthoptera, Thysanoptera, Araneida, Acarina, Lepidoptera and Isopoda. The abundance of arthropods was reduced to 44 and 47 families after the first and second sprayings, respectively. The percentage population of Collembola increased significantly after the first and second sprayings as compared to the number before treatment. The percentage population of Homoptera (Aleyrodidae) increased after the first spray but declined after the second spray. The family Isotomidae (Collembola) increased significantly after the first and second sprays. Some orders such as Isopoda and Lepidoptera disappeared after the plot was treated with fipronil.*

INTRODUCTION

Arthropoda belongs to a large phylum of invertebrate organisms that include crustaceas, mites, millipedes, centipedes, and insects such as springtails, proturans, diplurans, beetles, flies, ants, and termites. Some arthropods are beneficial (soil aeration, nutrient release), but some are considered pests to crops. Although only approximately 1% of the arthropods are pests to crops and flowers in the nursery, they can cause yield reduction between 5-15% (Davidson and Lyon 1987).

Pesticide application may not only kill the target organisms but also non-target and beneficial organisms. Mullie *et al.* (1999) reported that 26 species of arthropods such as Carabidae and Tenebrionidae were killed after the applications of Cyanox and Fenthion. Chlorpyrifos, an organophosphate insecticide, was reported to be very toxic to the parasitoid Hymenoptera (Pussey *et al.* 1994; Cohen *et al.* 1996; Viggiani 2000).

Horticultural nurseries are an ecosystem having a variety of arthropods, each with a specific role. The population of soil arthropods

has a positive correlation with soil properties, for instance, the Isopoda, Diplopoda and Staphylinidae have positive correlations with the availability of K and P in the soil (Danxiao *et al.* 1999). Furthermore, the populations of the arthropods are not necessarily the same in different ecosystems due to variability in abiotic factors.

Fipronil ((±)-5-amino-(2,6-dichloro-α,α,α-trifluro-p-tolyl-)-trifluoromethyl-sulfinylpyrazole-3-carbonitrilephenylpyrazole) is a phenylpyrazole insecticide developed by Rhone-Poulenc (Bobe and Cooper 1998). It is a highly effective and broad-spectrum insecticide against piercing-sucking, contact and chewing, pests and is widely used to control many species of soil and foliar insects on various crops such as rice, vegetables and fruits (Colliot and Kukorowski 1992; Balanca and de Visscher 1997).

Recent experiments showed that fipronil provided efficient protection against vegetable pests such as *Pieris rapae* and *Plutella xylostela* (Zhou and Wu 1995; Stevens and Helliwell 1998; Zhou *et al.* 2004). Since organophosphorus pesticides are gradually limited in application due to their high toxicity threaten human health and long-term residue in vegetables, it seems that organophosphorous pesticides would be replaced by fipronil in future.

Many horticultural nurseries in Malaysia especially those propagating *Cuphea ignea* use fipronil for pest control. Usually, this compound is used repeatedly to control pests but no reports on the effect of this insecticide on arthropods' populations has been documented. To the best of our knowledge, there are no published reports on the effects of fipronil on arthropod populations in Malaysia. The result of this study would provide a clear picture on the impact of this insecticide, particularly on arthropod populations. Therefore, the aim of this study was to determine the effects of repeated applications of fipronil on arthropod populations under experimental plot conditions.

MATERIALS AND METHODS

Study Site

The study was carried out at the experimental plots of the Universiti Kebangsaan Malaysia at Bangi, Selangor, Malaysia. Three month old *Cuphea ignea* seedlings were planted in black polybags and arranged at 30-cm intervals. Light intensity and humidity were measured by using a photometer (LI-COR Model LI-189) and Hygrometer (Hanna Instrument Model H18565), respectively. Soil and air temperatures, pH and organic contents of the soil used were also recorded.

Sampling of the Arthropods

Samplings of the arthropods were carried out for 4 months at 2-weekly intervals starting from 7 January 1999 prior to treatment. Arthropods were trapped using the pitfall trap and the yellow pan trap. The pitfall trap was used to trap invertebrates crawling on the soil surface. In our initial trials, it was found that very few specimens were collected from the soil samples, thus the pitfall trap and yellow pan traps were deemed suffieient and suitable for the purpose of this study. Ten holes were dug between the plastic bags in the plot and the trap comprising a glass bottle (4.5 x 4 cm) was placed in each hole. The yellow pan traps (petri dishes painted in yellow) were used for trapping flying arthropods and those crawling on leaves. The traps were placed randomly in the plots. Teepol (10%) was placed in each trap and left for 24 hours. The trap catches were brought back to the laboratory, sorted and identified to the family level using Borror *et al.* (1981) and Goulet and Huber (1993).

Fipronil (Regent 3G®, manufactured by Rhone Pholenc) was sprayed on 22 April 1999, at the rate of 0.03-kg a.i/ha. The spraying volume was 1 L per 10 m^2 using a knapsack sprayer at 12 kPa. Sampling recommended 1 WAT and continued at 2-weekly intervals. The second treatment was applied on 17 June 1999, and sampling was continued for another two months.

RESULTS AND DISCUSSSION

Fig. 1 shows the percentage of arthropods in the plot before and after treatment with fipronil. The four most dominant orders found before spraying were Hymenoptera (28.7%), Homoptera (19.1%), Collembola (17.8%) and Diptera (16.3%); the prespraying abundance of the orders Thysanoptera, Acarina, Coleoptera and Araneida were 7.7, 4.7, 2.3 and 2.1% of total arthropods, respectively. Four orders, namely Orthoptera, Hemiptera, Lepidoptera and Isopoda, were present at less than 1%. The percentage population of Collembola significantly increased after the first and second sprays, whilst the population of Hymenoptera significantly

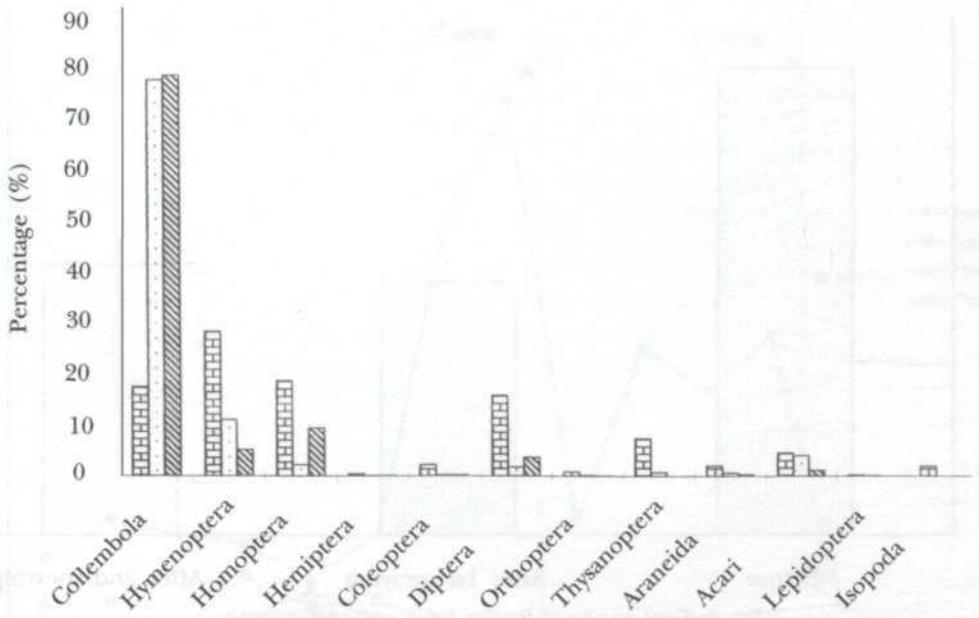

Fig. 1: Arthropod community in Cuphea ignea *before and after spraying with fiproni*

decreased after each treatment with fipronil. For Homoptera and Diptera, the population significantly decreased after the first spray but recovered slightly after the second spray. Generally, the percentage of the remaining arthropod populations decreased after the first and second treatments.

Fig. 2 shows the total number of arthropod families before and after treatment. The number of arthropod families was 81 before treatment, decreasing to 44 and 47 after the first and second sprays, respectively. The number of families belonging to Diptera and Hymenoptera recorded before treatments were 24 and 20, respectively, whilst Homoptera and Coleoptera had 9 families each.

Only 4 families of Collembola were found in the plot, but the number of individuals sampled was large. Hemiptera and Acarina were represented by 3 families each before treatment: Araneida, 6 families; and Lepidoptera, Thysanoptera and Isopoda, 1 each.

This paper reports in detail changes in the families representing only the four dominant orders (> 10% of the total populations), namely Hymenoptera, Homoptera, Collembola and Diptera. Some families of these orders were present only in small numbers during the sampling period.

Fig. 3 shows the population of Collembola families before treatment with fipronil and after the first and second treatments. After the first treatment, a larger number of Collembola were observed than of other orders (Fig. 1). Only four families of Collembola were trapped: Entomobyiidae, Isotomidae, Poduridae and Sminthuridae. Before treatment, the total number of Isotomidae was 60, but it increased to 785 after the first spraying and to 938 after the second spraying. The total number of Entomobyiidae before spraying with fipronil was 296, but numbers decreased to 190 and 87 after the first and second sprayings, respectively. The total numbers of Poduridae and Sminthuridae were low prior to spraying but increased drastically after both sprayings. The total number of Poduridae was 20 before spraying. However, the number increased to 639 after the first spray, and then decreased to 121 after the second spray. For Sminthuridae, the total number before spraying was 73, increasing to 394 after the first spraying, before decreasing to 140 after the second spray. The fluctuations in population abundance of Poduridae and Sminthuridae indicate the short term effects of fipronil on population increase of the two families of Collembola.

Fig. 4 shows the effect of the two applications of fipronil on the population of Hymenoptera.

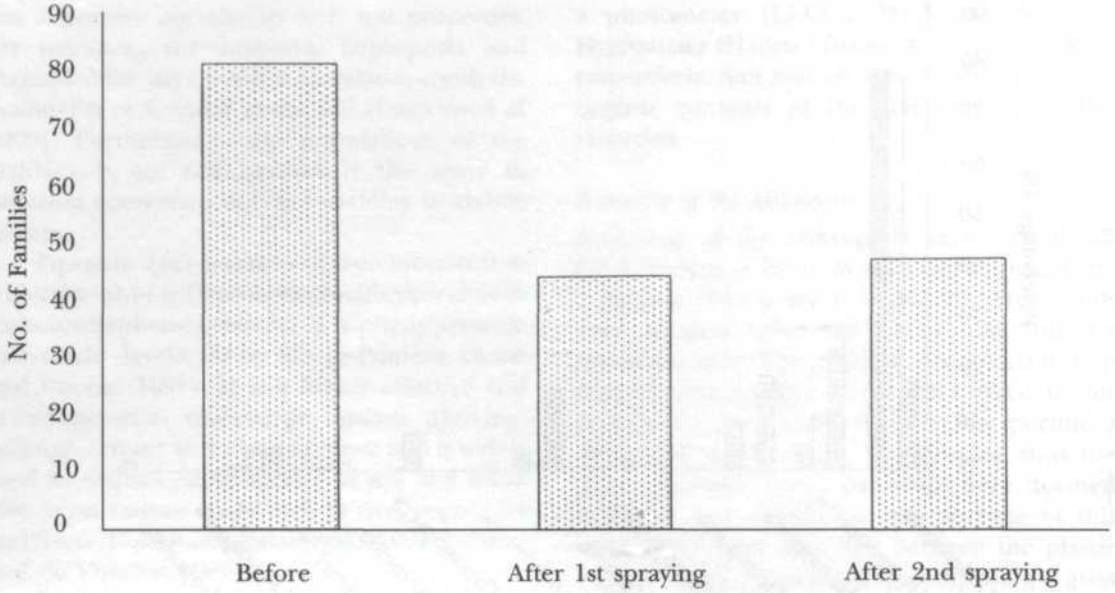

Fig. 2: Total number of families before and after spraying

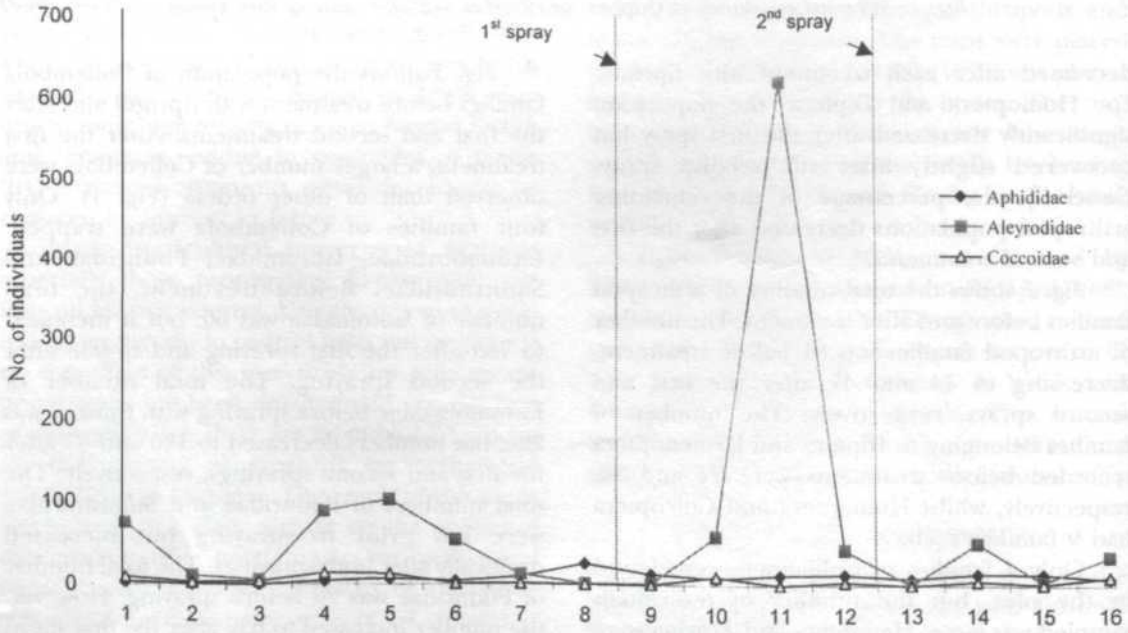

Fig. 3: Effect of repeated applications of fipronil on Collembola population

Twenty families of Hymenoptera were identified prior to the treatment, but this number decreased to 14 after the first spraying. The population of Formicidae was higher than that of other families of Hymenoptera. The highest number of Formicidae was observed at 8 weeks before treatment. The total number of Formicidae before treatment was 400, but it decreased to 254 and 34 after the first and second treatments respectively. Other families of Hymenoptera were present in small numbers before treatment, and their populations similarly decreased after the first and second treatments. Before spraying with fipronil, the total numbers of Scelionidae, Mymaridae and Ceraphronidae were 72, 38 and 33, respectively. The numbers of Mymaridae were reduced to 10 and 74, and of Scelionidae, to 8 and 7 after the first and second sprayings,

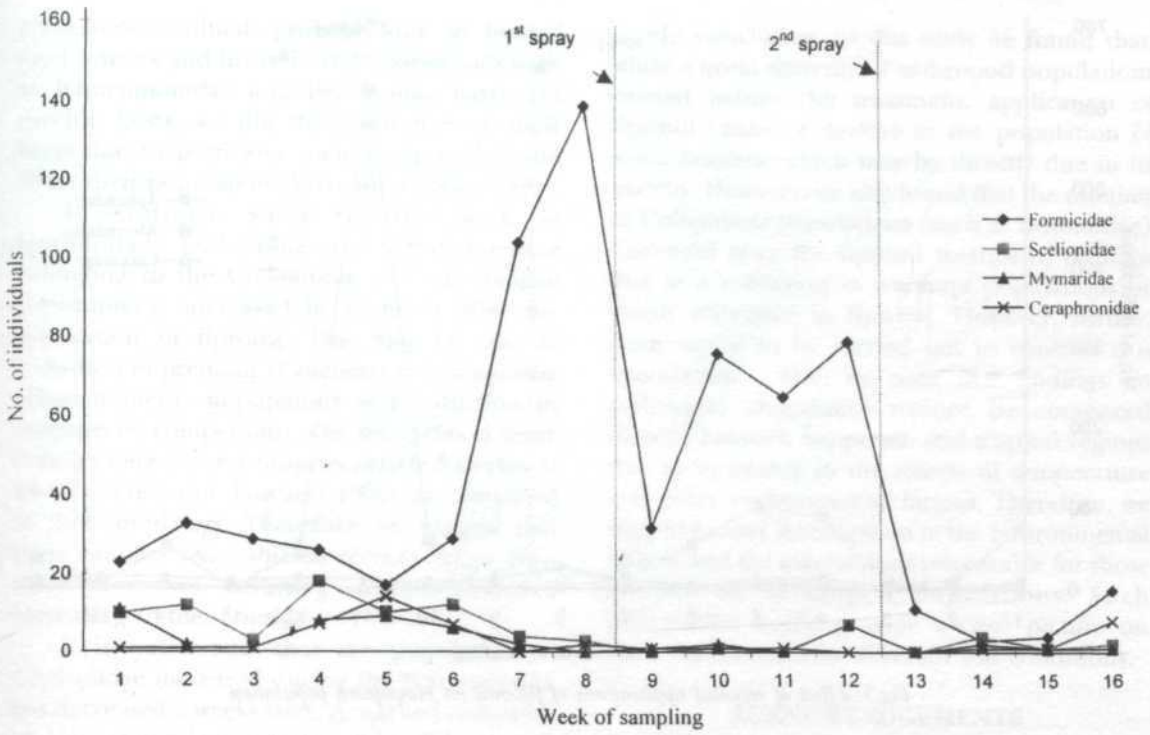

Fig. 4: Effect of repeated applications of fipronil on Hymenoptera population

respectively, whilst the Ceraphronidae were reduced from 33 prespraying to 3 after the first spraying, but this number increased after the second spraying.

Fig. 5 shows the effect of two applications of fipronil on Homoptera populations. The seven families of Homoptera found before the first spraying decreased to 3 after the first spray. The population of Aleyrodidae was comparatively higher than those of the other families after spraying with fipronil. The total number of Homopterans trapped before spraying was 353, but this number increased to 704 after the first spraying and subsequently decreased to 91 after the second spraying. Before treatment, the total numbers of Aphididae and Coccoidae were 55 and 47, respectively. After the first and second sprayings, the numbers were reduced to 46 and 44 for Aphididae and 14 and 17 for Coccoidae, respectively.

Fig. 6 shows the population of Diptera before and after treatment with fipronil. Diptera was represented by 24 families before treatment, but this number was reduced to 7 and 9 families after the first and second sprayings, respectively. However, many of the families existed in small numbers. The dominant family of Diptera was Phoridae, with a total of 157 individuals trapped before spraying. This was followed by Cecidomyiidae, Agromyzidae and Chironomidae (58, 52 and 49 individuals, respectively). After the first and second sprayings, many of the families disappeared. The total numbers of the dominant Phoridae were only 20 and 10 after the first and second sprayings, respectively.

Twelve orders and 81 families were represented in the 2,521 arthropods observed in the plot before spraying with fipronil. Most arthropod families belonged to the order of Hymenoptera, but many from other orders were also present in the plot. These results indicate that fipronil applications caused a shift in the population of arthropods in the experimental plot. The numbers of families decreased after applications of fipronil, and some families disappeared.

This work also shows that the number of individuals from the three families of Acarina (namely Tetranychidae, Ixodidae and Oribatei) declined significantly after the second spraying. The total numbers of Tetranychidae, Ixodidae and Oribatei were 97, 11 and 13, respectively, after the first spraying, but decreased significantly to 7, 6 and 5 after the second spraying.

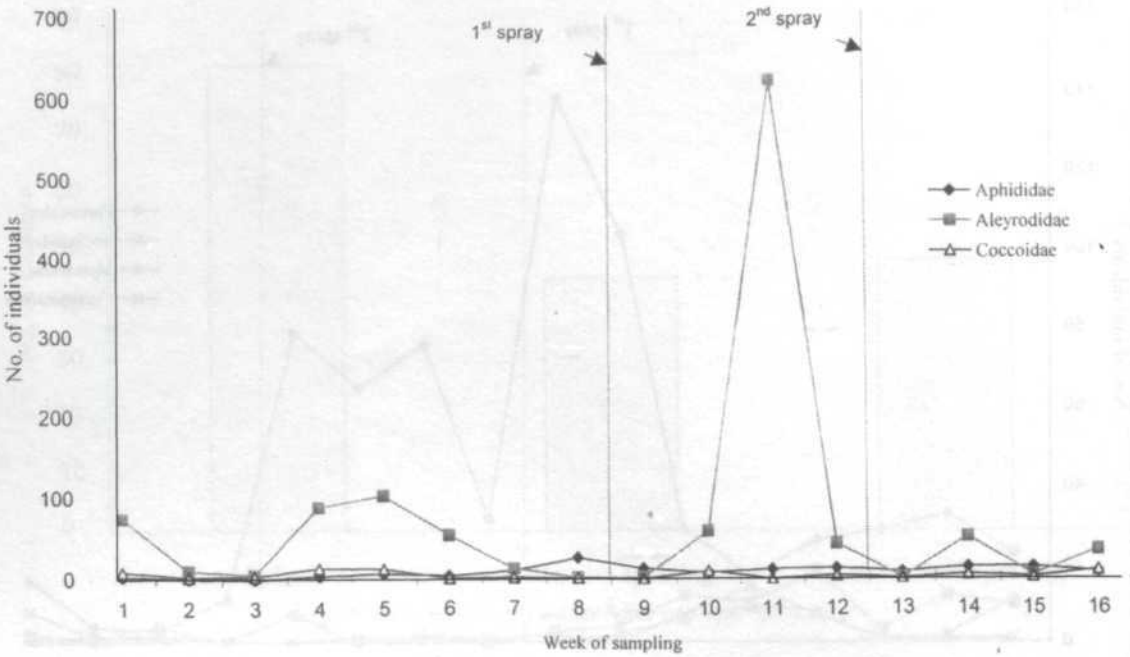

Fig.5: Effect of repeated applications of fipronil on Homoptera population

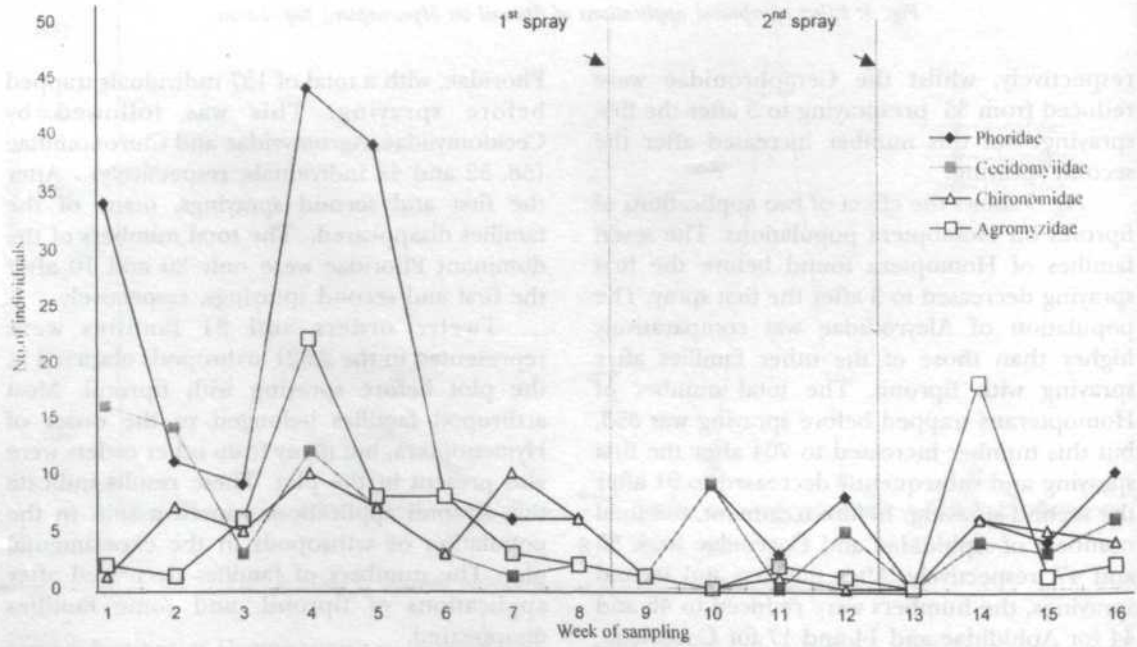

Fig. 6: Effect of repeated applications of fipronil on Diptera population

Application of fipronil may reduce some predators and parasitoid populations due to the reduction in host populations. For instance, a decrease in pest populations such as Homoptera, Hemiptera, Acarina and Orthoptera would cause a reduction in certain predators, such as Staphylinidae (Coleoptera), and in such parasitoids as Scelionidae and Pteromalidae (Hymenoptera).

Our results show that no Staphylinidae was detected after the second spraying, while for the Pteromalidae and Scelionidae, the total numbers obtained after the second spraying was 1 and 7, respectively. Populations of these predators or

parasitoids declined, probably due to limited food sources and hosts. Certain parasitoids such as Ichneumonidae and Braconidae have very specific hosts, so the disappearence of their hosts due to pesticides such as fipronil would affect their population (Idris and Grafius 1993).

In contrast, some families such as Isotomidae, Poduridae and Sminthuridae belonging to the Collembola and Aleyrodidae (Homoptera) increased in numbers after the application of fipronil. This may be due to reduction in predator (Coleoptera) and parasite (Hymenoptera) populations and reduction in interspecies competition. The life cycles of these families were shorter (approximately 3 weeks) at 24-30°C (Yee and Toscano 1996) as compared to their predators. Therefore, we suggest that these families were able to recover faster than their predators, resulting in their number increasing tremendously.

It is noteworthy that the population of Aleyrodidae increased during the first sampling but decreased 2 weeks later. A marked reduction of Aleyrodidae population could well be due to different numbers being in the larval stage during the two samplings. The two methods of sampling used were only able to trap adults, not larvae. A rise in the Aphelinidae population (Hymenoptera), which is a parasitoid for Aleyrodidae, may also have contributed to the reduction in the Aleyrodidae population.

The experimental results have shown that there is no correlation between abiotic factors (such as temperature and pH) and the abundance of arthropod populations (data not shown). This observation is different from results reported in similar research in temperate regions (Goulet and Huber 1993; Davey *et al.* 1998; Danxiao *et al.* 1999). These researchers report a positive correlation between soil arthropods (such as Isopoda and Formicidae) with salt content, available K and P and organic matter content of the soil. Abiotic factors, such as temperature during winter, may cause the life cycle of soil arthropods in temperate regions to be prolonged (Fujiyama 1996) when compared to the length of life cycles of soil arthropods in tropical regions. The temperature in the study plot ranged from 23° to 35°C, a temperature range suitable for their development. Therefore, the results concerning arthropod abundance cannot be compared directly between temperate and tropical regions due to variability in temperature effects.

In conclusion, in this study we found that, while a great diversity of arthropod populations existed before the treatment, application of fipronil caused a decline in the population of some families, which may be directly due to its toxicity. However, we also found that the number of Collembola populations (such as Isotomidae) increased after the fipronil treatment, perhaps due to a reduction in predator populations or family tolerance to fipronil. However, further study needs to be carried out to confirm this speculation. Also, we note that findings on arthropod abundance cannot be compared directly between temperate and tropical regions due to variability in the effects of temperature and other environmental factors. Therefore, we suggest further investigation of the environmental effects, and the mechanisms responsible for those effects, on arthropod populations. Such information would provide a clear picture on their distributions in different soil conditions.

ACKNOWLEDGEMENTS

This work was supported by research grant IRPA 08-02-02-0011 EA185 from the Ministry of Science, Technology and Innovation of Malaysia.

REFERENCES

BALANCA, G. and M.N. de VISSCHER. 1997. Effects of low doses of fipronil on grasshoppers and non-target insects following field trials for grasshopper control. *Crop Protection* **16:** 553-64.

BOBE, A. and J. F. COOPER. 1998. Behaviour of fipronil in soil under sahelian plain field conditions. *Pesticide Science* **52:** 275-81.

BORROR, D.J., D.M. DELONG and C.A. TRIPLEHORN. 1981. *An Introduction to the Study of Insects.* Philadelphia: CBS College Publishing.

COHEN, H., A.R. HOROWITZ, D. NESTEL and D. ROSEN. 1996. Susceptibility of the wooly apple aphid parasitoid, *Aphelinus mali* (Hymenoptera: Aphelinidae), to common pesticides used in apple orchards in Israel. *Entomophaga* **4:** 225-33.

COLLIOT, F. and K. A. KUKOROWSKI. 1992. Fipronil: a new soil and foliar broad spectrum insecticide. In *Proceedings of Brighton Crop Protection Conference, Pest and Diseases,* **II:** 29-34.

DANXIO, H., H. CHUNRU, X. YALING, H. BANWANG, H. LIYUAN and M.G. PAOLETTI. 1999. Relationship between soil arthropods and soil properties in the suburb of Qianjiang city, Hubei, China. *Critical Reviews in Plant Sciences* **18(3):** 467-73.

DAVEY, R.B., A.H. AHRENS, G.E. GEORGE, J.S. HUNTER and P.H. JEANNIN. 1998. Therapeutic and persistent efficacy of Fipronil against *Boophilus microplus* (Acari: Ixodidae) on cattle. *Veterinary Parasitology* **74:** 261-76.

DAVIDSON, R.H. and W.F. LYON. 1987. *Insect Pests of Farm, Garden and Orchard.* New York: John Wiley & Sons, Inc.

FUJIYAMA, S. 1996. Annual thermoperiod regulating and eight-year life cycle of a periodical diplopod, *Parafontaria laminata armigera* Verhoeff (Diplopod). *Pedobiologia* **40(6):** 541-7.

GOULET, H.J. and T.J. HUBER. 1993. *Hymenoptera of the World: An Identification Guide to Families.* Canada: Research Branch Agriculture Canada Publication. 1894/E.

IDRIS, A.B. and E. GRAFIUS. 1993. Field studies on the impact of pesticides on the diamondback moth, *Plutella xylostella* and parasitism by *Diadegma insulare. J. Econ. Entomol.* **86:** 1196-202.

MULLIE, W.C., A.O. DIALLO, B. GADJI and D. NDIAYE. 1999. Environmental hazards of mobile ground spraying with cyanophos and fenthion for quelea control in Senegal. *Ecotoxicology and Environmental Safety* **32:** 1-10.

PUSSEY, B.J., A.H. ARTHINGTON and J. MCLEAN. 1994. The effects of a pulse application of chlorpyrifos on microinvertebrate communities in an outdoor artificial stream system. *Ecotoxicology and Environmental Safety* **27:** 221-50.

STEVENS, M. M. and S. HELLIWELL. 1998. Fipronil seed treatments for control of chironomid larvae (Diptera: Chironomidae) in aerially-sown rice crops. *Field Crops Research* **57:** 195-207.

VIGGIANI, G. 2000. The role of parasitic Hymenoptera in integrated pest management in fruit orchards. *Crop Protection* **19:** 665-8.

YEE, W. L. and N.C. TOSCANO. 1996. Ovipositional preference and development of *Bemisia argentifolii* (Homoptera: Aleyrodidae) in relation to alfalfa. *J. Econ. Entomology* **89:** 870-876.

ZHAO, J.Z. and S.C. WU. 1995. Bioassays with recommended field concentrations of several insecticides for resistance monitoring in *Plutella xylostella. Resistant Pest Management* **7:** 13-4.

ZHAO, P., L. YITONG, L. BAOFENG and J.J. CHAN. 2004. Dynamics of fipronil residue in vegetable-field ecosystem. *Chemosphere* **57:** 1691-6.

(Received: 10 September 2003)
(Accepted: 3 August 2005)

Pertanika J. Trop. Agric. Sci. 27(2): 143 - 149 (2004) ISSN: 1511-3701

Effect of Partial Rootzone Drying (PRD) on Growth, Water Use Efficiency (WUE) and Yield of Tomatoes Grown in Soilless Culture

[1]HASSAN IBRAHIM ALI, [1]MOHD RAZI ISMAIL, HALIMI MOHD SAUD & [2]MOHD MOKHTARUDDIN MANAN

[1]Department of Crop Science,
[2]Department of Land Management,
Faculty of Agriculture,
Universiti Putra Malaysia 43400 UPM Serdang,
Selangor, Malaysia

Keywords: Partial rootzone drying (PRD), water use efficiency (WUE), yield, tomatoes

ABSTRAK

Satu kajian telah dijalankan di Jabatan Sains Tanaman, Universiti Putra Malaysia (UPM) untuk mengkaji pengaruh pengeringan separa akar menggunakan kultur tanpa tanah, campuran 70% habuk sabut kelapa dan gambut (3:2) ditambah dengan 30% kompos jerami padi. Tanaman tomato (Lycopersicon esculentum *Mill cv Red Rock) diberikan dua rawatan air, iaitu pengairan sepenuh (kawalan) dan pengairan secara pengeringan separa akar (PRD). Pengurangan kedapatan air dalam media secara PRD menyebabkan pengurangan signifikan bagi perkembangan daun, luas daun dan konduksi stomata. Prolina meningkat dengan PRD. Tidak terdapat pengurangan yang signifikan dalam pembahagian bahan kering tanaman dan hasil di antara pengairan penuh dan PRD. Kecekapan penggunaan air meningkat secara signifikan dengan PRD.*

ABSTRACT

An investigation was carried out at the Department of Crop Science, Universiti Putra Malaysia (UPM) to examine the effect of PRD using soilless media, a mixture of 70% coconut coir dust and peat (3:2 respectively) amended with 30% rice straw compost. Tomato (Lycopersicon esculentum *Mill cv Red Rock) plants were exposed to two different water treatments, which was either well-watered (control) or partially irrigated on half of the roots (PRD). Reduction in water availability in the media with PRD treatment caused a significant decrease in leaf expansion, leaf area and stomatal conductance. Proline was significantly increase with PRD. There was no significant reduction in dry matter partitioning and yield between well-watered and PRD-treated plants. Water use efficiency also was significantly increased with PRD.*

INTRODUCTION

Increasing water use efficiency (WUE) is one of the main strategic goals for the researchers as well as decision makers world wide due to water scarcity and continuing high demand of water for agricultural irrigation. The efficiency of utilization of irrigation water is often low leading to around 50% increase in the demand for water that could be met by increasing the effectiveness of irrigation. However, the agricultural irrigation uses over 70% of the world supplies of clean water and most of this clean water is especially used in the protected environments (Ismail and Razi 2002). The use of clean water and chemical solutions as fertilizers are very costly. In addition, the fast growing industrial sector competes with agriculture for water resources and the pollutants emitted were the source of underground water pollution and this will push the agricultural activities to remote areas where water and salinity are the major problems.

Tomato has more acreage than any vegetable crop in the world (Ho 1996) and is the second most common grown vegetable crop in Malaysia. Therefore, studying the effect of low cost irrigation technique such as partial rootzone drying (PRD) could make substantial contribution to saving water especially with soilless culture, since many studies conducted

under a protected environment showed the significance of the use of a soilless culture (Ismail and Razi 2002). Thus, the use of this low technological agronomic manipulation can also exploit recent understanding of plant functions and physiological basis of yield production under limited resources. In this way, yield can be sustained and resource use can be optimized.

PRD is a relatively new irrigation strategy, where at each irrigation time only a part of the root system is wetted with the complement being left to dry to a pre-determined level or time. It could save water by 50% and yet maintain yield as shown for some grape cultivars (Loveys *et al.* 2000). Implementing PRD technique is simple, requiring only the adaptation of irrigation systems to allow alternate wetting and drying part of the rootzone. Although the theory of PRD has been developed, little is known about how tomatoes growing under warm and humid climatic conditions will respond to this irrigation technique. However, it is also important to understand the basis of the plant's finely-tuned sensitivity to environmental stresses to overcome the problems by using either agronomic or genetic techniques and the advantages of crop growth and food production may be substantial. The objective of this study therefore, is to understand how PRD works within soilless media amended with rice straw compost through monitoring of water use efficiency; fruit yield and vegetative growth, as well as quantifying the impacts of PRD on proline accumulation within the leaf. The hypothesis is that PRD may decrease leaf area and growth of the plant without significant reduction in yield and hence, increase water use efficiency. Stomatal conductance will be significantly reduced and proline will be increased in response to PRD technique and that will be correlated with soil drying.

MATERIALS AND METHODS

Plant Materials

A study was conducted at the Department of Crop Science, Faculty of Agriculture, UPM, Malaysia. Tomato (*Lycopersicon esculentum* Mill.) cv Red Rock F_1 hybrid was used in this study. Seeds were sown on germination trays with media of peat amended with rice straw compost (3:1) and transplanted four weeks later. Seedlings with the same vigor were transplanted to double pots, where taproot was removed and the roots of each plant were approximately divided into two pots. The plants were placed under shade-house condition with daily average temperatures of 32 and 28°C day and night, respectively and average relative humidity of 65% and 80% day and night respectively. The plants were trained vertically, as single stems. Plants were also staked or trained by using raffia string tied to an overhead support. At weekly intervals, all auxiliary buds were removed. When the plants had produced a total of three trusses, the main growing stem was terminated at the point of two leaves after the final truss.

Treatments and Experimental Design

Soilless media (coconut coir dust and peat 3:2 v/v, respectively) 70%+ 30% rice straw compost were used in this study. Two treatments were used which were either irrigated to drip point daily (100% field capacity) with drip irrigation as a control (C) or partial irrigation PRD on half of the roots alternately (until the moisture content reached within 10% of the control plants). Each cycle of drying was 12 days. Each pot was irrigated with a single drip emitter, with one irrigation per day to maintain soil water close to field capacity. An auto timer was used and the amount of the water used for each treatment was monitored with flow meters placed in each irrigation line and calculated thereafter in kg. Half-Copper solution was used as fertigation fertilizer. The pots were raised to avoid direct contact with the ground and two weeks later the treatments started.

Parameters

Four pots from each replicate were selected randomly from either the both pots irrigated or half of the roots irrigated for determination of soil moisture content. The samples were taken from a depth of 0-5cm and oven dried at 90°C for 72 hours and the moisture content of the soil sample was determined.

Measurements were carried out on matured fully expanded leaves (leaf number four from the apex of the plant). The measurements were taken on the abaxial surface of the leaf daily between 11:00 and 14:00. All readings were accomplished within a one-hour period to avoid the diurnal pattern of variation in leaves, using a transit- time promoter (AP-4, Delta T Devices Ltd., Cambridge, UK). Similar leaves were used for leaf water potential determination using a pressure chamber (PMS, Soil Moisture

Equipment, Santa Barbara, USA). Leaf water potential was measured between 12:00 and 15:00.

Newly emerged leaves from four plants within each treatment were chosen randomly, labeled and tagged and the length of leaf blade was measured from the point of petiole insertion with the leaf blade to the tip of the leaf. The length was measured every two days at midday using a standard ruler.

Determination of free concentration of proline was based on the method described by Bates *et al.* (1973). Proline was extracted from liquid nitrogen-frozen tissue by homogenizing 0.5 g of sampled leaves with 10 ml of 3% aqueous solution of sulfosalsilic acid at 25°C. The homogenate was filtered through Whatman No. 2 filter paper. Two ml of the filtrate was reacted with two ml of glacial acetic acid and two-ml acidninhydrin in a test tube for one hour in a water bath at 95°C. The reaction mixture was then cooled in an ice bath. Following that, 4 ml of toluene was added to the reaction mixture and mixed vigorously with a test tube stirrer for 20 seconds. The toluene layer at the top, which is a pink-red color, was collected with a pipette. The absorbency of the toluene layer was read at 520 nm with a spectrophotometer using toluene as a blank. Standard curve was produced ranging from 0 to 30 g/ml of L-proline (Sigma Chemical Co., St. Louis, Mo.) dissolved in 3% sulfosalsilic acid. Proline standard curve was used to calculate proline concentrations in the samples on fresh weight basis.

A destructive sample method was used to determine leaf area and dry matter production (dry biomass). Total leaf area was measured in cm^2 using leaf area meter (Delta-T Cambridge, U.K.). Each plant part was put in a paper bag and placed in an oven at 85°C for 72 hr till a constant weight was reached for dry weight determination. Root to shoot ratio and total dry matter production in g per plant was calculated thereafter. Yield per plant was determined after each harvest from an average of ten plants.

Water use efficiency (WUE) was calculated for each treatment as function of the harvest yield and total dry biomass (shoot + roots) divided by the actual total amount of water irrigated as described by Kang *et al.* (2001).

$$WUE^1 = \frac{Yield(g)}{Gross\ irritation(kg)} \quad (1)$$

$$WUE^2 = \frac{Dry\ biomass(g)}{Gross\ irritation(kg)} \quad (2)$$

Statistical Design and Analysis of Data

The treatments (control (C) and PRD (P)) were arranged in a completely randomized design with three replicates. Data were analyzed using analysis of variance and means separation performed using least significant differences (LSD) at 0.05 levels. Both analyses were done using SAS (1997).

RESULTS AND DISCUSSION

Stomatal behavior varied significantly ($p< 0.05$) in response to PRD(*Fig.* 1). PRD significantly reduced the stomatal conductance gradually. This gradual reduction in stomatal was also shown in many studies with different crop species (Loveys *et al.* 1998; Stoll *et al.* 2000; Awad 2001) might be attributed to the signal coming from the dry part of the root system through the xylem stream. The signal, which may lead, to the partial closure of stomata of PRD plant may be attributed to root sourced chemical signals. Media drying presumably enhanced different hormones and enzymes such as proline and the accumulation resulted in stomatal closure and leaf growth restriction (*Fig. 6*).

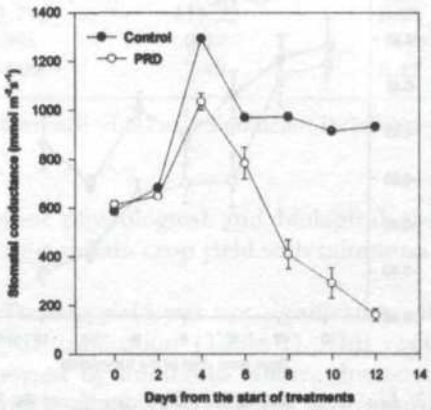

Fig. 1: Stomatal conductance as affected by PRD applications or tomato plants grown on soilless culture

Leaf water potential (LWP) showed different trend in response to PRD treatment (*Fig.* 2). This clearly suggested that LWP is strongly affected by plant age and the amount of water applied under environment of high evaporative demand occurred, although there were no visual symptoms due to desiccation in PRD treatment.

LWP values measured for control and PRD treatments as a mean for the whole cycle period were -0.41 and -0.51 MPa, respectively. Mean LWP value at day 0 was -0.39 MPa and reached –0.64 MPa on day 12. The minimum LWP observed with two tomato species under complete drying of media to all plant roots under water stress were –1.8 and –1.4 MPa for *L. esculentum* and *L. pennellii*, respectively after seven days of withholding water from the plants as stated by Torrecillas *et al.* (1995). In most split-root experiments, where half of the root was irrigated by 50% of the control, conducted under low evaporative demand (Ague' and Duan 1991; Blackman and Davies 1998; Zegbe-Dominguez *et al.* 2003) concluded that leaf water status did not change under water deficit. However, the maintenance of leaf water potential with decreasing soil water status is expected due to low evaporative demand of the atmosphere as reiterated by Hsiao (1990). This might be the reason why differences were not measurable or due to other limitations such as sensitivity of instrumentation, sporadic measurement of water status or the behavior of the stomata to maintain relatively stable leaf water potential during mild drought (Ague' and Moore 2002).

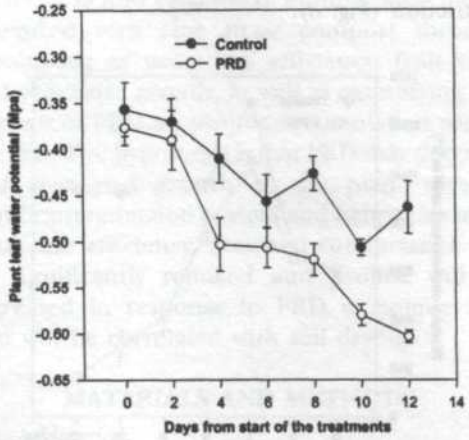

Fig. 2: Leaf water potential (LWP) as affected by PRD application for tomato plants grown on soilless culture

PRD significantly reduced leaf expansion (*Fig. 3*). There was also a significant relationship (r^2 = 0.98) between media drying and leaf expansion (*Fig. 6*). The concept of using PRD as a technique to control water deficit responses originated from observation that root-derived abscisic acid was an important factor in regulating grapevine stomatal conductance and leaf expansion in order to regulate shoot growth (Loveys 1991).

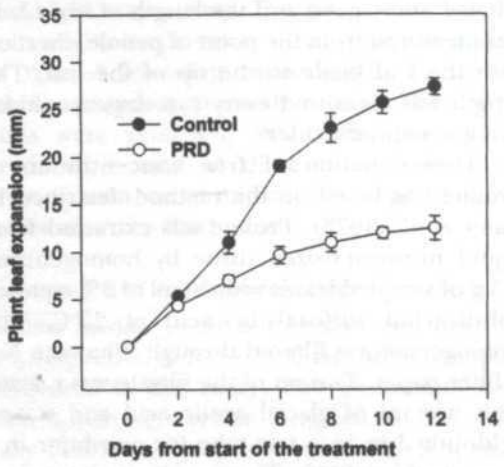

Fig. 3: Leaf expansion as affected by PRD application for tomato plants grown on soilless culture

Free proline accumulation seems to be a widespread stress response in higher plants, which can reach very high levels within a short time after stress induction (Gzik 1995). This accumulation is always induced by hydraulic stress for osmotic adaptation. However, little was known about the role of non-hydraulic signaling in responses to PRD in accumulation of the proline. However, proline increased significantly in response to PRD (*Fig. 4*). This indicates that proline accumulation was dramatically influenced by the root drying. The increase in proline content in stressed plants parts was predominantly due to *de novo* synthesis (Gzik 1995). Therefore, understanding the mechanism behind the accumulation of proline in response to PRD under non-hydraulic signaling needs further clarification.

PRD significantly reduced leaf area as shown in Table 1. The reduction of leaf area of PRD plant was almost 13% compared to control plants. These results were quite similar to those observed with PRD tomato plants (Davies *et al.* 2000). Dry biomass, dry shoot and root weights and root to shoot ratio were not significantly different between them for both PRD and control plants. There was very little data that suggested that root growth can actually be increased by soil drying in support of this present study. Authors that do (Sharp and Davies 1979), attribute such effects to a stress of particular magnitude which

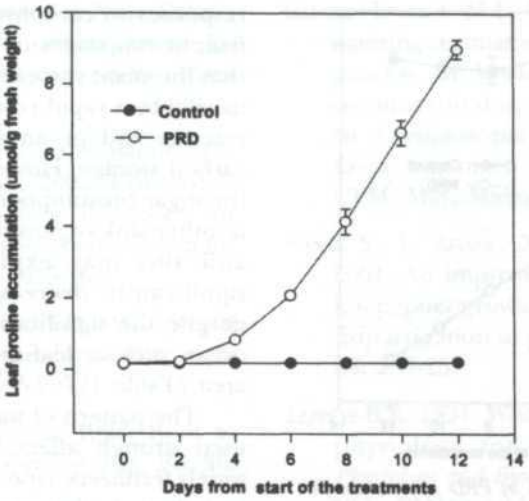

Fig. 4: Proline accumulation as affected by PRD application for tomato plants grown on soilless culture

TABLE 1
Effect of partial rootzone drying system (PRD) on leaf area, dry matter partitioning, total and marketable yields and water use efficiency (WUE) of tomato plant grown on soilless culture

Parameter	Control	PRD	LSD	C.V.
Leaf area (cm^2)	1739.53a	1507.45b	156.7	4.26
Dry whole plant biomass (g)	45.67a	41.67a	9.39	9.49
Dry shoot wt. (g)	40.00a	35.17a	6.58	7.72
Dry root wt. (g)	5.67a	6.50a	3.31	24.02
Root to shoot ratio	0.14a	0.19a	0.07	19.52
Total yield (g)	852.8a	744.30a	292.12	16.14
Marketable yield (g)	786.76a	721.77a	113.32	6.63
Water use efficiency1 (g/kg)	1.56b	2.39a	0.47	10.40
Water use efficiency2 (g/kg)	34.48b	43.99a	7.49	8.42

Means with the same letter in the same row is not significant difference with Least Significant Difference (LSD) at $p< 0.05$.

results in increased availability of assimilates to roots, as shoot growth is limited by water deficit in the absence of any effect on carbon gain. More recently, however, Mingo (2003) reported that root growth can be stimulated when roots are dehydrated after a drying episode, relative to roots in moist soil.

In the well-watered plants in which both sides of the roots were irrigated, moisture content remained high. Moisture content decreased progressively in PRD with time until 10% of the control plants (*Fig. 5*). This suggested that during the stress cycle, part of the plant received sufficient water when the other part received a sign of water deficit conditions resulting in different physiological and biological changes, and thus sustain crop yield with minimum water use.

Tomato yield was not significantly affected by PRD application (Table 1). This result was supported by numerous studies demonstrating that PRD application resulted in no significant reduction of crop yield (Loveys 1991). Recent evidence had showed that fruit growth was regulated by non-hydraulic regulations (Mingo *et al.* 2003). They concluded that restrictions in fruit growth rate in plant growing in a partial drying soil can occur in the absence of any changes in fruit cellular turgor. It was suggested that signals borne within the xylem can travel

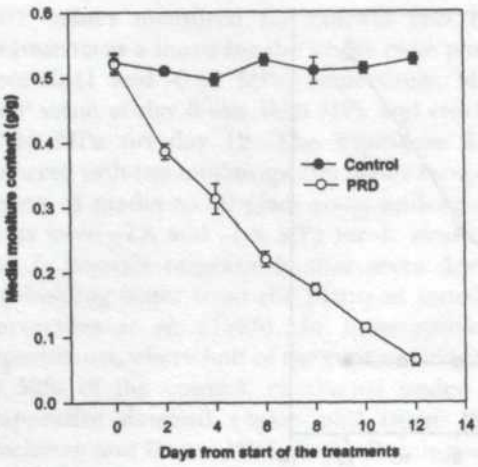

Fig. 5: Media drying as affected by PRD application for tomato plants grown on soilless culture

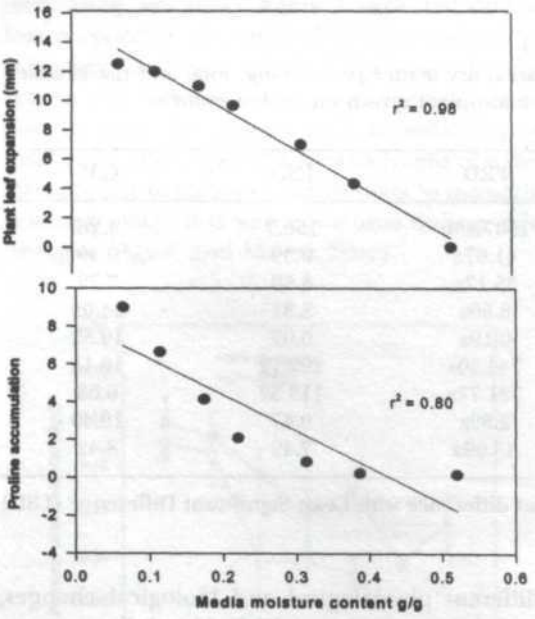

Fig. 6: Relationships between media drying, proline accumulation and leaf expansion as affected by PRD application for tomato plants grown on soilless culture

from root-to-shoot and shoot-to-fruit to elicit a powerful regulatory effect on fruit cell expansion. Other evidence might be that carbohydrate limitations, as showed in this study (data not shown) increased in both leaf and fruit in responses to PRD application. This might be due to maintenance of carbohydrate either directly by some active mechanism or indirectly via a relative increase in the sink strength of the fruit (Davies *et al.* 2000). This idea was strongly supported by Baldet *et al.* (2002) who contrasted responses to carbohydrate limitation in tomato fruit at two stages of development. They stated that the plant responses to sugar depletion were usually by a rapid consumption of carbohydrate reserves and/or an arrest of the processes of carbon storage. However, in tomato fruit where the sugar consumption is slowest when compared to other sink organs such as tomato young roots and this may explain why yield did not significantly decrease with PRD application despite the significant reduction of the source organ such as leaf expansion (*Fig. 3*) and leaf area (Table 1).

The pattern of water use by the crop can be used strongly affect by the agronomic means, widely fertilizers, type of soil, water and irrigation technique. In this present study PRD significantly increased WUE in the two different calculations as presented in Table 1. These results were in agreement with many findings dealing with PRD (Loveys *et al.* 1998; Stoll *et al.* 2000; Davies *et al.* 2000; Zegbe-Dominguez *et al.* 2003). These findings strongly support the idea behind using PRD with grapes to save and increase water use efficiency without significant reduction (Loveys 1991).

CONCLUSION

PRD decreased leaf expansion, stomatal conductance as well as plant leaf area, whereas it increased proline accumulation. PRD, on the other hand, increased water use efficiency (WUEs) of tomato plants by up to 50%and 28% compared to control plants as dry biomass and total yield espectively, without a significant reduction in yield.

REFERENCES

AGUE', R.M. and J.L. MOORE. 2002. Stomatal response to non-hydraulic root-to-shoot communication of partial soil drying in relation to foliar dehydration tolerance. *Environ, Exp. Bot.* **47**: 217-229.

AGUE', R.M. and X. DUAN. 1991. Mycorrhizal fungi and non-hydraulic root signals of soil drying. *Plant Physiol.* **97**: 821-824.

AWAD, M.H. 2001. Effect of water deficit on growth and leaf gas exchange of pepper plants (*Capsicum annuum*). Ph.D. Thesis, UPM, Malaysia, 178 p.

BACON, M.A., S.A. WILKINSON and W.J. DAVIES. 1998. pH regulated leaf cell expansion in droughted plant is ABA-dependent. *Plant Physiol.* **118:** 1507-1515.

BALDET, P., C. DEVAUX, C. CHEVALIER, R. BROUQUISSE, D. JUST and P. RAYMOND. 2002. Contrasted responses to carbohydrate limitation in tomato fruit at two stages of development. *Plant Cell Environ.* **25:** 1639-1649.

BATES, L. S., R.P. WALDREN and I.D. 1973. Rapid determination of proline for water stress studies. *Plant Soil* **39**: 205-207.

BLACKMAN, P.G. and W.J. DAVIES. 1998. Root to shoot communication. *Environ. Exp. Bot.* **36:** 39-48.

DAVIES, W.J., M.A. BACON, D.S. THOMPSON, W. SOBEH and L.G. RODRIGUEZ. 2000. Regulation of leaf and fruit growth in plants growing in dry soil: Exploitation of the plants' chemical signaling system and hydraulic architecture to increase the efficiency of water use in agriculture. *Environ Exp. Bot* **51:** 1617-1626.

DRY, P.R. and B.R. LOVEYS. 1999. Grapevine shoot growth and stomatal conductance are reduced when part of the root system is dried. *Vitis* **38:** 151-156.

GZIK, A. 1995. Accumulation of proline and pattern of amino acids in sugar beet plants in response to osmotic, water and salt stress. *Environ. Exp. Bot.* **36:** 29-38.

HO, L.C. 1996. Tomato. In *Photoassimilate Distribution in Plants and Crops: Source–Sink Relationships*, ed. E. ZEMASKI and A. A. SCHAFFER, p. 709–728. NY, USA: Marcel Dekker.

HSIAO, T.C., 1990. Plant–atmosphere interaction, evapotranspiration and irrigation scheduling. *Acta Hortic.* **278:** 55–66.

ISMAIL, I. and M.I. RAZI. 2002. Response of watering regimes on growth and physiological process of lowland tomatoes grown in coconut coir dust culture system. In *Workshop on Environmental Stress Impact on Tropical Crops*, organized by Faculty of Agriculture, UPM, May, Malaysia. IDEAL. 9 p.

KANG, S., L. ZHANG, X. HU, Z. LI and P. JERIE. 2001. An improved water use efficiency for hot pepper grown under controlled alternate drip irrigation in partial roots. *Scientia Hortic.* **89:** 257-267.

LOVEYS B.R. 1991. What use is knowledge of ABA physiology for crop improvement? In *Physiology and Biochemistry of Abscisic Acid*, ed. W.J. Davies and H.G. Jones, p. 245-259. Oxford, UK: Bio Scientific Publishers.

LOVEYS, B.R.M, STOLL, P.R. DRY, and M.G. MCCARTHY. 1998. Partial rootzone drying stimulates stress responses in grapes to improve water efficiency while maintaining crop yield and quality. *Australian Grapegrower and Winemaker*, Technical Issue **414:** 108-114.

LOVEYS, B.R.M. STOLL, P.R. DRY, and M.G. MCCARTHY. 2000. Using plant physiology to improve the water efficiency of horticultural crops. *Acta Hortic.* **537:** 187-197.

MINGO, D.M. 2003. Regulation of tomato plant growth in relation to soil water availability. Ph.D Thesis, Lancaster University, UK.

MINGO, D.M., M.A. BACON and W.J. DAVIES. 2003. Non-hydraulic regulation of fruit growth in tomato plants (*Lycopersicon esculentum* cv. Solario) growing in drying soil. *J. Exp. Bot.* **54:** 1205-1212.

SAS. 1997. Statistical Analysis System Institute, Inc., STAT procedure guide for personal computer version 6.04 SAS Institute, Inc., Cary, NC, 378 p.

SHARP, R.E. and W.J. DAVIES. 1979. Solute regulation and growth by roots and shoots of water stressed maize plants. *Planta* **147**: 43-49.

STOLL, M.B. LOVEYS and P. DRY. 2000. Hormonal changes induced by partial rootzone drying of irrigated grapevine. *J. Exp. Bot.* **51:** 1627-1634.

TORRECILLAS, A., C. GUILLAUME, J.J. ALARCÓN and M.C. RUIZ-SÁNCHEZ. 1995. Water relations of two tomato species under water stress and recovery. *Plant Sci.* **105:** 169-176.

ZEGBE-DOMINGUEZ, J. A., M. H. BEHBOUDIAN, A. LANG and B. E. CLOTHIER. 2003. Deficit irrigation and partial rootzone drying maintain fruit dry mass and enhance fruit quality in 'Petopride' processing tomato (*Lycopersicon esculentum* Mill.). *Scientia Hort.* **98**: 505-510.

(Received: 10 November 2003)
(Accepted: 29 November 2004)

Pertanika J. Trop. Agric. Sci. 27(2): 151 - 161 (2004) ISSN: 1511-3701

Biomonitoring of Heavy Metals in the West Coastal Waters of Peninsular Malaysia Using the Green-lipped Mussel *Perna viridis*: Present Status and What Next?

*YAP, C. K., ISMAIL, A. & TAN, S. G.
Department of Biology, Faculty of Science,
Universiti Putra Malaysia, 43400 UPM, Serdang, Selangor, Malaysia

Keywords: Green - lipped mussel *Perna viridis*, biomonitoring agent, heavy metals

ABSTRAK

Kertas kerja ini bertujuan untuk mengulas kembali kertas-kertas kerja yang telah diterbitkan menggunakan kupang Perna viridis *sebagai agen pemantauan bagi pencemaran logam berat di rantau ini dan khasnya dari Malaysia dan membincangkan pengetahuan yang sedia ada. Juga dicadangkan bahawa penyelidikan lanjutan yang berpotensi pada masa hadapan yang akan menambahkan pemahaman kita dan pengetahuan dalam ekotoksikologi logam berat di Malaysia dengan menggunakan kupang* P. viridis. *Kajian menggunakan kupang ini boleh dikatakan sebagai sebahagian daripada program 'Mussel Watch' di rantau ini. Kerja-kerja dalam membuktikan* P. viridis *sebagai agen pemantauan, yang berdasarkan kriteria yang telah dicadangkan bagi sesuatu agen pemantauan yang baik, boleh diterima pakai untuk spesies moluska yang lain.*

ABSTRACT

This paper aims to review the papers published using Perna viridis *as a biomonitoring agent of heavy metals in this region and particularly from Malaysia and to discuss the existing knowledge. Further research which will enhance our understanding and increase our knowledge on heavy metal ecotoxicology in Malaysia by using* P. viridis *is also suggested. This can be part of Mussel Watch monitoring program in this region. The work in establishing* P. viridis *for the biomonitoring of heavy metals, which is fundamentally based on the recommended criteria for a good biomonitoring agent, can be applied in other mollusks species.*

INTRODUCTION

Since the Mussel Watch monitoring approach was initially proposed by Goldberg (1975), the use of the green-lipped mussel *Perna viridis* as a biomonitoring agent for heavy metal pollution studies in the Asia-Pacific coastal waters has been reported in the literature for over a quarter of a century. In the Southeast Asian region, perhaps the earliest reported study was conducted in Thailand's coastal waters (Menasveta and Cheevaparanpiwat 1981). In Malaysia, the monitoring of contaminant levels in the coastal waters of Penang using Mussel Watch approach was reported in the 1980s (Sivalingam and Bhaskaran 1980; Sivalingam *et al.* 1982; Sivalingam 1985). However, there has been a long absence of reported data using this mussel species until Ismail (1993a) reported the general levels of heavy metals in *P. viridis* from the west coast of Peninsular Malaysia and Din and Jamaliah (1994) reported those from Penang Island. This paper aims to review the papers published in this region and particularly from Malaysia and to discuss the existing knowledge and potential research area for future studies. In this paper, most reported work on *P. viridis* particularly for biomonitoring of contaminants is reviewed from other regions and discussion is focused upon from those reported from Malaysia.

Overview of Biomonitoring Studies Using Perna viridis *from this Region*

The use of *P. viridis* for the biomonitoring studies in the coastal waters has been implemented in

Corresponding Author:
yapckong@hotmail.com

Asia Pacific coastal waters since 1980s (Tanabe 2000; Tanabe *et al.* 2000; Nicholson and Lam 2005) such as in China (Klumpp *et al.* 2002), Singapore (Bayen *et al.* 2004), Hong Kong (Nicholson and Szefer 2003), Malaysia (Ismail *et al.* 2003; Yap *et al.* 2003a) and Thailand, Philippines and India (Tanabe *et al.* 2000). The mussel species has been shown to be a good biomonitoring agent for contaminants such as organochlorines (Klumpp *et al.* 2002; Monirith *et al.* 2003; Bayen *et al.* 2004), buytltin compounds (Kan-atireklap *et al.* 1997; Fung *et al.* 2004; Sudaryanto *et al.* 2004), heavy metals (Wong *et al.* 2000; Nicholson and Szefer 2003; Yap *et al.* 2003a; Wang *et al.* 2004) and polychlorinated biphenyls (Tanabe *et al.* 1987). Other ecotoxicological studies using *P. viridis* include the uptake and assimilation of metals (Pan and Wang 2004; Wang *et al.* 2004), feeding behaviors (Wong and Cheung 1999; 2001), DNA strand breakage (Siu *et al.* 2003), metal exposure studies (Yap *et al.* 2004a; Shi and Wang 2004) and other physiological studies on its cytological, lysosomal, ecocytological and cardiac responses to metals (Nicholson 1999a, 1999b, 2001, 2003) and biomarkers (Lau and Wong 2003). All the mentioned work implied the importance of biomonitoring studies and *P. viridis* will continue to be used and focused upon for biomonitoring purposes in future.

WHY *PERNA VIRIDIS* ?

The state of metal pollution on the west coast of Peninsular Malaysia has been assessed by measuring metal levels in 1) sediments (Ismail 1993b; Ismail *et al.* 1993; Yap *et al.* 2002a, 2003b, 2003c) and 2) mollusks as biomonitoring agents. Based on our work done since 1998, we tested the suitability of *P. viridis* as a biomonitoring agent of heavy metals for Peninsular Malaysia based on the recommended criteria for a good biomonitoring agent (Phillips and Rainbow 1993; Tanabe 2000; Monirith *et al.* 2003). The eight criteria recommended are given below and the studies done so far to test each criterion are reviewed.

Criterion 1: Wide Geographical Distribution in the Coastal Waters

Mussels can easily be found in the west coast of Peninsular Malaysia during our field trip from 1998 to 2004. Background levels of Cd, Cu, Hg, Pb and Zn in the soft tissues of *P. viridis* were found and reported in the literature (Ismail *et al.* 2000, Yap *et al.* 2003a, 2003d) and this baseline is very important for future reference.

In April 2004, a survey and sampling was conducted from Tumpat to Mersing off the east coast of Peninsular Malaysia and the green-lipped mussels were found in very small numbers from Tumpat (Kelantan) and Nenasi (Pahang) and relatively high abundance of natural mussel population at Kuala Pontian (Pahang) (Yap *et al.* 2004b). This confirmed the fact that the abundance of *P. viridis* along the east coast is not as high as that in the west coast of the peninsula. Thus, the geographical distribution of mussel populations in the coastal waters of Peninsular Malaysia is mainly in the west coast, including the Straits of Johore. Since the natural habitats of mussels are found at Kuala Pontian, this information is important for mussel transplantation purpose because the east coast is regarded less contaminated than in the west coast.

Criterion 2: Sedentary Lifestyle after the Pelagic Lifespan

The biology of *P. viridis* is interesting since they experience a short (2-3 weeks) period of mobile pelagic larvae in the beginning of their life stage and become non-mobile after their attachment to substrata.

Criterion 3: Easy Sampling since They Can be Found in Abundance

Since most mussels can be collected under buoys, nibong poles or hanging ropes by raft (for mariculture sites), they are relatively easy for sampling.

Criteria 2 and 3 can be easily justified based on our field samplings and from the literature.

Criterion 4: Simple Correlations between the Metal Levels in the Mussels and Those in Their Environments

For this criterion, Yap *et al.* (2002b) found that significant ($P< 0.05$) correlations were observed between Cd in *P. viridis* and Cd in the sediment (EFLE fraction and total Cd), Cu in *P. viridis* and Cu in the sediment (EFLE and 'acid-reducible' fractions and total Cu) and Pb in *P. viridis* and Pb in the sediment ('oxidisable-organic' fraction and total Pb). No significant correlation ($P> 0.05$) was found between Zn in *P. viridis* and all the sediment geochemical fractions of Zn and total Zn in the sediment.

This indicates that Zn is possibly 'partially regulated' by the soft tissue of *P. viridis*. The results support the use of *P. viridis* as a suitable biomonitoring agent for Cd, Cu and Pb.

Criterion 5: Easy Identification of the Species due to Low Variability

We identified *P. viridis* by using morphological characters such as the green colour found specifically on the periphery of the shell and the total absence of the anterior adductor muscle (Siddall 1980). However, the question is 'Are they genetically similar so that the various geographical populations of the mussels can be used as a biomonitoring agent?' By using the isozyme approach, Yap *et al.* (2002c) studied the genetic structures of *P. viridis* collected from the west coast of Peninsular Malaysia and found an interesting phenomenon. From the eight geographical populations studied, fourteen polymorphic loci were observed. The observed mean heterozygosity ranged from 0.108 to 0.334, while the expected mean heterozygosity ranged from 0.133 to 0.301. The populations studied could be divided into two groups by the UPGMA dendrogram based on Nei's (1978) genetic similarities. The groupings seemed to indicate differentiation into local populations. These results suggested that *P. viridis* had a tendency to split into a number of geographical populations regardless of larval dispersal as a potential agent of gene flow. The mean *Fst* value of 0.149 indicated that the mussel populations showed a moderate degree of genetic differentiation. However, the mean genetic distance from the study (0.048 ± 0.004) fell within the range of genetic distances between conspecific populations of mussels (0.0-0.14). Therefore, the study supported the use of the local mussel *P. viridis* as a suitable biomonitoring agent for heavy metals (Yap *et al.* 2002d). The range of genetic distance values (0.004-0.091) that were obtained would also serve as baseline data with which results of similar studies in the future can be compared to determine whether genetic divergence of mussel populations from the west coast of Peninsular Malaysia is taking place. At the molecular level, Chua *et al.* (2003) reported that the population genetic structure of the green-lipped mussel *P. viridis* (L.) as indicated by Randomly Amplified Polymorphic (RAPD) molecular markers, was associated with the transplantation of mussels from Johore to Langkawi for mariculture purpose.

Criterion 6: Capacity to Accumulate Pollutants in the Tissues of the Mussels

The bioaccumulative properties of Cd, Cu, Pb and Zn were investigated. Although marine mussels are generally used as biomonitoring agents of heavy metals, regulative mechanisms of essential metals in the soft tissues of mussels have been reported (Phillips and Rainbow 1993). Earlier, Phillips (1985) and Chan (1988, 1989) studied the potential of *P. viridis* collected from Hong Kong coastal waters as a biomonitor of Cd, Cu, Pb and Zn. In Malaysia, Yap *et al.* (2002a, 2003e, 2003f) used similar mussel species for the same purpose but those mussels were collected from the coastal waters of Peninsular Malaysia and they used different methodology to find evidences to prove the suitability of *P. viridis* as a biomonitoring agent of heavy metals.

Yap *et al.* (2003e, 2003f) did ecotoxicological tests on *P. viridis* under laboratory conditions. Different rates of accumulation and depuration of Cd, Cu, Pb and Zn in the soft tissues were found and this might be due to different mechanisms for metal binding and regulation. At the end of depuration, Cd levels in the soft tissues of *P. viridis* were 10-30 times higher than those before exposure, while Zn levels in the soft tissues were almost similar to the levels before exposure (Yap *et al.* 2003e). These results indicate that *P. viridis* is a good biomonitoring organism for Cd but Zn levels may be actively regulated. It remains uncertain whether or not *P. viridis* is a good biomonitoring organism of environmental Zn contamination (Yap *et al.* 2003e). The high ratio of maximum to minimum Pb and Cu levels and the similar patterns (although at different rates) of accumulation and depuration in the different soft tissues for Pb and Cu indicate that *P. viridis* is a good biomonitoring organism for Cu and Pb (Yap *et al.* 2003f, 2004a). The conclusion on Cu and Pb was also supported by analysis of field samples collected from contaminated and uncontaminated sites (Yap *et al.* 2003f; Yap *et al.* 2004a). Recently, Yap *et al.* (2005) suggested that crystalline style and metal redistribution in the different soft tissues of *P. viridis* can be used as indicators of Cu and Pb bioavailabilities and contamination in coastal waters.

Criterion 7: They are Tolerant but Relatively Sensitive to Chemical Pollutants

To prove that they are tolerant but sensitive to heavy metal stress, studies on toxicities and tolerances of Cd, Cu, Pb and Zn in *P. viridis* were conducted by short-term bioassays using endpoint mortality (Yap *et al.* 2004c). The LC_{50} values for the mussels were 1.53 mg/L for Cd, 0.25 mg/L for Cu, 4.12 mg/L for Pb and 3.20 mg/L for Zn. These LC_{50} values were within the concentration ranges that were reported by other authors who used *P. viridis* as the test organism. Based on these LC_{50} values, the mussel seems to be most sensitive to Cu, followed by Cd, Zn and Pb.

In addition to endpoint mortality, toxicity tests of the effects of Cd, Cu, Pb and Zn based on the endpoint filtration rate (FR) of *P. viridis* were also conducted (Yap *et al.* 2003g). Probit analysis was used to calculate the metal concentrations that inhibited 50% of the FR (EC_{50}) of the experimental samples when compared to the control samples. For mussels with size ranging from 6-8 cm shell length (at salinity: 26 ppt, temperature: 28°C, pH: 7.87 and dissolved oxygen: 7.10 mg/L), the EC_{50} values were: 0.43 mg/L for Cd, 0.31 mg/L for Cu, 2.81 mg/L for Pb and 1.67 mg/L for Zn. The results suggest that the mussels are most sensitive to Cu, followed by Cd, Zn and Pb. This result agrees well with those that were obtained from the experiment which used mortality as an endpoint as has been mentioned earlier. Yap *et al.* (2004d) found that the smaller size group *P. viridis* is more sensitive, based on lower LC_{50} values, than those of the larger size group mussel.

To enhance our understanding of the sensitivity of *P. viridis* toward heavy metals (Cd and Pb), the relationships between condition index (CI) and accumulated concentrations of Cd and Pb were determined in field samples of *P. viridis* and those under experimental conditions. The field samples included those from uncontaminated and contaminated sites (Yap *et al.* 2002e). In the field samples, significant ($P< 0.05$) negative relationships between CI and metal concentrations were found. However, the results could also have been due to variations in nutritional state and reproductive status of the mussels. To clarify these uncertainties, an experimental study was conducted and the results agreed with that found in the field samples. Therefore, the results indicated that if the heavy metal levels of the field collected mussels were significantly ($P< 0.001$) different, the influences caused by the nutritional and reproductive states would be masked by the ecotoxicological effects of heavy metal contamination, provided that mussels with similar size range were selected for the CI determination. The CI can be used to show that *P. viridis* is a sensitive organism to Cd and Pb. This work also showed that the sensitivity of *P. viridis* to Cd and Pb when CI was used as a physiological index. Recently, Al-Barwani *et al.* (2004) found that CI can be used as a physiological index for the reproductive status of *P. viridis*.

Criterion 8: Public Concern from the Health Point of View Since They are a Commercially Important Protein Source

This criterion is important from the public health standpoint. The mussels collected between 1999 and 2001 from the wild and from aquacultural sites, off the west coast of Peninsular Malaysia were analysed for Cd, Cu, Hg, Pb and Zn concentrations and these values were compared to permissible limits from established guidelines for food safety in US, UK, Hong Kong, Australia, China and maximum permissible limits established by Malaysian Food Regulations 1985 (Yap *et al.* 2004e). The concentrations of these heavy metals should result in no acute toxicities of the metals since they were lower than the permissible limits for human consumption. In addition, these metal concentrations were also considered to be low when compared with regional data based on *P. viridis* as a biomonitoring agent. However, the risk would depend on the amount of mussels being consumed daily and weekly.

Other Studies on Perna viridis

Apart from the above criteria, related work that can enhance our understanding of the use of *P. viridis* as a biomonitoring agent includes the potential use of the occurrence of shell deformities (Yap *et al.* 2002f) as an indicator, and byssus (Yap *et al.* 2003h) and shell (Yap *et al.* 2003i, 2004f) as better biomonitoring materials of heavy metal contamination in coastal waters. Yap *et al.* (2003h) found that when compared to the soft tissues, the byssus was a more sensitive biomonitoring organ for Zn while it could be a complementary organ for Cd and Pb in the total soft tissues. The potential of the byssus of *P.*

viridis to monitor the metal pollution in the coastal waters was shown by Sivalingam *et al.* (1983). Since the total soft tissue of *P. viridis* had been reported to have a regulatory mechanism for Zn, the byssus can be used as a biomonitoring organ for the identification of coastal areas exposed to Zn pollution. Our suggestion is supported by the data reported by Nicholson and Szefer (2003) in which the byssus of *P. viridis* collected from a contaminated site at Kennedy Town of Hong Kong coastal waters accumulated about 3 times higher Zn concentration than that of an uncontaminated site at Kat O.

Apart from the soft tissues and byssus, the hard tissue, the shell of *P. viridis*, is also shown to be potential as a biomonitoring material of heavy metals (Yap *et al.* 2003i; Yap *et al.* 2004f). Yap *et al.* (2003i) found that the depuration of heavy metals in the shell was not affected by the physiological condition of the mussels. Although Zn could be regulated by the soft tissues, the incorporated Cd, Pb and Zn remained in the shell matrices. The present results supported the use of the total shell of *P. viridis* as a potential biomonitoring material for long-term contamination by Cd, Pb and Zn. By using correlation analysis between Zn levels in the soft tissue of *P. viridis* and those in the geochemical fractions of sediments, Yap *et al.* (2004f) found a higher correlation coefficient of Zn between shells-sediment than those of soft tissues-sediments. Therefore, the mussel shells can be used to monitor Zn pollution in the coastal waters more effectively than the soft tissue of *P. viridis*. Yap *et al.* (2004g) also suggested that allozyme polymorphism in *P. viridis* was a potential biomonitoring tool for heavy metal contamination.

In agreement with Boyden's formula (1977), Yap *et al.* (2003j) showed that the plotting of metal content against dry body flesh weight on a double logarithmic basis gave good positive straight lines. It was found that the smaller mussels (lower total soft tissue dry weight) had higher concentrations of Cd, Pb and Zn than the larger ones. Since shell thickness could be considered to be an estimate of the age of the mussel (Yap *et al.* 2003k), it was also found that the younger mussels accumulated more Cd, Pb and Zn than the older ones. This indicated that *P. viridis* has a different metabolic strategy for each of the studied metals which may be related to age. Nonetheless, the physiological conditions of the mussels, as indicated by the CI, was found not to be a significant factor affecting the accumulations of Cd, Cu, Pb and Zn in the mussel.

What are the Next Endeavors?

All the previous studies have contributed towards the use of *P. viridis* as a suitable biomonitoring agent of trace toxic contaminant levels in the west coast of Peninsular Malaysia. However, there are still a lot of unknowns to be looked into in the future. A lot of questions arise. We will again look into the previously mentioned criteria one after another and discuss what is still lacking in our knowledge of *P. viridis*.

For criterion 1, although *P. viridis* is widely found and distributed in the west coast of Peninsular Malaysia, the decline in spatfalls in certain areas has prompted massive transplantations from high spatfall coastal areas to low spatfall sites. This phenomenon needs further attention. Another question that arises is a) Why are there hardly (although they can still be found) any mussels found naturally in the east coast of Peninsular Malaysia although there have been reports in the literature that they are found in the Gulf of Thailand (Hungspreugs *et al.* 1984; Sukasem and Tabucanon 1993; Ruangwises and Ruangwises 1998) which is located just above the east coast of the peninsula.

Generally, we think that the topography, water quality, attachment substrates and the big waves of the east coast are factors that limit the growth of *P. viridis* on the peninsula's east coast. Therefore, detailed studies to find out the exact reasons for the rarity there should be undertaken.

For criterion 2, its sedentary adulthood is the determinant for the successful survival of *P. viridis* in coastal waters. This is much related to the attachment properties of the mussel byssus. a) What are the chemicals responsible for the detachment of byssus that would ultimately affect the abundance of mussels in coastal waters? b) What attachment substrates in spat collectors are more suitable to collect spats in the high spatfall waters? c) What is the composition in the byssus responsible for the attachment on hard substrates and d) Can the attachment plaque properties of the mussel byssus be applied for the benefit of humans?

For criterion 3, the occurrence in abundance of mussels in the west coastal waters of Peninsular

Malaysia is localized although they are widely found along this coast. This abundance is much related to the spatfalls in certain areas during transplantation. Even in the west coast, a) Is the spatfall density related to the attachment substrates? b) What are the reasons for the low spatfall in certain areas? c) What are the peak seasons of reproductive cycles in the west coast of Peninsular Malaysia (the Straits of Malacca)? d) How can we maintain the spatfalls of the high reproduction sites? e) How can we enhance high spat collection at a less productive spat area? and f) Could the low spatfall area be a result of pollution?

The success of mussel farming depends heavily on spatfalls in the natural environment. The origin of the spats is unknown and the supply is highly variable. Apart from the problem of insufficient supply of spats for seeding, genetic problems can arise from the use of wild stocks of unknown origins. Therefore, selection and improvement of cultured mussels may be hindered by the limited amount of genetic information available (Yap *et al.* 2002c, 2002d). Based on this aspect, information about the population genetic structure of *P. viridis* collected along the west coast of Peninsular Malaysia is undoubtedly very important. This particular aspect is now being looked into, using single-locus codominant microsatellite markers.

For criterion 4, simple correlations between the biomonitoring agent and its environment could only be found for certain metals such as Cd, Cu and Pb but not for Zn (Yap *et al.* 2002b, 2003h). This was due to the fact that not all metals are effectively accumulated in the soft tissues of *P. viridis*. 'Partial regulation' of Zn in the soft tissues of *P. viridis* was reported (Phillips 1985, Yap *et al.* 2003h) and this could affect the accuracy of the estimation of Zn pollution levels in coastal waters. Still, a lot of information on Zn levels in mussel soft tissues had been obtained from Hong Kong, Thailand, India and recently Malaysia. Perhaps, the question should be 'At what degree of metal regulation should the mussel be rejected as a biomonitoring agent of a particular metal?'

For criterion 5, low variability in morphological features and genetic variations need further validation. So far, only studies using isozyme markers have been reported to investigate levels of genetic variations of this species collected along the west coast of Peninsular Malaysia (Yap *et al.* 2002c). However, isozyme based studies are limited by low polymorphisms and the neutrality of isozymes is still doubtful (Jarne and Lagoda 1996). Therefore, hypervariable markers should be obtained at the DNA level. Polymorphisms detected at the DNA level are higher and give more information. Several questions could arise here; a) What are the DNA patterns of the local populations or indigenous species of *P. viridis*? b) What are the genetic variations of *P. viridis* collected along the west coast of Peninsular Malaysia, based on DNA markers (microsatellites)? c) How can the genetic information be used in maintaining the brookstocks of *P. viridis*? d) What are the useful genetic markers to distinguish between male and female mussels? e) What are the DNA level diagnostic markers to distinguish between contaminated and uncontaminated mussel populations?

For criterion 6, we found *P. viridis* is accumulative of Cd, Cu, Hg, Pb and Zn but we are lacking the knowledge and understanding on the chemical interactions among these metals in affecting the accumulation of metals in the mussel tissues. Also, other anthropogenic hazardous trace metals like As, Co, Ni, Fe and Se in the soft and hard tissues of *P. viridis* collected from Malaysia have not been studied yet to our knowledge.

For criterion 7, to further test the tolerances and sensitivities of *P. viridis* to heavy metals, other physiological indices should be tried and used. Also, the effects of single and multiple metals in affecting the tolerances and toxicities of metals that could cause 'synergistic' or 'additive' phenomenon are interesting research topics for future studies when this criterion is being focused upon.

For criterion 8, our risk assessment of heavy metal consumption through shellfish in Malaysia by comparison with established guidelines for food products from other countries should be improved. This could be done by establishing Malaysian guidelines based on our data spanning at least 10 years. To achieve this, the heavy metal concentrations in the soft tissues of *P. viridis* along the west coast of the peninsula should be studied yearly. 'What are the Malaysian hazardous indices/guidelines for shellfish consumption (rather than fish product in general)?' is also a question that should prompt further studies.

CONCLUSION

To establish an organism to be a good biomonitoring agent needs a lot of scientific research. Although workers from other regions have studied similar mussel species for the biomonitoring purpose, relatively similar studies should be conducted to test the local species and the existing information should be considered for comparison rather than extrapolation of the same phenomenon and results if the local similar species is used for biomonitoring studies. Our experience using the mussel *P. viridis* is interesting and by reviewing all the work, there is still a lot of interesting research areas to be investigated. Therefore, we suggest some of the potential work to be carried out in future studies but that would be greatly dependent on the numbers of researchers and the research funding available to study them. The work in establishing *P. viridis* for the biomonitoring of heavy metals, which is fundamentally based on the recommended criteria for a good biomonitoring agent, can be applied in other mollusks species such as the popular cockles and fish tilapia.

REFERENCES

AL-BARWANI, S., A. ARSHAD, B. JAPAR SIDIK, S. SITI SHAPOR and C. K. YAP. 2004. Comparison of condition index of the green-lipped mussel *Perna viridis* between Sebatu and Pasir Panjang. Presented at Second Biology Colloquium, Department of Biology, UPM. Biology: Unity and diversity. 22 December 2004, Marriot Putrajaya.

BAYEN, S., G.O. THOMAS, H.K. LEE and J.P. OBBARD. 2004. Organochlorine pesticides and heavy metals in green mussel, *Perna viridis* in Singapore. *Wat. Air Soil Poll.* **155:** 103-116.

BOYDEN, C.R. 1977. Effects of size upon metal contents of shellfish. *J. Mar. Biol. Assoc. U. K.* **57:** 675-714.

CHAN, H.M. 1988. Accumulation and tolerance to cadmium, copper, lead and zinc by the green-lipped mussel *Perna viridis*. *Mar. Ecol. Prog. Ser.* **48:** 295-303.

CHAN, H.M. 1989. Temporal and spatial fluctuations in trace metal concentrations in transplanted mussels in Hong Kong. *Mar. Poll. Bull.* **20:** 82-86.

CHUA, B. H., S. H. YONG, C. H. TEH, S. G. TAN, C. K. YAP and A. ISMAIL. 2003. Population genetic structure of the green-lipped mussel *Perna viridis* (L.) as indicated by Randomly Amplified Polymorphic (RAPD) molecular markers. In *Aquatic Resource and Environmental Studies of the Straits of Malacca: Managing the Straits Through Science and Technology*, ed. B. Japar Sidik, A. Arshad, H. H. Zakaria and A. Kawamura, p. 291-301. Serdang: MASDEC and JICA, UPM Malaysia.

DIN, Z.B. and M.R.S.N. JAMALIAH. 1994. Trace metal pollution in the coastal areas of Penang Island, Malaysia. In *ASEAN criteria and monitoring: Advances in marine environmental management and human health protection*, eds. D. Watson, S. O. Koh and G. Viger, p. 240-245. ASEAN-Canada Co-operative Programme on Marine Science.

FUNG, C.N., J.C. W. LAM, G.J. ZHENG, D.W. CONNELL, I. MONIRITH, S. TANABE, B.J. RICHARDSON and P.K.S. LAM. 2004. Mussel-based monitoring of trace metal and organic contaminants along the east coast of China using *Perna viridis* and *Mytilus edulis*. *Environ. Poll.* **127:** 203-216.

GOLDBERG, E. D. 1975. The Mussel Watch - A first step in global marine monitoring. *Mar. Poll. Bull.* **6:** 111.

HUNGSPREUGS, M., S. SILPIPAT, C. TONAPONG, R. F. LEE, H. L. WINDOM and K. R. TENORE. 1984. Heavy metals and polycyclic hydrocarbon compounds in benthic organisms of the Upper Gulf of Thailand. *Mar. Poll. Bull.* **15:** 213-218.

ISMAIL, A. 1993a. Heavy metals Cd, Cu, Pb and Zn in green mussel (*Perna viridis* L.) from the west coast of Peninsular Malaysia. 9th – 12th December. *IRPA/UKM Workshop and Seminar*, Allson Kelana, Seremban Malaysia.

ISMAIL, A. 1993b. Heavy metal concentration in sediments off Bintulu, Malaysia. *Mar. Poll. Bull.* **26:** 706-707.

ISMAIL, A., C. K. YAP, M.P. ZAKARIA, S. TANABE, H. TAKADA and A. RAHIM ISMAIL. 2000. Green-lipped mussel *Perna viridis* (L.) as a biomonitoring agent for heavy metals in the west coast of Peninsular Malaysia. In *Towards Sustainable Management of the Straits of Malacca,*

Technical and Financial Options, ed. M. Shariff, F. M. Yusoff, N. Gopinath, H. M. Ibrahim and A. Nik Mustapha, p. 553-559. MASDEC, Serdang, Malaysia: Universiti Putra Malaysia.

Ismail, A., M.A. Badri and M. N. Ramli. 1993. The background levels of heavy metal concentration in sediments of the west coast of Peninsular Malaysia. *Sci. Tot. Environ.* **Suppl. 1993:** 315-323.

Ismail, A., S. Tanabe and C.K. Yap. 2003. Mussel Watch: Heavy metals and tributyltin of the Straits of Malacca. Paper presented at *UPM-JICA Project Workshop: Managing the Straits Through Science and Technology*, 14-16 January. Renaissance Palm Garden Hotel Putrajaya.

Jarne, P. and P.J.L. Lagoda. 1996. Microsatellites, from molecules to populations and back. *TREE* **11:** 424-429.

Kan-atireklap, S., S. Tanabe, J. Sanguansin, M.S. Tabucanon and M. Hungsreugs. 1997. Contamination by butyltin compounds and organochlorine residues in green mussel (*Perna viridis*, L.) from Thailand coastal waters. *Environ. Poll.* **97:** 79-89.

Klumpp, D.W., H. Huasheng, C. Humphrey, W. Xinhong and S. Codi. 2002. Toxic contaminants and their biological effects in coastal waters of Xiamen, China. I. Organic pollutants in mussel and fish tissues. *Mar. Poll. Bull.* **44:** 752-760.

Lau, P.S. and H.L. Wong. 2003. Effect of size, tissue parts and location on six biochemical markers in the green-lipped mussel, *Perna viridis*. *Mar. Poll. Bull.* **46:** 1563-1572.

Menasveta, P. and V. Cheevaparanapiwat. 1981. Heavy metals, organochlorines, pesticides and PCBs in green mussels, mullets and sediments of river mouth in Thailand. *Mar. Poll. Bull.* **12:** 19-25.

Monirith, I., D. Ueno, S. Takahashi, H. Nakata, A. Sudaryanto, A. Subramanian, S. Karuppiah, A. Ismail, M. Muchtar, J.S. Zheng, B.J. Richardson, M. Prudente, N.D. Hue, T.S. Tana, A.V. Tkalin and S. Tanabe. 2003. Asia-Pacific mussel watch: monitoring contamination of persistent organochlorine compounds in coastal waters of Asian countries. *Mar. Poll. Bull.* **46:** 281-300.

Nei, M. 1978. Estimation of average heterozygosity and genetic distance from a small number of individuals. *Genetics* **89:** 583-590.

Nicholson, S. 1999a. Cytological and physiological biomarker responses from green mussels, *Perna viridis* (L.) transplanted to contaminated sites in Hong Kong coastal waters. *Mar. Poll. Bull.* **39:** 261-268.

Nicholson, S. 1999b. Cardiac and lysosomal responses to periodic copper in the mussel *Perna viridis* (Bivalvia: Mytiloidae). *Mar. Poll. Bull.* **38:** 1157-1162.

Nicholson, S. 2001. Ecocytological and toxicological responses to copper in *Perna viridis* (L.) (Bivalve: Mytilidae) haemocyte lysosomal membranes. *Chemosphere* **45:** 399-407.

Nicholson, S. 2003. Cardiac and branchial physiology associated with copper accumulation and detoxication in the mytilid mussel *Perna viridis* (L.). *Journal of Experimental Marine Biology and Ecology* **295:** 157-171.

Nicholson, S. and P.K.S. Lam. 2005. Pollution monitoring in Southeast Asia using biomarkers in the mytilid mussel *Perna viridis* (Mytilidae: Bivalvia). *Environ. Int.* **31:** 121-132.

Nicholson, S. and P. Szefer. 2003. Accumulation of metals in the soft tissues, byssus and shell of the mytilid mussel *Perna viridis* (Bivalvia: Mytilidae) from polluted and uncontaminated locations in Hong Kong coastal waters. *Mar. Poll. Bul.* **46:** 1040-1043.

Pan, J-F. and W-X. Wang. 2004. Influences of dissolved and colloidal organic carbon on the uptake of Ag, Cd, and Cr by the marine mussel *Perna viridis*. *Environ. Poll.* **129:** 467-477.

Phillips, D.J.H. 1985. Organochlorines and trace metals in green-lipped mussels *Perna viridis* from Hong Kong waters: A test of indicator ability. *Mar. Ecol. Prog. Ser.* **21:** 251-258.

Phillips, D.J.H. and P.S. Rainbow. 1993. *Biomonitoring of Trace Aquatic Contaminants.* 371 pp. London: Elsevier Science.

Ruangwises, N. and S. Ruangwises. 1998. Heavy metals in green mussels (*Perna viridis*) from

the Gulf of Thailand. *J. Food Protection* **61:** 94-97.

SHI, D. and W-X. WANG. 2004. Modification of trace metal accumulation in the green mussel *Perna viridis* by exposure to Ag, Cu, and Zn. *Environ. Poll.* **132:** 265-277

SIDDALL, S.E. 1980. A clarification of the genus *Perna* (Mytilidae). *Bull. Mar. Sci.* **30:** 858-870.

SIU, W.H.L., C.L.H. HUNG, H.L. WONG, B.J. RICHARDSON and P.K.S. LAM. 2003. Exposure and time dependent DNA strand breakage in hepatopancreas of green-lipped mussels (*Perna viridis*) exposed to Aroclor 1254, and mixtures of B[*a*]P and Aroclor 1254. *Mar. Poll. Bul.* **46:** 1285-1293.

SIVALINGAM, P.M. 1985. An overview of the Mussel Watch monitoring programme in conjunction with the Westpac programme. *J. Asian Environ.* **6:** 39-50.

SIVALINGAM, P.M. and B. BHASKARAN. 1980. Experimental insight of trace metal environmental pollution problems in mussel farming. *Aquaculture* **20:** 291-303.

SIVALINGAM, P.M., H. SCHWENKE and J. KNOTH. 1983. Mussel byssus as biomonitor of heavy metals. *J. Singapore Nat. Acad. Sci.* **10-12:** 34-37.

SIVALINGAM, P.M., I. ALLAPITCHAY, H. KOJIMA and T. YOSHIDA. 1982. Mussel watch of PCBs and persistent pesticides residues in *Perna viridis* Linnaeus from Malaysian and Singapore waters. *Appl. Geography* **2:** 231-237.

SUDARYANTO, A., S. TAKAHASHI, H. IWATA, S. TANABE and A. ISMAIL. 2004. Contamination of butyltin compounds in Malaysian marine environments. *Environ. Poll.* **130:** 347-358

SUKASEM, P. and M. TABUCANON. 1993. Monitoring heavy metals in the Gulf of Thailand using Mussel Watch approach. *Sci. Tot. Environ.* **139/140:** 297-305.

TANABE, S. 2000. Asia-Pacific Mussel Watch progress report. *Mar. Poll. Bull.* **40:** 651.

TANABE, S., M.S.S. PRUDENTE, KAN-ATIREKLAP and A. SUBRAMANIAN. 2000. Mussel watch: marine pollution monitoring of butyltin and organochlorines in coastal waters of Thailand, Phillips and India. *Ocean Coast. Manage.* **43:** 819-939.

TANABE, S., R. TATSUKAWA and D.J.H. PHILLIPS. 1987. Mussels as bioindicators of PCB pollution: A case study on uptake and release of PCB isomers and congeners in green-lipped mussel (*Perna viridis*) in Hong Kong waters. *Environ. Poll.* **47:** 41-62.

WANG, W-X., R.S.K. WONG, J. WANG and Y-F. YEN. 2004. Influences of different selenium species on the uptake and assimilation of Hg(II) and methylmercury by diatoms and green mussels. *Aquat. Toxicol.* **68:** 39-50.

WONG, C.K., R.Y.H. CHEUNG and M.H. WONG. 2000. Heavy metal concentrations in green-lipped mussels collected from Tolo Harbour and markets in Hong Kong and Shenzhen. *Environ. Poll.* **109:** 165-171.

WONG, W.H. and S.G. CHEUNG. 1999. Feeding behaviour of the green mussel, *Perna viridis* (L.): Responses to variation in seston quantity and quality. *J. Exp. Mar. Biol. Ecol.* **236:** 191-207.

WONG, W.H. and S.G. CHEUNG. 2001. Feeding rates and scope for growth of green mussels, *Perna viridis* (L.) and their relationship with food availability in Kat O, Hong Kong. *Aquaculture* **193:** 123-137.

YAP, C.K., A. ISMAIL, S.G. TAN and H. OMAR. 2002a. Concentrations of Cu and Pb in the offshore and intertidal sediments of the west coast of Peninsular Malaysia. *Environ. Int.* **28:** 467-479.

YAP, C.K., A. ISMAIL, S.G. TAN and H. OMAR. 2002b. Correlations between speciation of Cd, Cu, Pb and Zn in sediment and their concentrations in total soft tissue of green-lipped mussel *Perna viridis* from the west coast of Peninsular Malaysia. *Environ. Int.* **28:** 117-126.

YAP, C.K., S.G. TAN, A. ISMAIL and H. OMAR. 2002c. Genetic variation of the green-lipped mussel *Perna viridis* (L.) (Mytilidae: Mytiloida: Mytilicae) from the west coast of Peninsular Malaysia. *Zool. Stud.* **41:** 376-387.

YAP, C.K., A. ISMAIL, S.G. TAN and H. OMAR. 2002d. Genetic structures of populations of the green-lipped mussel *Perna viridis* (Linnaeus): A biomonitoring agent for the

Straits of Malacca. In *Tropical Marine Environment: Charting Strategies for the Millennium*, ed. F.M. Fatimah, M. Shariff, H.M. Ibrahim, S.G. Tan and S.Y. Tan, p. 641-650. Serdang, Malaysia: MASDEC, Universiti Putra Malaysia.

YAP, C.K., A. ISMAIL and S.G. TAN. 2002e. Condition index of green-lipped mussel *Perna viridis* (Linnaeus) as a potential physiological indicator of ecotoxicological effects of heavy metals (Cd and Pb). *Malaysian Appl. Biol.* **31:** 37-45.

YAP, C.K., A. ISMAIL and S.G. TAN. 2002f. The occurrence of shell deformities in green-lipped mussel *Perna viridis* (Linnaeus) collected from Malaysian coastal waters. *Bull. Environ. Contam. Toxicol.* **69:** 877-884.

YAP, C.K., A. ISMAIL and S.G. TAN. 2003a. Background concentrations of Cd, Cu, Pb and Zn in the green-lipped mussel *Perna viridis* (Linnaeus) from Peninsular Malaysia. *Mar. Poll. Bull.* **46:** 1035-1048.

YAP, C.K., A. ISMAIL and S.G. TAN. 2003b. Cd and Zn in the straits of Malacca and intertidal sediments of the west coast of Peninsular Malaysia. *Mar. Poll. Bull.* **46:** 1348-1353.

YAP, C.K., A. ISMAIL and S.G. TAN. 2003c. Mercury levels of the surface sediments of the intertidal area along the west coast of Peninsular Malaysia. *Toxicol. Environ. Chem.* **85:** 13-21.

YAP, C.K., A. ISMAIL and S.G. TAN. 2003d. Mercury levels in the green-lipped mussel *Perna viridis* (Linnaeus) from the west coast of Peninsular Malaysia. *Bull. Environ. Contam. Toxicol.* **71:** 570-576.

YAP, C.K., A. ISMAIL, H. OMAR and S.G. TAN. 2003e. Accumulation, depuration and distribution of cadmium and zinc in the green-lipped mussel *Perna viridis* (Linnaeus) under laboratory conditions. *Hydrobiologia* **498:** 151-160.

YAP, C.K., A. ISMAIL and S.G. TAN. 2003f. Different soft tissues of the green-lipped mussel *Perna viridis* (Linnaeus) as biomonitoring agents of copper: field and laboratory studies. *Malay. Appl. Biol.* **32:** 9-18.

YAP, C.K., A. ISMAIL, S.G. TAN and H. OMAR. 2003g. Effects of heavy metals (Cd, Cu, Pb and Zn) on the filtration rate of the green-lipped mussel *Perna viridis* (Linnaeus). *Malaysian Appl. Biol.* **32:** 7-13.

YAP, C.K., A. ISMAIL, S.G. TAN and H. OMAR. 2003h. Can the byssus of green-lipped mussel *Perna viridis* (Linnaeus) from the west coast of Peninsular Malaysia be a biomonitoring organ for Cd, Pb and Zn? Field and laboratory studies. *Environ. Int.* **29:** 521-528.

YAP, C.K., A. ISMAIL, S.G. TAN and I. ABDUL RAHIM. 2003i. Can the shell of the green-lipped mussel *Perna viridis* (Linnaeus) from the west coast of Peninsular Malaysia be a potential biomonitoring material for Cd, Pb and Zn ? *Estuar. Coast. Shelf Sci.* **57:** 623-630.

YAP, C.K., A. ISMAIL and S.G. TAN. 2003j. Effects of body size (total soft tissue) and shell thickness on the accumulation of heavy metals (Cd, Cu, Pb and Zn) in the green-lipped mussel *Perna viridis* (Linnaeus). *Russian J. Mar. Biol.* **29:** 323-327.

YAP, C.K., A. ISMAIL and S.G. TAN. 2003k. Water content, conversion factor, shell thickness and condition index of the green-lipped mussel *Perna viridis* (Linnaeus) from Malaysian coastal waters. *Malay. Nature J.* **56(4):** 387-396.

YAP, C.K, A. ISMAIL and S.G. TAN. 2004a. Assessment of different soft tissues of the green-lipped mussel *Perna viridis* (Linnaeus) as biomonitoring agents of Pb: Field and laboratory studies. *Wat. Air Soil Poll.* **153:** 253-268.

YAP, C.K., W. H. CHENG, A. ISMAIL and S.G. TAN. 2004b. Tissue distribution of heavy metals (Cd, Cu, Pb and Zn) in the green-lipped mussel *Perna viridis* from Nenasi and Kuala Pontian, east coast of Peninsular Malaysia. Presented at *Second Biology Colloquium*, Department of Biology, UPM. 22 December. Biology: Unity and diversity. Marriot Putrajaya.

YAP, C.K., A. ISMAIL, H. OMAR and S.G. TAN. 2004c. Toxicities and tolerances of Cd, Cu, Pb and Zn in a primary producer (*Isochrysis galbana*) and in a primary consumer (*Perna viridis*). *Environ. Int.* **29:** 1097-1104.

YAP, C.K., A. ISMAIL and S.G. TAN. 2004d. Tolerance and accumulation of cadmium, copper, lead and zinc by two different size groups of the green-lipped mussel *Perna viridis* (Linnaeus). *Pertanika J. Sci. Technol.* **12(2):** 235-248.

YAP, C. K., A. ISMAIL and S.G. TAN. 2004e. Heavy metal (Cd, Cu, Pb and Zn) concentrations in the green-lipped mussel *Perna viridis* (Linnaeus) collected from some wild and aquacultural sites in the west coast of Peninsular Malaysia. *Food Chem.* **84:** 569-575.

YAP, C.K, A. ISMAIL and S.G. TAN. 2004f. The shell of the green-lipped mussel *Perna viridis* as a biomonitoring material for Zn: Correlations of shells and geochemical fractions of surface sediments. *Malaysian Appl. Biol.* **33:** 79-88.

YAP, C.K., S.G. TAN, A. ISMAIL and H. OMAR. 2004g. Allozyme polymorphisms and heavy metal levels in the green-lipped mussel *Perna viridis* (Linnaeus) collected from contaminated and uncontaminated sites in Malaysia. *Environ. Int.* **30:** 39-46.

YAP, C.K., A. ISMAIL, W.H. CHENG and S.G. TAN. 2005. Crystalline style and tissue redistribution in *Perna viridis* as indicators of Cu and Pb bioavailabilities and contamination in coastal waters. *Ecotoxicol. Environ. Safety.* (In Press).

(Received: 2 August 2004)
(Accepted: 19 July 2005)

Pertanika J. Trop. Agric. Sci. 27(2): 163 - 171 (2004) ISSN: 1511-3701

Preliminary Study on Isozyme Variation in Silkworm Germplasm of *Bombyx mori* (L.) and its Implication for Conservation

P. SOMASUNDARAM, K. ASHOK KUMAR, *K.THANGAVELU, P.K. KAR & R.K. SINHA
Central Sericultural Germplasm Resources Centre
Hosur-635 109,Tamil Nadu, India

Keywords: *Bombyx mori*, genetic diversity, isozyme

ABSTRAK

Diversiti genetik di dalam dan di kalangan dua belas ulat sutera ras Bombyx mori *telah dikaji menggunakan enzim metabolisme iaitu, a-esterase (a- EST), b-esterase (b-EST), glukosa 6-fosfat dehidrogenase (G6PD) dan asid fosfatase (ACP). Satu penelitian diversiti genetik di dalam dan di kalangan ras menunjukkan bahawa 28% variasi yang diperhatikan berlaku di kalangan ras dan variasi selebihnya (72%) di dalam ras. Kadar purata aliran gen antara bandingan pasangan demi pasangan ras dua belas didapati sangat tinggi (0.62). Perkaitan genetik ras dua belas diperlihatkan oleh dendogram UPGMA, menunjukkan pengelompokan genetik ras dalam 6 kelompok. NB4D2 dan NB18 adalah secara genetiknya ras serupa manakala BL-24 dan Nistari pula secara genetiknya jauh. Populasi ras ulat sutera J-112 dan NB4D2 masing-masing menunjukkan diversiti genetik tertinggi, % polimorfisme dan lebih alel. Diversiti genetik mereka yang kaya perlu dieksploitasi dalam program pemuliharaan dan pembiakbakaan.*

ABSTRACT

Genetic diversity within and among twelve silkworm Bombyx mori *races was investigated using metabolic enzymes viz., a- esterase (a- EST), b-esterase (b-EST), glucose 6-phosphate dehydrogenase (G6PD) and acid phosphatase (ACP). A perusal of genetic diversity within and among races indicated that 28% of the observed variation occurred among races and the rest of the variation (72%) within races. The average rate of gene flow between pair wise comparisons of the twelve races was found to be very high (0.62). Genetic relatedness of the twelve races revealed by the UPGMA dendrogram, showed genetic grouping of races in six clusters. NB4D2 and NB18 are genetically similar while BL-24 and Nistari are genetically distant races. Populations of silkworm races J-112 and NB4D2 showed the highest genetic diversity, % polymorphism and more alleles, respectively. Their rich genetic diversity needs to be exploited in conservation and breeding programme.*

INTRODUCTION

Central Sericultural Germplasm Resources Centre (CSGRC), Hosur, India, maintains a large number of silkworm (*Bombyx mori* L.) genetic resources totaling 398 (65 multivoltine, 313 bivoltine and 20 mutant) silkworm accessions. Genetic evaluation of such resources is useful in the context of conservation and utilization in the silkworm breeding programme. Many of the accessions have been characterized for morphological characters as well as for important economic traits such cocoon weight, shell weight, silk ratio, cocoon filament length, etc (Thangavelu *et al.* 1997; Thangavelu *et al.* 2001).

Biochemical characterization through protein profiling of isozyme is needed to know the genetic constitution of an individual and thereby the genetic diversity among and within the races. The isozyme technique is one of the molecular tools in biochemical methods to ascertain the extent of variability available among the species, subspecies and races (Pushpalatha and Vijayan 1999).

Understanding the genetic constitution of an individual in the population of races and

* Corresponding Author

allelic variations through isozyme studies is known to reflect the differential catalytic ability of allelic genes and their significant role in the adaptive strategy of the genotypes (Parkash *et al.* 1992). Among the various known isozymes, esterases, amylase, acid phosphatase and alkaline phosphatase have been studied extensively since they are the groups of enzymes involved in the metabolic rate of fat body of silkworm involved in gonadal maturation, maintenance of cell viability, metabolic activities of silk gland and defense functions (Yoshitake and Eguchi 1965; Yoshitake and Akiyama 1965; Eguchi *et al.* 1965; Eguchi and Yoshitake 1967; Battacharaya *et al.* 1990).

Knowledge on genetic variability is important for breeding (Frankel and Brown 1983; Frey *et al.* 1983) and useful to improve the specific set of characters in low yielding silkworm stocks. Maintenance of variability within population is one of the most important aspects in the conservation of genetic resources (Zeng *et al.* 2003). This in mind, four isozymes viz., a- esterase (a- EST), b-esterase (b-EST), glucose 6 phosphate dehydrogenase (G6PD) and acid phosphatase (ACP)), were utilized to characterize the individuals in the population of 12 silkworm races for studying the genetic variation and their polymorphic features for maintenance and genetic conservation strategies in the silkworm germplasm stocks.

MATERIALS AND METHODS

Twelve silkworm races (6 Multivoltines and 6 Bivoltines) from the germplasm stocks of CSGRC, Hosur, India were selected for isozyme characterization (Table I). Ten individual moths from each of 12 silkworm races were homogenized in extraction buffer (200 mM Tris-Cl, pH 7.5; 5mM $MgCl_2$; and 250 mM NaCl)) and centrifuged at 10,000 rpm for 10 min (Joy and Gopinathan,1995). The supernatant was subjected to electrophoresis as described by Laemmli (1970) on 7.5 % non-denaturing polyacrylamide gel.

Enzyme Visualization

The gels were incubated in respective stains for a-EST (E.C.Number 3.1.1.1), b - EST (E.C.Number. 3.1.1.1), G6PD (E.C.Number. 1.1.1.49) and ACP (E.C.Number.3.1.3.1) for 30 minutes at 37°C (Richardson *et al.* 1986). The stained gels were visualized under a bright illuminator and photographed using Kodak DC4800 gel documentation system (Biovis, India).The isozymes were numbered in the order of decreasing mobility from the cathode. The locus that specifies the isozyme with the least cathodal migration was labeled as A, the next as B ,C, D, E and so on (*Fig. 1.*).

Statistical Analysis

Genetic Variation within Races

Within each race, the observed number of alleles per locus (N_a), the effective number of alleles per locus (N_e), observed heterozygosity (Ho), expected heterozygosity (He) (Nei, 1973) and the number and per cent of polymorphic loci were determined. In addition, for each locus the observed and the expected heterozygosity of an individual over the total analyzed races were estimated (Nei, 1973). Wright's F-statistics (Wright, 1951) viz., F_{IS}-inbreeding coefficient

TABLE 1
Details of the silkworm races, their origin and class for isozyme analysis

Sl.No	Accessions	Races	Origin	Class
1	BMI-0058	BL-23	India	Evolved breed
2	BMI-0059	BL-24	India	Evolved breed
3	BMI-0017	Nistari	WestBengal	Original
4	BMI-0027	O	WestBengal	Evolved breed
5	BMI-0001	Pure Mysore	Karnataka	Original
6	BMI-0009	Kollegal Jawan	Karnataka	Evolved breed
7	BBI-0083	CC-1	Karnataka	Evolved breed
8	BBI-0082	NB7	Karnataka	Evolved breed
9	BBE-0010	J-112	Japan	Evolved breed
10	BBE-0009	B-40	Japan	Evolved breed
11	BBI-0081	NB18	India	Evolved breed
12	BBI-0044	NB4D2	India	Evolved breed

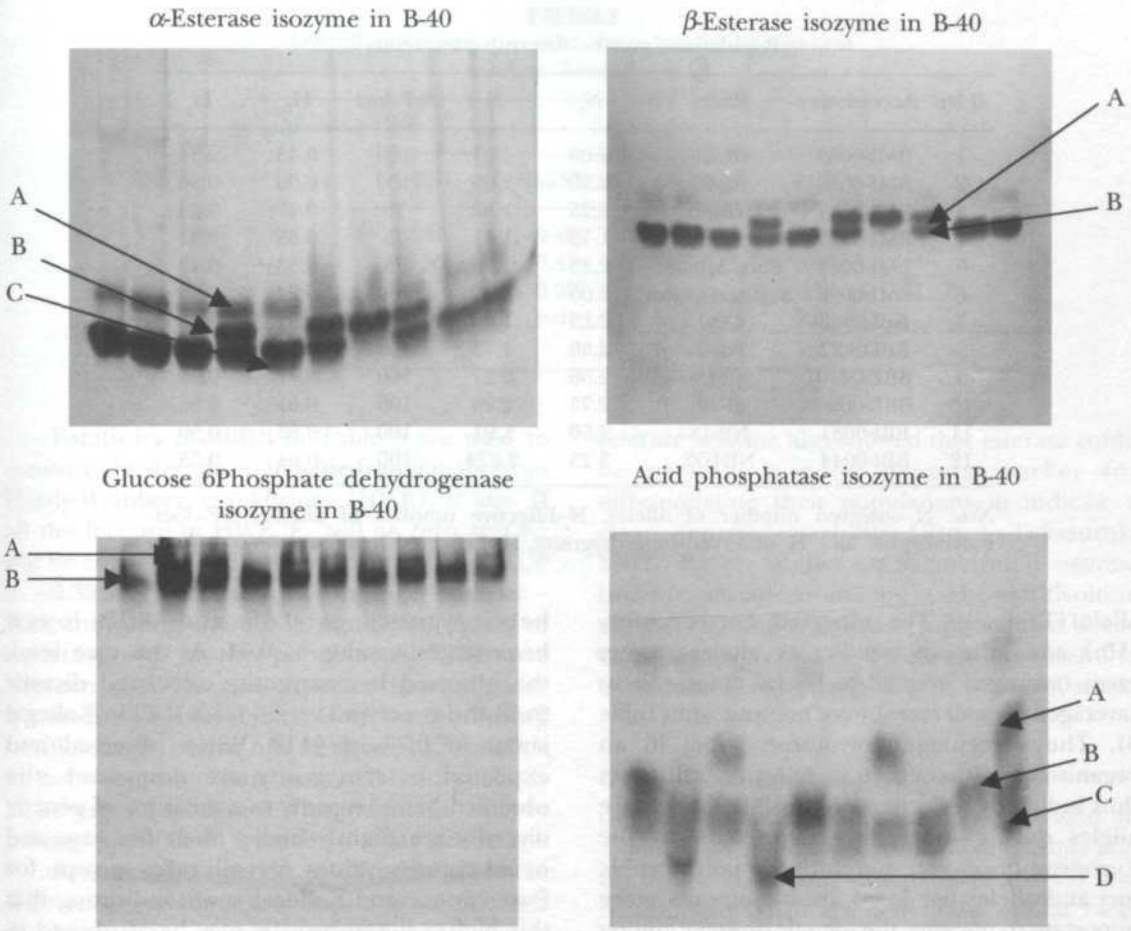

Fig. 1: Isozyme polymorphism in four loci in the B-40 race of Bombyx mori. A,B,C and D are alleles

within races; F_{IT}- inbreeding coefficient over the total analyzed races; F_{ST} - reduction in inbreeding coefficients due to differences among races or genetic differentiation of races at the level of all the loci, were also analyzed. All these statistical measures were determined using the POPGENE version 1.31 (Yeh *et al.* 1999).

Genetic Variation between Races

Estimates of Nei's genetic distance unbiased for sample size (Nei 1978) for each pair wise combination of races were calculated using POPGENE version 1.31 and an unweighted pair group method analysis (UPGMA) of dendrogram was constructed (Yeh *et al.* 1999).

RESULTS AND DISCUSSION

Twelve silkworm races were selected for studies on genetic variability. Four isozymes, *viz.*, α-EST, β- EST, G6PD and ACP were analyzed in the larval heamolymph samples of 10 individuals from each race. Results are presented in Table 2. The value of N_a (number of allele/locus) ranged from 1.75 (O race) to 3.25 (NB4D2 race). The value of observed heterozygosity ranged from 0.22 (Kollegal jawan) to 0.77(J-112).

Genetic Variation within Races

Four-enzyme system representing four loci was resolved with sufficient consistency and clarity (*Fig. 1*). All the four loci were found to be polymorphic. The per cent of polymorphic loci was found to vary from 75% in the races of Nistari, O and Pure Mysore and 100% in BL-23, BL-24, Kollegal jawan, CC-1, NB-7, J-112, B-40, NB18 and NB4D2 (Table 2). The genetic "richness" as measured by the observed number of alleles indicated that all loci were equally rich with 4 to 5 alleles except for G6PD (3

TABLE 2
Population genetic diversity parameters

Sl.No	Accessions	Races	N_a	N_e	%P loci	H_O	H_e
1	BMI-0058	BL-23	2.00	1.51	100	0.43	0.34
2	BMI-0059	BL-24	2.50	2.05	100	0.59	0.56
3	BMI-0017	Nistari	2.25	1.82	75	0.47	0.34
4	BMI-0027	O	1.75	1.51	75	0.35	0.31
5	BMI-0001	Pure Mysore	2.25	1.98	75	0.33	0.42
6	BMI-0009	Kollegal jawan	2.00	1.40	100	0.22	0.30
7	BBI-0083	CC-1	2.25	1.85	100	0.57	0.45
8	BBI-0082	NB-7	2.50	1.73	100	0.55	0.40
9	BBE-0010	J-112	2.50	2.27	100	0.77	0.56
10	BBE-0009	B-40	2.75	2.29	100	0.65	0.56
11	BBI-0081	NB-18	2.50	1.91	100	0.53	0.50
12	BBI-0044	NB4D2	3.25	2.274	100	0.64	0.55

Note: N_a-observed number of alleles, N_e-Effective number of alleles, %P loci-Polymorphic loci, H_o-observed heterozygosity, H_e-Expected heterozygosity.

allele) (Table 4). The observed heterozygosity (Ho) and effective number of alleles among races (averaged over all loci) and among locus (averaged over all races) were not uniform (Table 3). The percentage polymorphic loci in an organism are susceptible to selective influences thus indirectly reflecting on possible loss of rare alleles during inbreeding depression in the European bison and therefore the polymorphic loci and alleles per locus are biologically more important to measure the genetic diversity among any species (Hartl and Pucek 1994). All the four loci were polymorphic in all twelve-silkworm races.

Observed average heterozygosity, expected average heterozygosity, and F-statistics (F_{IS}, F_{it} and F_{st}) each are listed in Table 3. Four loci viz., α-EST, β-EST, G6PD and ACP showed heterozygosity. α-EST showed highest heterozygosity of 0.66 and ACP lowest heterozygosity value of 0.41. At the race level, the observed heterozygosity calculated directly from the genotypes varied from 0.22 in Kollegal jawan to 0.77 in J-112. When observed and expected heterozygosity are compared, the observed heterozygosity as a measure of genetic diversity are slightly higher than the expected heterozygosity values for all races except for Pure Mysore and Kollegal jawan indicating that this higher heterozygosity may be attributed to different geographic origin of the twelve silkworm races involved in this study (Table 1). It is reported that such genetic diversity expressed either as average number of alleles or heterozygosity is usually higher in any species with wider geographic ranges, higher fecundity, greater longevity and larger population sizes (Nevo *et al.* 1984).

TABLE 3
Genetic diversity measures based on each locus over all population

LOCUS	N_a	N_e	H_0	H_e	F_{IS}	F_{IT}	F_{ST}	Nm
α-EST	5.00	2.98	0.66	0.66	-0.33	0.01	0.26	0.70
β-EST	4.00	2.37	0.54	0.58	-0.27	0.01	0.22	0.84
G6PD	3.00	1.97	0.48	0.49	-0.29	0.03	0.25	0.73
ACP	4.00	2.73	0.41	0.63	0.02	0.39	0.38	0.40
Mean	4.00	2.51	0.52	0.59	-0.22	0.12	0.28	0.62

Note: N_a-observed number of alleles, N_e-Effective number of alleles, H_O-observed heterozygosity, H_e-Expected heterozygosity, F_{IS}-inbreeding coefficient within population, F_{IT}-inbreeding coefficient over the population, F_{ST}-genetic differentiation and Nm –Gene flow.

TABLE 4
Overall allele frequencies at four loci in 12 silkworm races of *Bombyx mori*

Alleles	Locus			
	a-EST	b-EST	G6PD	ACP
A	0.45	0.48	0.38	0.41
B	0.32	0.41	0.60	0.40
C	0.11	0.08	0.01	0.15
D	0.04	0.01	-	0.02
E	0.05	-	-	-

F-statistics provided in Table 3 are used to measure the deviations in gene frequencies from Hardy-Weinberg equilibrium (HWE), if any. If all the loci are in HWE, F_{IS} will be zero and F_{IT} will be equal to F_{ST}. F_{IS} ranged from 0.02 for ACP to –0.33 for α-EST and the average value as –0.22, indicating there is a significant excess of heterozygosity in the populations. Difference between observed and expected heterozygosity is a measure of inbreeding. Here the inbreeding coefficient (F_{IT}) value of 0.12 showed 12% deficit in heterozygosity than expected under Hardy Weinberg Equilibrium. This might be due to mating (gene flow) between similar genotypes. F_{ST} measures the fixation of different alleles in different populations and is used as an indicator of divergence among populations. Genetic differentiation among races was low with mean F_{ST} = 0.2842 implying that 28.42 % of total variation was between races and 71.58% of total genetic variation was within races. The rate of gene flow (Nm=0.6297) was found to be very high among races. The high rate of gene flow might be the reason for the low level of genetic differentiation among the populations. Thus it is found that the silkworm races in Germplasm stocks of Hosur, India exhibit a pattern of genetic diversity characterized by a moderate degree of inter-population genetic diversity and a rather high intra-population genetic diversity. Further a high level of heterozygosity was found only in a-esterase. The important aspect of the present work is that the observation of high level of heterozygosity (0.66) at a- EST locus indicates that this esterase may be considered as a good genetic marker for studies of genetic variability in silkworm genetic resources. Similar studies on the population genetic variability among the sugarcane borer *Diatraea saccharalis* through esterase isozyme also showed that esterase could be used as a good genetic marker for differentiating their populations to indicate a clear genetic variability (Ruvolo-Takasusuki 2002). Earlier studies on haemolymph esterase isozyme variations among eight multivoltine silkworm germplasm stocks indicate that allelic variations expressed in the isozyme are useful to determine genetic variability in silkworm stocks (Das *et al.* 1992).

Genetic Variation between Races

Genetic distance coefficients are provided in Table 5. Of the 12 silkworm races, Nistari and BL-24 are genetically distant races (0.949) and NB4D2 and NB18 are genetically similar races (0.029). This is in agreement with earlier studies on microsatellites in thirteen diverse silkworm races that the above breeds viz., NB4D2 and NB18 were also found in one cluster group because they are derived from a common Japanese double hybrid (Damodar Reddy *et al.* 1999). The genetic relatedness of the twelve silkworm races as revealed by the UPGMA dendrogram (*Fig. 2*) showed relatedness among the evolved breeds (NB4D2 and NB18) while this was not found among local races (Pure Mysore and Nistari). The branches in the dendrogram reflect the genetic differentiation among populations of different races. The populations of twelve silkworm races covered a broad geographical range and thus enzymatic differentiation is expected to exist among them. An understanding of the genetic diversity and population genetic structure is not only important for the conservation of species but is also essential for maintenance of genetic diversity within the populations and improvement of races through breeding (Millar and Westfall 1992).

TABLE 5
Genetic relationships among 12 races of silkworm based on Nei's genetic distance

Sl.No	Races	BMI-0058	BMI-0059	BMI-0017	BMI-0027	BMI-0001	BMI-0009	BBI-0083	BBI-0082	BBE-0010	BBE-0009	BBI-0081	BBI-0044
1	BMI-0058	****											
2	BMI-0059	0.265	*****										
3	BMI-0017	0.445	0.949	****									
4	BMI-0027	0.089	0.449	0.386	****								
5	BMI-0001	0.396	0.484	0.6152	0.751	****							
6	BMI-0009	0.148	0.532	0.672	0.152	0.401	****						
7	BBI-0083	0.139	0.038	0.945	0.317	0.353	0.267	****					
8	BBI-0082	0.662	0.442	0.939	0.565	0.360	0.366	0.328	****				
9	BBE-0010	0.281	0.087	0.568	0.267	0.403	0.320	0.106	0.188	****			
10	BBE-0009	0.446	0.240	0.763	0.363	0.535	0.538	0.248	0.240	0.122	****		
11	BBI-0081	0.139	0.148	0.680	0.356	0.179	0.247	0.036	0.234	0.184	0.281	****	
12	BBI-0044	0.074	0.250	0.584	0.240	0.216	0.206	0.090	0.406	0.290	0.299	0.029	****

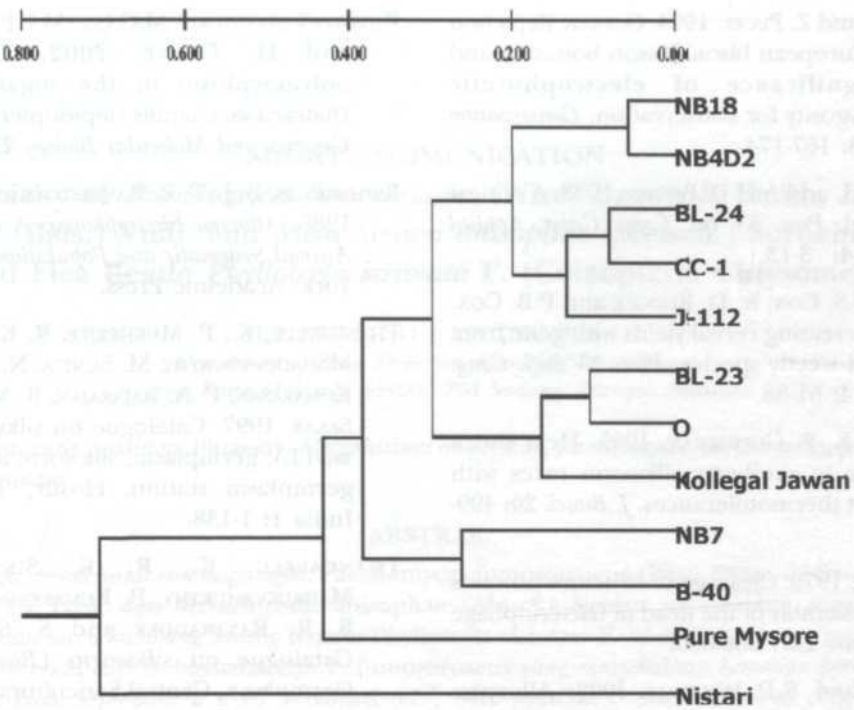

Fig. 2. Dendrogram showing clustering of populations of twelve silkworm races based on Nei's (1978) genetic distance: method =UPGMA

CONCLUSION

The genetic diversity and population genetic structure of twelve silkworm races revealed in this study would be utilized for an effective conservation plan and breeding strategies. Based on the results observed in this study, it is inferred that populations of silkworm races J-112 and NB4D2 would be very useful in a breeding programme, because they harbour higher levels of genetic diversity and more alleles.

ACKNOWLEDGEMENT

The authors wish to express their thanks to Shri. A. L. Deole, Senior Statistician and Shri. S. Sekar, Computer Programmer of Central Sericultural Germplasm Resources Centre, Hosur, India, for their help in statistical analysis of data. Also we express our gratitude to the two unknown referees for their valuable suggestions.

REFERENCES

AYALA, F.J. 1983. Enzyme as taxonomic characters. In *Protein Polymorphism: Adaptive and Taxonomic Significance,* ed. S. Oxford and D. Pollinson. p. 3-26. New York: Academic Press.

BATTACHARYYA, A., S. K. SARKAR, S. GANGULY and S.K. BASU. 1990. Studies on the isozymes patterns of G-6-PD and LDH in male pupa of Eri-silkworm, *Philosamia ricini. Science and Culture* **56:** 131-132.

DAMODAR REDDY, K., J. NAGARAJU and E. G. ABRAHAM. 1999. Genetic characterization of the Silkworm, *Bombyx mori* by simple sequence repeat (SSR)-anchored PCR. Heredity **83:** 681-687.

DAS, S. K., P. K. CHINYA, S. PATTNAIK, S. K. SEN and G. SUBBA RAO. 1992. Studies on genetic variability of heamolymph esterases in some genetic stocks of mulberry silkworm *Bombyx mori* L. *Perspectives in Cytology and Genetics* **7:** 421-425.

EGUCHI, M., N. YOSHITAKE and H. KAI. 1965. Types and inheritance of the blood esterase in the silkworm, *Bombyx mori* L. *J.Genet.* **40:** 15-19.

EGUCHI, M. and. N. YOSHITAKE. 1967. Interrelation of non-specific esterase among various tissues in the silkworm, *Bombyx mori* L. *Jpn. J. Seri cult.* **36:** 193-198.

HARTL., G.B and Z. PUCEL. 1994. Genetic depiction in the European bison (Bison bonasus) and the significance of electrophoretic heterozygosity for conservation. *Conservation Biology* **8:** 167-174.

FRANKEL, O. H. and A.H.D. BROWN. 1983. A critical appraisal. *Proc. XV Int. Cong. Genet. Applied Genetics* **4:** 3-13.

FREY, K. J., T.S. COX, R. D. RODGER and P.B. COX. 1983. Increasing cereal yields with gene from wild and weedy species. *Proc. XV Intl. Cong. Genetics* **4:** 51-68.

JOY, O. and K. P. GOPINTHAN. 1995. Heat shock response in mulberry silkworm races with different thermotolerances. *J. Biosci.* **20:** 499-513.

LAEMMLI, E.K. 1970. Cleavage of structure proteins during assembly of the head of bacteriophage T4. *Nature* **227:** 680-685.

MILLAR, C.I. and R.D. WESTFALL. 1992. Allozyme markers in forest genetic conservation. *New Forest* **6:** 347-372.

NEI, M. 1973. Analysis of gene diversity in subdivided populaltions. *Proc. Natl. Acad. Sci.* **70:** 3321-3323.

NEI, M. 1978. Estimation of average heterozygosity and genetic distance from a small number of individuals. *Genetics* **89:** 583-590.

NEVO, E., A. BELLES and R. BEN-SHLOMO. 1984. The evolutionary significance of genetic diversity: ecological, demographic and life history correlates. In *Evolutionary 'Dynamics of Genetic Diversity'*, ed. G.S. Mani, pp. 11-132. New York: Springer-Verlag.

PUSHPALATHA, N. and V. A. VIJAYAN. 1999. Isozyme profiles in relation to ecological status in two Japanese encephalitis vectors. *Culex vishnui* and *Culex Fuscocephala* (Diptera: Culicidae). *Entomon* **24:** 297-306.

PARKASH, R., J. P. YADAV and M. VASHISHT. 1992. Allozymic variation at ADH locus in some Drosophila species. *Perspectives in Cytology and Genetics* **8:** 495-502.

RUVOLO-TAKASUSUKI, M.C.C., M.F.P.S. MACHADO and H. CONTE. 2002. Esterase –3 polymorphism in the sugarcane borer Diatraea saccharalis (lepidoptera: Pyralidae). *Genetics and Molecular Biology* **25:** 61-64.

RICHARDSON, B. J., P. R. BAVERSTOCK and M. ADAMS. 1986. *Allozyme Electrophoresis A Hand book for Animal Systematic and Population Studies.* New York: Academic Press.

THANGAVELU, K., P. MUKHERJEE, R. K. SINHA, T. S. MAHADEVAMURTHY, M. SUNITA, N. K. SAHANI, P. KUMARESAN, P. A. RAJARAJAN, B. MOHAN and S. SEKAR. 1997. Catalogue on silkworm (*Bomyx mori* L), germplasm, silkworm and mulberry germplasm station, Hosur, Tamil Nadu, India **1:** 1-138.

THANGAVELU, K., R. K. SINHA, T. S. MAHADEVAMURTHY, P. KUMARESAN, B. MOHAN, F. R. RAYARADDER and S. SEKAR. 2001. Catalogue on silkworm (*Bomyx mori* L), Germplasm, Central Sericultural Germplasm Resources Centre, Hosur, Tamil Nadu, India **2:** 1-138.

WRIGHT, S. 1951. The genetical structure of populations. *Annals of Eugenics* **15:** 323-354.

YEH, F.C., R. YANG and T. BOYLE. 1999. Population genetic analysis POPGENE version 1.31: Microsoft window-based freeware for population genetic analysis. A quick user's guide. A joint project developed by Centre for International Forestry Research and University of Alberta, Canada.

YOSHITAKE, N. and M. EGUCHI. 1965. Distribution of blood esterase types in various strains of the silkworm *Bombyx mori* L (in Japanse) *Jpn. Sericult. Sci.* **34:** 95-98.

YOSHITAKE, N. and M. AKIYAMA. 1965. Genetic aspect on the esterase activities of the egg in the silkworm *Bombyx mori* L (in Japanese) *Jpn. Seri cult. Sci.* **34:** 327-332.

ZENG, ZHONGREN WANG, JIE, SHILIANG ZHOU, JIAYU BAI and HAISHUI ZHENG. 2003. Allozyme variation and population genetic structure of *Betula alnoides* from Guangxi, China. *Biochemical Genetics* **41:** 61-75.

(Received: 9 March 2005)
(Accpeted: 14 October 2005)

Pertanika J. Trop. Agric. Sci. 27(2): 171-177 (2004) ISSN: 1511-3701

SHORT COMMUNICATION

Pathogenicity of *Paecilomyces fumosoroseus* (Wise) Brown & Smith, *Beauveria bassiana* (Bals.) Vuill. and *Metarhizium anisopliae* (Metsch.) Sorokin on the Striped Flea Beetle *Phyllotreta striolata* F. (Coleoptera: Chrysomelidae)

T.P. PRIYATNO & Y.B. IBRAHIM
Department of Plant Protection, Faculty of Agriculture
Universiti Putra Malaysia, 43400 UPM Serdang, Selangor, Malaysia

Keywords: ***Beauveria bassiana*, bioassay, *Metarhizium anisopliae*, *Paecilomyces fumosoroseus*, pathogenicity, *Phyllotreta striolata***

ABSTRAK

Keupayaan tiga spesies kulat entomopatogen, Paecilomyces fumosoroseus *(Wise) Brown & Smith,* Beauveria bassiana *(Bals.) Vuill. dan* Metarhizium anisopliae *(Metsch.) Sorokin menyebabkan jangkitan ke atas dewasa, telur dan larva kumbang lenting berjalur* Phyllotreta striolata F. *telah diuji. Hanya satu pencilan* M. anisopliae *dan tiada bagi* B. bassiana *dan* P. fumosoroseus *yang menyebabkan kematian peringkat dewasa melebihi 50% pada kepekatan 2 x 10^7 conidium mL^{-1}. Satu pencilan* P. fumosoroseus *(Pf), satu bagi* B. bassiana *(Wls) dan dua bagi* M. anisopliae *(MPs dan Cy3) didapati patogenik ke atas larva instar pertama* P. striolata *menyebabkan lebih daripada 50% kematian pada kepekatan 2 x 10^6 conidium mL^{-1}. Kadar kematian larva meningkat mengikut peningkatan kepekatan. Nilai anggaran LT_{50} untuk Pf, Wls, MPs dan Cy3 masing-masing bagi larva pada kadar 2 x 10^6 conidium mL^{-1} ialah 2.9, 3.5, 3.0 dan 3.0 hari. Kedua-dua pencilan* M. anisopliae *ini juga didapati amat patogenik ke atas telur mengakibatkan kerencatan penetasan yang signifikan, manakala* B. bassiana *dan* P. fumosoroseus *didapati kurang patogenik. Anggaran median kepekatan maut masing-masing bagi Cy3 dan MPs ialah 13.0 x 10^5 dan 5.03 x 10^5 conidium mL^{-1}.*

ABSTRAK

The ability of three species of entomopathogenic fungi, Paecilomyces fumosoroseus *(Wise) Brown & Smith,* Beauveria bassiana *(Bals.) Vuill. and* Metarhizium anisopliae *(Metsch.) Sorokin to cause infection on the adults, eggs and larvae of the striped flea beetle* Phyllotreta striolata F. *was tested. Only one isolate of* M. anisopliae *and none of the* B. bassiana *and* P. fumosoroseus *caused adult mortality in excess of 50% at a concentration of 2 x 10^7 conidia mL^{-1}. One isolate of* P. fumosoroseus *(Pf), one* B. bassiana *(Wls) and two of* M. anisopliae *(MPs and Cy3) were pathogenic to the first instar larvae of* P. striolata *causing more than 50% mortality at a concentration of 2 x 10^6 conidia mL^{-1}. The rate of larval mortality increased with increase in conidia concentration. The respective estimated LT_{50} values for Pf, Wls, MPs and Cy3 for the larvae at 2 x 10^6 conidia mL^{-1} were2.9, 3.5, 3.0 and 3.0 days. The two isolates of* M. anisopliae *were also highly pathogenic to the eggs causing significant inhibition of hatching, while* B. bassiana *and* P. fumosoroseus *were less pathogenic. Estimates of the median lethal concentration for Cy3 and MPs were 13.0 x 10^5 and 5.03 x 10^5 conidia mL^{-1} respectively.*

INTRODUCTION

The striped flea beetle, *Phyllotreta striolata* F., is not only a serious pest of canola and mustard but also feeds on a wide range of other brassicas (Ibrahim and Khoo 1989; Bartlet and Williams 1991). Injury to plants is caused by beetles and larvae feeding on leaves, or by larvae mining within stems or leaves, or feeding on the roots. They can kill plants directly by severing the hypocotyls or by eating the newly emerged meristem (Soroka and Pitchard 1987). The implementation of effective IPM in crop systems as the extension of biological control in a much more effective way is not yet established because to date the effectiveness of biocontrol agents such as predators, parasites or *Bacillus thuringiensis*

seems rather limited (Burgess 1982; Wylie 1984; Hazzard and Ferro 1991). Consequently, majority of the growers preferred applying broad spectrum chemical insecticides instead. For a strongly bio-based IPM to be successful, dependence upon chemical insecticides must be minimized and some suitable and safe alternative control measures are needed.

Entomopathogenic fungi, the common pathogen of soil-associating coleopterans such as the flea beetle, are promising agents for biological control and are gaining increasing attention worldwide as mycoinsecticides. The use of fungus as a biological control agent against flea beetle was first reported by Butt *et al.*(1992) when *M. anisopliae* isolate V90 was found to be pathogenic to the cabbage stem flea beetle, *Psylliodes chrysochephala*. Miranpuri and Kachatourians (1995) also reported the pathogenicity of *B. bassiana* against the cabbage flea beetle, *Phyllotreta cruciferae*. Similarly, *M. anisopliae* isolate with high pathogenicity against *P. cochleariae* demonstrated little or no pathogenicity for *P. chrysocephala* (Butt *et al.* 1992).

The aim of this study was to determine the susceptibility of the striped flea beetle adults, eggs and larvae to three species of entomopathogenic fungi and to evaluate the relative potency of these fungi against the beetles.

MATERIALS AND METHODS

Adult flea beetles collected using an aspirator from Chinese mustard, *Brassica juncea*, grown in the vegetable plot at the Universiti Putra Malaysia (UPM) Agricultural Park were used in the study. The beetles were maintained in plastic containers (38 x 23 x 38 cm) provided with fresh Chinese mustard leaves in a room at an ambient environment of 28 ± 2°C, a 12 h photoperiod and 85 ± 15% RH. To obtain eggs, gravid females were confined overnight in plastic containers (38 x 23x 38 cm) each lined with moist filter paper and provided with fresh Chinese mustard leaves. The eggs were then collected from these filter papers and left to incubate for 24 h in separate plastic cups (6 cm diameter). The first instar larvae were obtained the following day.

The original hosts and countries of origin for the fungal isolates used in this study are listed in Table 1. All isolates were maintained at room temperature of 28 ± 2°C on potato dextrose agar (PDA, Oxoid, Basingstoke, UK) containing 0.5% yeast extract (Difco) which had been sterilised for 20 minutes and 121°C and 1.05 kg/cm^2. To prepare fungal inocula, conidia from 15 day-old sporulating cultures were scraped from the surface of the plates with a sterile scalpel and suspended in a 0.05% aqueous Tween 80. A Neubauer haemocytometer was used to estimate the conidial concentration and subsequent appropriate dilutions were made.

A preliminary test on adult flea beetles was made using a dose of 2 x 10^7 conidia mL^{-1}. Inoculation was done by spraying 0.5 mL of the conidial suspension with a Sigma hand atomiser. Control insects were treated similarly with 0.05% aqueous Tween 80. After inoculation, the insects were transferred to a Petri dish (15 cm diameter) lined with moist filter paper and supplied with a fresh Chinese mustard leaf. Mortality was recorded 10 days after inoculation. Each assay consisted of five replicates with 10 adults per replicate.

The preliminary assays identified isolate *M. anisopliae* (Mps) (see Table 1) as worthy of further investigation, and it was passaged through and reisolated from the host onto PDA. Further bioassays were conducted using conidial concentrations ranging from 2 x 10^3 to 2 x 10^9 conidia mL^{-1} to determine the LC_{50} and LT_{50} values. Adult flea beetle mortalities were recorded daily for 10 days. Each assay was replicated five times with 10 adults per replicate.

The isolates tested for pathogenicity against the flea beetle eggs and first instar larvae were *M. anisopliae* (Cy3 and MPs), *B. bassiana* (Wls) and *P. fumosoroseus* (Pf). Bioassays were using conidial concentrations ranging from 2 x 10^3 to 2 x 10^8 conidia mL^{-1}. A 24-h old egg cluster (10-15 eggs) placed on a leaf section was inoculated by a drop (5 ml) of conidial suspension using a Gilson micropipette (P20). Larvae were inoculated by dipping them into conidial suspension for five seconds. The eggs or the larvae were transferred to Petri dishes (9 cm diameter) lined with moist filter paper and fresh Chinese mustard seedlings were supplied for the larvae. Number of eggs and larvae infected were recorded for five days. Each assay consisted of five replicates with 10 eggs or larvae per replicate. The final proportions of dead adults, larvae and infected eggs for each concentration were analysed using probit analysis (S103, Statistical Research Service, Canada Department of Agriculture, unpublished) based on Finney (1971).

TABLE 1
Isolates of *Metarhizium anisopliae, Beauveria bassiana* and *Paecilomyces fumosoroseus,* their original hosts and countries of origin

Species	Code	Insect host	Country of origin
M. anisopliae	*Cy3*	*Cylas formicarius* (Curculionidae)	Indonesia
	MPs	*Phyllotreta striolata* (Chrysomelidae)	Malaysia
B. bassiana	*Wls*	*Leptocorisa oratorius* (Alydidae)	Indonesia
P. fumosoroseus	*Pf*	*Pteroma pendula* (Psychidae)	Malaysia

TABLE 2
Pathogenicity[a] of *M. anisopliae, B. bassiana* and *P. fumosoroseus* isolates on adult *P. striolata*

Species	Code	%Mortality
M. anisopliae	Cy3	8
	PPs	58
B. bassiana	Wls	0
P. fumosoroseus	Pf	26

[a] at a dose of 2 x 10^7 conidia mL^{-1}

RESULT AND DISCUSSION

The pathogenicity of *M. anisopliae* on the flea beetles differed between isolates. Only one isolate of *M. anisopliae* caused mortality in excess of 50% (Table 2). The isolate of MPs was found to be the most pathogenic. No mortality was observed in the control.

The mortality of *P. striolata* was dose-dependent using the *M. anisopliae* (MPs) isolate. The estimated LC_{50} value was 6.77 x 10^7 conidia mL^{-1} while the LT_{50} values of *M. anisopliae* (MPs) at 2 x 10^9 and 2 x 10^8 conidia mL^{-1} were 4.7 and 5.8 days respectively. A 10-fold increase in dose did not result in any marked decrease in median lethal time. The LT_{50} values were not obtained at doses 2 x 10^7 conidia mL^{-1} and below because less than 50% mortality of the beetles was observed within the 10-days period (Table 3). The earliest death at the higher dose (2 x 10^9 conidia mL^{-1}) occurred on the third day. Fungal development was observed for at least 60% of the cadavers within 4-6 days. Sporulation occurred by the 7th day. Butt *et al.* (1992) also reported over 70% fungal growth within 2-5 days after the cabbage stem flea beetle, *P. chroscephala,* was exposed with 1 x 10^{10} conidia mL^{-1} of a highly pathogenic isolate of *M. anisopliae.*

Two isolates of *M. anisopliae* (MPs and Cy3), one *B. bassiana* (Wls) and one *P. fumosoroseus* (Pf) were pathogenic to the first instar larvae of *P. striolata.* All isolates caused more than 50% mortality at a dose of 2 x 10^6 mL^{-1}. No mortality was recorded in the control (Table 4). This indicated that the flea beetle larvae were more susceptible than the adult. Butt *et al.*(1992) also reported that adult flea beetles were less susceptible than the aphids *Myzus persicae* and *Lipaphis erydimi* which have softer bodies. The heavily sclerotised beetle cuticle, as opposed to a thinner integument for the larvae, could be the probable factor affording resistance to infection by entomopathogenic fungi.

Dose-related mortality was evident in all the isolates tested. The values of LC_{50s} and LT_{50s} for

TABLE 3
Median lethal concentration an median lethal time for varying dosages of *M. anisopliae* on adult *P. striolata*

Isolate	LC_{50} (95% FL) x 10^7 (conidia mL^{-1})	LT_{50}(95%FL) (days)	
		2 x 10^9 (conidia mL^{-1})	2 x 10^8 (conidia mL^{-1})
MPs	6.77 (3.25 – 18.16)	4.7(4.1 – 5.2)	5.8(3.9 – 13.1)

TABLE 4
Mean percent infection on first instar larvae of *P. striolata* upon treatment with varying dosages of *M. anisopliae*, *B. bassiana* and *P. fumosoroseus*

Doses (conidia mL^{-1})	*M. anisopliae*		*B. bassiana* (Wls)	*P.fumosoroseus* (Pf)
	(Cy3)	(MPs)		
2 x 10^8	74	86	82	80
2 x 10^7	64	78	68	64
2 x 10^6	66	72	54	70
2 x 10^5	18	20	22	26
2 x 10^4	8	12	12	10
2 x 10^3	2	0	0	4
Control	0	0	0	0

TABLE 5
Median lethal concentration of *M. anisopliae* (Cy3, MPs), *B bassiana* (Wls) and *P. fumosoroseus* (Pf) on first instar larvae of *P. striolata*

Isolate	Intercept	Slope ± SE	LC_{50}(95% FL) x 10^6 (conidia m^L1)
Cy3	1.408	0.545 ± 0.126	3.92 (0.22 – 57.29)
MPs	0.851	0.673 ± 0.138	1.47 (0.88 – 6.91)
W ls	1.222	0.579 ± 0.059	3.32 (1.70 – 6.91)
Pf	1.517	0.544 ± 0.111	2.20 (0.15 ± 74.93)

isolates MPs, Cy3, Wls and Pf are presented in Table 5. Dose-dependent relationship have similarly been reported for flea beetles in crucifers (Butt *et al.* 1992). The LT_{50} values of MPs, Cy3, Wls and Pf for *P. striolata* larvae at 2 x 106 conidia mL^{-1} were 3.0, 3.0, 3.5 and 2.9 days. A 10- to 100-fold increase in dosage significantly reduced the LT_{50} values of MPs and Wls, while for Cy3 and Pf a 100-fold increase occurred (Table 6).

Both the isolates of *M. anisopliae* were also highly pathogenic to flea beetle eggs, while *B. bassiana* and *P. fumosoroseus* were less pathogenic. *M. anisopliae* achieved infection in excess of 50% with the highest being a 97.03% and 100% infection by Cy3 and MPs respectively at a dose of 2 x 10^8 conidia mL^{-1}. Fungal infection to eggs significantly inhibited hatching (Table 7). Estimates of median lethal dose for isolates Cy3 and MPs are presented in Table 8. Zimmermann (1982) reported that *M. anisopliae* could infect the eggs or *Otiorhincus sulcatus* F. (Coleoptera: Curculionidae) only at the early stage before the eggs were melanised. Species reported to have

TABLE 6
Median lethal time of *M. anisopliae* (Cy3, MPs), *B. bassiana* (Wls) and *P. fumosoroseus* (Pf) on first instar larvae of *P. striolata*

Isolate	Dose (conidia mL^{-1})	Intercept	Slope ± SE	LT50(95%FL) days
Cy3	2 x 10^6	2.947	4.261 ± 0.899	3.0 (2.7 – 3.5)
	2 x 10^7	3.139	3.791 ± 0.887	3.1 (2.7 – 3.7)
	2 x 10^8	2.042	5.804 ± 1.397	3.2 (2.9 – 3.7)
MPs	2 x 10^6	3.569	3.003 ± 0.632	3.0 (2.5 – 3.4)
	2 x 10^7	3.318	3.861±0.659	2.7 (2.3 – 3.1)
	2 x 10^8	2.836	4.858 ± 0.699	2.8 (2.5 – 3.1)
Wls	2 x 10^6	2.991	3.639 ± 0.905	3.5 (3.1 – 4.5)
	2 x 10^7	2.802	4.620 ± 0.916	3.1(2.6 –3.5)
	2 x 10^8	2.679	6.317 ± 0.983	2.7 (2.4 – 2.9)
Pf	2 x 10^6	3.003	4.388 ± 0.898	2.9 (2.4 – 3.3)
	2 x 10^7	2.748	4.539 ± 1.842	3.1 (2.8 – 3.5)
	2 x 10^8	2.084	5.452 ± 0.935	2.9 (2.7 – 3.2)

TABLE 7
Mean percent infection of *P. striolata* eggs against doses used in assays of isolates of *M. anisopliae, B. bassiana* and *P. fumosoroseus*

Doses (conidia mL^{-1})	*M.anisopliae*		*B. bassiana* (Wls)	*P.fumosoroseus* (Pf)
	(Cy3)	(MPs)		
2 x 10^8	97.0 (0)	100.0 (0)	48.8 (46.8)	35.9 (40.5)
2 x 10^7	83.2 (2.4)	97.4 (4.2)	13.9 (76.6)	9.3 (63.9)
2 x 10^6	59.2 (20.3)	92.7 (3.0)	0 (75.3)	0 (82.3)
2 x 10^5	34.9 (38.9)	85.6 (5.6)	0 (84.0)	0 (95.5)
2 x 10^4	18.7 (74.5)	47.4 (42.5)	0 (80.7)	0 (82.6)
2 x 10^3	0 (85.5)	0 (91.1)	0 (83.1)	0 (86.3)
Control	0 (81.0)	0 (94.3)	0 (87.5)	0 (87.3)

( ) % eggs hatched.

TABLE 8
Median lethal concentration of isolates of *M. anisopliae* on the eggs of *P. striolata*

Isolate	Intercept	Slope ± SE	LC_{50} (95% FL) x 10^5 (conidia mL^{-1})
Cy3	1.564	0.562±0.045	13.00 (7.62 – 22.76)
MPs	0.558	0.945+0.209	5.03 (0.46 – 8.38)

caused egg mycosis were *Oospora ovorum* on locust and others that were limited to *Aspergillus, Fusarium,* and *Penicillium* (Madelin 1963; Fransen 1987).

Treatment at the egg stage could be advantageous because newly emerged larvae could quickly become infected by the persisting conidia in the immediate vicinity. Hence, for

soil-borne pest such as the larvae and eggs of the flea beetles treated with fungal spores, either prophylactically or curatively, could be applied as suggested by Charnley (1997). Moorhouse *et al.* (1993) reported that prior incorporation of *M. anisopliae* conidia into compost gave year-long protection to impatiens plant *(Impatients wallerana)* against the vine weevil. By direct drilling of *M. anisopliae* conidia using existing crop-sowing machinery to a depth of 20-25 cm, a long-term control of the redheaded cockchafer *Adophorus couloni* in the pasture was achieved (Rath 1992). Application of dried mycelial pellets were also particularly suitable as soil treatment (Stenzel *et al. 1992).* Krueger *et al.* (1991) used standard and lyophilized mycelial particles of *M. anisopliae* against scarab grubs. They concluded that the grub mortality occurred significantly quicker in mycelium-inoculated compared with conidia-inoculated soil.

CONCLUSION

This study has demonstrated that the three fungal isolates were infective against the larval stage and one of these isolates was highly pathogenic against the eggs. Further tests using formulated mycelial particles of these three fungi isolates are being planned against the flea beetle larvae in the field with the rational that the ambient soil moisture and temperature in Malaysia would promote profuse sporulation of the mycelium.

ACKNOWLEDGEMENT

The authors are grateful to Universiti Putra Malaysia for all the research facilities.

REFERENCES

BARTLET, E. and I.H. WILLIAMS. 1991. Factors restricting the feeding of the cabbage stem flea beetle *(Psylliodes chrysocephala). Entomologia Experimentalis et Applicata* **60:** 233-238.

BURGESS, L. 1982. Occurrence of some flea beetle pests of parkland rapeseed crops in open prairie and forest in Saskatchewan (Coleoptera: Chrysomelidae). *Canadian Entomologist* **114:** 623-627.

BUTT, T.M., M. BARRISEVER, J. DURMMOND, T.H. SCHULER, T.F. TILLEMAS and N. WILDING. 1992. Pathogenicity of the entomogenous, hypomycete fungus, *Metarhizium anisopliae* against the chrysomelid beetles *Psylliodes chrysocephala* and *Phaedon cochleariae. Biocontrol Science and Technology* **2:** 327-334.

CHARNLEY, A.K. 1997. Entomopathogenic fungi and their role in pest control. In *The Mycota a Comprehensive Treatise on Fungi as Experimental Systems for Basic and Applied Research,* ed. K. Esser and P.A. Lemke, **14**: 185-201. Berlin Heideldberg: Springer-Verlag.

FINNEY, D.J. 1971. *Probit Analysis.* Cambridge Univ. Press, Cambridge.

FRANSEN, J.J. 1987. Control of greenhouse whitefly, *Trialeurodes vaporiourum,* by the fungus *Aschersonia aleyrodis. Bulletin SROP* **10:** 57-61.

HAZZARD, R.V. and D.N. FERRO. 1991. Feeding responses of adult *Colemegilla maculate* (Coleoptera: Coccinellidae) to eggs of Colarado potato beetle (Coleoptera: Chrysomelidae) and green peach aphids (Homoptera: Aphididae). *Environmental Entomology* **20:** 644-651.

IBRAHIM, Y.B and K.C. KHOO. 1989. *Major Pests of Malaysian Crops.* Kuala Lumpur: Dewan Bahasa & Pustaka. Pp. 125-127. (in Bahasa Malaysia)

KRUEGER, S.R., M.G. VILLANI, A.S. MARTINS and D.W. ROBERTS. 1992. Efficacy of soil applications of *Metarhizium anisopliae* (Metch) Sorokin conidia, and standard and lyophilized mycelial particles against scarab grubs. *Journal of Invertebrate Pathology* **59:** 54-60.

MADELIN, M.F. 1963. Disease caused by hypomycetous fungi. In *Insect Pathology, an Advanced Treatise,* ed. E.A. Steinhaus, **2:** 233-272. New York: Academy Press.

MIRANPURI, G.S. and G.G. KACHATOURIANS. 1995. Entomopathogenicity of *Bearuveria bassiana toward* flea beetle, *Phyllotreta cruciferae* Goeze (Coleoptera: Chrysomelidae). *Journal of Applied Entomology* **119:** 167-170.

MOORHOUSE, E.R., A.T. GILLESPIE and A.K. CHARNLEY. 1993. Application of *Metarhizium anisopliae* (Metsch) Sorokin conidia to control *Otiorhynchus sulcatus* (F.) (Coleoptera: Curculionidae) larva on glasshouse pot plants. *Annals of Applied Biology* **122:** 623-636.

RATH, A.W. 1992. *Metarhizium anisopliae* for control of the Tasmanian pasture scarab *Adoryphorus couloni*. In *User of the Pathogens in Scarab Pests Management*, ed. T.A Jackson and T.R. Glare, pp. 217-227. Andover: Intercept.

SOROKA, J.J. and M.K. PRITCHARD. 1987. Effects of flea beetle feeding on transplanted and direct-seeded broccoli. *Canadian Journal of Plant Science* **67:** 549-557.

STENZEL, K., JHOLTERS, W. ANDERSCH, and T.A.M. SMIT. 1992. Bio 1020: granular *Metarhizium* - a new product for biocontrol of soil pests. In *Brighton Crop Protection Conf., Pests and Diseases*, Pp. 363-368. British Crop Protection Council. British crop Protection Council, Farnham.

WYLIE, H.G. 1984. Oviposition and curvival of three Nearctic euphorine braconids in crucifer-infesting flea beetles (Coleoptera: Chrysomelidae). *Canadian Entomologist* **116:** 1-4.

ZIMMERMANN, G. 1982. Investigation on efficacy of *Metarhizium anisopliae* (Metsch) Sorokon on egg and hatching first instar larvae of *Otiorhyncus sulcatus* F. (Coleoptera: Curculionidae). *Zeitschrift fur Angewandte Entomologie* **93:** 476-482.

(Received: 3 July 2001)
(Accepted: 9 October 2002)

Pertanika Journal of Tropical Agricultural Science

Subject Index for Volume 27 Nos. 1 & 2, 2004

Pertanika Journal of Tropical Agricultural Science

Author Index for Volume 27 Nos. 1 & 2, 2004

Acknowledgements

The Editorial Board acknowledges the assistance of the following reviewers in the preparation of Volume 27, Numbers 1 & 2 of this journal

Prof. Dr. Datin Ann Anton
Prof. Dr. Ahmad Said Sajap
Dr. Abdol Shukor Juraimi
Prof. Dr. Abdul Razak Alimon
Dr. Azmi Man
Assoc. Prof. Dr. Che Teh Fatimah Iskandar
Assoc. Prof. Dr. Fauziah Ishak
Prof. Dr. Hor Yue Luan
Dr. Izham Ahmad
Dr. Lee Pat Moi
Assoc. Prof. Dr. Luang Jun Boo
Assoc. Prof. Dr. Mahmud Tengku Muda Muhamad
Dr. Masri Muhamad
Prof. Dr. Dennis B. McConnell
Prof. Dr. Mohd. Nasir Saadon
Prof. Dr. Mohd. Zamri Saad
Prof. Dr. P.S. Motooka
Assoc. Prof. Dr. Patimah Ismail
Prof. Dr. P.S Rainbow
Assoc. Prof. Dr. Rajan Amartalingam
Assoc. Prof. Ramlah Hamid
Assoc. Prof. Dr. Rita Muhammad
Dr. Rusea Go
Prof. Dr. Johnnes Scholberg
Prof. Dr. Shamsudin Jusop
Assoc. Prof. Dr. Siti Azizah Mohd. Noor
Dr. S. Sivapragasam
Prof. Dr. S. Tanabe
Dr. Tan Yee How
Assoc. Prof. Dr. Umi Kalsom Yusof
Dr. Yap Chee Kong
Prof. Dr. Yusof Ibrahim
Dr. Zainudin Meon
Prof. Dr. Zubir Din
Prof. Dr. Zulkarnain Zainal

Preparation of Manuscript

A full article should not exceed 10 printed pages (one printed page is roughly equivalent to 3 type-written pages) including figures and tables.

A short communication, not exceeding two printed pages, is intended for rapid publication.

Typing and Paper
Manuscripts should be typewritten on A4 paper, double spaced, and of letter quality with 4cm margins on all sides.

Title page
This page should bear the title of the paper with the full name of the author(s), followed immediately by the address. Author citation should also be provided. A short title not exceeding 60 characters should be provided for the running headline.

Abstract
Abstracts in Bahasa Melayu and English, each not exceeding 200 words, should follow immediately after the names and affiliation of author(s). Papers from outside of Malaysia may be submitted with an English abstract only.

Keywords
Up to a maximum of 10 keywords are required and they should be placed directly below the abstract.

Illustrations & Photographs
Illustrations including diagrams and graphs will be referred to as 'figures' and photographs as 'plates' in the text and numbered consecutively in Arabic numerals. All photographs (glossy black and white prints) should be supplied with appropriate scales.

Illustrations should be of print quality; outputs from dotmatrix printers are not acceptable, Illustrations should be on separate sheets, about twice the size finished size in print. all letters, numbers and legends must be included on the illustration with the author's name, short title of the paper, and figure number written on the verso. A list of captions should be provided on a separate sheet.

Tables
Tables should conform to page size. Vertical lines should be avoided.

Measurements
Metric units must be used for all measurements.

Equations and Formulae
These must be set up clearly and should be typed triplespaced. Numbers identifying equations should be in square brackets and placed on the right margin of the text.

Scientific Names
Scientific names should be given for all organisms.

Abbreviations
Standard abbreviations should be used.

Citations and References
Items in the reference list should be referred to in the text by inserting, in parentheses, the year of publication after the author's name. If there are more than two authors, the first author should be cited followed by '*et al*'. The names of all authors, however, will appear in the reference list.

In case of citing an author(s) who has published more than one paper in the same year, the papers should be distinguished by addition of a small letter, e.g. Choa (1979a); Choa (1979b); Choa (1979c).

References should be arranged alphabetically according to first author. Serial titles are to be given in full.

Examples of reference citations are provided:

Monographs
Turner, H.N. and S.S.Y. Yong. 1969. Quantitative Genetics in Sheep Breeding. Ithaca: Cornell University Press.

Serials
Ho, Y.W. and A. Nawawi. 1991. Effect of carbon and nitrogen sources on growth of *Ganoderma boninense* from oil palm. *Journal of Plant Protection in the Tropics* **8**: 37-43

Chapter in Edited Book
Roberts, D.W. 1980. Toxins of entomopathogenic fungi. In *Microbial Control of Pests and Plant Diseases*, ed. H.D. Burgess, p. 441-463. New York: Academic Press.

Proceedings
Hussein, M.Y. 1986. Biological control of aphids on potatoes by inundative releases of predators. In *Biological Control in the Tropics*, ed. M.Y. Hussein and A.G. Ibrahim, p. 137-147. Serdang: Universiti Pertanian Malaysia Press.

Unpublished Materials (e.g. theses, reports & documents)
Normah, M.N. 1987. Effects of temperature on rubber (*Hevea brasiliensis* Muell - Arg.) Seed storage. Ph.D. Thesis, 206p. Universiti Pertanian Malaysia.

The abbreviation for Pertanika Journal of Tropical Agricultural Science is *Pertanika J. Trop. Agric. Sci.*

Pertanika Journal of Tropical Agricultural Science

Volume 27 Number 2 (September) 2004

Contents

ISSN 1511-3701

09

9 771511 370234