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Ammonium (NH_4^+): Nitrate (NO_3^-) Ratio and its Relation to the Changes in Solution pH, Growth, Mineral Nutrition and Yield of Tomatoes Grown in Nutrient Film Technique

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Keywords: NH_4^+: NO_3^- ratio: growth, pH, water uptake, yield, blossom-end rot, mineral nutrition

ABSTRAK

Pengaruh nisbah NH_4^+:NO_3^- terhadap pertumbuhan, pengambilan air, pH larutan, pemakanan tanaman dan hasil tanaman tomato telah dikaji menggunakan teknik nutrien cetek. Enam perlakuan rawatan nisbah NH_4^+:NO_3^- telah diberikan kepada tanaman: 0:100 dengan dan tanpa kawalan pH; 12.5:87.5, 25:75, 37.5:62.5 dan 50:50 tanpa kawalan pH. Kajian mendapati pH didalam larutan meningkat apabila berada di dalam NH_4^+:NO_3^- dengan nisbah 0:100 dan 12.5:87.5, tetapi menurun pada nisbah 50:50. Nisbah NH_4^+:NO_3^- yang tinggi mengurangkan pertumbuhan daun dan akar tanaman. Pengurangan ini mungkin di sebabkan oleh pengurangan pengambilan air. Berat basah buah juga dikurangkan dan peratus kejadian reput hujung buah meningkat apabila tanaman didedahkan pada nisbah NH_4^+:NO_3^- yang tinggi. Nisbah NH_4^+:NO_3^- yang tinggi meningkatkan kandungan N dan mengurangkan kandungan Ca dalam bahagian tisu tanaman. Kandungan P, K dan Mg menurun didalam tisu daun dengan peningkatan nisbah NH_4^+: NO_3^-.

ABSTRACT

The effects of NH_4^+:NO_3^- ratio on growth, water uptake, solution pH, mineral nutrition and yield of tomatoes were investigated using the nutrient film technique. There were six NH_4^+:NO_3^- ratios: 0:100 with and without pH control; 12.5:87.5, 25:75, 37.5:62.5 and 50:50 without pH control. There was an increase in pH of the nutrient solution with 0:100 and 12.5:87.5 ratios, but the pH decreased with 50:50 ratio. Higher NH_4^+:NO_3^- ratio reduced leaf and root growth. The reduction in leaf and root growth could be attributed to reduction of plant water uptake. Fruit fresh weight was reduced and the percentage of blossom-end rot increased with higher ratio of NH_4^+:NO_3^- in the solution. Increased ratio of NH_4^+:NO_3^- increased N content and decreased Ca content in all the plant tissues. P, K and Mg content decreased in leaf tissue with increasing NH_4^+:NO_3^- ratio.

INTRODUCTION

Cultivation of tomatoes by conventional methods on soil in lowland areas of Malaysia is limited due to the unfavourable weather and occurrence of soil-borne pathogens. To overcome these limitations, the technology of soilless crop production has been developed and has proved advantageous (Lim 1985). Nutrient film technique (NFT) is one of the soilless culture systems used for cultivation of horticultural crops.

One factor that may contribute to the effectiveness of mineral nutrition in NFT system is the form of nitrogen added to the nutrient formulation. In common with conventional methods, nitrogen is supplied to the plants in the form of ammonium ion (NH_4^+) and/or nitrate ion (NO_3^-). Cooper (1979), Iwata (1983) and Ikeda and Yamada (1984) reported that growth and plant development are enhanced by the use of NO_3^- in the fertilizer formulation. However, there are also reports indicating better

growth if both NH_4^+ and NO_3^- are used as the N source (Cox and Reisenauer 1973; Follett and Doaglas 1987). Costellane *et al.* (1987) reported maximum growth of tomatoes when 25% NH_4^+ was used in the liquid feed. Similarly, Monnerat *et al.* (1982) reported that NH_4^+:NO_3^- ratio of 60:40 resulted in increased dry weight accumulation. NH_4^+ salts are cheaper sources of nitrogen than NO_3^- salts. Furthermore, uptake of NH_4^+ is usually coupled with H^+ enrichment in the nutrient solution which consequently minimises the rise in solution pH.

This study was undertaken to investigate growth, water uptake, changes in solution pH, mineral nutrition and yield of tomatoes grown in NFT-trough system.

MATERIALS AND METHODS

The experiment was conducted at the Hydroponic Unit, Universiti Pertanian Malaysia. The plants were grown under glasshouse conditions with air temperatures ranging from 27-35°C and relative humidity of 65-80%.

Four-week-old uniform-sized tomato (*Lycopersicon esculentum* Mill) plants var. Sweet Chelsea were transplanted into the NFT-trough system. The plants were grown in the recirculating water for one week and then subjected to treatments of six NH_4^+:NO_3^- treatments (Table 1)

The NH_4^+:NO_3^- ratio was calculated based on the concentration of salts used in the nutrient formulation. Cooper formulation full strength solution (Cooper 1979) was modified so that the desired NH_4^+:NO_3^- ratio was achieved while the N level was maintained. Chloride and sulphate salts were used to replace specific cations and anions and to maintain a constant solution conductivity. The treatments were arranged in a randomized design with four replicates. Seven plants spaced 40 cm apart in a trough represented a replicate. Each trough had its own catchment tank supplying nutrient solution to the plants (Jarret and Charter 1981). Troughs were spaced 55 cm apart.

As the relative concentration of salts was not continuously monitored, the nutrient solution in the catchment tank was replenished fortnightly. When new nutrient solution was prepared, pH was adjusted to 6.0 using sulphuric acid, after which it was not readjusted. Changes in pH were monitored daily.

After 14 weeks, plants were sampled for leaf, root and stem dry weight by oven drying at 80°C for 48 hours. Leaf area was determined using an automatic leaf area meter (Delta-T Devices). Leaf area index (LAI) was recorded in week 7 using a 'Plant Canopy Analyser' (LiCor 2000).

Plant water uptake was recorded over 24 hours by measuring water loss from the catchment tank. Accumulation of radiant energy was also recorded concurrently with water loss from the catchment tank using solarimeters attached to a microvolt integrator (MV2, Delta-T Devices).

Flower number was recorded to determine fruit set in the various treatments. Fruits were harvested at the orange to red stage and the number of fruits and their fresh weight were recorded. Fruit physical characters were also recorded. Fruit diameter was recorded using a vernier caliper. Total soluble solids were determined from 2-3 drops of expressed fruit juice using a hand refractometer. Fruit dry weight was determined after 72 hours oven drying at 80°C.

Nutrient analysis was performed on dry samples of plant parts according to the standard procedure described by Mohd. Haniff *et al.* (1990). Plant parts were sampled in week 5 and 12 for mineral nutrition determination. Total N, P and K were determined using an autoanalyser

TABLE 1
NH_4^+: NO_3^- ratios of fertiliser used in nutrient film technique experiments on tomato

Treatment	NH_4^+	NO_3^-	pH
T1	0	100.0	maintained at 6.0
T2	12.5	87.5	not controlled
T3	25.0	75.0	not controlled
T4	37.5	62.5	not controlled
T5	50.0	50.0	not controlled
T6	0	100.0	not controlled

(Technicon Auto Analyser). Ca and Mg were determined using an atomic absorption spectrophotometer.

RESULTS AND DISCUSSION

pH of Nutrient Solution

The pH fluctuated within a narrow range in weeks 3 and 4 (*Fig. 1a*). At this stage, plants were probably capable of absorbing the nutrients actively, which would result in less imbalance of nutrients in the catchment tank. By weeks 5 and 6, pH of 100% NO_3^- in T6, showed a marked increase (*Fig. 1b*). On the other hand, nutrient solution containing NH_4^+ ratio of more than 37.5% resulted in a decline in the pH level, but did not fall below pH 5.5. In weeks 7 and 8, the pH for T6 exceeded 7.3 (*Fig. 1c*). In contrast, when the proportion of NH_4^+ was 50% (T5) the pH in the nutrient solution did fall below 5.5. The changes in pH determined in weeks 10 and 11 followed a similar trend as weeks 5 and 6 (*Fig. 1d*). The higher pH values obtained with higher proportions of NO_3^- agree with those observed by Ikeda and Osawa (1981). In contrast, higher proportions of NH_4^+ (T4, T5) resulted in decreased pH in the nutrient solution, which is attributable to acidification of the nutrient solution due to the release of H^+ in the active transport of nutrients, a phenomenon reported by other investigators (Maynard and Barker 1969; Qasem and Hill 1993).

Plant Vegetative Growth

Table 1 illustrates leaf, stem and root growth as influenced by different NH_4^+:NO_3^- ratios. Leaf area and dry weight were significantly reduced with NH_4^+ higher than 37.5% in the nutrient solution. For leaf area, increasing the proportion of NH_4^+ to 37.5 and 50% caused a 17% and 20% reduction in leaf area compared with the 100% NO_3^- treatment.

Treatments with higher proportions of NH_4^+ reduced plant dry weight, the reduction being greatest in the roots, followed by stems and leaves. The NH_4^+ ions hasten breakdown of carbohydrates (Barker *et al.* 1965), uncouple photosynthetic phosphorylation (Gibbs and Colo 1959) and play a significant role in the disruption of chloroplast membrane (Purich and Barker 1967).

The present study did not attempt to confirm the above-mentioned role of NH_4^+, but it provided evidence that there may be a possible effect on plant-water relations which caused reductions in leaf area and dry weight. *Fig. 2* illustrates the of influence NH_4^+:NO_3^- ratio on plant water uptake. Water uptake was reduced with higher proportions of NH_4^+; the effect was particularly obvious with increasing irradiance. The role of water relations in influencing growth when plants are subjected to increasing NH_4^+ in the nutrient solution agrees with reports by Pill and Lambeth (1977) and Pill *et al.* (1978). Quebedeaux and Ozbun (1973) suggested NH_4^+ N alters the physiological mechanisms involved in uptake and movement of water. The inhibitory effect of NH_4^+ on water uptake may involve two mechanisms: NH_4^+ may directly interfere with water uptake, and NH_4^+ may cause an anatomical and physiological change requiring a longer period for recovery.

Yield

The effect of NH_4^+:NO_3^- ratios on fruit fresh weight is consistent with fruit yield being reduced as NH_4^+ ratio increases. Increasing the proportion of NH_4^+ to 25, 37.5 and 50% resulted in reductions in fruit fresh weight compared with 100% NO_3^- (Table 2). This reduction in fruit fresh weight may result from reduced assimilate being translocated due to reduced leaf area when the proportion of NH_4^+ is higher. Increasing the proportion of NH_4^+ to more than 25% significantly increased the percentage of fruits with blossom-end rot (BER) (Table 3). It is well known that this disorder in tomatoes is associated with reduced Ca^{++} translocation to the growing region of the fruit (Cerda *et al.* 1979; Ehret and Ho 1986). Moreover, the partitioning of Ca^{++} concentration in different regions of leaves and fruit shows a clear involvement of NH_4^+ in suppressing the translocation of Ca^{++} to growing region (*Fig. 3*). Ca^{++} concentration in the root did not differ indicating that Ca^{++} uptake at the root surface was not inhibited by the presence of NH_4^+ at early stages of plant growth, but deficiency in Ca^{++} may arise from translocation to the actively growing regions. The NH_4^+:NO_3^- ratio did not produce an appreciable effect on fruit size, total soluble solids and percentage of fruit dry matter.

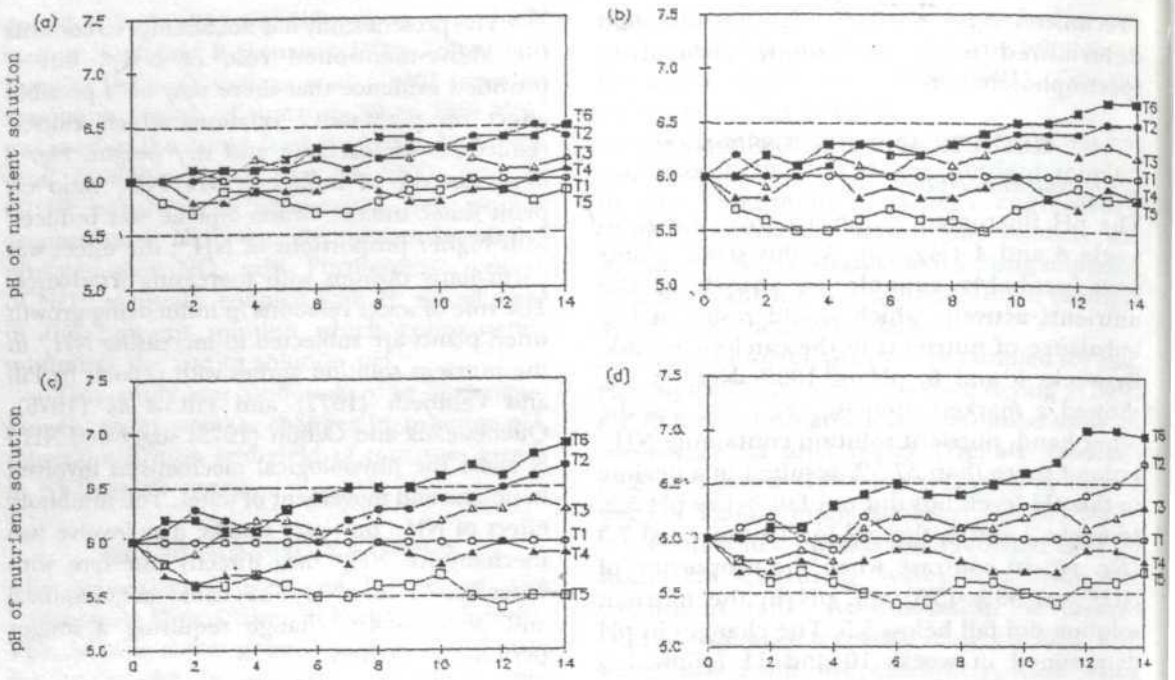

Fig. 1: Changes of pH in the nutrient solution influenced by NH_4^+:NO_3^- ratio at various durations a) weeks 3-4 b) weeks 5-6 c) weeks 7-8 d) weeks 9-10

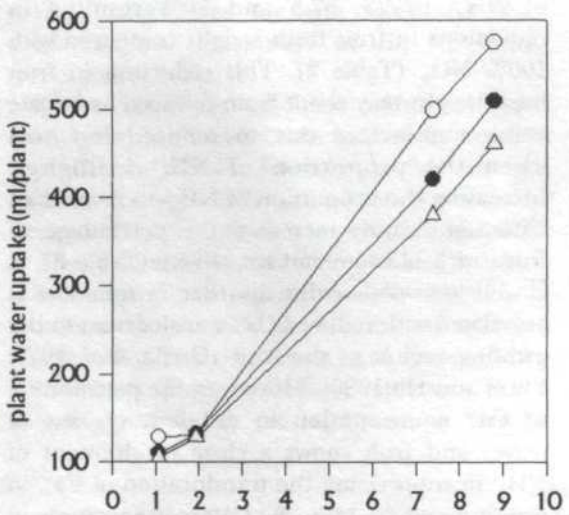

Fig. 2: Plant water uptake (ml/plant) as influenced by accumulated radiant energy at different NH_4^+:NO_3^- ratios. O=0.100; •=25:75 and Δ= 50:50. Measurement of radiant energy was done conecurrently with the plant water uptake throughout the day therefore radiant energy is not replicated

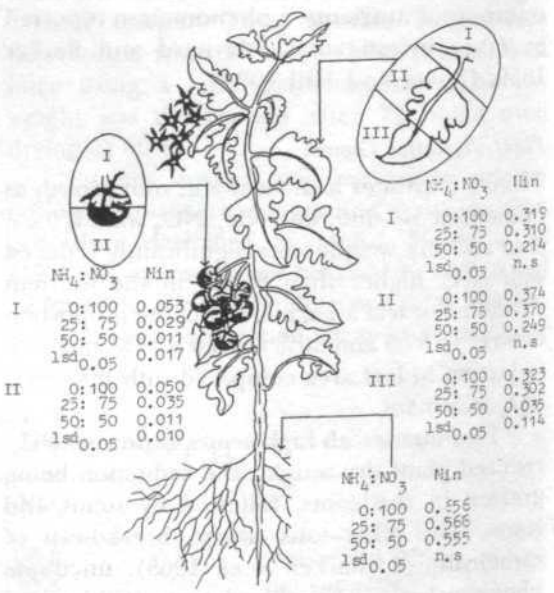

Fig. 3: Distribution of Ca in leaves, fruits and roots at week 5

TABLE 2
Leaf area, leaf, root and stem dry weight of tomato plants subjected to different NH_4^+:NO_3^- ratios. T1=0:100 (pH adjusted to 6.0), T2=12.5:87.5, T3=25:75, T4=37.5:62.5, T5=50:50, T6=0:100; T2-T6 (pH not adjusted)

Treatment	Leaf area (cm^2)	Dry weight (g/plant) Leaf	Stem	Root
T1	6309.30	40.2	32.6	21.7
T2	5933.00	38.8	31.1	20.0
T3	6061.30	38.6	28.9	20.1
T4	5252.50	36.6	29.3	18.5
T5	5110.30	38.1	29.7	20.6
$LSD_{0.05}$	761.20	1.9	ns	2.1

TABLE 3
Effects of NH_4^+:NO_3^- ratio in the nutrient solution on fresh weight production of tomatoes

Treatment	Flower number (unit)	Fruit number (unit)	Fresh weight (g/plant)	% of BER	Fruit diameter (mm)	% dry matter	Total soluble solids (%)
T1	62	36	915.55	0	35.27	5.51	4.90
T2	62	34	886.35	2.57	34.15	5.37	4.97
T3	64	34	688.67	17.97	34.27	5.53	5.00
T4	60	33	623.91	24.37	34.65	5.48	5.05
T5	62	34	533.72	37.75	34.70	5.42	5.07
T6	61	36	857.42	2.72	34.70	5.30	4.72
LSD0.05	ns	ns	118.12	4.35	ns	ns	ns

Means of 4 replication; NH_4^+:NO_3^- ratio of; T1 & T6=0:100 (with and without pH controlled); T2=12.5:87.5; T3=25:75, T4=37.5:62.5 and T5=50:50; T2-T5 (without pH controlled)

Mineral Nutrition

Fig. 4-8 illustrate the partitioning of total N, P, K, Ca and Mg in different parts of the plant. In week 5, N concentration in young leaves and fruits generally increased with the concentration of NH_4^+ in the solution (*Fig. 4*). Similarly, by week 12, increase in N ratio significantly increased N in all parts of the plant except in the stem. The percentage of P in the young leaves at both harvest dates increased with increase of NH_4^+ in the N ratio (*Fig. 5*).

Changes in the percentage of P are associated with the mechanism of active uptake where anions such as P are present in higher concentration when NH_4^+ is used as nitrogen source (Costellane *et al.* 1987). Similar mechanisms also apply when referring to K level (*Fig. 6*) in the plant parts where inorganic cations such as K+ is depressed with increasing NH_4^+ (Mengel and Kirkby 1982).

The effect of increasing the proportion of NH_4^+ on Ca^{++} at both harvest dates is illustrated in *Fig. 7*. Increasing the proportion of NH_4^+ to more than 35% significantly reduced the percentage of Ca^{++} in the young leaves at both harvest dates and in mature leaves, stems, roots and fruits at week 12. Pill *et al.* (1978) indicated that NH_4^+ uptake must be accompanied by either inorganic anion uptake and/or higher organic anion production, or reduced uptake or inorganic cations. Furthermore, higher soluble salt concentration of substrate under NH_4^+ nutrition may render divalent ions less available than monovalent ions. Our results showed that there were no significant differences (P>0.05) between treatments on Ca^{++} level in roots when

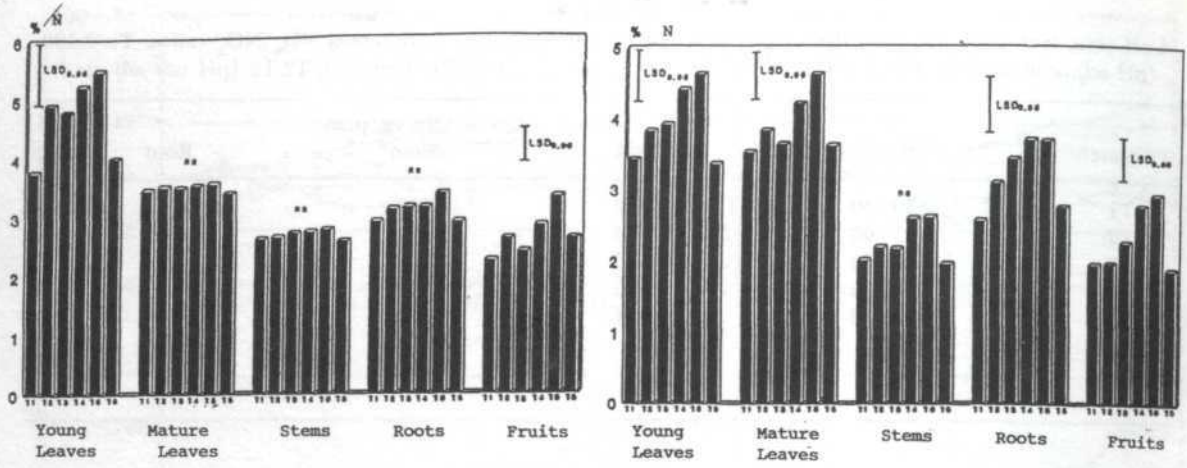

Fig. 4: Effects of NH_4^+:NO_3^- ratio on the N content in various parts of the plant a) week 5 b) week 12.

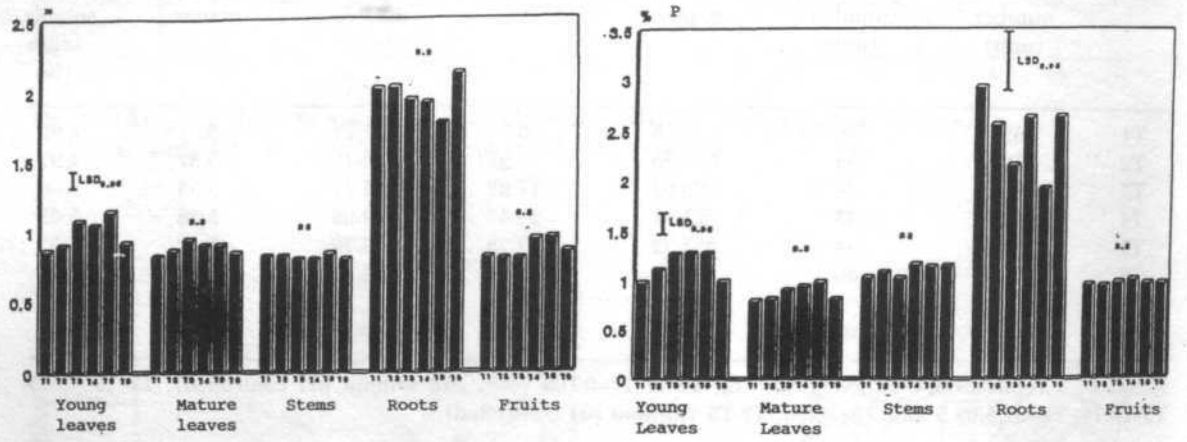

Fig. 5: Effects of NH_4^+:NO_3^- ratio on the P content in various parts of the plant a) week 5 b) week 12.

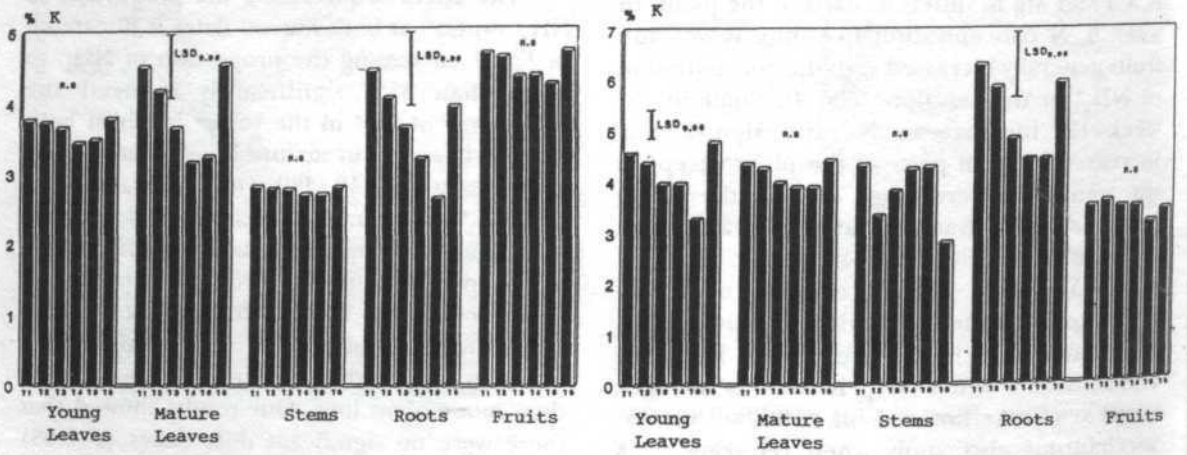

Fig. 6: Effects of NH_4^+:NO_3^- ratio on the K content in various parts of the plant a) week 5 b) week 12.

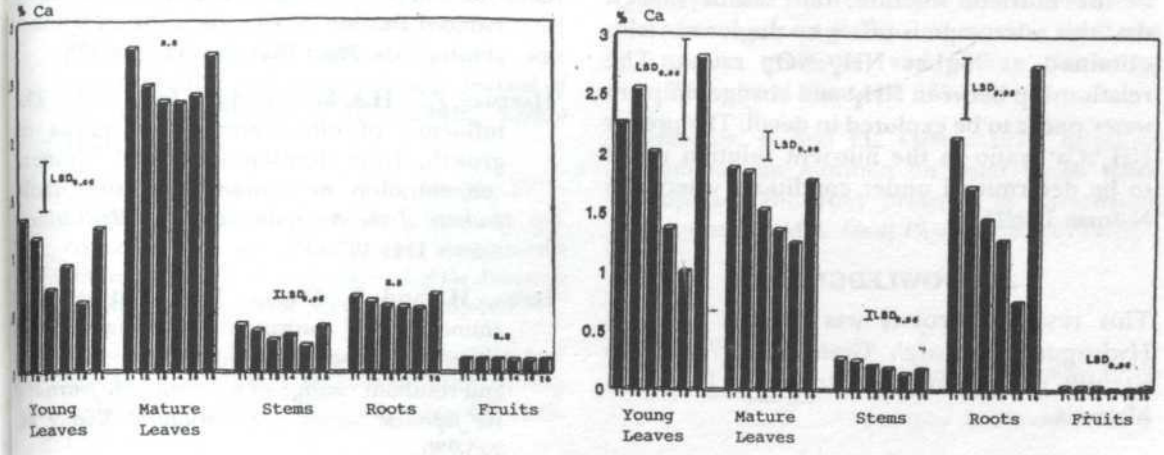

Fig. 7: Effects of NH_4^+:NO_3^- ratio on the Ca content in various parts of the plant a) week 5 b) week 12.

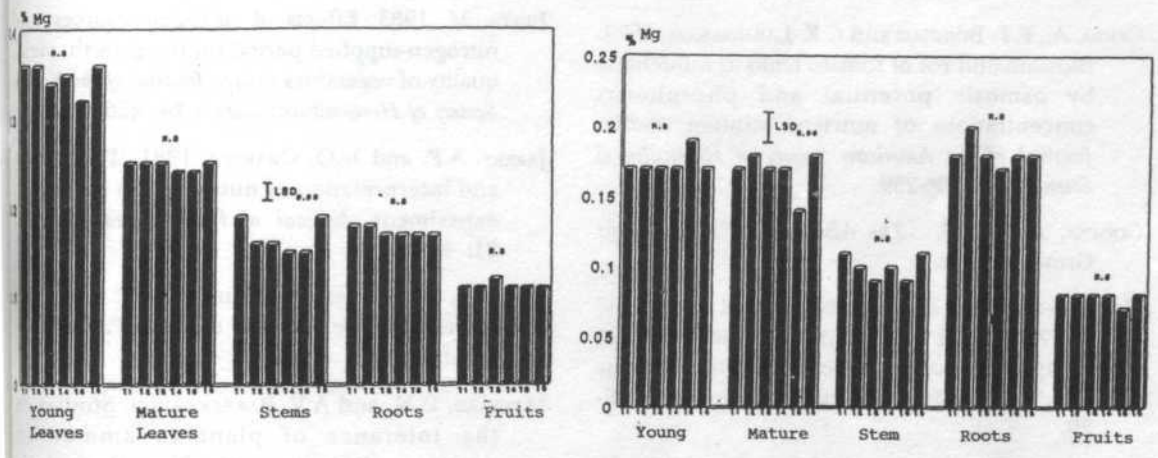

Fig. 8. Effects of NH_4^+:NO_3^- ratio on Mg content in various parts of the plant a) week 5 b) week 12

sampling was done in week 5. Evans and Troxler (1953) suggested that higher rates of organic acid synthesis as a result of NH_4^+ may immobilize Ca^{++} within the roots. However, sampling in week 12 saw Ca^{++} levels significantly reduced with increased proportion of NH_4^+.

The effect of the NH_4^+:NO_3^+ ratio on the percentage of Mg^{++} was not pronounced except for mature leaves when sampled in week 12 (*Fig. 8*). Leaf Mg^{++} decreased at the highest NH_4^+:NO_3^- ratios. The mechanism of this reduction may be similar to those of Ca and K.

CONCLUSION

The reduction in plant growth with increase in the NH_4^+:NO_3^- ratio could be due to the imbalance of nutrient uptake resulting from changes in the plant-water relationship. There was a clear increase in fruits with BER with increase of NH_4^+ in the nutrient solution. Since the occurrence of BER is related to Ca^{++} concentration, it may be necessary to increase application of Ca when fertilizer containing a high proportion of NH_4^+ is used in order to reduce the severity of NH_4^+ related BER. Although NH_4^+ is useful in controlling pH level

in the nutrient solution, our results suggest that this advantage is offset by the lower yields obtained at higher NH_4^+:NO_3^- ratios. The relationship between NH_4^+ and changes in plant water needs to be explored in detail. The proper NH_4^+:Ca^{++} ratio in the nutrient solution needs to be determined under conditions where the N form is NH_4^+.

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The Biology of the Mango Leafhopper, *Idioscopus nitidulus* in Malaysia

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Keywords: **mango leafhopper, *Idioscopus nitidulus*, biology, mango**

ABSTRAK

Eksperimen di ladang telah dijalankan untuk mengkaji biologi Idioscopus nitidulus *Walk. selepas letusan di utara Semenanjung Malaysia pada tahun 1986 dan 1987. Pada tangkai bunga jangkamasa perkembangan jantan ialah 13.77±0.25 hari dan betina 13.50±0.60 hari; tempoh inkubasi telur adalah 3.85±2.00 hari. Lelompat daun yang dibiak di atas tangkai bunga menghasilkan 277±110 biji telur dengan kadar penetasan telur 90.2±8.4%; manakala lelompat daun yang dibiak pada pucuk daun menghasilkan 149±57 biji telur dan kadar penetasan sebanyak 54.8±22.0%. Betina yang mengawan hanya sekali menghasilkan 176±72 biji telur, manakala betina yang mengawan beberapa kali menghasilkan 149±57 biji telur. Longeviti betina (69.8±9.8 hari) tidak berbeza dengan bererti daripada jantan (60.5±8.5 hari) pada pucuk daun mangga di ladang.*

ABSTRACT

Field experiments were conducted to study the biology of the mango leafhopper, Idioscopus nitidulus *Walk., following outbreaks in north Peninsular Malaysia in 1986 and 1987. The developmental period on inflorescense was 13.77±0.25 days for males and 13.50±0.60 days for females, and mean incubation period of eggs was 3.85±2.00 days. Hoppers reared on the inflorescence produced 277±110 eggs with a hatchability rate of 90.2±8.4%; those on shoots produced 149±57 eggs and had a hatchability rate of 54.8±22.0%. A female mating only once laid 176±72 eggs, whereas multiple mated females produced 149±57 eggs. On shoots in the field, the longevity of females (69.8±9.8 days) was not significantly different from that of males (60.5±8.5 days).*

INTRODUCTION

Mango, *Mangifera indica* L., in particular the variety Harumanis (MA 128) is grown extensively in north Peninsular Malaysia. Several important insect pests are associated with mango production in Malaysia (Khoo *et al.* 1991). The mango leafhopper, *Idioscopus nitidulus* Walk. (Homoptera: Cicadellidae) is an important pest of mangoes in Malaysia and Indonesia (Reddy 1975; Tandon and Varghese 1985). Although species of *Idioscopus* were recorded in Malaysia as early as 1924 (Gater 1924) little investigation has been carried out. These hoppers can pose a serious threat to the mango industry since their feeding activity can result in loss of flowers and reduce fruit set.

Little information is available on mango leafhoppers, particularly the species *I. nitidulus;* an exception is the species *I. clypealis* (Bato 1978; Corey 1986). Following outbreaks of *I. nitidulus* in 1986 and 1987, a study on the biology of this species was conducted.

MATERIALS AND METHODS

The study was conducted in a mango orchard growing the variety Harumanis in Perlis.

Twenty pairs of adult leafhoppers were collected at random from the mango orchard and

released into a cage which enclosed a one-week-old mango inflorescence on a mango tree; the cage, of dimensions 15 cm length by 18 cm diameter, was made from fine nylon mesh. Twenty pairs of leafhoppers were introduced into a similar cage enclosing a single mango shoot. The leafhoppers were allowed to lay their eggs for 12 h, after which they were removed. The development of the leafhoppers was monitored daily.

Larvae for morphological examination were fixed overnight in KAAD and AAD solutions (Peterson 1943). The preserved specimens were measured for width of head capsule, length of mouth sheath and body length.

Fecundity and Longevity

A pair of newly emerged adult male and female hoppers were placed inside a cage enclosing either an inflorescence or a shoot. The pair were transferred to another inflorescence or shoot every 24 h. The fecundity and longevity of 20 pairs were determined.

Single vs Multiple Mating

The effect of mating incidence on egg production was studied. For single mating, a newly emerged female was caged with two males until mating occurred, after which the males were removed. For multiple mating, the female was kept in captivity with two males throughout her life time. The fecundity and adult longevity of both single-mated and multiple-mated females were compared. There were 20 replications.

RESULTS AND DISCUSSION

Egg Development

Eggs were deposited along the rachis of the inflorescence in clusters averaging 65 eggs/cluster; each cluster consisting of several rows. The eggs were partially embedded in the plant tissue with the anterior end protruding. The stalked aeropyle, which is a respiratory horn (Hinton 1981), was clearly visible.

The eggs measured 0.95±0.05 mm in length. The egg was translucent, smooth and shining. In the later stage of egg development, the eye spots of the embryo were visible. The incubation period on the inflorescence was 3.85±2.00 days. Hatching took place between 0500 and 0900 h.

Nymphal Development

Newly emerged nymph were stationary for 20±5.5 min, after which they began to look for feeding sites. The sex of the nymph could be differentiated by the shape and size of the sheath surrounding the stylet. In males, the tip of the stylet is broader. On inflorescence, rudimentary wing pads appeared in the 3rd instar and by the 4th and final instar it resembled the adult. The number of nymphal instars for both males and females was based on the width of head capsules (Table 1). There were five nymphal instars on shoots, compared with only four on inflorescence (Table 2). This phenomenon of variation in number of instars was also recorded for *I. clypealis* in the Philippines (Bato 1978).

TABLE 1

Mean width (mm ± SD) of head capsules for determining the nymphal instars of *I. nitidulus* caged on the inflorescence and shoots of mango var. Harumanis in the field (n=20)

Nymphal instar	Inflorescence		Shoots	
	Female	Male	Female	Male
1	0.47±0.02	0.47±0.02	0.46±0.02	0.46±0.02
2	0.72±0.09	0.70±0.07	0.65±0.03	0.68±0.10
3	1.14±0.25	1.07±0.21	1.00±0.15	0.92±0.14
4	1.59±0.23	1.45±0.18	1.38±0.23	1.43±0.26
5			1.64±0.20	1.65±0.17

TABLE 2
Mean duration (days ± SD) of eggs and nymphal instars of *I. nitidulus* reared on inflorescence and shoots of mango var. Harumanis in the field (n=20)

Stage	Inflorescence		Shoots	
	Female	Male	Female	Male
Egg	3.85±2.00	3.85±2.00	3.76±2.00	3.76±2.00
Nymphal instar				
1	2.06±0.25	2.05±0.24	2.00±0.63	1.77±0.44
2	2.20±0.56	2.29±0.58	2.00±0.73	2.54±2.31
3	2.06±0.59	2.29±1.15	1.63±0.62	2.31±1.03
4	3.60±0.63	2.80±0.56	2.50±0.89	3.15±1.46
5			3.18±1.17	3.57±2.49
Total (nymph)	9.90±0.25	9.65±0.60	10.07±1.63	11.92±2.92

Ecdysis between the last instar and the adult took 30±5 minutes. This process occurred between 0700 - 0800 h. The total developmental periods of nymphs on inflorescence and shoot were not significantly different (Table 2).

Adult Mango Leafhoppers

The colour of the newly emerged adult was pale cream with weak venation; 30 min after emergence the wings changed to testaceous brown with prominent black veins. The scutellum was brownish with elongated triangular patches. The body lengths of male and female hoppers on inflorescence were 4.72±0.59 mm and 5.07±0.26 mm respectively (Table 3). The adult is equipped with a mouth sheath made of lipoprotein substance (Backus *et al.* 1988). The mouth sheath of the female is tubular and rounded at the tip. Its broadest end measured 0.31±0.05 mm. The mouth sheath of the adult male is tubular but broad and flattened at the tip with the broadest end at the tip measuring 0.39±0.05 mm. However, both males and females have the same mouth sheath length of 0.88±0.55 mm.

Reproductive Capacity

Leafhoppers reared on shoots produced half the number of eggs of those reared on the inflorescence. The flower sap may contain as much as 36% protein (Corey 1986) and insects that feed on protein food either as nymphs, adults, or both produce more eggs (Engelmann 1984).

The leafhoppers begin to mate 4.75±1.67 days after adult emergence. Oviposition took

TABLE 3
Mean width (mm ± SE) and mean body length (mm ± SE) of adults of *I. nitidulus* caged on inflorescence and shoots of mango var. Harumanis in the field (n=20)

Food source	Head capsule		Body length	
	Male	Female	Male	Female
Inflorescence	1.89±0.09a	1.99±0.090a	4.72±0.59a	5.07±0.26a
Shoot	1.87±0.15a	1.88±0.15a	4.73±0.61a	4.88±0.26a

Means within a column followed by the same letters are not significantly different at P>0.05 according to LSD

TABLE 4
Fecundity, hatchability and longevity of female *I. nitidulus* reared on inflorescence and shoots of mango var. Harumanis in the field (n=20)

Food source	Fecundity	Hatchability (%)	Longevity (days)
Shoot (±SE)	149.0±57a	54.8±22.0a	59.6±21.8a
Inflorescence (±SE)	277.1±110b	90.2±8.4b	50.8±17.5a

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

place shortly after mating as is usual of females of many related species (Engelmann 1984).

The number of eggs produced by single- and multiple-mated females fed on mango shoots were 176±72 and 149±57 eggs respectively. However, this difference was not significant..

Adult Longevity

The longevity of males feeding on shoots in the field was 60.5±8.5 days and females 69.8±9.8 days; the difference was not significant. However, Miller and Delzer (1960) emphasised that females, especially if mated, live longer than males of the same age. The shorter life span of male hoppers has also been reported by other workers (Severin 1924; Harries and Douglas 1948).

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The Effect of Shade on Leaf Characteristics of *Mikania micrantha* (Compositae) and Their Influence on Retention of Imazapyr

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Keywords: ***Mikania micrantha*, shade, imazapyr, leaf surface**

ABSTRAK

Lindungan telah mengakibatkan perubahan terhadap ciri-ciri histologi daun Mikania micrantha *H.B.K., dimana daun yang berada pada intensiti cahaya yang tinggi adalah lebih tebal daripada daun dari intensiti cahaya yang rendah. Peningkatan dengan kadar yang bermakna bagi luas semburan dan retensi imazapyr di permukaan atas daun dalam intensiti cahaya yang rendah menunjukan lindungan telah menukarkan topografi permukaan atas dan kuantiti lilin daun.*

ABSTRACT

Shading led to changes in the leaf histological characteristics of Mikania micrantha *H.B.K., leaves at higher light intensity being thicker than those at lower light intensity. There was a significant increase in the area of spread of imazapyr droplets and retention on the upper leaf surface at lower light intensity, suggesting that shading had changed the upper surface topography and the amount of epicuticular wax of the leaves.*

INTRODUCTION

Mikania micrantha H.B.K. is a pernicious weed in crops such as rubber, cacao, oil palm, coconut, banana, pepper and tea. It usually grows profusely in places receiving high rainfall or in humid habitats (Holm *et al.* 1977). The obnoxious character of this weed is mainly due to its rapid growth and spread, which smothers neighbouring plants, as well as its ability to root at the nodes when the stem comes in contact with soil (Macalpine 1959). Ipor (1991) reported that *M. micrantha* is a shade-tolerant species and persists in sites receiving 25% of full sunlight.

Imazapyr (isoproypylamine salt of 2-(4-isopropyl-4-methyl-5-oxo-2-imidazoin-2-yl) nicotic acid) is a broad spectrum herbicide commonly used for controlling both annual and perennial weeds (Fine *et al.* 1983). It is a systemic herbicide and is readily absorbed and translocated in plant tissues (Mallipudi *et al.* 1986). The uptake, translocation and activity of imazapyr increases significantly as light intensity decreases (Ipor and Price 1990).

It is well known that level of light intensities during growth can markedly alter the morphological, anatomical, physiological and biochemical properties of leaves. Ipor (1989) found that growth pattern, rate of expansion, final leaf area and specific area of individual leaves of *M. micrantha* were greatly influenced by shade or light intensities.

Daubenmire (1970) reported that epicuticular wax and distribution of crystalline wax had a significant effect on herbicide penetration. Hence, the objective of this study was to determine the role of light intensity in altering the topographical characteristics of wax, epidermal cell size and morphological structure of *M. micrantha*, which are likely to influence the retention of imazapyr.

MATERIALS AND METHODS

Midrib Sectioning for Light Microscopy

Plants were grown by using the procedures described by Price and Ipor (1990). The middle part of the second youngest lamina of

M. micrantha of plants grown under three levels of shade (0, 50 and 75%) was cut into a piece of 7 x 7 mm and fixed in formalin-acetic acid (FAA) under reduced pressure for a week. The samples were dehydrated in a graded ethyl alcohol series. After dehydration, the tissue was infiltrated and embedded in paraffin, which allowed to solidify. Sections 10 μm thick were stained with alcian blue and safranin (50% alcohol) (Sass 1958).

For stomata counting, the middle portion of the preserved leaves was cut into pieces 10 x 10 mm. The tissues were thoroughly washed with distilled water then gently boiled in 45 ml of 15% nitric acid until the adaxial and abaxial surfaces separated. The tissues were gently transferred into a petri dish and washed with two changes of distilled water. The remaining mesophyll was brushed away with a fine brush. The cuticles were then treated with 5% acetic acid for 30 sec and immediately transferred into sodium hypochlorite (NaOCl) for 30 min until the tissues became clear. They were then washed twice with distilled water before being transferred into 50% ethanol for 2 min followed by staining with 50% safranin for 10 min. After dehydration, the cuticles were mounted in Canada balsam or euparal and the slides dried on a hot plate for two weeks. The stomata were counted under a light microscope.

Scanning Electron Microscopy of Upper Leaf Surface

Leaf pieces 0.75 x 0.75 cm were taken from the middle portion (avoiding the midrib) of the second youngest leaf of plants grown at the three levels of shade mentioned above as well as from plants growing naturally in an open habitat in an oil palm plantation in Malaysia. The leaf pieces were affixed to aluminium stubs with colloidal silver adhesive and immediately cooled in liquid nitrogen. Specimens were then freeze dried between at -40 – -60°C for 48 h.

Specimens were coated with gold and examined and photographed using a Jeol T20 scanning electron microscope (SEM) at 3200x magnification.

Wax Deposits on the Leaf Surface

The amount of epicuticular wax was estimated gravimetrically in a chloroform extract, using a method similar to that of Souza and Williams (1986). Fully expanded leaves from the three shade regimes were excised and their surface areas determined using a photomax tracer. The leaves were then dipped in chloroform twice for 10 sec, which was then filtered through Whatman No. 1 paper into a pre-weighed test tube. Test tubes with extracts were placed in a fume hood to evaporate the chloroform and then in a forced air oven at 45°C before being transferred into a vacuum desiccator and dried to constant weight.

Droplet Spread on Leaf Surface

A Buckard microapplicator was used to apply 0.2 μl droplets of lisamine red formulations on the upper leaf surfaces (Mabb and Price 1986). The eighth and second youngest leaves of plants at the ten-leaf growth stage were used. The diameter of the droplet deposit was measured after 24 hours with a calibrated graticule eyepiece and the area calculated. The experiment was carried out with thirty replicates and repeated twice.

Spray Retention of Imazapyr

Fifty-leaf stage plants were used. The plants were sprayed with solutions containing the soluble dye lisamine (1% w/v) with imazapyr (0.3 kg a.i/ha) and distilled water. A maldrive spraying system was used to deliver 211 l/ha.

After the sprayed deposits had dried, the leaves were detached from each plant and the sprayed deposits were washed off with 25 ml of distilled water. The lisamine concentration was measured in a spectrophotometer at wavelength 460 μm. The value of the peak point was compared with a standard concentration curve for the calculation of the equivalent amount of herbicide in the sprayed deposit. Data were expressed in μg herbicide per cm^2 of leaf. The experiment was repeated twice with thirty replicates.

RESULTS

Histology of M. micrantha

The effect of shading on the leaf characteristics and anatomy are presented in *Plate 1* and Table 1. Leaves grown at 0% shade in both glasshouse and the open area in the field were significantly thicker than those at other light regimes. The number of cells between the upper and lower leaf surfaces increased with increase in light intensity.

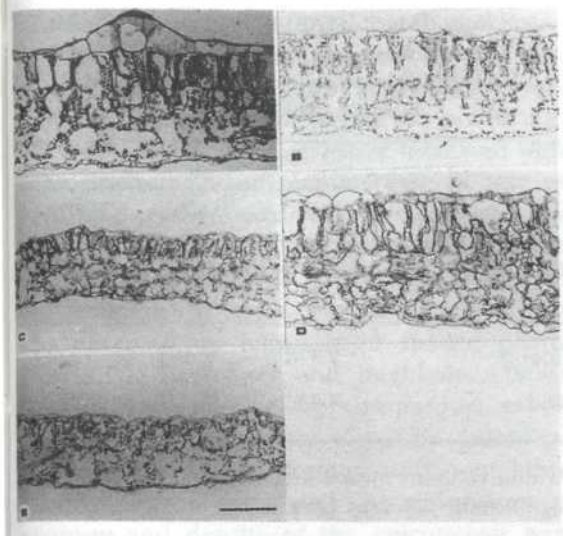

Plate 1: Transverse sections of Mikania micrantha *leaves grown under (A) 0%; (B) 50%; (C) 75% shade; (D) open areas; (E) in mature oil palm plantation. (Scale bar = 60mm)*

The size of epidermal cell of leaves grown under low light intensity increased appreciably. Palisade cell diameter also increased significantly with light intensity.

Upper Leaf Surface of M. micrantha

The width of epidermal cells on the upper surface decreased appreciably with increasing light intensity (*Plate 2*). The epidermal cells of leaves from 0% shade in the glasshouse and open areas in the field were fewer than of leaves from 75% shade (Table 1). The wax on the upper leaf surface was generally flattened.

Wax Deposits on Leaf Surfaces

The average amount of chloroform-soluble wax from leaves under different shade levels is presented in Table 2. The quantity of epicuticular wax per unit area of leaves increased as the level of shade decreased: the amount from leaves under 0% shade was more than twice that from 75% shade.

Droplet Spread on Upper Leaf Surface

The area of spread of droplets on both young and old leaves at 75% shade was significantly greater than that at 0 and 50% shade (Table 3),

TABLE 1
Effect of shading on the histological characteristics of leaves of *Mikania micrantha*

	Glasshouse			Field	
Leaf cell characters	0%	50%	75%	Open	Shaded
Number of stomata per mm^2					
Upper surface	142a	73c	10d	101b	12d
Lower surface	597a	381b	295c	298c	234d
Leaf thickness (μm)	207a	194b	136c	210a	105d
Number of cells from upper to lower leaf surface	7.8a	6.1bc	5.5cd	6.4b	4.8d
Average width of:					
Epidermal cell (μm)	32.6b	38.5a	42.7a	28.1b	22.5c
Xylem (μm)	13.4b	20.9a	18.9a	19.8b	13.3b
Phloem (x 10^{-3} μm)	7.0a	8.5a	9.1a	7.7a	8.8a
Mesophyll cell (μm)	25.2b	32.8a	25.4b	31.1a	23.3b
Palisade cell (μm)	60.6a	54.4b	40.0d	46.3c	25.6e

Within each row, values sharing the same letter are not significantly different at 5% level, according to Duncan's multiple range test.

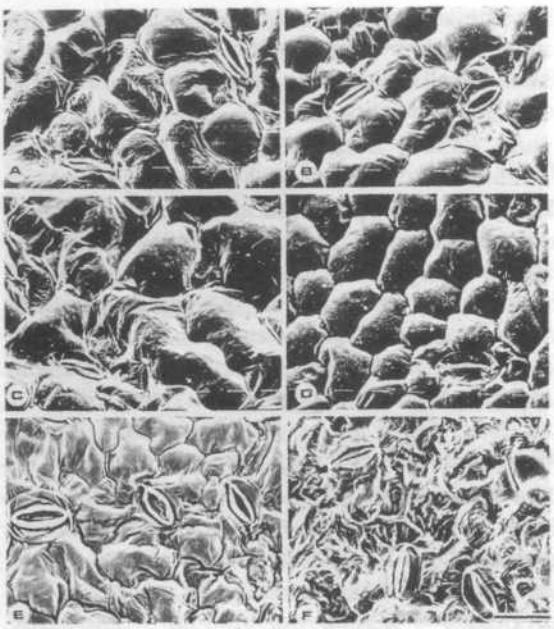

Plate 2: EM micrographs showing leaf surface of Mikania micrantha *at different levels of shade (A) 0%; (B) 50%; (C) 75% shade; (D) open areas; (E) in mature oil palm plantation: (F) without epicuticular wax after stripping with cellulose acetate. (Magnification = 3200x, Scale bar = 25cm)*

TABLE 2
Effect of shading on the quantity of epicuticular wax deposits on the leaf surface of *Mikania micrantha*

Shade Level	Mean Weight of Epicuticular Wax ($\mu m\ cm^{-2}$)
0%	8.2a
50%	5.2b
75%	3.4a

Within each column, values sharing the same letter are not significantly different according to Duncan's multiple range test.

but there was no significant difference between 0 and 50% shade on young leaves.

Spray Retention of Imazapyr on M. micrantha *Leaves*

There was a trend towards increased retention of imazapyr with shade (Table 4). Plants under 75% shade retained more than three times that from 0% shade (Table 4).

TABLE 3
Effect of shade levels on area of spread of imazapyr drops on the upper surface of *Mikania mircrantha* leaves

	Shade level		
	0%	50%	75%
Leaf stage	Area of droplet spread (mm^2)		
Young	2.87d	2.43d	12.52c
Old	4.44d	31.01b	62.50a

Within columns means with the same letter are not significantly different (P>0.05) according to DMRT

TABLE 4
Amount of imazapyr retained on leaves of *Mikania micrantha* grown under different shade levels

Shade level (%)	Imazapyr spray retained ($\mu g\ cm^{-2}$)*
0	0.95b
50	1.17b
75	3.16a

*Means with the same letter within the column are not significantly different (P>0.05) according to DMRT

DISCUSSION

Plants grown under high light intensities showed differences in leaf surface structure from those grown at low light intensities (Table 1). High light intensity reduces leaf expansion resulting in thicker leaves. Boardman (1977) found that leaves of *Atriplex patula* grown at 20 mw cm^{-2} were seven cells thick compared with three or four cells in leaves grown at 2 mw cm^{-2}. The mesophyll cells grown under low light intensity were smaller and more densely packed and there were fewer vascular strands. The size of the epidermal cell of *M. micrantha* was considerably smaller under high light intensity.

Shade was observed to play an important role in the development of the epicuticular waxes of *M. micrantha*. A greater deposit of epicuticular wax was found under higher light intensities

(Table 2). Skoss (1955) found that shaded leaves of ivy (*Hedera helix*) had less cuticle and wax than those exposed to full sunlight. Martin and Juniper (1970) also reported that wax production on soybean (*Glycine max*) leaves increased with light intensity. Significant increase in wax of field bindweed (*Convolvulus arvensis*) leaves was also reported under high light intensities (Steward *et al.* 1986).

A significant increase in the droplet spread was observed on leaves from shaded plants (Table 3). Dorschner and Buchholtz (1956) found that shading by companion crops increased the wettability of alfalfa (*Medicago sativa*) leaves. In the present study, wettability was significantly correlated with differences in quantity and density of the epicuticular wax crystals, and to the size of the epidermal cells of *M. micrantha*.

There was a trend toward greater retention of imazapyr on *M. micrantha* leaf surfaces grown under shade (Table 4). Differential spray retention is dependent on leaf surface characteristics and the angle of incident of the spray droplet to the leaf (Ennis *et al.* 1952; Brunskill 1956; Blackman 1958). Ennis *et al.* (1952) reported that the waxy layer on the leaf was an important characteristic affecting spray droplet repulsion. In this study, the waxy layer of *M. micrantha* leaves grown at high light intensities repelled spray droplets more effectively than those grown at lower light intensity. In heavily waxed leaves, Brunskill (1956) showed that spray droplets bounced off the leaf because the angle of incidence had decreased. This may be the main explanation why more imazapyr is retained on the leaf surface of *M. micrantha* grown under lower light intensity and which are less waxy. In addition *M. micrantha* at 0 and 50% shade levels had leaves which were slightly erect and facilitated runoff, whereas at 75% shade, the leaves were oriented horizontally, bigger and proportionally longer. Less bounce of droplets should occur at 75% shade, which may contribute to the greater spray retention observed at 75% shade.

Changes in morphology, surface structure and histological characteristics influence retention of imazapyr on plants exposed to different shade levels. Price and Ipor (1990) reported that leaves of *Paspalum conjugatum* grown under low light intensity (75% shade) had significantly increased uptake and translocation of imazapyr, and ascribed this to the thinner leaves with higher permeability. Uptake and translocation have repeatedly been shown to account for the effectiveness of herbicides (Jensen 1982). The greater susceptibility of plants growing under higher shade levels means that a smaller quantity of the herbicide and less frequent application of herbicide are needed.

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Water Relations of Melon *(Cucumis melo)* Plants in Soilless Culture

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Keywords: ***Cucumis melo*, water availability, growth, relative water content, stomatal resistance, photosynthesis rate, yield**

ABSTRAK

Tanaman tembikai wangi (Cucumis melo) di tanam didalam campuran gambut dan pasir dengan diberikan beberapa kedapatan air iaitu 25, 50, 166% dan muatan ladang. Isipadu air yang ditambah pada substrat adalah 300, 600, 2000 dan 1200 ml setiap hari menyamai keperluan air yang dinyatakan. Pertumbuhan vegetatif dan hasil berkurangan secara berkadaran dengan kedapatan air. Pemberian air diatas paras muatan ladang substrat menghasilkan pertumbuhan dan hasil yang tinggi disebabkan tanaman mengubahsuaikan pengaruh evaporasi tinggi didalam iklim mikro. Jumlah bahan terlarut didalam buah meningkat cepat semasa perkembangan buah didalam keadaan kedapatan air rendah. Peningkatan kedapatan air memperbaiki status air daun, respon stomata dan kadar fotosintesis. Pada tahap kedapatan air yang rendah, pengurangan status air daun menyebabkan kadar fotosintesis mengurang sehingga mencapai nilai negatif pada akhir perkembangan tanaman. Perkaitan di antara status air daun dan rintangan stomata di hasilkan dan dibincang berdasarkan pengaruh hidrolik dan tanpa hidrolik terhadap stomata.

ABSTRACT

Melon (Cucumis melo) plants were grown in a peat and sand mixture under water availability of 25, 50, 166% and field capacity. The respective amount of water added to substrate was 300, 600, 2000 and 1200 ml per day. Vegetative growth and yield decreased proportionately according to water availability. Overwatering above substrate field capacity resulted in the highest growth and yield as the plants compensated for the influence of high evaporative demand in the microclimate. Total soluble solids in the fruit increased rapidly during fruit development under reduced water availability. Increased water availability improved leaf water status, stomatal response and photosynthesis rate. At lowest water availability, a reduction in leaf water status caused photosynthesis rate to decline and to reach negative values by the end of the growth period. A relationship between leaf water status and stomatal resistance was established and is discussed with reference to hydraulic and non-hydraulic causes controlling stomatal responses.

INTRODUCTION

Cultivation of crops using soilless culture in a protected environment has proven beneficial compared to open field cultivation (Mohd Razi 1994). An important feature in the management of aggregate soilless culture is to optimise production through efficient use of water and nutrients. As plants grown in soilless culture are normally grown in a protected structure, changes in plant microclimate, especially temperature and humidity, can subject them to water stress, as measurable by various indicators including leaf water potential, relative water content, hydraulic resistance and transpiration rate. Most physiological processes are affected by the water status of a plant (Hsiao 1973). The relationship between leaf water status and plant physiological processes needs to be established for efficient irrigation management, especially when available water is scarce. Schulze (1994) indicated that in

sunflower, the daily water loss from leaves may be equivalent to several times their total fresh weight under conditions of open stomata and high photosynthesis rates. In contrast, a plant water deficit equivalent to only a small fraction of its total fresh weight would cause severe metabolic disorders due to water stress.

In the present study, the sensitivity of melon plants to the changes in water status of plants grown in a peat:sand mixture in a protected environment was investigated relating to growth, physiological processes and yield.

MATERIALS AND METHODS

The experiment was conducted in the Hydroponics Glasshouse Unit at Universiti Pertanian Malaysia. Throughout the experiment, the mean maximum air temperature was 33.6 ± 5.7°C and the mean minimum temperature was 26 ± 2.1°C; mean day relative humidity was 56 ± 6.2%. The plants were generally grown at an atmospheric vapour pressure deficit of 2.3 ± 0.5 kPa.

Seeds of melon *(Cucumis melo)* cv Birdie were sown in compost. After 14 days seedlings were transferred to polybags containing 10 kg of a peat and sand mixture (3:1 peat:sand). The seedlings were grown in the mixture for a further 2 weeks with regular watering before uniform plants were chosen.

Four irrigation regimes were used in the experiment. Field capacity, determined as the moisture held by the substrate after free drainage for 24 h, was 0.12 g water/g substrate. The irrigation regimes were 25, 50 (restricted watering), 100 (field capacity) and 166% (overwatering) of field capacity arranged in a completely randomized design with 4 replicates. The respective volumes of water added to the substrate every day were 300, 600, 1200 and 2000 ml. The plants were fertilized with the constituents of Cooper formulation (Cooper 1979) at 20CF. Other standard management procedures for melon cultivation were followed (Mohd Razi 1994).

Dry matter accumulation was assessed from seven sequential destructive samplings. At each sampling, four plants were selected at random from each treatment except the guard rows. During each harvest, the plants were fractionated into the following parts: leaves, stems, roots and fruit. Leaves were enclosed in polythene bags for leaf area determinations using an automatic leaf area meter (Delta-T Cambridge, UK). All samples were dried to constant weight for at least 48 h in a forced draught oven at 80°C.

Relative water content, stomatal diffusive resistance and photosynthesis rate were determined 1, 3, 5 and 7 weeks after each treatment. Relative water content was determined according to Barrs and Weatherley (1962). Stomatal resistance was measured with a diffusion porometer (MKIII, Delta-T Devices Ltd, Cambridge, UK) on the mature leaves which were exposed to full sunlight and which were adjacent to leaves sampled for relative water content. Leaf photosynthesis rate of attached leaves was measured using a portable infrared gas analyser (ADC2-The Analytical Development Co. Ltd, Hoddesdon, UK) on the same leaves as used for the diffusive resistance measurements. For each treatment, at least four readings were taken from different leaves. Measurements were made 4-5 h after sunrise when PFD was between 750-860 μmol $m^{-2}s^{-1}$.

Fruit dry weight accumulation was followed by sequential harvesting. Total soluble solids were determined on each of the harvested fruit using a hand refractometer. The experiment was terminated when fruits on the plants reached maturity, determined by small cracks at the base of the fruits.

RESULTS

Plant Vegetative Growth

Fig. 1 shows the dry matter accumulation in leaf, stem and root parts of melon plants as influenced by different water availability. Leaf dry weight increased proportionately to the available water in the substrate. In general, leaf dry matter accumulation of plants receiving 2000 ml water was 4-6, 12-18, and 14-22 g higher than in plants receiving 1200, 600 and 300 ml water, respectively. The difference between treatments was noticeable by the third week of growth. Similarly, stem dry weight was higher in the plants receiving 2000 ml water per day, while differences between plants receiving water less than 1200 ml was not apparent after the 4th week. The difference in root dry weight of plants receiving 2000 ml was apparent by the first week, but no difference was registered between plants receiving less than 1200 ml of water each day. The differences between plants receiving 1200 and 600 or 300 ml water were only apparent by the fifth week. Root growth of plants receiving

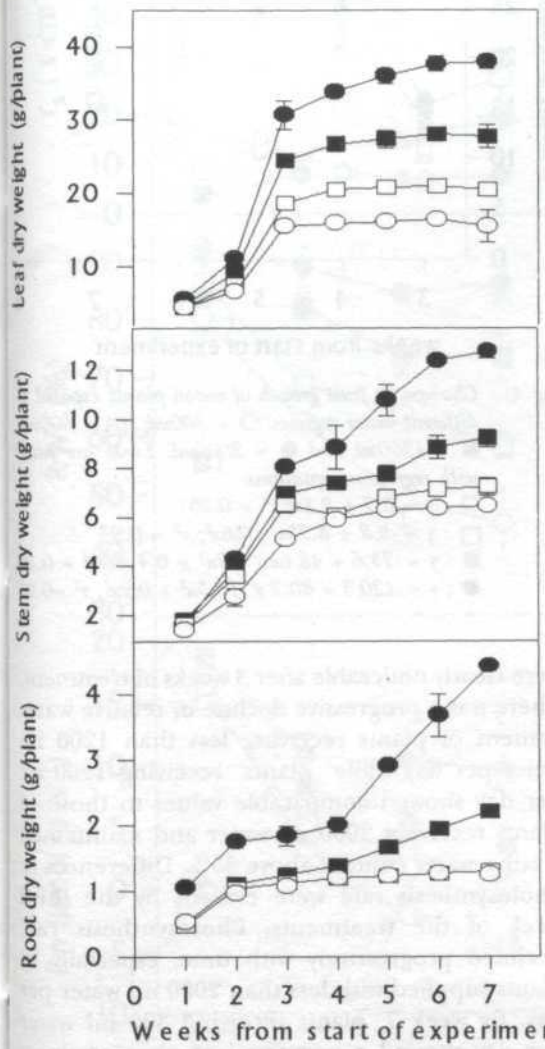

Fig 1: Leaf, stem and root dry weight of melon plants grown in different water regimes. ○ = 300ml; □ = 600ml; ■ = 1200ml and ● = 2000ml. Values given are means of ± SE of 4 replicates. Some SE marks reside within symbols

less than 1200 ml water was almost constant throughout the growth period. At final harvest, root dry matter accumulation in plants receiving 2000 ml water perday was twice and four times higher than plants receiving 1200 and 600 or 300 ml, respectively.

Fig. 2 shows the relationship between leaf area and the duration of plants under various water regimes. In general, the relationship was almost sigmoidal for the two parameters, except for plants receiving 300 ml water. The reduction in leaf area by the end of the growth period was due to senescence of the older leaves during fruit maturity. At the period of maximum growth, the leaf area of plants receiving 2000 ml water was 1.3, 2 and 5 times greater than for plants receiving 1200, 600 and 300 ml, respectively

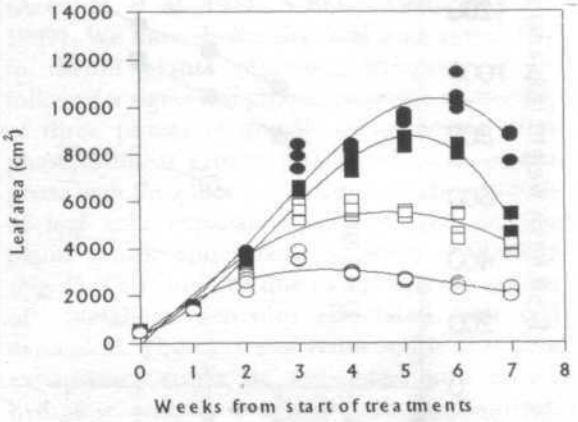

Fig 2: Leaf area of melon plants as influenced by different water regimes. Lines are fitted with regression equation: ○ = 300ml; $y = 282.3 + 1853x - 375.8x^2 + 1x^3$; $r^2 = 0.88$
□ = 600ml; $y = -112.0 + 2400x - 256.2x^2$; $r^2 = 0.91$
■ = 1200ml; $y = 400.0 + 522.2x + 748.4x^2 - 104.0x^3$; $r^2 = 0.98$
● = 2000ml; $y = 257.8 + 1090.6x + 583.4x^2 - 81.5x^3$; $r^2 = 0.96$

Fruit Development

Fig. 3 shows changes in total soluble solids and fresh weight of fruits exposed to different water regimes. The differences in total soluble solids values between treatments were only apparent by the fifth week. A reduction in water availability to the plants increased the total soluble solids content of fruit. Fruit fresh weight was consistently higher on plants receiving 2000 ml water. At final harvest, fresh weight of fruit from plants receiving 1200, 600 and 300 ml was 15, 42 and 70% respectively, lower than plants receiving 2000 ml water. The change in fruit

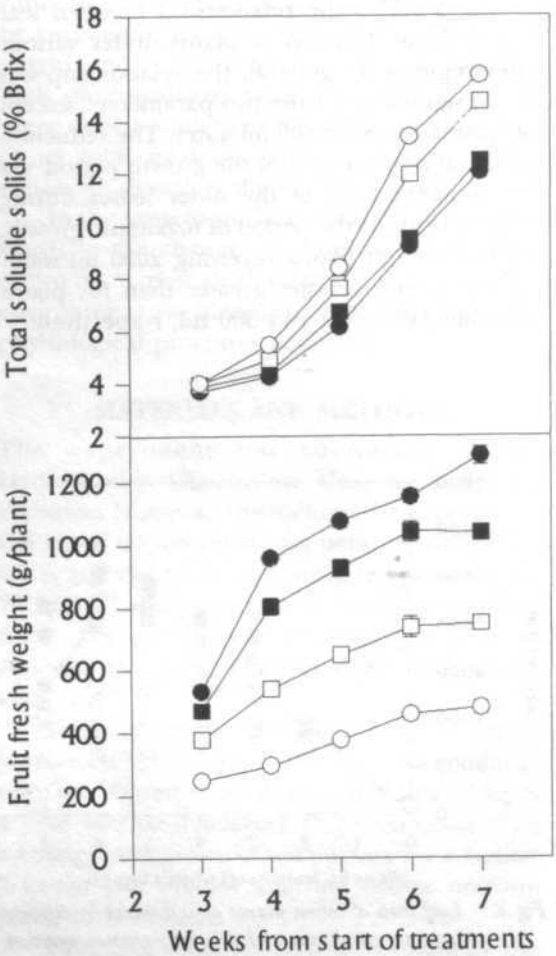

Fig 3: Fruit growth and total soluble solids of melon plants exposed to different water regimes. ○ = 300ml; □= 600ml; ■= 1200ml and ●= 2000ml. Values given are means of ± SE of four replicates. (Most SE marks reside within symbols)

dry weight followed a similar pattern *(Fig. 4)* and there was also a close correlation between the accumulation of dry matter in the fruit and the duration of treatments.

Relative Water Content, Stomatal Resistance and Rate

Changes in stomatal resistance, relative water content and photosynthesis rate are illustrated in *Fig. 5*. Stomatal resistance was increased with reduced water availability. Plants provided with only 300 ml water per day showed a marked increase in stomatal resistance and displayed complete stomatal closure by the fifth week. Reducing water availability resulted in decreased relative water content of leaves; the differences

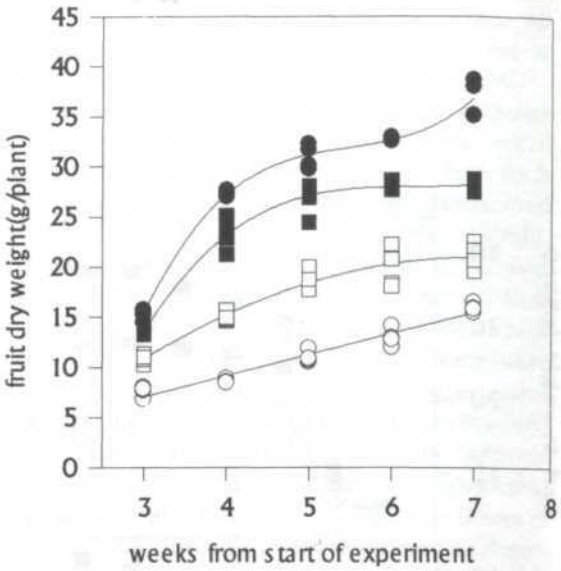

Fig 4: Changes in fruit growth of melon plants exposed to different water regimes. ○ = 300ml; □ = 600ml; ■ = 1200ml and ● = 2000ml. Lines are fitted with regression equations:
○ : $y = 0.7 + 2.1x$; $r^2 = 0.95$
□ : $y = -9.8 + 8.75x - 0.6x^2$; $r^2 = 0.93$
■ : $y = -73.6 + 48.6x - 7.7x^2 + 0.4x^3$; $r^2 = 0.97$
● : $y = -120.3 + 80.7x - 14.5x^2 + 0.9x^3$; $r^2 = 0.98$

were clearly noticeable after 3 weeks of treatment. There was a progressive decline in relative water content of plants receiving less than 1200 ml water per day while plants receiving 1200 ml per day showed comparable values to those of plants receiving 2000 ml water and maintained relative water content above 85%. Differences in photosynthesis rate were evident by the third week of the treatments. Photosynthesis rate declined progressively with time, especially in plants supplied with less than 2000 ml water per day. By week 7, plants receiving 300 ml water per day showed a negative leaf photosynthesis rate.

DISCUSSION

As reported for several other plant species (starfruit; Mohd Razi *et al.* 1994; pepper, Aloni *et al.* 1991; tomatoes, Mohd Razi *et al.* 1993), reduced water availability in melon plants retards vegetative growth and fruit development. This is particularly evident for plants grown under high temperature with low air humidity conditions, which often results in high atmospheric vapour pressure deficits in the plant microclimate.

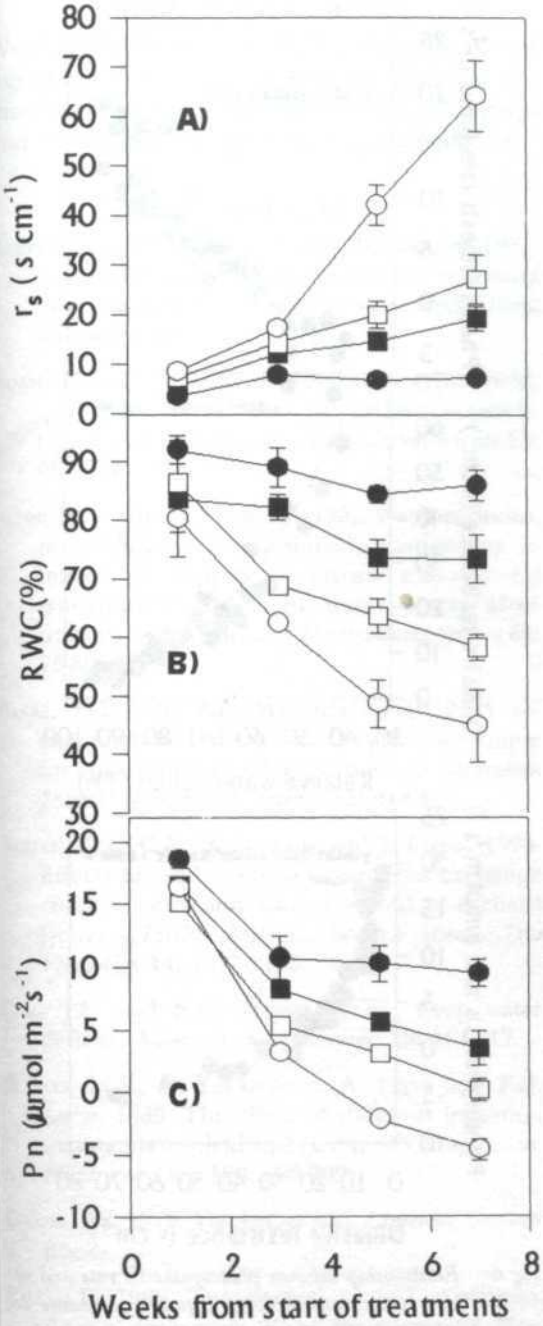

Fig 5: Stomatal diffusive resistance, Rs(A), relative water content, RRWCB(B) and photosynthesis rate, Pn(C) of melon plants as influenced by water regimes. ○ = 300ml; □ = 600ml; ■ = 1200ml and ● = 2000ml. Bars represent SE with 4 replicates, some marks reside within symbols

Smith (1989), working with oil palm, argues that such conditions would limit production even if plants were grown under adequate moisture. We have demonstrated in this study that irrigating plants to field capacity level (1200 ml water) under similar conditions also resulted in a decrease in dry matter accumulation after 3 weeks. Further reductions in water availability to the plants have resulted to a decrease in leaf and root growth. Leaf area expansion, particularly, was greatly reduced in plants receiving 1200 ml or less water compared to 2000 ml water per day.

It has been reported by many workers that the primary effect of slight to moderate water stress is either at the cell extension phase or at both the cell division and cell extension phases of leaf growth depending upon the plant species (Acevedo *et al.* 1971; Schulze 1986; Jefferies 1989). We have shown that leaf area expansion in melon plants receiving adequate water followed a sigmoidal growth response consisting of three phases of growth i.e an acceleration phase, a linear growth phase and a senescent phase with the older leaves dying. Early cessation of leaf area expansion was observed on the plants grown under reduced water availability *(Fig. 2)*. This could be due to an early disruption of metabolic activities associated with cell expansion. The causes of reduction in leaf area expansion could be associated with either hydraulic and/or non-hydraulic mechanisms. The hydraulic process is associated with changes in turgor pressure which act as a driving force for cell expansion and hence leaf growth (Acevedo *et al.* 1971; Begg and Turner 1976: Dale 1988). Non-hydraulic signals generated from roots growing under reduced water availability have been reported to directly inhibit effect on leaf growth in the absence of detectable shoot water deficit as related to the latter mechanism (Passioura 1988; Gowing *et al.* 1990). Zhang and Davies (1991) have proposed that abscisic acid plays the role of a chemical signal in root to shoot communication and can bring about a retardation of leaf growth in plants grown at reduced water availability.

The study also demonstrated the importance of water availability for fruit development. The reduction in fruit growth is a common response in plants exposed to reduced water availability (Blanco *et al.* 1989; Batten *et al.* 1994), though some other researchers showed a beneficial regulated deficit irrigation in perennial fruit (Mitchell and Chalmers 1982; Van den Ende *et al.* 1987). Adam (1990), working with tomatoes,

reported a decrease in fruit growth but an increase in fruit total soluble solids under reduced water availability conditions prevailing on peat moss. This fruit fresh weight and total soluble solids pattern is also observed in the present study *(Fig. 3)*.

Photosynthesis rate decreased with decreasing water content *(Fig. 6)* so that respiration appears to exceed photosynthesis rate when relative water content was reduced to less than 60%. Under such conditions, stomatal diffusive resistance also showed a substantial increase. Although the role of guard cell turgor in regulating stomatal closure could be a causative factor for this phenomenon, the effect of non-hydraulic signals cannot be ruled out. If leaf internal water status solely influenced stomatal closure, there would be a clear linear relationship between these two parameters. The correlation analysis shows such linearity only when relative water content is low, so that there must be another factor triggering early stomatal closure during slight or undetectable changes in leaf water status *(Fig. 6)*.The responses of stomata to a root signal may be regarded as a feedforward response, in which roots in dry soil produce a chemical signal to reduce water loss even before plants experience internal water deficits (Schulze 1994). However, this chemical signal controlling the root-shoot communication has yet to be identified. According to Davies *et al.* (1994), there seems to be quite compelling evidence for a central role for abscisic acid in chemical signalling between roots and shoots in controlling stomatal responses. Some other workers, however, disagree (Munns and King 1988; Trejo and Davies 1991).

This biphasic evidence on leaf internal water status and stomatal resistance observed in the present study with melon plants needs to be further examined to ascertain the role of hydraulic and non- hydraulic factors influencing plants under conditions of water stress. The relationship between stomatal resistance and rate shows a drastic (50%) reduction in photosynthesis rate is coincident with even a small increase in stomatal resistance from 4.5 to 10 s cm^{-1}. It is speculated that photosynthesis apparatus may be inhibited before any effect on the stomatal apparatus. The influence of such stomatal and non-stomatal factors in regulating rates has also been reported by other workers (Ogren and Oquist 1985; Ephrath *et al.* 1993). The present study further

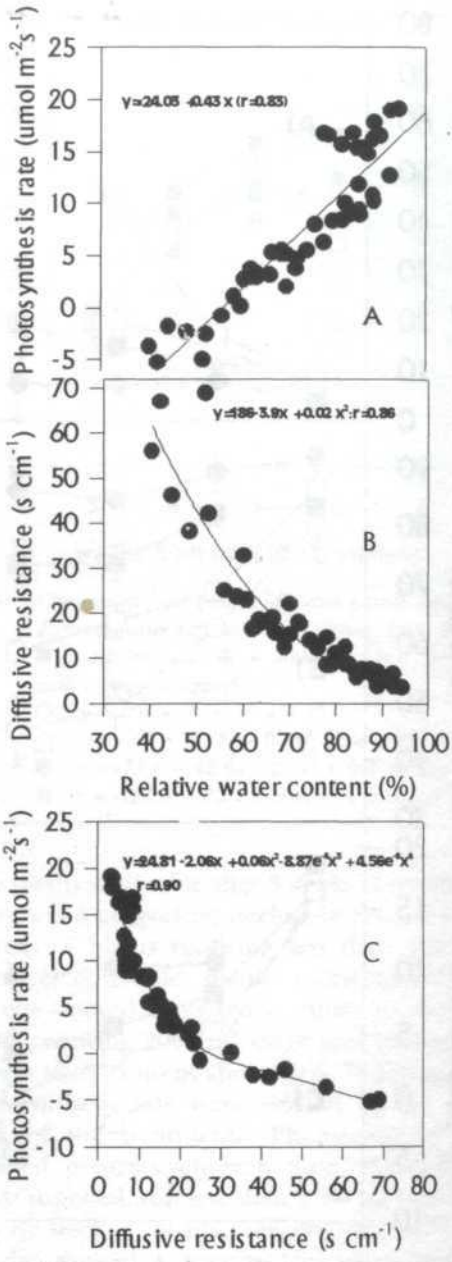

Fig 6: Relationship between photosynthestis rate and relative water content (A), diffusive resistance and relative water content (B) and diffusive resistance and photosynthesis rate (C) of melon plants exposed to different water regimes

shows that when stomatal resistance increased to more than 20 s cm^{-1}, photosynthesis rates declined to negative values. This threshold value is particularly important in future studies to improve water use efficiency of melon plants under reduced water availability.

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Two-year Performance of *Acacia crassicarpa* Provenances at Serdang, Malaysia

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Keywords: ***Acacia crassicarpa*, provenance, survival, growth, provenance selection**

ABSTRAK

Satu percubaan yang melibatkan lapan provenans Acacia crassicarpa *A. Cunn. ex Benth. diukur kemandirian dan pertumbuhan pada umur dua tahun. Dari kesemua provenans ini, tiga berasal dari Queensland utara, Australia, empat dari Papua New Guinea dan satu dari Irian Jaya, Indonesia. Kesemua provenans menunjuk kemandirian baik (>94%), tetapi berbeza dengan bererti ($p < 0.01$) dari segi pertumbuhan. Kesemua provenans mempunyai lebih dari 43% pokok yang berbatang satu. Untuk pengeluaran kayu, provenans dari Irian Jaya (Samlleberr) dan 2 provenans dari Queensland (Olive River dan Jardine River-Bamaga) dikenalpasti berpotensi baik.*

ABSTRACT

A trial of eight provenances of Acacia crassicarpa *A. Cunn. ex Benth. was assessed for survival and growth at age two years. Three provenances were from northern Queensland, Australia, four from Papua New Guinea and one from Irian Jaya, Indonesia. All provenances survived well (> 94%), but they differed significantly ($p < 0.01$) in their growth performance. All provenances had more than 43% of their trees with single stems. For timber production, the provenance from Irian Jaya (Samlleberr) and two provenances from Queensland (Olive River and Jardine River-Bamaga) were identified as promising.*

INTRODUCTION

Acacia crassicarpa A. Cunn. ex Benth., native to northeastern Queensland, Australia, southwestern Papua New Guinea, and southeastern Irian Jaya, Indonesia, is one of the humid/subhumid tropical acacias with potential for wood production for fuelwood, timber and pulp (Harwood 1992; Thomson 1994). It fixes nitrogen, grows rapidly, and competes effectively with weedy grasses. It appears able to tolerate a wide range of soil textures, with pH ranging from 4 to 8, and a dry season up to six months and annual rainfall as low as about 900 mm.

However, many of the acacia plantations in the Asian tropical regions are based on *Acacia mangium* and *Acacia auriculiformis* (Pinyopusarerk 1992). Early reports on the evaluation of several *A. crassicarpa* provenances in Thailand (Chittachumnonk and Sirilak 1991), Malaysia (Sim and Gan 1991), Sri Lanka (Weerawardane and Vivekanandan 1991), Hainan Island, China (Yang and Zeng 1991), Vietnam (Kha and Nghia 1991), and Laos (Latsamay 1991) indicate that their growth is either better or comparable to those of *A. mangium* and *A. auriculiformis*. These provenance trials of *A. crassicarpa* have also demonstrated the superior vigour of provenances from Western Province, Papua New Guinea over those from north Queensland (Thomson 1994). In Malaysia, the introduction of *A. crassicarpa* has been limited to Sabah (Sim and Gan 1991). This paper reports on the survival and growth of eight provenances of 2-year-old *A. crassicarpa* in a trial at Serdang, Peninsular Malaysia.

MATERIALS AND METHODS

Seedling Establishment

Eight seedlots of *A. crassicarpa* provided by the Australian Tree Seed Centre of Commonwealth Scientific and Industrial Research Organisation (CSIRO) were used. The seeds were collected from the species' natural distribution in northern Queensland, Australia, Papua New Guinea and Irian Jaya, Indonesia. This is one of the first provenance trials to include a seedlot from Irian Jaya, Indonesia for comparison with Papua New Guinean and Australian seed sources. Table 1 provides details of seed origin.

The seeds were pretreated by soaking in hot water at 80°C for 30 seconds and then in water at room temperature for 10 minutes. The procedure was repeated three times. The seeds were then air dried, sown in containers filled with washed river sand, and later transplanted into polythene bags. Inoculation with *Rhizobium* was not made in the nursery. The seedlings were about four months old when planted out.

Field Establishment

The field trial was established in January 1992 at Universiti Pertanian Malaysia (UPM) Farm, Serdang (latitude 3° 02'N, longitude 101° 42'E, altitude 32 m) representing a humid site under *Imperata cylindrica* grass. Mean annual rainfall is 2140 mm and mean annual temperature 26°C. The site experiences an average windspeed of 0.86 m/sec, receiving a daily average of 5.8 h of sunshine and an annual evaporation of 1527 mm. The soil is fine-loamy, mixed, Typic Hapludults, isohyperthermic and udic, with a pH of 4.4. The site was fully cultivated before planting.

A randomised complete block design with six replicates was used. Each replicated plot consisted of 16 trees (4 x 4) spaced at 3m x 3m. The plots were weeded every three months during the first year, and less frequently thereafter.

Assessment and Analysis

Measurements of height, diameter at breast height (dbh) and survival were made for all trees every six months after planting. Square root of the sum of the squares of each individual stem was used to calculate dbh of multi-stemmed trees. At 24 months, trees were also individually assessed for form following three classes:

Class 1: Tree with one main leading stem up to the tip. Branches are small, with a basal diameter less than 50% of the principal bole at the same height.

Class 2: Tree with more than one leading stem originating at a height more than 50 cm above the ground. The branching bole is considered a stem if its basal diameter is equal to or greater than 50% of the diameter of the principal bole at the same height.

Class 3: Tree with more than one leading stem originating below a height of 50 cm above the ground. The distinction of a branching bole is the same as for Class 2.

TABLE 1
Details of the eight provenances of seedlots of *Acacia crassicarpa*

No.	CSIRO Seedlot No.	Provenance		Lat. (S)	Long. (E)	Alt. (m)	No. parents
1	16128	Jardine River - Bamaga	QLD	11° 02'	142° 22'	20	15
2	17943	Olive River	QLD	12° 19'	142° 50'	60	5
3	17944	Claudie River	QLD	12° 48'	143° 18'	20	4
4	16598	Bimadebun Village	PNG	8° 37'	141° 55'	25	40
5	17548	Oriomo Old Zim	PNG	8° 48'	143° 06'	20	5
6	17552	Bensbach	PNG	8° 53'	141° 17'	25	35
7	17561	Limal-Malam	PNG	8° 40'	142° 43'	40	30
8	17849	Samlleberr, Irian Jaya	IND	8° 20'	141° 00'	40	5

QLD = Queensland, Australia; PNG = Papua New Guinea; IND = Indonesia

The two years' data were analysed for variance, and provenance means were compared using studentised range test. MPTStat, a statistical package developed by the Forestry/Fuelwood Research and Development Project of Winrock International, was used for the analyses.

RESULTS

Survival for all the provenances was high, percentages ranging from 94.5 to 100%, and showed no statistical differences among provenances (Tables 2 and 3). Lowest survival was recorded for the Bimadebun Village provenance from Papua New Guinea, while the Jardine River provenance from Queensland had 100%. survival.

However, the provenances showed significant differences in their height and diameter growth (Table 2). Significant differences were also recorded among provenances from Queensland and Papua New Guinea (Table 3). The overall ranking based on the mean of the ranks assigned for each parameter indicates that the Samlleberr provenance from Irian Jaya, Indonesia was the best performer, followed by two provenances from Queenland (Olive River and Jardine River). The poorest provenance was from Claudie River, Queensland. The four provenances from Papua New Guinea (Bimadebun Village, Oriomo Old Zim, Bensbach and Limal-Malam) were intermediate in their performance.

TABLE 2
Analysis of variance of survival, height, and diameter breast height (Dbh) of 2-year-old *Acacia crassicarpa* provenances

Parameter	Source of variation	df	Mean square	P. value	C.V. (%)
Survival	Provenance	7	15.283	0.5205	
	Replication	5	27.471	0.1831	
	Residual	35	17.061		
					4.2
Height	Provenance	7	4.896	0.0025	
	Replication	5	9.737	0.0000	
	Residual	35	1.216		
					13.2
Dbh	Provenance	7	5.627	0.0000	
	Replication	5	0.883	0.3773	
	Residual	35	0.802		
					10.2

TABLE 3
Performance of 2-year-old *Acacia crassicarpa* provenances

Provenance		Survival (%)	Height (m)	Diameter breast height (cm)	Composite ranking
Jardine River - Bamaga	QLD	100.0 a	8.8 abc	8.5 abd	3
Olive River	QLD	97.7 a	9.4 abcd	9.6 ae	2
Claudie River	QLD	97.7 a	6.7	7.1 bc	7
Bimadebun Village	PNG	94.5 a	7.4 ab	7.5 bc	8
Oriomo Old Zim	PNG	97.8 a	8.2 ab	7.8 b	5
Bensbach	PNG	97.7 a	8.9 abcde	8.4 abdf	4
Limal-Malam	PNG	96.7 a	8.6 a	9.4 a	5
Samlleberr, Irian Jaya	IND	98.8 a	9.0 abcde	9.6 ae	1

Means having the same letter are not significantly different at p = 0.05
Composite ranking = Means of survival, height and diameter breast height

TABLE 4
Percentage of trees in tree form classes of various provenances of *Acacia crassicarpa*

Provenance		Class 1	Class 2	Class 3
Jardine River - Bamaga	QLD	49.0	16.7	34.3
Olive River	QLD	56.0	14.9	28.5
Claudie River	QLD	48.5	36.9	14.6
Bimadebun Village	PNG	46.3	24.2	29.5
Oriomo, Old Zim	PNG	43.9	40.6	15.5
Bensbach	PNG	46.5	25.8	27.7
Limal-Malam	PNG	45.6	26.7	27.7
Samlleberr, Irian Jaya	IND	64.4	15.8	19.8

Tree form also differed markedly among the provenances (Table 4). Single-stemmed trees (Class 1) were the most prominent among the provenances. However, the number of trees within this class ranged only between 43.9 and 64.4%. The top three most vigorous provenances (Samlleberr, Indonesia, Olive River and Jardine River, Queensland) also had the highest percentage of single-stemmed trees with value of 64.4, 56.6 and 49% respectively.

DISCUSSION

The results indicate that all provenances survived well, with survival rate ranging from 94 to 100%, but differed markedly in their growth in terms of height, diameter and tree form. These differences were associated with both inter- and intra-variations from the two provenance regions in New Guinea/Irian Jaya and northern Queensland. Although the results are preliminary, this variation indicates the presence of genetic diversity in the species within its distributional range. Based on vigour and tree form, the provenance from Indonesia (Samlleberr, Irian Jaya) and two from Queensland (Olive River and Jardine River-Bamaga) are the most promising, and could be selected for further planting.

Comparison of the results obtained here with those from other sites such as at Ba Vi, Vietnam (Kha and Nghia 1991; Thomson 1994) suggests that the provenances evaluated exhibit strong genotype x environment interaction effect. That is, the performance of a particular provenance with respect to the others is not the same across sites. For example, the Jardine River-Bamaga from Queensland was the poorest performer at Ba Vi, while the Bimadebun Village provenance from Papua New Guinea, which was the poorest performer here, was the second top performer at Ba Vi. Williams and Luangviriyasaeng (1989) also found genotype-environment interaction with this species in Thailand. Therefore, further planting of the provenances recommended here should be restricted to sites similar to the trial site. It also implies that further testing of selected, promising provenances on other sites with different environmental conditions is needed. This should draw on the results obtained here, and those reported from similar trials in other countries.

Although the results indicate that there are clear differences in provenance means for different parameters, the potential of individual provenance in contributing towards the gene pool for future breeding programmes must not be discounted. It would be prudent to thin the plot, retaining superior individuals not only from those good provenances but also from poor ones based on plot means. This could prevent the exclusion of other desirable traits such as high wood basic density and resistance to diseases. It also implies that these parameters need to be further assessed for the purpose of a breeding programme to meet the needs of different end users.

One striking aspect about the overall results is the high growth rates obtained with the species. The ranges of calculated mean annual increments of height and dbh were 3.4-4.7 m and 3.6-4.8 cm respectively. In comparison, similar ranges for the top ten of the 28 provenances of *A. auriculiformis* tested in adjacent

adjacent plots were 3.0-3.5 m and 2.7-3.2 cm (Kamis Awang *et al.* 1994). Sim and Gan (1991) also reported the superiority of growth of *A. crassicarpa* over *A. auriculiformis, A. mangium, A.aulacocarpa* and *A. mearnsii* on four sites in Sabah, Malaysia. Similarly, Pinyopusarerk (1989) reported that a Papua New Guinean provenance averaged 10.8 m in height and 10.3 cm dbh at 2 years of age at Saitong, Thailand, slightly greater than the best provenance in this trial. This reinforces the view that *A. crassicarpa* has potential for industrial planting.

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Correlation between Volumetric Oxygen Transfer Coefficient and Power Requirement in Citric Acid Fermentation by *Aspergillus niger*

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Keywords: volumetric oxygen transfer coefficient, power requirement

ABSTRAK

Satu sistem kultur sesekelumpuk bagi penghasilan asid sitrik oleh Aspergillus niger *TISTR 3089 telah dikaji untuk melihat kesan pengudaraan dan pengadukan keatas pekali pemindahan oksigen isipadu (k_La) dan penggunaan kuasa. Tangki berpengaduk 2 liter digunakan dengan halaju pengaduk 500-900 psm dan kadar pengudaraan 0.65 iim dengan glukosa sebagai sumber karbon. k_La dan penggunaan kuasa berubah dengan tempoh fermentasi. Data yang diperolehi dipadankan dengan korelasi matematik antara k_La dan keperluan kuasa semasa pengassan bagi sunit isipadu cecair (P_g/V) dan halaju superfisial gas yang dilapurkan oleh penyelidik terdahulu. Dengan memasukkan halaju pengaduk, korelasi-korelasi yang lebih baik didapati, menandakan kepentingan halaju pengaduk di dalam korelasi berkenaan. Perhubungan yang dicadangkan ialah: $k_La = k\ (P_g/V)^{0.95}\ U^{0.67}\ N^{0.5}$, dimana U ialah halaju superfisial gas dan N ialah halaju putaran pengaduk.*

ABSTRACT

A batch culture system for the production of citric acid by Aspergillus niger *TISTR 3089 was studied to determine the effect of aeration and mixing on the volumetric oxygen transfer coefficient (k_La) and power requirement. A 2-l batch stirred-tank was used with impeller speeds of 500-900 rpm at an aeration rate of 0.65 vvm with glucose as the carbon source. k_La and power consumption varied with duration of fermentation. The data obtained were fitted to mathematical correlations between k_La and power requirement during gassing per unit volume of liquid (P_g/V) and superficial gas velocity reported by previous researchers. By including the agitator speed, better correlations were obtained, indicating the importance of stirrer speed in such correlations. The proposed relationship is: $k_La = k\ (P_g/V)^{0.95}\ U^{0.67}\ N^{0.5}$, where U is the superficial gas velocity and N is the stirrer speed.*

INTRODUCTION

Citric acid fermentation is usually done in a batch system, using moulds such as *Aspergillus niger*. During the fermentation process, the effect of environmental factors such as pH, temperature, aeration and mixing are critical (Berry *et al.* 1977). The degree of mixing significantly influences the efficiency of oxygen transfer, as the bubble size can be reduced and gas hold-up increased. Power consumption is closely related to degree of mixing. The relationship between volumetric oxygen transfer coefficient and power consumption is useful and important in the design and scaling-up of bioreactors. Various correlations have been suggested, especially for newtonian liquids (Cooper *et al.* 1944; Bartholomew 1960; Richards 1961; van't Riet 1983) and non-newtonian systems (Blakebrough and Sambamurthy 1966; Manfredini and Cavallera 1983; Kargi and Moo-Young 1985). Taguchi and Humphrey (1966) studied the relationship between oxygen transfer rate and power consumption in an Endomyces fermentation system which is pseudoplastic. The

objective of this study is to investigate the relationship between power consumption and oxygen transfer in citric acid fermentation by *Aspergillus niger*.

MATERIALS AND METHODS

Microorganism

The microorganism used was *Aspergillus niger* TISTR 3089 obtained from Scientific and Technological Research Institute, Thailand. The culture was grown in potato dextrose agar (PDA) slants and kept at 4°C.

Medium

The medium was made up of 180 g/l glucose, 2.0 g/l ammonium nitrate, 2.0 g/l potassium hydrogen phosphate, 0.5 g/l magnesium sulphate, 0.1 mg/l ferric sulphate, 0.1 mg/l zinc sulphate and 0.06 mg/l copper sulphate. The pH was adjusted to pH 4.5. and the medium was autoclaved at 121°C for 15 minutes. Glucose was autoclaved separately and added later.

Inoculum

The culture from the agar slant was transferred to a petri dish containing PDA, and incubated at 30°C for 7-10 days. Sterile water was added to the petri dish containing the spores. The spore suspension was then collected aseptically and its optical density was determined at 565 nm to be within 0.8-0.85.

The spore suspension was then transferred to a shake flask containing the fermentation medium. It was incubated on an orbital shaker at 200 rpm and 30°C for 1-2 days to obtain the inoculum for the fermenter; 10% (v/v) inoculum was used.

Fermentation

The fermenter used was a 2-l Braun Biostat M stirred-tank reactor with a 6-bladed Rushton turbine impeller. The reactor was equipped with ports for air inlet and outlet, acid and alkali, antifoam, inoculation, sampling and pH and oxygen probes.

Pure oxygen was used for aeration. Impeller speeds ranged from 500-900 rpm. Sterile silicone oil was used to control foaming. Fermentation proceeded for 7-8 days. Sampling was done daily. Power requirement, k_La, pH, glucose, citric acid and dry cell weight were determined throughout the fermentation process.

Glucose was determined by the dinitrosalicylic acid method (Miller 1959). Citric acid was determined by HPLC using Lichrosorb RP-18 column with 8 mM sulphuric acid as the mobile phase and UV detector at 210 nm. For the dry weight, the sample was centrifuged, re-suspended with distilled water and centifuged again before drying in an oven at 105°C overnight. The k_La was measured by the dynamic gassing-out technique. A torsion dynamometer was used for power measurement.

RESULTS AND DISCUSSION

Table 1 shows the effect of stirrer speed on biomass (cell weight), citric acid production, glucose consumption, pH changes, k_La and power consumption. The kinetic data showed the expected pattern for any batch fermentation, although the citric acid yield is rather low.

Table 2 compares the experimental fit of correlations based on current experimental data in the literature as well as the new correlation proposed. Using the correlation suggested by Cooper *et al.* (1944), i.e. $k_La = k\ (P_g/V)^{0.95}\ U^{0.67}$; the regression coefficient R^2 is 0.78. When the square root of the stirrer speed is added inside the correlation, a better fit is obtained, with $R^2 = 0.83$.

Richards (1961) suggested $k_La = k\ (P_g/V)^{0.4}\ U^{0.5}$; using 0.4 as the exponent on the power per unit volume. Fitting the current experimental data using that relationship, $R^2 = 0.78$. By including the term for the stirrer speed $N^{0.5}$, there was better correlation with $R^2 = 0.82$.

Taguchi *et al.* (1968) suggested the correlation $k_La = k\ (P_g^{0.33}/V)\ U^{0.56}$. Using this relationship, $R^2 = 0.77$. When $N^{0.5}$ is included inside the relationship, again a better fit of the current experimental data is obtained ($R^2 = 0.81$). This further reinforces the suggestion that the stirrer speed is an important factor in the correlation.

Richards (1961) showed that the liquid mass transfer coefficient (k_L) is proportional to the square root of the stirrer speed. From Calderbank (1967), under constant surface tension and terminal gas velocity, the volumetric gas-liquid interfacial area (a) would be proportional to $(P_g/V)^{0.4}\ U^{0.5}$. Combining these two for an expression for the volumetric oxygen transfer coefficient, k_La is then proportional to $(P_g/V)0.4\ U^{0.5}\ N^{0.5}$. This gives the theoretical foundation for the proposed correlation in this study.

TABLE 1
Volumetric oxygen transfer coefficient, power and kinetic data for citric acid fermentation by *Aspergillus niger* TISTR 3089

Stirrer Speed (rpm)	Time (days)	k_La (/h)	Power (kW)	Glucose (g/l)	Citric Acid (g/l)	Cell Wt. (g/l)	pH
500	0	1.69	0.44	180	0.0	1.7	3.0
	1	1.73	0.70	165	0.0	6.5	1.9
	2	1.78	0.79	140	0.6	10.4	1.8
	3	2.10	1.01	99	2.3	12.4	1.7
	4	2.21	1.14	95	3.4	14.3	1.4
	5	2.69	1.27	88	4.2	17.1	1.4
	6	3.11	1.40	70	4.6	17.3	1.4
	7	3.38	1.44	39	4.7	18.4	1.4
	8	4.01	1.44	21	4.8	18.6	1.4
700	0	1.89	0.61	180	0.0	1.8	3.1
	1	2.26	0.80	161	0.0	8.9	2.2
	2	3.81	1.04	130	0.8	12.3	2.0
	3	4.93	1.23	88	2.9	14.8	1.9
	4	5.24	1.41	57	3.8	16.6	1.8
	5	5.54	1.65	56	4.7	18.5	1.8
	6	5.89	1.84	45	5.4	20.6	1.8
	7	5.99	1.86	33	5.9	22.4	1.8
	8	6.01	1.90	20	5.9	22.4	1.8
900	0	1.97	0.79	180	0.0	1.8	3.1
	1	2.56	0.79	159	0.0	9.9	2.7
	2	3.42	0.87	140	0.9	13.9	2.5
	3	3.94	1.02	85	3.0	16.5	2.2
	4	4.75	1.26	47	3.9	20.0	2.1
	5	5.88	1.58	25	4.9	25.4	2.0
	6	6.11	1.97	18	5.8	25.8	2.0
	7	6.13	2.36	16	6.1	25.8	1.9
	8	6.1 4	2.36	15	6.2	25.7	1.9

TABLE 2
Experimental fit of current results to various correlations

			R^2
1.	Cooper *et al.* (1944)	$k_La = k\ (P_g/V)^{0.95}\ U^{0.67}$	0.77
	New correlation	$k_La = k\ (P_g/V)^{0.95}\ U^{0.67}\ N^{0.5}$	0.83
2.	Richards (1961)	$k_La = k\ (P_g/V)^{0.4}\ U^{0.5}$	0.78
	New correlation	$k_La = k\ (P_g/V)^{0.4}\ U^{0.5}\ N^{0.5}$	0.82
3.	Taguchi *et al.* (1968)	$k_La = k\ (P_g^{0.33}/V)\ U^{0.56}$	0.77
	New correlation	$k_La = k\ (P_g^{0.33}/V)\ U^{0.56}\ N^{0.5}$	0.81

CONCLUSION

The results of this study show that in all cases, by including the square root of the stirrer speed inside the equations for correlations suggested by previous researchers, a better correlation between the volumetric oxygen transfer coefficient and power requirement during citric acid fermentation by *Aspergillus niger* was obtained. Thus it is suggested that the stirrer speed should be taken into consideration in such correlations.

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Effect of Interactions of Three Growth-promoting Microorganisms on VAM Colonization, Spore Density, Plant Growth and Nutrient Accumulation in Tomato *(Lycopersicon esculentum)* Seedlings

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ABSTRAK

Kajian dibuat terhadap interaksi Azospirillum brasilense *dan* Bacillus megaterium *var.* phospharicum *dan* Glomus fasciculatum *dalam rizosfera tanaman tomato. Tidak terdapat perbezaan yang signifikan dalam parameter pertumbuhan tanaman antara rawatan-rawatan inokulat - VAM. Tanaman-tanaman yang dinokulat dengan fosfobakteria jelas mempunyai tunas yang lebih panjang yang mana bersamaan dengan VAM dan cantuman VAM + fosfobakteria yang lain. Cuma* Azospirullum *atau fostobakteria sahaja menambahkan biojisim tanaman dibandingkan dengan kawalan tak inokulat. Indeks VAM jelas menurun dengan penambahan fosfobakteria. Pemekatan nutrien tisu tidak berbeza antara rawatan.*

ABSTRACT

Interactions of Azospirillum brasilense *and* Bacillus megaterium *var.* phosphaticum *and* Glomus fasciculatum *in the rhizosphere of tomato plants were studied. There was no significant difference in plant growth parameters between VAM-inoculated treatments. Plants inoculated with the phosphobacteria had significantly higher shoot length, which was equivalent to VAM and other VAM + phosphobacteria combinations.* Azospirullum *or phosphobacteria alone increased plant biomass compared with the uninoculated control. VAM index was significantly reduced with the addition of phosphobacteria. There was no difference in tissue nutrient concentrations between treatments.*

INTRODUCTION

Interactions of growth-promoting microbial populations in the rhizosphere of VA-mycorrhizal plants have been studied by many workers (Barea *et al.* 1983; Pacovsky and Fuller 1985; Linderman 1988; Baas 1990). Subba Rao *et al.* (1985) reported that the synergistic interactions of VAM and *Azospirillum brasilense* significantly increased dry matter production and grain yield of barley. Response of plants to colonization by mycorrhizas depends on many biotic and environmental factors. Plant-available P is considered to influence the degree of mycorrhizal symbiosis (Bethlenfalvay *et al.* 1982). Among the many soil microorganisms known to solubilize unavailable forms of P, phosphobacteria have been used as bacterial fertilizer (Bagyaraj 1984). These bacteria survive for a longer period in the rhizosphere of mycorrhizal roots (Linderman 1988). Hence this trial aimed to study the interactions of VAM fungus with *Azospirillum* and phosphobacteria in rhizosphere soils of tomato seedlings and their effect on plant growth, tissue nutrient concentration, VAM colonization and spore density.

MATERIALS AND METHODS

The soil used was a nutrient deficient (N 225, P 22.5, K 780, Zn 0.20 and Cu 0.78 kg/ha^{-1}) alluvial deposit of sandy loam with pH 7.2 and EC 0.2 milli S/cm^{-1} from the Bharathiar University

Campus, Coimbatore. A mixture of equal parts of soil and sand autoclaved at 121°C and 15lb/inch^2 (1 h each on three consecutive days, followed by 1 week incubation at room temperature) was used to fill 30 x 12 cm polyethylene bags (about 3 kg per bag). As bacteria require an organic substratum for initial establishment in the soil (Lynch 1983; Subba Rao 1993), 50 g of autoclaved, (121°C, 15 lb/inch^2) dried cowdung was added to the topsoil in each bag.

A stock culture of *Glomus fasciculatum* was used as VAM inoculum, since it is known that this species is most effective in enhancing growth and P uptake (Sulochana *et al.* 1989; Sivaprasad *et al.* 1992). It was maintained in a pot culture of 90-day-old maize. Fresh cultures of *Azospirillum brasilense* and phosphobacteria, *Bacillus megaterium* var. *phosphaticum* (obtained from the Tamil Nadu Agricultural University, Coimbatore) were used as bacterial inocula. Ten grams of VAM inoculum soil, containing approx. 644 spores (64 spores/g dry soil) along with lyphae and infected root fragments/10 g charcoal base containing about 10^9 bacterial cells, (1 g charcoal base containing 10^8 bacterial cells) were placed as a thin layer about 2 cm below the soil surface in the bags. The control bags received autoclaved inocula. The treatments used were: (i) VAM-free (control), (ii) *Azospirillum*, (iii) phosphobacteria, (iv) VAM, (v) VAM + *Azospirillum*, (vii) VAM+ *Azospirillum* + phosphobacteria. Seeds of tomato *(Lycopersicon esculentum* Mill.) cv. Co 1 were sown in all the bags at the rate of 10 seeds/bag^{-1}. The bags were kept in a greenhouse, watered regularly and the seedlings were thinned on the 5th day after emergence (DAE) to maintain one seedling per bag. Each treatment was replicated four times.

At 60 DAE, the plants were harvested and growth parameters such as shoot and root length, leaf area, biomass, tissue nutrient (N, P, K, Zn and Cu) concentrations, VAM colonization index (VAMI) and spore density were determined. Leaf area was measured using a leaf area meter. Plant biomass was recorded after drying at 60°C for 12 h. Determination of VAMI was done after staining the root samples following the method of Phillips and Hayman (1970) and using the scoring method of Edathil *et al.* (1994). Spore density was assessed using the modified wet-sieving and decanting method (Gerdemann and Nicolson 1963) and expressed as the number of spores per gram of dry soil. Tissue nutrient concentration was determined following the standard methods of Jackson (1973).

RESULTS

There was no significant difference in the growth parameters of tomato seedlings between VAM treatments. Phosphobacteria-inoculated seedlings exhibited the highest shoot length (73 cm), which was equivalent to VAM (67 cm) and VAM + phosphobacteria (64 cm) combinations. Seedlings inoculated with *Azospirillum* or phosphobacteria alone had higher biomass than the uninoculated control. Leaf area and root length were more or less equal in all treatments (Table 1).

TABLE 1
Effect of interactions of microorganisms in the rhizosphere on the growth of tomato plants

Treatment	Leaf area (cm^2)	Shoot length (cm)	Root length (cm)	Biomass (g)
VAM-free (control)	23.05^a	61.00^{bc}	46.75^{ab}	2.48^d
Azospirillum	17.77^a	60.25^{bc}	33.25^b	4.23^a
Phosphobacteria	22.22^a	73.00^a	43.75^{ab}	4.12^a
VAM	20.81^a	67.00^{ab}	42.00^{ab}	3.87^{ab}
VAM + *Azospirillum*	19.32^a	60.75^{bc}	40.25^{bc}	3.01^{bd}
VAM + Phosphobacteria	18.20^a	64.75^{ab}	46.75^{ab}	3.05^{bcd}
VAM + *Azospirillum* + Phosphobacteria	23.15^a	64.50^{ab}	58.75^a	3.39^{abd}

Values are mean of four replications.
Values with the same letter are not significantly different P> 0.05 according to Duncan's new multiple range test.

The VAM and VAM + *Azospirillum*-inoculated plants registered higher VAMI than phosphobacteria-inoculated treatments. However, spore density was equal in all VAM treatments (Table 2). The accumulation of N, K, Zn and Cu in plant tissue was equal in all treatments. In the case of P accumulation, there was no regular trend (Table 3).

DISCUSSION

The enhancement of plant growth with the addition of VAM fungi (Nicolson 1960; Koske *et al.* 1975; Tinker 1975, 1978; Menge *et al.* 1978; Koske 1981; Abbott and Robson 1982), *Azospirillum* (Barea *et al.* 1983; Pacovsky and Fuller 1985; Palanisami 1985; Subba Rao *et al.* 1985) and phosphobacterium (Graeves and

TABLE 2
Effect of interactions of microorganisms in the rhizosphere of tomato plants on VAM colonization and spore density

Treatment	VAMI (%)	Spore Density (individuals/g^{-1} dry soil)
VAM-free (control)	-	-
Azospirillum	-	-
Phosphobacteria	-	-
VAM	62.25[a]	12.61[a]
VAM + *Azospirillum*	69.67[a]	11.34[a]
VAM + Phosphobacteria	49.10[bc]	11.16[a]
VAM + *Azospirillum* + Phosphobacteria	42.51[ab]	9.23[a]

Values are means of four replications
Values with the same letter are not significantly different at P>0.05 according to Duncan's new multiple range test.

TABLE 3
Effect of interactions of microorganisms in the rhizosphere on tissue nutrient concentrations in tomato plants

Treatment	N (%)	P (%)	K (%)	Zn (%)	Cu (%)
VAM-free (control)	1.82[a]	0.14[b]	4.3[a]	0.011[a]	0.001[a]
Azospirillum	1.99[a]	0.15[ab]	4.2[a]	0.01[a]	0.0013[a]
Phosphobacteria	2.10[a]	0.16[a]	3.6[a]	0.01[a]	0.0012[a]
VAM	1.67[a]	0.14[b]	4.1[a]	0.01[a]	0.001[a]
VAM + *Azospirillum*	1.74[a]	0.16[a]	4.4[a]	0.01[a]	0.0011[a]
VAM + Phosphobacteria	1.81[a]	0.12[c]	4.1[a]	0.01[a]	0.0011[a]
VAM + *Azospirillum* + Phosphobacteria	2.01[a]	0.17[a]	4.2[a]	0.01[a]	0.0014[a]

Values are means of four replications
Values with the same letter are not significantly different at P>0.05 according to Duncan's new multiple range test.

Webley 1965; Bagyaraj 1984; Meyer and Linderman 1986) has been well documented. Abbott and Robson (1982) reported that VAM fungi would manifest their performance to a greater extent in low nutrient soils. As sufficient nutrients are available in soil, the test plants could directly absorb the nutrients rather than depending on VAM fungi or other rhizosphere microorganisms. Futhermore, the addition of organic manure will result in increased soil microbial population releasing organic acids such as lactic acid, all of which have chelating properties which will ultimately promote P solubilization (Sperber 1958a, 1958b; Louw and Webley 1959; Duff *et al.* 1963; Banik and Dey 1982). If nutrient availability, especially phosporus, is high, the host plant may show a negative growth response to VAM fungi and VAM colonization can be reduced (Hayman *et al.* 1975; Johnson 1976; Sparling and Tinker 1978; Koide 1991). Data in Table 2 substantiate this observation because addition of phosphobacteria significantly reduced VAM index in tomato plants. The VAMI was significantly higher in VAM and VAM + *Azospirillum* treatments.

Higher shoot length was observed in the treatment with phosphobacteria or its combination with VAM fungus (Table 1). Shoot elongation may be a function of the excretion of certain growth promoting substances by the bacteria, because researchers have proved that the growth promotion by B. megaterium is mainly due to their excretion of growth-promoting hormones and vitamins (Banik and Dey 1982; Meyer and Linderman 1986).

Tien *et al.* (1979) reported the production of plant hormones by *Azospirillum.* The plant hormones present in bacterial cultures may improve the formation and development of VA mycorrhiza (Azcon *et al.* 1978).

The unaffected nature of VAMF spore density in the rhizosphere of tomato seedlings co-inoculated with either Azospirillum or phosphobacteria probably indicates the positive interactions among these growth-promoting micro-organisms (Table 2).

Since there is no difference in the tissue concentrations between the treatments (Table 3), it may not serve as an indication of the beneficial microbial interactions in a nutrient-rich (organic manure amended) soil with regard to nutrient accumulation.

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Influence of Seed Ripeness, Sarcotesta, Drying and Storage on Germinability of Papaya (*Carica papaya* L.) Seed

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Keywords: papaya, seeds, germination, fruit maturity, sarcotesta, drying

ABSTRAK

*Betik (*Carica papaya *L.) adalah buah-buahan tropikal terkenal yang dibiakkan oleh biji benih. Walau bagaimanapun, percambahan dan cara mendapatkan anak benih yang baik dalam spesis ini adalah rumit kerana keadaan biji benihnya. Sehingga kini, satu siri kajian untuk menilai kesan kematangan buah, sarkotesta dan kekeringan terhadap percambahan dan penghasilan anak benih yang baik masih diteruskan. Ciri-ciri biji benih buah betik bertukar bersama kematangan buah. Kecepatan percambahan meningkat bersama kematangan buah. Setakat ini, biji benih yang terbaik untuk percambahan dan untuk pemerolehan anak benih yang subur di dapati dari buah-buah masak atau yang terlebih masak. Kewujudan sarkotesta mengurangkan percambahan dan meningkatkan bilangan anak benih yang luar biasa. Gabungan sarkotesta dalam kesederhanaan percambahan biji benih betik atau padi tidak menghalang percambahannya. Ini bermakna bahawa halangan lebih berpunca dari sarkotesta yang tidak tersentuh bukan dari penghalang-penghalang yang diperolehi daripadanya. Mengering biji benih betik di bawah suhu berudara dan teduh, akan mengekalkan proses percambahan ke darjah yang lebih tinggi daripada bila biji benih dikeringkan di dalam oven. Penurunan kelembapan biji benih di bawah 10% yang mengurangkan percambahan, menunjukkan corak sifat yang sederhana untuk biji benih betik dibandingkan biji benih ortodok atau degil.*

ABSTRACT

Papaya (Carica papaya *L.) is a popular tropical fruit which is propagated by seed. However, germination and the procurement of good seedlings are difficult in this species due to the nature of the seed. Thus a series of studies to evaluate the influence of fruit maturity, sarcotesta and drying on germination and production of healthy seedlings was carried out. The characteristics of papaya seed change with fruit maturity. Speed of germination increases with fruit maturity. Thus, the best seed for germination and for the the procurement of healthy seedlings is obtained from ripe or over-ripe fruits. The presence of the sarcotesta reduces germination and increases the number of abnormal seedlings. Incorporation of the sarcotesta in the germination medium of papaya or rice seeds did not inhibit their germination. This suggests that inhibition is caused by the intact sarcotesta rather than inhibitors derived from it. Drying papaya seeds under shade and ambient temperature maintained germinability to a greater degree than when seeds were desiccated in ovens. The reduction of seed moisture below 10% reduced germination significantly, indicating an intermediate behaviour pattern for papaya seeds in contrast to orthodox or recalcitrant seeds.*

INTRODUCTION

Seeds are a primary source of plant propagation in agriculture, horticulture and forestry, as they are dispersal units consisting of an embryo, food reserves and protective structure (Roberts and King 1989). Seeds of most cultivated species can be dehydrated and stored under conditions of low humidity and temperatures for varying lengths of time, without loss of germinability (Ellis 1991). Such seeds are classified as orthodox types (Roberts and King 1989; Hofmann and Steiner 1989).

Another group of seeds retains high moisture contents during maturation. These seeds do not withstand desiccation and need to be stored at a high moisture content. The seed

moisture content at which germinability is lost varies from species to species and according to the drying regime (Farrant *et al.* 1988). These seeds are classfied as recalcitrant and are common among tropical and subtropical perennial species (Chin and Roberts 1980; Roberts *et al.* 1984).

An intermediate category of seed has been identified (Ellis *et al.* 1990); these survive desiccation to approximately 10% moisture content, but further drying reduces germinability (Ellis 1991). Seeds of several important food crops, e.g. coffee (Ellis *et al.* 1990) are of this type.

Papaya seeds have been classified as recalcitrant (Chin *et al.* 1984; Hofmann and Steiner 1989) and more recently as the intermediate type (Ellis *et al.* 1990).

Propagation of papaya by seed is difficult due to rapid seed deterioration after harvest. This is attributed to microbial degeneration of the sarcotesta, which reduces viability (Begum *et al.* 1987), although Gherardi and Valio (1976) had reported the presence of growth-inhibiting substances in the mucilage covering the seed. However, these studies do not clearly identify the influence of fruit maturity and the presence or the sarcotesta on the germinability of papaya seeds, before and after drying. Thus three experiments were carried out with the objective of determining the importance of fruit maturity, the sarcotesta and the process of drying on the germinability of papaya.

MATERIALS AND METHODS

The experiments used seeds of commonly available papaya ecotypes in Sri Lanka, which are a mixture of the Hawaiian and Indian varieties.

Experiment 1. Influence of Fruit Maturity and the Presence of the Sarcostesta on Germination of Papaya Seeds

Seeds were removed from mature, ripe and over-ripe papayas. These stages corresponded to green fruits with a yellow tinge, with hard pink flesh (mature), yellow-green fruit with soft edible red flesh (ripe) and yellow fruit with pulpy red flesh not suitable for consumption (over-ripe) respectively.

Soon after extraction, four replicates of 100 seeds from each maturity stage were planted at a depth of 2 cm in washed river sand (diameter 0.5-0.6 mm). Similar replicates of seeds from the three maturity stages were planted in the same manner after removal of the sarcotesta by rubbing with sand. The fresh and dry weights of seed and sarcotesta were recorded. Germination and the percentage of abnormal seedlings were determined beginning from day 5 after planting, up to day 30.

Experiment 2. Effects of Drying on Storability and Germination of Papaya Seeds

Based on the results of the experiment, eight replicates, each of 600 seeds of ripe and over-ripe fruits, 50% with the sarcotesta removed as described above were dried either under partial shade at a mean ambient temperature of 28°C ± 2.6° or oven dried at 40°C ± 1.5°.

Subsamples (150 seeds) from each replicate for each of the four treatments were dried to moisture contents of 25, 10 and 5% and stored in sealed containers. Germination was determined at 0, 30 and 90 days after storage using 50 seeds per replicate.

Experiment 3. Influence of the Sarcotesta on the Germination of Papaya and Rice Seeds

Seeds obtained from ripe and over-ripe papaya fruits were divided into nine seedlots, each containing 300 seeds. The sarcotesta of seeds in each seedlot were carefully separated and placed in individual petri dishes, while the clean seeds were washed in distilled water.

Within the same maturity category, the sarcotesta from one seedlot were mixed with clean seeds of another sample, which were planted in trays and germination determined as described in Experiment 1. This was carried out on all six groups.

The sarcotesta of the remaining three seedlots were mixed with three replicates of rice seeds (variety BG 34-8), each containing 300 seeds, and planted in sand at a depth of 2 cm. Another three replicates of rice seeds were planted without mixing with the papaya sarcotesta. Control treatments of seeds with and without the sarcotesta were also maintained for comparison. Germination and numbers of abnormal seedlings were determined on day 21 after planting.

The data of all experiments were analysed statistically to determine the significance of the different treatments.

RESULTS AND DISCUSSION

The stage of maturity of papaya fruits had a significant influence on seed characteristics (Table 1). Seeds of mature unripe fruits had the lowest fresh weight. The sarcotesta accounted for 50% of fresh weight, and the seeds had a very high moisture content. Thus in comparison dry weight was low.

Fresh weight of seeds increased in ripe fruits but did not change significantly in over-ripe fruits. The weight of the sarcotesta also increased in seeds of ripe fruits, but this constituted only 45% of the seed fresh weight, and it was lower than in seeds of mature fruits. Further ripening did not change these parameters.

Seed moisture content declined with fruit maturity, and the dry weights increased, culminating in a 100-seed dry weight of 2.41 g in ripe fruits. Accumulation of photosynthates in the endosperm and loss of seed moisture are characteristic of seed maturity (Hanson 1984).

Stage of maturity and the presence of the sarcotesta had a significant impact on germination and development of abnormal seedlings (Table 2). The interaction between these two variables was significant in all samples.

Germination of seeds from mature fruits was lowest and the percentage of abnormal seedlings was the highest. In contrast, there were no differences in these parameters between seeds obtained from ripe and over-ripe fruits. This clearly shows the importance of stage of fruit maturity in determining the germinability of papaya seeds and the procurement of healthy normal seedlings. It also confirmed the unsuitability of using seed from mature but unripe fruit for the propagation of papaya. This could be attributed to incomplete development and high moisture content of the seeds, both of which affect germination and seedling development.

Germination of seeds of mature fruits was low on the 10th day, but increased significantly thereafter (Table 2), in contrast to that of the other two categories. Stage of fruit maturity had a direct impact on speed of germination. Although the number of abnormal seedlings was greater from seeds of mature fruits, the increments in number between the 10th and 30th day was similar (approximately 72%) in all seedlots. Thus development of abnormal seedlings was not affected by fruit maturity, in contrast to germination.

The presence of the sarcotesta reduced germination, and echanced the number of abnormal seedlings (Table 2). This confirms earlier reports of the detrimental effects of the sarcotesta on germination of papaya seeds (Gherardi and Valio 1976; Begum *et al.* 1987). However, the effect of the sarcotesta in reducing germination and enhancing the number of abnormal seedlings differed with fruit maturity, having a greater inhibitory effect on the germination of seeds from ripe and over-ripe fruit, while the number of abnormal seedlings was greatest in seeds of mature fruits on the 30th day. Reyes *et al.* (1980) have suggested the presence of growth inhibitors in the sarcotesta as the causal agent.

TABLE 1
Characteristics of seeds obtained from mature, ripe and over-ripe papaya

Characteristic	Mature Fruit	Ripe Fruit	Over-ripe Fruit	LSD
Fresh wt. per 100 seeds (g)	9.45	11.84	11.59	0.152
Fresh wt. of sarcotesta of 100 seeds (g)	4.81	5.42	4.99	0.029
Dry wt. per 100 seeds without sarcotesta (g)	1.15	1.94	2.41	0.008
Moisture content (%)	76.0	64.2	51.7	2.619

TABLE. 2

Influence of fruit maturity and presence of sarcotesta on germination and occurrence of abnormal seedlings in papaya

Fruit Type	Sarcotesta	Observation (Days after planting)					
		10		20		30	
		A	B	A	B	A	B*
Mature	Absent	30.1%	10.6%	42.5%	16.2%	48.2%	18.5%
	Present	24.2%	22.6%	36.0%	32.0%	42.1%	38.0%
Ripe	Absent	75.4%	8.4%	82.0%	9.5%	87.5%	10.0%
	Present	37.4%	12.5%	46.0%	17.2%	56.8%	21.0%
Over-ripe	Absent	78.2%	9.0%	84.5%	7.2%	89.0%	7.0%
	Present	39.5%	14.0%	50.2%	18.0%	57.0%	24.5%
LSD (P=0.05)	Fruit type	9.59	14.24	4.98	9.07	6.91	2.91
	Presence of Sarcotesta	2.51	8.55	1.95	2.80	1.92	4.90
	Interaction	*	*	*	*	*	*

* A - Percentage germination; B - Percentage abnormal seedlings.

The influence of the sarcotesta and method of drying on storability and germinability of papaya seeds is presented in Table 3. Seeds from ripe and over-ripe fruits were used due to the poor germinability of those from mature fruit (Table 2). The germination response was similar in both types of seed and drying significantly reduced germination, confirming the poor response of papaya seeds to desiccation (Chin *et al.* 1984). Thus the highest percentage germination was observed at a seed moisture content of 25%, irrespective of other treatments. Lowering of seed moisture to 10% reduced germination in both categories of seed, although the decrease was not excessive. In contrast, drying seeds to 5% moisture decreased germination significantly. Thus, as reported by Ellis *et al.* (1990), seeds of ripe and over-ripe papaya fruits seen to tolerate desiccation to 10% seed moisture content without considerable loss of germination. Thus, these seeds could be dried to a greater degree than most recalcitrant seeds, which lose viability at approximately 30% moisture content, thereby exhibiting intermediate characteristics, as suggested by Ellis *et al.* (1990).

The absence of the sarcotesta increased germinability significantly irrespective of fruit maturity, drying regime or seed moisture content. This confirms earlier results of the adverse effects of the sarcotesta on germination, due to the presence of inhibiting compounds (Begum *et al.* 1987). However, when compared with germination values at 25% moisture content, its effect in reducing germination is lower at reduced seed moisture contents. This indicates that drying, ecpecially to 5% seed moisture content, may deactivate the germination inhibitors. However, drying to this low seed moisture level reduced germination of all seeds significantly. Thus the beneficial impact of removing the inhibitory effect of the sarcotesta is minimal in dried seeds. Storage of seeds does not negate the adverse effect of the sarcotesta, thus illustrating the value of removing it prior to planting.

Oven drying decreases germination irrespective of fruit maturity and the presence of the sarcotesta (Table 3). This is due to the higher temperature regime, which could destroy the embryo and/or cause detrimental changes to the endosperm reserves. Drying at moderate temperatures under shade allows the process of desiccation to progress gradually, thus causing minimal changes to the seed. However, germination decreases with length of storage for both categories of seed dried to three moisture levels. The decline in germination is most significant in seeds dried to a moisture content of 5%. This again confirms the adverse effect of low moisture in papaya. The decline in germination with length of storage is greater when seeds are dried with the sarcotesta, again showing the importance of removing it.

TABLE 3
Influence of sarcotesta and drying regime on storability and germinability of papaya seed at various levels of seed moisture content

Fruit Type	Sarcotesta	Method of Drying	Storage Period (days) 0	30	90
			Germination (%)		
25% Seed Moisture					
Ripe	absent	shade	84	75	70
		oven	65	52	42
	present	shade	50	41	36
		oven	34	27	20
	LSD (P=0.05)		2.1	3.8	1.9
Over-ripe	absent	shade	85	76	70
		oven	67	55	44
	present	shade	47	44	35
		oven	36	27	21
	LSD (P=0.05)		0.9	1.13	0.7
10% Seed Moisture					
Ripe	absent	shade	74	64	61
		oven	60	47	40
	present	shade	40	32	26
		oven	30	24	20
	LSD (P= 0.05)		1.3	0.4	0.3
Over-ripe	absent	shade	76	70	65
		oven	64	57	42
	present	shade	45	40	32
		oven	35	30	24
	LSD (P = 0.05)		0.8	1.2	2.9
5% Seed Moisture					
Ripe	absent	shade	27	18	16
		oven	22	14	8
	present	shade	16	10	9
		oven	10	8	6
	LSD (P = 0.05)		0.3	1.1	0.3
Over-ripe	absent	shade	22	26	12
		oven	18	12	7
	present	shade	16	9	5
		oven	9	6	5
	LSD (P = 0.05)		0.2	0.4	0.2

The adverse effect of the sarcotesta in inhibiting seed germination of other seeds in seen in Table 4. Application of extracted sarcotesta of a similar quantity as the papaya seed of the same maturity stage or to rice seed did not reduce germination or result in abnormal seedlings. This clearly illustrates that the inhibitory effect of the sarcotesta occurs only when kept intact. Thus, the possibility that the sarcotesta acts as a barrier to germination and healthy seedling development cannot be ignored, although the presence of germination inhibitors has been reported (Begum *et al.* 1987).

CONCLUSION

Fruit maturity affects seed quality in papaya. Seed development is complete at fruit ripening and not at maturity as was observed from seed dry weight and the contribution of the sarcotesta to this parameter.

The stage of maturity, the presence of sarcotesta and drying method affected seed quality. Seeds of ripe and over-ripe fruits are most suitable for propagation purposes. As in recalcitrant seeds (e.g. Sangakkara 1993), drying under shade at ambient temperatures maintains germinability in contrast to forced desiccation in ovens. Desiccation of seeds to a moisture content of less than 10% reduces germination significantly. Length of storage also decreased germination, especially when seeds were oven-dried.

Presence of the sarcotesta, which could be considered a protective cover, significantly inhibits germination of papaya seeds and increases the number of abnormal seedlings. Thus, removal of the sarcotesta increases germination. Addition of the extracted sarcotesta was only inhibitory when left intact; this aspect requires elucidation. High rates of germination and development of healthy seedlings can be obtained by using seeds from ripe or over-ripe fruits and from which sarcotesta is removed. If desiccation is required, this process should be carried out gradually under ambient temperatures.

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TABLE 4
Influence of sarcotesta of seed from ripe and over-ripe papaya fruits on germination of rice or other papaya seeds

Seed Type	Treatment	Germination (%) at 21 Days	Abnormal Seedlings (%) 21 Days
Ripe Papaya	with sarcotesta intact	47.2	31.0
	without sarcotesta	85.6	4.5
	with sarcotesta from other seeds	80.4	5.6
	LSD (P= 0.05)	4.03	5.73
Over-ripe Papaya	with sarcotesta intact	51.7	37.6
	without sarcotesta	88.0	5.8
	with sarcotesta from other seeds	83.6	6.1
	LSD (P = 0.05)	2.77	1.84
Rice	with sarcotesta from ripe papaya seeds	91.6	4.86
	with sarcotesta from over-ripe papaya seeds	94.2	3.96
	without sarcotesta	95.1	4.22
	LSD (P = 0.05)	1.23	1.04

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Component Analyses and their Implication on the Breeding of Soya Bean (*Glycine max* (L.) Merr)

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Keywords: soya bean breeding, correlation, factor analysis, selection index

ABSTRAK

Sepuluh ciri yang digabungkan dengan hasil biji benih dalam 20 genotip kacang soya, Glycine max *(L) merr, telah dianalisis menggunakan teknik-teknik korelasi, regresi dan analisis-analisis faktor. Korelasi linear berganda (R) 0.99 dengan koefisien penentuan 0.97 adalah direkodkan. Analisis regresi menunjukkan bahawa hari-hari mengeluarkan bunga, hari-hari kematangan, ketinggian untuk menuai, bilangan dahan setiap pokok, bilangan biji setiap lenggai, pembintilan dan panjang lenggai yang banyak dihasilkan kepada variasi adalah disebabkan oleh regresi. Hanya ketinggian untuk menuai dan hari-hari kematangan, secara positif dan signifikan berkaitan dengan hasil biji benih. Analisis faktor menghasilkan keputusan yang sama dengan analisis regresi dan kolerasi ciri-ciri tanaman. Empat faktor pertama diambil kira untuk 82.53% variasi dalam struktur bergantungan.*

ABSTRACT

The characters associated with seed yield in 20 genotypes of soya bean, Glycine max *(L) Merr. were analysed using techniques of correlation, regression and factor analyses. The multiple linear correlation (R) of 0.99 with a coefficient of determination of 0.97 was recorded. The regression analysis indicated that days to flowering, days to maturity, height at harvest, number of branches per plant, number of seeds per pod, nodulation and pod length contributed substantially to the variation due to regression. Only height at harvest and days to maturity were positively and significantly correlated with seed yield. Factor analysis produced a similar result to those of plant character correlation and regression analysis. The first four factors accounted for 82.53% of the variation in the dependence structure.*

INTRODUCTION

Seed yield in soya bean is a complex character influenced by the interplay of many other characters. Knowledge of the relationship of yield with its main components is important in plant breeding, particularly for indirect selection for quantitative traits, such as seed yield, that exhibit low heritability. Beside the yield components, physiological and morphological characters of soya bean plants are known to play a major and interdependent role in determining seed yield (Denis and Adams 1978; Bartual *et al.* 1985).

Correlation studies between characters and the use of multivariate analysis to determine the relative contribution of different characters to the total variation are of great value in determining the most effective breeding procedures (Bhatt (1976) in wheat; Ghaderi *et al.* (1979) in mung bean; Broich and Palmer (1980) and Ariyo (1995) in soya bean; Ariyo (1991a, 1991b, 1993 in okra).

The breeder has a number of desirable characters in mind when carrying out selection. To maximize improvement in the character of choice, selection is generally applied simultaneously to several other characters that influence the character of choice. Falconer (1960) reported that the most rapid improvement of economic value was expected from selection applied simultaneously to all the components. Use of selection index gives adequate weight to

each of the desirable characters identified. Hazel and Lush (1942) and Falconer (1981) reported that selection based on such an index was more efficient than selecting individuals on various characters. In addition, Ariyo (1991a) reported that the selection index should be used in conjunction with yield data preferably obtained across contrasting environments to produce valuable results.

The objectives of this study were:

(a) to determine the relative importance of various characters of soya bean and the relationship among them, and
(b) to construct selection indices for seed yield.

MATERIALS AND METHODS

Twenty genotypes, consisting of early, medium and late maturing varieties of soya bean from the International Institute of Tropical Agriculture (IITA), Ibadan were grown in a randomized complete block design with three replications. The planting was done at the University of Agriculture, Abeokuta, in July, 1991. Each entry was grown in four-row plots of 6 x 3m but only the competitive plants in the two inner rows were observed. Following planting, a mixture of 4 l Galex and 1 l Gramaxone in water was sprayed per hectare to control weeds. Subsequent weeding was done manually.

The number of days to flowering was recorded as the date the plants of a genotype attained 50% flowering. Maturity was when the pods had turned brown just before shattering. Shattering was taken as the proportion of guard rows that shattered two weeks after the two inner competitive plants had been harvested to determine seed yield; nodulation was assessed on the size and number of nodules at full bloom. Lodging was scored on the proportion of the plants that fell down at harvest. Genotype means, averaged across replications, were used for statistical analysis.

Phenotypic a coefficients of correlation were calculated among all the characters evaluated following the procedure of Steel and Torrie (1968). Step-wise multiple regression analysis (forward selection) was performed as outlined by Draper and Smith (1966) by which the multiple-regression equation and multiple coefficient of determination (R2) were obtained by adding independnet variables, one at a time depending on their relative importance, in determining dependent variables. Analysis was terminated when the proportion of dependent variance explained by adding each of the remaining variables was not significant at 0.05 level of probability. In this study, seed yield was fitted as a linear function of the other ten characters. The sequential contribution of each character to the total variation in seed yield was determined by the forward selection procedure. On the basis of the relative importance of each character to seed yield, selection indices were contructed.

Data were also subjected to factor analysis according to the procedure of Cattell (1965). The analysis produced factor loading as well as communality for each character from the variance-covariance matrix of the 20 genotypes. The factors were ranked on the basis of the magnitude of variability explained in the dependent structure. When the contribution of a factor to the variability was less than 10%, the process was terminated. The particular combination of variables that form a factor accounted for more of the variance of the data as a whole than any other linear combination variable. Therefore, Factor 1 was the best combination of the linear relationships in the data. Factor 2 was the best linear combination of variables that accounted for most of the residual variance after the effect of Factor 1 had been removed. Subsequent factors contributed progressively less to the total variance.

Communality is the amount of variance of a variable accounted for by all factors collectively and it is the R^2 value obtained by regressing a variable on all other variables in the model (Lee and Kaltsikes 1973; Eckert and Westfall 1975).

RESULTS

Table 1 presents the correlation coefficients between the various characters evaluated. Only height at harvest was strongly correlated with seed yield (r=0.65), while number of days to maturity was moderately correlated with seed yield (r=0.44). Height at harvest was also positively correlated with days to flowering (r=0.78) and maturity (r=0.57) while pod shattering was negatively correlated with days to maturity (r=0.75) and height at harvest (r=0.58). Number of branches per plant exhibited a positive relationship with days to flowering (r=0.59), days to maturity (r=0.55), height at harvest (r=0.58), and lodging at harvest (r=0.44) but was negatively correlated with pod shattering (r=-0.62). On the

TABLE 1
Evaluation of correlations among the eleven characters of soya bean

Characters	Seed Yield/ plot (g)	Days to Flowering	Days to Maturity	Height at Harvest (cm)	Lodging at Harvest	Pod Shattering	Number of Branches/ Plant	Number of Pods/Plant	Number of Seeds/Pod	Nodulation
Days to Flowering	0.30									
Days to Maturity	0.44*	0.35								
Height at Harvest (cm)	0.65**	0.78**	0.57**							
Lodging at Harvest	0.09	0.22	0.34	0.06						
Shattering	-0.35	-0.30	-0.75**	-0.58**	-0.01					
Number of Branches/Plant	0.22	0.59**	0.55*	0.58**	0.44*	-0.62**				
Number of Pods/Plant	-0.10	0.52*	0.38	0.29	0.61**	-0.28	0.80**			
Number of Seeds/Pods	0.34	0.08	0.07	0.19	-0.49*	0.00	-0.13	-0.36		
Nodulation	0.28	0.12	0.46*	0.28	0.01	-0.53*	0.44*	0.39	-0.19	
Pod Length (cm)	0.29	-0.14	-0.45*	0.10	-0.10	0.14	-0.27	-0.31	-0.27	-0.24

* Significant at P = 0.05; ** Significant at P = 0.01

other hand, number of pods per plant showed a correlation of r=0.52, 0.61 and 0.80 with days to flowering, lodging at harvest and number of branches per plant respectively. Nodulation was positively correlated with days to maturity and number of branches per plant, but negatively correlated with shattering. Pod length was negatively correlated with days to maturity (r=-0.45).

Four factors were obtained by factor analysis (Table 2); together these accounted for 82.53% of the variance for all the 11 characters. This implies that the factor-analysis model used in this study was effective in illuminating the unique variance of each variable. Communalities ranged from 0.6728 to 0.9360. Generally, factor loadings of 0.7 - 0.9 would be considered high loadings and those from 0 to 0.2 as low loadings (Denis and Adams 1978). In this study, however, for the purpose of interpretation only characters exhibiting factor loadings of 0.5 or larger were considered important and no character was loaded on more than one factor. It should be noted that the choice of loading of 0.5 or greater is arbitrary and does not imply biological significance. Biological interpretation of factors depends largely on the genotypes evaluated, the particular sets of characters measured, and how well the researcher understands the biology of the organism (Fakorede 1979). These are limitations of factor analysis.

Factor 1, which accounted for 39.44% of the total variance, contained days to flowering, days to maturity, height at harvest, shattering, number of branches per plant and number of pods per plant. The influence of a factor on a trait is determined by the square of the factor loading for that trait (Lee and Kaltsikes 1973). Therefore, Factor 1 accounted for 33% of the variance due to seed yield in this study. In addition, Factor 1 related essentially to the physiological aspect of the crop. Apart from number of pods per plant, other characters loaded in Factor 1 had direct bearing on a crop's growth and development.

Lodging at harvest and number of seeds per pod were grouped under Factor 2, and this factor accounted for 19.34% of the variance in the data as a whole. Factor 3 comprised only pod length, while Factor 4 was nodualtion; most characters in Factor 1 were correlated with each

TABLE 2
Evaluation of communalities and the factor loadings of eleven characters

Traits	Communalities	Factor Loadings			
		1	2	3	4
Factor 1					
Days to Flowering	0.8380	0.6940	0.1088	0.2378	0.5371
Days to Maturity	0.7694	0.8130	0.1252	-0.2680	-0.1441
Plant Height	0.9331	0.7724	0.4738	0.2929	0.1621
Shattering	0.7938	-0.7530	-0.2327	0.1695	0.3792
Number of Branches/Plant	0.8258	0.8678	-0.2480	-0.0034	0.1061
Number of Pods/Plant	0.8759	0.7017	-0.5956	0.0384	0.1654
Factor 2					
Lodging at Harvest	-0.6102	0.4136	-0.2327	0.3402	0.1172
Seed Yield/Plant	0.7885	0.4371	0.6715	0.3542	-0.1450
Seeds/Pod	0.8923	-0.0681	0.7234	-0.4168	0.4333
Factor 3					
Pod Length	0.9359	-0.2913	0.2312	0.8469	-0.2834
Factor 4					
Nodulation	0.7518	0.5794	-0.0260	-0.2484	-0.5948
Percentage of Total Variation		39.44	19.34	13.07	10.68
Cumulative Percentage		39.44	58.78	71.85	82.53
Eigen Value		4.33	2.13	1.43	1.17

other while correlations between characters were observed across factors.

Table 3 presents linear regression analysis of yield components. The analysis showed that all except lodging at harvest, shattering and number of pods per plant contributed significantly to the total variation due to regression. The multiple linear correlation coefficient between seed yield and the other ten characters was R=0.99 given a coefficient of determination of 0.97.

Table 4 gives the selection indices, using various character combinations and their relative effectiveness. The high value of the coefficient of determination indicated that the ten characters accounted for most of the variation in seed yield. The step-wise regression indicated that nine characters accounted for 97% of the total variation. The remaining character, shattering, did not meet the a = 0.5 significance level for entry into the model. Plant height alone accounted for 42.46% of the total variation due to regression. By inclusion of number of days to flowering, 53.62% of the total variation was explained. Adding number of branches per plant, lodging, number of seeds per pod, nodulation and pod length, 92.47% of the total variation was accounted for. Inclusion of days to maturity and number of pods per plant separately occasioned a marginal increase of about 2% in each case. It is generally accepted that correlation may not always adequately indicate the realtionship among variables. This is why multivariate rather than bivariate statistical methods are preferred.

TABLE 3

Multiple linear regression analysis of components of seed yield in soya bean

Source	DF	MS
Regression	10	205,820**
Days to flowering	1	194,393**
Days to maturity	1	271,429**
Height at harvest (cm)	1	673,161**
Lodging at harvest	1	46,325
Shattering	1	55,814
Number of branches/pods	1	179,221**
Number of pods/plant	1	4,476
Number of seeds/pod	1	188,524**
Nodulation	1	252,840**
Pod length (cm)	1	192,021**

DISCUSSION

The high correlation between days to flowering, days to maturity and height at harvest suggests that short varieties flowered earlier and matured earlier. However, high correlation between height at harvest and seed yield indicates that tall varieties yielded more than short varieties. The correlation between branches per plant, pods per plant and days to flowering indicates that late flowering plants produced more seed-bearing branches, which were possibly responsible for correlation between height at harvest and seed yield as only tall varieties could accommodate many branches. Late flowering varieties matured late and grew taller,

TABLE 4

Selection indices in soya bean for yield. Multiple regression equation

	R^2(%)
$Y1 = -11.62 + 0.45X_1$	42.46
$Y2 = 12.02 - 0.74X_1 - 0.86X_2$	53.62
$Y3 = 13.07 + 0.85X_1 - 0.69X_2 - 1.64X_3$	59.08
$Y4 = 6.86 + 0.9X_1 - 0.82X_2 - 2.61X_3 + 12.99X_4$	66.75
$Y5 = -8.29 + 0.85X_1 - 0.84X_2 - 2.44X_3 + 18.54X_4 + 4.95X_5$	73.10
$Y6 = -24.47 + 0.76X_1 - 0.62X_2 - 3.62X_3 + 23.39X_4 + 6.85X_5 + 3.13X_6$	82.04
$Y7 = -96.54 + 0.43X_1 - 0.15X_2 - 2.83X_3 + 17.35X_4 + 11.92X_5 + 4.92X_6 + 9.70X_7$	92.47
$Y8 = -222.87 + 0.06X_1 + 0.35X_2 - 2.54X_3 + 22.06X_4 + 13.17X_5 + 4.83X_6 + 15.73X_7 + 1.03X_8$	95.13
$Y9 = -243.86 - 0.08X_1 + 0.64X_2 - 1.41X_3 + 24.80X_4 + 13.01X_5 + 5.39X_6 + 16.38X_7 + 1.01X_8 + 13.01X_9$	97.10

X_1= Height at harvest; X_2 = Days to flowering; X_3 = Number of branches/plant; X_4 = lodging; X_5 = Number of seeds/pod; X_6 = Nodulation; X_7= Pod length; X_8= Days to maturity; X_9 = Number of pods/plant

besides producing more branches. That such varieties are susceptible to lodging suggests that a compromise must be struck between high yield and loss due to lodging at harvest. Correlation between pods per plant and days to flowering indicated that late flowering varieties which were also late maturing produced more pod-bearing branches. Of interest is the correlation between pods per plant and lodging at harvest. This implied that top-heavy varieties were likely to lodge under the weight of the pods.

The study identified days to flowering, days to maturity, number of branches per plant, height at harvest and pods per plant, some of these characters correlated among themselves, as important yield components in soya bean.

The results of regression analysis were complementary to the correlation studies by highlighting the relative weight of the various characters to the total variation. Height at harvest contributed the highest variance to yield followed by days to maturity, days to flowering, pod length and number of branches per plant in diminishing order of contribution. Similarly, the factor model identified the contribution of all these characters to the dependence structure. Days to flowering, days to maturity, height at harvest, number of branches per plant, number of pods per plant and pod length had high communalities, suggesting their importance in the dependence structure.

The various selection indices showed that height at harvest alone accounted for 42.46% of the total variation due to regression, demonstrating a multiple correlation coefficient (R) of 0.66. A selection index based on height at harvest and days to flowering gave the coefficient of determination of 53.62% of the total variable. The coefficient of determination continued to increase as more characters were entered, and terminated at R^2 of 97.10%. However, there was a marginal decrease in R^2 value as more characters were progressively added, indicating that earlier characters were more important than later entries. Although number of pods per plant, days to maturity and pod length were ranked higher in importance than lodging at harvest, nodulation and number of seeds per pod, these were nonetheless also important components of pod yield.

In breeding soya bean for high yield, therefore days to flowering, which to a large extent determines days to maturity and height at harvest, is a premium character. Other characters such as number of branches per plant, pod length and nodulation should also be considered. Similar observations were also reported by Denis and Adams (1978). Since it is better to apply selection generally to several characters that influence yield, a knowledge of the inheritance of the various characters in the selection index is required.

This study gives an insight into the association among traits observed in a set of genotypes. A statistical demonstration of association, whether by correlation, regression or factor analysis, however, does not provide information on the causative agents (genetic, physiological, morphological or environmental) (Fakorede 1979). Genetic analysis is necessary whenever the deterministic relationship is of interest.

Factor analysis has the limitations of the arbitrary and subjective nature of interpretation of factors and the dependence on the number and loadings of factors on the particular set of genotypes and variables. Despite these limitations, however, its data-reducing capacity gives it an advantage over correlation and regression analyses.

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Input and Output of Energy in Processing Gizzard Pickle

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ABSTRAK

Data dikumpul dari tujuh contoh untuk menentukan input tenaga komersil dalam pemprosesan jeruk daging ayam secara manual berasaskan minyak dan juga yang berasaskan cuka (VB) serta out put nilai-nilai kalori daripada pengeluaran yang juga diambil kira. Sejumlah elektrik (0.691 kWh) dan tenaga (9.792 MJ) yang lebih tinggi dengan input tenaga manusia yang sedikit diperlukan dalam menyediakan jeruk berasaskan cuka jika dibandingkan yang berasaskan minyak (0.597 kWh) dan 8.731 MJ/kg daging mentah). Walau bagaimanapun, lebih output kalori diperolehi dari pengeluaran berasaskan minyak (282 Cal/100 g) berbanding dengan jeruk berasaskan cuka (261 Cal/100 g). Walaupun kelebihan jeruk daging berasaskan minyak ini adalah pada warna, kebasahan dan kelembutannya, namun penerimaan menyeluruh terhadap jeruk ini ternyata berbeza. Analisis kehampiran menunjukkan variasi dalam pH, kandungan kelembapan dan protein mentah dalam kedua-kedua jeruk adalah tidak jelas. Berasaskan hubungan tenaga input output, disimpulkan bahawa pemprosesan jeruk daging berasaskan cuka adalah lebih ekonomik dari segi kos (67 Cal/rupee) berbanding jeruk daging ayam berasaskan minyak (56 Cal/rupee).

ABSTRACT

Data were collected from seven replicates to quantify the input of commercial energy in manual processing of oil-based (OB) as well as vinegar-based (VB) chicken gizzard pickles and output of caloric values from the products was calculated. Higher amounts of electrical (0.691 kWh) and total energy (9.792 kWh) with lesser inputs of human energy were required in preparation of VB pickle compared with 0.3 (0.597 kWh) and 8.731 MJ/kg of raw gizzard products. However, more caloric outputs were obtained from OB product (282 Cal/100g) compared with VB (261 Cal/100g) pickle. Despite significant superiority of oil-based gizzard pickle for colour, juiciness and tenderness, the overall acceptability of pickles was insignificantly different. Proximate analysis revealed nonsignificant variations in pH, moisture content and crude protein of the two pickles. Based on the input output energy relations, it is concluded that processing of experimental VB gizzard pickle is economically cost effective (67 Cal/rupee) over the oil-based (56 Cal/rupee) chicken gizzard pickle.

INTRODUCTION

With the growing popularity and demand for fast foods, it is becoming obligatory for the industry to spend higher exchange rates for energy inputs required in preparation of desired products. A substantial amount of information has been published on application of energy in food processing (Unger 1975; Schwartzberg 1977; Carroad *et al.* 1980; Ostrander 1980; Singh and Dhingra 1987). Various studies were also made on the preparation and storage of gizzard pickles (Arfa 1977; Charoenpong and Chen 1980; Sharma *et al.* 1986), but no work has so far been reported on the energy utilization patterns in the processing of pickled gizzards. In view of the need for such information, data have been collected through methodical evaluation and audit on quantification in input energy during processing of chicken gizzard pickles and a comparative study has been made on the cost effectiveness of two pickles in relation to the output of the caloric energy yields.

MATERIALS AND METHODS

A total of seven replicates utilizing 63.6 kg of gizzards, collected from the pilot poultry processing plant of the Central Avian Research Institute, were prepared by removing the adipose tissue and slicing each gizzard into 3-4 pieces. The oil-based (OB) and vinegar-based (VB) pickles were made using the procedures of Chatterjee *et al.* (1969) and Panda (1988). Formulations are given in Table 1. While processing the products, condiments were weighed on a Sartorious top-pan electronic balance. The frying of condiments was undertaken by using a 1500W hot plate. Gizzards were pressure cooked (15 lb/inch^2) for 10-12 minutes. Cooked gizzards were separated and the water discarded.

Estimation of Input Energy

The labour force comprised an unskilled adult man. Quantification of human energy (hE), electrical inputs (EI) and total energy (TE) was calculated by the following formulae:

$$\text{Manhour (hE)/kg of raw gizzards} = \frac{\text{Average time taken in the process}}{60 \times \text{Average quantity of gizzards processed}}$$

$$\text{EI (kWh)} = \frac{\text{Watt (W)} \times \text{Time (min)}}{1000 \times 60}$$

$$\text{EI/kg raw gizzards} = \frac{\text{kWh}}{\text{Average quantity of gizzards processed}}$$

TE (total energy) was determined from hE and EI by using the following standards (Panesar and Bhatnagar 1987):

1 manhour/kg = 1.96MJ
1 kWh/kg = 11.93 MJ

Output Energy

The nutritional energy (Cal/100g) of pickles was determined using the formula of Shackelford *et al.* (1989)

Sensory and Physico-chemical Traits

Sensory properties including colour, flavour, juiciness, tenderness, texture and overall acceptability of OB and VB pickles were estimated after 72 hours of ageing at ambient temperature. Seven experimental panellists from the professional staff of the institute were requested to judge the products for the above traits on the

TABLE 1
Formulation of gizzard pickle per kilogram of gizzards

Oil-based		Vinegar-based	
Ingredients	Quantity (g)	Ingredients	Quantity (g)
Table salt	38.0	Table salt	90
Sodium nitrite	0.2	Peeled garlic	32
Monosodium glutamate	0.5	Peeled ginger	32
Red chilli	15.0	Cumin	6
Black pepper	8.0	Red chilli	6
Caraway	3.0	Aniseed	3
Clove	1.0	Caraway	3
Cinnamon	1.0	Cinnamon	2
Pelled garlic	6.0	Clove	2
		Turmeric	3
		Black pepper	2
			ml
Vinegar	190.0	Refined mustard oil	10
Refined mustard oil	200.0	Vinegar	195
		Water	195

10-point Hedonic scale. In all seven replicates, pH was measured by a ELICO pH meter as per AOAC (1985). Shear force of the pickled gizzards was determined in lb/inch^2 by using Warner Bratzler Shear Press (Model 13806). Moisture, crude protein and ether extract (EE) were estimated as per AOAC (1985). A minimum of three samples was taken for recording observations of these traits for each replicate.

Cost of Production

Based on the input energy consumed in processing chicken gizzard pickles, prevailing market rates, bank interest (15% per annum), depreciation on appliances (8%) and cost of raw gizzards (Rs.12.00 per kg), the cost of production for OB and VB pickles was calculated. However, fluctuations in rates at various locations and other market conditions cannot be overlooked.

Statistical Analysis

Data related to time consumed for common steps of processing the two products were subjected to 't' tests for determination of significant differences. Observations on proximate analysis were transformed into arcsine values prior to statistical analysis. Data on sensory traits were subjected to statistical corrections before adoption of standard procedures by Snedecor and Cochran (1967).

RESULTS AND DISCUSSION

Input Energy

The means ± SE of the human energy (hE) consumed at various steps of processing OB and VB gizzard pickles are presented in Tables 2 and 3. The basic data on time taken were converted into manhour/kg as well as total energy required for the purpose. Obviously, maximum time was utilized for cooking,

TABLE 2
Energy consumption profile in processing of oil-based gizzard pickle

Parameters	Time taken *(min/4.56 kg)	%	Manhour/ (kg)	Energy consumption (MJ/kg)
A *Human Energy*				
1. Cleaning and slicing of gizzards	51.43 ± 4.48	22.78	0.188	0.368
2. Weighing condiments	23.57 ± 0.85	10.43	0.086	0.169
3. Putting gizzards in vinegar	30.00 ± 0.00	13.28	0.109	0.214
4. Frying condiments	38.14 ± 2.54	16.76	0.139	0.272
5. Cooking of gizzards	53.57 ± 2.61	23.72	0.195	0.382
6. Separating cooked gizzards	10.86 ± 0.82	4.80	0.039	0.076
7. Heating pickle	12.14 ± 1.02	5.38	0.044	0.086
8. Tranferring pickle to glass jar	6.43 ± 0.44	2.85	0.022	0.043
Total	226.14	100.00	0.822	1.610
B. *Electrical Energy*	kWh	% kWh	kWh/kg	MJ/kg
1. Frying condiments	0.96	35.29	0.211	2.517
2. Cooking gizzards	1.45	53.31	0.318	3.793
3. Heating pickle	0.31	11.40	0.068	0.811
Total	2.72	100.00	0.597	7.121

Grand total energy consumed 8.731 MJ/kg
* Mean ± standard error

TABLE 3
Energy consumption profile in processing of oil-based gizzard pickle

Parameters	Time taken *(min/4.53 kg)	%	Manhour/ (kg)	Energy consumption (MJ/kg)
A *Human Energy*				
1. Cleaning and slicing of gizzards	51.42 ± 4.80	23.93	0.189	0.370
2. Weighing condiments	21.42 ± 0.85	9.98	0.079	0.155
3. Heating vinegar and water	17.14 ± 1.37	7.98	0.063	0.123
4. Frying condiments	19.28 ± 2.12	8.98	0.071	0.139
5. Preparation of pickle solution	36.57 ± 1.82	17.03	0.135	0.265
6. Cooking of gizzards	51.42 ± 1.94	23.93	0.189	0.370
7. Separating cooked gizzards and putting in pickle solution	12.14 ± 0.93	5.65	0.045	0.088
8. Tranferring pickle to glass jar	5.42 ± 0.27	2.52	0.020	0.039
Total	214.81	100.00	0.791	1.549
B. *Electrical Energy*	kWh	% kWh	kWh/kg	MJ/kg
1. Heating vinegar and water	0.43	13.74	0.095	1.133
2. Frying condiments	0.49	15.65	0.108	1.288
3. Preparation of pickle solution	0.92	29.40	0.203	2.422
4. Cooking of gizzards	1.29	41.21	0.285	3.400
Total	3.13	100.00	0.691	8.243

Grand total energy consumed 9.792 MJ/kg
* Mean ± standard error

followed by cleaning and slicing raw gizzards. However, there were no significant variations in time consumed for common steps *viz.* cleaning and slicing of gizzards; weighing and frying of condiments; cooking gizzards and transferring pickle into glass jars with plastic lids. Differences in hE requirements for the two pickles were probably due to variations in formulation procedures.

EI requirements were significantly ($P<0.05$) higher for VB than OB pickle (Tables 2 and 3).

TE requirements (sum of hE and EI) were higher for processing VB (9.72 MJ/kg raw gizzards) than OB (8.731 MJ/kg) gizzard pickle.

Output Energy

Calculations revealed higher caloric output from OB (282 Cal/100 g) pickle than VB product (262 Cal/100 g). The obvious reasons reason is the greater amount of fat available in oil-based gizzard pickle

Sensory and Physico-chemical Traits

Significant differences were observed in colour, juiciness, tenderness, shear force value and EE of the OB and VB pickles (Table 4). However, no significant variations were recorded for flavour, texture, overall aceptability, pH moisture and CP.

TABLE 4
Means ± SE of sensory and physico-chemical characters in chicken gizzard pickle

Parameters	Gizzard Pickle					
	Oil-based			Vinegar-based		
1. Colour	7.55	±	0.22[a**]	6.48	+	0.14[b]
2. Flavour	6.78	±	0.26	6.73	+	0.17
3. Juiciness	7.30	±	0.21[a*]	6.45	+	0.19[b]
4. Tenderness	7.28	±	0.20[a*]	6.44	+	0.30[b]
5. Texture	7.02	±	0.15	6.50	+	0.23
6. Acceptability	7.04	±	0.19	6.58	±	0.25
7. pH	4.71	±	0.13	4.33	±	0.18
8. Shear force (lb/inch^2)	3.06	±	0.21[b*]	4.01	±	0.25[a]
9. Moisture	50.95	±	0.33	51.24	±	0.45
		(60.30)			(60.80)	
10. Crude protein (CP)	27.83	±	0.38	27.97	±	0.29
		(21.80)			(22.00)	
11. Ether extract (EE)	19.45	±	1.76[a**]	11.51	±	1.10[b]
		(11.10)			(4.00)	

Figures bearing same or no superscripts did not differ significantly for treatment effects. Observations at S1. No. 9, 10 & 11 are analysed in arcsine values. Percentages are reported in parenthesis.

Coast of Production

Based on the standards already mentioned and cost of input energy, the coast of producing OB gizzard pickle was higher than that of VB pickle. The main contributor to this effect was the cost of additional mustard oil. Comparioson of input output energies from the two kinds of pickles revealed higher yield of calories per Indian rupee from VB (67 Cal) than OB gizzard pickle (56 Cal).

CONCLUSIONS

Observations were recorded on the input and output energies of oil-based as well as vinegar-based chicken gizzard pickle. Results showed that processing of VB pickle required lower amounts of human and electrical inputs and resulted in more nutritional energy per rupee. Therefore processing of VB chicken gizzard pickle is more cost effective than oil-based gizzard pickle.

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Sensory, Biochemical and Microbiological Changes of Farmed Catfish (*Clarias batrachus*, Linnaeus) and Red Tilapia (*Oreochromis* sp.) at Ambient Storage

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Keyword: catfish, tilapia, quality changes, TRARS value, K_1-value, sensory evaluation

ABSTRAK

Ikan keli (Clarias batrachus, *Linnaeus*) *dan tilapia merah* (Oreochromis *sp.*) *disimpan di suhu persekitaran (28±2°C) untuk selama 24 jam. Sampel di nilai bagi penerimaan, perubahan kesegaran, kehadiran ketengitan dan perubahan bilangan mikrob. Ikan keli masih diterima sehingga 20 jam dan ikan tilapia selama 15 jam penyimpanan. Ketika ditolak nilai K_1 ialah 70.0 dan 42.7% bagi tilapia dan keli masing-masing. Sampel ikan keli tidak melampaui bilangan mikrob 10^7 cfu/g hingga ke akhir masa penyimpanan; walaubagaimanapun, sampel tilapia telah melebihi bilangan mikrob dalam masa 20 jam.*

ABSTRACT

Farmed catfish (Clarias batrachus, *Linnaeus*) *and red tilapia* (Oreochromis *sp.*) *were stored at ambient temperature (28±2°C) for a period of 24 h. They were evaluated for acceptability, freshness deterioration, rancidity development and microbiological changes. Raw catfish was acceptable up to 20 h and that of tilapia up to 15 h. Upon rejection their K_1 values were 70.0 and 42.7% for tilapia and catfish respectively. Microbiologically, catfish samples did not exceed the 10^7 cfu/g limit until the end of the storage period; however, tilapia was not acceptable by the 20th hour.*

INTRODUCTION

Freshwater fish in Asian countries are distributed both in live and fresh form. No icing is practised when they are handled fresh. It could be that certain fishes, e.g. the catfish, are still alive 1-2 h after catch and can survive for 6-8 h in the open provided they are kept wet (Mohammad Mohsin and Ambak 1983). The effect of immediate icing on fish is well documented but less for tropical cultured fish. The lack of information on the quality deterioration of fish at ambient temperature, especially those of commercial significance in Malaysia makes it less conducive for the expansion and diversification of aquacultural activities. Therefore, this study was carried out to determine the chemical, microbial and sensory changes in farmed catfish *(Clarias batrachus)* and red tilapia *(Oreochromis* sp.*)* under ambient storage. The information gathered in this study could give some insight into the handling characteristics of these fishes prior to further processing.

MATERIALS AND METHODS

Sample

Farmed catfish and red tilapia were obtained from a nearby aquafarm and brought live to the faculty's laboratory. The catfish and tilapia were of commercial size, weighing 100-200 g and 500-1000 g respectively. They were not separated according to sexes.

Storage and Sampling

Upon arrival, the fishes were placed in dry trays at ambient temperature (28±2°C) and sampled at 4-h intervals. At each sampling time, eight fish

of each species were sampled at random. Sampling for microbial count was first carried out on one side of the fish, followed by sampling for chemical indices. Out of the eight fish, three were put aside for sensory evaluation of raw samples.

Proximate Analyses for Protein, Fat and Moisture Content

The crude protein, moisture and lipid contents of the fish muscle were determined according to the procedure of Pearson (1976).

Sensory Evaluation of Raw and Cooked Samples

Sensory evaluation of the raw and cooked samples was carried out by eight semi-trained panellists (laboratory staff and students at the faculty). The raw samples were evaluated for changes in the eyes, gills, odour, and overall acceptability based on a 3-point hedonic score according to the procedure of Gorcyzca *et al.* (1985).

To evaluate cooked fish, a 2 cm cube of raw fish was placed in a glass petri dish with a cover. The sample was steamed for 10 min and served to the panellists while still warm. The sample was evaluated for odour (by sniffing when the cover was first opened partially), taste and texture based on a 7-point scoring system recommended by Kosmark (1986).

Thiobarbituric Acid Reactive Substances (TBARS)

The TBARS values in fish muscle were determined according to the procedure of Ke *et al.* (1984). The distillates collected were reacted with thiobarbituric reagent, heated to 100°C for 45 min and cooled under running tap water. The absorbance was read at 538 nm within 30 min and malonaldehyde (MA) concentration was obtained from a standard curve and reported as μmol MA/kg sample.

Quantification of ATP Catabolites and Determination of K_1-value

The nucleotides were extracted and prepared for high performance liquid chromatography (HPLC) following the procedure of Ryder (1985). The HPLC system used (LDC Analytical, CM 4000, Australia) was equipped with two pumps and an ultraviolet (UV) detector. The standards and the unknown were detected at 254 nm. Separation of standards was achieved on a reverse phase Lichrosorb RP-18 column (5 μm, 4.0 mm I.D x 25 cm, Merck, Germany). All nucleotide standards were obtained from Sigma (St. Louis, Missouri, USA). The buffer used was 0.04 M KH_2PO_4 and 0.06 M KH_2PO_4 in the ratio of 75:25 with a flow rate of 1.5 ml/min. The maximum absorbance sensitivity was set at 0.2 aufs. The K_1 -value was defined as the percentage of the sum of hypoxanthine (Hx) and inosine (HxR) to the sum of inosine monophosphate (IMP), HxR and Hx (Watanabe and Karube 1986).

Microbial Analysis

The total plate count of the samples was carried out according to AOAC (1984) using the pour plate method. Plates were incubated at 37°C for 48 h.

Statistical Analysis

Analysis of variance, Duncan's multiple range test and other relevant statistical analyses were carried out using the Statistical Analysis System (SAS) programme.

RESULTS AND DISCUSSION

The moisture content obtained for the catfish was 74.5±1.5% and that of tilapia was 77.2±0.5%. Their protein contents were 14.4.8±1.6% for catfish and 12.9±1.1% for tilapia. The lipid content of catfish was 2.2±0.4% and that of tilapia was 1.6±0.2%.

Fig. 1 and *Fig. 2* show the sensory scores for eyes, gills, odour and acceptability of the whole catfish and tilapia respectively. Very little changes in the sensory scores occurred (except for the gills) for the first 4 h in tilapia and for the first 8 h in catfish. Rapid decline in the scores for the eyes, gills, odour and acceptability were observed thereafter for both fish though the rate of decline was not similar. With the overall acceptability cut-off point set at 1.5, it could be said that tilapia was acceptable up to approximately 15 h and that of catfish up to 20 h of storage (though the acceptable condition of the eyes was only up to 12 h). Nile tilapia *(Oreochromis niloticus)* was reported to be rejected after 16.5 h of ambient storage with the development of putrid, bitter and itchy flavour, but no softening of the texture (Estrada *et al.* 1985). Jamilah and Yusoff (1993),

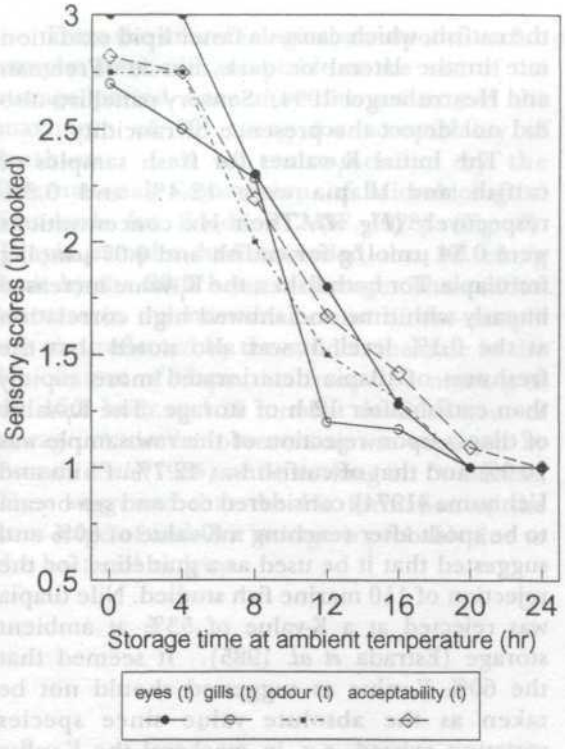

Fig. 1: Sensory scores of red tilapia kept at ambient temperature

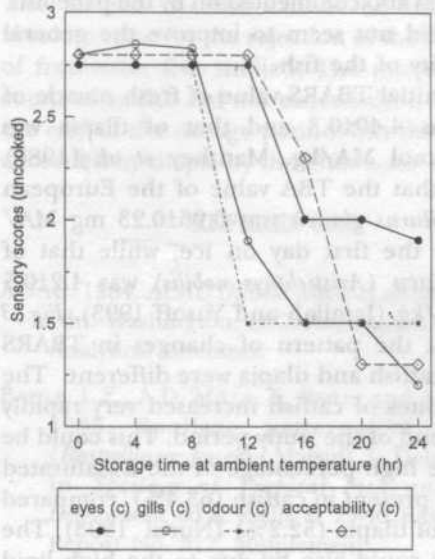

Fig. 2: Sensory scores of catfish kept at ambient temperature

in a study of bighead carp *(Aristichthys nobilis)* also found the acceptability of the fish to be at 12 h of storage at ambient temperature. Acceptable shelf-life of common carp *(Cyprinus carpio)* was reported to be 13 h (Gelman *et al.* 1990). From the few reports available, it seems that the shelf-life for freshwater fish at ambient storage (25-30°C) was 12-16 h. Development of the ammonical odour was obvious in both fish upon prolonged storage.

The results of the sensory scores for cooked samples were as in Table 1. The initial acceptability scores for tilapia were higher than for catfish. This could be due to the strong influence of the odour since odour in catfish was scored significantly lower than tilapia. Based on

TABLE 1
Sensory scores of cooked samples of tilapia and catfish after storage at ambient temperature

Storage time	Tilipia				Catfish			
(h)	Odour	Taste	Texture	Accept.*	Odour	Taste	Texture	Accept.*
0	6.0	5.5	6.0	5.8	4.8	6.3	5.0	5.3
4	5.3	5.5	6.5	5.8	4.5	4.6	6.0	5.0
8	4.5	4.8	5.5	4.9	4.0	4.3	5.3	4.5
12	4.3	4.5	5.3	4.7	4.2	4.3	4.8	4.5
16	2.5	1.8	5.0	3.1	3.8	4.2	4.0	4.0
20	1.8	1.3	3.6	2.2	2.5	3.8	3.7	3.3
24	1.5	1.3	3.1	2.0	2.3	3.2	3.2	2.9

* Overall acceptability

the rejection value of 3.5, the cooked samples of tilapia were acceptable up to slightly less than 16 h and that of catfish up to approximately 20 h. Odour seemed to be the most rapidly deteriorated parameter for both fish; this was also commented on by the panellists. Cooking did not seem to improve the general acceptability of the fish.

The initial TBARS value of fresh muscle of catfish was 4.40±0.3 and that of tilapia was 3.7±0.2 μmol MA/kg. Manthey *et al.* (1988) reported that the TBA value of the European catfish (*Silurus glanis*) was 0.96±0.23 mg MA/100 g for the first day on ice, while that of bighead carp (*Aristichthys nobilis*) was 4.2±0.5 μmol MA/kg (Jamilah and Yusoff 1993). *Fig. 3* shows that the pattern of changes in TBARS values of catfish and tilapia were different. The TBARS values of catfish increased very rapidly until the end of the study period. This could be due to the high percentage of the unsaturated fatty acids present in catfish (63.4%) compared with that of tilapia (52.2%) (Nurul 1993). The difference could also be due to the high lipid content and the presence of oxidation catalyst (Fe and Cu) in the dark muscle and the skin of the catfish, which causes a faster lipid oxidation rate in the lateral or dark muscle (Freeman and Hearnsberger 1994). Sensory panellists also did not detect the presence of rancidity.

The initial K_1-values for fresh samples of catfish and tilapia were 12.4% and 6.3% respectively (*Fig. 4*). Their Hx concentrations were 0.34 μmol/g for catfish and 0.07 μmol/g for tilapia. For both fishes, the K_1-value increased linearly with time and showed high correlation at the 0.1% level. It was also noted that the freshness of tilapia deteriorated more rapidly than catfish after 12 h of storage. The K_1-value of tilapia upon rejection of the raw sample was 70.0% and that of catfish was 42.7%. Ehira and Uchiyama (1974) considered cod and sea bream to be spoilt after reaching a K-value of 60% and suggested that it be used as a guideline for the rejection of 110 marine fish studied. Nile tilapia was rejected at a K-value of 53% at ambient storage (Estrada *et al.* 1985). It seemed that the 60% K-value as suggested should not be taken as the absolute value since species variation existed, e.g. in mackerel the K-value for the rejection was reported to be 53-76% (Barile *et al.* 1985).

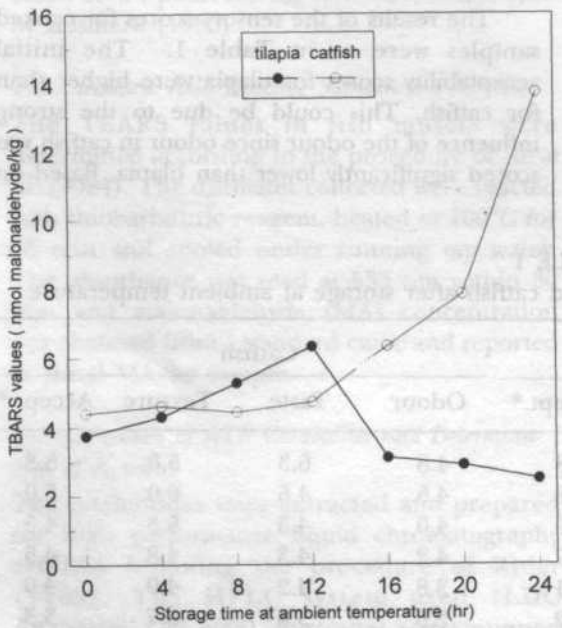

Fig. 3: TBARS values of tilapia and catfish kept at ambient temperature

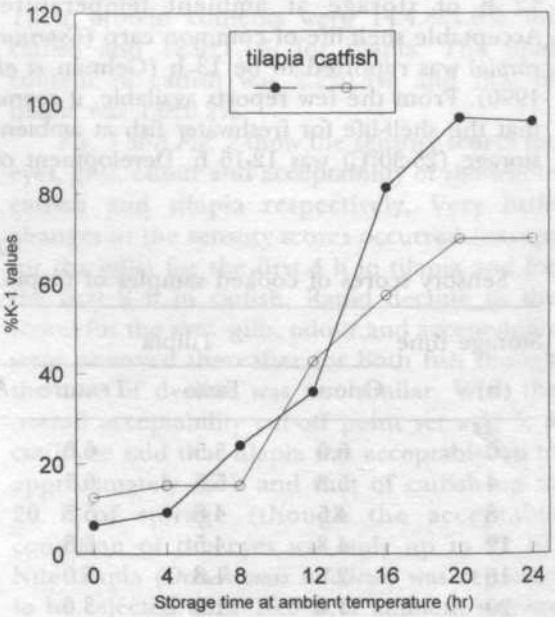

Fig. 4: K_1-values of tilapia and catfish kept at ambient temperature

From the microbiological standpoint, catfish samples were still acceptable at the end of the storage period since they did not exceed the maximum of 10^7 cfu/g for acceptability of freshwater fish as recommended by the International Commission of Microbiological Standards for Foods (ICMSF 1978) (*Fig. 5*). Tilapia samples had exceeded the 10^7 cfu/g limit by the 20th hour of storage. The sudden increase in the microbial count of the sample was also reflected by the sudden increase in the Hx content of tilapia, i.e. from 0.51 nmol/g at the 16th hour to 1.19 nmol at the 20th hour of storage. A similar observation was reported by Estrada *et al.* (1985) while working on *O. niloticus*. They suggested that the Hx formation at the later stage of ambient storage is probably due to the bacterial activity.

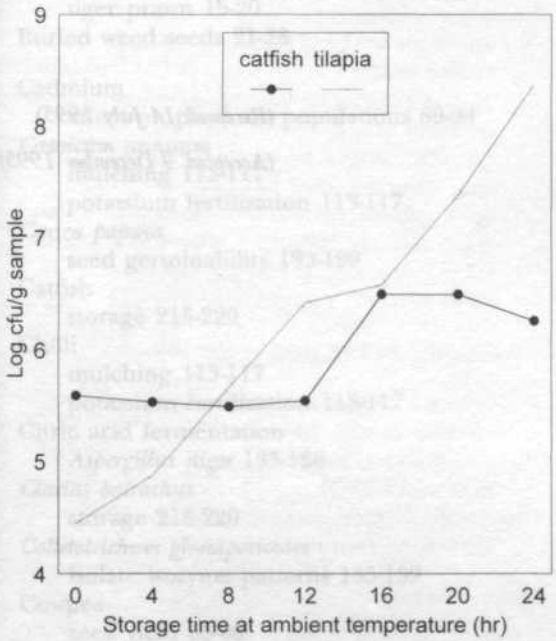

Fig. 5: Microbial count of tilapia and catfish kept at ambient temperature

CONCLUSION

The shelf-life of catfish and tilapia at ambient storage was 20 and 15 h respectively. Catfish with the initial TBARS value of 4.40 µmol MA/kg developed rancidity more rapidly than tilapia though sensory panellists did not indicate any detection of rancidity thoughout the study. However, tilapia showed a faster rate of freshness deterioration with a K_1-value of 70% upon rejection. Catfish was rejected at 42.7% K_1-value. The K-value of 60% cannot be used as the absolute value for the rejection of the two species of freshwater fish studied. The microbiological count of catfish did not exceed the 10^7 cfu/g at the end of the storage period, but the value was exceeded by tilapia by the 20th hour of storage.

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