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The Phytotoxic Effects of Palm Oil Dry Solids on Plant Growth

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Keywords: palm oil mill effluent (POME), palm oil dry solids (PODS), phytotoxicity, decomposition, bioassay, sandy tailing soil

ABSTRAK

Kajian di rumahkaca dan di makmal bertujuan mengkaji kefitotoksikan pepejal kering kelapa sawit (PODS) terhadap pertumbuhan sayuran dan kesan penguraian terhadap kefitotoksikan. PODS mentah dan reput pada kadar 0, 1, 3, 6, 9, 15 dan 21% (b/b) digaul ke dalam tanah pasir bekas lombong dan ditanam dengan anak benih tomato dan bayam. Sampel PODS mentah dieramkan pada suhu 30°C selama 0, 1, 2, 3, 4, 6 dan 8 minggu dan ekstrak akueus setiap sampel dibiocerakinkan untuk mengesan perencatan pertumbuhan akar tomato. Keputusan kajian rumahkaca menunjukkan pertumbuhan tomato dan bayam dipengaruhi oleh jenis (mentah atau reput) dan kadar PODS yang digunakan. Tumbesaran kedua-dua sayuran terencat dengan pemberian > 1% PODS mentah. Sebaliknya, pemberian 1 - 21% PODS reput meningkatkan tumbesaran tanaman dengan penghasilan bahan kering maksimum pada paras 6%. Pada paras ini, berat kering pucuk tomato dan bayam meningkat 7 dan 178 kali berturutan, manakala berat kering akar meningkat 1.6 dan 62 kali berturutan berbanding tanaman pada PODS mentah. Kandungan N, P dan K tanah serta pH dan konduktiviti elektrik tanah juga meningkat dengan peningkatan paras PODS. Kajian pengeraman menunjukkan kesan fitotoksik PODS mentah berkurangan apabila PODS telah mereput > 4 minggu.

ABSTRACT

Glasshouse and laboratory experiments were conducted to evaluate the phytotoxicity of palm oil dry solids (PODS) on growth of vegetables and the effect of decomposition on the reduction of PODS phytotoxicity. Raw and decomposed PODS was applied to sandy tailing soil at the levels of 0, 1, 3, 6, 9, 15 and 21% (w/w) and planted with tomato and spinach seedlings. Samples of raw PODS were incubated at 30°C for 1, 2, 3, 4, 6 and 8 weeks and the aqueous extract of each sample was bioassayed for growth inhibition of tomato radicles. Results from the glasshouse experiment showed that growth of tomato and spinach was strongly affected by the type (raw or decomposed) and amount of PODS applied. Growth of both plants was inhibited by application of >1% raw PODS. In contrast, application of 1- 21% decomposed PODS increased plant growth, with maximum dry matter production at 6% level. At this level, shoot dry weights of tomato and spinach increased 7 and 178 times, respectively, while root dry weights increased 1.6 and 62 times, respectively, compared to plants in raw PODS. Soil N, P and K contents, pH and electrical conductivity also increased with increase in PODS levels. The incubation study showed that the phytotoxicity of raw PODS was reduced when PODS was decomposed for > 4 weeks.

INTRODUCTION

Palm oil mill effluent (POME) contributes a large proportion of the agricultural waste in Malaysia. If not treated correctly, this waste can cause environmental pollution. As a step to minimizing this problem, POME is currently being utilized in agriculture as organic fertilizer. Application of POME to soil has been shown to increase the growth of oil palm and other crops (Lim *et al.* 1984). However, application of high levels of raw POME to soil can adversely affect growth of plants. Direct application of such effluent was observed to reduce growth of oil palm seedlings (Mohd Nazeeb *et al.* 1984) and

the production of oil palm fresh fruit bunch (FFB) (Chan *et al.* 1981). Application of undecomposed POME to sandy tailings also reduced the growth of mustard greens (Zulkifli and Rosmin 1990). The inhibitory effect of raw POME on plant growth has been associated with the presence of lipid and some volatile substances, which indirectly inhibit the development of plant's roots (Lim 1986). This inhibitory effect is however only temporary, since the toxic compounds are rapidly decomposed and eliminated from the soil.

The inhibitory effects of POME on plant growth are similar to the phytotoxicity exhibited by other types of crop residues (Rice 1984). Inhibition of plant growth has been closely associated with the presence of phenolic substances. These toxic substances could be leached out in high amounts from the organic residues into the soil or produced by microorganisms during residue decomposition. The degree of inhibition from these compounds depends on the type of organic residue and the sensitivity of the plant root system (Zucconi and de Bertoldi 1987). Phytotoxicity has been found to be maximum at the early stage of residue decomposition and it disappears with prolonged decomposition (Guenzi *et al.* 1967; Kimber 1973).

Several types of POME generated in palm oil mills are being utilized as organic fertilizer One of the commonly used types is the decanter dried sludge. This is the solids remaining after the decanter sludge has been dried to a constant weight at 105°C in the mill and is referred to as palm oil dry solids (PODS) (Zakaria and Hassan 1993). It is usually applied to soil a few weeks before seeding or transplanting. This material may also contain water-soluble compounds which are phytotoxic to plant growth. Continuous application of the raw PODS can result in accumulation of these toxic compounds in soils. Although inhibition of plant growth with the application of raw palm oil mill effluent has been frequently observed, only a few studies have been conducted on this phenomenon. It is therefore of great importance to assess the phytotoxicity of PODS, quantify the amount of PODS to be applied and determine the degree of its decomposition, in order to utilize the effluent as an organic fertilizer. The experiments conducted aimed to determine the phytotoxic effect of raw and decomposed PODS on growth of two types of vegetables, and to evaluate the effect of decomposition period on the growth of tomato radicles.

MATERIALS AND METHODS

Effect of Raw and Decomposed PODS on Plant Growth

Raw PODS was collected from the Rantau Palm Oil Mill, Negeri Sembilan. It was a dried material containing 8% moisture and granular in shape, with size range 2-5 mm. The material was kept in plastic bags and stored in a cold room (8°C) before use.

Decomposed PODS was prepared by placing the raw effluent in dark plastic bags, moistening with (100%) distilled water and leaving to decompose aerobically in the glasshouse for 6 months at temperatures of 28-35°C. The material was turned over every month and the moisture content was maintained throughout the incubation period. The decomposed PODS was then air-dried, ground and sieved (2-mm mesh). The chemical properties of raw and decomposed PODS are shown in Table 1.

TABLE 1
Chemical characteristics of palm oil dry solids

	Raw	Decomposed
C (%)	19.43	14.40
N (%)	1.44	2.31
P (%)	0.32	0.35
K (%)	1.32	1.75
Ca (%)	1.46	2.36
Mg (%)	0.32	0.81
Lipid (%)	12.0	0
pH(1:5 in H_2O)	5.0	6.7

Soil Preparation and Treatments

Sandy tailing soil used in the study was collected from ex-mining land located at Universiti Putra Malaysia, Serdang. The infertile soil contained 89.7% sand, 7.5% silt and 1.7% clay, pH 6.3 (1:2.5 in H_2O), 1.4 g kg^{-1} organic carbon, 0.01 g kg^{-1} P, 0.01 cmol (+) K kg^{-1} soil and traces of total N. The soil was air-dried and passed through a 2-mm sieve. The soil was subsequently used to fill (1 kg/pot) 60 undrained pots with top diameter 19 cm and height of 15 cm.

The sandy soil was then treated with different levels of raw and decomposed PODS. The levels of PODS used were: 0, 1, 3, 6, 12, 15, and 21% (w/w air-dried basis). The treated soil was mixed thoroughly and watered to field capacity.

The two test plants used were tomato (*Lycopersicum esculentum*) and spinach (*Amaranthus viridis*). Tomato and spinach seeds were germinated on sandy soil for one month, after which two uniform seedlings from each plant species were transplanted to the respective pots. The experiments were laid out as a randomized complete block design (RCBD) with four replications per treatment. All the plants were watered to field capacity daily and harvested 30 days after transplanting (DAT). At harvest, plant shoots were cut 1 cm above ground level. The roots were freed from the soil and washed clean of adhering soil particles with tap water. Both shoots and roots were oven-dried at 60°C to constant weights and their dry weights were recorded.

Soil in each pot was sampled, air-dried and analysed for total N using the microkjeldahl procedure (Bremner 1965), exchangeable P using the method of Bray and Kurtz (1945), exchangeable K using the method of Singh and Ratnasingam (1970), soil pH (1:2.5 in H_2O) using pH meter and soil electrical conductivity (EC) (1:2.5; in H_2O) using the portable EC meter.

Effects of PODS Decomposition Period on Growth of Tomato Radicle

Incubation study. One hundred grams of raw PODS were placed in separate 500-ml conical flasks. The contents were moistened with 100 ml distilled water and incubated at room temperature (28-30°C) for 0, 1, 2, 3, 4, 5, 6 and 8 weeks. At the end of each sampling period, 10 g PODS from each flask was mixed with 100 ml distilled water and vigorously shaken on a rotary shaker for 6 hours. The suspension was left to settle in a cold room (8°C) for 30 min. The suspension was then decanted into a clean tube and centrifuged at 1000 (rpm) for 1 h. The clear brown supernatant was vacuum-filtered through Whatman No. 2 filter paper and the filtrate was subsequently bioassayed to assess its phytotoxicity.

Bioassay technique and seed selection. Several seeds, *viz* rice, mungbean, cucumber, spinach and tomato were tested for their sensitivity to the inhibitory compounds present in PODS extract (Table 2). The degree of inhibitory effect of the aqueous extract was evaluated by measuring the length of radicles in treated versus control. Tomato seed was found to be most sensitive to the bioassay tests conducted. This seed was easy to handle and consistent in response to PODS extract. Tomato seed was subsequently used in further bioassay tests conducted.

TABLE 2
Growth of seedling radicles in PODS extract

Seedlings	Radicle length (mm)	
	Distilled H_2O (control)	PODS extract
Tomato	61.9	10.8
Spinach	37.6	19.2
Mungbean	51.6	30.9
Cucumber	43.8	65.2
Rice	49.4	54.3

One gram of tomato seeds (400 seeds) were successively washed 5 times with sterilized distilled water. Initially, the seeds were sterilized in 0.1% NaOCl. However, this was discontinued as 0.1% NaOCl concentration was found to inhibit seed germination. The seeds were then germinated in glass petri dish lined with Whatman No.1 filter paper and kept in the dark at 30°C for 48 h. Uniformly germinated seeds with radicle length of about 1 mm were then used in the bioassay.

One millilitre of the PODS extract was pipetted into sterile glass petri dishes (90 x 10 mm) lined with double layer Whatman No.1 filter paper. Two millilitres of distilled water were added to make up a total of 3 ml solution per dish. The control dish was only given 3 ml sterilized distilled water. Ten pregerminated seeds were then placed at equidistant points in the dish. A similar procedure was used to determine the effect of lipids (oils and fats) extracted from PODS on growth of tomato radicles. The lipids were extracted earlier from PODS (raw and decomposed for 4 weeks). Five milligrams of the lipids were dissolved in 5 ml chloroform to form a concentration of 1 mg ml^{-1}. One millilitre of the solution was then placed in sterilized petri dishes as described previously. The chloroform in the dish was allowed to evaporate overnight, before 3 ml of sterilized distilled water was added to the dish. The control dish was given 1 ml chloroform which evaporated off overnight, before adding 3 ml of water. Ten pregerminated seeds were then placed at equidistant points in the dish. The bioassay was replicated three times.

All the petri dishes were then incubated in the dark at 30°C. Radicle growth was determined

by measuring the length of the radicles three days after incubation. The presence of inhibitory compounds in the treatment was indicated by stunted radicle length as compared to the normal radicle in control. The radicle growth in PODS extract was then expressed as the percentage of radicle growth in control (distilled water).

PODS analysis. The lipid content in PODS was determined by fluxing 10 g PODS with 100 ml petroleum ether for 2 h using the Buchi Sohxlet fat extractor. The percentage of lipid was calculated from the weight of dried residue in the collecting dish. The pH and electrical conductivity (EC) of the PODS extract (1:10; PODS: water (w:v)) were also determined. All data obtained were subjected to the analysis of variance using the SAS (1987) procedures.

RESULTS

Effect of Raw and Decomposed PODS on Plant Growth

Shoot and root dry weights. Results showed that shoot and root dry weights of tomato and spinach were significantly ($P \leq 0.01$) affected by the type (raw or decomposed) and amount of PODS applied (*Fig. 1A, B*). In general, application of decomposed PODS significantly increased both the dry weight of shoots and roots of both plants compared to raw PODS. Plant growth was also affected by the level of PODS applied. Maximum growth of tomato and spinach obtained in soils with an application of 6% decomposed PODS. At this level, the dry weight of tomato shoots and roots increased by 7 and 1.6 times, respectively, compared to plants in raw PODS. Spinach shoots and roots increased by 178 and 62 times respectively, in decomposed PODS compared to those in raw PODS.

Application of decomposed PODS at levels > 6%, however, decreased the growth of both plants. Shoot dry weight of tomato decreased by 43% when the level of decomposed PODS was increased from 6 to 9% (*Fig. 1A*). Shoot dry weight of spinach decreased by 50% when given 15% PODS (*Fig. 1B*). Similarly, the root growth of both plants was also reduced with the application of > 6% decomposed PODS.

Soil nutrients. Application of both types of PODS significantly ($P \leq 0.01$) affected N, P and K

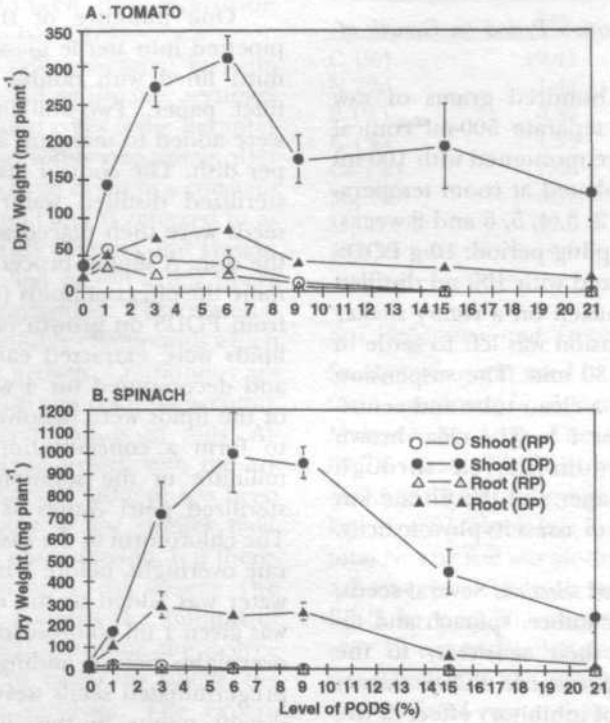

Fig 1. Effect of raw (RP) and decomposed PODS (DP) on dry weight of shoots and roots of tomato (A) and spinach (B) (Means ± Standard error)

contents of the sandy soil cultivated with tomato and spinach. In general, the soil N, P and K increased with increase in the level of PODS. Total N and available P were higher in soils treated with decomposed than with raw PODS (Table 3A, B). This was true for all levels of PODS applied. The available K in soil did not differ significantly between treatments with raw or decomposed PODS.

Soil pH and electrical conductivity (EC) were significantly ($P \leq 0.01$) influenced by the type and level of PODS applied (Table 4A, B). In general, the soil pH increased with increase in the levels of PODS applied. The pH was slightly higher in soil treated with decomposed PODS than soil treated with raw PODS. The EC, which measures the total soluble salts in soil, also increased with increase in PODS level. At ≤ 9% level, EC value in soils treated with raw PODS increased to > 5.5 mS cm^{-1} and to > 7.0 mS cm^{-1} with decomposed PODS.

Effect of PODS Decomposition Period on Growth of Tomato Radicles

Growth of tomato radicle. The results showed that the period of PODS decomposition significantly ($P \leq 0.01$) affected radicle length of tomato seedlings (*Fig. 2*). The percentage of radicle growth for tomato seedlings increased with decomposition period. Growth of the radicle was severely inhibited at weeks 0 and 1 with 30 and 18.7% inhibition, respectively, compared to the control. The tomato radicle was stunted and severely browned, indicating necrosis of the root cells resulting from the effect of growth inhibitors. Toxicity of PODS was found to decline rapidly after 2 weeks of incubation, resulting in rapid increase in growth of radicles up to 84.1% four weeks after PODS decomposition.

The bioassay results also showed that growth of tomato radicles was inhibited by the presence of high amounts of lipids in PODS, and the inhibition was reduced with PODS decomposi-

TABLE 3
Nutrient content of sandy tailings under (A) tomato and (B) spinach

(A) Tomato

Level of PODS (%)	N (g kg^{-1})		P (mg kg^{-1})		K (cmol(+)kg^{-1})	
	Raw	Decomposed	Raw	Decomposed	Raw	Decomposed
0	0.55	0.55	13.27	13.27	0.02	0.02
1	0.33	0.60	19.61	28.17	0.30	0.32
3	0.38	0.73	41.04	52.75	0.82	0.70
6	0.63	1.03	76.15	105.47	1.36	1.32
9	1.98	1.23	100.67	136.16	1.72	1.68
15	1.33	2.38	151.19	211.85	2.43	2.43
21	2.73	3.40	255.69	295.96	3.26	2.81
LSD (0.05)	0.08	0.58	17.93	15.57	2.57	0.34

(B) Spinach

Level of PODS (%)	N (g kg^{-1})		P (mg kg^{-1})		K (cmol(+)kg^{-1})	
	Raw	Decomposed	Raw	Decomposed	Raw	Decomposed
0	0.40	0.40	11.62	11.62	0.02	0.02
1	0.60	0.80	17.30	17.62	0.03	0.32
3	0.60	0.80	42.75	40.94	0.80	0.70
6	0.80	1.10	71.00	81.06	1.30	1.40
9	0.90	1.19	101.28	121.13	1.10	1.85
15	1.00	2.40	151.61	211.60	2.40	2.18
21	2.10	3.60	251.95	294.39	2.70	2.90
LSD (0.05)	0.12	0.16	16.79	10.13	0.33	0.53

TABLE 4
pH and EC of sandy tailings under (A) tomato and (B) spinach

(A) Tomato

Level of PODS (%)	pH		EC (mS cm^{-1})	
	Raw	Decomposed	Raw	Decomposed
0	5.8	5.8	0	0
1	6.2	6.5	1.0	1.0
3	6.3	6.8	2.8	2.5
6	6.3	7.1	3.3	2.8
9	6.1	7.2	5.5	7.0
15	6.1	7.3	7.3	10.0
21	6.0	7.3	11.3	16.0
LSD (0.05)	NS	0.1	0.3	1.0

(B) Spinach

Level of PODS (%)	pH		EC (mS cm^{-1})	
	Raw	Decomposed	Raw	Decomposed
0	5.8	5.7	0	0
1	6.6	6.9	1.0	1.0
3	6.9	6.9	2.3	2.3
6	7.0	7.1	3.3	2.7
9	7.0	7.0	5.7	7.0
15	7.0	7.1	7.7	9.0
21	0.2	7.2	10.0	16.3
LSD (0.05)	0.2	0.2	0.5	1.5

NS - not significant at $P \leq 0.05$

tion (Table 5). It was observed that the lipid content in PODS was reduced from 10.1% to 0.4% after 4 weeks of decomposition. The radicle growth was subsequently observed to increase from 78.7% at week 0 to 88.2% at week 4.

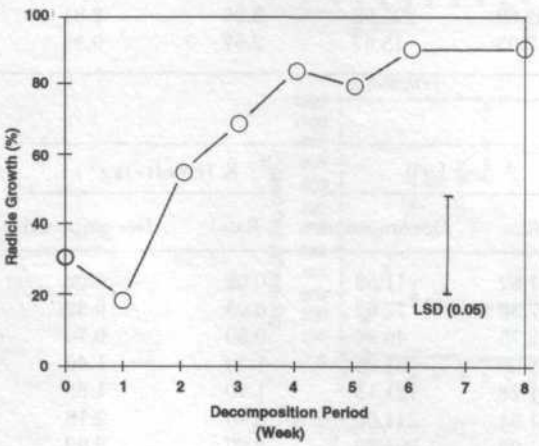

Fig 2. Effect of decomposition period of PODS on growth of tomato radicles

Changes in lipid, pH and EC of PODS. Decomposition of PODS was found to significantly ($P \leq 0.01$) influence the lipid content and pH, but not the electrical conductivity (EC) of PODS (Table 6). The lipid content in PODS decreased rapidly with increase in decomposition time. The lipid content was reduced by 96% after 4 weeks of incubation. The decrease in lipid content paralleled the increase in radicle growth of tomato seedlings as observed in Table 5. The pH of PODS extract increased significantly ($P \leq 0.01$) with increase in decomposition time. The pH increased from 5.0 at week 0 to 7.3 at week 4

TABLE 5
Effect of lipid on growth of tomato radicles

Decomposition period (week)	Lipid content in PODS (%)	Radicle (% of control)
0	10.1	78.7
4	0.4	88.2

TABLE 6
Lipid content, pH and EC of PODS extract as affected by decomposition period

Decomposition period (week)	Lipid content (%)	pH	Electrical conductivity (mS cm^{-1})
0	10.1	5.0	3.9
1	7.3	5.6	3.4
2	2.8	5.6	3.4
3	1.1	6.3	3.6
4	0.4	7.3	3.8
5	0.2	7.4	3.5
6	0.1	7.5	3.5
8	0.1	7.6	3.6
LSD (0.05)	1.9	0.4	NS

NS - not significant at $P \leq 0.05$

and remained almost constant thereafter. The EC of PODS remained almost constant (3.4 - 3.9 mS cm^{-1}) throughout the decomposition periods.

DISCUSSION

Results from the present study indicated the strong influence of PODS on growth of vegetable seedlings. Application of sandy tailing soil with raw PODS inhibited plant growth. Both tomato and spinach plants exhibited little shoot and root growth. In contrast, application of decomposed PODS benefited plant growth in sandy soil. Maximum plant growth was observed in soil treated with 6% decomposed PODS. At this level, growth of shoots and roots of tomato in decomposed PODS was higher than growth in raw PODS. There were differences in the plant's sensitivity to PODS. Spinach was more sensitive to the toxic compounds present in raw PODS then tomato.

Severe reduction in plant growth in soil treated with raw PODS strongly indicates the presence of soluble toxic compounds. The inhibition in plant growth was similar to that observed by earlier studies using other types of crop residues. Growth of wheat and oat seedlings was inhibited when grown in extract of wheat residue (Kimber 1973). Residues of wheat, barley and bluegrass were also inhibitory to wheat seedlings (Cochran *et al.* 1997). Isolation of the compounds revealed that phenolic compounds, especially the free phenolic acids, were responsible for causing plant growth inhibition (Kuwatsuka and Shindo 1973; Lodhi *et al.* 1987; Wójcik-Wojtkowiak *et al.* 1990).

The phytotoxic effect has also been shown to differ with plant types, the amount of toxins present and the amount of roots in contact with the toxic compound (Zucconi and de Bertoldi 1987). The toxins have often been found to affect the growth of the root system more severely than the vegetative parts. In the present study, development of plant roots in the raw PODS was severely retarded with the roots becoming dark brown, indicating death of the root cells. This effect was probably due to the presence of the toxic compounds. Poor root growth would then lead to poor development of the entire plant. Although the presence of high concentrations of nutrients could also give similar toxicity to plant root system (Reuter and Robison 1986), such effect was however not observed in soils given the decomposed PODS.

The results obtained do not illustrate clearly the relationship between plant toxicity and excessive availability of soil nutrients and pH. It was observed that soil N, P and K increased with increase in levels of either raw or decomposed PODS (Table 4). Application of 6% decomposed PODS was the optimum level for plant growth. In contrast, application of the same level of raw PODS inhibited growth, even though the nutrient contents in the soil were similar to those present in soil treated with decomposed PODS. This strongly suggested that plant growth inhibition could be due to other factors. Zucconi and de Bertoldi (1987) had earlier shown that plant growth could be inhibited by the phytotoxin, even in the presence of nutrients. The inhibitory effect could not be related to the soil pH as there was no extreme change in the pH with PODS application. The addition of 6% raw PODS to soil resulted in soil pH of 6.3-6.9, i.e. a soil pH which is considered normal for most plants (Table 4A, B) as compared to pH 7.1 for soils applied with 6% decomposed PODS. Application of high amounts (> 6%) of either raw or decomposed PODS resulted in increase in soil soluble salts measured as the electrical conductivity (EC), which could cause some problems to plant growth. Most plants have been shown to be adversely affected by salt content of > 8 mS cm^{-1} (Mengel and Kirby 1982). Differences in salt concentration will lead to differences in the osmotic pressure around the root cells and will subsequently inhibit the physiological activities of the plant, thus hindering uptake of water by the root cells (Wild 1988). The de-

composition period of PODS did not significantly affect the EC value. This probably indicates that the soluble salts would be an unlikely factor causing growth inhibition in tomato radicles as observed at weeks 0-1 after PODS decomposition. Inhibition of plant growth on soil applied with high doses of raw palm oil mill effluent could also result in waterlogging, which reduces the soil aeration (Chan *et al.* 1981).

Results from the bioassay study showed a rapid decrease in the degree of phytotoxicity as the decomposition period increased. The growth of tomato radicles in the first week of decomposition was severely inhibited, with only an 18.7% growth as compared to control. A prolonged decomposition period of 4 weeks resulted in reduced inhibition and a subsequent radicle growth increment of 84.1% (*Fig. 2*). The water-soluble toxic compounds could be the factor involved in the inhibition of radicle growth. The toxic compounds could rapidly be leached out from PODS immediately after it was mixed with water. This probably caused the radicle inhibition observed at week 0. A slight increase in toxicity after one week of decomposition was probably due to the presence of toxins produced through microbial metabolism of PODS. Several species of bacteria, fungi and actinomycetes have been isolated from other types of POME decomposing in soil (Palaniappan *et al.* 1984; Radziah 1994). These groups of microorganisms could also be responsible for producing the phytotoxic effects observed in raw PODS. However, the phytotoxic effect is temporary as the toxic compounds are rapidly decomposed by microorganisms. There are other microbial communities which metabolize toxins such as phenolic acids as their source of carbon and energy for growth (Blum and Shafer 1988). Such reduction in phytotoxicity of PODS was evident when PODS was allowed to decompose for > 4 weeks.

Apart from the soluble compounds, the lipid (oils and fats) component in PODS extract was also found to inhibit growth of tomato radicles (Table 5). Results obtained showed that crude lipid extract from raw PODS caused a 21.3% growth inhibition. This resultant effect could have intensified the overall phytotoxicity of PODS. However, the toxicity decreased with increase in decomposition period. Earlier studies have indicated that lipids in PODS were responsible for inhibiting root growth of some vegetables (Zulkifli and Rosmin 1990). The presence of fatty acids which are the glyceride components of the oils and fats in oil palm (Azis and Tan 1990) could be inhibitory to radicle growth. Braids and Miller (1975) have shown that a number of short chain fatty acids inhibited growth of wheat roots. However, these fatty acids have also been proven to be rapidly decomposed by soil microorganisms (MouCawi *et al.* 1981).

Decomposition of PODS was found to be beneficial to growth of plants, especially on sandy tailing soil. Application of 6% decomposed PODS to soils tremendously increased growth of spinach and tomato. The absence of phytotoxicity in decomposed PODS can probably be attributed to the breakdown of the toxins by soil microorganisms. The identity of the toxic compound and the significant role of these microorganisms in breaking down the inhibitory compounds present in the PODS need further study.

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Reduction in Leaf Growth and Stomatal Conductance of Capsicum (*Capsicum annuum*) Grown in Flooded Soil and Its Relation to Abscisic Acid

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ABSTRAK

*Pengaruh banjir ke atas pertumbuhan daun, kaitan air, perubahan stomata dan kandungan asid absisik (ABA) pada tanaman cili (*Capsicum annuum *L.) di kaji di bawah keadaan persekitaran terkawal. Kebanjiran tanah mempercepatkan penutupan stomata dan pengurangan perkembangan daun tanpa pengurangan defisit air daun. Pengurangan potensi air daun tidak berlaku selama 4 hari rawatan banjir. Seterusnya, potensi air daun berkurangan kepada tahap minima. Kepekatan asid absisik di dalam xilem bertambah selepas 24 jam banjir, dan bertambah secara progresif dengan masa rawatan banjir. Kepekatan asid absisik di dalam daun dan bunga lebih tinggi pada pokok yang di dedahkan kepada banjir di bandingkan dengan pokok yang di beri pengairan berterusan. Dalam kedua-dua keadaan persekitaran, kandungan asid absisik di dalam bunga adalah lebih tinggi daripada daun*

ABSTRACT

*The effects of soil flooding on leaf growth, water relations, stomatal responses and abscisic acid (ABA) content in young capsicum (*Capsicum annuum *L.) plants were studied under controlled environmental conditions. Soil flooding induced early stomatal closure and leaf growth reduction without any reduction in leaf water deficit. The undetectable changes in leaf water potential of plants grown in flooded soil persisted for 4 d. Thereafter, leaf water potential was reduced to the minimum values. Xylem sap abscisic acid concentration was increased after 24 h of soil flooding, and increased rapidly with duration of flooding. Plants grown in flooded soil had higher concentration of abscisic acid in leaves and flowers than the well watered plants. Under both conditions, abscisic acid concentrations was higher in flowers than in leaves.*

INTRODUCTION

Soil flooding may result in many detrimental morphological and physiological changes, such effects having been reported on many horticultural crop species (Schaffer *et al.* 1992; Else *et al.* 1995). Capsicum (*Capsicum annuum* L.) is considered a flood-sensitive vegetable species as the reduction in stomatal conductance and photosynthesis rate occurs after 24 h of soil flooding (Pezeshki and Sundstrom 1988). Many plant species respond to flooding much as they do to drought through reduced growth, chlorosis and subsequently leaf senescence (Hurng *et al.* 1994).

Reid *et al.* (1991) indicated that flooding of roots causes variable and complex changes in plant water relations. The reports on the changes in the water relations of plants exposed to root hypoxia are contradictory. Some reports suggest the reduction in stomatal conductance can be related to the leaf water deficit by the effects of flooding (Wadman-van-Schravendijk and van Andel 1986; Sanchez-Blanco *et al.* 1994). In

contrast, other reports suggest that flooding induced stomatal closure prior to alteration in leaf water potential (Andersen *et al.* 1984; Zhang and Davies 1986; Smit *et al.* 1989; Neuman and Smit 1991). Everard and Drew (1989) suggested that a reduced capacity of water flow through roots of herbaceous plants deprived of O_2 is only a partial explanation for flood-induced reduction in leaf growth and stomatal closure. In cases where leaves respond independently to the changes in internal water deficit, a chemical signal is considered to mediate the response. There have already been extensive studies on the action of ABA as a chemical signal that induces stomatal closure of flooded plants (Zhang and Davies 1987; Jackson and Hall 1987), but there is still controversy over whether only ABA or other chemical signals mediate plant responses.

The present study examined changes in leaf growth, water relations and stomatal conductance influenced by soil flooding and determined the role of xylem sap ABA in regulating leaf responses in flooded capsicum plants. The effect of flooding on ABA content in leaves and flowers was also examined.

MATERIALS AND METHODS

Seeds of capsicum (*Capsicum annuum* L.) cv Bell Boy were germinated and raised under glasshouse conditions at the Department of Biological Science, University of Lancaster, England during summer 1994. Seedlings were transferred to pots containing 3.3 l of John Innes II compost. At the reproductive stage, plants were transferred into a growth cabinet at a temperature of 22-25°C (day) and 18°C (night), relative humidity of 48% and photoperiod of 14 h with photo flux density averaging 320 (mol m^{-2} s^{-1}. The plants were allowed to grow for 7 days in the growth cabinet prior flooding treatments. The plants were randomly divided into two groups with five replicates each of 3 plants. Control plants were watered daily to the drip point. Another group of plants was flooded by closing the drainage holes of the plastic container. Flooding was maintained at 3 cm above the soil surface. The surface of each pot was covered with black polythene plastic in order to reduce evaporation.

Over the following 6 days, measurements of leaf length, leaf breadth, stomatal conductance, leaf water potential and abscisic acid (ABA) in xylem, leaf and flower were made.

Leaf Length and Breadth

The length and breadth of the youngest leaf from each plant was measured and tagged prior to flooding treatments. On every sampling date, leaf expansion was recorded by measuring the differences between the measured and the initial leaf length and breadth values.

Stomatal Conductance

Stomatal conductance was determined on the abaxial surface of the youngest fully expanding and mature (4th leaf from top canopy) leaves on four plants, using a diffusion porometer (AP4, Delta-T Devices Ltd., Cambridge, UK). Measurements were performed at 4 h into the light period.

Leaf Water Potential

Leaf water potential was determined after measuring stomatal conductance on the same leaf used for stomatal conductance. Leaf water potential was measured using a pressure chamber (Soil Moisture Equipment Co., Santa Barbara, CA, USA).

Estimation of Abscisic acid Concentration

ABA concentration was estimated in leaves, flower and xylem sap using a radioimmunoassay technique (RIA). Samples of leaves and flowers were placed in open plastic vials, wrapped in aluminium foil and frozen in liquid nitrogen. They were then freeze dried, finely ground and extracted overnight at 5°C with distilled, deionised water. Samples were extracted using a ratio of 1:25 (leaf dry weight:solvent volume). Xylem sap was collected by pressurizing the cut stem portion of the plants. The extruded sap was collected in a capillary tubing and transferred to eppendorf vials, and then frozen in liquid nitrogen. Analysis of ABA was carried out using monoclonal antibody (McAb) that is specific for (+)-ABA (AFR MAC62) (Quarrie *et al.* 1988).

RESULTS

Leaf expansion, measured as leaf length and breadth, was markedly reduced with soil flooding; a greater reduction was observed for leaf length. As illustrated in *Fig. 1*, leaf length shows greater differences after day 1 of plants in the treatment. For the first 3 days of soil flooding, leaves on soil-flooded plants elongated at an aver-

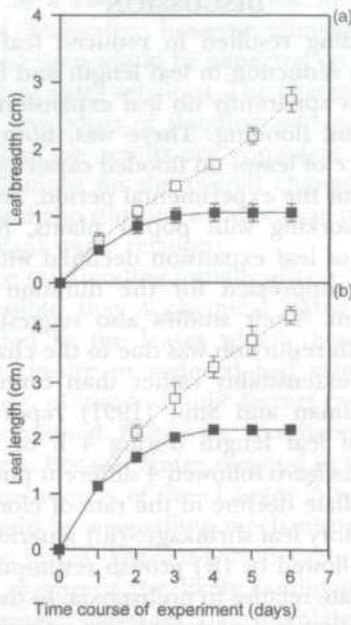

Fig 1. Cumulative leaf length and breadth of capsium plants when subjected to soil flooding ■ or well watered □ conditions. Bars represent ± SE means of 5 replicates. Bars were not indicated if less than SE values.

age rate of approximately 0.8 cm d^{-1} compared with 0.6 cm d^{-1} for leaves grown in soil flooding. After day 3, the differences were exacerbated when leaf expansion ceased in flooded plants.

Fig. 2 shows that soil flooding induced early stomatal closure irrespective of leaf age. After 2 days of soil flooding, stomatal conductance in both young and old leaves had declined to less than 100 mmol m^{-2} s^{-1}. Further exposure to soil flooding caused the stomatal conductance to decline to minimum values on day 3 and day 5 on old and young leaves, respectively. As illustrated in *Fig. 2b*, leaf water potential of flooded plants began to decline after day 4 of soil flooding, suggesting evidence of non-hydraulic factors that regulate the reduction in leaf expansion and stomatal conductance. We observed no symptoms of leaf wilting on plants in the early hours of flooding, which failed to suggest the occurrence of transient water stress on rapidly transpiring plants when immediately exposed to the oxygen deficient environment. After day 4, leaf water potential declined rapidly to reach a minimum value of approximately -1.2 MPa by day 6.

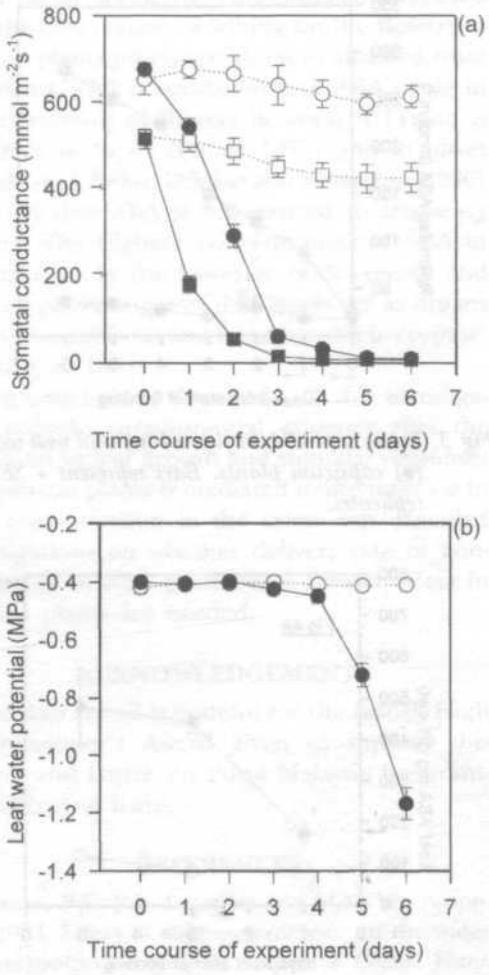

Fig 2 Stomatal conductance and leaf water potential of mature sapsicum plants when exposed to soil flooding (closed symbol) and well watered (open symbol). For stomatal conductance; ○ young, expanding leaves and □ old leaves. Bars represent ± SE means of 4-5 replicantes. Bars were not indicated if less than SE values.

When plants were exposed to soil flooding, xylem sap ABA increased by day 1, and then increased progressively with the duration of flooding (*Fig. 3*). Similarly, there was an increase in ABA concentration in leaf extrudate, which was apparent by day 3 of soil flooding. The ABA concentration in flower extrudate was also increased with 5 days of soil flooding. The concentration of ABA was 1.5-2.3 times higher in flowers than in leaf extrudates under flooded conditions (*Fig. 4*).

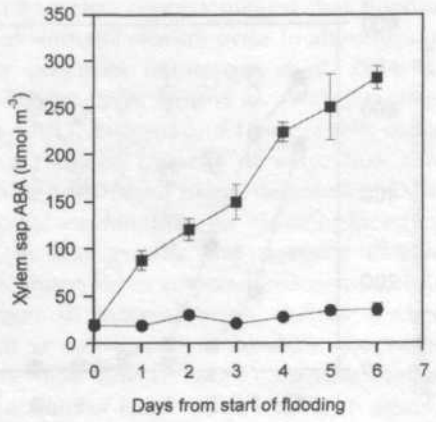

Fig 3. Xylem sap ABA in flooded (■) and well watered (●) capsicum plants. Bars represent + SE of 4 replicates.

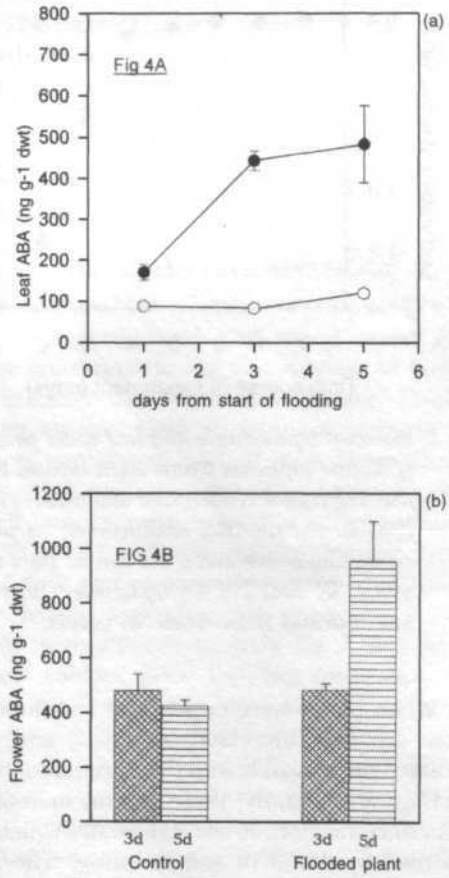

Fig 4. ABA in leaf (Fig 4a) and flower extrudate (Fig 4b) of capsicum plants when exposed to soil flooding. In Fig 4a ○ = well watered and ● = flooded plants. Bars represent ± SE means of 4 replicates.

DISCUSSION

Soil flooding resulted in reduced leaf expansion, by a reduction in leaf length and breadth. There was apparently no leaf expansion after 2 days of soil flooding. There was, however, no senescence of leaves on flooded capsicum plants throughout the experimental period. Smit *et al.* (1989), working with poplar plants, reported that rate of leaf expansion declined within 8 h and was suppressed for the duration of the experiment. Their studies also suggested that leaf growth reduction was due to the changes in cell wall extensibility rather than turgor pressure. Neuman and Smit (1991) reported the pattern of leaf length during 4 h of flooded *Phaseolus vulgaris* followed 4 different phases: (i) an immediate decline in the rate of elongation; (ii) transitory leaf shrinkage; (iii) a period of no growth followed by (iv) growth resumption at a reduced rate relative to prehypoxia. In this study, the reduction in leaf growth was only apparent after 24 h of flooding. The reduction in leaf growth relative to the control may have occurred immediately in the present study. Measurements using a differential transformer, which was not done in this study, might detect immediate changes in capsicum plants.

The present study showed no reduction in leaf water potential within 4 days of flooding. The results suggest flooding reduced leaf growth independently to the development of internal water deficit. Davies *et al.* (1987) suggested that when leaf water relations are unaffected by root hypoxia, another mechanism might be involved in affecting the metabolic effect in the roots. Flooding induced a progressive decrease of stomatal conductance in both young and old leaves. By day 3, stomatal conductance values were very low in flooded plants on both leaves. Pezeshki and Sundstrom (1988) showed a similar trend in early reduction in stomatal conductance in capsicum. There are, however, uncertainties on the role of water relations affecting the stomatal response. There are reports which indicate that stomatal closing in response to flooding occurred both in the presence and in the absence of water deficit (Jackson and Hall 1987). The role of water deficit is considered transient and the shoot regains its water balance within 24 h (Bradford and Hsiao 1982; Everard and Drew 1989; Else *et al.* 1995). Schildwacht (1989) suggested that during the first hour of root anoxia, the leaf elongation rate was

reduced as a consequence of the lower water potential, Thereafter, however, elongation rates were lower than would be expected on the basis of the plant water relations. The changes during the first few hours of flooding were not determined, but the results are consistent with the earlier findings on the overall changes in leaf growth and also stomatal conductance independent of plant water relations.

Since soil flooding initially affects the roots, it is possible that a chemical signal may be transmitted to the leaves which then triggers stomatal closure or reduced leaf growth. The mechanism of non-hydraulic factors causing the above-mentioned responses has been studied extensively. Because leaves respond to soil flooding independently of the changes in water flux, there could be a signalling mechanism between roots and leaves. There is considerable evidence of ABA as one of the likely candidates in the non-hydraulic messages that regulate leaf responses. The results from the present study may support the role of ABA in mediating the leaf responses. There was a four-fold increase in xylem sap ABA in flooded plants by day 1, which increased progressively with the duration of flooding. Similar to the observations of Jackson and Hall (1987), the increase in ABA was not preceded or accompanied by loss in leaf turgor. Neuman and Smit (1993) showed that ABA has no role in regulating leaf growth in poplars. They found only a small and transient increase in leaf ABA in poplars with hypoxix roots, and endogenous ABA in the xylem sap was found to be much lower than the concentration required to mimic the leaf response to root hypoxia. The discrepancies may be due to species and environmental differences. The possibilities of other chemical signals causing reductions in leaf growth and stomatal conductance to plants were not excluded exposed to flooding, however, were not excluded. Reid *et al.* (1991) suggested that ABA is unlikely to act alone as the effects of other pytohormones on stomatal functioning such as the existence of non-ABA in non flood -induced elevations of ethylene and lowered cytokinins. Else *et al.* (1995) found a greater involvement of ethylene precursor 1-aminocylopropane 1-carboxlic acid (ACC) within 24 h of soil flooding rather than ABA or nitrate in tomato plants.

The present study shows an elevated ABA concentration in flower extrudates of flooded plants on day 5 (*Fig. 4*). This could be associated with the observation of wilting on the flowers of flooded plants which are about to abscised from the plants. This is consistent with ABA's role in the senescence of flowers in oranges (Talon *et al.* 1990) in lupin (Porter 1977) and in olives (Kitsaki *et al.* 1995). Dunlap and Robacker (1990) reported that ABA is transported to senescing flowers. The highere concentration of ABA in flowers than in the leaves in both control and flooded plants suggests that leaves act as organs of ABA biosynthesis and flowers as ABA receptors (Hein *et al.* 1984).

In conclusion, the results of this investigation provide circumstantial evidence that the reduction in leaf growth and stomatal responses in capsicum plants is mediated to the increase in ABA concentration in the xylem sap. Detailed investigations on whether delivery rate or concentration of ABA produced a greater effect in flooded plants are needed.

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The Effect of Tamarind (*Tamarindus indica*) and Lime (*Citrus medica*) Juice Washing on the Sensory Attributes and the Rancidity Development in Breaded Tilapia - A Preliminary Study

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Keywords: muddy flavour or odour, breaded tilapia fillet, storage

ABSTRAK

Keberkesanan bagi menyah bau dan citarasa lumpur di dalam ikan tilapia hitam (Oreochromis mossambicus) *dengan perlakuan pembasuhan (15.% b/isipadu jus asam jawa, 1.5% jus limau nipis dan campuran: 0.75% jus limau dan 0.75% jus asam jawa) telah dikaji. Kesan pembasuhan terhadap ketengitan di dalam ikan tilipia bersalut yang disimpan di suhu -20°C juga dibuat. Pembasuhan dengan larutan asid berjaya mengurangkan bau dan citarasa lumpur mengikut ahli panel. Sampel yang dibasuh dengan asam jawa menunjukkan perbezaaan daripada sampel lain dan mendapat lebih tinggi yang ketara bagi citarasa, bau dan warna. Kesemua sampel yang diberi perlakuan menunjukkan perbezaan kekenyalan yang ketara dari sampel kawalan. Walaubagaimanapun, proses ketengitan di dalam sampel berdasarkan nilai peratus asid thiobarbituric (TBARS) yang di simpan tidak dapat direncatkan.*

ABSTRACT

The feasibility of removing the muddy flavour and odour of black tilapia (Oreochromis mossambicus) *with natural acid extracts from tamarind and lime by washing treatment (1.5% w/v tamarind juice, 1.5% v/v lime juice and a mixture of lime juice 0.75% and tamarind juice 0.75% was investigated. The effect of the washing treatment on rancidity development in breaded tilapia kept at -20°C was also monitored. Acid washing improved the acceptability for flavour and odour as detected by sensory panellists. Samples treated with tamarind juice were different from other samples and were scored significantly higher for flavour, odour and colour than other treatments. All treated samples had a significantly tougher texture than the control. However, the rancidity development as indicated by the percentage of thiobarbituric acid reactive substances (TBARS) values in stored samples was not retarded.*

INTRODUCTION

Fish is a major source of protein, especially in the Asian region. However, fish consumption is mainly limited to marine species as freshwater fish is still less acceptable. One deterrent to the acceptability of freshwater fish is its characteristic muddy or earthy flavour and aroma which are mainly due to the presence of geosmin and 2-methylisoborneol (Yurkowski and Tabachek 1974, 1980; Kuusi and Suihko 1983). Geosmin is a volatile compound (Tyler *et al.* 1978), which can be transformed into an odourless compound by acids (Marshall and Hochstetler 1968). The marketability of fish can also be increased if it is formulated as a convenience food such as a breaded product. Attempts to remove the muddy flavour from live fish in holding tanks have been reported in pond-cultured channel catfish (Lovell 1983) and rainbow trout (Yurkowski and Tabachek 1974). Van Allen and Pessoney (1982) demonstrated that the off-flavour in cultured catfish can be reduced by the addition of potassium ricinoleate to the pond water to inhibit the growth of the blue-green algae believed to be

the source of the compound that causes the muddy flavour. In either case, it takes time to be effective, e.g. 14 days for rainbow trout (Yurkowski and Tabachek 1974). Hence it is time consuming compared with a method that removes the smell during the processing itself. However, Rohani and Yunus (1994) reported that soaking for 30 min in 5% salt solution leached out some of the muddy attribute as reflected by the sensory scores of the deboned red tilapia meat.

Traditional practices, such as washing whole fish with lime juice, tamarind juice and flour, are said to remove the muddy flavour and thus increase the acceptability of the fish. The effectiveness of this procedure has not been scientifically tested, but may have some basic since tamarind and lime juice are rich tartaric and citric acids, respectively. We here report the results of a preliminary study on the effect of washing tilapia fillets with tamarind juice, lime juice or a mixture to removal its muddy flavour, to evaluate the sensory attributes of the fillets and to monitor rancidity development in the breaded product. The rancidity factor is taken into account since the breaded tilapia is a deep-fried product and the development of rancidity may be induced by the presence of the additional oil in the fish and breading.

MATERIALS AND METHODS

Live black tilapia (*Oreochromis mossambicus*) weighing approximately 500-600 g each were procured from a nearby farm and brought alive to the laboratory. The procedure for their preparation is shown in *Fig. 1.*

Preparation of Washing Solutions

The lime juice solution was prepared by squeezing ripe fresh limes (*Citrus medica*) and diluting the juice to the percentage (v/v) with distilled water. The tamarind (*Tamarindus indica*) paste (the form used locally) was purchased from the nearby retail shop. The percentage of tamarind juice for washing was prepared on w/v basis (excluding the seeds). The pH of the juice extracts was not determined. A higher percentage of the juice is not desirable as a preliminary study showed it caused excessive gaping and toughening of the muscle and also imparted a slightly acidified flavour to the cooked samples.

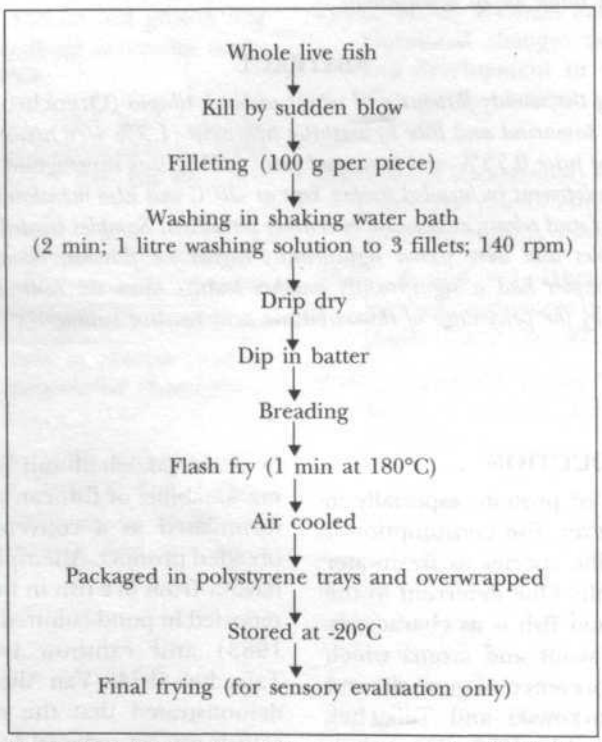

Fig. 1. Flowchart for the sample preparation of breaded tilapia

Titrable Acidity

The titrable acidity of both juice was determined by the method used by Ranganna (1986).

pH Determination

Determination of pH of unbreaded fillets was carried out after the drip-drying process. Ten grams of the samples were homogenized in 100 ml of distilled water and the pH was determined by a pH probe. Readings were done in triplicates.

Sensory Evaluation

The sensory evaluation was carried out by 12 untrained panellists according to the scale of Kosmark (1986) where 7 = like very much; 4 = neither like nor dislike and 1 = dislike very much. It is presumed that samples that were liked did not have a muddy odour or flavour, which if present would have been scored as less than 4, i.e. are disliked. The attributes scored were flavour, odour, colour, texture and acceptability. It was done fully cooked samples, i.e. after frozen storage they were further deep-fried to golden brown prior to evaluation.

Thiobarbituric Acid Reactive Substances (% TBARS)

The percentage of thiobarbituric acid reactive substances (% TBARS) values (Ramanathan and Das 1992) was measured after samples had been stored at -20°C. Storage was carried out mainly to observe the possible effect of the washing treatment on the rancidity development of the product.

RESULTS AND DISCUSSION

The titrable acidity of both tamarind and lime juice was 2.0 ± 0.1%. The fillets washed with LIJ had a pH significantly lower than those that were unwashed (control), washed with TAJ and washed with mix (Table 1). This lower pH of the LIJ washed samples may be the reason why these samples were slightly springy in texture as compared to the others (Kramer 1971).

Table 2 shows the sensory scores of cooked breaded tilapia. The TAJ samples scored significantly higher ($P<0.05$) for flavour and odour compared to other treatments. However, fillets washed in TAJ, LIJ were scored significantly higher for colour. The samples looked more bleached than the control and those washed with the MIX. The texture of the control scored significantly lower than all treated samples. A slight toughening of the fillets was noted. TAJ received the highest acceptability score. Hence, the effectiveness of the treatment can be ranked as TAJ > LIJ > control, mix.

TABLE 1
pH of tilapia fillets

Item*	pH**
fish muscle (unwashed)	6.67[a]
Control	6.68[a]
TAJ	6.68[a]
LIJ	6.57[b]
MIX	6.73[c]

* Control - washing with water; TAJ - washing with 1.5% tamarind juice; LIJ - washing with 1.5% lime juice; MIX - washing with 0.75% lime juice + 0.75% tamarind juice

** Means followed by a different superscript are significantly different at 5% level.

TABLE 2
Sensory scores of cooked breaded tilapia

Treatment*	Flavour	Odour	Colour	Texture	Acceptability**
Control	5.7[b]	6.0a[b]	5.9[b]	5.3[a]	5.7[b]
TAJ	6.1[a]	6.3[a]	6.5[a]	5.9[b]	6.2[a]
LIJ	5.8[ab]	5.8[b]	6.8[a]	5.7[b]	6.0[ab]
MIX	5.5[b]	5.9[b]	6.0[b]	5.7[b]	5.7[b]

* Control - washing with water; TAJ - washing with 1.5% tamarind juice; LIJ - washing with 1.5% lime juice; MIX - washing with 0.75% lime juice + 0.75% tamarind juice

** Based on the average scores of flacour, odour, colour and texture

\- Means followed by a different superscript are significantly different at 5% level.

The % TBARS values (*Fig. 2*) in breaded tilapia increased linearly with storage time. Washing with either lime juice or tamarind juice did not retard the rancidity development in frozen samples. Ramanathan and Das (1992) indicated that samples with % TBARS > 100% are indicative of the presence of rapid lipid oxidation. This value was achieved by all samples after about eight weeks of frozen storage. However, no samples were scored unfavourably or rejected for off-flavour development (*Fig.3*). Similarly, no samples was rejected for off-odour development (*Fig 4.*) The rapid decline in both flavour and odour scores of the samples indicates they may be rejected after a few more weeks' storage at -20°C.

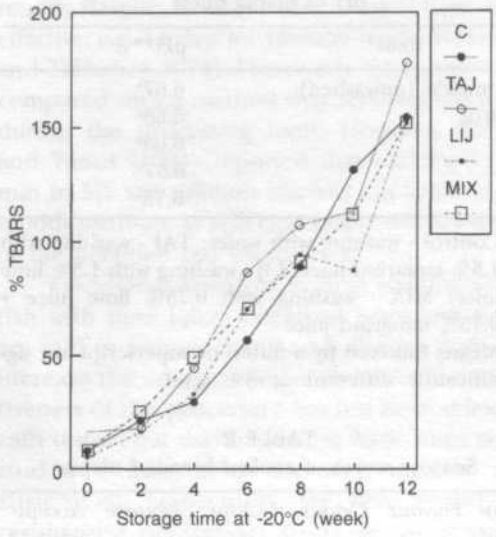

Fig. 2. % TBARS values of breaded tilapia stored at -20 °C

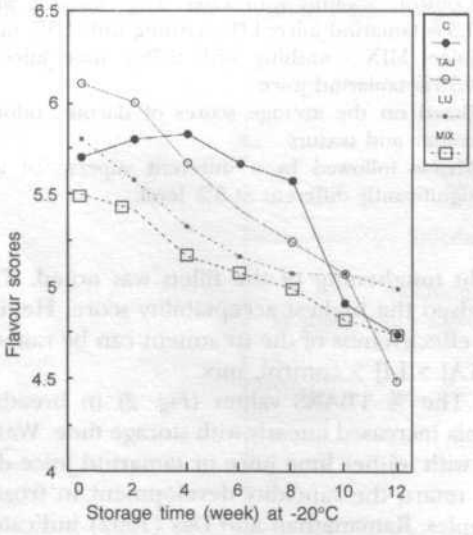

Fig. 3. Flavour scores of breaded tilapia kept at -20 °C

CONCLUSION

Washing with TAJ, LIJ or the MIX improved acceptability score for the odour and flavour of tilapia fillets. Tamarind juice washing scored highest for overall acceptability. None of the washing treatments retarded rancidity development in breaded fillets during storage as measured by the % TBARS value.

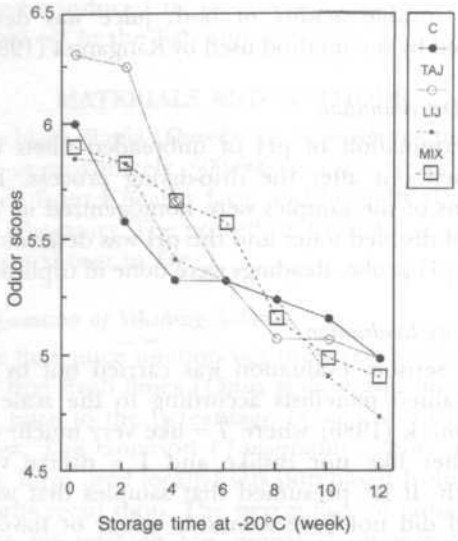

Fig. 4. Odour scores of breaded tilapia kept at -20 °C

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An Evaluation of Cation Exchange Capacity Methods for Acid Tropical Soils

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ABSTRAK

Keupayaan pertukaran kation (KPK) adalah kriteria penting bagi menilaikan kesuburan tanah selain daripada digunakan untuk pengelasan tanah. Tujuh kaedah telah dikaji untuk penentuan dan perbandingan nilai KPK tanah berasid tropika, iaitu, (1) $BaCl_2$ - triethanolamine atau $BaCl_2$ -TEA (pH 8.2), (2) NH_4OAc (pH 7.0) - larutlesap, (3) NH_4OAc (pH 7.0) -digoncang, (4) kaedah pertukaran mendadak oleh Gillman, 1979 (KPK_{PM}), (5) kaedah pertukaran mendadak diubahsuaikan oleh Gillman, 1986 (KPK_B), (6) kaedah pencampuran Ca dari kaedah 5 dengan Al tukarganti menggunakan larutan 1M NH_4NO_3 (KPK_{jumlah}), dan, (7) kaedah pencampuran bes tukarganti dari NH_4OAc (pH 7.0) dengan Al tukarganti dari 1M KCl (KPK_{campur}). Semua kaedah memberikan nilai KPK yang berbeza, iaitu, $BaCl_2$ - TEA > NH_4OAc digoncang - NH_4OAc dilarutlesap > KPK_{campur} = KPK_{jumlah} > KPK_{PM} = KPK_B. Kaedah yang hampir serupa dengan keadaan pH di ladang memberikan nilai KPK yang lebih rendah dari kaedah menggunakan larutan penampan. Ini adalah disebabkan oleh pertambahan cas pada koloid cas berubah oleh larutan penampan. Oleh itu, kaedah larutan penampan memberikan nilai KPK yang tidak benar. Korelasi yang tinggi diperolehi di antara kaedah $BaCl_2$ - TEA dan NH_4OAc larutlesap; KPK_{PM} dan KPK_{campur}; dan, KPK_B dan KPK_{jumlah}. Daripada kaedah-kaedah yang dinilai, kaedah NH_4OAc (pH 7.0) ialah kaedah yang disyorkan sebagai kaedah rutin untuk tujuan pengelasan tanah manakala KPK_{campur} disyorkan untuk penilaian agronomi.

ABSTRACT

The cation exchange capacity (CEC) of soil is an important criteria for assessing soil fertility beside its use in soil classification. Seven methods, namely, (1) $BaCl_2$ - triethanolamine of $BaCl_2$ - TEA (pH 8.2), (2) NH_4OAc (pH 7.0) - leaching, (3) NH_4OAc (pH 7.0) - shaking, (4) compulsive exchange method of Gillman (1979) (CEC_{CE}), (5) modified compulsive exchange method of Gillman (1986) (CEC_B), (6) Summation of Ca from method 5 with 1 M NH_4NO_3 exchangeable Al (CEC_{total}, and (7) summation of NH_4OAc (pH 7.0) exchangeable bases with 1 M KCl exchangeable Al (CEC_{sum}), were used to determine and compare the CEC values of five acid tropical soils. All methods gave different CEC values which followed the order $BaCl_2$ - TEA > NH_4OAc shaking = NH_4OAc leaching > CEC_{sum} = CEC_{total} > CEC_{CE} = CEC_B. Methods with pH conditions close to field situations gave much lower CEC values than the buffered methods. The buffered methods generate charge on the variable-charge colloids, thus resulting in inflated CEC values, while the unbuffered methods do not. There is a high correlation between $BaCl_2$ - TEA and NH_4OAc (pH 7.0) leaching method; CEC_{CE} and CEC_{sum}; and, CEC_B and CEC_{total}. Amongst the methods evaluated, the NH_4OAc (pH 7.0) leaching is recommended in routine soil analyses for classification purposes while CEC_{sum} is recommended for agronomic evaluation.

INTRODUCTION

Cation exchange capacity (CEC) is used in characterizing soils for soil classification as well as in assessing their fertility status. Several procedures have been established, modified and officially accepted as standard methods for CEC determination. Some methods determine CEC under conditions of pH and ionic strength close

to the natural state of the soil while others do not (Bache 1976). The CEC values obtained are highly dependent on methods used and therefore it is essential to evaluate these methods. It is also desirable that the methods selected should measure exchangeable bases as well as the anion exchange capacity (AEC) in the complete characterization of the charge properties of soils.

The humid tropical climate with high rainfall and temperatures favours rapid dissolution and leaching of weatherable minerals. As such, resultant soils are rich in kaolinitic clays and sesquioxides, which possess pH-dependent charges. Therefore, CEC of most Malaysian soils depends largely on the pH at which CEC of the soil is determined, the ionic strength, dielectric constant and the counter ion valency of the solutions used (Tessens and Shamshuddin 1982). If the objective of the CEC determination is to assess the ability of the soil to retain cation nutrients for plant use or to study other reactions that may be affected by CEC, then the measurement should be made on the soil at its natural acidity. If, on the other hand, the objective is to use CEC as an aid to soil classification, then there are strong grounds to determine it at a standard pH. An example of such a method is NH_4OAc method buffered at pH 7.0. This method has some very definite advantages: (i) the method is used worldwide, thus the CEC values obtained can be compared with those measured elsewhere, and (ii) in soil survey and classification work, soils of the same series, which have different pH values as a result of liming or fertilizer application, will have the same CEC in a buffered system (Bache 1976).

The objective of this study was to compare the CEC values determined by various methods and to suggest a suitable method for use in soil classification and fertility status evaluation. For soil classification purposes, a method which is widely practised as well as easy to perform and does not require sophisticated instrumentation will be recommended, whereas a method which closely reflect CEC at field condition will be recommended for fertility evaluation.

MATERIALS AND METHODS

Six soils commonly found in Peninsular Malaysia, that is, Bungor, Holyrood, Munchong, Rengam and Serdang, were used in this study. The soils were air-dried, ground and sieved through a 2.0-mm sieve before use. Seven methods of CEC determination were studied. The first three determinations (methods 1 - 3) were at the buffered soil pH, whereas the last four (methods 4 - 7) were determined close to the soil natural pH. The summary of procedures for all the methods is given in Table 1.

RESULTS AND DISCUSSION

The classification and relevant characteristics of the soils used are shown in Table 2. The CEC depends not only on clay content but also on clay types, that is, on specific surface and charge density. All the soils, except Munchong, have kaolinite as the dominant clay type. The dominant clay type for Munchong series soil is oxides of Fe and Al. Both these type of clays are variable charge colloids. Therefore, the charges of these clays will be affected by pH changes. This is exhibited in the increase in CEC values where the pH of determination has been increased, that is, using $BaCl_2$ - TEA (pH 8.2) and NH_4OAc (pH 7.0) methods (Table 3). Also, soils with a high percentage of clay, such as the Bungor, Munchong and Rengam series soils have higher CEC values than the Holyrood and Serdang series soils. Besides clay type and content, the pH dependence of soil CEC is also a function of organic matter. However, in this case, the amounts of organic C among these 5 soils are low and relatively similar; thus, the charge contribution from organic matter can be considered minimal.

The average values of CEC obtained by the different methods are given in Table 3. Generally, the CEC values of all five soils are rather low. This can be expected of soils dominant in kaolinitic clay (Birrell and Gradwell 1956). The CEC values determined under conditions close to natural soil pH, that is, CEC_{CE}, CEC_B, CEC_{total} and CEC_{sum} are much lower than those obtained by the $BaCl_2$ - TEA (pH 8.2) and NH_4OAc (pH 7.0) methods.

The $BaCl_2$ - TEA (pH 8.2) method gave the highest CEC values for all the soils. The buffering of the $BaCl_2$ - triethanolamine solution to pH 8.2 causes further dissociation of weakly acidic groups comprising the pH-dependent charges in soils (Peech 1965). Therefore, this procedure results in inflated CEC values for acid soils. The inflated CEC values can also be explained in terms of the valency of the displacing cation. Tan (1970) showed that CEC values determined by leaching with monovalent cati-

TABLE 1
CEC methods used in the evaluation study

Method		Solution used for saturated	Method of displacement	Solution used for displacement	Reference
Buffered					
1	$BaCl_2$-TEA (pH 8.2)	0.5 M $BaCl_2$ (pH 7.0)	Compulsive exchange	0.025M $MgSO_4$	Bascomb (1964)
2	NH_4OAc (pH 7.0)	1M NH_4OAc (PH 7.0)	Direct displacement	0.05M K_2SO_4	Soil Survey Staff (1972)
3	NH_4OAc (pH7.0) shaking for 10 minutes	1M NH_4OAc (pH 7.0)	Direct displacement	0.05M K_2SO_4	
Unbuffered					
4	Compulsive exchange of Gillman (1979) (CEC_{CE})	0.1M $BaCl_2$ and 0.1M NH_4Cl	Compulsive exchange	0.005M $MgSO_4$	Gillman(1979)
5	Modified compulsive exch. of Gillman (1986) (CEC_B)	0.1M $CaCl_2$	Compulsive exchange	1M NH_4NO_3	Gillman and Sumpter (1986)
6	Summation method of Gillman (1986) (CEC_{total})	0.1M $CaCl_2$ for bases and 1M NH_4NO_3 for Al	-	-	Gillman and Sumpter (1986)
7	Summation of NH_4OAc exch. bases and KCl exch. Al (CEC_{sum})	1M NH_4OAc (pH 7.0) for bases and 1M KCl for Al	-	-	Coleman and Thomas (1967) Kamprath (1970)

TABLE 2
Some characteristics of the soils studied

Soil	Depth (cm)	Classification*	pH_{H20} (1:2.5)	pH_{KCl} (1:2:5)	Org. C (%)	Exch Al ($cmol(+)kg^{-1}$)	Clay (%)
Bungor	0-20	Fine clayey, kaolinitic, isohyperthermic, Typic Paleudult	5.1	4.1	0.92	1.02	50
Holyrood	0-20	Fine loamy, kaolinitic, isohyperthermic, Typic Dystropept	4.7	3.8	0.97	0.96	15
Munchong	0-20	Clayey, oxidic, isohyperthermic, Tropeptic, Hapludox	5.0	4.0	1.00	0.60	71
Rengam	0-15	Clayey, kaolinitic, isohyperthermic	4.4	3.8	1.28	2.31	69
	15-30	Typic Paleudult	4.4	4.0	0.53	1.34	72
Serdang	0-20	Fine loamy, kaolinitic, isohyperthermic, Typic Paleudult	4.8	3.8	0.94	0.77	25

*Soil Taxonomy USA (Soil Survey Staff 1975)

TABLE 3
CEC values of soils determined by the seven methods ($cmol\ (+)kg^{-1}$)

Soil series	*BaCl-TEA	NH_4OAc *Leaching	(pH 7.0) *Shaking	$*CEC_{CE}$	$**CEC_{B}$	$**CEC_{total}$	$**CEC_{sum}$
Bungor	14.10	7.32	7.48	2.93	2.45	2.92	4.65
Holyrood	10.04	4.65	3.97	0.61	1.26	2.18	1.28
Munchong	13.35	6.35	8.40	1.26	1.56	2.23	1.55
Rengam							
- top soil	15.74	7.58	9.80	1.72	1.94	2.82	2.78
- subsoil	11.58	5.65	7.87	1.53	1.60	2.06	1.64
Serdang	12.90	5.70	6.57	2.22	3.25	4.21	3.21

*CEC values are average of 6 replicates
#CEC values are average of 3 replicates
**No replicates

ons such as NH_4^+, is lower than that obtained with divalent cations such as Ba^{2+}. According to the lyotropic series, the higher the valency of the cations, the more difficult it is for these cations to be replaced from the exchange sites colloids by cations of lower valency (Bohn *et al.* 1985).

The NH_4OAc (pH 7.0) leaching method has been widely accepted for the determination of CEC for soil classification purposes. The shaking procedure as compared with leaching will help to minimize the analysis time and hence large numbers of samples can be determined. From Table 3, it can be seen that the CEC values for the shaking are greater than for the leaching method. The shaking method results in the rupturing of some clay surfaces and hence produces greater CEC values. A correlation study between these two techniques showed quite a significant correlation, r = 0.83 (Table 4).

The CEC values obtained by the CEC_{CE} method are on the average about 27% of the NH_4OAc (leaching) CEC values and this demonstrates the need for caution in CEC determination at a pH value higher than the soil pH, using solutions of relatively high ionic strength. The $BaCl_2$ - TEA (pH 8.2) and NH_4OAc (pH 7.0) methods produce higher CEC values due to an increase in the adsorption of Ba^{2+} and NH_4^+ as a result of the increase in the negative charge on variable charge colloids. Soils extracted with unbuffered soil solutions as in CEC_{CE}, depict the true CEC values (Bache 1976; Gillman 1979). Since the solutions have little effect on soil pH values, the pH-dependent charge will remain unchanged. However, the CEC_{CE} method is laborious and unsuitable for large-scale routine work, where only 64 samples per week can be determined (Gillman 1979).

The CEC_B and CEC_{total} is a modification of the CEC_{CE} method. CEC_B measures only the Ca^{2+} adsorbed after saturating the soil with $CaCl_2$. Below pH 5.0, Al^{3+} is measured in the 1M NH_4NO_3 solution which was used to extract the Ca^{2+}. CEC_{total} is a measure of the amount of Ca^{2+} and Al^{3+} adsorbed. This modified technique is less tedious than the CEC_{CE} method. The CEC_B is not significantly correlated to CEC_{CE} and CEC_{total} with r = 0.77 and 0.60, respectively (Table 4). According to Gillman and Sumpter, 1986, CEC_B will give the true CEC value of soils under natural condition even if free lime is present. This method could also be used for calcareous and saline soils.

CEC_{sum} is an easy way to obtain CEC values. With this method, it is assumed that all the cations extracted with NH_4OAc are exchangeable, and this might not always be so. Apparently, the size of NH_4^+ allows more complete displacement of K^+ from the micaceous clay mineral wedge zone (Rich and Black 1964). The K^+ released from highly specific sites by the NH_4^+ ions are generally considered as fixed or unavailable to plants (Donahue *et al.* 1983; Mengel 1985; Sawhney 1972). Therefore, it is incorrect to include this K^+ as part of the exchangeable cations at the colloidal surfaces. In general, this will not be a problem to the mineral soils of the tropics since micaceous clay is not abundant in these soils. In the CEC_{sum} method, it is further assumed that all of

TABLE 4
Correlation study between different CEC methods

	$BaCl_2$-TEA	NH_4OAc (Leaching)	NH_4OAc (Shaking)	CEC_{CE}	CEC_B	CEC_{total}	CEC_{sum}
1) $BaCl_2$-TEA		0.96**	0.85*	0.59^{ns}	0.39^{ns}	0.34^{ns}	0.59^{ns}
2) NH_4OAc (Leaching)			0.83*	0.65^{ns}	0.27^{ns}	0.17^{ns}	0.65^{ns}
3) NH_4OAc (Shaking)				0.37^{ns}	0.11^{ns}	0.01^{ns}	0.24^{ns}
4) CEC_{CE}					0.77^{ns}	0.60^{ns}	0.95^{**}
5) CEC_B						0.96^{**}	0.74^{ns}
6) CEC_{total}							0.62^{ns}
7) CEC_{sum}							

The r values labelled*,**, are significant at the 5% and 1% levels, respectively, ns = non-significant

the acidic cations extracted with 1M KCl are exchangeable. However, Amedee and Peech (1976) showed that this is not true for some highly weathered tropical soils. An increase in solution electrolyte concentration induces a greater negative charge on variable charge surfaces by the release of surface protons, which then cause dissolution of amorphous oxide coatings. Hence, not all of the aluminium extracted is truly exchangeable (Gillman and Sumpter 1985). The values of CEC_{CE} and CEC_{sum} differ (Table 3), that is, $CEC_{CE} < CEC_{sum}$ although it can be predicted well from the CEC_{CE}, r = 0.95 (Table 4). The difference in CEC value could be because CEC_{total} also measures aluminium that are not truly exchangeable. Thus CEC_{sum} and CEC_{total} slightly overestimate the true CEC values of the soils. However, the limitation of CEC_{sum} is that it does not measure the AEC of the soil and might not be accurate if used for freshly fertilized or limed soils, unless the non-exchangeable cations can be separated from the basic exchangeable cations.

CONCLUSION

The nature of the soil and the purpose of determination are two main factors to consider when selecting a method for CEC determination. The $BaCl_2$ - TEA (pH 8.2) and NH_4AOc (pH 7.0) methods overestimate the ability of variable charge soils to retain cations under field conditions. It is recommended that methods which represent the maximum amount of basic cations that the soil can retain, such as CEC_{CE} and CEC_{sum} may be used for agronomic evaluation. However, the CEC_{CE} method is tedious and therefore not feasible for routine advisory purposes where speed and simplicity of operations are important. The CEC_{sum} method appears to be a suitable choice for fertility evaluation because it is easier to perform and can be carried out on a routine basis. However, for soil classification purposes, the NH_4OAc (pH 7.0) leaching is still the method of preference.

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Prey Spectra of Bornean *Nepenthes* Species (Nepenthaceae) in Relation to their Habitat

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Keywords: Prey spectra, Bornean Nepeuthes species, habitat

ABSTRAK

Kajian terhadap kandungan mangsa periuk 18 spesis Nepenthe*s di Borneo menunjukkan terdapat 17 kumpulan fauna utama. Hasil kajian mendapati periuk lantai dan udara memerangkap kedua-dua fauna rayap dan fauna terbang, pada umumnya periuk lantai memerangkap lebih banyak fauna rayap sementara periuk udara memerangkap lebih banyak fauna terbang. Kandungan jenis mangsa adalah berkorelasi dengan habitat, spesis yang tumbuh pada habitat berbeza dalam satu lokasi yang sama kadangkala menunjukkan kandungan jenis mangsa yang berbeza. Formicidae didapati jenis mangsa yang kerap ditemui dan yang paling melimpah, dan periuk yang dikutip pada altitud bawah daripada 100 m altitud mengandungi bilangan individu semut yang tinggi tetapi bilangannya didapati menurun dengan peningkatan altitud. Walau bagaimanapun, bilangan semut diperangkap dalam setiap periuk adalah berbeza, contohnya bilangan semut yang ditemui dalam periuk* N. rafflesiana *adalah daripada beberapa individu sehingga 700 individu per periuk. Spesis* Nepenthes *tanah tinggi memerangkap pelbagai jenis mangsa berbanding dengan spesis* Nepenthes *tanah pamah. Spektra mangsa yang pelbagai ini menyediakan lebihan makanan kepada pemangsa menghuni periuk, terutama Arachnida, dan ianya didapati kerap ditemui pada spesis tanah tinggi.*

ABSTRACT

Examination of pitcher prey contents of 18 Bornean pitcher plants showed that pitchers attract 17 fauna groups. The upper and lower pitchers trap both flying and creeping fauna but generally the lower pitchers trap more creeping fauna and upper pitchers more flying prey species. Prey composition is generally correlated with habitat; species occupying different habitats in the same locality sometimes show striking differences in prey composition. Formicidae are the most abundant and frequently trapped, and pitchers collected below 100 m altitude contained enormous numbers of ants but their number generally decreased with altitude. However, the number of ants caught per pitcher was variable, e.g. from a few ants to 700 per pitcher in N. rafflesiana. Nepenthes *species growing at high altitudes trapped a broader spectrum of prey than species at lower altitudes. This broad spectrum of prey provides an ample food source for the pitcher-inhabiting predators, particularly Arachnida, which are common in high altitude species.*

INTRODUCTION

Nepenthes species, commonly known as pitcher plants, are tropical carnivorous plants, which generally grow in areas of infertile soil such as in heath forests, swamp forests, forests on ultrabasic soils, and in limestone forests. *Nepenthes burbidgeae, N. kinabaluensis, N. rajah* and *N. villosa* grow on serpentinized ultrabasic rocks and on acid soils on Mt. Kinabalu (Meijer 1965; Mackinnon 1975; Kurata 1976; Kaul 1982). *Nepenthes northiana* (Anderson 1965; Adam *et al.* 1992), *N. clipeata* (Danser 1928; Adam *et al.* 1992) and *N. mapuluensis* (Adam and Wilcock 1990) are endemic to limestone in Borneo. The ability of *Nepenthes* species to thrive on poor soils is largely attributed to their carnivorous habit of trapping prey in the pitchers, which are a modification of the leaf tip.

Nepenthes species display a carnivorous syndrome, i.e. they attract, retain, trap, kill, digest, and absorb useful substances (Juniper 1986).

The pitchers act as pitfall or passive traps (Lloyd 1942), increasing their efficiency by a seductive device (Slack 1980), the secretion of nectar by numerous glands on the under-surface of the lid and the margin of the inner peristome. The effectiveness of the trap is enhanced by the presence of a waxy, slippery surface on the upper half of the inner surface of the pitcher and sharp descending inner peristome teeth. These teeth are very well developed in some species, for example, in *Nepenthes bicalcarata, N. edwardsiana, N. kinabaluensis, N. rafflesiana, N. rajah,* and *N. villosa.* The pitcher wall secretes a digestive fluid containing enzymes and the products of digestion are absorbed by the same glands (Lloyd 1942).

The prey content of pitchers is varied and includes insects like Hymenoptera (including Formicidae), Isoptera, Coleoptera, Plecoptera, and Dermaptera, and other faunal groups such as millipedes, and snails (Jensen 1910; Adam and Wilcock 1994). Pitcher contents of *Nepenthes* from Borneo have not been extensively studied (Spencer 1860; Slack 1980; Phillipps and Lamb 1988). Slack (1980) noted that pitchers, not referring to any particular species, contained digestive bodies of large insects, such as cockroaches and centipedes as well as scorpions, small mammals and reptiles. Spencer (1860) and Phillipps and Lamb (1988), in an extreme case, mentioned a large rat being trapped in pitchers of *Nepenthes rajah.* Jensen (1910) mentioned the horrible odour arising from pitchers loaded with centipedes, cockroaches, butterflies, and scorpions found in *Nepenthes* near Tjibodas, Java.

The objectives of this study were as follows: (1) to investigate the prey spectra in pitchers of 18 Bornean *Nepenthes* species; (2) to correlate their occurrence and abundance with altitude, habitat and pitcher morphology; (3) to investigate the prey-partitioning between upper and lower pitchers of 9 *Nepenthes* species; (4) to study prey-partitioning between 18 *Nepenthes* species occupying the same locality but growing in different habitat types.

MATERIALS AND METHODS

The localities, habitat, altitude, number of pitchers sampled per species for prey pitcher contents and prey partitioning of 18 Bornean *Nepenthes* are listed in Table 1. Prey pitcher contents were sampled 2 weeks after the pitcher opened. The prey found in the pitchers are listed in Table 2. A total of 255 pitchers were sampled, ranging from 4-47 pitchers per species. Eighteen species and nine species were investigated respectively for prey-partitioning between species (Table 3) and between upper and lower pitchers within a species (Tables 4, 5 and 6).

Using a stereo microscope, the intact fauna were carefully separated from the soils and decayed insect debris from each sample and subsequently preserved in 70% alcohol. Prey was identified to class, order, or family level. Keys to the class of Arthropoda or orders of Insecta (Borror *et al.* 1954), and keys to the class and families of British Insecta (Unwin 1981, 1984) were used. The number of individuals of each taxon in each sample was counted and numbered accordingly.

Duplicate specimens were sent to the Sarawak Forest Department Entomology Section and National Institute of Health, Kyoto. Specimens are deposited in Biology Department Museum, Universiti Kebangsaan Malaysia Sabah Campus and Sarawak Forest Entomology Section. Analysis of principal component analysis (PCA) used a computer statistical programme and Sorenson coefficients similarity (CC) were used to determine fauna group similarity between *Nepenthes* species (Mueller-Dombois and Ellenberg 1974; Brower and Zar 1977).

$$CC = \frac{2c}{s1 + s2} \times 100$$

where s1 and s2 are the number of fauna groups in *Nepenthes* species 1 and 2, and c is the number of faunal groups common in both species.

RESULTS

Diversity of Prey

Of the total 690 taxa collected from 255 pitchers of 18 *Nepenthes* species sampled, 53.9% and 1.2% were identified to family and generic level respectively. A total of 6384 individuals were recorded of which 79.5% (5077 individuals) were Formicidae (excluding other Hymenoptera), 8.4% Isoptera, 3.8% Diptera, 2.6% in other miscellaneous groups, 2.0% Coleoptera, 1.2% Hymenoptera; and less than 1% in each of the other 11 groups (Table 2).

The mean number of individuals per pitcher was 25, but varied greatly between species, ranging from 1.7 individuals in *N. bicalcarata* to 168.9 individuals in *N. macrovulgaris.* Eight *Nepenthes* species had a mean less than 10 individuals per

TABLE 1
Locality, altitude and habitat of pitcher prey sampling sites

No	*Nepenthes* species	Locality	Altitude (m)	Habitat	No. pitchers sampled
1	*N. albomarginata*	Weston, Sabah	5-30	HF	16
2	*N. ampullaria*+	Weston, Sabah	5-30	DDF	7
3	*N. bicalcarata****	Weston, Sabah	5	DDF	7
4	*N. gracilis**	Weston, Sabah	5-30	SWF	20
5	*N. rafflesiana****	Weston, Sabah	5-30	HF	16
6	*N. mirabilis**	Telupid, Sabah	150	SV	10
7	*N. hookeriana***	Telupid, Sabah	150	SV	4
8	*N. macrovulgaris**	Mt. Silam, Sabah	520	RC	7
9	*N. sandakanensis**	Mt. Silam, Sabah	700-800	MF	14
10	*N. lowii***	Mt. Mulu, Sarawak	1680	MF	12
11	*N. muluensis**	Mt. Mulu, Sarawak	1800-2300	MF	16
12	*N. tentaculata**	Mt. Mulu, Sarawak	1600-2300	MF	23
13	*N. x alisaputraiana****	Mt. Kinabalu, Sabah	1900-1930	UF	14
14	*N. curtisii***	Mt. Kinabalu, Sabah	1400	SB	6
15	*N. kinabaluensis****	Mt. Kinabalu, Sabah	2850	UF	16
16	*N. rajah****	Mt. Kinabalu, Sabah	1950	UF	10
17	*N. reinwarditiana**	Mt. Kinabalu, Sabah	960	RC	10
18	*N. villosa****	Mt. Kinabalu, Sabah	1600-2300	UF	47

* Inner surface of lower and upper pitcher partly glandular
** Inner surface of upper and lower pitcher wholly glandular, lower pitcher partly glandular
*** Inner surface of upper and lower pitcher wholly glandular
\+ The only known species to have lower pitcher only, wholly glandular
HF Heath forest
SWF Swamp forest
SV Secondary vegetation
MF Mossy forest
RC Roadside clearing
DDF Disturbed dipterocarp forest
UF Forest on ultrabasic soil

TABLE 2
Prey spectra of eight Bornean *Nepenthes* species

Nepenthes species*	Fauna group	1	2	3	4	5	6	7	8	9
(1)	%	0	0	0	0	0	0	0	11.4	0
(1)	No.	0	0	0	0	0	0	0	71	0
(2)	%	0	0	23	0	0	0	0	69.2	0
(2)	No.	0	0	3	0	0	0	0	9	0
(3)	%	0	0	0	0	0	0	0	91.7	0
(3)	No.	0	0	0	0	0	0	0	11	0
(4)	%	1	0	1	0	0	0	5.9	90	0
(4)	No.	4	0	4	0	0	0	24	367	0
(5)	%	0	0	0	0	0	0	1.9	96.2	0
(5)	No.	0	0	0	0	0	0	43	2239	0
(6)	%	0	0	0	0	1.9	0	0	94.5	0
(6)	No.	0	0	0	0	6	0	0	291	0
(7)	%	0	0	0	0	0	0	0	100	0
(7)	No.	0	0	0	0	0	0	0	60	0
(8)	%	0	0	0	0	0	0	0	99.1	0

Table 2 (cont'd)

Nepenthes species*	Fauna group	1	2	3	4	5	6	7	8	9
(8)	No.	0	0	0	0	0	0	0	1171	0
(9)	%	1.9	0	6.2	0	0	0	31	42.5	1
(9)	No.	3	0	10	0	0	0	50	69	2
(10)	%	16.7	0	17	0	0	0	8.3	45.8	0
(10)	No.	4	0	4	0	0	0	2	11	0
(11)	%	0	0	17	0	0	0	23	36.5	0
(11)	No.	0	0	9	0	0	0	12	19	0
(12)	%	7.5	0	20	0	0	3.7	6	41.2	10
(12)	No.	6	0	16	0	0	3	5	33	8
(13)	%	6	0	5	0	0	0	0	77	0
(13)	No.	6	0	5	0	0	0	0	77	0
(14)	%	0	0	0	6.2	1	0	0	84.5	0
(14)	No.	0	0	0	6	1	0	0	81	0
(15)	%	13.8	4	11	0	3	0	57	0	0
(15)	No.	14	4	11	0	3	0	58	0	0
(16)	%	2.5	0	1	0	0	0	5.9	85.5	0
(16)	No.	8	0	4	0	0	0	19	275	0
(17)	%	0	0	3	0	0	0	3.1	81.7	2
(17)	No.	0	0	11	0	0	0	11	293	7
(18)	%	13.4	8	33	1	3	0	9.2	0	0
(18)	No.	22	13	53	2	5	0	15	0	0

Table 2 (cont'd)

Nepenthes species*	Fauna group	10	11	12	13	14	15	16	17	Total	Mean
(1)	%	0	0	86.3	0	0	0	0	2.3	100	-
(1)	No.	0	0	534	0	0	0	0	14	619	38.7
(2)	%	0	0	0	0	7.1	0	0	0	100	-
(2)	No.	0	0	0	0	1	0	0	0	13	1.9
(3)	%	0	0	0	0	8.3	0	0	0	100	-
(3)	No.	0	0	0	0	1	0	0	0	12	1.7
(4)	%	0	0	0	0	0	0	0	1.9	100	-
(4)	No.	0	0	0	0	0	0	0	8	409	20.4
(5)	%	0	0	0	0	0	0	0	1.9	100	-
(5)	No.	0	0	0	0	0	0	0	43	2325	145.3
(6)	%	0	0	0	0	0	0	0	3.6	100	-
(6)	No.	0	0	0	0	0	0	0	11	308	30.8
(7)	%	0	0	0	0	0	0	0	0	100	-
(7)	No.	0	0	0	0	0	0	0	0	60	15.0
(8)	%	0	0	0	0	0	0	0	0.9	100	-
(8)	No.	0	0	0	0	0	0	0	11	1182	168.9
(9)	%	0	16	0	0	0	0	0	1.2	100	-
(9)	No.	0	26	0	0	0	0	0	2	162	11.6
(10)	%	8.3	0	0	0	4.2	0	0	0	100	-
(10)	No.	2	0	0	0	1	0	0	0	24	2.0
(11)	%	0	23	0	0	0	0	0	0	100	-
(11)	No.	0	12	0	0	0	0	0	0	52	3.3
(12)	%	0	6.2	0	0	0	1.3	1.3	2.5	100	-
(12)	No.	0	5	0	0	0	1	3	2	80	3.5
(13)	%	2	0	4	2	2	0	0	2	100	-

Table 2 (cont'd)

Nepenthes species*	Fauna group	10	11	12	13	14	15	16	17	Total	Mean
(13)	No.	2	0	4	2	2	0	0	2	100	7.1
(14)	%	3.1	0	0	3.1	2.1	0	0	0	100	-
(14)	No.	3	0	0	3	2	0	0	0	96	16.0
(15)	%	0	3	0	0	5	0	0	3	100	-
(15)	No.	0	3	0	0	5	0	0	3	104	6.3
(16)	%	0	1.2	0	0	1.2	0	0	2.5	100	-
(16)	No.	0	4	0	0	4	0	0	8	322	32.2
(17)	%	0	6	0	0	0	0	0	3.9	100	-
(17)	No.	0	22	0	0	0	0	0	14	358	35.8
(18)	%	3.1	3.1	0	0	0	0	0	26.4	100	-
(18)	No.	5	5	0	0	0	0	0	43	162	3.5

Key

Fauna group
1 Arachnida
2 Chilopoda
3 Coleoptera
4 Dermaptera
5 Dictyoptera
6 Diplopoda
7 Diptera
8 Formicidae
9 Homoptera
10 Heteroptera
11 Hymenopters (excluding 8)
12 Isoptera
13 Mollusca
14 Orthoptera
15 Plecoptera
16 Pscoptera
17 Others

* *Nepenthes* species numbered as in Table 1

pitcher but two had means of 145.3 (*N. rafflesiana*) and 168.9 (*N. macrovulgaris*). Fifteen prey groups were identified to family level, one prey group each to order and unknown faunal group respectively (Table 2). Six of these prey groups, Formicidae (which excludes other flying hymenopteran taxa), Dictyoptera, Isoptera, Chilopoda, Diplopoda and Mollusca are creeping fauna. The other prey groups are flying insects.

The commonest group was Formicidae, found in abundance in pitchers of 16 *Nepenthes* species. Although the Formicidae were overall the most common group, they were better represented in lowland habitats where they formed 69-100% of pitcher prey in seven of eight species found below 500 m above sea level compared with 3-87% for species growing between 700-2300 m, and they were totally absent from pitchers in *N. villosa* (1600-2300 m) and *N. kinabaluensis* (2850 m).

Diptera were the second most common group although in much lower abundance. In ten of the species studied, they comprised 57% of the prey in pitchers of *N. kinabaluensis*, 31% in *N. sandakanensis*, and 23% in *N. muluensis*. While the pitchers of the remaining seven species contained between 2-9% Diptera. Sixty-five Dipteron taxa were recognized, belonging to families such as Phoridae, Syrphidae, Cecidomyiidae and Sciaridae.

Coleoptera, the third most common prey group encountered, were recorded in ten species, being most abundant in pitchers of *N. villosa* (33%), *N. ampullaria* (23%), *N. tentaculata* (20%), *N. lowii* (17%), *N. muluensis* (17%) and *N. kinabaluensis* (11%). Eighty-two coleopteran taxa were recognized belonging to families such as Curculionidae, Scarabaeidae, Elateridae, Chrysomilidae, Pyrochoridae, Odemeridae, Carabidae, Passalidae, Nitidulidae, Buprestidae, Cerambycidae and Lucanidae.

Hymenoptera (excluding Formicidae) were found in ten species studied, being abundant in *Nepenthes muluensis* (23%) and *N. sandakanensis* (16%). Forty-six Hymenopteran taxa recognized belong to families such as Chacidoidae, Apidae, Vespoidae, Trichgrammatidae and *Icheu monidae*. Hymenopteran species recognized included *Apis cerana* (in *N. muluensis*), *Trigona* sp. (in *N.* x *alisaputraiana*, *N. gracilis*, and *N. rajah*), and *Dacus* sp. (in *N. gracilis*). *Bulbitermis* sp. (Isoptera) were present in abundance in *Nepenthes albomarginata* and comprised 86% of the prey.

The other 12 fauna groups were less common, and each group never comprised more than 10% of the pitcher prey of the 18 species of *Nepenthes* recorded (Table 2).

Fig. 1-3 give the results of principal component analysis (PCA) of the faunal contents of 67 pitchers collected from 18 species from 30-2970 m

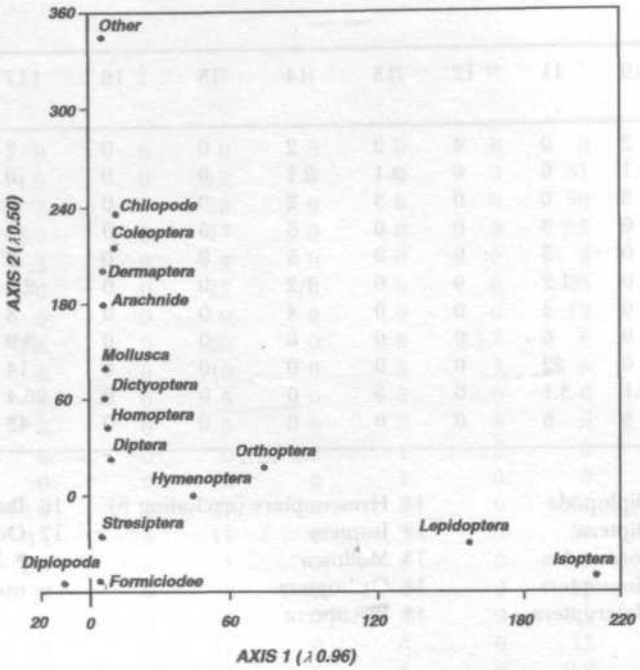

Fig 1. Principal compenent analysis (PCA) of prey type in 18 Bornean Nepenthes

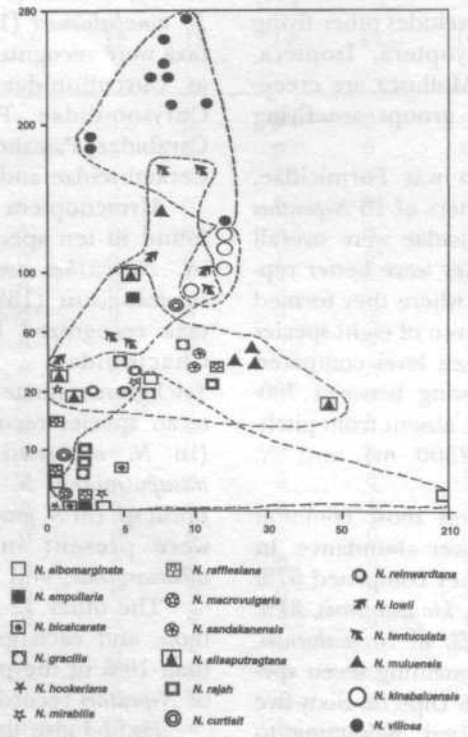

Fig 2. Principal component analysis for 67 pitcher samples from 18 Nepenthes *species*

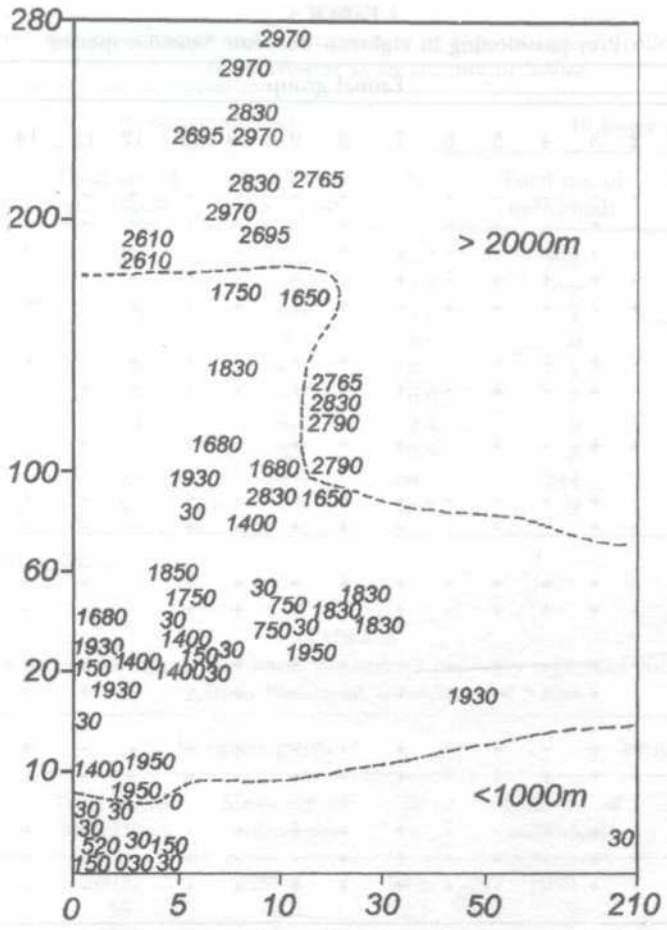

Fig 3. Principal component analysis of Nepenthes *pitcher samples*

above sea level. Apart from the unusual prey composition of *N. albomarginata*, which had an abundance of *Bulbitermis* sp. (Isoptera), the principal variation relates to altitude (*Fig. 3*). *Nepenthes* species at higher altitudes have a greater diversity of fauna prey (Table 1) and *Fig 1* shows that Isoptera and Formicidae are much commoner, while Isoptera and Lepidoptera are confined to low altitudes. Although there is a considerable overlap, some species have fairly distinct prey spectra, particularly those at higher altitudes.

The prey spectra of 18 Bornean *Nepenthes* species studied showed similarity with prey spectra of *N. mirabilis* sampled in New Guinea (Table 3). Sorenson's similarity coefficient (Mueller-Dombois and Ellenberg 1974; Brower and Zar 1977) of the prey spectra between species studied (Table 3) is more than 30% in all species and average 64%. Formicidae (excluding other Hymenopteran), Coleoptera, Diptera and Arachnida are common components of *Nepenthes* prey spectra. They are recorded in 16, 11, 10 and 8 *Nepenthes* species studied (Tables 2, 3).

Partitioning between and within Species

Different *Nepenthes* species often occupy different habitats within a single site (Table 1). For example, in Telupid, *Nepenthes mirabilis* and *N. hookeriana* grow in wetter and drier habitats respectively. The prey composition of these two species shows differences, although two of the eight faunal groups (Coleoptera and Formicidae) recorded are present in both species and calculated Sorenson's similarity coefficient value between them is 36% (Table 3). *Nepenthes sandakanensis* and *N. macrovulgaris* collected from mossy forest and roadside clear-

TABLE 3
Prey partitioning in eighteen Bornean *Nepenthes* species

	Faunal group																	
Localities/Species	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	CC(%)
Weston																		
N. albomarginata	+	-	-	-	-	-	-	+	-	-	-	+	-	-	-	-	-	
N. ampullaria	-	-	+	-	-	-	-	+	-	-	-	-	-	+	-	-	-	
N. bicalcarata	-	-	-	-	-	-	-	+	-	-	-	-	-	+	-	+	-	
N. gracilis	+	-	+	+	+	-	+	+	+	-	+	-	-	-	-	-	-	37
N. rafflesiana	+	+	-	-	-	+	+	+	+	+	+	-	-	+	-	-	+	
Telupid																		
N. hookeriana	-	-	+	-	-	-	-	+	-	-	-	-	-	-	-	-	-	
N. mirabilis	+	-	+	-	+	-	+	+	+	-	-	-	+	-	-	-	+	36
PNG																		
N. mirabilis	+	+	+	-	+	-	+	+	+	+	+	-	+	-	-	-	-	
Mt. Silam																		
N. macrovulgaris	-	-	+	-	-	-	+	+	-	-	+	-	-	+	-	-	+	
N. sandakanensis	+	+	+	-	-	-	+	+	+	-	+	-	-	-	-	-	-	61
Mamut																		
N. curtisii	-	-	+	+	+	-	+	+	+	+	+	-	-	+	-	-	+	
N. reinwardtiana	+	-	+	+	+	-	+	+	+	+	+	-	-	-	-	-	-	94
Pig Hill																		
N. x alisaputraiana	+	-	+	-	+	-	+	+	+	+	-	-	+	+	+	-	-	
N. rajah	+	-	+	-	+	+	+	+	-	+	-	-	+	-	-	-	+	73
Mt. Kinabalu																		
N. kinabaluensis	+	+	+	-	+	+	+	-	-	-	+	-	-	+	-	-	-	
N. villosa	+	+	+	+	+	+	+	-	+	+	+	+	-	-	-	-	-	74
Mt. Mulu																		
N. lowii	+	-	+	-	-	-	+	+	+	+	-	-	-	+	-	-	-	
N. muluensis	+	-	+	-	-	-	+	+	-	-	+	-	-	-	-	-	-	74
N. tentaculata	+	-	+	-	-	+	+	+	+	+	+	-	-	-	-	-	-	

+ - present – absent CC= Sorenson coefficient similarity (refer to method)
PNG - Papua New Guinea (Jebb 1989)
Key to faunal group: refer in TABLE 1

ing respectively on Mt. Silam in Lahad Datu, Sabah provides another example. Four of the nine faunal groups (Coleoptera, Diptera, Formicidae and Hymenoptera) were found in both species.

However, several species pairs found on the same site and occupying the same habitat show markedly similarly prey composition (Tables 1, 3), namely *N. reinwardtiana* and *N. curtisii* collected from a roadside clearing or secondary vegetation at Mamut, *N. rajah* and *N. x alisaputraiana* growing in mossy forest on Mt. Kinabalu, and *N. villosa* and *N. kinabaluensis* occupying gnarled forest on ultrabasic soil on the summit trail of Mt. Kinabalu. Full analysis of prey-partitioning was not possible because no sampled *Nepenthes* was common to more than one site.

Comparison between upper and lower pitchers in *Nepenthes gracilis* (Table 4) and *N. rafflesiana* (Table 5) show that flying taxa are more numerous in upper pitchers. However, the principal prey in both species are ants and in *N. rafflesiana* these are more numerous in the lower pitchers than the upper pitchers. Similar comparisons between upper and lower pitchers of *Nepenthes* species are summarized in Table 6. Six of these seven species (except *Nepenthes tentaculata*) contained higher mean numbers of individuals of crawling taxa in the lower pitchers than in the upper pitchers.

DISCUSSION

This study of 18 *Nepenthes* species shows that they possess various characters which may act as an attractant to potential prey. All the 18 species

TABLE 4
Prey spectra of upper and lower pitchers of *Nepenthes gracilis* collected from Weston at an altitude of 5-30m

Faunal group	10 upper pitchers			10 lower pitchers		
	Total no. of individuals	Mean no. of individuals	%	Total no. of individuals	Mean no. of individuals	%
Formicidae*	134	13.4	80.7	232	23.2	95.2
Diptera+	22	2.2	13.2	5	0.5	2.1
Coleoptera+	3	0.3	1.8	1	0.1	0.4
Dermaptera+	1	0.1	0.6	0	0	0
Homoptera+	0	0	0	1	0.1	0.4
Hymenoptera+	1	0.1	0.6	2	0.2	0.8
Dictyoptera+	4	0.4	2.4	0	0	0
Arachnida*	1	0.1	0.6	3	0.3	1.2
Total	166	16.6	100	244	24.4	100
Total no. of flying fauna	27	2.7	16.3	9	0.9	3.7

*creeping Insects; + flying fauna

TABLE 5
Prey spectra of upper and lower pitchers of *Nepenthes rafflesiana* collected from Weston at an altitude of 5-30m

Faunal group	10 upper pitchers			10 lower pitchers		
	Total no. of individuals	Mean no. of individuals	%	Total no. of individuals	Mean no. of individuals	%
Formicidae+	2003	250.4	94.4	1036	129.5	98.8
Diptera*	39	4.9	3.1	5	0.6	0.5
Orthoptera+	10	1.3	0.8	0	0	0
Coleoptera+	10	1.3	0.8	1	0.13	0.09
Lepidoptera+	2	0.3	0.2	1	0.13	0.09
Homoptera	2	0.3	0.2	1	0.13	0.09
Diplura+	2	0.3	0.2	0	0	0
Hymenoptera+	1	0.1	0.1	2	0.25	0.19
Arachnida*	5	0.6	0.4	2	0.25	0.19
Chilopoda	1	0.1	0.1	1	0.13	0.09
Total	2075	259.4	100	1049	131.1	100
Total of flying fauna	66	8.3	3.2	10	1.25	1.0

*creeping Insects; + flying fauna

studied produced both upper and lower pitchers, which, however, display varying shapes and colours. The shapes of the pitcher ranged from tubulose (*N. x alisaputraiana* and *N. rajah*; *Plate 1A and 1D*), infundibulate (*N. kinabaluensis*; *Plate 1B*), infundibulate-globose (*N. lowii*; *Plate 1C*) tubulose-ventricose, infundibulate-ventricose, ovate, globose and urceolate; and the colour of the pitchers ranges from green with mottling purple, green, dark red or scarlet to yellowish-red. It has been suggested by Lloyd (1942), Heslop-Harrison (1978); Joel (1984, 1988) that the various pattern of pitcher shapes and bright colouring are among the common mechanisms to attract potential prey. Joel (1988) reported that the pitchers of the same *Nepenthes* species are conspicuous to insects due to the overall shining colour, such as scarlet or golden yellow in *N. bicalcarata*, or deep red as in *N. ampullaria*. The pitcher is a seductive, alluring or attractive device; insects are attracted by the nectar secreted by the glands covering the inner surface

TABLE 6
Prey spectra of lower and upper pitchers of seven Bornean *Nepenthes* species

Nepenthes species	Pitcher	No. of pitchers	Mean no. of individuals per pitcher		
			flying taxa	crawling taxa	Total
N. albomarginata	Upper	10	1.0	6.4	7.4
	Lower	6	0.2	90.6	90.8
N. kinabaluensis	Upper	10	4.6	1.6	6.2
	Lower	6	5.3	1.7	7.0
N. lowii	Upper	8	0.6	1.1	1.7
	Lower	4	1.0	1.5	2.5
N. mirabilis	Upper	7	0.7	7.7	8.4
	Lower	3	1.3	81.6	82.9
N. muluensis	Upper	10	2.3	0.5	2.8
	Lower	6	1.5	2.3	3.8
N. rajah	Upper	5	4.8	24.5	29.3
	Lower	5	0.8	34.4	35.2
N. tentaculata	Upper	12	1.3	2.8	4.1
	Lower	12	1.5	1.3	2.8

of the lid (*Plate 2A-F*) and inner peristome margin (*Plate 3A-F*). In this study, 17 of the 18 species studied had numerous nectar glands covering the underside of the lid.

Jebb (1989) suggested a certain degree of prey partitioning between the upper and lower pitchers of the same species. This study shows that the upper pitchers trap enormous numbers of ants, comparable to the number of ants caught by the lower pitchers. A possible explanation is related to the behaviour of the ants. Ants in the tropics nest on shrubs or small trees, and they often move from the nesting site to the ground level. The upper and lower pitchers, particularly of *N. gracilis* and *N. rafflesiana*, are located within the vertical foraging zone of ants and can thus potentially trap enormous numbers. Such a trapping phenomenon is called by Juniper *et al.* (1989) episodic capture of prey. Such an episodic capture of *Bulbitermis* sp. (Isoptera) was observed in a single lower pitcher of *N. albomarginata* from Weston.

Jenzen (1977) suggested that in lowland tropical habitats, ants are omnipresent visitors, guardians, and sugar collectors at most sugar-source floral nectaries, hymenopteran exudates, broken fruits, etc. Hotta (1989) suggested that mossy forest at high altitudes is too moist throughout the year to offer good habitats for ants. This study demonstrates that ants are the main prey component in pitchers of the lowland *Nepenthes* species, decreasing in number with increasing altitude and totally absent in species found in mossy forest located at very high altitudes.

CONCLUSION

This study of fauna pitcher contents of 18 Bornean *Nepenthes* species shows that the pitchers attracted a broad spectrum of prey, which included 17 fauna groups. The upper and lower pitchers trap both flying and creeping fauna, and lower pitchers trap more creeping prey and upper pitchers more flying fauna. The prey composition is correlated with locality; and the species occupying different habitats of the same locality sometimes show striking differences. Formicidae or ants (excluding other Hymenopteran) are the most abundant and frequent prey trapped. The pitcher contents below 100 m altitude contained enormous numbers of ants, generally decreasing in number with increasing altitude; no ants were caught in pitchers of *N. villosa* collected at 1600-2300 m altitude, and pitchers of *N. kinabaluensis* at 2850 m altitude.

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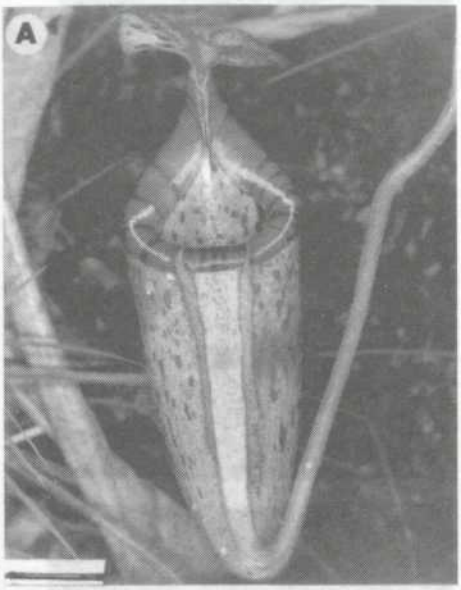

Plate 1A. Lower pitcher of Nepenthes x alisaputraiana
Scale bar 4 cm

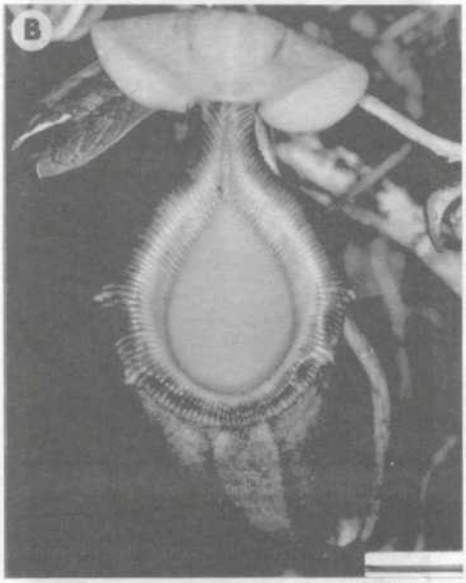

Plate 1B. Upper pitcher of Nepenthes kinabaluensis
Scale bar 6 cm

Plate 1C. Upper pitcher of Nepenthes lowii
Scale bar 6 cm

Plate 1D. Ground pitcher of Nepenthes rajah
Scale bar 8 cm

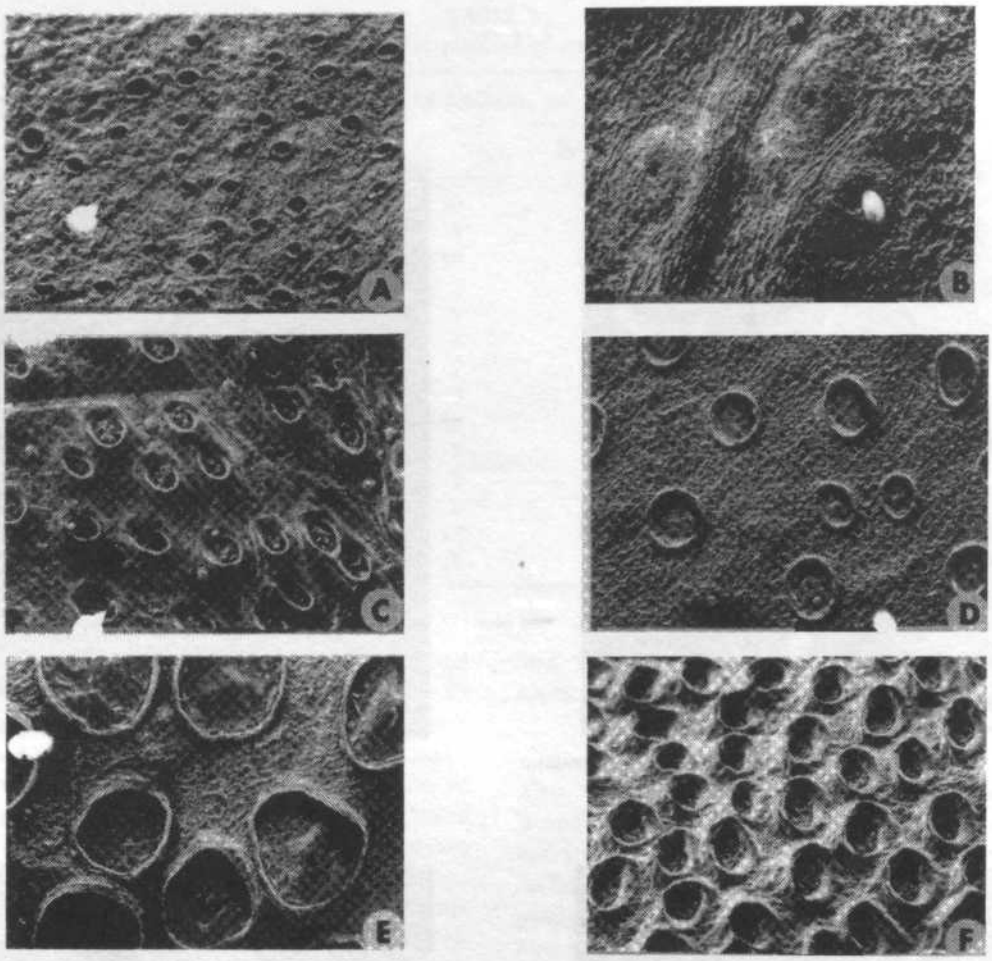

Plate 2A. Lid nectar glands of Nepenthes albomarginata *Scale Bar 400 μm*
Plate 2B. Lid nectar glands of Nepenthes gracilis *Scale Bar 400 μm*
Plate 2C. Lid nectar glands of Nepenthes kinabaluensis *Scale Bar 400 μm*
Plate 2D. Lid nectar glands of Nepenthes mirabilis *Scale Bar 400 μm*
Plate 2E. Lid nectar glands of Nepenthes rafflesiana *Scale Bar 400 μm*
Plate 2F. Lid nectar glands of Nepenthes villosa *Scale Bar 400 μm*

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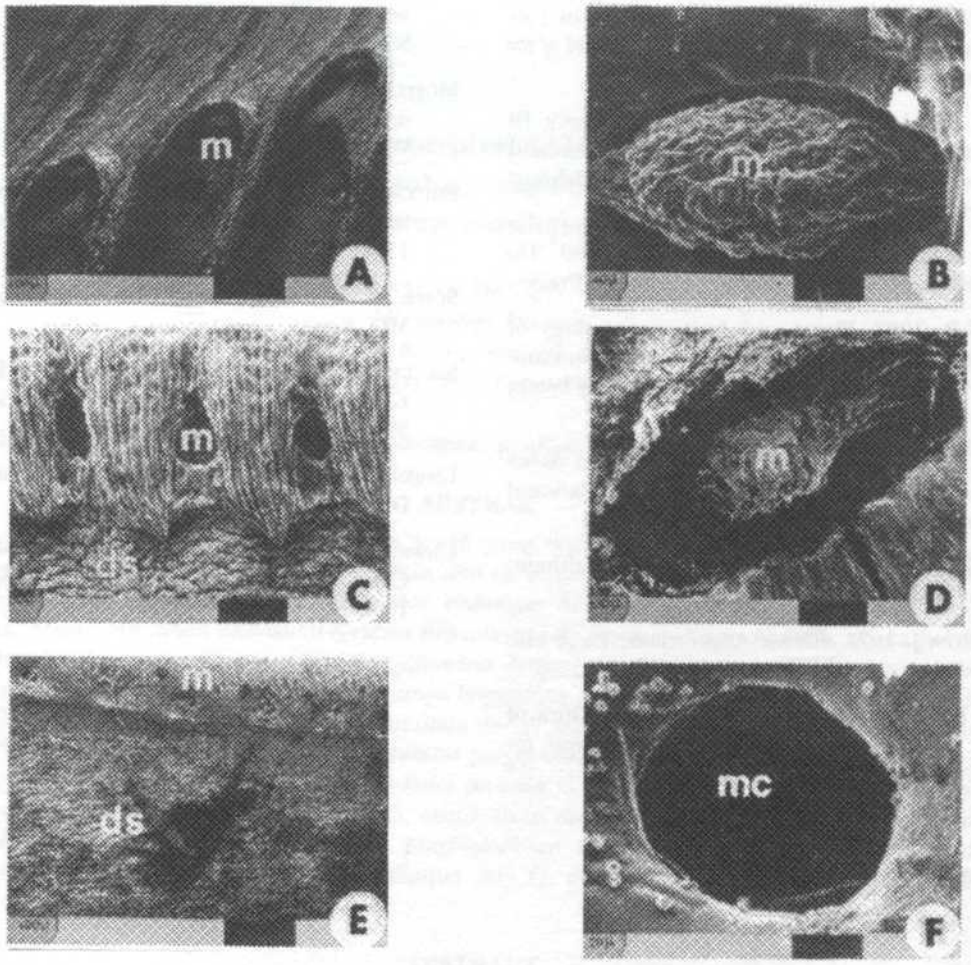

Plate 3A. Marginal glands (m) of Nepenthes rafflesiana *Scale bar 400 μm*
Plate 3B. Marginal glands (m) of Nepenthes rafflesiana *Scale bar 40 μm*
Plate 3C. Marginal glands (m) of Nepenthes gracilis *Scale bar 200 μm*
Plate 3D. Marginal glands (m) of Nepenthes rajah *Scale bar 100 μm*
Plate 3E. Marginal glands (m) of Nepenthes reinwardtiana *Scale bar 400 μm*
Plate 3F. Marginal gland cavity (mc) of Nepenthes reinwardtiana *Scale bar 20 μm*

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Possible Role of Arbuscular Mycorrhizal (AM) Fungi on Drought Tolerance in *Vigna unguiculata* subsp. *unguiculata* (L.) Walp and *Leucaena latisiliqua* L.

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ABSTRAK

Ujian terhadap kultur pot telah dibuat dalam tanah yang disteril bagi menentukan kesan tiga kulat AM, Acaulospora scrobiculata, Glomas aggregatum *dan* G. etunicatum, *kedua-duanya secara bersendiri serta dicantumkan, pada pertumbuhan tanaman serta rintangan kemarau* Vigna unguiculata *dan* Leucaena latisiliqua. *Semua tumbuhan diinokulat bersama* Rhizobium *sp. Semasa 45 hari pertama, semua pot diairkan kepada kapasiti lapangan dan tanaman kemudiannya bergantung kepada pusingan kemarau melalui air tertahan selama 7 hari. Tanaman tersebut seterusnya bergantung kepada 7 pusingan kemarau. Penginokulatan endofit tunggal* G. etunicatum *dalam* V. unguiculata *dan* A. scrobiculata *dalam* L. latisliqua *menghasilkan pertumbuhan, biojisim, bilangan nodula, dan peratus pengkolonian akar yang lebih baik. Kandungan prolina lebih tinggi dalam semua tanaman yang diinokulat bersama* G.etunicatum. *Sebaliknya penginokulatan* G. aggregatum *dalam* Vigna unguiculata *dan* G. etunicatum *dalam* L. latisliqua *terhasil dalam kandungan tisu N, P dan K yang lebih tinggi. Dalam penginokulatan endofit berganda,* A. scrobiculata *dan* G. aggregatum *dalam* V. unguiculata; A. latisilaqua *dan* G. etunicatum *dalam cantuman* L. latisiliqua *didapati menjadi sangat berkesan.*

ABSTRACT

Pot culture experiments were carried out in sterilized soil to determine the effects of three AM fungi, Acaulospora scrobiculata, Glomus aggregatum *and* G. etunicatum, *both individually as well as in combinations, on the plant growth and drought resistance of* Vigna unguiculata *and* Leucaena latisiliqua. *All plants were inoculated with* Rhizobium *sp. During the first 45 days all pots were watered to field capacity and the plants were then subjected to drought cycles by withholding water for 7 days. The plants were further subjected to 7 drought cycles. Inoculation of single endophyte* G. etunicatum *in* V. unguiculata *and* A. scrobiculata *in* L. latisiliqua *produced higher growth, biomass, nodule number, and percentage of root colonization. Proline content was higher in all the plants inoculated with* G. etunicatum, *whereas inoculation of* G. aggregatum *in* Vigna unguiculata *and* G. etunicatum *in* L. latisiliqua *resulted in higher tissue N, P and K contents. In double endophyte inoculations,* A. scrobiculata *and* G. aggregatum *in* V. unguiculata; A. scrobiculata *and* G. etunicatum *in* L. latisiliqua *combinations were found to be highly effective.*

INTRODUCTION

Arbuscular mycorrhizas (AM) confer several benefits on host plants. The AM fungi help plants not only in the better utilization of soil phosphorus (Hayman 1982; Koide 1991) through increased uptake but also of other elements such as N, K, Zn, Mg, Cu and S (Lambert *et al.* 1979; Abbott and Robson 1984; Stribley 1987; Barea 1991). Arbuscular mycorrhizas are also known to increase resistance of plants to conditions such as drought (Safir *et al.* 1972) and extreme soil acidity (Mosse 1973). The improvement in the water relations of plants as a result of mycorrhizal infection has been reviewed by Cooper (1983) and Harley and Smith (1983). But it is difficult to distinguish direct mycorrhizal effects from those that could be mediated via improved mineral nutrition (Nye and Tinker

1977; Nelson and Safir 1982). In addition, other factors associated with AM colonization include changes in leaf elasticity, improved leaf water and turgor potential and maintenance of stomatal opening and transpiration (Auge *et al.* 1987). Increased root length and development of external hyphae may influence water relations of mycorrhizal plants (Kothari *et al.* 1990).

Although most studies on AM fungi have concentrated on their effect on plant nutrition, there is substantial evidence that the mycorrhizal associations may alter plant-water relations. Higher transpiration rates have been found in arbuscular mycorrhizal red clover, rangeland grass (*Bouteloua gracilis*), rose and apple, cow pea and cassava plants (Hardie and Leyton 1981; Allen 1982; Pai *et al.* 1994; Sundaresan and Sudhakaran 1996). In addition, more rapid recovery from water stress and higher soil moisture extraction at low soil water potential have also been observed in mycorrhizal plants (Safir *et al.* 1971; Hardie and Leyton 1981). These changes were broadly attributed to higher root hydraulic conductivity because of improved P nutrition of mycorrhizal plants, although the sizes of mycorrhizal and non-mycorrhizal plants were altogether different (Hardie and Leyton 1981). However, only a few studies have compared the role of different AM fungal species/isolates on imparting drought tolerance to host plants (Simpson and Daft 1990). The purpose of the present study was to compare the effects of AM fungal species, both individually as well as in combinations, in imparting drought resistance and also growth enhancement in *Vigna unguiculata* subsp. *unguiculata* and *Leucaena latisiliqua*.

MATERIALS AND METHODS

Plant Sources

Seeds of *Vigna unguiculata* subsp. *unguiculata* (L.) Walp and *Leucaena latisiliqua* (L.) Gills were procured from Tamil Nadu Agricultural University and Institute of Forest Genetics and Tree Breeding, Coimbatore, Tamil Nadu, India, respectively. Seeds were weighed and selected for uniformity, surface sterilized in 5% H_2O_2 for 5 min, treated with boiling water for 30 sec (*V. unguiculata*) or scarified in concentrated H_2O_2 for 30 min (*L. latisiliqua*) and then soaked in water for 24 h.

Substrate

Seeds were directly sown into 10 x 15 cm polythene bags each filled with ca 1.5 kg sterilized soil:sand mixture (3:1). The soil had pH 8.1 and electric conductivity of 0.2 $mScm^{-1}$. The content of nitrogen, phosphorus and potassium was 104, 4 and 380 kg ha^{-1} respectively.

Inoculum

Soil with infected root bits, hyphae and spores was collected from the pot cultures of AM fungi, *Acaulospora scrobiculata* Trappe, *Glomus aggregatum* Schenck and Smith Emend. Koske and *Glomus etunicatum* Becker and Gerde., maintained in sterilized sand:soil (1:1 by volume) mixture, with cowpea as the host plant in the greenhouse of the Department of Botany, Bharathiar University, Coimbatore - 46, Tamil Nadu, India. Inocula of single and double and triple combinations were added respectively at the rate of 20, 10 + 10 and 6.7 + 6.7 + 6.7 gm soil per pot 5 cm below the soil surface. All the plants were additionally inoculated with a 20-ml suspension of nodulating bacteria which were grown in a yeast extract mannitol broth (Subbarao 1986). The bacteria were obtained from nodules of *Vigna unguiculata* and *Leucaena latisiliqua* growing at Bharathiar University campus, Coimbatore.

Water Regime

During the first 45 days of the experiment, all plants were watered at field capacity. The plants were subjected to drought cycle by withholding water for 7 days. Similarly, plants were subjected to seven drought cycles.

Treatments

The pot culture experiments comprised a 2 x 8 x 5 factorial of the following treatments: 2 sources of plant materials, 8 combinations of endophytes and 5 replicates. The treatments were arranged in randomized block design.

Plants were harvested along with their entire root system 101 days after sowing. Growth parameters such as root length, shoot length, leaf area, nodule and plant dry weights were measured.

Known weight of fresh soil sample (50 g) was dried in an oven at 105 °C until a constant weight was attained. It was cooled in a desiccator and weighed. The loss in weight denotes the moisture content

$$\text{Percentage moisture content} = \frac{\text{Wt. of fresh soil - Wt. of dry soil}}{\text{Turgid wt. - Dry wt.}} \times 100$$

Relative Water Content (RWC)

$$\text{Relative water content (RWC)} = \frac{\text{Fresh wt. - Dry wt.}}{\text{Turgid wt. - Dry wt.}} \times 100$$

Proline Estimation (Chinard 1952)

Fifty mg. of plant materials were homogenized in 10 ml of 3% aqueous sulphosalicylic acid, and filtered through Whatman No. 1 filter paper. To 2 ml of the filtrate, 2 ml of acid-ninhydrin and 2 ml of glacial acetic acid were added. The mixture was heated in a water bath at 100°C for 1 h and cooled in running water. Then 4 ml of toluene was added and mixed vigorously with a test tube stirrer for 15 to 20 seconds and read with a spectrophotometer at 520 nm.

Plant Tissue N, P and K

Dry matter of 101-day-old plants was ground and digested in a triple acid mixture, and tissue P was determined by the molybdenum blue method as described by Jackson (1973). N was estimated following micro-kjeldahl digestion of the samples (Humphries 1956) and K was estimated by a flame photometric method (David 1962).

Root Colonization

The roots were cleared in 2.5% KOH at 90°C and stained with trypan blue (0.05% in lactophenol). The percentage of root length infected was evaluated using the magnified intersection method as described by McGonigle *et al.* (1990).

RESULTS

Growth and Biomass

Growth, as measured by root and shoot lengths and dry weights, was invariably enhanced by endophyte inoculations, although the increase was not always statistically significant. Infection with either *Glomus etunicatum* or *Acaulospora scrobiculata* significantly increased the growth of *Vigna unguiculata* and *Leucaena latisiliqua*, respectively (Table 1, 2) while in double inoculations, *A. scrobiculata* and *G. etunicatum* on *V. unguiculata; G. aggregatum* and *G. etunicatum* on *L. latisiliqua* combinations gave maximum plant growth and biomass. The double and triple combinations of endophytes tend to eliminate the poor performance by a single species although some single species produced the largest plants. In general, the more consistent results were obtained from plants with mixed inoculations.

Root: Shoot Ratio

Though the double and triple endophyte inoculation for *V. unguiculata* (Table 1) and single endophyte *G. etunicatum* for *L. latisiliqua* (Table 2) increased root to shoot ratio, it generally decreased with endophyte inoculations but were

TABLE 1
Growth and biomass of *Vigna unguiculata* subsp. *unguiculata* treated with various combinations of AMF and *Rhizobium* sp. under drought-stressed conditions

Treatment	Shoot Length (cm) (plant^{-1})	Root Length (cm) (plant^{-1})	Leaf area (cm^2) (plant^{-1})	Dry Weight (g plant^{-1})			R/S ratio
				Leaf	Shoot	Root	
Control	21.90d	24.00 d	4.65 c	0.024 d	0.298 c	0.049 b	0.178 a
V1	52.30 a	30.20 abcd	6.83 cd	0.040 c	0.638 b	0.088 b	0.142 a
V2	31.40 c	36.60 a	6.14 de	0.430 bc	0.588 b	0.092 b	0.157 a
V3	52.80 a	34.80 ab	9.26 ab	0.470 ab	0.767 a	0.109 b	0.141 a
V1 + V2	30.40 c	28.00 cd	8.39 abc	0.048 ab	0.526 b	0.198 a	0.200 a
V1 + V3	52.66 a	33.20 abc	7.57 bcd	0.044 bc	0.867 a	0.198 a	0.200 a
V2 + V3	22.54 d	35.30 ab	8.05 abc	0.045 abc	0.542 b	0.109 b	0.170 a
V1 + V2 + V3	37.60 b	29.60 cd	9.58 a	0.052 a	0.615 b	0.094 b	0.200 a

(V1: *Acaulospora scrobiculata;* V2: *Glomus aggregatum;* V3: *G. etunicatum*)

Mean values followed by the same letter are not significant according to Duncan's new multiple range test at $P< 0.05$.

TABLE 2
Growth and biomass of *Leucaena latisiliqua* treated with various combinations of AMF and *Rhizobium* sp. under drought-stressed conditions

Treatment	Shoot Length (cm) (plant^{-1})	Root Length (cm) (plant^{-1})	Leaf area (cm^2) (plant^{-1})	Dry Weight (g plant^{-1})			R/S ratio
				Leaf	Shoot	Root	
Control	12.58 d	32.80 c	9.00 ab	0.044 a	0.176 c	0.160 c	0.950 a
VI	16.30 a	40.86 ab	9.54 a	0.052 a	0.297 ab	0.264 b	0.910 a
V2	13.90 bcd	37.80 b	5.82 e	0.042 a	0.265 ab	0.236 c	0.940 a
V3	14.80 abc	42.80 a	6.22 de	0.048 a	0.285 ab	0.268 b	1.010 a
V1 + V2	13.20 cd	41.60 ab	5.83 de	0.049 a	0.222 bc	0.213 d	0.960 a
V1 + V3	13.80 bcd	42.60 a	8.30 abc	0.057 a	0.324 a	0.238 c	0.730 a
V2 + V3	15.30 ab	39.50 ab	8.00 bc	0.058 a	0.319 a	0.296 a	0.940 a
V1 + V2 + V3	13.40 cd	37.60 cd	7.50 cd	0.056 a	0.310 a	0.266 b	0.890 a

(V1: *Acaulospora scrobiculata*; V2: *Glomus aggregatum;* V3: *G. etunicatum*)

Mean values followed by the same letter are not significant according to Duncans' new multiple range test at $P< 0.05$.

not statistically significant. Single inoculation of *G. etunicatum* on *V. unguiculata* and double inoculation of *A. scrobiculata* and *G. etunicatum* combinations on *L. latisiliqua* resulted in the lowest root to shoot ratio.

Moisture Content (Leaf, Shoot and Soil)

Leaf moisture content was lower in endophyte-noculated *V. unguiculata* than the control plants (*Fig. 1*) whereas in *L. latisiliqua* (*Fig. 2*) double endophyte inoculations, with *A. scrobiculata* and *G. aggregatum* combinations, had higher leaf moisture content.

Single endophyte *A. scrobiculata* in *V. unguiculata* and double endophyte combinations, especially *A. scrobiculata* and *G. etunicatum* in *L. latisiliqua,* had higher shoot moisture content than other inoculations but was statistically significant (*Fig. 1, 2*).

Higher soil moisture content was observed in plants with single endophyte inoculation: *A. scrobiculata* on *V. unguiculata* and double endophytes, *G. aggregatum* and *G. etunicatum,* on *L. latisiliqua* (*Fig. 1, 2*).

Proline Accumulation

Proline accumulation was significantly higher in *G. etunicatum*-inoculated *V. unguiculata* and *L. latisiliqua.* While double endophytes *G. aggregatum* and *G. etunicatum* on *V. unguiculata* (*Fig. 1*) and triple endophytes *A. scrobiculata, G. aggregatum* and *G. etunicatum* on *L. latisiliqua* (*Fig. 2*) resulted in higher accumulation of proline.

Nodulation

Nodule number was higher in *G. etunicatum* and *A. scrobiculata*-inoculated *V. unguiculata* (Table 3) and *L. latisiliqua* (Table 4) seedlings respectively. Double endophytes, *G. aggregatum* and *G. etunicatum* and triple endophytes *A. scrobiculata, G. aggregatum* and *G. etunicatum,* combinations inoculated seedlings had significantly higher nodule number in *V. unguiculata,* but not in *L. latisiliqua.*

Root Colonization

Mycorrhizal root colonization varied amongst different endophyte inoculations and host plants. Infection levels in *V. unguiculata* (Table 3) ranged from 20.0-97.7% of total root length. Higher root colonization was observed in single endophyte, *G. etunicatum* inoculation, whereas *L. latisiliqua* (Table 4) infection levels ranged from 10.4-48.7%. Combination of *A. scrobiculata, G. aggregatum* and *G. etunicatum* gave higher root colonization. No AM fungal contamination was evident in the control plants.

Mycorrhizal Dependency (MD)

The mycorrhizal dependency of the host species varied depending upon the endophytes and their combinations (Table 3 and 4). In a single endophyte, inoculation with *G. etunicatum*

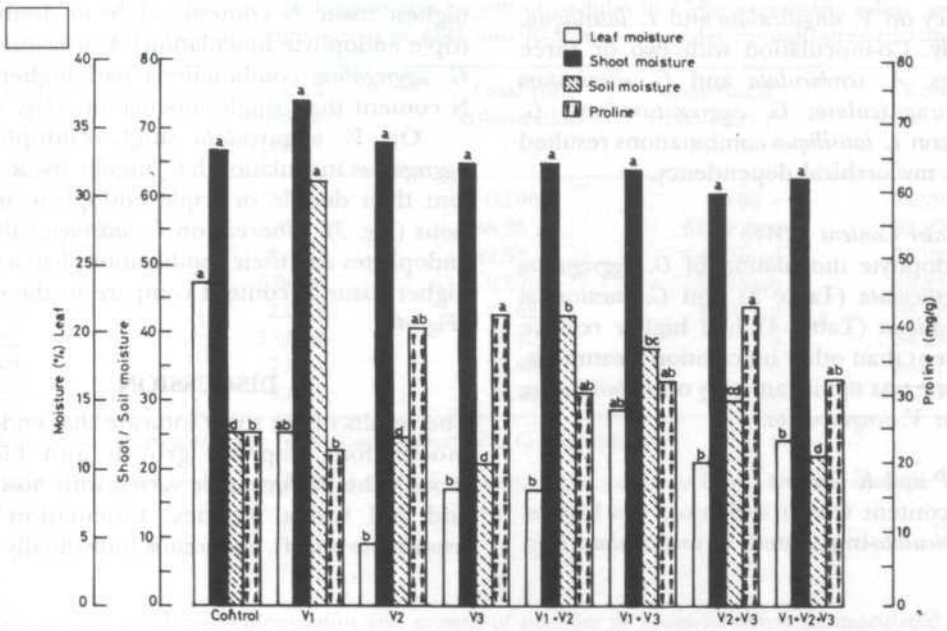

Fig. 1 Effect of VAM fungi (V1, Acaulospora scrobiculata; V_2 Glomus aggregatum; V_3 G. etunicatum*) with* Rhizobium *sp. in different combinations on plant / soil moisture content and proline accumulation in* Vigna unguiculata. *subsp.* unguiculata. *Bars with the same letter(s) are not statistically significant according to DMRT at* $P \leq 0.05$

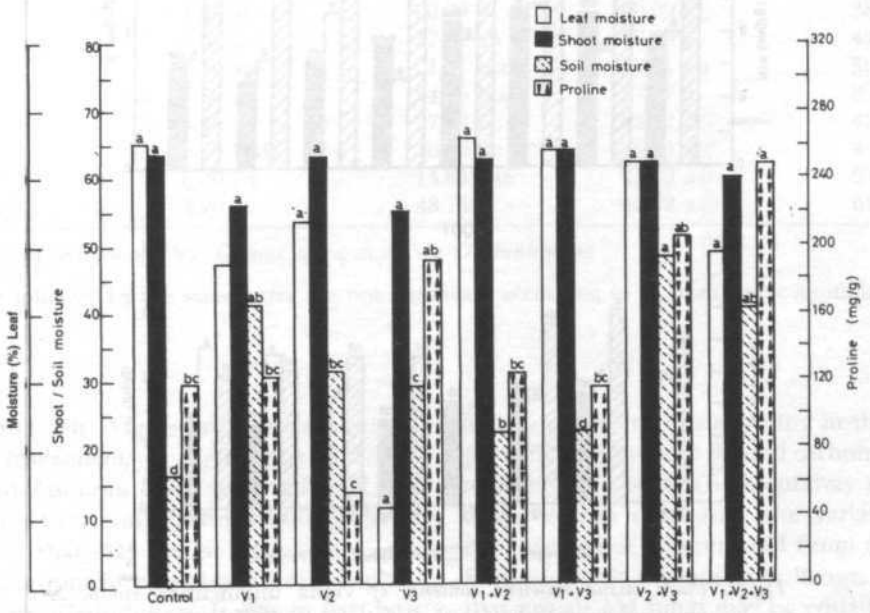

Fig. 2 Effect of VAM fungi (V1, Acaulospora scrobiculata; V_2 Glomus aggregatum; V_3 G. etunicatum*) with* Rhizobium *sp. in different combinations on plant / soil moisture content and proline accumulation in* Leucaena latisiliqua. *Bars with the same letter(s) are not statistically significant according to DMRT at* $P \leq 0.05$

and *A. scrobiculata*, there was higher mycorrhizal dependency on *V. unguiculata* and *L. latisiliqua*, respectively. Co-inoculation with two or three endophytes, *A. scrobiculata* and *G. etunicatum* onto *V. unguiculata*; *G. aggregatum* and *G. etunicatum* on *L. latisiliqua* combinations resulted in highest mycorrhizal dependency.

Relative Water Content (RWC)

Single endophyte inoculation of *G. aggregatum* on *V. unguiculata* (Table 3) and *G. etunicatum* on *L. latisiliqua* (Table 4) had higher relative water content than other inoculation treatments. This increase was significant only on *L. latisiliqua*, but not on *V. unguiculata*.

Tissue N, P and K Content

Nitrogen content (shoot and root) was highest in *A. scrobiculata*-inoculated *V. unguiculata* (*Fig. 3*) plants. On *L. latisiliqua*, *G. etunicatum* had highest tissue N content, while in double and triple endophyte inoculations *A. scrobiculata* and *G. aggregatum* combinations had higher tissue N content than single inoculation (*Fig. 4*).

On *V. unguiculata* single endophyte *G. aggregatum* inoculation had higher tissue K content than double or triple endophyte inoculations (*Fig. 3*). Whereas on *L. latisiliqua* all single endophytes and their combinations had a slightly higher tissue K content compare to the control (*Fig. 4*).

DISCUSSION

The results of the study indicate that endophyte inoculations improve growth and biomass, though the performance varied with host plants and AM fungal species. Inoculation of *A. scrobiculata* and *G. etunicatum* individually and in

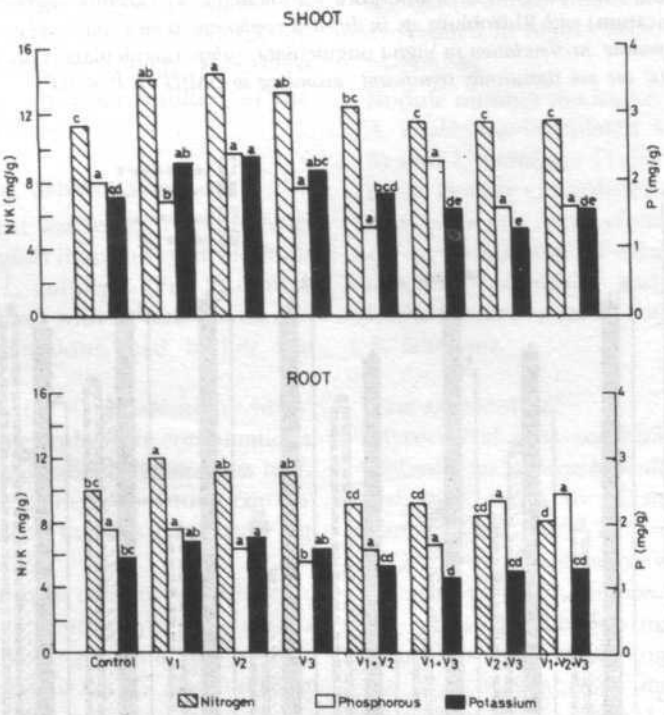

Fig. 3. Plant tissue nutrient contents of Vigna unguiculata *subsp.* unguiculata *inoculated with (V*$_1$, Acaulospora scrobiculata; *V*$_2$ Glomus aggregatum; *V*$_3$ g. Etunicatum*) with* Rhizobium *sp. in different combination on plant / soil moisture content and proline accumulation in* Vigna unguiculata *subsp.* unguiculata. *Bars having the same letter(s) are not statistically significant according to DMRT at P ≤ 0.05*

TABLE 3
Estimate of total VAM root colonization and growth of nodules in *Vigna unguiculata* subsp. *unguiculata* inoculated with various combinations of AMF and *Rhizobium* sp. under drought-stressed conditions

Treatment	No. of nodules (plant^{-1})	Total root colonization (%)	Mycorrhizal dependency (%)	Relative water content (%)
Control	2.00 d	00.00 e	00.00 e	71.29 a
VI	5.80 c	88.35 ab	51.72 bcd	83.97 a
V2	3.00 d	82.52 ab	47.26 cd	93.02 a
V3	6.00 bc	97.70 a	59.75 ab	88.23 a
V1 + V2	2.20 d	20.62 d	42.91 cd	88.72 a
V1 + V3	7.00 ab	78.72 b	67.15 a	80.55 a
V2 + V3	7.80 a	78.68 b	41.26 d	86.47 a
V1 + V2 + V3	7.80 a	48.62 c	52.90 bc	86.47 a

(V1: *Acaulospora scrobiculata;* V2: *Glomus aggregatum;* V3: *G. etunicatum*)

Mean values followed by the same letter are not significant according to Duncan's new multiple range test at P< 0.05.

TABLE 4
Estimate of total VAM root colonization and growth of nodules in *Leucaena latisiliqua* inoculated with various combinations of AMF and *Rhizobium* sp. under drought-stressed conditions

Treatment	No. of nodules (plant^{-1})	Total root colonization (%)	Mycorrhizal dependency (%)	Relative water content (%)
Control	7.40 ab	00.00 e	00.00 c	38.93 b
VI	8.00 a	37.72 ab	39.39 a	42.83 b
V2	6.80 abc	21.67 bcd	32.71 ab	56.17 b
V3	7.80 a	24.56 bcd	37.97 a	89.74 a
V1 + V2	5.40 de	27.98 bc	22.72 b	42.84 b
V1 + V3	6.40 bcd	10.49 de	39.84 a	44.87 b
V2 + V3	6.00 cd	14.85 cde	45.89 a	51.47 b
V1 + V2 + V3	4.20 e	48.73 a	40.82 a	61.16 b

(V1: *Acaulospora scrobiculata;* V2: *Glomus aggregatum;* V3: *G. etunicatum*)

Mean values followed by the same letter are not significant according to Duncan's new multiple range test at P< 0.05

combinations on *Vigna unguiculata* and *Acaulospora scrobiculata, G. aggregatum* and *G. etunicatum* on *Leucaena latisiliqua* was found to be highly effective. Simpson and Daft (1990) also reported that AM fungi inoculation on maize and sorghum increased plant dry weight under water-stress conditions. It may in part be due to improvements in mineral nutrition and partly to the direct water-absorbing capacity of the mycelium, which acts to increase the conductivity of the plant root system and to maintain water flow to the plant even under conditions of water stress. Some inocula reduced leaf area and dry weight suggesting in these cases, a parasitic effect due to limited carbohydrate availability in the plant (Bethlenfalvay *et al.* 1982). These results emphasize the variation in the effectiveness of different AM fungi (Abbott and Robson 1978; Carling and Brown 1980) and that not all AM fungi may be equally beneficial to all host species.

The root to shoot ratio of *V. unguiculata* reflects the high resistance of this species to drought stress. *V. unguiculata* is able to significantly increase the root to shoot ratio under drought stress, which is considered to be im-

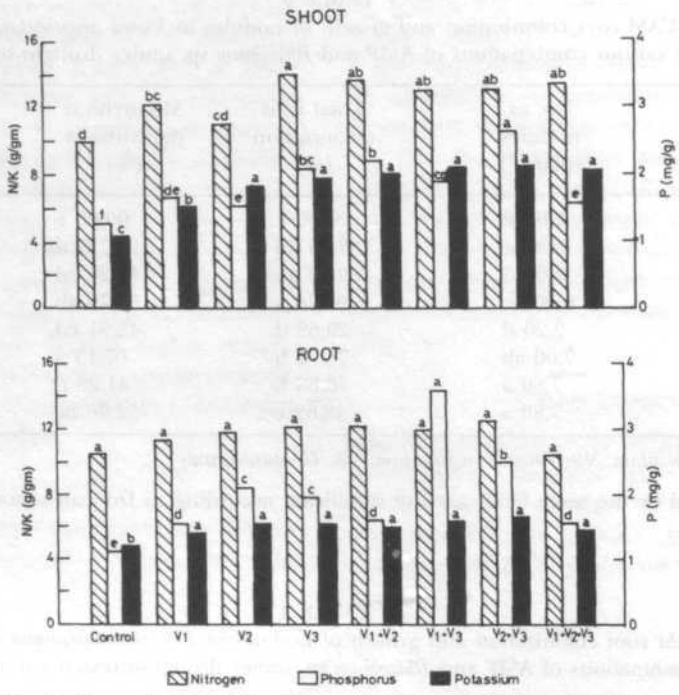

Fig. 4. Plant tissue nutrient contents of Vigna unguiculata *subsp.* unguiculata *inoculated with (V_1,* Acaulospora scrobiculata; V_2 Glomus aggregatum; V_3 G. etunicatum*) with* Rhizobium *sp. in different combination on plant / soil moisture content and proline accumulation in* Leucaena latisiliqua. *Bars having the same letter (s) are not statistically significant according to DMRT at $P \leq 0.05$*

portant for drought prone areas. The root to shoot ratio of *L. latisiliqua* was high, but this species showed no capability to adapt to the environment. AM-inoculated seedlings of both plant species had a lower root to shoot ratio than uninfected plants, but the decreased root mass of the inoculated plants was probably functionally substituted by the external mycelium of the AM fungi. The length and biomass of the extraradical mycelium have been shown to increase under drought stress (Bethlenfalvay *et al.* 1988), and this could be a key factor in AM mediated drought stress.

The shoot and soil moisture contents were higher in mycorrhizal plants. Mycorrhizal plants, which frequently appear to be less prone to wilting and transplanting shock than uninfected plants (Barrows and Roncadori 1977; Levy and Krikun 1980; Janos 1980; Hardie and Leyton 1981; Cooper 1983). Safir *et al.* (1972) reported that soybean root resistance to water uptake was reduced by about 40% with mycorrhizal infection. This was made possible as a result of increase in surface area of the root system and absorption of the hygroscopic water by external hyphae. Nye and Tinker (1977) also suggested that hyphae ramifying into the soil are likely to increase the absorbing area for water uptake even further, and may also be able to bypass the dry zones that often surround the slow-growing roots during periods of drought. Allen (1982) calculated that the rate of fungus root water transport was 2.5 x 10^{-5} mg S^{-1} per hyphal entry point. Tisdall (1994) suggested that the hyphae present in the soil are produced by extracellular polysaccharides, to which microaggregates are attached and bound into stable macroaggregates, so that they do not collapse in water.

It is known that a number of physio-chemical factors are associated with drought tolerance in plants. Proline accumulation correlates with resistance to water stress in various plant species (Thakur 1980; Singh and Rai 1981: Fukutoku and Yoshio 1981). In the present

study, proline accumulation was higher in *G. etunicatum-inoculated V. unguiculata* and *L. latisiliqua* plants. Ramakrishnan *et al.* (1988) also reported that mycorrhizal maize plants yielded considerably greater amounts of proline accumulation under water stress. Different mechanisms have been suggested for proline accumulation, which occurred in desiccated leaves due to protein degradation. However, Singh *et al.* (1973) observed that the net protein synthesis continued slowly even as proline was accumulating under osmotic stress, suggesting that pre-existing protein is not the major source of proline. Therefore, some de novo synthesis seems to take place in water stressed leaves through inter conversion from other amino acids, especially glutamic acid (Srivastava and Kooner 1974). Some inocula yielded lower proline accumulation, suggesting that under moisture stress conditions, abscisic acid accumulation increases and transpiration rate is reduced, thus increasing the level of water in plants. Indirectly, proline has been shown to increase that water level by binding its active groups with water (Palfi *et al.* 1974).

Nodule water potential plays an important role in the nodular activity. Soil based stress, either directly affects the infection process and/or nodule functioning or indirectly plant growth and available photosynthates by acting upon the symbiosis (Singleton 1983). Plants inoculated with *G. etunicatum* and in combination with other endophytes on *V. unguiculata* and *A. scrobiculata* on *L. latisiliqua* had higher nodule number. However, the higher number of nodules formed in control plants were smaller. Similar results were reported in *Medicago sativa* and *Trifolium alexandricum* (Patterson *et al.* 1990). It may be due to the absorption of water by the external hyphae beyond the water depletion zone around roots and root hairs.

Percentage of AMF root colonization varied with different endophyte inoculations. In general, mycorrhizal inoculation had a higher root colonization than uninoculated control. Simpson and Daft (1990) reported similar increases in mycorrhizal root colonization in inoculated maize and sorghum plants. Higher root colonization makes more fungal-host contact and exchange of nutrients and water for better plant growth.

The mycorrhizal dependency varied among endophyte inoculations. Similar differences in mycorrhizal dependency of other plant species such as citrus, wheat, forest plants, hardwood trees and crop plants have been reported by other workers (Menge *et al.* 1978; Janos 1980; Azcon and Ocampo 1981; Plenchette *et al.* 1983; Pope *et al.* 1983). It has been proposed that the length of the root hairs is indicative of the degree of mycorrhizal dependency (Baylis 1975). However, in the present study, it was observed that although mycorrhizal plants had higher root length, they were more dependent on mycorrhiza. This clearly indicates that root length alone can not be used as an index for assessing mycorrhizal dependency. Root production, root fibrosity and root geometry may be even more important factors in mycorrhizal dependency (Mosse *et al.* 1973; Plenchette *et al.* 1983).

Inoculation of *A. scrobiculata* in *V. unguiculata* and *G. etunicatum* in *L. latisiliqua* resulted in higher tissue P content. Michelsen and Rosendahl (1990) also reported similar results in *Acacia nilotica* and *L. leucocephala* under drought stress conditions. This can be attributed to the increase in surface area for absorption due to the extensive extramatrical network of mycelium produced by the mycorrhizal fungi in association with the host root system (Hayman 1978; Howeler *et al.* 1981).

Increased uptake of other nutrients, especially N and K, by mycorrhizal plants has also been reported by some workers. Dhillion and Ampornpan (1992) reported that inoculation of the AM fungi significantly increased the concentrations of N in rice than the control. The present study, in addition to confirming the above findings, adds that uptake of N and K by plants is greatly influenced by AM fungal species colonizing the roots. Stribley (1987) reported that P seems to be the most important nutrient involved, Other nutrients, such as N, K, S, Zn, Cu and Mn, are translocated along AM hyphae. Besides this increasing content of tissue N by direct absorption of extramatrical hyphae (Johanson *et al.* 1992, 1993; Frey and Schuepp 1993) which may also be increased indirectly through *Rhizobium*. The decrease in the nutrient content of *V. unguiculata* inoculated with double and triple endophytes could be due to the dilution effect of growth because dry matter usually accumulates faster than nutrient uptake (Jarrel and Beverly 1981).

The plants inoculated with multiple AM fungi in general had higher growth, proline and

nutrient content. Similar results were reported by Daft and Hogarth (1983) in maize and onion. In these cases, two situations may occur; two or more AM fungal species colonize the roots and add their P uptake abilities to give the plant a better phosphorus nutrition and water uptake. An alternative suggested by Daft and Hogarth (1983) claimed that each endophyte may be important at different times during the growing season. The results of the present study suggest that the inoculations of selected AM fungi offer resistance to drought. The introduced endophytes have to compete with the indigenous flora. Using a mixed inoculum, containing endophytes with differing strategies, might reduce variation and give more consistent benefits to the host plants. An ideal endophyte would need to possess several properties. For example, the ability to infect plants early in their growth period, efficiency in exploiting the soil, easy transfer of materials to the host quick, spread and multiplication, effective infection on a range of host plants under different environmental conditions. All these properties may not be found in one single endophyte and so a multiple inoculum would perhaps give more effective compromise.

CONCLUSION

The present study clearly reveals that inoculation with *Glomus etunicatum* in *Vigna unguiculata* and *Acaulospora scrobiculata* in *Leucaena latisiliqua* would be the best for producing better growth, nodule number and percentage of root colonization. Proline content was higher in both plants inoculated with *Glomus etunicatum*. Multiple AM fungi inoculation had a positive role on drought tolerance in both plants in terms of growth, proline and nutrient content. Work is in progress to select the most effective AMF for drought tolerance under field conditions.

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Phytotoxicity of Phenolic Acids Extracted from Palm Oil Dry Solids

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Keywords: palm oil dry solids (PODS), phytotoxicity, bioassay, phenolic acid, vanillic acid

ABSTRAK

Pengekstrakan ke atas pepejal kering kelapa sawit (PODS) telah dilakukan bagi mengenalpasti kompaun terlarutkan air yang terlibat dalam kefitotoksikan PODS. Ekstrak akueus PODS telah dilakukan sekatan berturutan dengan beberapa pelarut organik. Setiap ekstrak kering telah dibioceraki nkan untuk aktiviti perencatan terhadap pertumbuhan radikel tomato. Perencatan maksimum ke atas pertumbuhan radikel diperolehi pada ekstrak dietil eter, yang menghasilkan 53.3% pertumbuhan berbanding kawalan. Pemisahan pecahan eter menggunakan kromatografi turus menghasilkan satu pecahan toksik, RM10, yang hanya dapat menampung 30% pertumbuhan radikel. Perbandingan pecahan tersebut dengan 14 kompaun fenolik sintetik ke atas kromatograf lapisan nipis menunjukkan persamaan antara empat kompaun tersebut. Analisis lanjut menggunakan kromatografi cecair berkeupayaan tinggi menunjukkan pecahan RM10 terdiri dari asid vanilik. Namun demikian, pecahan RM10 lebih merencatkan pertumbuhan radikel tomato berbanding asid vanilik sintetik.

ABSTRACT

A study on the extraction of palm oil dry solids (PODS) was conducted to identify the water-soluble compounds involved in the phytotoxicity of PODS. The aqueous extract of PODS was sequentially partitioned using various organic solvents. Each of the dried extracts was bioassayed for inhibitory activity on the growth of tomato radicles. Maximum inhibition of radicle growth was observed in the diethyl ether extract, resulting in 53.3% growth compared with control. Further separation of the ether fraction using column chromatography resulted in a single toxic fraction, RM10, which caused only 30% radicle growth. The fraction was compared with 14 synthetic phenolic compounds using thin-layer chromatography and was observed to be similar to four of the compounds. Further analysis by high performance liquid chromatography revealed that the RM10 fraction comprised vanillic acid. However, the RM10 fraction was more inhibitory to the growth of tomato radicles than synthetic vanillic acid.

INTRODUCTION

The increase in palm oil production has generated abundant waste effluent from the palm oil mills. Due to the pollution problem from the effluent, several treatment methods have been developed, producing different types of by-products. Palm oil dry solids (PODS) is one of the by-products generated from mills equipped with a decanter drier system. It contains substantial amounts of plant nutrients and is currently being applied to soil as an organic fertilizer. However, PODS must be properly decomposed before it can be applied to soil. Growth of plants, especially vegetables, has been shown to be adversely affected by undecomposed PODS or other forms of palm of mill effluent (POME). Application of raw or partially decomposed PODS to sandy tailing soil has been shown to inhibit growth of tomato and spinach seedlings (Radziah *et al.* 1997). Zulkifli and Rosmin (1990) observed low yields of cowpea and mustard greens grown on sandy tailing soil with undecomposed POME added. The inhibitory effect of raw effluent has been associated with the presence of lipid and volatile substances (Lim 1986). However, such effects are reduced when the material is completely decomposed. Growth of vegetable seedlings increased when

grown on soil containing decomposed PODS (Radziah *et al.* 1997).

It has been shown that decomposed PODS contains higher quantities of plant nutrients, especially nitrogen, and low lipid contents. Interactions of nutrients and improved physical and biological properties finally lead to improved plant growth. However, there is little information which explains the inhibitory effects of undecomposed PODS or other forms of POME.

The phytotoxicity of plant residue which reduces growth of plants has been associated with the presence of various organic compounds including phenolic compounds (Rice 1984) which are widely distributed in various plant species. These compounds are either leached from plant residues or are formed as by-products during residue decomposition in soil. Several phenolic acids such as ferulic, *p*-coumaric, *p*-hydroxybenzoic, syringic, vanillic acids and *p*-hydroxybenzaldehyde have been identified in extracts of wheat mulch (Lodhi *et al.* 1987), rice residues (Chou and Lin 1976) and sorghum-sudan grass hybrid (Weston *et al.* 1989). The activities of various compounds in soil are often attributed to the water-soluble fractions. The allelopathic effects of phenolic compounds could also be due to both the water-soluble and free form compounds (Whitehead *et al.* 1983). Growth is adversely affected as these water-soluble compounds are taken up by plant roots which inhibit nutrient uptake and affect various physiological processes (Rice 1984). Currently, the potential phytotoxicity of soluble compounds in raw PODS has not been properly characterized. The soluble compounds in PODS could be similar to those in other crop residues since PODS is a by-product originating from plant material i.e. oil palm fruit. Identification of these water-soluble compounds is essential in order to better understand the nature of phytotoxicity and mechanisms involved in its formation, thus enabling proper management of the effluent. Therefore, the following studies were conducted to extract and identify the water-soluble compounds present in raw PODS which are inhibitory to plant growth.

MATERIALS AND METHODS

Extraction of Water-soluble Phytotoxic Compounds

Raw PODS was collected from the Rantau palm oil mill, Negeri Sembilan. The dark granular material, previously described by Radziah *et al.* (1997), contained 19.4% C, 1.44% N, 0.32% P, 1.32% K, 1.46% Ca, 0.32 Mg, 12.0% lipid and the pH was 5.0 (1:5 in H_2O). It was kept dry in the cold room at 8 ± 1°C before use to prevent microbial decomposition. The method of extracting the water-soluble compounds was adapted from that of Weston *et al.* (1989). The flow diagram of the extraction and partitioning procedures used is shown in *Fig. 1.* One hundred gram batches of dried PODS were extracted for 6 hours with 500 ml distilled water in a 1-l conical flask on an orbital shaker, and the suspension was left to stand in the refrigerator for one hour at 4 ± 1°C. The supernatant was decanted and centrifuged at 5,000 rpm for 15 min and the clear brown solution obtained transferred into a clean flask, and the residue returned to the original flask and re-extracted using 250 ml water. A total of 750 ml of water was used to extract every 100 g PODS. The fine

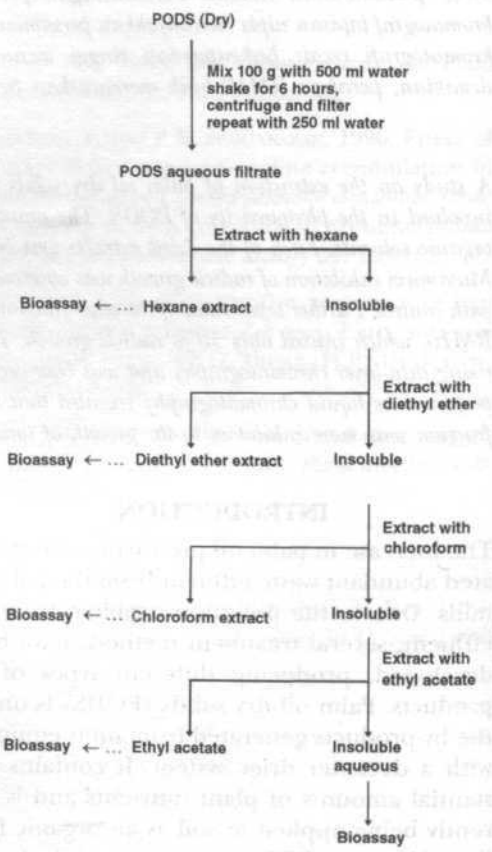

Fig. 1. Flow diagram of the extraction and partitioning procedure of PODS

particulates in the aqueous extract were removed by vacuum filtration through Whatman No. 4, 1 and 42 filter papers sequentially. The extraction procedure was repeated until sufficient aqueous extract was collected for further use. A total of 2.2 kg PODS was used during the extraction process, giving 16.5 l of aqueous extract.

Partitioning of Aqueous PODS Extract

The aqueous PODS extract was sequentially partitioned with hexane, diethyl ether, chloroform and ethyl acetate. The solvents were used to separate the polar and non-polar compounds present in the aqueous PODS. The separation procedure was conducted in batches using 1 l of aqueous extract each time. Each litre of the aqueous extract in a 2-l separatory flask was partitioned six times with 200 ml of the respective four solvents. The extracts were transferred to clean flasks, dried with anhydrous $MgSO_4$ and filtered. The solvent was subsequently removed from each extract by rotary evaporation at 35-40°C. The degree of inhibitory properties of each extract was then evaluated on growth of tomato radicles as described below.

Bioassay of the Solvent Soluble Fractions

Each extract (yellowish brown residue) was weighed and redissolved in chloroform to form a solution with a concentration of 1.0 mg residue ml^{-1} chloroform. Ten millilitres of the remaining aqueous solution were then freeze-dried and redissolved in water to form a similar concentration of 1 mg ml^{-1}. One millilitre of each extract was then placed in separate sterile glass petri dishes (90 x 15 mm), lined with a double layer of Whatman No. 1 filter paper. The chloroform was allowed to evaporate overnight before adding 3 ml sterilized distilled water, to a final concentration of 334 µg residue ml^{-1}. The control dish was given 1 ml chloroform which was allowed to evaporate overnight before the addition of 3 ml distilled water. Ten uniform pregerminated tomato seeds were then placed equidistantly in each dish and incubated in the dark at 30°C. Radicle length of each seedling was measured 72 h after placement in the dish. The activity of the extracts was expressed as percentage radicle growth of control. The bioassay for each extract was replicated three times.

Separation of the Active Fractions by Column Chromatography

The bioassay conducted on the different extracts showed that the diethyl ether extract was the most toxic to growth of tomato radicles (Table 1). This extract was further separated using column chromatography. The ether extract (4.4 g) was precoated with silica gel (60, Baker, 200-250 mesh) and loaded into a silica gel column (30 cm x 6 cm). The column was eluted with petroleum ether, gradually increas-

TABLE 1
Quantity of solvent extracts partitioned from 2.2 kg PODS and their effects on growth of tomato radicles

Solvent extract	Quantity extracted (g)	Recovery (%)	Radicle length ± standard error	Radicle growth (% of control)
Hexane	7.26	0.33	35.8 ± 5.0	115.0
Diethyl ether	4.40	0.20	16.6 ± 1.3	53.3
Chloroform	1.54	0.07	24.0 ± 1.4	77.1
Ethyl acetate	1.98	0.08	30.7 ± 1.9	98.7
Remaining aqueous	ND	ND	41.4 ± 3.1	132.1
Control (H_2O)	-	-	31.1 ± 2.8	100.0
LSD (0.05)			8.4	20.9

ND - not determined

ing the amount of chloroform and methanol. Two hundred fractions of 100 ml each collected from the column were monitored using thin-layer chromatography (TLC) (Kieselgel 60 F_{254} by Merck, 0.2 mm layer) and the profiles were visualized under UV light at 254 nm. Fractions with similar profiles under the UV light were combined to form 11 fractions. During the combination process, precipitation was observed in fractions 9, 10 and 11. These fractions were then separated further into chloroform and methanol soluble fractions. The respective fractions at the concentration of 334 µg ml^{-1} was bioassayed for their inhibitory activity using two controls. Chloroform was used as control for fractions soluble in chloroform and methanol for fractions soluble in methanol. There was strong inhibitory activity in one of the fractions (Table 2), which gave a single spot on the TLC, was active under UV light and was hence selected for further characterization

Comparison of the Unknown Fraction with Standard Phenolic Compounds

The active unknown fraction obtained (designated as RM10) was tentatively identified by comparing its migration profile with the other synthetic (standard) phenolic compounds. The RM10 fraction and the standards were subsequently dissolved in methanol, spotted on TLC plates using a mobile phase of 6:4 acetone-chloroform, and their profiles visualized under UV (254 nm) light. The 14 standard compounds used were: 4-hydroxybenzaldehyde, vanillin, acids of *p*-hydroxybenzoic, ferulic, *p*-coumaric, vanillic, caffeic, gentisic, *p*-hydroxyphenyl acetic, sinapic, gallic, salicylic, *trans*-cinnamic and syringic (Fluka Chemical Company).

HPLC Profile of the Unknown Fraction

The identity of the unknown fraction RM10 was further confirmed by using high performance liquid chromatography (HPLC). The RM10 fraction and the four standard samples (ferulic, *p*-hydroxybenzoic, vanillic and *p*-coumaric acid) were dissolved singly in methanol to form a concentration of 1 mg ml^{-1}, and filtered through 0.45 µm Millipore membrane. Ten microlitres of the sample was injected into the Waters HPLC system fitted with an absorbance detector model 460 set at 254 nm, a pump model 510 and a reverse-phase µ Bondapak C_{18} column (300 mm x 3.9 mm). The isocratic elution solvent used consisted of a mixture of methanol, ethyl acetate and acetic acid in the

TABLE 2
Recovery of different fractions from column chromatography of diethyl ether extract and their effects on growth of tomato radicles

Fractions	Quantity recovered (mg/4.4 g)	Recovery (%)	Radicle length (mm) ± standard error	Radicle growth (% of control)
R1-R3	126	2.9	ND	ND
R4	488	11.1	37.0 ± 3.8	92.5
R5	752	17.1	31.5 ± 4.4	78.7
R6	1271	28.9	38.2 ± 2.5	95.7
R7	869	19.8	34.0 ± 1.3	85.1
R8	158	3.6	35.6 ± 2.2	88.9
R9 $CHCl_3$	194	4.4	15.7 ± 0.4	39.2
R9 MeOH	45	1.0	30.0 ± 2.6	68.7
R10 $CHCl_3$	27	0.6	13.9 ± 1.7	34.8
R10 MeOH	184	4.2	13.2 ± 4.3	30.2
R11 $CHCl_3$	3	0.1	39.0 ± 1.0	97.5
R11 MeOH	31	0.7	24.9 ± 4.8	56.9
Control ($CHCl_3$)[a]			36.6 ± 3.3	100.00
Control (MeOH)[b]			41.7 ± 4.6	100.00
LSD (0.05)				18.8

ND - not determined
a for fractions dissolved in $CHCl_3$
b for fractions dissolved in MeOH

ratio of 35:1:2, at a flow rate of 2 ml min^{-1} (Blum *et al.* 1984).

Effect of Various Concentrations of RM10 Fraction and Standard Phenolic Acids on Growth of Tomato Radicles

The bioassay technique described previously was used to compare the toxicity of RM10 fraction with standard phenolic acids. The activity of RM10 was assessed with four standard phenolic acids of similar TLC profiles. The standards used were: ferulic, *p*-hydroxybenzoic, vanillic and *p*-coumaric acids. The concentrations used were; 0, 250, 500, 750 and 1000 µg ml^{-1}. Growth response curves were then drawn to determine the concentration of each compound responsible for causing 50% inhibition (I_{50}) on elongation of tomato radicles.

RESULTS

Extraction of Water-soluble Phytotoxic Compounds

Various amounts of water-soluble organic fractions were extracted from PODS. The highest amount of dry fraction recovered from the partitioning procedure was the hexane extract (7.26 g), followed by diethyl ether extract (4.4 g) (Table 1). The hexane extract probably consisted of lipids and non-polar compounds which were present in PODS in substantial amounts. The hexane extract made up 0.33% and diethyl ether extract 0.20% of the total dried PODS used. Smaller amounts of residues were obtained from the chloroform and ethyl acetate extracts.

Results from the bioassay studies showed that different solvent extracts significantly ($P \leq 0.01$) influenced elongation of tomato radicles. The maximum inhibitory activity of radicle growth was observed in the diethyl ether extract where radicle elongation was significantly reduced to 53.3% of the distilled water control. This indicated that the ether had extracted out most of the inhibitory compounds present in the aqueous PODS. The chloroform and ethyl acetate extracts exhibited lesser inhibition on radicle growth. The hexane extract as well as the remaining crude aqueous extract, however, stimulated growth of tomato radicles. Improvement in radicle elongation, especially in the remaining aqueous fractions, indicated absence of the toxic compounds.

Isolation and Characterization of Phytotoxins

Since the greatest inhibitory effect was obtained from the diethyl extract, this extract was further separated by column chromatography and each new fraction recovered was bioassayed for inhibitory activity. Two hundred fractions were collected from the separation process and were monitored by using TLC. Fractions with similar TLC profiles were then combined to form 11 distinct fractions. Fractions R9, R10 and R11 which formed precipitations were further extracted by dissolving the individual fraction in chloroform and methanol. Three additional fractions were obtained from this extraction (Table 2). The flow chart of the separation procedure is shown in *Fig. 2.*

Results of the bioassay of all fractions (R4-R11) showed significant ($P \leq 0.01$) differences in their inhibitory activities (Table 2). High inhibition on radicle growth was concentrated in three fractions, R9 which is soluble in $CHCl_3$, R10 which is soluble in $CHCl_3$ and R10 which is soluble in MeOH. Among these three fractions, the R10 in MeOH (designated as RM10) had the lowest radicle growth of 30.2%. This brown, viscous fraction was found to produce a single

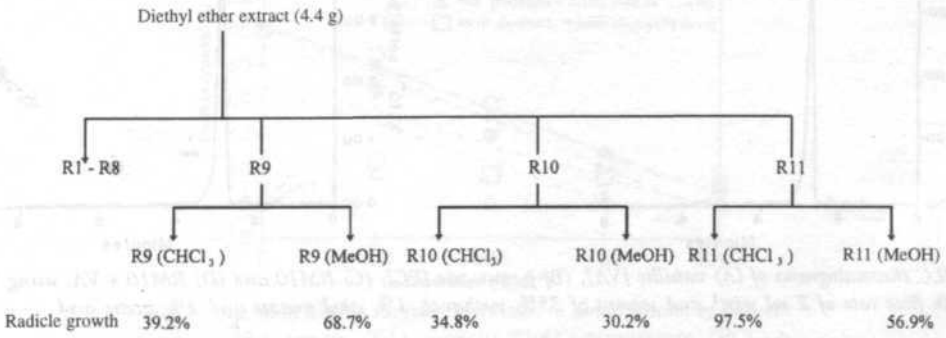

Fig. 2. Separation of the diethyl ether extract and the effect on growth of tomato radicles

spot on the TLC and was strongly visible under UV light (254 nm). Although the other two fractions (R9-$CHCl_3$ and R10-$CHCl_3$) showed almost similar activity to RM10, they did not give a distinct spot on the TLC, and thus required further separation for identification. No attempt was made to identify them in this study.

The amount of RM10 fraction recovered from the crude diethyl ether extract using the column chromatographic procedure was very small (only 184 mg/4.4 g or 4.2%) (Table 2). This indicates that the active RM10 fraction which could be toxic to plant growth was only 0.0084% (dry weight) of the original raw PODS.

The unknown RM10 fraction was tentatively identified by comparing its TLC profile with the standard phenolic acids. The results showed that this fraction was similar to 4 of the standards: ferulic, *p*-hydroxybenzoic, *p*-coumaric and vanillic acids (*Fig. 3*). The identity of RM10 was further confirmed by injecting the sample into HPLC using the reverse-phase μ Bondapack C_{18} column. A single peak at the retention time of 2.89 min was obtained (*Fig. 4*). Further coelution with known standards indicated that

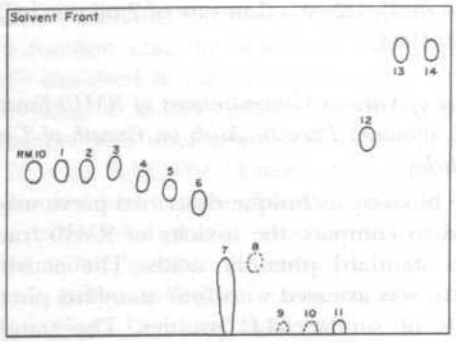

Fig 3. Thin-layer chromatography profiles of fraction RM10 and standard phenolic acids with mobile phase of acetone : chloroform (6:4)

**Key: 1) p-hydroxybenzoic acid; 2) ferulic acid; 3) p-coumaric Acid; 4) vanillic acid; 5) sinapic acid; 6) syringic acid; 7) caffeic acid; 8) p-hydroxyphenyl acetic acid; 9) gentisic acid; 10) salicylic acid; (11) trans-cinnamic acid; 13) 4-hydroxybenzaldehyde; 14) vanillin*

RM10 was similar to vanillic acid. The retention times of the respective standards are shown in Table 3.

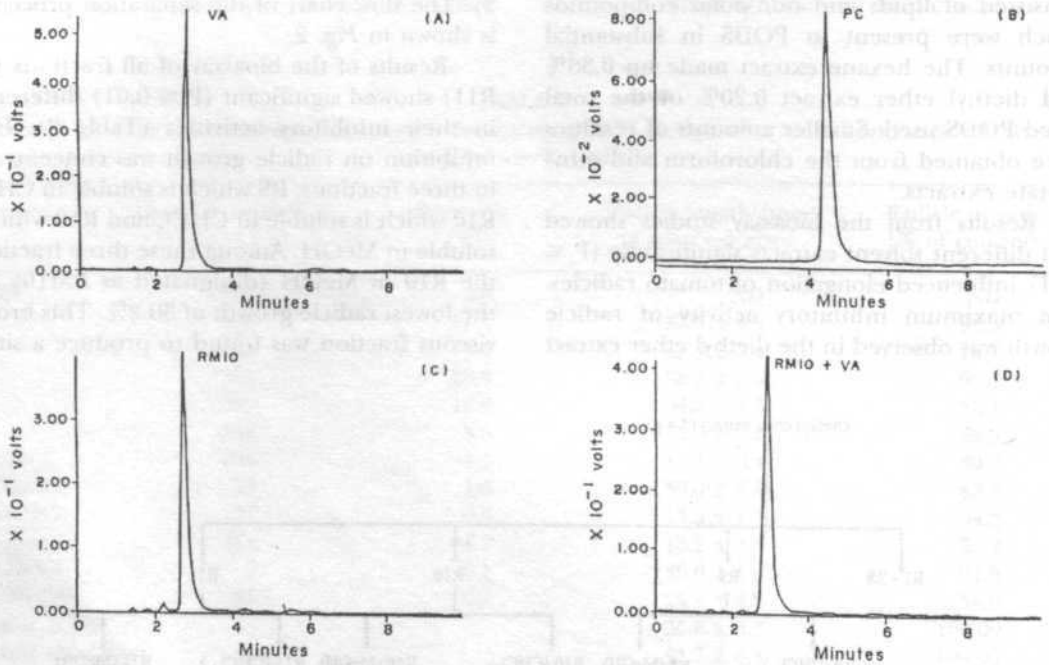

Fig 4. HPLC chromatograms of (A) vanillic [VA], (B) p-coumaric [PC], (C) RM10 and (D) RM10 + VA, using C_{18} column with flow rate of 2 ml min^{-1} and solvent of 35% methanol, 1% ethyl acetate and 2% acetic acid

TABLE 3
Retention times of the unknown and standard phenolic compounds

Phenolic acid	Retention time (min)
Ferulic	5.40
p-coumaric	4.64
p-hydroxybenzoic	3.02
Vanillic	2.89
Extract RM10	2.89

Growth Response of Tomato Radicles to Various Concentrations of RM10 and Standard Phenolic Acids

The length of tomato radicles was found to be significantly ($P \leq 0.01$) affected by the concentrations of RM10 and the standard phenolic acids (ferulic, vanillic, *p*-coumaric and *p*-hydroxybenzoic acids) (*Fig. 5*). Radicle elongation was reduced with increase in concentration of RM10 and phenolic acids. This indicated that the toxicity of these compounds increased with increase in concentration. The percentage of radicle growth showed negative liner responses to increasing concentration of the compounds.

p-coumaric acid was found to be the most toxic to growth of tomato radicles of all the compounds. Total inhibition (no growth) on radicle elongation was observed at 500 µg ml^{-1} *p*-coumaric acid concentration. Ferulic acid was least toxic to the growth of tomato radicles. At low concentrations, the activity of RM10 was close to that of *p*-coumaric acid. However, the activities differed as the concentration of compounds increased to 500 µg ml^{-1}. Total lack of growth of tomato radicles was observed as the concentration of these compounds increased to 1000 µg ml^{-1}, i.e. the highest concentration studied.

The 50% radicle growth inhibition (I_{50}), i.e. the compound concentration at which 50% of the radicle elongation was inhibited, was also calculated from the growth response curves in *Fig. 5*. The RM10 fraction was more toxic then *p*-hydroxybenzoic, vanillic and ferulic acids. The descending order of toxicity of the compounds with increasing I_{50} values recorded was *p*-coumaric acid (194 µg ml^{-1}) > RM10 (292 µg ml^{-1}) > *p*-hydroxybenzoic acid (434 µg ml^{-1}) > vanillic acid (466 µg ml^{-1}) > ferulic acid (524 µg ml^{-1}).

DISCUSSION

Results from the extraction procedures used showed that most soluble toxic compounds present in raw PODS were concentrated in the diethyl ether extract. This extract was found to reduce the growth of tomato radicles by 46.7% of control (Table 1). Most of the earlier findings have also shown that the toxic compounds found in other crop residues are soluble in polar solvents such as ether (Weston *et al.* 1984). It was also observed that the toxicity of the remaining aqueous PODS was completely eliminated after removal of the toxic extracts, where growth of tomato radicles was found to be stimulated. Further separation of the ether extract using column chromatography recovered a more toxic RM10 fraction. The amount of this phytotoxic compound present in raw PODS was small (0.0084%). Hence, under normal conditions the application of a low amount of raw PODS to soil would probably cause minimum inhibition on

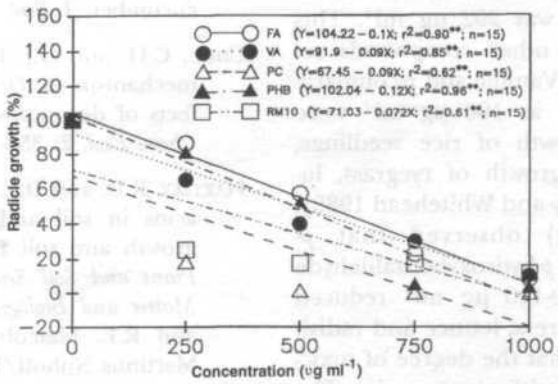

Fig 5. Growth of tomato radicles in relation to concentration of phenolic acids: ferulic (FA), vanilic (VA), p-coumaric (PC), p-hydroxybenzonic (PHB) and fraction RM10

growth of plants. Degradation of these compounds is rapid in well-drained soils. However, continuous application of raw PODS to clayey soil with long periods of water saturation could probably lead to accumulation of these phytotoxic compounds.

Although RM10 was confirmed to be vanillic acid on the HPLC, its inhibitory effect on growth of tomato radicles was higher than that caused by the synthetic vanillic acid. The higher toxic effect of RM10 was probably a result of being bound to other compounds which have higher toxic effect. No attempt was made to purify RM10 in the present study.

The activity of RM10 was also compared to the activity of the other toxic phenolic compounds (besides vanillic acid) commonly found in soil: ferulic, *p*-coumaric and *p*-hydroxybenzoic acids (Lodhi *et al.* 1975). The inhibitory effect exhibited by RM10 was found to be intermediate between that of *p*-coumaric and vanillic acid (*Fig. 5*). This indicates that the phytotoxic compound in PODS is present in mixed form. This is to be expected since under natural conditions, phenolic acids usually occur in mixed rather than in single form. The phytotoxic effects of organic residues as observed is probably the result of the strengthening effect of complex organic mixtures (Rasmussen and Einhellig 1977; Blum 1996).

Results of the present study showed that the RM10 fraction was more toxic then standard ferulic, vanillic, and *p*-hydroxybenzoic acids, but less toxic than *p*-coumaric acid on the growth of tomato seedlings. However, the inhibitory activity of RM10 extracted from PODS was low compared to the activity of some other standard phenolic compounds. The concentration of RM10 which caused 50% reduction in radicle growth (I_{50}) of tomato was 292 µg ml^{-1}. This value was higher than other compounds reported to be phytotoxic. Vanillic and *p*-coumaric acids at as low a level as 100 µg ml^{-1} were reported to inhibit growth of rice seedlings, seed germination and growth of ryegrass, lucerne and wheat (Hartley and Whitehead 1985). Weston *et al.* (1989) observed that *p*-hydroxybenzoic acid and *p*-hydroxybenzaldehyde at concentrations of 70-140 µg ml^{-1} reduced radicle growth of curly cress, lettuce and radish by 50%. This indicates that the degree of toxicity of compounds differs with plant species. The tomato seeds used in the study were more tolerant to the presence of phenolic acids than other seeds. The choice of plant species in the bioassay technique is crucial in determining the inhibitory activity of phenolic compounds.

The amount of the inhibitory compound extracted from PODS in the present study was only an estimate and could not therefore reflect the actual amount present in the entire PODS. This study could only focus on compounds that are soluble in water and not on the total compounds present. The amount of toxic compounds may also vary with the type of POME. There are variations in the physical, chemical and biological properties of POME generated from different palm oil mills which use different methods of effluent treatment (Lim 1986). Raw POME needs to be decomposed in order to eliminate the inhibitory effect on plant growth. The proper management of POME applied to soils is important in order to reduce its phytotoxic effect, while obtaining optimum benefits for plant growth. Decomposition is important to reduce the phytotoxicity of various types of POME in order to convert them to valuable organic fertilizer.

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Changes in Tannin and Pectic Substances at Different Positions within a Bunch of Cavendish Banana (*Musa cavendishii* L. var. Montel) during Development and Maturation

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Keywords: Cavendish banana, tannin, pectic substances, hands, fingers, development, maturation

ABSTRAK

Kajian telah dijalankan ke atas perubahan kandungan tanin, pepejal taklarut alkohol (AIS) dan pecahan pektin (air, ammonia oksalat dan natrium hidroksida larut) bagi pisang Cavendish varieti Montel pada kedudukan yang berlainan dalam satu tandan semasa kematangan dan kemasakan. Terdapat penurunan yang bererti ($P<0.01$) dalam kandungan tanin di antara kedudukan sikat yang berlainan (atas, tengah dan bawah) dalam satu tandan dan juga di antara buah (atas dan bawah) dalam satu sikat semasa kematangan. Kandungan AIS meningkat pada peringkat awal, tetapi menurun dengan bererti ($P<0.05$) apabila mencapai peringkat kematangan (minggu 12) bagi buah-buah yang masak. Bahan-bahan pektik juga menunjukkan perbezaan bererti ($P<0.05$) pada kedudukan-kedudukan yang berlainan (sikat dalam satu tandan dan buah dalam satu sikat) bagi setiap tandan semasa kematangan. Pektin meningkat pada peringkat awal sehingga ke peringkat maksimum pada minggu 12 dan kemudian menurun secara perlahan-lahan. Pada peringkat akhir kematangan, bahan-bahan pektik mulai meningkat dengan perlahan sehingga ke peringkat kemasakan. Terdapat juga perbezaan bererti ($P<0.05$) di antara kedudukan sikat dan buah yang berlainan bagi kandungan tanin , AIS dan bahan-bahan pektik semasa kematangan. Walau bagaimanapun, terdapat perbezaan yang bererti ($P<0.05$) di antara kedudukan-kedudukan sikat-sikat dan buah-buah yang berlainan dalam satu tandan di mana kedudukan sikat atas dan kedudukan buah atas dalam sikat memberikan nilai yang tinggi bagi kandungan-kandungan tanin, AIS dan bahan-bahan pektik berbanding dengan kedudukan-kedudukan sikat tengah dan bawah dalam satu tandan dan kedudukan buah bawah dalam sikat masing-masing.

ABSTRACT

Studies were carried out on changes in the tannin content, alcohol insoluble solids (AIS) and pectin fractions (water, ammonium oxalate and sodium hydroxide solubles) of Cavendish banana variety Montel at different positions within a bunch during maturation and ripening. There was a significant decrease ($P<0.01$) in the tannin content between the different portions of hands within a bunch and between different fingers within a hand during maturity. AIS increased at the early stages, but it decreased significantly ($P<0.05$) in the ripe fruits (week 12). There was a significant difference ($P<0.05$) in the pectic substances at different positions within a bunch during maturity. The pectins increased at the early stages, reaching a maximum at week 12 and then slowly decreased. At the end of maturation, the pectic substances started to increase slowly until ripening. There were also significant differences ($P<0.05$) in the tannin content, AIS and pectic substances during maturity between different portions of hands and fingers. However, significant differences ($P<0.05$) were observed between portions of hands within a bunch and between fingers within a hand during maturity stage; the top hands and upper fingers were higher in tannin, AIS and pectic substances contents than the middle and bottom hands within a bunch and lower fingers within a hand respectively.

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INTRODUCTION

Pectic substances play a significant role in maturation, softening and textural changes in some fruits (Pilnik and Voragen 1970). Ripening and maturation in fruit involve the breakdown of these compounds to sugars and acids (Doesburg 1973). In the USA, commercial pectin is mainly produced from citrus peel and apple pomace (Rouse 1967) with other fruits such as guava (Verma and Srivastava 1966), purple passion fruit (Pruthi 1965) and banana (Von Loesecke 1930) also good sources. During the ripening of many edible fruits (Goldstein and Swain 1963), both tannins and astringency are reduced and the changes in astringency are a reflection of changes in the molecular size of the tannins. Effects of tannin on browning in fruit and fruit products (Swain 1962) and cloudiness in many fruit juices (Cash *et al.* 1976) have long been recognized. Tannins have also been associated with astringency in many fruits, especially less ripe ones (Swain 1962; Joslyn and Goldstein 1964; Ismail and Mamat 1984). Goldstein and Swain (1963) believed that the physiology of the astringency sensation was due to the interaction of these polyphenols with salivary proteins and glycoproteins in the mouth. The objective of this study was to determine the changes in pectic substances and tannin content of 'Montel' banana (*Musa cavendishii* L.) from different hand positions (top, middle and bottom) and different finger positions (upper and lower) within a hand in a bunch.

MATERIALS AND METHODS

Fruit Source

One hundred banana plants (cv. Montel) were tagged randomly during flower emergence at the experimental plot, Universiti Pertanian Malaysia (UPM), Serdang, Selangor. Fruit growth was observed weekly. Banana bunches were harvested each week from week 3 to week 15 (fruit started to ripen). Observations were done in triplicates with one bunch per replicate. The harvested fruits were immediately transported to the laboratory of the Faculty of Food Science and Biotechnology, UPM for further evaluation.

Sampling

The hands from each bunch were divided into top (1st hand from the top), middle (5th hand from the top) and bottom (2nd hand from the bottom) portions. The fingers from each hand of the bunch were divided into upper and lower portions. Observations of chemical parameters were made on composite samples of six fruits. Experiments were done in triplicate.

Determination of Tannin Content

Five grams of homogenized fruit material was used for tannin analysis according to the AOAC (1980) method. The sample was boiled for 30 min with 400 ml of distilled water, then transferred to a 500-ml volumetric flask and filtered. The standard tannic acid solutions of 0-10 ml aliquots were prepared. The absorbance of standard and samples was determined at 760 nm after 30 min of mixing against experimental blank adjusted to 0 absorbency using UV-Vis Spectrophotometer model Shimadzu.

Determination of Pectic Substances

The alcohol insoluble solids (AIS) obtained after extraction of sugars were used to measure pectin. The residue was washed in acetone and dried at 60 °C to a constant weight. The dry residue was ground finely and weighed. The sample was recorded as the alcohol insoluble solids (AIS) fraction and later used for pectin estimation. The AIS preparation was separated into three types of pectic substances by successive extraction with distilled water, 0.75% ammonium oxalate and 0.05 N sodium hydroxide (Roe and Bruemmer 1981). Each pectin fraction was then analysed colorimetrically after reaction between carbazole and anhydrogalacturonic acids of the pectin as described by Rouse and Atkins (1955).

Statistical Analysis

For data analyses, the SAS programme (Statistical Analysis System) was used. The values obtained were subjected to analyses of variance and tested using the Duncan's multiple range test (DUNCAN).

RESULTS AND DISCUSSION

The tannin content of 'Montel' banana from different positions was significantly different at all the different stages of maturity (*Fig. 1*). Tannin content of fruits from all positions decreased rapidly at the early stages and then slowly increased, for fruits from the top lower, middle upper and bottom lower positions, after week 9.

From week 12 onwards, the tannin content of fruits from all positions decreased rapidly during ripening (*Fig. 1*). When green and in the early stages of ripening, the banana fruit is astringent (Barnell and Barnell 1945). These results supported those of Von Loesecke (1949) who noted that green bananas have soluble tannin, the content of which decreases during ripening. The decrease in astringency of banana was correlated with the decrease in tannin content (Slocum 1933). Tannin is one of the sources from the polyphenol group which cause the astringent taste in fruits (Goldstein and Swain 1963; Ranganna 1977). Goldstein and Swain (1963) found that the tannin content and astringency of banana decreases rapidly during ripening.

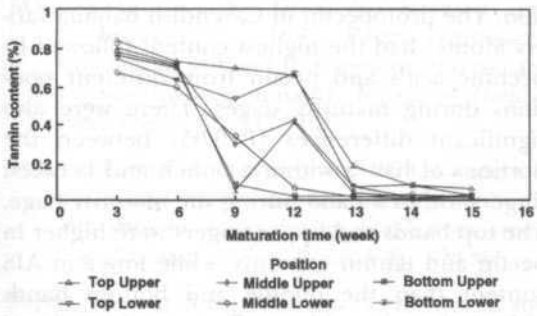

Fig 1. Effect of different positions of 'Montel' banana on tannin content during development and maturation

Pectin is a polysaccharide found in plant tissues (Kertesz 1951). There was a significant difference ($P<0.01$) in the total pectin, which is the sum of protopectin (NaOH soluble fraction), pectin (water soluble fraction, and pectinic acids (oxalate soluble fraction), of 'Montel' banana from different positions, indicating at different stages of maturity of the bunch (*Figs. 2-5*). There was also a significant difference ($P<0.01$) in the AIS content during maturity (*Fig. 6*). The AIS content exhibited an irregular pattern, decreasing rapidly at the early stages and then slowly increasing. At week 12, the AIS content (21.82%) started to decrease rapidly during ripening from 20.77% at week 12 to 18.12 and 12.56% at weeks 14 and 15 respectively (*Fig. 6*). These results support those of Subramanyam *et al.* (1972) and Roe and Bruemmer (1981) who reported that the AIS content decreased markedly during ripening. In spite of AIS content, the total pectin increased rapidly at the early stages until week

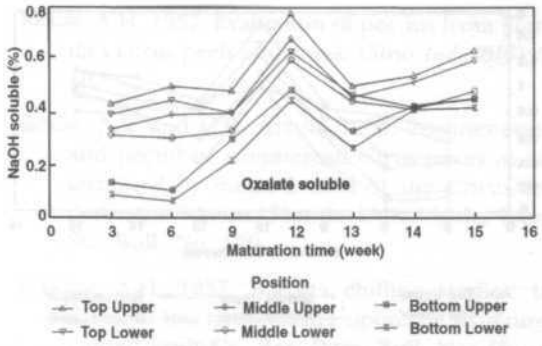

Fig 2. Effect of different positions of 'Montel' banana on NaOH soluble pectin fraction during development and maturation

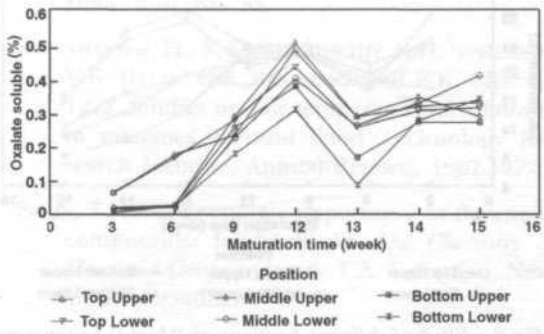

Fig 3. Effect of different positions of 'Montel' banana on oxalate soluble pectin during development and maturation

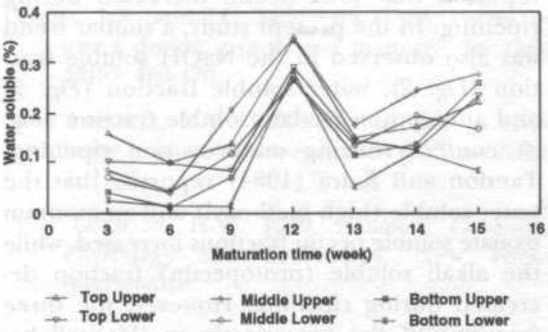

Fig 4. Effect of different positions of 'Montel' banana on water soluble pectin during development and maturation

12 (1.32%), which was the optimum period for harvesting (*Fig. 5*). It also exhibited an irregular pattern, decreasing slowly at week 13 (0.75%) and then increasing during ripening. According to Brady (1976), pectinesterase enzyme is involved in the changes in total pectin during ripening. This supported the findings of Stratton and Von Loesecke (1930) who

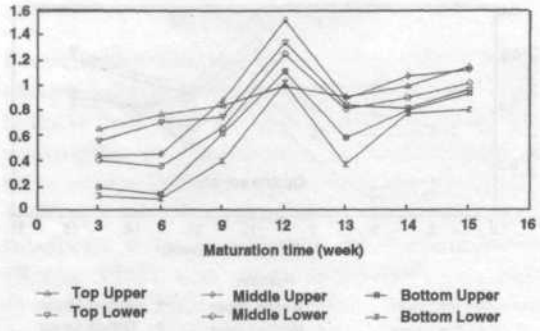

Fig 5. Effect of different positions of 'Montel' banana on total pectin during development and maturation

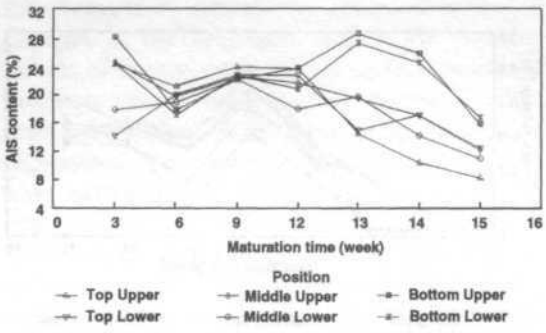

Fig 6. Effect of different positions of 'Montel' banana on AIS content during development and maturation

reported that total pectin increased during ripening. In the present study, a similar trend was also observed in the NaOH soluble fraction (*Fig. 2*), water soluble fraction (*Fig. 3*) and ammonium oxalate soluble fraction (*Fig. 4*) contents during maturity and ripening. Tandon and Kalra (1984) reported that the water soluble (high methoxyl) and ammonium oxalate soluble pectin fractions increased, while the alkali soluble (protopectin) fraction decreased during ripening. However, the three fractions of the protopectin in 'Montel' banana increased (*Fig. 2*). This supported the findings of Heimann (1980) who found that protopectin, upon hydrolysis, yields water soluble pectin and its content increases in ripe mango fruit (Mizuta and Subramanyam 1973). Recently, similar results were reported by Malisarad *et al.* (1983) in ripening tomato fruit. It seems that protopectin is an important pectin fraction which increases before physiological maturity and then decreases with fruit ripening and softening. Tandon and Kalra (1984) also noted that alkali soluble fraction increased again 70 days after fruit set but decreased thereafter. *Fig. 2* shows that the protopectin in 'Montel' banana had the highest content followed by pectinic acids and pectin from different positions during maturity stages.

CONCLUSION

From this study it was found that there is a highly significant difference ($P<0.01$) in tannin and pectin contents during maturity stages between the various portions of hands and fingers. The tannin content of Cavendish banana decreased rapidly, while the pectic substances increased during harvesting. There is a significant difference ($P<0.05$) in total pectin, tannin and AIS contents between the different portions of hands and fingers of the bunch during maturation. The protopectin in Cavendish banana variety Montel had the highest content followed by pectinic acids and pectin from different positions during maturity stages. There were also significant differences ($P<0.05$) between the portions of hands within a bunch and between fingers within a hand during the maturity stage. The top hands and upper fingers were higher in pectin and tannin contents, while lower in AIS content than the middle and bottom hands within a bunch and lower fingers within a hand respectively. The positional effects seen in the present study could possibly be due to the different physiological maturity of the fruit.

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Substitution of Maize with Cassava and Sweet Potato Meal as the Energy Source in the Rations of Layer Birds

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ABSTRAK

Tiga ratus enam puluh ayam telur (berusia 36 minggu - Isa Brown) diberi makanan berasakan jagung. Semasa 12 minggu tempoh percubaan, ubi keledek dan ubi kayu menggantikan jagung sebagai makanan asas. Pengeluaran telur ayam pada siang hari signifikan (P<0.05) merosot melalui makanan ubi kayu sementara burung-burung yang diberi makanan jagung dan ubi keledek tidak signifikan antara satu sama lain dalam pengeluaran telur ayam hari siang. Makanan ubi kayu secara signifikan mengurangkan (P<0.05) pengambilan tenaga metabolisme dan tiada perbezaan signifikan dalam pengambilan protein mentah oleh burung-burung yang memakan ubi keledek dan ubi kayu. Walau bagaimanapun penggantian jagung kepada ubi keledek dan ubi kayu meninggalkan kesan signifikan terhadap saiz telur, berat, pengambilan makanna, ketebalan kulit, unit Haugh. telur dozen/makanan atau telur kg/makanan (p<0.05). Makanan kawalan jagung adalah lebih baik (p<0.05) dalam mecerna protein mentah sementara ketercenaan tenaga merosot (p<0.05) dengan makanan ubi kayu.

ABSTRACT

Three hundred and sixty layer hens (36 weeks old Isa Brown) were fed a maize based diet in which sweet potato meal and cassava meal replaced maize in the basal diet during a 12-week trial period. Hen-day egg production was significantly (p<0.05) depressed by cassava meal while birds fed diets with maize and sweet potato meal were not significantly different from each other in hen-day eeg production. Cassava meal significantly reduced (p<0.05) metabolizable energy intake while there was no significant difference in the crude protein intake of birds fed sweet potato and cassava meal. There was, however, no significant effect from replacing maize with sweet potato or cassava meal on egg, weight, feed intake, shell thickness, Haugh unit, feed/dozen eggs or feed/kg egg (p<0.05). The maize control diet was superior (P<0.05) in the digestibility of crude protein while energy digestibility was depressed (P<0.05) with the cassava diet.

INTRODUCTION

Going by the fundatemntasl role of energy in animal nutrition and the fact that it constitutes 35-60% of rations for different species and classes of livestock, the sustenance and expansion of the livestock industry, particularly in developing countries, depends to a large extent on the availability of the dietary energy source used in compounding concentrated rations for various classes of livestock. Its energy value is generally used as a standard of comparison for others.

Cassava constitutes a major staple food which is widely cultivated in the lowland humid tropics. Hence, its use as a replacement for maize in animal feed has been advocated. However, it contains a cyanogenic glucoside, linamarin, which is toxic to animals. Studies with poultry and pigs have shown that performance declines progressively as the amount of cassava root meal is increased in the rations, unless such diets are supplemented with additional protein, methionine, lysine and oils (Job *et al.* 1980; Tewe 1982. Tewe (1981) showed that replacement of 30% of maize with sundried cassava peel does not reduce egg production.

The cultivation and use of sweet potato as a replacement for maize in livestock rations has also been advocated in the tropics because there

is less competition for this tuber than cassava. Sweet potato is a good source of carbohydrate for livestock, being highly digestible and soluble (Oyenuga and Fetuga 1975). However, it contains some anti-nutritional factors (tannins, phytins, oxalates, etc) which may affect nutrient utilization. Its use in poultry rations has shown that performance is depressed at levels of inclusion above 10% for broilers and 30% for growers (Job *et al.* 1978; Tewe 1984).

This study was carried out to assess the relative feeding values of cassava and sweet potato meal as a replacement for maize in the diet of layer birds.

MATERIALS AND METHODS

Diets

The proximate composition and gross energy of feed ingredients are presented in Table 1 while Table 2 shows the composition and chemical analysis of the diets. Maize in diet 1 was replaced by sundried sweet potato meal and cassava meal in diets 2 and 3, respectively. The unpeeled cassava and sweet potato tubers were chopped and sundried for five days in the dry month of January before grinding.

Animals

A total of 360 36-week-old Isa Brown birds was allotted to three experimental diets. They were housed in pairs in laying cages in an open-sided poultry house and fed the experimental diets shown in Table 2. There were three treatment, each with three replicates of forty birds. The experimental diets and water were supplied *ad libitum* during the trial period of 12 weeks. Data were collected on daily feed intake and egg production, while egg weight measured twice each week was calculated individually to the nearest 0.01 g using a sensitive electronic balance.

Individual eggs were broken on a flat glass plate. Height of the thick albumen was measured, to the nearest 0.01 mm, using a tripod micrometer. Haugh units of individual eggs were later calculated using the formula of Haugh. Shell thickness was measured at the blunt and pointed ends as well as the middle using a micrometer screw gauge. Mean of the three values was taken as shell thickness per egg. Feed efficiency was measured in terms of kg feed/dozen eggs or kg feed/kg egg while the egg size was estimated by the oblong and horizontal circumferences. The circumference was measured using a thin thread and thereafter measuring such lengths along a graduated ruler in centimetres.

A nutrient retention trial was carried out after the birds had been on the diet for two weeks. Hence the two weeks formed the preliminary adjustment period prior to fecal collection. Weighed quantities of feed were supplied and excreta samples collected in weighed aluminium foil spread under the cages during a 72-hour period, using the total collection procedure. Excreta samples were oven dried at 70°C, weighed and ground prior to chemical analysis.

Chemical Analysis

Ingredients, feed and excreta samples were subjected to chemical analysis using the methods of AOAC (1990). Nitrogen was determined using the Kjeldahl procedure while fat was determined by petroleum ether (bp 40-60°C) extraction in a soxhlet apparatus. Gross energy values were determined using the ballistic bomb calorimetre while the hydrocyamic acid contents of the unpeeled cassava were determined using the modification of an earlier established method (Tewe 1975).

TABLE 1
Chemical composition of feed ingredients (%)

Ingredients	DM	CP	Ash	EE	CF	NFE	ME Kcal/g
Maize	91.80	7.40	1.85	4.41	1.32	85.03	3.40
Sweet potato meal	90.86	5.54	7.33	2.31	3.82	81.00	3.20
Cassava meal	90.00	3.50	3.80	0.80	4.20	87.70	3.10
Fish meal	93.45	61.18	2.74	19.34	0.35	15.89	2.80
Groundnut meal	91.85	42.20	6.69	12.00	4.76	31.35	2.50
Dried brewer's grains	92.00	20.00	4.20	6.00	18.00	51.80	2.00

DM = dry matter; CP = crude protein; CF = crude fibre; EE = ether extract; ME = metabolizable energy

TABLE 2
Composition of experimental diets (%)

Ingredients	Diets		
	1	2	3
Maize	52.3	-	-
Dried cassava meal	-	-	52.3
Dried sweet potato meal	-	52.3	-
Groundnut meal	17.5	17.5	17.5
Fishmeal	5.0	5.0	5.0
Dried brewer's grains	15.1	15.1	15.1
Oyster shell	7.2	7.2	7.2
Bonemeal	2.4	2.4	2.4
Salt	0.25	0.25	0.25
Min-Vit premix*	0.25	0.25	0.25
Total	100.00	100.00	100.00
Chemical analysis (dry matter) basis			
Crude protein	17.86	16.85	15.85
Crude fibre	4.50	5.72	5.98
Ether extract	6.33	5.25	4.11
Calculated			
ME (Kcal/g)	2.66	2.55	2.50
ME/CP ratio	148.94	151.34	157.73

* Supplied per kg diet: Vit. A, 15,000 i.u.; Vit D_3, 2,000 i.u; Vit. E, 25 mg; riboflavin, 3 mg; Vit B_{12}, 0.01 mg; Vit. K, 2.0 mg; niacin, 20 mg; choline chloride, 500 mg; folic acid, 0.25 mg; Co, 0.25 mg; I, 1.0 mg; Cu, 1.0 mg; Fe, 10 mg; Zn, 30 mg; Mn, 50 mg

Statistical Analysis

Data collected were subjected to analysis of variance using the methods of Steel and Torrie (1980). Where significant differences were observed, treatment means were further subjected to Duncan's multiple range test (Duncan 1955).

RESULTS AND DISCUSSION

Performance and egg quality characteristics of the birds are summarized in Table 3. Hen-day egg production was significantly ($P<0.05$) depressed by cassava meal while birds fed diets with maize and sweet potato meal were not significantly ($P<0.05$) different from each other in hen-day egg production. This reflects more on the composition of the diets in terms of adequacy of dietary crude protein and metabolizable energy. As earlier reported for pigs by Tewe and Maner (1980), it appears in this study that dietary crude protein and amino acid balance, but not cyanide, were the major factors of concern in the feed intake and thereby affecting the egg production. The value of total hydrocyanic acid content of the cassava diet was 10.24 ppm while Tewe *et al.* (1987) showed that dietary cyanide up to 117.3 ppm did not play any appreciable role in performance and carcass traits of pigs. The results of this present study are also in line with the findings of Job *et al* (1978; 1980) and Tewe (1984).

Egg size, measured by oblong and horizontal circumferences, was not significant ($P<0.05$). Egg weight, Haugh unit, kg feed/dozen eggs, kg feed/kg egg, energy intake, protein intake and shell thickness were not significantly ($P<0.05$) influenced by the dietary treatment although birds on the maize diet had higher protein and energy intake.

The egg weight and shell thickness fell within the range reported by Oluyemi and Roberts (1979). Efficiency of conversion of feed to eggs expressed either in kg of feed per dozen eggs or ratio of kg of feed to kg of egg was not significantly different ($P<0.05$) in the three diets.

The better digestibility of the maize diet may be due to the differences in crude fibre of the diets. As fibre increases, the content of starch and other readily available carbohdyrates

TABLE 3
Performance characteristics of layers fed maize, cassava or sweet potato-based diets

Parameters	1	2	3	SEM[1]
Average hen-day production (%)	88.61[a]	80.27[ab]	75.71[b]	0.94
Feed intake/bird/day (gm)	110.0	108.0	108.4	1.90
ME intake/bird/day (Kcal/g)	292.6[a]	277.95[b]	273.5[b]	0.64
Crude protein intake/ bird/day (gm)	19.65[a]	18.20[ab]	17.18b	0.80
Feed/dozen eggs (kg)	2.10	2.15	2.25	0.04
Feed/kg egg (kg)	1.84	1.94	1.85	0.06
Average egg oblong circumference (cm)	15.98	16.12	16.25	0.33
Average egg horizontol circumference (cm)	13.72	13.90	13.27	0.29
Average egg weight (gm)	58.95	56.16	59.18	0.24
Average shell thickness (mm)	0.35	0.35	0.35	0.34
Haugh unit	71.1	71.3	70.2	0.33
DM digestibility(%)	73.1	72.3	70.9	0.54
Energy digestibility (%)	80.5	78.1	74.1[b]	0.05
Crude protein digestibility	81.6	76.4[b]	73.5[b]	0.33

[1]SEM = Standard error of mean
[ab] Means in the same row with the same superscripts are not significantly different (P<0.05)

decreases (Tewe 1988). In addition, cassava and sweet potato both contain antinutritional factors which may affect digestibility. The digestibility of protein has been shown to be adversely affected by the presence of crude fibre (Sauer *et al.* 1980; Clandinin *et al.* 1981) and tannin (Clandinin and Heard 1968). Also, the physical nature of sweet potato and cassava meals, being rather powdery, might have affected their utilization (Fashina-Bombata and Fanimo 1994). Efforts should therefore be made to improve the physical nature of sweet potato and cassava meal to enhance intake and utilization.

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Sheep–Oil Palm Integration: Growth Performance of Dorset x Malin and Dorset x Siamese Long Tail Sheep

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ABSTRAK

*Kajian ini melibatkan integrasi bebiri dengan peladangan kelapa sawit. Penilaian kadar pertumbuhan bebiri jenis 25% Dorset x 75% Malin (DMalin) dan 25% Dorset x 75% Siamese Long Tail (DSLT) dipelihara di dalam ladang kelapa sawit berumur 9, 13, 16 dan 21 tahun telah dijalankan. Ukuran-ukuran telah diambil secara rawak dari kumpulan bebiri pada umur lahir 2, 4, 6, 8 dan 10 bulan. Pertalian antara berat badan dan umur ditentukan oleh model Brody. Model pertumbuhan bebiri adalah 1) BW = 17.9715 (1-0.9113 exp (0.0049*AGE)) untuk DMalin jantan, 2) BW = 17.7792 (1-0.9230 exp(-0.005*AGE)) untuk DMalin betina, 3) BW = 21 (1-0.8778 exp(-0.0049*AGE)) untuk DSLT jantan dan 4) BW = 18.7301 (1-0.8613 exp(-0.0059*AGE)) untuk DSLT betina. Kenaikan berat harian bebiri DMalin dan DSLT dari umur lahir ke 10 bulan ialah 41.0 dan 54.1 gram/ekor/hari, berturutan. Bebiri jantan adalah lebih berat sedikit daripada bebiri betina. Kenaikan berat harian bebiri adalah berhubungkait dengan masa ragutan yang kurang dan kualiti rumpai yang rendah didalam ladang kelapa sawit tua.*

ABSTRACT

*This study involved the integration of sheep into oil palm plantations. The growth rates of 25% Dorset x 75% Malin (DMalin) and 25% Dorset x 75% Siamese Long Tail (DSLT) sheep raised in 9, 13, 16 and 21 year-old oil palm plantations were evaluated. The measurements were taken by randomly sampling from the flock at birth, 2, 4, 6, 8 and 10 months of age. The relationship between body weight and age was determined by Brody's model. Consequently, the growth models of sheep were 1) BW = 17.9715 (1-0.9113 exp(-0.0049*AGE)) for DMalin male, 2) BW = 17.7792 (1-0.9230 exp(-0.005*AGE)) for DMalin female, 3) BW = 21 (1-0.8778 exp(-0.0049*AGE)) for DSLT male and 4) BW = 18.7301 (1-0.8613 exp(-0.0059*AGE)) for DSLT female. The average daily gain of DMalin and DSLT sheep from birth to 10 months of age was 41.0 and 54.1 gm/head/day, respectively. The males were slightly heavier than the females. Daily weight gains of sheep were related to the limited grazing period and low quantity of herbage available in old oil palm plantations.*

INTRODUCTION

Sheep production under oil palm is a viable venture in Malaysia (Chen *et al.* 1996). This production system is popular because of its symbiotic nature. The integrated system has two main objectives, namely, to convert the unwanted herbage under the oil palm canopy into a useful feed resource (Pillai *et al.* 1985), and to

utilize free space for animal production (Chen and Dahlan 1996). The breeds of sheep such as Malin (Malaysia indigenous breed), Siamese Long Tail and its crosses with the Dorset used for this integrated system have performed well in the Malaysian environment and management systems (Tajuddin and Chong 1988; Rajion *et al.* 1993). However, information on the productivity of an integrated sheep-oil palm production system is very limited and is mostly related to fertility, mortality and management problems. Recently, the interest of plantation owners towards sheep-oil palm integration is declining because of the poor performance of the animals in the plantation and inadequate information on the relevant technology in livestock production (Chen *et al.* 1996). More information on this system is needed through proper data collection and field experiments.

This study was conducted to evaluate the growth performance of 25% Dorset x 75% Malin (DMalin) and 25% Dorset x 75% Siamese Long Tail (DSLT) sheep integrated with oil palm.

MATERIALS AND METHODS

Animals and Management

The research was conducted at Sungai Seraya Plantation belonging to Far East Holdings Bhd at Keratong, Pahang. The size of the plantation was 376 ha and the ages of oil palms were 9, 13, 16 and 21 years. The plantation raised 1,460 head of sheep. Lambs were kept in the sheds and fed with natural herbage *ad libitum* through a cut and carry system supplemented with commercial pellets containing 16.7% crude protein, 7.5% ash, 6.9% crude fibre, and 6.5% ether extract at 80 gm/head/day from 3 weeks of age until weaning at four months of age. After weaning, the lambs were divided into two groups according to sex. They were kept in the sheds in groups of 35-45 until six months of age when they were allowed to graze under oil palms from 0900 to 1400 hours without supplementation. The grazing area was rotated every day.

Data Collection

Measurements of body weight related to age were randomly sampled from 220 DMalin and 275 DSLT sheep. The relationship between weight and age was determined using the model of Brody (Brown *et al.* 1976). The following model was used; body weight $(BW) = AWT * (1 - CON * exp(-MR * AGE))$; where AWT = the asymptotic weight, CON = constant, MR = rate of maturity, and AGE = age of sheep (days).

Herbage was sampled from experimental plots measuring 3.5 x 3.5 m under 9-year-old oil palm canopy and from experimental plots measuring 4 x 4 m under 16-21 year-old oil palm canopy. Herbage was cut about 1.5 inches above the ground at monthly intervals. The samples were separated into 2 groups to analyse for dry matter yield and chemical composition (AOAC 1984).

Statistical Analysis

The relationship between body weight and age was determined by a non-linear procedure. Statistical analysis was performed according to SAS (1988).

RESULTS AND DISCUSSION

Nutritive Value of Herbage Yield under Oil Palm

The herbage in the oil palms plantation was a mixture of mainly grasses, broad leaves, legumes and ferns (Dahlan *et al.* 1993; Chen *et al.* 1996). Table 1 shows the chemical composition of the herbage. The crude protein content of herbage under oil palm of 7.6-12.7%, and gross energy of 16.0-16.3 MJ/kg dry matter were similar to the report of Dahlan *et al.* (1993). The average dry matter (DM) yield of herbage under oil palm from 9-21 years was 74.8 kg/ha/month. This DM yield was higher than that of Dahlan *et al.* (1993) (76.1 vs. 66.7 kg/ha/month). Nevertheless, the herbage DM yield (DMHYA) decreased as the age of the oil palms increased. This corresponded with lower light penetration under the oil palm canopy (Dahlan *et al.* 1993; Chen *et al.* 1996). The average DM yield of herbage was 1.7-3.7 kg/ha/day (0.17–0.37g/m^2/day). The DM intake requirement for maintenance and growth by sheep in the tropics is about 74.9 g/head/day/kg $W^{0.75}$ (Kearl 1982). Thus, the estimated DM intake per W $kg^{0.75}$ of sheep aged 2-10 months in this present study was 256.3-545.0 g/head/day for DMalin and 359.6-662.7 g/head/day for DSLT, respectively. However, the available herbage in the old oil palm plantation cannot supply sufficient daily DM intake for the sheep.

Growth Performance

Table 2 shows the growth performance of DMalin and DSLT sheep from birth to 10 months of age. The DMalin grew significantly slower than

TABLE 1
Chemical composition of herbage under oil palm

Palm age	%	% of DM[1/]				GE[5/]	ME[6/]	DMYHA[7/]
(years)	DM	CP[2/]	ADF[3/]	CF[4/]	Ash	(MJ/kg)	(MJ/kg)	(kg/ha/mo)
9	18.9	12.7	44.3	24.9	12.7	16.0	8.9	110.9
13	19.8	10.7	45.7	26.9	12.5	16.2	8.0	75.4
16	22.8	9.2	48.4	23.2	10.7	16.1	6.4	62.2
21	25.0	7.6	46.5	28.4	11.7	16.3	6.0	50.8
Mean	21.6	10.1	46.2	25.9	11.9	16.2	7.3	74.8

1/ DM = dry matter; 2/ CP = crude protein; 3/ ADF = acid detergent fibre; 4/ CF = crude fibre; 5/ GE = gross energy; 6/ ME = metabolisable energy; DMYHA = DM yield per ha per month

TABLE 2
Growth performance of DMalin and DSLT sheep under oil palm

Items	Sex	DSLT (MeanSD)	n	DMalin (MeanSD)	n	Level of Significance
Birth wt. (kg)	M	2.1 ± 0.2[a]	45	1.7 ± 0.2[b]	30	0.01
	F	2.0 ± 0.3[a]	45	1.6 ± 0.3[b]	20	0.01
	MeanSE	2.1 ± 0.1		1.7 ± 0.1		
Wt. at 60 days (kg)	M	8.1 ± 0.5[a]	20	5.4 ± 0.6[b]	20	0.01
	F	8.0 ± 0.4[a]	20	4.9 ± 0.5[b]	20	0.01
	MeanSE	8.1 ± 0.1		5.1 ± 0.3		
Weaning wt (120 days) (kg)	M	10.8 ± 1.1[a]	20	8.9 ± 1.9[b]	20	0.05
	F	9.0 ± 0.5	20	8.6 ± 1.1	20	NS
	MeanSE	10.3 ± 0.7		8.8 ± 0.2		
Wt. at 180 days (kg)	M	13.1 ± 0.3[a]	20	11.9 ± 0.4[b]	20	0.05
	F	12.7 ± 0.5[a]	20	11.3 ± 0.4[b]	20	0.05
	MeanSE	13.1 ± 0.7		11.6 ± 0.4		
Wt. at 240 days (kg)	M	15.4 ± 0.4[a]	20	13.0 ± 0.6[b]	15	0.01
	F	14.7 ± 0.6[a]	20	13.0 ± 0.9[b]	15	0.05
	MeanSE	15.0 ± 0.6		13.0 ± 0.0		
Wt. at 300 days (kg)	M	18.9 ± 0.4[a]	10	14.7 ± 0.4[b]	10	0.01
	F	17.6 ± 0.5[a]	15	13.5 ± 1.0[b]	10	0.01
	MeanSE	18.3 ± 0.9		14.1 ± 0.8		
ADG, gm/day						
– At weaning	M	72.5[a]		57.1[b]		0.01
(birth-4M)	F	58.3		60.5		NS
	MeanSD	65.4 ±10.0		58.8 ± 2.4		
– After birth	M	56.2[a]		43.3[b]		0.05
to 10 M.	F	51.9[a]		39.5[b]		0.01
	MeanSD	54.1 ± 3.0		41.4 ± 2.7		

Means within column with different superscripts differ significantly

DSLT in both sexes (P<0.01). The birth weight of the DMalin lamb was lighter than the weight of DSLT (1.7 vs. 2.1 kg, respectively). The weaning weight of DMalin was lower than DSLT lambs (8.8 vs. 10.3 kg, respectively). The ADG from birth until 10 months of age of the DMalin and the DSLT male was 42.4 and 56.2 gm/day, respectively, while the female was 39.5 and 52.0 gm/head/day, respectively. In both breeds, the males tended to grow faster than the females.

The performance data of DMalin from this study were similar to those of Devendra (1975), Tajuddin and Chong (1988) and Khusahry and Gayah (1991) although the management practices were different. The growth pattern of the DSLT was similar to the report of Schrader

TABLE 3
Comparison of actual weight and estimated weight (kg) of sheep under oil palm

Age	DSLT, male		DSLT, female		DMalin, male		DMalin, female	
(days)	Actual	Estimate	Actual	Estimate	Actual	Estimate	Actual	Estimate
Birth	2.1	2.7	2.0	2.7	1.8	1.7	1.6	1.5
60	8.1	7.5	8.0	7.4	5.4	5.8	4.9	5.6
120	10.8	11.1	9.0	10.8	8.9	8.9	8.6	8.8
180	13.1	13.8	12.7	13.2	11.9	11.2	11.3	11.1
240	15.4	15.8	14.7	14.8	12.9	12.9	13.0	12.8
300	18.9	17.3	17.6	16.0	14.5	14.2	13.5	14.1

(1994) who observed that the SLT sheep grazing under oil palm with no supplements grew about 52.9 gm/day. However, Ramakrishnam *et al.* (1992) reported that the growth rate from birth until 12 months of male SLT sheep months grazing on native grasses and weeds under fruit trees from 0900 to 1700 hours each day was 82.2 gm/head/day.

Genetically, the Siamese Long Tail sheep is bigger than the Malin. When crossed with the Dorset, the DSLT grew faster than the DMalin sheep. The inferior growth performance of the DMalin in this study was similar to that in studies of Wan Mohamad (1977) and Davis *et al.* (1993). Thus, under the integrated system, the DSLT sheep were 29.8% heavier than the DMalin at 10 months of age.

The poor growth performance of both crossbreeds was probably due to insufficient feed from the plantation. During the pre-weaning stage (3-16 weeks of age) when supplementation was given, the animals showed faster growth rates (58.8 and 65.4 gm/head/day for DMalin and DSLT, respectively). These results were similar to those of Batubara *et al.* (1996) who reported that the ADG of North Sumatra sheep under oil palm was 45.3 gm/head/day. However, the results of the present study contrasted with the report of Rajion *et al.* (1994) who showed that Wiltshire x Malin sheep showed good performance when grazing for 7 hours under oil palm due to the availability of preferred digestible forage of high nutritive value.

Table 3 shows the actual weight per age of sheep compared with the estimated weight from Brody's model. The relationship between body weight and age of sheep in this study determined by the model of Brody were 1) DMalin male, $BW = 17.9715\ (1\text{-}0.9113\ exp(\text{-}0.0049*AGE))$, 2) DMalin female, $BW = 17.7792\ (1\text{-}0.9230\ exp(\text{-}0.005*AGE))$, 3) DSLT male, $BW = 21.6869\ (1\text{-}0.8778\ exp\ (\text{-}0.0049*AGE))$, and 4) DSLT female, $BW = 18.7301\ (1\text{-}0.8613\ exp\ (\text{-}0.0059*AGE))$. The asymptotic weight (*AWT*) of all breeds was higher than the actual weight at 10 months of age. The *AWT* of males was higher than that of females while DMalin was lower than the DSLT sheep. The study indicated that the maturity rates (*MR*) of both crossbreeds were low, showing the late maturing of the sheep. *MR* was 0.0049-0.005 for DMalin and 0.0049-0.0059 for DSLT. The females showed slightly higher *MR* than the males but not significantly different ($P>0.05$). However, the models derived from this study are applicable to sheep from birth until 300 days of age.

CONCLUSION

The growth rates of DMalin and DSLT sheep at Sungai Seraya plantation were low due to the lack of quality feed and a limited grazing period. The inferiority of genes was also a factor to consider. In order to improve the growth performance, supplementation is needed to provide sufficient nutrients for maintenance and production. The results of this study indicated that the grazing period should be increased. The model of Brody can be used to estimate the growth of sheep. However, the constant values in Brody's equations will change according to the breed type and feed supply.

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