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# Pertanika Journal of Tropical Agricultural Science

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## Contents

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# The Effect of Dosage and Storage Time on the Formation of Bound Residues in Paddy, Milled Rice and Maize

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**Keywords: malathion, dose, storage time, grains, bound residues**

## ABSTRAK

*Padi, beras dan jagung yang dirawat dengan malathion berlabel membentuk sisabaki $^{14}C$ terikat. Setelah disimpan selama 3 bulan, amoun sisabaki terikat yang dihasilkan dalam padi, beras dan jagung yang dirawat dengan 10µg/g malathion masing-masing adalah 10.8, 6.5 dan 13.3% daripada dos yang digunakan. Setelah 6 bulan, amaunnya ialah 14.2, 4 dan 17.7% masing-masing. Peratusan sisabaki terikat yang dihasilkan tidak meningkat apabila dos rawatan malathion dinaikkan kepada 50µg/g. Walau bagaimanapun jumlah sebenar sisabaki terikat yang dihasilkan meningkat lebih dari 4 kali ganda dalam semua bijiran yang diuji.*

## ABSTRACT

*Stored unmilled rice (paddy), milled rice and maize treated with radiolabelled malathion formed bound $^{14}C$ residues. After 3 months of storage, the bound residues in the grains treated with 10µg/g malathion accounted for 10.8, 6.5 and 13.3% of the applied dose in paddy, rice and maize respectively. After 6 months, the corresponding values of bound residues were 14.2, 4 and 17.7% respectively. Increasing the dosage of malathion to 50µg/g does not significantly increase the percentage of the bound residues formed. However, the absolute amount increased by more than 4-fold in all the grains tested.*

## INTRODUCTION

Organophosphorus insecticides are widely used in agriculture. The toxicity of this class of insecticide is due mainly to its inhibitory effect on the enzyme cholinesterase (Eto 1974). Malathion is still being used in the local granaries to control storage pests although its usage has been reduced. Spraying of malathion in the local godown is normally conducted using a thermal fogger or mist blower. The milled or unmilled rice (paddy) is stored in jute sacks or polyethylene bags. It has been shown that malathion applied to bagged milled rice results in penetration of malathion through the jute sacks into the rice/paddy grains (Arshad and Salehudin 1988). Due to the insecticide's low mammalian toxicity, in some countries malathion is mixed directly with the grains (Zayed *et al.* 1992). It has been shown that insecticides in general form non-extractable residues in grains (Khan 1982; Kovacs 1986). The chemical nature of the non-extractable malathion residues in grains and its biovailability to mammals is generally unknown. Therefore, information on the amount and nature of bound pesticide residues on the grains under controlled conditions is important in managing their use.

The present study reports on the formation of bound malathion residues in paddy (unmilled rice), milled rice and maize. The effects of insecticide dosage and storage time on the formation of bound residues are also investigated.

## MATERIALS AND METHODS

### *Chemicals*

$^{14}C$-malathion (o,o-dimethyl S,1,2-diethoxy carbonyl,1,2-$^{14}C$ ethyl phosphorodithioate) (1,2-ethyl-$^{14}C$ label) with specific activity of 112 mCi/mg was purchased from Amersham Corporation. The insecticide was approximately 98% pure as determined by thin-layer chromatography. Technical non-labelled malathion of 99.5% purity was obtained from the American Cyanamid Company.

*Treatment of Samples*

Freshly harvested paddy and milled rice, variety MR 71, were obtained from a godown in Malacca. Maize was obtained from the University farm. All the grains were obtained from crops that had not been treated with insecticide.

Samples of 0.5 kg each of milled rice, paddy and maize were treated with 10µg/g equivalent of non-labelled malathion and $^{14}$C-malathion of specific activity 4.43 x $10^7$ dpm/mg. This was prepared by dissolving 0.89 mg of labelled malathion and 4.11 mg of non-labelled pure malathion in hexane (residue grade). One ml of the insecticide solution was pipetted at a time into the glass jar containing the grains. The jars were then capped and the grains thoroughly mixed by manually shaking and turning the jars end over end. The jars were then uncapped and placed in a fume cupboard to allow the hexane to evaporate completely. Grains were also treated using 50µg/g equivalent of malathion. Portions were removed at 0, 1, 3 and 6 m for analysis. All samples were analysed in triplicate.

*Residue Analysis*

*External Residue (washable residue)*

Twenty-five grams of the grain samples were washed with 250 ml of distilled water. The washing was collected and analysed for radioactivity. The process was repeated several times and each washing was counted individually.

*Extractable Residue*

The washed grains were dried and ground thoroughly using a mortar and pestle. These were then extracted with 400 ml of methanol (residue grade) using a Soxhlet apparatus. Extraction was carried out for 24 h. The methanolic extract was then tested for radioactivity.

*Bound Residue*

The grains left in the thimble of the Soxhlet apparatus were dried in the oven and then taken for dry combustion in a Harvey Biological Oxidizer to determine the quantity of bound residue.

*Total Residue*

The levels of the total residue in the grains at the stated time intervals (i.e. 0, 1, 3 and 6 m) were determined by the dry combustion procedure using the Harvey Biological Oxidizer.

*Determination of Radioactivity*

Liquid samples (e.g. solvent extracts) were assayed in a Hewlett Packard Liquid Scintillation Counter, Tri-carb Model 460C using a standard cocktail (333 ml Triton X-100, 5.5 g PPO, 100 mg POPOP and 667 toluene). Radioactivity in the solid samples was determined after combustion in the Harvey Biological Oxidizer using the cocktail supplied by R.J.Harvey Instruments Corporation, New Jersey.

## RESULTS AND DISCUSSION

The moisture content of the rice grain, paddy and maize was 13.1, 12.3 and 12.5% respectively. This was determined by oven drying the grains at 130°C for 18 h.

Table 1 shows the results of the level of malathion residues in the untreated paddy (unmilled rice). At 0 time, the level of bound residue was 4.7% of the total applied radioactivity. After 3 m, the level rose to 10.8% of the total radioactivity applied. Longer storage time (6 m) resulted in a slight increase in the percentage of bound residues formed. The amount of bound residues formed was 14.2% of the applied dose.

The table also shows the level of malathion residues in the treated milled rice grains determined at various time intervals. The results indicate that very little of the pesticide is bound to the grains. Most of the residues are found in the extractable or washable and the methanolic extracts. After 6 m of storage, the bound residues account for only about 4% of the total residues. Thus longer storage time does not increase the total amount of bound residues formed.

A similar pattern is observed in maize. The amount of external residues obtained is the total of extractable and external residues. The amount of bound residues obtained after 3 m was 13.3%. After 6 m of storage, the level increased slightly to 17.7%.

The low level of bound malathion residues in milled rice which comprised mainly starch and endosperm may be due to the removal of the hull/bran and germ region during the milling process. It has been suggested that bound residues are associated with the hull/bran and germ region of the grains (Arshad and Salehudin 1988).

TABLE 1

Distribution of external, extractable and bound malathion residues in paddy, milled rice and maize treated with 10μg/g malathion as a function of storage time. Results are expressed as percentages of applied dose. Results are the mean of triplicates

| Grains | | Storage Time (months) | | | |
|---|---|---|---|---|---|
| | | 0 | 1 | 3 | 6 |
| | | Residues (% of the applied dose) | | | |
| External | Unmilled rice | 65 ± 2.80 | 52.6 ± 3.68 | 39.8 ± 0.59 | 40 ± 2.94 |
| | Milled rice | 90 ± 6.37 | 91 ± 8.49 | 84.3 ± 3.18 | 80 ± 4.55 |
| Extractable | Paddy | 23.6 ± 2.22 | 35 ± 2.2 | 45 ± 3.08 | 44.7 ± 4.77 |
| | Milled rice | 4.0 ± 0.37 | 4.8 ± 0.64 | 8.0 ± 0.96 | 11 ± 1.08 |
| | Maize (external + extractable) | 93 ± 5.72 | 86 ± 58.4 | 76 ± 3.91 | 75.8 ± 8.24 |
| Bound | Paddy | 4.7 ± 1.2 | 5.4 ± 0.63 | 10.8 ± 2.62 | 14.2 ± 3.12 |
| | Milled rice | 1.5 ± 0.72 | 0.5 ± 0.14 | 6.5 ± 0.40 | 4.0 ± 1.10 |
| | Maize | 1.4 ± 0.71 | 5.6 ± 1.87 | 13.3 ± 2.87 | 17.7 ± 2.81 |

Rowlands and Bramhall (1977) reported that malathion residues were found chiefly in the germ and scutellum which probably explains the relatively high amount of bound residues formed in maize.

Table 2 shows that the amount of bound residues formed is dose dependent. The absolute amounts of bound residues formed in paddy, milled rice and maize using 10μg/g labelled malathion were 1.7, 0.69 and 1.33μg/g respectively. The amounts formed when the applied dose was increased to 50μg/g were 5.99, 2.91 and 7.25μg/g respectively. These correspond to 5.4, 4.2 and 5.5 fold increases in the amount of bound residues formed in paddy, milled rice and maize respectively. It appears, therefore, that although the percentage of the applied dose does not increase significantly, the absolute amounts of bound residues formed increased by more than four times. Therefore, it is important that the correct amounts of malathion be used in the control of pests as indiscriminate use may result

TABLE 2

Amount of bound residues formed after 3 months storage as a function of dose applied in unmilled rice, milled rice and maize. Results are the mean of triplicates. Bound residues are formed as described in the text

| Grains | Malathion Applied | | | |
|---|---|---|---|---|
| | 10 μg/g | | 50 μg/g | |
| | Bound Residues Formed | | | |
| | μg/g | % | μg/g | % |
| Unmilled rice | 1.1 ± 0.49 | 10.8 ± 2.08 | 5.99 ± 1.62 | 12 ± 3.26 |
| Milled rice | 0.69 ± 0.26 | 6.9 ± 2.68 | 2.91 ± 0.75 | 5.8 ± 0.82 |
| Maize | 1.33 ± 0.12 | 13.3 ± 1.2 | 7.25 ± 1.24 | 14.5 ± 2.49 |

in the formation of bound residues that may be potentially hazardous to humans.

Although relatively low amounts are bound in milled rice, little is known on its biovailability and hence its potential hazard to humans. Unmilled rice (paddy) is not normally consumed by humans directly. Since very little residues are found in milled rice after treated paddy is milled, bound residues are only significant if the bran is used for consumption. However, the amount of bound residues obtained in maize is substantially higher. Therefore, it is essential that further studies be carried out to determine its biological activity and bioavailability in animals.

## ACKNOWLEDGEMENTS

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# Growth, Water Relations and Physiological Changes of Young Durian (*Durio zibenthinus* Murr) as Influenced by Water Availability

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**Keywords: growth, water relations, *Durio zibenthinus***

## ABSTRAK

*Kesan air ke atas klon-klon durian D24, D99 dan MDUR79 dikaji di dalam dua eksperimen berbeza. Di dalam eksperimen pertama, pokok didedahkan kepada kadar air yang berbeza; 80%, 40% dan 10% daripada kapasiti ladang. Kekurangan air menurunkan kadar pertumbuhan, status air dan kadar fotosintesis pokok. Terdapat bukti kesan klonal ke atas kadar fotosintesis di mana klon D99 menunjukkan kadar fotosintesis yang lebih tinggi daripada klon D24. Di dalam eksperimen yang kedua, klon-klon D24, D99 dan MDUR79 didedahkan kepada suatu jangkamasa kekurangan air daripada 7 hingga 21 hari. Status air pokok dan kadar fotosintesis didapati lebih rendah oleh kerana kekurangan air pada klon D24 jika dibandingkan dengan klon D99 atau MDUR 79. Penambahan proline yang lebih tinggi pada klon-klon D24 dan MDUR79 mencadangkan bahawa kedua-dua klon tersebut lebih toleran terhadap kekurangan air jika dibandingkan dengan klon D24.*

## ABSTRACT

*The effects of water availability on durian clones D24, D99 and MDUR79 were investigated in two different experiments. In the first experiment, plants were exposed to different water availability: 80% ; 40% and 10% of the field capacity. Water deficit reduced vegetative growth, water status and rate of photosynthesis in the plants. There was evidence of clonal effect on photosynthesis rate where clone D99 showed higher photosynthesis values than clone D24. In the second experiment, plants of D24, D99 and MDUR 79 were exposed to a duration of water stress ranging from 7 to 21 days. Plant water status and photosynthesis rate were more reduced by water deficit in the D24 than D99 or MDUR79. Higher proline accumulation in D99 and MDUR 79 clones suggested that both clones were more tolerant to water stress than clone D24.*

## INTRODUCTION

The percentage of survival of young grafted clonal durian plants after transplanting to the field differs in dry conditions. The main problem is related to a lack of water for root development. Plant-water relations are important in controlling stomatal aperture and photosynthesis rate (Hsiao and Acevedo 1984). Plant responses like these will contribute to the differences in the drought tolerance in the plants. In drought-susceptible clones, the physiological processes are adversely affected even by a small reduction in tissue hydration, while those that are drought tolerant possess morphological hydration even under limited water supply (Hanson and Nelsen 1980).

Little has been published on the mechanism contributing to the tolerance of young grafted durian plants to water stress. This study was undertaken to quantify the level of stress that affects the plant vegetative growth, water relations, stomal response and photosynthesis rate; and to determine the accumulation of proline in the leaves of different clones so as to identify the degree of osmotic adjustment in the plants as it has been proposed that proline acts as an osmoticum during water stress (Kauss 1977).

## MATERIALS AND METHODS

The experiments were conducted in the greenhouse unit, Faculty of Agriculture, UPM, Serdang, Selangor, Malaysia. The mean daily air temperature was 28.6± 6°C and the relative humidity was 78± 4% RH. *Fig. 1* shows the typical diurnal changes of air temperature and relative humidity

in the greenhouse unit on a dry and sunny day. The experimental materials were young durian clones D24, D99, and MDUR79.

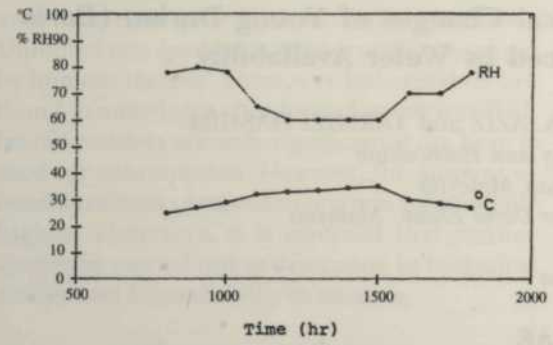



*Fig. 1: Diurnal changes in air temperature and relative humidity (RH) in the greenhouse unit on a clear day.*

In the first experiment, four-month-old plants D24 and D99 wedge grafted on D8 stock plants were grown in plastic pots containing 17 kg of soil mixture 3:2:1 (top soil: organic manure: sand). The soil mixture was sterilised before planting. Plants were regularly watered for the first two weeks after transplanting. Two levels of water stress were imposed on the plants, i.e. 40% (moderate water stress) and 10% (severe water stress) of the field capacity. The plants were grown in the treatments for 7 weeks. For the control, plants were watered to 80% of the field capacity predetermined by the gravimetric method. The experiment was conducted in a completely randomised design with five replications in a factorial arrangement.

In the second experiment, five-month-old plants of clones D24, D99 and MDUR79, grafted on to unselected seedling stocks (supplied by MARDI Serdang) were grown in pots containing 15 kg of soil mixture. Throughout the experimental period, 8 plants from each clone were regularly watered and 8 others had their irrigation stopped to induce progressive dehydration for a period ranging from 7 to 21 d.

Plant height, leaf area and dry weight of leaf, root and stem were determined at harvest. Root length and volume were also recorded. Leaf area was recorded using a leaf meter (Delta T-Cambridge Ltd, UK). Leaf length was measured weekly with a ruler. Plant parts were oven dried for 48 h at 80°C and the dry weight determined.

Relative water content was determined according to the method of Weatherley (1950). Leaf discs (10 cm in diameter) were floated for 12 h before the turgid weight was recorded. Canopy transpiration was determined by recording the amount of water loss during each consecutive weighing of the plants. The pots were covered with black polythene sheets to minimise soil evaporation. Stomal diffusive resistance was recorded using a transit time porometer (Delta - T device porometer Model MK3 Cambridge, UK). Stomatal diffusive resistance was calculated from a calibration curve obtained on a perforated plate with known resistance prior to making measurements. Leaf photosynthesis rate was recorded using an infrared gas analyser Model LCA-2 (ADC, Hoddesdon, UK). Four measurements of different leaves were made for each treatment. The measurements were made when radiation level was within the range of 600-800 W/m$^2$ for clone D99 and 500-700 W/m$^2$ for clone D24. Light response curves for these two clones are illustrated in Appendix 1.

In the second experiment, chlorophyll content was determined from the leaf extract following the method outlined by Nose (1987). Leaves were sampled for relative water content, while the adjacent leaves were tested for proline content on day 21. Determination of proline content was based on the method described by Bates *et al.* (1973). Half gram of leaf was ground in 10 ml of 3% sulphosalicylic acid and the extract filtered through a Whatman No. 2 filter paper. Two ml aliquots were sampled for proline estimation by the acid ninhydrin method, and determined by using a spectrophotometer (Shimadzu UV-160A Visible Recording Spectrophotometer).

## RESULTS

There was no significant interaction between clones and water availability with respect to plant vegetative growth. There were no clonal differences in plant height, leaf elongation, leaf area and dry weight of leaves and roots (Table 1; *Fig. 2*). Root length and root volume, however, were significantly ($P < 0.05$) higher in D99 than in D24 plants.

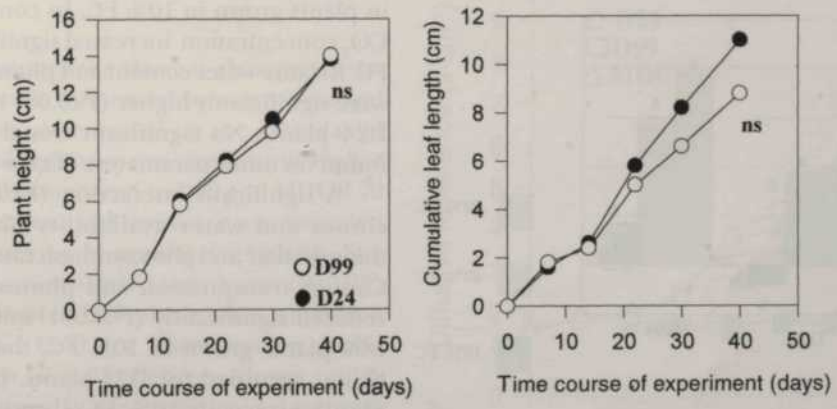
Water stress had considerable effect on the plant vegetative growth (Table 1). Plants grown in 10% of field capacity (FC) had significantly lower leaf area, leaf and root dry weight, root length and root volume. At harvest, leaf area and dry weight of plants grown in 10% FC were 50% less than those of the control at 80% FC.

TABLE 1
Effects of water availability on plant vegetative growth at the end of experiment 1 (after 7 weeks in treatment)

| | Leaf area ($cm^2$) | Leaf dry wt (g/plant) | Root dry wt (g/plant) | Root length (cm) | Root volume ($cm^3$) |
|---|---|---|---|---|---|
| Clone | | | | | |
| D24 | 1057a | 18.14 a | 8.90 a | 18.8 a | 16.1 a |
| D99 | 1034 a | 16.32 a | 9.96 a | 33.3 b | 21.8 b |
| Water availability (FC) | | | | | |
| 80% | 1258 a | 20.80 a | 12.68 a | 33.4 a | 24.5 a |
| 40% | 1219 a | 19.88 a | 10.97a | 28.1 b | 22.1 a |
| 10% | 602 b | 9.14 b | 4.04 b | 16.2 b | 9.5 b |
| Interaction (P<0.05) | ns | ns | ns | ns | ns |

Mean separation by DMRT at 5% level

**a)clone**




**b)water availability**

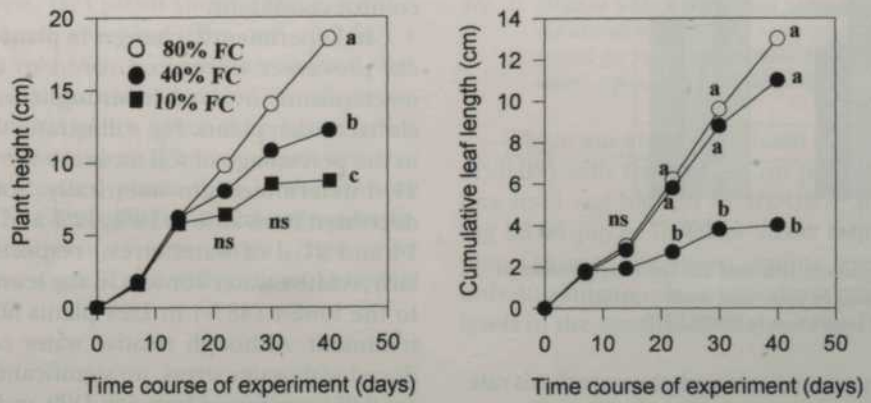



*Fig. 2: Plant height and cumulative leaf length as influenced by clone and water availability Means separation by DMRT5%*

TABLE 2
Effects of clone and water availability on relative water content (RWC), canopy transpiration (Tr), internal $CO_2$ concentration (int $CO_2$) and photosynthesis rate (Pn)

| Clone | RWC (%) | Tr (ml/day) | int $CO_2$ (μmol/m²/s) | Pn (μmol/m²/s) |
|---|---|---|---|---|
| D24 | 75.4a | 11.2a | 333a | 2.2a |
| D99 | 81.3b | 12.2a | 326a | 5.0b |
| Water availability (FC) | | | | |
| 80% | 78.8 | 12.8a | 324a | 5.81a |
| 40% | 81.6a | 13.7a | 321a | 4.92a |
| 10% | 73.3b | 8.6b | 344b | 0.03b |
| Interaction (P<0.05) | ns | * | ns | * |

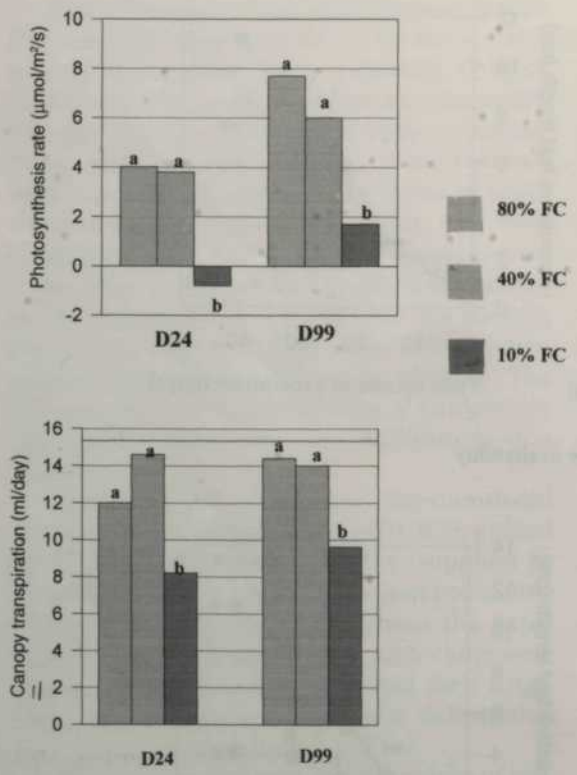



*Fig. 3: Photosynthesis rate and canopy transpiration as influenced by clone and water availability Means separation by DMRT5%*

The plant water status and photosynthesis rate were determined on the 28th day of treatment. Relative water content, canopy transpiration and photosynthesis rate reduced significantly(<0.05) in plants grown in 10% FC. In contrast, internal $CO_2$ concentration increased significantly at 10% FC. Relative water content and photosynthesis rate were significantly higher (P<0.05) in D99 than in D24 plants. No significant clonal effects were found on other parameters (Table 2).

A significant interaction (P<0.05) between clones and water availability on the canopy transpiration and photosynthesis rate was observed. Canopy transpiration and photosynthesis rate reduced significantly (P<0.05) in both D24 and D99 plants grown in 10% FC, the lowest value being recorded for D24 plants. Leaves of D24 plants grown in 10% FC showed a negative photosynthesis rate of -0.8 μmol/m²/s (*Fig. 3*). The highest photosynthesis rate, reaching 7.7 μmol/m²/s was recorded for D99 plants under control conditions.

In Experiment 2, changes in plant physiological processes were monitored to determine mechanisms involved in drought tolerance of clonal durian plants. *Fig. 4* illustrates the changes in the percentage of soil moisture for a period of 21 d determined gravimetrically. Soil moisture decreased from 23% to 19%, 12% and 9% after 7, 14 and 21 d of water stress, respectively. Similarly, relative water content in the leaves declined to the lowest (48%) in D24 plants after 21 d of treatment. Although relative water content reduced with water stress, no significant difference (p>0.05) was found between D99 and MDUR 79 plants after 14 and 21 d of treatment (*Fig. 5*).

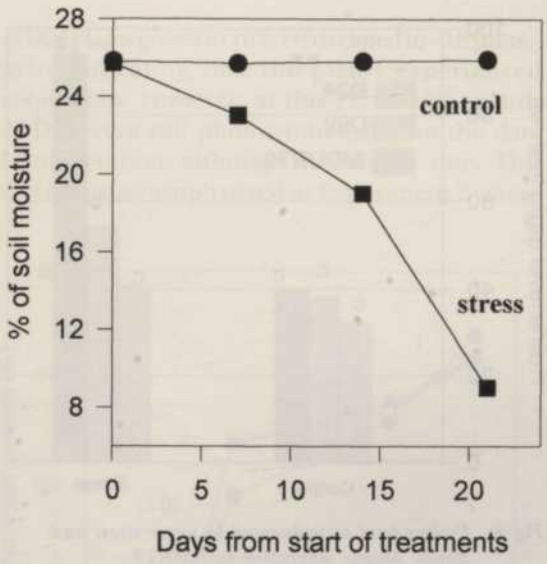



*Fig. 4: Changes in soil moisture (%) over stress period*

*Fig. 6* illustrates the diurnal changes in stomatal resistance as influenced by water stress and between clones, determined on the 18th day of stress. Stomatal resistance was significantly higher ($P<0.05$) in D24 compared to D99 and MDUR 79 plants. Highest stomatal resistance was recorded at 1500 hrs reaching 11 s $cm^{-1}$ in D24 plants. Stomatal resistance was significantly lower ($P<0.05$) in MDUR 79 than in D99 plants when measured at 0900 hrs and 1100 hrs. There was no significant difference ($P<0.05$) between clones under control conditions. Water stress for 14 d resulted in a significant difference in stomatal resistance between clones; D24 plants showed higher resistance than D99 and MDUR79 plants. After 21 d of water stress, D24 plants showed increased stomatal resistance reaching 6.3 s $cm^{-1}$ and 6.01 s $cm^{-1}$ respectively (*Fig. 5*). The study showed that higher stomatal resistance reduced the photosynthesis rate of D24 plants to less than 1µmol/$m^2$/s after 20 d of water stress. Photosynthesis rate was 70% less in stressed plants than in the control plants (*Fig.*7). Chlorophyll content reduced with increasing duration of water stress. There was, however, no significant differences between clones (*Fig.*7).

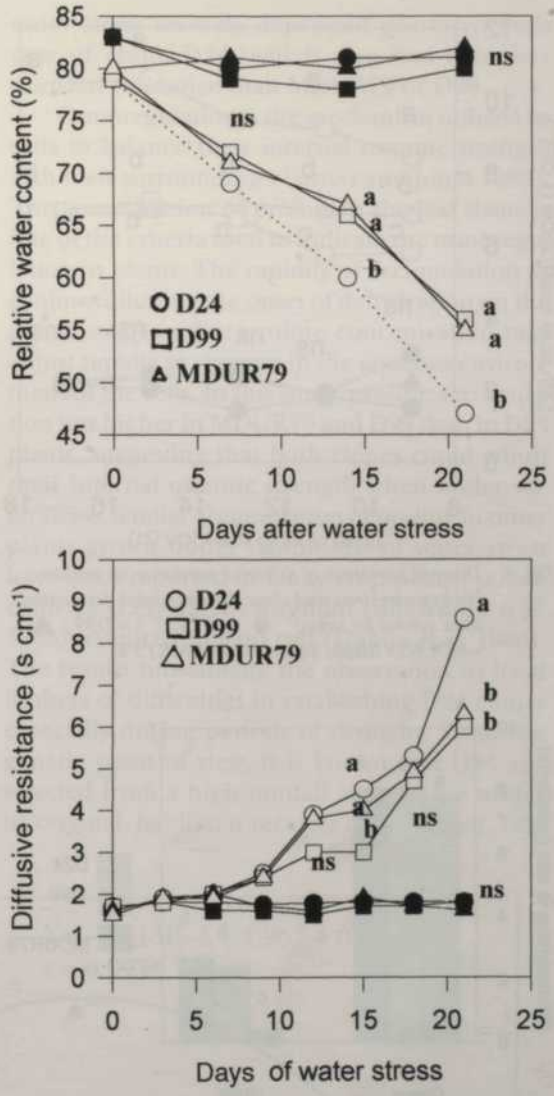



*Fig. 5: Relative water content and stomatal resistance as influenced by clone and water availability. Open symbol for stress; closed symbol for well watered. means separation by DMRT5%*

There was also a significant clonal difference ($P<0.05$) with water stress on proline level. Proline level was highest in MDUR79 plants reaching 93.09 µg/g. In water stress treatment, D24 plants showed the lowest proline level which was only 40.37 µg/g, which was almost similar to the levels in the unstressed plants (*Fig. 8*).

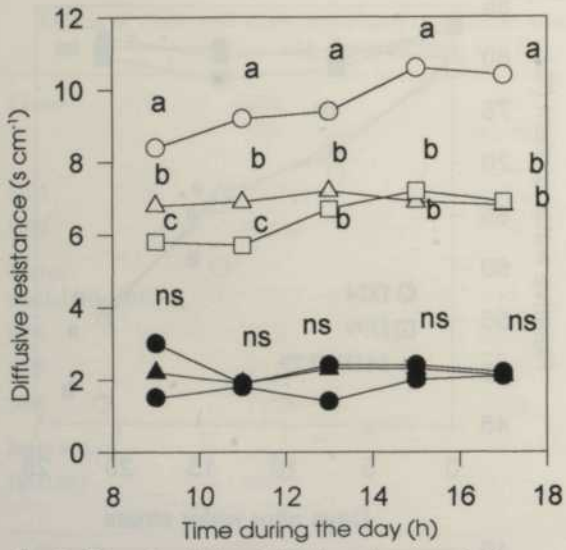



*Fig. 6: Diurnal variation of diffusive resistance as influenced by water stress and clone. Closed symbol for control; open symbol for stress; ● = D24; □ = D99; ▲ =MDUR79 Means separation by DMRT5%*

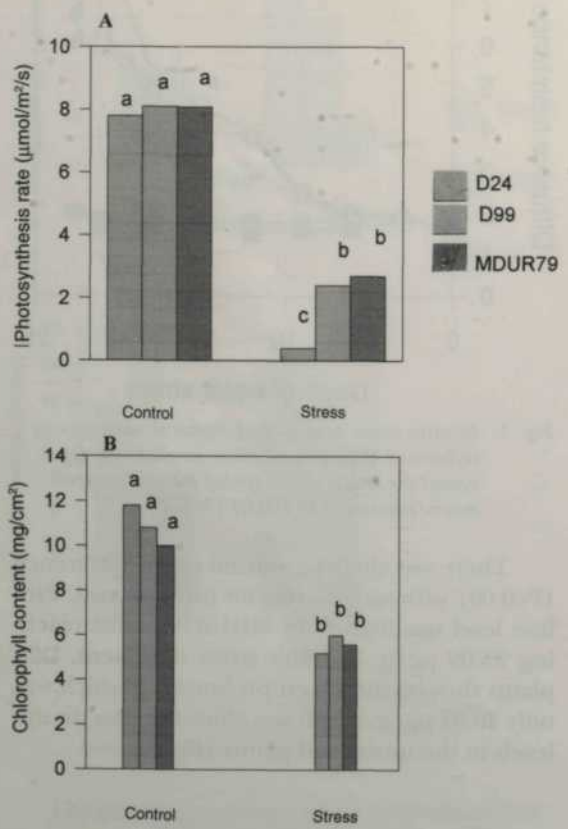



*Fig. 7: Photosynthesis rate and chlorophyll content as influenced by water stress and clone. Means separation by DMRT 5%*

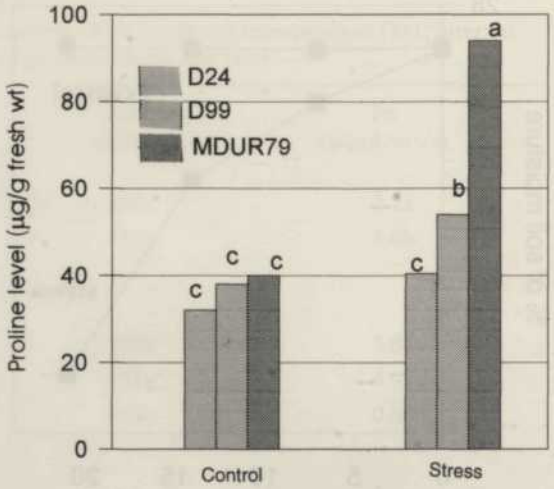



*Fig 8: Proline level as influenced by water stress and clones. Means separation by DMRT5*

## DISCUSSION

The fundamental effect of water stress on plants is the reduction in leaf area and shoot elongation. As shown in Table 1 and *Fig. 1*, leaf area reduced to 50% when plans were grown in the 10% FC. This experiment, however, showed that leaf area and dry weight of durian plants were not affected under moderate water stress. Begg and Turner (1976) suggested that one of the most important consequences of the sensitivity of cell enlargement to water stress is marked reduction in leaf area which will reduce crop growth rate particularly during early stages of plant growth. The decrease in leaf dry weight (Table 1) is the consequences of reduced leaf area accumulation. Reduction in root growth was also observed in plants grown at 10% FC. Zahner (1968) suggested that the decline in root growth with water stress is due to the limited cell division, cell enlargement and tissue differentiation. The reduction in root growth observed in this study agrees with the findings of Sharpe *et al.* (1988).

In both the experiments, relative water content and activities of physiological processes reduced with water stress. *Fig. 9* illustrates a close relationship between soil water status and plant water status indicated by the relative water content in the leaves. The trend is in agreement with the observation by Maruyama and Toyama (1987) on deciduous plants indicating an exponential reduction in xylem pressure with the depletion in soil water. The photosynthesis rate

of D24 plants grown in 10% FC declined to -0.8 µmol/$m^2$/s indicating that the plants experienced respiration. However, at this FC of 10%, plants of D99 were still photosynthesizing on the date of observation, although at a slower rate. This fact is further emphasized in Experiment 2 where water stress severely depressed photosynthesis rate of clone D24, which also had a higher stomatal resistance than MDUR79 or D99.

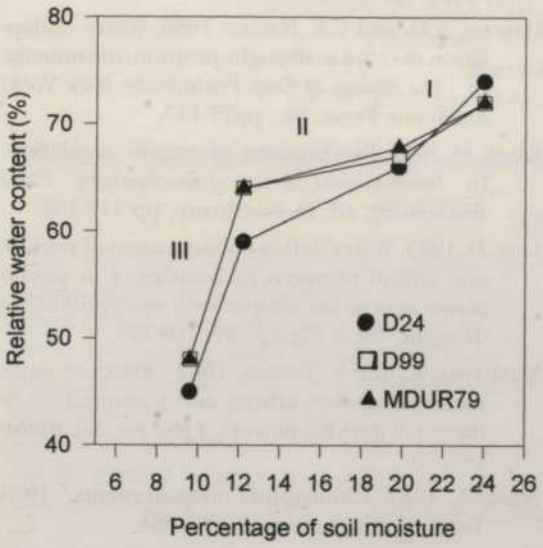
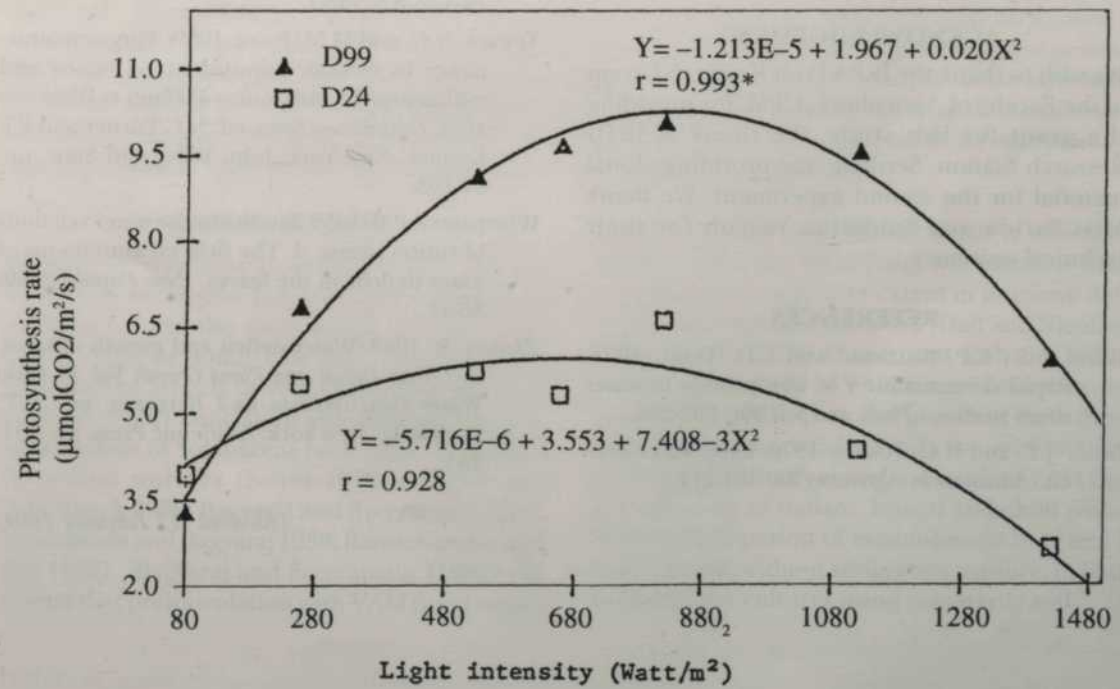



*Fig. 9: Relationship between relative water content and % of soil moisture*

Osmoregulation is the mechanism utilized by cells to balance their internal osmotic strength with their surrounding (Turner and Jones 1980). The accumulation of proline in the leaf tissue is one of the criteria used to indicate the osmoregulation in plants. The rapidity of accumulation of proline following the onset of dehydration on the plants suggests that proline concentration may adjust rapidly to changes in the aqueous environment of the cells. In this study, proline accumulation was higher in MDUR79 and D99 than in D24 plants, suggesting that both clones could adjust their internal osmotic strength when under water stress. Similar accumulation of proline in other plants grown under conditions of water stress have been reported in many crop plants: potato cultivars (Levy 1983); sorghum (Bhaskaran *et al.* 1985); cultured tomato cell (Handa *et al.* 1986). The results substantiate the observation by local farmers of difficulties in establishing D24 clones especially during periods of drought. From the genetic point of view, it is known that D24 was selected from a high rainfall zone where under its original habitat, it receives daily rainfall. D99




*Appendix 1: Light response curves of D24 and D99 young clonal plants*

is a clone from a dry zone and at a higher latitude and it was selected to suit the drier environment while clone MDUR79 is a hybrid having D10 parent obtained from a zone that experiences periods of long water stress. Further analyses of genetic involvement in these clones may clarify the mechanism of such responses to water stress.

## CONCLUSION

The results in this study demonstrate a clear difference in water relations, stomatal responses, photosynthesis rate and accumulation of proline in D24 plants compared to D99 and MDUR79 plants when exposed to water stress. Because of the magnitude of the differences in the parameters examined it would be useful to incorporate the screening of such characters in the early stages of any breeding programme for drought tolerance. The understanding of the clonal responses to water stress could also be beneficial in irrigation planning and management, especially when cultivation is done within an area where water is a limiting factor, or irrigation is a major problem due to topographical differences. Studies also need to be conducted on the improvement of water relation in plants such as the use of water retaining polymers to improve water use efficiency of the plants.

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# Early Establishment of Native Vesicular-Arbuscular Mycorrhizas in Three Vegetable Crops of South India - A Comparative Study

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**Keywords: vesicular-arbuscular mycorrhizas, early establishment, tomato, brinjal, chilli**

## ABSTRAK

*Status mikroiza resrikula-arbuskula, (VAM) tiga tanaman sayuran seperti tomato, terung dan cili dinilai semasa tempoh awal pertumbuhan dalam keadaan kawasan semulajadi dan juga dalam bekas kultur yang menggunakan tanah tak-steril. Tanah tersebut mempunyai status nutrien yang rendah dan tiada pembajaan semasa 60 d kajian dibuat. Pengkolonian fungi VAM dalam akar adalah maksimum pada 45, 50, dan 60 d selepas percambahan setiap benih terung, tomato dan cili di bawah keadaan padang, dan atas d ke 60 dalam percubaan kultur bekas. Jika tiada masalah, 100 peratus pengkolonian akan diperolehi. Kenaikan dan penurunan arah aliran dalam pengkolonian ini adalah diawasi. Dalam semua kes, bilangan spoa asal 3.90 (±0.30) g tanah kering ternyata meningkat dalam tanah rizosfera selepas pertumbuhan tanaman. Jumlah spora lebih nyata dalam bekas daripada yang di kawasan lapang.*

## ABSTRACT

*The vesicular-arbuscular mycorrhizal (VAM) status of three vegetable crops viz., tomato, brinjal and chilli was assessed during the initial establishment period in natural field conditions as well as in pot culture using non-sterile soil. The soil had low nutrient status and no manurial application was made during the 60 d course of the study. VAM fungal colonization in the roots was maximum at 45, 50, and 60 d after the respective germination of brinjals, tomato and chilli seeds under field conditions, and on the 60th d in the pot culture experiment. In no case was 100 per cent root colonization obtained. Ascending and descending trends in colonization were observed. In all cases, the original spore count of 3.90 (±0.30) g dry soil increased markedly in the rhizosphere soil after plant growth. Spore number was more pronounced in pot than in field culture.*

## INTRODUCTION

Tomato (*Lycopersicon esculentum* Mill.), brinjal (*Solanum melongena* L.) and chilli (*Capsicum annuum* L.) are the most widely accepted vegetable crops in South East Asia. Investigations on different aspects of vesicular-arbuscular mycorrhizal (VAM) association with these three popular members of Solanaceae have been carried out by several workers (Sanni 1976; Dehene and Schonbeck 1979; Bagyaraj and Sreeramalu 1982; Sreeramalu and Bagyaraj 1986; Ramachandra and Rai 1987). Bagyaraj and Sreeramalu (1982) observed that preinoculation with VAM fungi would improve growth and yield of chilli. It is well established that the VAM fungi will manifest their performance to a greater extent in nutrient-deficient or low nutrient status soil (Daft and Nicolson 1966; Mosse 1973; Abbott and Robson 1982). However, the study of VAM fungal infectivity, especially in the early period of growth of these three crops in nutrient deficient soils, has seldom been reported. This study aimed to elucidate VAM fungal infectivity of tomato, brinjal and chilli plants in their early period of establishment in N and P deficient soil, without adding any manure, in both field and pot cultures using non-sterile soil.

TABLE 1
Physico-chemico properties of soil used in this study

| Nature | Moisture content (%) | pH | ECse | (kg/hectare$^{-1}$*) N | PO | KO | Zn | Fe | Cu | Mn |
|---|---|---|---|---|---|---|---|---|---|---|
| Sandy loam | 16.50 | 7.20 | 0.20 | 225 (9.00) | 22.50 (0.90) | 780 (31.20) | 0.20 (0.08) | 0.78 (1.00) | 25 (0.03) | 22.50 (0.90) |

* Figures in parentheses are values in mg kg$^{-1}$ soil sample

## MATERIALS AND METHODS

*Field Experiment*

The experiments were carried out in N and P deficient alluvial deposits of sandy loam (Table 1) in Bharathiar University campus, Coimbatore. The soil contained VAM fungal spores predominantly of *Acaulospora bireticulata*, Rothwell and Trappe, *A. sporocarpium*, Berch, *Glomus deserticola* Trappe, Bloss and Menge, *G. fasciculatum* Gerdemann and Trappe, *G. geosporum* Nicolson and Gerdemann and *Sclerocystis pakistanica* Iqbal and Bushra, with an average spore count of 3.90 (10.30) g$^{-1}$ dry soil. Seeds of tomato cv. Co.l, brinjal cv. RR and chilli cv. K were sown in the field, in the normal planting season (monsoon) of June 1992 without any fertilizer but watered regularly. Five hundred seeds were sown per bed each measuring 2 x 0.5 metre, where three complete randomized beds were maintained for each crop in the open field. Ten randomly selected seedlings per crop were carefully removed from the planting beds, from the first day of germination up to 60d at 5-day intervals. VAM colonization was assessed after staining the root samples following the method of Phillips and Hayman (1970) and using the scoring method of Edathil *et al.* (1994). For every sampling, 100 root pieces (10 pieces of young branches from each seedling) measuring 1 cm each were assessed for VAM colonization and the mean value was expressed in percent colonization. The mycorrhizal spore density was estimated using the modified wet-sieving and decanting method (Gerdemann and Nicolson 1963). VAM fungal species were identified by means of morphological characteristics of the spores and sporocarps using the synoptic keys (Hall 1984; Schenck and Perez 1987; Morton 1988). The number of VAM fungal spores in the soil before sowing the seeds and after the completion of the experiment were estimated. Spore count was made on 100g dry soil and recorded as number of spores g$^{-1}$ soil. Each treatment was replicated four times.

*Pot Culture Experiment*

Five seeds per crop were sown in pots of 18 cm diameter filled with 6 kg field soil. A total of 140 pots were used for each crop. No fertilizer was added and the pots were maintained under natural light and watered regularly. After germination, seedlings were thinned to one seedling per pot. Ten randomly selected seedlings were carefully removed each time and VAM colonization was assessed at 5-day intervals from day 1 to day 60 after germination. The number of VAM fungal spores in the soil, before and after the experiment, was also determined.

## RESULTS

Under field conditions, tomato and brinjal seeds germinated on day 8 after sowing while chilli seeds germinated on day 13. Percent root colonization of tomato roots on day one was 24.6 (±5.3)%, brinjal 15.75 (±1.5)% and chilli 18.63 (±3.86)%. In all the three crops, arbuscules or vesicles were readily observed from day 5 onwards until the end of the study (60 d). For tomato VAM colonization was maximum (97.5 ± 0.98%) on day 50 after germination and declined thereafter. In brinjal peak colonization was observed on day 55 after germination (87 ± 2.94%). In the case of chilli the peak was attained on day 60 (97.7 ± 1.07%) (*Fig.1*).

In pot culture, seeds of tomato and brinjal germinated on day 5 after sowing, and on day 8 for chilli. Percentage root colonization on the day after germination was: in tomato 10.55 (±1.74)%, brinjal 8.54 (±2.89)% and chilli 3.42 (±0.90)%. In pot culture, arbuscules and vesicles

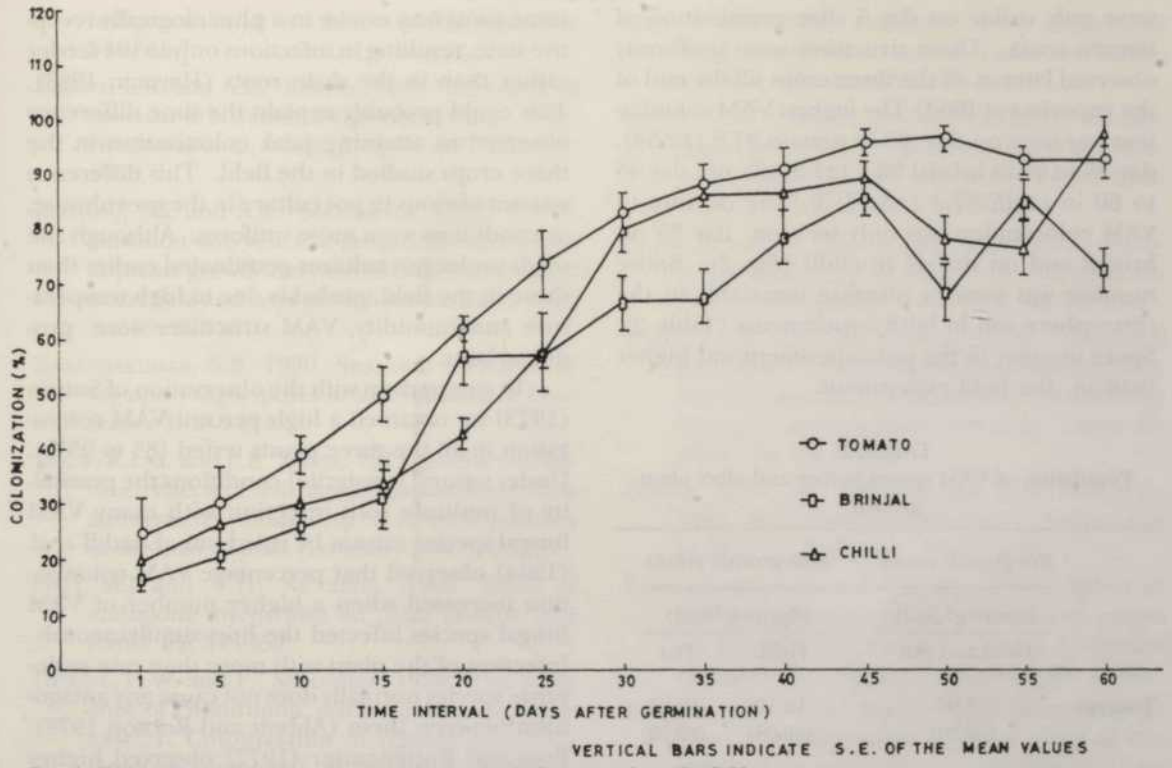


*Fig. 1: VAM colonization in various time intervals-of plant growth in the field*

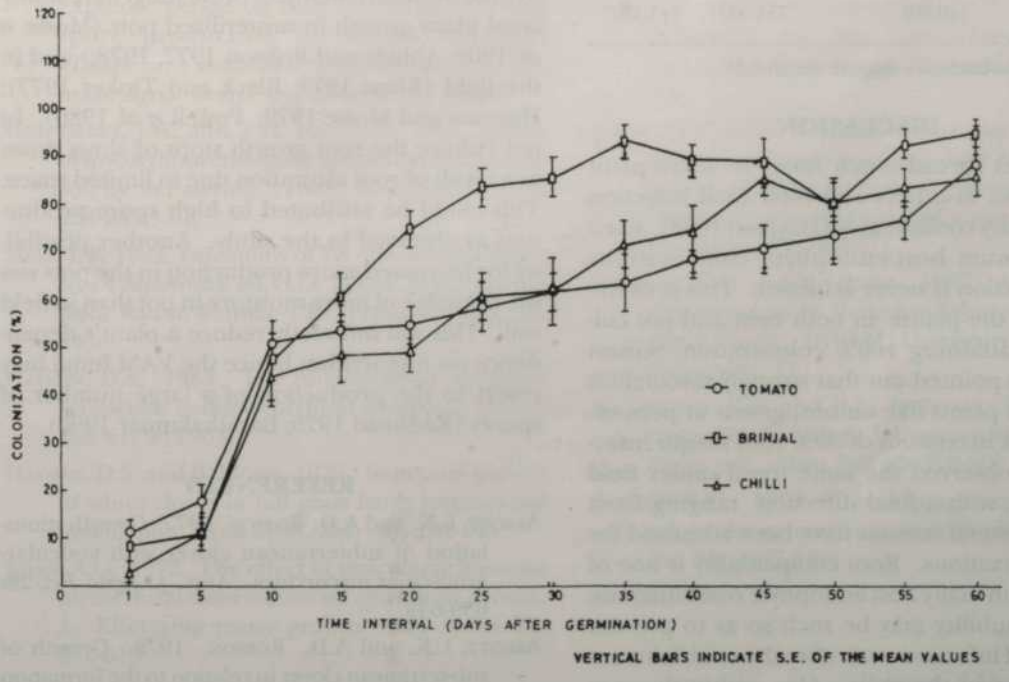


*Fig. 2: VAM colonization in various time intervals of plant growth in pot culture*

were only visible on day 5 after germination of tomato roots. These structures were uniformly observed later in all the three crops till the end of the experiment (60d).The highest VAM colonization was seen on day 60 in tomato 87.8 (±2.54), day 35 to 60 in brinjal 96.1 (±1.39)% and day 45 to 60 in chilli 87.4 (±3.47)%. The decline in VAM colonization was only seen on day 35 in brinjal and on day 45 in chilli (*Fig. 2*). Spore number was seen to increase invariably in the rhizosphere soil in both experiments (Table 2). Spore number in the pot experiment was higher than in the field experiment.

TABLE 2
Population of VAM spores before and after plant growth

| | Pre-growth count | Post-growth count | |
|---|---|---|---|
| | (Spore $g^{-1}$ Soil) | (Spore $g^{-1}$ Soil) | |
| | Field and Pot | Field | Pot |
| Tomato | 3.90 (±0.30) | 16.10 (±0.98) | 52.25 (±3.98) |
| Brinjal | 3.90 (±0.30) | 9.81 (±0.80) | 63.86 (±3.14) |
| Chilli | 3.90 (±0.30) | 26.59 (±1.48) | 43.21 (±1.19) |

Figures in parentheses denote mean S.D.

## DISCUSSION

Endophytes spread much faster in some plant species than in others and their final infection level may vary considerably (Hayman 1983). Even with optimum host-endophyte combinations 100% infection is never achieved. This is exemplified by the plants in both field and pot cultures not attaining 100% colonization. Sutton (1973) has pointed out that strongly mycorrhiza dependent plants like onions, grown in pots, often reach a maximum of 80% root length infection. He observed the same trend under field conditions, with a final infection ranging from 48-84%. Several reasons have been advanced for these observations. Root compatibility is one of the factors in many host endophyte combinations; the compatibility may be such so as to prevent widespread infection even after the establishment of the fungi in the roots. Also, within the root system of a strongly mycorrhiza-dependent plant, some roots may not be in a physiologically receptive state, resulting in infections only in the feeder rather than in the main roots (Hayman 1983). This could probably explain the time difference observed in attaining peak colonization in the three crops studied in the field. This difference was not obvious in pot culture in the greenhouse, as conditions were more uniform. Although the seeds under pot cultures germinated earlier than those in the field, probably due to high temperature and humidity, VAM structures were produced later.

In comparison with the observation of Sutton (1973) we obtained a high percent VAM colonization in all the three plants tested (85 to 95%). Under natural (unsterile) conditions the possibility of multiple root infection with many VAM fungal species cannot be ruled out. Edathil *et al.* (1994) observed that percentage VAM colonization increased when a higher number of VAM fungal species infected the host simultaneously. Infection of the plant with more than one endophyte species normally does not cause any antagonism between them (Abbott and Robson 1978). Ross and Ruttencutter (1977) observed higher infection in peanuts and soybean when inoculated with *Glomus* and *Gigarspora* simulatenously than when inoculated with *Glomus* alone. It is relevant to note that efficient mycorrhizal fungi have stimulated plant growth in unsterilized pots (Mosse *et al.* 1969; Abbott and Robson 1977; 1978;) and in the field (Khan 1972; Black and Tinker 1977); Hayman and Mosse 1979; Powell *et al.* 1980). In pot culture the root growth stops or slows down as a result of root saturation due to limited space. This could be attributed to high spore production as observed in the study. Another possibility for increased spore production in the pots was the presence of more moisture in pot than in field soil. This will indirectly reduce a plant's dependence on mycorrhiza; hence the VAM fungi may resort to the production of a large number of spores (Redhead 1975; Barathakumar 1990).

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# Genotypic Differences in Dry Weight Accumulation, N Assimilation and Redistribution, and the Effects on Seed Yield and Protein Content in Faba Beans

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**Keywords: faba bean, N assimilation and redistribution, protein content, seed yield**

## ABSTRAK

*Kajian ini dijalankan untuk mengkaji kesan-kesan perbezaan genotip dalam penumpukan berat kering, asimilasi dan pengagihan semula N, dan keberkesanan pengagihan semula N ke atas hasil dan kandungan protein di antara tiga kultivar kacang faba dengan menggunakan rekabentuk blok rawak lengkap. Jumlah penumpukan berat kering dalam berbagai peringkat pertumbuhan menunjukkan perbezaan yang bererti di antara kultivar semasa pertumbuhan vegetatif dan pembungaan awal, dan semasa pembungaan dan pengisian biji awal, tetapi tidak pada pengisian biji. Asimilasi N berlaku di sepanjang pertumbuhan tetapi sebahagian besar diasimilasikan semasa pengisian biji dengan julat 47.66 hingga 56.50% jumlah N dalam tumbuhan. Pengagihan semula N daripada daun, batang dan kulit lenggai kepada biji adalah masing-masing dengan julat 26.66 - 31.42%, 6.89 - 11.05% dan 12.24 - 20.51% kandungan N dalam biji. Kandungan N dalam biji yang selebihnya adalah diasimilasikan semasa pengisian biji yang terdiri daripada 40.04 - 52.15%. Kandungan protein yang tinggi dalam biji nampaknya disebabkan oleh asimilasi N yang banyak dalam masa pengisian biji dan bukan keberkesanan pengagihan semula N daripada bahagian-bahagian vegetatif tumbuhan.*

## ABSTRACT

*This experiment was conducted to study the effects of genotypic differences in dry weight accumulation, N assimilation and redistribution, and N redistribution efficiency of different plant parts on seed yield and protein content among three faba bean cultivars, using a randomized complete block design. The total dry weight accumulated in different growth stages showed significant differences among cultivars during vegetative growth and early flowering, and during flowering and early seed filling, but not during seed filling. N was assimilated throughout growth but a large quantity was assimilated during seed filling ranging from 47.66 to 56.50% of the total plant N. The redistribution of N from leaves, stems and pod walls to the seeds ranged from 26.66 - 31.42%, 6.89 - 11.05% and 12.24 - 20.51% of the seed N content, respectively. The remaining seed N content was assimilated during seed filling which accounted for 40.04 - 52.15%. High protein content in seeds seemed to be due to greqter N assimilation during seed filling rather than to the N redistribution efficiency from the vegetative plant parts.*

## INTRODUCTION

The important role of nitrogen (N) in plant productivity is obvious. Many studies in leguminous plants have shown that the loss of N in the vegetative tissues relates to the accumulation of N in the seed (Hanway and Weber 1971; Salado-Navaro *et al.* 1985; Westermann *et al.* 1985).

The proportion of seed N obtained from redistribution varies from 20% to essentially 100% in leguminous plants, depending on enviromental conditions (Egli *et al.* 1978; Minchin *et al.* 1980; Neves *et al.* 1981; Zeiher *et al.* 1982; Venekamp and Koot 1984). Although some

studies have been carried out on the assimilation and redistribution of N in faba bean (e.g. Cooper *et al.* 1976; Dekhuijzen *et al.* 1981; Dekhuijzen and Verkerke 1984; Venekamp and Koot 1984), they are all based on single genotypes.

The present work was carried out to study the genotypic differences in dry weight accumulation, N assimilation and redistribution, and N redistribution efficiency of different plant parts, and the effects of these on seed yield and protein content among three faba bean (*Vicia faba* L.) cultivars.

## MATERIALS AND METHODS

Three faba bean cultivars: Maris Bead, Maris Beagle and Aquadulce were chosen due to the difference in their protein content and seed size. They were sown in the Wye College Experimental Plot on 9 March 1984 in six-row plots at a density of 24 plants per $m^2$ using a randomized complete block design with three replicates. Each plot measured 3.6 x 1.8 m with 22 plants per row. No fertilizer or *Rhizobium* inoculation was given, since the plots were routinely fertilized with organic manure.

Samples were taken at weekly intervals beginning at the ninth week after sowing until maturity. At each sampling period, four plants, one from each of the inner rows, were harvested except for the first two samplings for Maris Bead and Maris Beagle where eight plants were harvested to obtain sufficient materials for the near infrared (NIR) analyses.

The plants were separated into leaves (leaflets + stipules), stems (including petioles), flowers, pod walls and seeds where available. All the plant parts except the matured seeds was dried at 80°C, weighed and carefully mixed to give reasonable representative samples for grinding. The seeds were air dried. The N content in leaves, stems and matured seeds was analysed using the NIR analyser, after suitable calibration for each component was obtained. The N content in flowers, pod walls and developing seeds was analysed using the semi-micro Kjeldahl technique.

The amount of N redistributed was estimated from the change in total N in each plant part between the maximum N content and that at the final harvest at maturity (Cregan 1983). Thus the N redistributed from the leaves was represented by the total maximum N in the leaves minus the total N in leaves at maturity disregarding the fallen leaves. The calculation of N redistribution in leaves therefore overestimated the actual amount, since fallen leaves were not recovered at maturity. The extent of this bias due to fallen leaves was calculated by estimating the amount of fallen leaves. The difference between the maximum leaf dry weight and that at the final harvest was assumed to result from fallen leaves. It was also assumed that the fallen leaves were due to senescence and contained the minimum level of N which was considered equal to that at the final harvest. The amount of N redistributed from the stems and pod walls were also calculated, and the three values summed to give the total of redistributed N. The percentage of redistributed N from each plant part was designated as N redistribution efficiency. The loss of N from the vegetative plant parts and pod walls was assumed to be redistributed to seeds. If the total redistributed N from the vegetative plant parts and pod walls was less than the total seed N, then the difference was assumed to be derived from assimilation during seed filling (Pate and Minchin 1980).

From the above measurements, harvest index and N harvest index were calculated. Harvest index was calculated as the proportion of seed yield over the total above-ground yield, while the N harvest index was calculated as the proportion of seed N over the total above-ground N.

## RESULTS

### *Dry Weight Accumulation*

The total dry weight accumulation in the three faba bean cultivars, i.e. Aquadulce, Maris Bead and Maris Beagle showed a similar trend throughout growth but with higher dry weight accumulation for Aquadulce up to early seed filling stage (*Fig. 1*). The higher dry weight accumulation in Aquadulce compared to the other two cultivars was probably due to the influence of its large seed size (Table 1). However, at maturity, the total dry weight and seed yield were not significantly different among the three cultivars.

The total dry weight accumulated in different growth stages showed significant differences among cultivars during vegetative growth and early flowering, and during flowering and early seed filling but not during seed filling (Table 2). The proportion of dry weight accumulation ranged from 9.50 to 16.52%, 32.88 to 52.45% and 38.05 to 50.60% among the cultivars at three different growth stages respectively. *Fig. 2* shows

TABLE 1
Mean performance of three faba bean cultivars at maturity

| Cultivar | Total dry wt (g/plant) | Seed yield (g/plant) | 100-seed weight (g) | Seed protein (%) | Seed N yield (mg/plant) | Total N yield (mg/plant) | Harvest index (%) | N harvest index (%) |
|---|---|---|---|---|---|---|---|---|
| Aquadulce | 62.81a (78.25a)[1] | 31.84a | 154.94c | 30.00ab | 1534.64a | 1828.24a (1838.53a) | 50.69a (42.78a) | 83.94a (75.09a) |
| Maris Bead | 68.76a (79.85a) | 32.43a | 43.90a | 31.33b | 1626.30a | 1945.14a (1952.08a) | 47.16a (42.06a) | 83.61a (77.95a) |
| Maris Beagle | 79.62a (88.73a) | 34.71a | 76.96a | 29.34a | 1629.64a | 1957.08a (1964.76a) | 43.60a (39.73a) | 83.27a (76.94a) |

[1]Values in parentheses indicate the inclusion of fallen leaves.
All means in a column followed by the same letter were not significantly different from another at 5% level of probability as determined by LSD test.

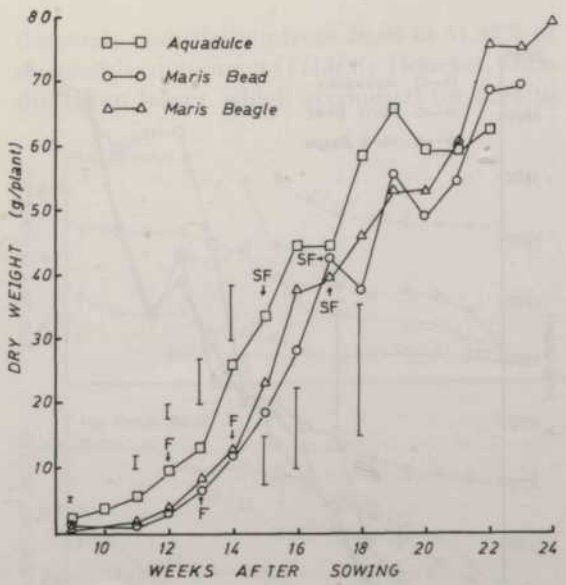


*Fig. 1: Changes in total plant dry weight accumulation of three faba bean cultivars. F and SF indicate the beginning of flowering and seed filling respectively. The vertical bars represent the LSD at the 5% level of significance*

the changes in total dry weight of different plant parts throughout growth and apparently indicated that most of the plant parts except seed attained maximum weight during early seed filling. Therefore, most of the dry weight accumulated during seed filling was devoted to seeds. Moreover, other plant parts showed decreasing dry weight during seed filling as is clearly shown

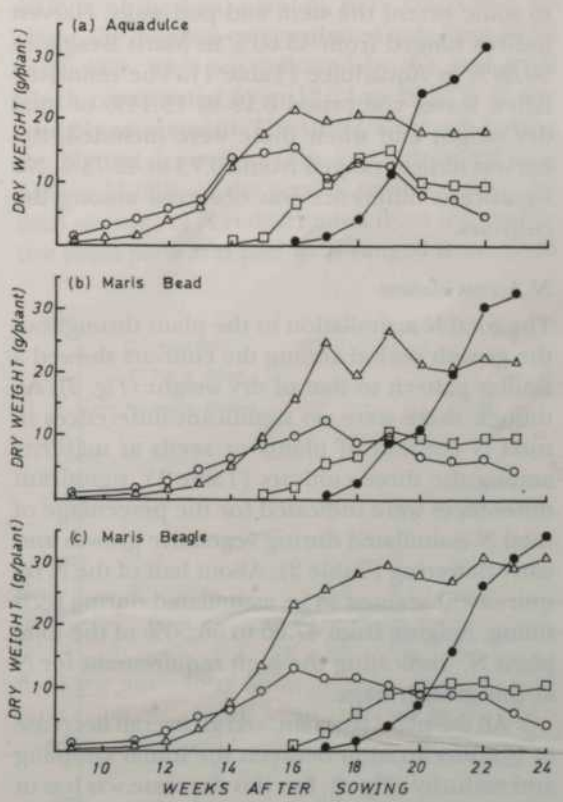


*Fig. 2: Changes in dry weight of different plants parts of three faba bean cultivars.* ( ○ ) leaves; ( △ ) stems; ( ❑ ) pod walls; ( ● ) seeds

TABLE 2
Ontogenetics of dry weight (DW) and N accumulation in three faba bean cultivars

| Cultivar | Character | Percentage of total DW and N accumulation during: | | |
|---|---|---|---|---|
| | | vegetative growth and early flowering | flowering and early seed filling | seed filling |
| Aquadulce | DW | 15.71b | 38.03a | 46.26a |
| | N | 19.16b | 24.35a | 56.50a |
| Maris Bead | DW | 9.50a | 52.45b | 38.05a |
| | N | 13.92a | 38.42a | 47.66a |
| Maris Beagle | DW | 16.52b | 32.88a | 50.60a |
| | N | 23.36b | 21.42a | 55.22a |

All means in a column followed by the same letter were not significantly different at 5% level of probability as determined by LSD test for DW and N respectively.

by leaves (most probably due to fallen leaves) and to some extent the stem and pod walls. Harvest indices ranged from 43.60% in Maris Beagle to 50.69% in Aquadulce (Table 1). The estimated fallen leaves comprised 8.19 to 15.44% of total dry weight and when these were included, the harvest indices ranged from 39.73 to 42.78%. No significant difference was observed among the cultivars.

*N Accumulation*

The total N assimilation in the plant throughout the growth period among the cultivars showed a similar pattern to that of dry weight (*Fig. 3*). Although there were no significant differences in total N content of plants or seeds at maturity among the three cultivars (Table 1), significant differences were indicated for the percentage of total N assimilated during vegetative growth and early flowering (Table 2). About half of the N requirement seemed to be assimilated during seed filling, ranging from 47.66 to 56.50% of the total plant N, indicating the high requirement for N at this growth stage.

All the plant parts showed an overall decrease in N concentration between the initial sampling and maturity (*Fig. 4*), but this decrease was less in the seeds than in leaves, stems or pod walls. Except during early seed filling, seeds of Maris Bead exhibited consistently higher N concentrations throughout seed development compared to Aquadulce or Maris Beagle. At maturity, the seed protein content of Maris Bead was significantly

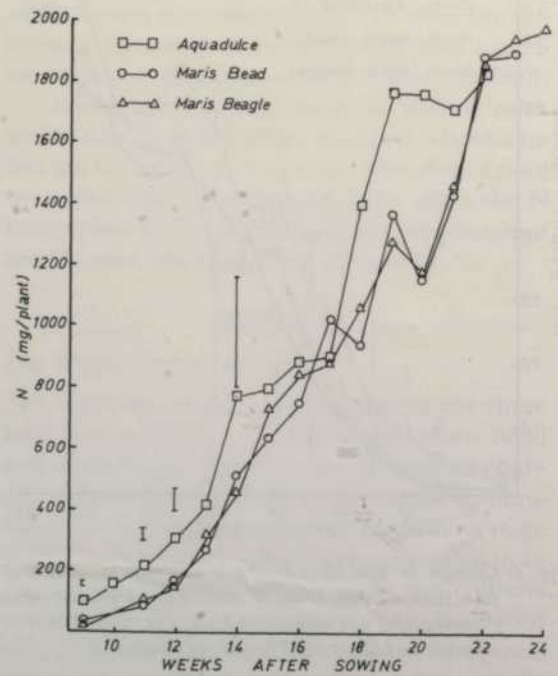


*Fig. 3: Changes in total plant N accumulation of three faba bean cultivars. The verticals bars represent the LSD at 5% level of significance*

higher than that of Maris Beagle but not with Aquadulce.

The decrease of total N in the leaves, stems and pod walls was assumed to be caused by redistribution of N to the seeds during seed filling. The leaves seemed to be the major source of N to

TABLE 3
Sources of N to seeds in three faba bean cultivars. All values expressed as percentage of total N yield of seeds at final harvest

| Source | Aquadulce | Maris Bead | Maris Beagle |
|---|---|---|---|
| Redistribution during seed filling from: | | | |
| leaves + stipules | 30.86a(16.98a)[1] | 26.66a(17.50a) | 31.42a(20.42a) |
| stems + petioles | 8.59a | 6.89a | 11.05a |
| pod walls | 20.51a | 14.30a | 12.24a |
| Total redistribution | 59.96a(46.08a) | 47.85a(38.69a) | 55.69a(43.71a) |
| Assimilation of N during seed filling | 40.04a(53.92a) | 52.15a(61.31a) | 44.31a(56.29a) |

[1]Values in parentheses indicate the percentage when fallen leaves were included.
All means in a row followed by the same letter were not significantly different at 5% level of probability as determined by LSD test.

the seeds, contributing from 26.66 to 31.42% of the seeds' requirement (Table 3). However, when the fallen leaves, which accounted for 6.94 to 10.29% of the total plant N, were taken into account, the total leaves contributed only from 16.98 to 20.42%. This was followed by the pod walls which contributed from 12.24 to 20.51% of the seed N requirement. The stems, although having the highest dry weight (*Fig. 2*), contributed only 6.89 to 11.05% of the seed N requirement. The total amount of N redistributed from the vegetative plant parts and pod walls ranged from 47.85

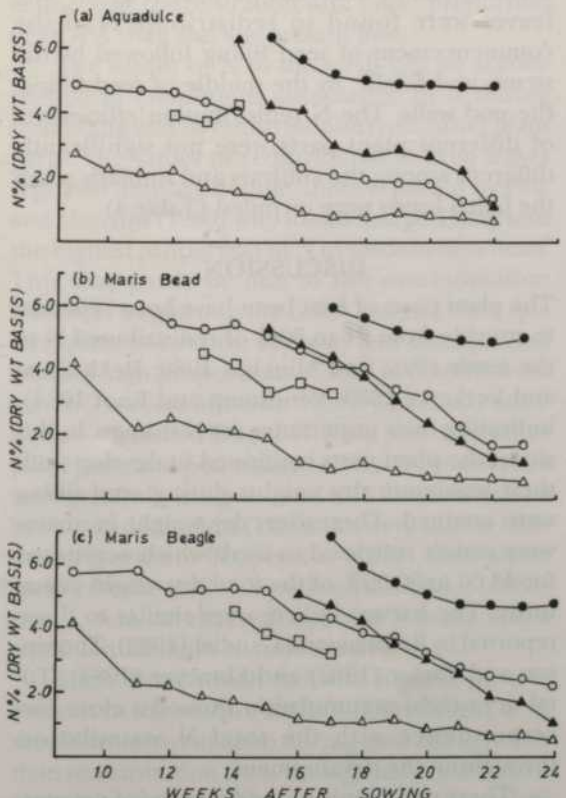


*Fig.4: Changes in N content of different plant parts of three faba bean cultivars.* ( ○ ) leaves; ( △) stems; ( ❑ ) flowers; ( ● ) seeds; ( ▲ ) pod walls

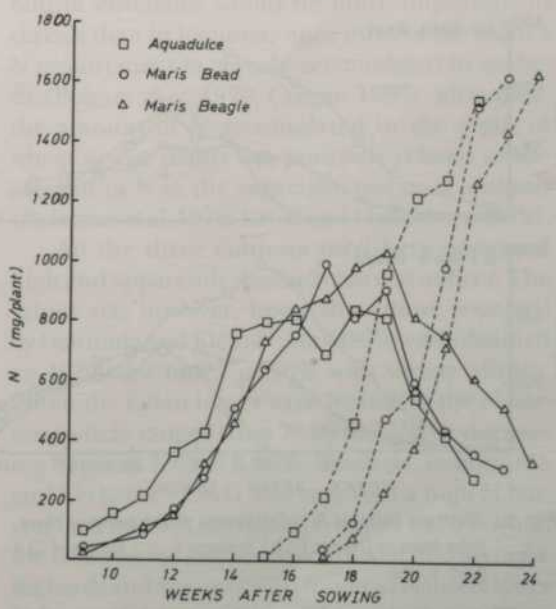


*Fig.5: The relationship of total N in vegetative parts + pod walls and seeds throughout growth of three faba bean cultivars. —— vegetative parts + pod walls;.....seeds*

TABLE 4
N redistribution efficiency (%) of different plant parts in three faba bean cultivars

| Plant part | Aquadulce | Maris Bead | Maris Beagle |
|---|---|---|---|
| Leaves + stipules | 85.95a(47.76a)[1] | 84.70a(57.56a) | 86.24a(56.49a) |
| Stems + petioles | 49.03a | 46.23a | 53.28a |
| Pod walls | 76.30a | 65.07a | 65.75a |
| Total N redistribution efficiency | 75.79a(58.51a) | 70.94a(57.88a) | 72.57a(58.48a) |

[1]Values in parentheses indicate the percentage when fallen leaves were included. All means in a row followed by the same letter were not significantly different at 5% level of probability as determined by LSD test.

to 59.96% but only 38.69 to 46.08% when fallen leaves were included. The remainder of the N in the seeds was therefore assumed to be assimilated during seed filling, and accounted for 40.04 to 52.15% among the three cultivars and with even higher proportions (53.92 to 61.31%) when the fallen leaves were taken into account.

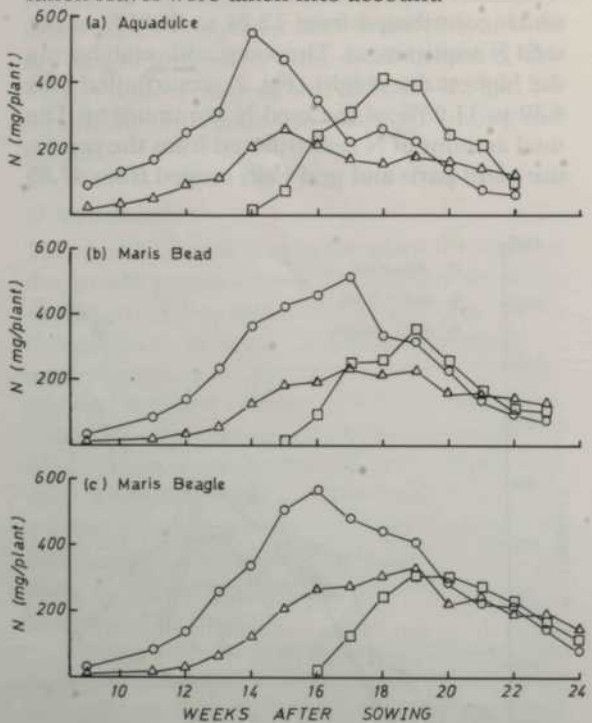


*Fig. 6: Changes in total N of different plant parts of three faba bean cultivars.* ( ○ ) leaves; ( △ ) stems; ( ❑ ) pod walls;

The relationship of the total N in the vegetative plant parts and pod walls and the assimilation of N in the seeds is shown in *Fig. 5.* The total N in the vegetative plant parts and pod walls reached maximum values during early seed filling and then showed a rapid decline which coincided with the linear phase of N assimilation in the seeds. When the total N in each plant part, i.e. leaves, stems and pod walls was examined separately, it was found that the redistribution commenced at different periods (*Fig. 6*). The leaves were found to redistribute N at the commencement of seed filling followed by the stems, and finally, by the middle of seed filling, the pod walls. The N redistribution efficiencies of different plant parts were not significantly different among the cultivars and similarly when the fallen leaves were included (Table 4).

## DISCUSSION

The plant parts of faba bean have been reported to provide from 21 to 50% of redistributed N to the seeds (Pate and Minchin 1980; Dekhuijzen and Verkerke 1983; Venekamp and Koot 1984), indicating their importance for N storage. In this study, the plant parts continued to develop until their maximum dry weights during seed filling were attained. Thereafter, dry weight increases were mainly restricted to seeds which accounted for 43.60 to 50.69% of the total dry weight at maturity. The harvest indices were similar to those reported by Redshaw and Gaudiel (1982), Thompson and Taylor (1982) and Ebmeyer (1984). Total dry weight accumulation showed a close correspondence with the total N assimilation throughout the development.

There were significant differences of percentage N assimilated among the three cultivars only up to early flowering. By early seed filling about

half of the total N had been assimilated and the remainder during seed filling which ranged from 47.66% in Maris Bead to 56.50% in Aquadulce, indicating some differences in the efficiency of assimilating N at different growing periods.

All the plant parts showed decreasing N concentrations throughout growth, most notably during seed filling. Maris Bead which had a significantly higher protein content in mature seeds than Maris Beagle generally had a higher seed N concentration throughout development. Barrat (1982) reported that a line with higher protein concentration in the mature seed maintains a higher percentage of total N in the seed throughout development than a low protein line of the same cultivar. The higher seed N concentration in a high protein plant when compared with a low protein plant is presumably caused by a higher N supply. Barrat and Pullen (1984) studied the changes in the total amino acid and 3,4-dehydroxyphenylalanine (DOPA) content of faba bean pod phloem sap during development and found that the high protein line had a greater amino acid concentration with lower proportion of DOPA than the low protein line.

The supply of N to the seeds can be accomplished by continuous assimilation of N during seed filling and its redistribution from other plant parts. The values of redistributed N from leaves reported here are double those reported by Pate and Minchin (1980) who found that pod walls sent the highest proportion of N to seeds in faba bean. This may partly be due to the overestimation caused by neglecting the fallen leaves. Other species, such as cowpea, soybean and chickpea, all showed high proportions of seed N derived from leaves (Pate and Minchin 1980). The total contribution of redistributed N from the vegetative plant parts and pod walls in all the three cultivars were generally in agreement with values obtained by Pate and Minchin (1980) but were higher than those reported by Cooper *et al.* (1976), Dekhuijzen and Verkerke (1984), Venekamp and Koot (1984) who concluded that redistributed N made only a small contribution to total seed N.

Maris Bead which had the highest protein content in the mature seeds obtained most of its seed N from assimilation during seed filling rather than redistribution from the vegetative plant parts and pod walls; the estimate was even higher when fallen leaves were taken into account. However, the effects of N assimilation during seed filling and N redistribution efficiency on seed protein content could not be ascertained as both were not significantly different among the cultivars. But a low N redistribution efficiency might have the possibility of delaying the decline in N assimilatory processes, nitrate reduction and $N_2$ fixation and therefore enabling the extra assimilation of N during seed filling, since faba bean has the capability of fixing $N_2$ throughout seed filling (Schilling 1983). The result is in accordance with that of Westermann *et al.* (1985) who found that the photosynthetic and $N_2$ fixation activities during seed filling can have a significant influence on total seed N concentration and yield in *Phaseolus vulgaris*. A similar assumption was made by Dekhuijzen and Verkerke (1984), who found that more $^{15}N$ was recovered in the seed at maturity when it was applied to the soil at early pod filling than when applied during early flowering. This indicated that assimilated N during seed filling was preferentially distributed to the developing seeds and the more N was assimilated during this period the less was redistributed from the vegetative plant parts and pod walls.

The present results are in contrast to those of Dickson and Hackler (1975) who found that high protein content in seeds of *Phaseolus vulgaris* was due to greater transfer efficiency from other plant parts. It is assumed that a high N redistribution efficiency would be more important in cereals than in legumes, since most of the plant's N requirement in cereals accumulated by anthesis (Polmer *et al.* 1979; Cregan 1983). Moreover, the amount of N accumulated in the grain of wheat or oat plants was positively related to the amount of N in the vegetative tissues at anthesis (Peterson *et al.* 1975; Cross and Haslemore 1979).

All the three cultivars used here possessed high and apparently similar N harvest indices. The values are, however, lower than those reported by Dantuma and Klein-Hulze (1979) who obtained an N harvest index of 90% with variety Minica. When the fallen leaves were included, the N harvest indices ranged from 75.09 to 77.95%, decreasing between 5.66 to 8.85%. Similarly, Dekhuijzen and Verkerke (1984) also reported a high N harvest index which decreased from 86 to 70% when the fallen leaves and roots were taken into account. Richards and Soper (1979) found a relatively lower N harvest index of 60%. These various reports suggest that there is some variation within faba beans for N harvest indices, although some dif-

ferences may be due to changing environmental conditions.

The results of this study indicate the presence of genotypic differences for N assimilation and redistribution within faba beans. The leaves were the main contributor of N to the seeds followed by pod walls and stem. However, high protein content in seeds seemed to be due to greater N assimilation during seed filling rather than N redistribution efficiency from the vegetative plant parts. The effects on yield could not be generalised due to the small variation of yield between the cultivars.

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# Contribution of Nitrogen to Growth of Maize in Legume-maize Rotation on Limed Ultisols

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**Keywords: N contribution, legume-maize rotation, Ultisols**

## ABSTRAK

*Pengunaan kapur dan sisa tanaman kekacang merupakan pendekatan yang sesuai untuk mengatasi masalah kandungan aluminium yang tinggi dan kandungan N setempat yang rendah menghadkan produktiviti tanaman makanan jangkapendek pada tanah Ultisol. Dengan menggunakan kaedah ini, nilai sumbangan nitrogen kepada tanaman jagong di dalam tiga sistem giliran kacang tanah (L)-jagong (M) (LMLM) telah ditaksirkan. Taksiran dilakukan dengan menolak jumlah pengambilan N oleh jagong pada petak tanpa baja N dalam sistem tanaman tunggal daripada jumlah pengambilan N oleh jagong pada petak sistem tanaman giliran kacang tanah-jagong yang tertentu. Keputusan menunjukkan tanah Ultisol yang dikapur berkeupayaan menampung peningkatan hasil tanaman kacang tanah dan jagong. Dalam sistem tanaman giliran kacang tanah-jagong, purata anggaran N yang disumbangkan kepada jagong selepas satu, dua dan tiga kali tanaman kacang tanah berturutan adalah sebanyak 19, 33, dan 56 kg N ha$^{-1}$. Anggaran 56 kg N ha$^{-1}$ merupakan satu per tiga daripada syor baru keperluan N (159 kg ha$^{-1}$) untuk tanaman jagong.*

## ABSTRACT

*Liming of soils and the use of legume residues in a crop rotation are considered good approaches to reduce the constraints of high aluminium and low native nitrogen affecting annual food crop production in Ultisols. Using this approach the values of nitrogen contributed to maize in three groundnut (L)-maize (M) sequences (LMLM, MLLM and LLLM) in an intensive rotational cropping system were estimated. The estimates were made by subtracting the total N-uptake by maize in the zero N-fertilized monocropping from the total N-uptake by maize under the respective legume-maize rotation. The results showed that it is possible to sustain an increased yield of groundnut and maize on limed Ultisols. In the groundnut-maize cropping system, the average N contribution was after one, two and three successive groundnut crops was 19, 33, and 56 kg N ha$^{-1}$. The latter estimate is about one-third of the newly recommended total N requirement of maize, 159 kg N ha$^{-1}$.*

## INTRODUCTION

In Malaysia about 120,000 ha of highly weathered Oxisols and Ultisols under rubber or oil palm are replanted annually. Annual crops such as groundnut and maize are often used as cover crops, which can replace non-food legume cover crops, during the first four years under the replanting programme. The acidic nature associated with high aluminium concentration and low native nitrogen status often are severe constraints to the growth and productivity of annual food crops on these Ultisols.

The value of atmospheric nitrogen ($N_2$) fixed by groundnut ranges from 72 to 297 kg ha$^{-1}$ (Nutman 1966: Gibson *et al.* 1982; FAO 1984) while the value of N contributed to the soil has been estimated to be between 26 and 60 kg ha$^{-1}$ (Jones 1974; Giri and De 1980). These values were derived from estimations based on $N_2$ fixed over one season of groundnut which almost entirely depended on the native rhizobia. Seed inoculation with specific *Bradyrhizobium* strains has been shown to increase growth and yield of groundnut (Faizah *et al.* 1985). Consequently, the prac-

tice is useful in a crop rotation system involving groundnut and maize.

In Malaysia the high acidity and Al saturation of Ultisols have been shown to reduce growth and yield of groundnut (Foster *et al.* 1980). Earlier research has not, however, provided sufficient information on the N economy of a groundnut-based crop production system for soils with low pH and high Al saturation.

A field study was undertaken, using maize yield data, to estimate the amount of N contributed by inoculated groundnut grown on limed Ultisols.

## MATERIALS AND METHODS

Two long-term field experiments were conducted from 1981 to 1984. Two cropping systems were carried out simultaneously: (1) groundnut-maize rotational cropping system and (2) inorganic N-fertilized maize monocropping system.

1. *Groundnut-maize rotational cropping system*

The experimental site, a former secondary forest, was ploughed twice and rotovated three times before lime was applied at an equivalent rate of 4 mt GML $ha^{-1}$ to raise the soil pH (1:2.5 with $H_2O$) from 4.2 to 5.5. The Bungor soil (Typic Paleudult) was limed after each cropping to maintain the same initial pH for all subsequent croppings throughout the experiment. This was followed by basal dressing with 56 kg $P_2O_5$ as TSP and 56 kg $K_2O$ as MOP. No nitrogenous fertilizer was applied.

A pre-emergent herbicide, Lasso, was sprayed immediately after sowing while the insecticide Malathion was regularly applied after crop emergence. Weeding was done manually when necessary.

Groundnut seeds cv. V13, which were previously treated with the fungicide Thiram, were inoculated with *Bradyrhizobium* strain CB756 and sown in prepared plots, each measuring 5 m by 8 m. Two seeds were planted in each planting hole at 25 cm x 30 cm spacing and thinned a week later to a final plant population of *ca.* 133,000 $ha^{-1}$. The experimental plots were arranged in randomized complete block design with 6 replications.

The legume crop was harvested 106 d after planting ($D_{106}$). Pods were removed and the total weight of residue taken after each harvest. N concentrations were determined at each harvest. Similar measurements were done to all other subsequent groundnut crops during the study period. The residues were returned to the soil as green manure to provide nitrogen for the subsequent crop. Prior to each planting, ten random soil cores (0-20 cm) were collected from each plot. They were bulked, dried, ground, sieved (2 mm) and stored in polythene bags at 4°C. The available soil N ($NH_4^+ + NO_3^-$) from these core samples was determined before cropping using the method outlined by Bremner (1965).

After a fallow period of about three to four weeks, the same plot was prepared for another crop of either maize or groundnut, according to the cropping sequence (Table 1). In either case, the subsequent crop would receive similar basal fertilizers and, where applicable, N from groundnut residues returned to the respective plot at harvest ($D_{106}$). The maize residue, however, was not returned to the soil but its total N uptake along with that of the grain was determined at each harvest using the methods outlined by Chapman and Pratt (1961). Final harvest of maize took place 78 d ($D_{78}$) after planting.

TABLE 1
Groundnut-maize sequence used in the rotational cropping system on limed Ultisols (1981-1984)

| fertilizer rate (kg $ha^{-1}$) | | | groundnut-maize cropping sequence | |
|---|---|---|---|---|
| N | $P_2O_5$ | $K_2O$ | 1st cycle (1981-1982) | 2nd cycle (1983-1984) |
| 0 | 56 | 56 | L M L M | L M L M |
| 0 | 56 | 56 | M L L M | M L L M |
| 0 | 56 | 56 | L L L M | L L L M |

L: legume M: maize

2. *Nitrogen-fertilized Maize Cropping System*

An inorganic nitrogen-fertilized maize monocrop was established simultaneously with the groundnut-maize rotational cropping system on an area separated by 3 m wide grass border from the latter. All field operations including liming to pH 5.5 were similar to those in the rotational cropping system. Five levels of N: 0, 45, 90, 135 and 180 kg N $ha^{-1}$ ($N_0$, $N_1$, $N_2$, $N_3$ and $N_4$) were applied to the respective maize plots; half of these rates were applied together with the basal P and K fertilizers two weeks before sowing. The remainder of the fertilizer N was applied approxi-

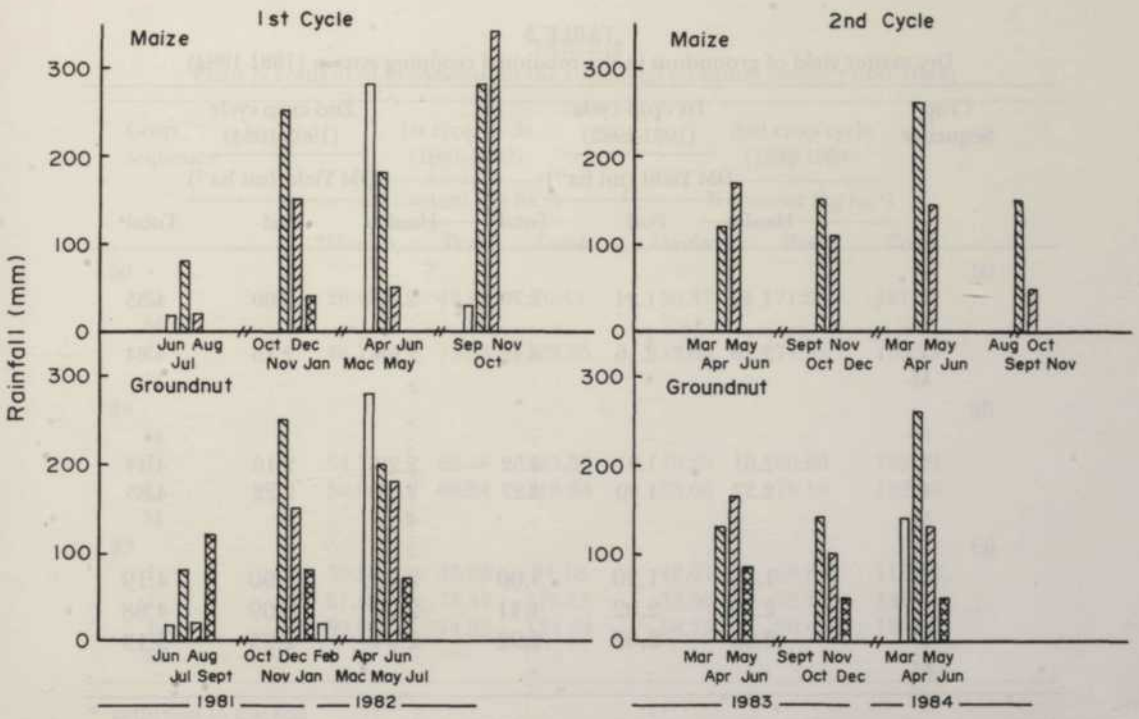


*Fig. 1: Cropping season and rainfall pattern during the field trial*

mately 10 d before tasselling. Thiram-treated maize seeds (var. Sg. Buluh 3) were planted at a spacing of 25 cm x 75 cm to give a plant population of *ca.* 53000 $ha^{-1}$. Experimental plots were arranged in a randomized complete block design with 4 replications with regular applications of insecticide and manual weeding. Maize cobs were harvested at $D_{78}$ after which 10 random maize stubbles from each plot were chopped, dried at 60°C for seven days, weighed, and analysed for N. The grain and stover samples were ground and their N concentrations determined by micro-Kjeldahl method (Bremner 1965).

TABLE 2
Selected chemical characteristics of Bungor sandy clay loam (Typic Paleudult) at the experimental site

| | |
|---|---|
| C ($g\ kg^{-1}$) | 16.83 |
| pH (1:2.5 in $H_2O$) | 4.23 |
| pH (1:2.5 in 1M KCL) | 3.34 |
| N ($g\ kg^{-1}$) | 1.10 |
| P ($mg\ kg^{-1}$) | 10.81 |
| K [$cmol(+)kg^{-1}$] | 0.19 |
| Ca [$cmol(+)kg^{-1}$] | 0.34 |
| Mg [$cmol(+)kg^{-1}$] | 0.24 |
| Al [$cmol(+)kg^{-1}$] | 3.48 |
| ECEC [$cmol(+)kg^{-1}$] | 4.25 |
| *Al saturation (%) | 82.00 |

* Based on effective cation exchange capacity (ECEC)

Two cropping seasons were carried out each year. All crops, except those in the first season of 1981, were planted at the onset of the rainy season to avoid the drought period. The rainfall pattern recorded at the experimental site is presented in *Fig. 1.*

*Plant and Soil Analyses*

All plant tissues were prepared for analysis by the dry-ashing method outlined by Chapman and Pratt (1961). Concentrations of N were determined with the Technicon Auto Analyzer.

Composite soil cores (0-20 cm) from all cropped plots were collected randomly. These soil cores were bulked, air-dried, ground, sieved (2 mm) and analysed for N. The selected chemical characteristics of the soil before the experiment are given in Table 2.

*Estimates of N Contributed by Groundnut*

The N contributed by 1, 2 or 3 successive groundnut crops to maize was estimated by subtracting the total N-uptake of maize in the zero N-fertilized ($N_0$) monocropping experiment from the total N-uptake by maize succeeding

TABLE 3
Dry matter yield of groundnut in the rotational cropping system (1981-1984)

| Crop Sequence | | 1st crop cycle (1981-1982) | | | 2nd crop cycle (1983-1984) | | |
|---|---|---|---|---|---|---|---|
| | | DM Yield (mt ha$^{-1}$) | | | DM Yield (mt ha$^{-1}$) | | |
| | | Haulm | Pod | Total | Haulm | Pod | Total |
| (a) | | | | | | | |
| | L | 1.46 | 1.24 | 2.70 | 2.35 | 2.00 | 4.35 |
| | M | - | - | - | - | - | - |
| | L | 2.26 | 2.16 | 4.42 | 2.68 | 2.16 | 4.84 |
| | M | - | - | - | - | - | - |
| (b) | | | | | | | |
| | M | - | - | - | - | - | - |
| | L | 2.61 | 1.91 | 4.52 | 2.28 | 2.16 | 4.44 |
| | L | 2.37 | 1.90 | 4.27 | 2.63 | 2.22 | 4.85 |
| | M | - | - | - | - | - | - |
| (c) | | | | | | | |
| | L | 1.80 | 1.20 | 3.00 | 2.29 | 1.90 | 4.19 |
| | L | 2.39 | 2.02 | 4.41 | 2.79 | 2.09 | 4.88 |
| | L | 2.85 | 2.07 | 4.92 | 2.94 | 2.25 | 5.19 |
| | M | - | - | - | - | - | - |

groundnut under the respective legume-maize rotation. An estimate was also made by comparing the performance of this maize crop with the response curve developed using the total N-uptake data obtained from the N-fertilized monocropping experiment (MacColl 1989b).

*Statistical Analyses*

Statistical analyses on all data collected and polynomial curve fitting were conducted using the Statistical Analysis System (SAS) Package. An analysis of variance and significant differences was determined using the general linear model (GLM) (SAS, 1979) and contrast method respectively (Snedecor and Cochran 1973; Steel and Torrie 1980).

## RESULTS AND DISCUSSION

### (a) Crop Yield under Rotational and Monocropped Systems

*Dry Matter Yield of Groundnut*

In the cropping sequence where groundnut was grown alternately with maize (LMLM) for four years (1981-1984), total dry matter (DM) yield of groundnut (inoculated with *Bradyrhizobium* CB756) growing on limed Ultisols ranged from 2.70 to 4.84 mt ha$^{-1}$ per season (Table 3a). From this total DM yield the haulm ranged from 1.46 to 2.68, and pods from 1.24 to 2.16 mt ha$^{-1}$. In the cropping sequence where maize was the first crop on the limed Ultisols, followed by two groundnut crops before being put to another maize crop (MLLM), the total DM (Table 3b) fell within the same upper range as in the earlier sequence (LMLM).

In the sequence where three successive groundnut crops were grown, followed by a maize crop (LLLM), the total DM ranged from 3.00 to 5.19; haulm 1.80 to 2.94 and pod yield 1.20 to 2.25 mt ha$^{-1}$ (Table 3c). The pod yield obtained in this study was comparable with that obtained elsewhere in Malaysia (Halim and Ramli 1980; Foster *et al.* 1980). Except for the last sequence (LLLM), there was no clear legume crop phase as originally done by Yaacob and Blair (1979). Nevertheless, the total DM reflects the actual conditions as they exist under field situations in Malaysia. It was most likely that the maintenance of appropriate edaphic conditions through liming had produced the rather consistent DM yield of groundnut. Apart from the first legume crop in the sequence LMLM and LLLM, about 2.1 mt ha$^{-1}$ of pod and 2.5 mt ha$^{-1}$ haulm were obtained. The latter was returned to the soil after each crop as a source of N in the crop rotation.

TABLE 4
Plant N content of groundnut in the rotational cropping system (1981-1984)

| Crop sequence | 1st crop cycle (1981-1982) | | | 2nd crop cycle (1983-1984) | | |
|---|---|---|---|---|---|---|
| | N content (kg ha$^{-1}$) | | | N content (kg ha$^{-1}$) | | |
| | *Haulm | Pod | Total | Haulm | Pod | Total |
| (a) | | | | | | |
| L | 26.49 | 43.95 | 70.44 | 50.37 | 71.62 | 121.99 |
| M | - | - | - | - | - | - |
| L | 48.71 | 73.84 | 122.55 | 53.08 | 77.25 | 130.33 |
| M | - | - | - | - | - | - |
| (b) | | | | | | |
| M | - | - | - | - | - | - |
| L | 54.75 | 68.58 | 123.33 | 49.29 | 80.50 | 129.79 |
| L | 50.90 | 68.94 | 119.84 | 53.08 | 79.18 | 132.80 |
| M | - | - | - | - | - | - |
| (c) | | | | | | |
| L | 30.35 | 43.83 | 74.18 | 49.07 | 69.81 | 118.88 |
| L | 51.47 | 74.47 | 125.65 | 57.50 | 75.45 | 132.95 |
| L | 59.86 | 74.23 | 134.09 | 58.75 | 80.41 | 139.16 |
| M | - | - | - | - | - | - |

* returned to the soil

*Plant Nitrogen*

In the LMLM sequence, the first legume crop did not produce much plant N (Table 4a). The relatively low production could be due to the low rainfall during the growing season (*Fig. 1*). Apart from this first legume crop, legume N was maintained between 122.0 to 130.3 kg ha$^{-1}$ throughout the four-year study period (Table 4). Similarly, except for the first crop, more than 45 kg N ha$^{-1}$ was returned to the soil through the legume residues. Another 74 kg N ha$^{-1}$ in nuts representing 59% of total legume N was removed in the form of pods.

When the same soil from different plots was first cropped to maize in the MLLM sequence, the following two successive legume crops produced a consistent amount of legume N returned to the soil during the same four-year period (Table 4b). Around 53 kg N ha$^{-1}$ was returned to the soil; nearly 69 kg N ha$^{-1}$ was removed in the pod for the first two-year crop cycle and around 80 kg N ha$^{-1}$ in the second crop cycle two years later (Table 4b). In terms of total legume N produced, the amount ranged from 120 to 133 kg ha$^{-1}$, which was about the same as in the previous crop sequence (LMLM).

In the sequence where the soil remained longer under the legume phase (LLLM), the total legume N yield varied from 74 to 139 kg N ha$^{-1}$ (Table 4c). Except for the first legume crop in the first crop cycle, which was low yielding and comparable to the first crop of the LMLM sequence due to lack of rainfall (*Fig. 1*), there was a steady increase in legume N as the legume cropping intensity increased especially in the second two-year crop cycle (1983-1984). This suggested that some of the N returned to the soil in residues had been utilized by the following legume crop, and part of this may have also been removed in the nuts. If so, this followed the usual pattern whereby grain legumes removed a large proportion of their fixed N in nuts. In these three successive crops, the pods, which contained about 55 - 62% of total plant N, were removed from the soil-plant system and, therefore contributed nothing to the succeeding maize crop. The remaining 38 to 45% of plant N contained in the residues was returned to the soil at the end of each groundnut season.

*Soil Nitrogen*

There was a relatively high available N level of 89 to 95 kg ha$^{-1}$ in all plots prior to the first planting (Table 5). In the LMLM plots, available N remained at a high level (87 kg N ha$^{-1}$) before the planting of maize due to the addition of 26

TABLE 5
Available N in soil before sowing in each season in the rotational cropping system (1981 - 1984)

| Crop sequence | 1st crop cycle (1981-1982) | 2nd crop cycle (1983-1984) |
|---|---|---|
| | Available N (kg ha$^{-1}$) | Available N (kg ha$^{-1}$) |
| (a) | | |
| L | 95.40 | 57.32 |
| M | 86.72 | 72.89 |
| L | 65.86 | 65.71 |
| M | 72.09 | 76.42 |
| (b) | | |
| M | 88.56 | 58.69 |
| L | 62.58 | 56.72 |
| L | 68.57 | 61.48 |
| M | 73.73 | 76.89 |
| (c) | | |
| L | 94.82 | 51.66 |
| L | 79.88 | 75.34 |
| L | 82.47 | 80.83 |
| M | 86.48 | 90.41 |

kg N ha$^{-1}$ from the first legume crop (Table 5a). After the first maize crop, the level of available N before the planting of groundnut in the third season was 66 kg N ha$^{-1}$, a lower amount since no residual N was added. A similar trend was observed for the remaining seasons throughout the four-year period. The same trend was also observed in the MLLM sequence after the first maize crop where lower amounts of available N were detected before the planting of groundnut. The available N in the same plot had increased to 74 and 77 kg N ha$^{-1}$ after two successive legume crops in the first (1981-1982) and second (1983-1984) crop cycles, respectively (Table 5b). This was possible because the accumulated legume N from successive legume crops when subjected to periodic wetting and drying cycles under field conditions would stimulate the decomposition of N-rich legume residues (Wetselaar 1967; Yaacob and Blair 1980). The total available N before the second maize planting was about the same for both crop sequences (LMLM and MLLM). Higher available N (86 and 90 kg N ha$^{-1}$) was found in plots after three successive groundnut crops (LLLM) and prior to planting with maize (Table 5c). In all the three crop sequences, the component of legume residual N in the available N before each maize crop was considered the main source of N. The available N represents the organic N of recently added as well as accumulated plant N that was mineralized under field conditions imposed in this experiment. It was observed that every time after the incorporation of the legume residue into the soil, there was an increase in available N before each subsequent crop was planted. However, after each maize crop the available N prior to the next crop planting was low as expected due mainly to no addition of a crop residue and removal by the maize. Under this N depleted condition, whenever groundnut was used in the preceding crop more N accumulated in the plant system, the extra N must have been obtained through $N_2$ fixation.

*Total Dry Matter Yield of Maize*

In the LMLM cropping sequence, the total DM yield of maize after a legume crop ranged from 6.1 to 7.2 mt ha$^{-1}$ per season (Table 6a). In the MLLM sequence, the maize DM yield after two successive legume crops was relatively higher (7.1 and 7.9 mt ha$^{-1}$). The DM yield of maize after three successive legume crops (LLLM cropping sequence) was the highest , 8.5 and 9.9 mt ha$^{-1}$ (Table 6c). The total DM yield increased with increase in legume cropping intensity indicating the influence of cumulative residual legume N. Since no fertilizer N was applied in the rotational

TABLE 6
Dry matter yield of maize under different groundnut-maize rotational cropping systems (1981-1984)

| Crop sequence | 1st crop cycle (1981-1982) | | | 2nd crop cycle (1983-1984) | | |
|---|---|---|---|---|---|---|
| | DM Yield (mt ha$^{-1}$) | | | DM Yield (mt ha$^{-1}$) | | |
| | Stover | Grain | Total | Stover | Grain | Total |
| (a) | | | | | | |
| L | - | - | - | - | - | - |
| M | 2.56 | 3.91 | 6.47 | 2.52 | 3.58 | 6.10 |
| L | - | - | - | - | - | - |
| M | 3.13 | 3.86 | 6.99 | 2.94 | 4.22 | 7.16 |
| (b) | | | | | | |
| M | 2.82 | 3.32 | 6.14 | 2.26 | 2.77 | 5.03 |
| L | - | - | - | - | - | - |
| L | - | - | - | - | - | - |
| M | 3.14 | 3.99 | 7.13 | 3.63 | 4.29 | 7.92 |
| (c) | | | | | | |
| L | - | - | - | - | - | - |
| L | - | - | - | - | - | - |
| L | - | - | - | - | - | - |
| M | 4.14 | 4.36 | 8.50 | 4.77 | 5.09 | 9.86 |

system, the yield data reflected the supply of N derived from native soil N and decomposition of the preceding legume crop. This in turn depended on the total dry matter yield or the vegetative vigour of the legume crops, its seed yield in relation to its vegetative growth, and the possible loss of available N before it could be absorbed by the following maize crop. While it was possible for groundnut to leave a zero residual N (MacColl 1989a), the opposite was true in this study, despite a considerable leaching expected during the four-year cropping period.

In the monocropped maize experiment, maize showed a positive response to N application except for the first season which was erratic due to poor rainfall (*Fig. 1*). This indicated that after the first season of maize, the N present in the soil was insufficient for satisfactory yield. Increments in fertilizer-N up to 135 kg ha$^{-1}$ produced a corresponding increase in yield (*Figs.* 2a and 2b). Beyond this rate, the maize plant experienced a luxury consumption which led to excessive growth of vegetative parts and possibly delayed plant maturity. This subsequently lowered the yield, since harvest was fixed at 78 d for all maize crops (Gauch 1972), and increased the

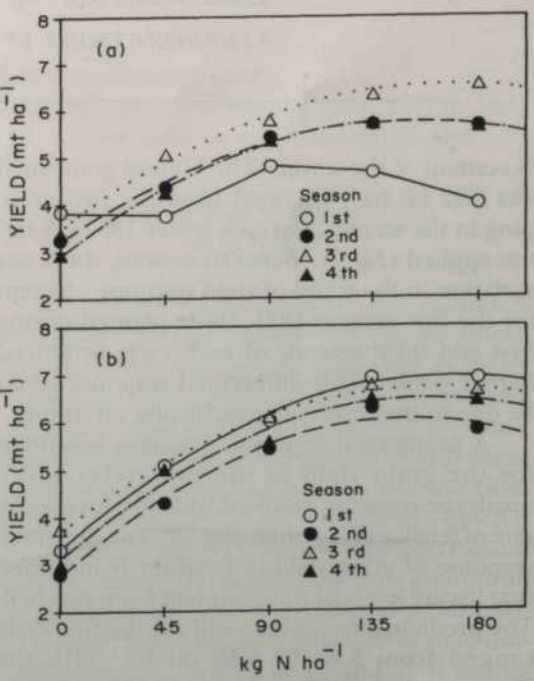


*Fig. 2: Influence of fertilizer-N on the grain yield of maize during (a) 1st (1981-1982) and (b) 2nd (1983-1984) crop cycles*

TABLE 7
Regression analysis of grain yield of maize during (a) 1st (1981-82) and (b) 2nd (1983-1984) crop cycles in monoculture cropping system

(a) 1st Crop Cycle

| Season | Regression | Predicted maximum yield | Quantity of N required to produce maximum yield |
|---|---|---|---|
| | | mt $ha^{-1}$ | kg $ha^{-1}$ |
| 2 | 3.22+0.033X-0.11x10-3 $X^2$ | 5.69 | 153.15 |
| 3 | 3.48+0.037X-0.11x10-3 $X^2$ | 6.59 | 166.19 |
| 4 | 2.92+0.038X-0.12x10-3 $X^2$ | 5.92 | 151.70 |

(b) 2nd Crop Cycle

| Season | Regression | Predicted maximum yield | Quantity of N required to produce maximum yield |
|---|---|---|---|
| | | mt $ha^{-1}$ | kg $ha^{-1}$ |
| 1 | 3.33+0.042X-0.12x10-3 $X^2$ | 7.00 | 172.91 |
| 2 | 2.72+0.044X-0.15x10-3 $X^2$ | 5.95 | 149.72 |
| 3 | 3.69+0.037X-0.11x10-3 $X^2$ | 6.80 | 163.45 |
| 4 | 3.12+0.042X-0.13x10-3 $X^2$ | 6.51 | 158.69 |

N content of the stover. The highest grain yield was 6.92 mt $ha^{-1}$, obtained from the first cropping in the second crop cycle where 135 kg N $ha^{-1}$ was applied (*Fig.* 2). Between seasons, there was variation in the trend of yield response. Except for the first crop in 1981, those planted during first and third seasons of each cycle produced higher yields. This differential response could be due to the result of agroclimatic variations.

A polynomial response equation was fitted for the grain yield of the two cycles and a quadratic response resulted with the increasing rate of fertilizer N applied (*Fig.* 2). The quadratic response of grain yield to fertilizer N indicated that luxury levels of nitrogen had been reached. The predicted maximum yield for the first cycle ranged from 5.69 to 6.59 mt $ha^{-1}$ with the corresponding fertilizer N rate of 152 to 166 kg N $ha^{-1}$. For the second crop cycle it ranged 5.95 to 7.00 mt $ha^{-1}$ when fertilized at 150 to 173 kg N $ha^{-1}$ (Table 7). These fertilizer N rates are higher than those recommended by the Department of Agriculture of Malaysia for maize on sedentary soils (Jabatan Pertanian Malaysia 1982). The data suggest a re-examination of what constitutes a recommended fertilizer N rate for maize on Bungor sandy clay loam. Average grain yields for the plot receiving no fertilizer N were 3.37 mt $ha^{-1}$ (58.9% relative to maximum yield) and 3.22 mt $ha^{-1}$ (48.8% relative to maximum yield) for the first and second cycles, respectively.

**(b) N Contribution by Groundnut**

The total DM yield (grain and stover) and total N uptake by maize cropped on soil to which varying amounts of legume residues were added were used to estimate the amount of N contributed by the legume crop. This estimate was further compared to values for using the same parameters in maize from the inorganic N-fertilized monocropping experiment.

A closer examination of the total dry matter (DM) and N uptake (NU) data from maize grown on soils under different crop sequence compared to those from the zero N-fertilized monocropped experiment showed significant positive trends in

TABLE 8
Total DM (mt $ha^{-1}$) and N uptake (kg N $ha^{-1}$) of maize on limed Ultisols receiving varying number of groundnut crops or rates of fertilizer-N at Puchong, Malaysia

| No. of previous legume crops | rotation (Legume N) | | monoculture [Fertilizer N (kg $ha^{-1}$)] | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | 0 (Control) | | 45 | | 90 | | 135 | | 180 | |
| | DM | NU | DM | NU | DM | NU | DM | NU | DM | NU | DM | NU |
| *1st crop cycle* | | | | | | | | | | | | |
| ┌ | 6.47 | 68.05 | 6.17 | 56.25 | 8.47 | 88.94 | 10.24 | 111.90 | 11.65 | 118.25 | 10.98 | 119.31 |
| LMLM ── | 6.99 | 65.28 | | | | | | | | | | |
| LLLM ── | 8.50 | 87.67 | 5.76 | 50.78 | 8.57 | 77.15 | 10.17 | 105.93 | 11.98 | 123.26 | 11.16 | 118.75 |
| MLLM ── | 7.13 | 73.80 | | | | | | | | | | |
| └ | 6.14 | 71.74 | 7.54 | 71.79 | 7.48 | 70.71 | 9.31 | 97.74 | 8.80 | 88.90 | 7.82 | 76.02 |
| *2nd crop cycle* | | | | | | | | | | | | |
| ┌ | 6.10 | 63.04 | 5.10 | 45.43 | 8.60 | 79.37 | 10.02 | 104.39 | 11.87 | 124.07 | 10.89 | 114.38 |
| LMLM ── | 7.16 | 74.24 | | | | | | | | | | |
| LLLM ── | 9.86 | 97.40 | 6.02 | 50.35 | 9.32 | 89.27 | 10.80 | 110.40 | 12.65 | 134.05 | 12.20 | 131.14 |
| MLLM ── | 7.92 | 79.55 | | | | | | | | | | |
| └ | 5.03 | 61.18 | 6.50 | 57.92 | 9.74 | 99.58 | 11.00 | 123.70 | 13.02 | 153.42 | 13.20 | 155.69 |

DM: total DM yield NU: total N uptake

the N-balance due to the groundnut-maize rotational system on limed Ultisols (Table 8).

Where maize was grown after one legume crop (LMLM) for four years (1981-1984), the total DM yield values (6.47 and 6.99 mt $ha^{-1}$) were equivalent to those between control and 45 kg N $ha^{-1}$ plots (6.17 and 8.47 mt $ha^{-1}$, respectively) in the monocropping experiment. Similar results were obtained with the maize yield after two successive groundnut crops (7.13 mt $ha^{-1}$) as observed in the MLLM crop sequence. A similar trend was observed in the N-uptake values. However, when three groundnut crops were grown and later followed by a maize crop (LLLM) a higher total DM yield (8.50 mt $ha^{-1}$) and N uptake value (87.67 kg N $ha^{-1}$) were obtained (Table 8). From these data, it is possible to estimate the percentage of legume-N contributed by groundnut under the various rotational cropping sequence. For example, in the LMLM cropping sequence:

$$\% \text{ increase in total yield} = \frac{\text{Total yield after one legume crop (6.47 mt ha}^{-1}\text{)} - \text{Total yield in zero N-fertilized plot (6.17 mt ha}^{-1}\text{)}}{\text{Total yield in zero N-fertilized plot (6.17 mt ha}^{-1}\text{)}} \times 100$$

In the 1981 cropping season, the legume N contributed by one groundnut crop produced about 5 % increase in total DM yield of maize (Table 9a). This was expected as the growing season experienced a poor rainfall (*Fig. 1*). Since then (1982-84), groundnut alternated with maize (LMLM) contributed an average increase close to 20 % total maize DM yield (Table 9a), which is comparable to that estimated by MacColl (1989b). In the cropping sequence where two groundnut crops were grown prior to a maize crop (MLLM), the latter produced a 24 % increase in yield during the first crop cycle and around 32 % during

TABLE 9
Percentage increase in, and fertilizer equivalent of DM yield of maize in the rotational cropping system

| Crop sequence | 1st crop cycle (1981-1982) | | 2nd crop cycle (1983-1984) | |
|---|---|---|---|---|
| | % Increase in Total DM | Fertilizer N equivalent (kg ha$^{-1}$) | % Increase in Total DM | Fertilizer N equivalent (kg ha$^{-1}$) |
| a) | | | | |
| L | - | - | - | - |
| M | 4.86 | 5.87 | 19.60 | 19.70 |
| L | - | - | - | - |
| M | 19.61 | 12.86 | 18.94 | 15.55 |
| b) | | | | |
| M | - | - | - | - |
| L | - | - | - | - |
| L | - | - | - | - |
| M | 23.78 | 21.94 | 31.56 | 25.91 |
| c) | | | | |
| L | - | - | - | - |
| L | - | - | - | - |
| L | - | - | - | - |
| M | 47.57 | 43.88 | 63.79 | 61.42 |

the second crop cycle (Table 9b). In a crop sequence where three successive legume crops were grown (LLLM), the legume N contributed towards an increase of 48% and 64% in maize DM yield during the first and second crop cycle, respectively (Table 9c).

These percentages can be equated to the fertilizer equivalent without referring to the response curves developed from the inorganic N-fertilizer monocropped experiment but by a direct comparison using the response of DM up to 90 kg N ha$^{-1}$ which showed a highly correlated relationship. All the maize yield responses in the rotational crop were observed to fall within this range. Beyond this fertilizer rate the specific yield response increased at a reduced rate. For the LMLM sequence, the fertilizer-N (DM) equivalent derived after the first and second legume crop were 6 and 20 kg ha$^{-1}$, respectively, for the first crop cycle, and 13 and 16 kg ha$^{-1}$, respectively, for the second crop cycle (Table 9a). For the MLLM, the fertilizer-N (DM) equivalent derived after two successive legume crops in the first and second crop cycles were 22 and 26 kg ha$^{-1}$, respectively (Table 9b). With three succes-

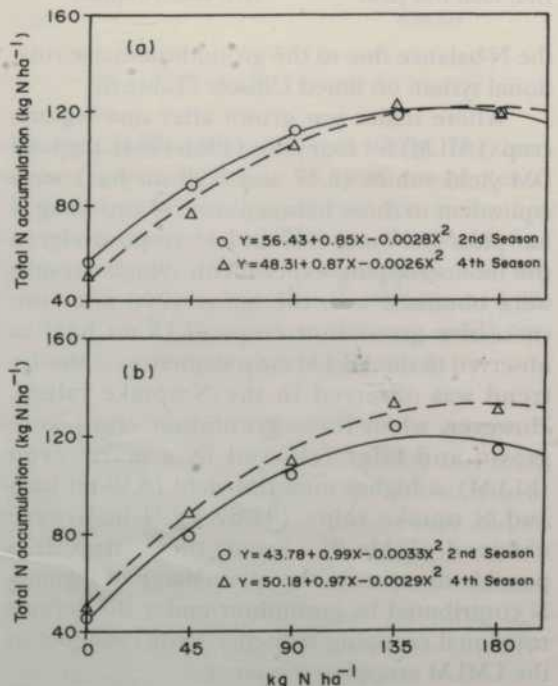


*Fig. 3 : Effect of fertilizer-N on the total-N accumulation during (a) 1st (1981-1982) and (b) 2nd crop cycles*

sive legume crops (LLLM) the amount of fertilizer-N (DM) equivalent was 44 kg $ha^{-1}$ for the first crop cycle and 61 kg $ha^{-1}$ for the second crop cycle (Table 9c).

In addition to the N estimates obtained by direct comparison, the estimates contributed by the legume can also be equated to the fertilizer-N equivalent, obtained by comparing them with the response curves developed from the N-fertilized monocrop experiment. Using the higher coefficient of determination of the response curves for N uptake until 180 kg N $ha^{-1}$ (*Fig.* 3a and 3b), it was possible to estimate the amount of N contributed by groundnut, a technique similar to that used by MacColl (1989b). The fertilizer-N estimates obtained after the first and second legume crop (LMLM) during the first crop cycle was 14.30 and 20.81 kg N $ha^{-1}$, respectively (*Fig.* 3a). During the second cycle in the next two years (1983-1984), the same crop sequence (LMLM) produced the respective fertilizer-N estimates: 13.39 and 26.82 kg N $ha^{-1}$ (*Fig. 3b*). In the MLLM sequence the amounts of fertilizer-N estimates derived after two successive legume crops were 32.45 and 33.46 kg N $ha^{-1}$ for the first and second crop cycle, respectively. The fertilizer-N equivalent produced after three successive legume crops (LLLM) was 53.9 for the first cycle and 58.6 kg $ha^{-1}$ for the second crop cycle.

In practical terms, it is the fertilizer estimate contributed by the groundnut (or other annual grain legumes) that becomes important in a crop rotational practice. This N contributions was determined by averaging the estimates from the first (1981-1982) and second (1983-1984) crop cycles in all three groundnut-based sequences. The average N contribution in the LMLM, MLLM and LLLM sequence were 19, 33 and 56 kg N $ha^{-1}$, respectively. The amount of N contributed increased with increase in the number of successive groundnut crops which produced a cumulative effect of legume residual N. When more than one legume crop was grown it is expected that the N would be derived partly from the previous and newly added legume residue (Yaacob and Blair 1979). From *Figs 2a and 2b*, the average fertilizer-N required to produce maximum grain yield was 159 kg N $ha^{-1}$. The result implied that a substantial amount of N (56 kg N $ha^{-1}$) could be contributed by a legume residue especially after three successive groundnut crops. The N contributed which amounted to 35 % of the N requirement of maize is substantial in the nitrogen economy of a groundnut-based cropping system.

## CONCLUSION

The data from this long-term (1981-1984) field experiment show that it is possible to sustain an increased yield of groundnut and maize on limed Ultisols. In the groundnut-maize cropping system, the average N contribution after one, two and three successive groundnut crops were 19, 33, and 56 kg N $ha^{-1}$. The last figure is about one-third of the recommended total N requirement of maize of 159 kg N $ha^{-1}$ for Bungor soil.

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# Heritability and Response to Recurrent Selection in Two Sweet Corn Varieties

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## ABSTRAK

*Kajian pemilihan berulang mudah dan pemilihan berulang salingan penuh-sib di dalam pusingan pertama telah dijalankan ke atas dua varieti jagung manis, Manis Madu dan Bakti-1, di Universiti Pertanian Malaysia. Objektif kajian ini ialah untuk membandingkan kesan kedua-dua prosedur pemilihan tersebut, dan menganggarkan kebolehwarisan untuk ciri-ciri penting dalam kedua-dua populasi. Kewujudan varian genetik yang lebih tinggi di dalam populasi Bakti-1 $S_1$ ($B1S_1$) berbanding dengan populasi Manis Madu $S_1$ ($MMS_1$) menunjukkan bahawa $B1S_1$ mengandungi variabiliti genetik yang lebih tinggi dari $MMS_1$. Anggaran kebolehwarisan-luas untuk ciri-ciri yang dikaji adalah sederhana hingga tinggi (42.6% hingga 65.7%) dalam populasi progeni penyendirian, tetapi rendah hingga sederhana (20.0% hingga 49.2%) dalam populasi progeni kacukan. Selepas satu pusingan pemilihan, pemilihan berulang mudah didapati lebih berkesan di dalam meningkatkan hasil dalam kedua-dua populasi. Pemilihan berulang mudah telah meningkatkan hasil tongkol segar dalam Bakti-1 dan Manis Madu, masing-masing sebanyak 16.7% dan 10.2%, manakala pemilihan berulang salingan penuh-sib telah meningkatkannya dalam Bakti-1 sebanyak 5.9%, tetapi mengurangkannya dalam Manis Madu sebanyak 6.4%.*

## ABSTRACT

*One cycle of simple and full-sib reciprocal recurrent selection programme was conducted on two sweet corn varieties, Manis Madu and Bakti-1, at Universiti Pertanian Malaysia. The objectives of the study were to compare the response to the two selection procedures, and to estimate heritability of some important characters in the two populations. The presence of higher genetic variance in Bakti-1 $S_1$ population ($B1S_1$) compared to that of Manis Madu ($MMS_1$) showed that $B1S_1$ possessed higher genetic variability than $MMS_1$. Broad-sense heritability estimates for the characters studied were moderate to high (42.6% to 65.7%) in the selfed progeny populations, but were low to moderate (20.0% to 49.2%) in the crossed progeny populations. After one cycle of selection, simple recurrent selection was found to be more effective in increasing yield in both populations. Simple recurrent selection increased fresh ear yield in Bakti-1 and Manis Madu by 16.7% and 10.2% respectively, while full-sib reciprocal recurrent selection increased it in Bakti-1 by 5.9%, but decreased it in Manis Madu by 6.4%.*

## INTRODUCTION

In Malaysia, growing of corn (*Zea mays* (L.)) for human consumption of the fresh ears started with the use of field corn varieties, Local Flint and Metro. The beginning of the utilization of sweet corn varieties in the country was marked by the introduction of the variety Chinta in the sixties. Subsequently, many varieties were introduced, developed and selected for local utilization, among which were Bakti-1, Manis Madu and Mas Madu. The country's average yield production, however, has been low i.e. less than 5 metric tons of fresh

ears per ha. (Abdul Rahman *et al.* 1987). Research efforts are continously carried out to develop high yielding varieties, with acceptable eating quality and suitable to the local conditions.

Selection based on $S_1$ progeny performance in simple recurrent selection was found effective in increasing the frequency of favourable genes in corn populations by Comstock (1964) and Wright (1980). The full-sib reciprocal recurrent selection was found effective in population improvement involving crosses between two diverse populations (Hallauer 1967). West *et al.* (1980) found that after two cycles, selection based on $S_1$ progeny performance was more effective than that based on full-sib progeny performance in increasing yield of selfed populations; but both methods gave the same effects in populations developed from crosses between the improved populations. In another study, after five cycles, selection based on the $S_1$ progeny performance was found to be more effective in increasing yield in crossed populations between the improved populations (Odhiambo and Compton 1989).

This study was conducted to compare the response to one cycle of simple recurrent selection ($S_1$ progeny) and full-sib reciprocal recurrent selection in improving the base population of each of the two sweet corn varieties, Manis Madu and Bakti-1, and to determine the genetic variation and heritability of some important traits in the populations.

## MATERIALS AND METHODS

The study was conducted at the Faculty of Agriculture Research Farm, Universiti Pertanian Malaysia, Serdang, Selangor. The source populations were two open-pollinated local sweet corn varieties, Bakti-1 and Manis Madu.

One cycle of simple recurrent selection based on the $S_1$ progeny performance and reciprocal recurrent selection based on the full-sib progenies was performed on the two source populations, involving four phases of planting: selfing and full-sib crossing in the first phase; evaluation of $S_1$ and full-sib progenies in the second; intermating among the selected $S_1$ families based on the $S_1$ or full-sib progeny performance in the third; and evaluation of the improved populations in the fourth phase. In the first phase about 200 selfed and 200 full-sib families were formed from each population. After the second phase, 20% of the $S_1$ families in each evaluation (the male $S_1$ families in case of full-sib evaluation) were selected based on their superiority in fresh largest ear weight per plant, for intermating in the third phase. At the end of the first cycle, four improved populations were formed, i.e. two from simple recurrent selection: $B1SC_1$ from Bakti-1 and $MMSC_1$ from Manis Madu, and two from the full-sib reciprocal recurrent selection: $B1RFSC_1$ from Bakti-1 and $MMRFSC_1$ from Manis Madu. Evaluations of the improved populations were conducted in comparison with the respective source populations Bakti-1 ($B1C_0$) and Manis Madu ($MMC_0$) to determine the improvement in performance of each population *per se*. The randomised complete block design was used in all evaluations. The $S_1$ and the full-sib progeny evaluations in the second phase were conducted separately, each in two replications at one environment, Field 2. Each progeny family was planted in three-metre-long, one-row plots, with a planting density of 0.75 m x 0.25 m. Six plants were sampled randomly from each plot for the measurements. The improved population evaluations in the fourth phase were conducted in four replications each at two environments, Field 2 and Field 10B. Each plot comprised eight four-meter-long rows of plants, with the same planting density as in the progeny evaluations. Plants in the middle portion of the inner six rows in each plot were used for data collection.

Broad-sense heritability was estimated using the variance components in the analysis of variance table in the selfed and full-sib progeny evaluations following the equation:

$$h_B^2(\%) = \frac{\acute{o}_F^2}{(\acute{o}_e^2 + r\acute{o}_F^2)/r} \times 100 ;$$

where $h_B^2$, $\acute{o}_F^2$, $\acute{o}_e^2$ and r are broad-sense heritability, variance among families, error variance and number of replications, respectively.

Response to selection was calculated using the following equation:

$$\text{Selection response } (\%) = \frac{C_1 - C_0}{C_0} \times 100 ;$$

where $C_1$ and $C_0$ are means for the respective improved and source populations, respectively.

TABLE 1
Genotypic and phenotypic variances, and heritability ($h_B^2$) for characters measured in $B1S_1$ and $MMS_1$ populations

| Character | Population | Mean squares | | Variance | | $h_B^2$(%) |
|---|---|---|---|---|---|---|
| | | Family | Error | $\acute{o}_g^2$ | $\acute{o}_p^2$ | |
| Fresh largest-ear weight per plant (g) | $B1S_1$ | 2641.2** | 1376.7 | 632.3 | 1320.6 | 47.9 |
| | $MMS_1$ | na | na | na | na | na |
| Fresh dehusked largest-ear weight per plant (g) | $B1S_1$ | na | na | na | na | na |
| | $MMS_1$ | 1244.8** | 553.9 | 345.4 | 622.4 | 55.5 |
| Days to tasselling (days) | $B1S_1$ | 6.1** | 2.2 | 1.9 | 3.1 | 63.3 |
| | $MMS_1$ | 6.1** | 2.7 | 1.7 | 3.0 | 55.3 |
| Ear diameter (mm) | $B1S_1$ | 14.0** | 5.6 | 4.2 | 7.0 | 60.1 |
| | $MMS_1$ | 15.5** | 6.4 | 4.6 | 7.7 | 58.9 |
| Ear length (cm) | $B1S_1$ | 4.2** | 2.2 | 1.1 | 2.1 | 48.3 |
| | $MMS_1$ | 7.3** | 2.8 | 2.2 | 3.6 | 61.6 |
| Plant height (cm) | $B1S_1$ | 468.1** | 268.6 | 99.8 | 226.0 | 65.7 |
| | $MMS_1$ | na | na | na | na | na |
| Ear height (cm) | $B1S_1$ | 240.6** | 122.1 | 59.3 | 120.3 | 49.3 |
| | $MMS_1$ | 261.5** | 100.3 | 80.6 | 130.8 | 61.7 |

$\acute{o}_g^2$, $\acute{o}_p^2$, $h_B^2$ = genotypic variance, phenotypic variance, and broad-sense heritability, respectively.

** Significant at $p < 0.01$.

na =Data not available.

TABLE 2
Genotypic and phenotypic variances, and heritability ($h_B^2$) for characters measured in MMB1 and B1MM populations

| Character | Population | Mean squares | | Variance | | $h_B^2$(%) |
|---|---|---|---|---|---|---|
| | | Family | Error | $\acute{o}_g^2$ | $\acute{o}_p^2$ | |
| Fresh largest-ear weight per plant (g) | MMB1 | 2379.2* | 1654.3 | 362.4 | 1189.6 | 30.5 |
| | B1MM | 4281.2** | 2567.8 | 856.7 | 2140.6 | 40.0 |
| Fresh dehusked largest-ear weight per plant (g) | MMB1 | 1214.6* | 866.8 | 173.9 | 607.3 | 28.6 |
| | B1MM | 1953.2* | 1413.0 | 270.1 | 976.6 | 27.7 |
| Days to tasselling (days) | MMB1 | 4.7** | 2.4 | 1.2 | 2.4 | 49.2 |
| | B1MM | 10.2** | 6.6 | 1.8 | 5.1 | 35.4 |
| Ear diameter(mm) | MMB1 | 34.7** | 48.4 | 6.9 | 24.2 | 28.4 |
| | B1MM | 14.2** | 8.8 | 2.7 | 7.1 | 38.2 |
| Ear length (cm) | MMB1 | 4.9** | 2.6 | 1.2 | 2.5 | 47.7 |
| | B1MM | 7.9** | 4.2 | 1.9 | 4.0 | 47.1 |
| Plant height (cm) | MMB1 | 453.8ns | 363.3 | 45.3 | 226.9 | 20.0 |
| | B1MM | 709.4** | 357.1 | 176.2 | 354.7 | 49.7 |
| Ear height (cm) | MMB1 | 211.5** | 120.7 | 45.4 | 105.7 | 42.9 |
| | B1MM | 318.8** | 175.9 | 71.5 | 159.4 | 44.8 |

$\acute{o}_g^2$, $\acute{o}_p^2$, $h_B^2$ = genotypic variance, phenotypic variance, and broad-sense heritability, respectively.

**; *; ns = Significant at $p < 0.01$; significant at $p < 0.05$; and non-significant, respectively.

## RESULTS

*Heritability*

A summary of the results of the analyses of variance and estimates of broad-sense heritability from the $S_1$ and full-sib progeny evaluations is shown in Tables 1 and 2. Broad-sense heritability values estimated from the $B1S_1$ progeny population were high for days to tasselling (63.3%) and ear diameter (60.1%); moderate for ear height (49.3%), ear length (48.3%) and fresh largest ear weight per plant (47.9%). In $MMS_1$ progeny population, estimates of broad-sense heritability were high for plant height (65.7%), ear height (61.7%), ear length (61.6%), ear diameter (58.9%), fresh dehusked largest ear weight per plant (55.5%) and days to tasselling (55.3%) (Table 1).

Broad-sense heritability estimates in the MMB1 progeny population were moderate for days to tasselling (49.2%), ear length (47.7%), ear height (42.9%), fresh largest ear weight per plant (30.5%) and fresh dehusked largest ear weight per plant (28.6%). Heritability for plant height was, however, low (20.0%). In the B1MM progeny population, all traits showed moderate broad-sense heritability estimates, ranging from 27.7% to 49.7% (Table 2).

*Response to Selection*

Selection responses on fresh ear yield, fresh dehusked ear yield and number of ears per hectare are shown in Table 3.

TABLE 3
Response to one cycle of simple and full-sib reciprocal recurrent selection on populations $B1C_0$ and $MMC_0$, for yield characters

| Population | At Field 2 | | At Field 10B | | Average Response (%) |
|---|---|---|---|---|---|
| | Mean | Response (%) | Mean | Response (%) | |
| **Fresh ear yield (ton/ha)** | | | | | |
| $B1C_0$* | 6.11c | | 6.60a | | |
| $B1SC_1$ | 8.21a | 34.2 | 6.55a | -0.8 | 16.7 |
| $B1RFSC_1$ | 7.63ab | 24.8 | 5.74a | -13.1 | 5.9 |
| $MMC_0$* | 7.12abc | | 5.59a | | |
| $MMSC_1$ | 7.35abc | 3.2 | 6.56a | 17.2 | 10.2 |
| $MMRFSC_1$ | 6.61bc | -7.2 | 5.29a | -5.5 | -6.4 |
| Mean | 7.17 | | 6.05 | | |
| **Fresh dehusked ear yield (ton/ha)** | | | | | |
| $B1C_0$* | 4.12c | | 4.55a | | |
| $B1SC_1$ | 5.76a | 39.8 | 4.69a | 3.2 | 21.5 |
| $B1RFSC_1$ | 5.25ab | 27.6 | 3.96a | -12.9 | 7.4 |
| $MMC_0$* | 5.17abc | | 3.97a | | |
| $MMSC_1$ | 5.07abc | -2.0 | 4.56a | 14.6 | 6.3 |
| $MMRFSC_1$ | 4.55bc | -12.1 | 3.87a | -2.7 | -7.4 |
| Mean | 4.99 | | 4.27 | | |
| **Number of ears/ha** | | | | | |
| $B1C_0$* | 34223b | | 40148ab | | |
| $B1SC_1$ | 42667a | 24.7 | 41926ab | 4.4 | 14.6 |
| $B1RFSC_1$ | 41556a | 21.4 | 33778b | -15.9 | 2.8 |
| $MMC_0$* | 40222a | | 36741ab | | |
| $MMSC_1$ | 39889a | -0.8 | 43408a | 18.1 | 8.7 |
| $MMRFSC_1$ | 39445a | -1.9 | 36000ab | -2.0 | -2.0 |
| Mean | 39667 | | 38667 | | |

* source population

Means followed by the same letter in the same column of each character are not significantly different at $p < 0.05$, following DNMRT.

*Fresh Ear Yield*

Evaluation at Field 2 showed that both populations $B1SC_1$ and $B1RFSC_1$ had fresh ear yield significantly higher than that of the source population, $B1C_0$, with increases of 34.2% and 24.8% , respectively. Evaluation at Field 10B, however, showed that both populations had lower yields than the source population, with decreases of 0.8% and 13.1%, respectively. The mean of both locations for fresh ear yield indicated that there were increases of 16.7% and 5.9% in B1SC and $B1RFSC_1$, respectively, over the source population $B1C_0$.

For Manis Madu, results at Field 2 showed that population $MMSC_1$ had a fresh ear yield 3.2% higher than the source population $MMC_0$, but population $MMRFSC_1$ had a fresh ear yield 7.2% lower than the source population. At Field 10B, fresh ear yield of $MMSC_1$ was 17.2% higher, but fresh ear yield of $MMRFSC_1$ was 5.5% lower than that of $MMC_0$. The means of both locations for $MMSC_1$ and $MMRFSC_1$ increased by 10.2% and decreased by 6.4% respectively, compared with the yields of the source populations.

*Fresh Dehusked Ear Yield*

For the Bakti-1 population, evaluation at Field 2 showed that both $B1SC_1$ and $B1RFSC_1$ had higher yields than the source population, $B1C_0$, with 39.8% and 27.6% increases, respectively. At Field 10B, however, fresh dehusked ear yield of $B1SC_1$ was 3.2% higher than that of the source population, but the yield of $B1RFSC_1$ was 12.9% lower than that of the source population. The means of the yield increments were 21.5% in $B1SC_1$ and 7.4% in $B1RFSC_1$.

For selection on Manis Madu, from evaluation at Field 2, it was found that both $MMSC_1$ and $MMRFSC_1$ had a fresh dehusked ear yield lower than that of the source population, $MMC_0$, with decreases of 2.0% and 12.1%, respectively. At Field 10B, fresh dehusked ear yield was 14.6% higher in $MMSC_1$, but was 2.7% lower in $MMRFSC_1$, compared to the yield of the source population. The means were 6.3% higher in $MMSC_1$ but 7.4% lower in $MMRFSC_1$.

*Number of Ears per Hectare*

For selection on Bakti-1, evaluation at Field 2 showed that both $B1SC_1$ and $B1RFSC_1$ had a higher number of ears per hectare than the source population, $B1C_0$, with increases of 24.7% and 21.4%, respectively. At Field 10B, the number of ears per hectare was 4.4% higher in $B1SC_1$, but was 15.9% lower in $B1RFSC_1$, compared to that of the source population. The mean values, however, showed increases of 4.6% and 2.8%, respectively for $B1SC_1$ and $B1RFSC_1$.

For selection on Manis Madu at Field 2 the number of ears per hectare in both the improved populations, $MMSC_1$ and $MMRFSC_1$ were reduced by 0.8% and 1.9%, respectively, when compared to that of the source population, $MMC_0$. At Field 10B, however, the number of ears per hectare was 18.1% higher in $MMSC_1$, but was 2.0% lower in $MMRFSC_1$, compared to that of the source population. The mean values were 8.7% higher in $MMSC_1$, but 2.0% lower in $MMRFSC_1$, when compared to the source population.

## DISCUSSION

The moderate to high heritability estimates obtained from the $B1S_1$ and $MMS_1$ progeny evaluations indicated the presence of a substantial amount of genetic variability in the populations; and selection carried out on the populations should be able to isolate favourable genes to be recombined in the new genetic background. Similar estimates of heritability were also obtained in similar studies on other corn populations (Obilana and Hallauer 1974; Silva 1974; Bartual and Hallauer 1976). The generally higher heritability estimates obtained in the $B1S_1$ population compared to those of the $MMS_1$ population, might have led to the generally higher response to the simple recurrent selection revealed by the Bakti-1 as compared to Manis Madu.

In this study, comparisons of selection response were only made between the improved populations and their respective source populations, and not between the crosses of the improved populations and the respective source populations. This was because the main intention was to observe the direct effect of the selection, and to make inferences on the superiority of one selection method over the other in accumulating additive genes in the populations.

The significant increase in fresh ear yield in $B1SC_1$ and $B1RFSC_1$ also indirectly increased fresh dehusked ear yield and the number of ears per hectare, indicating the presence of positive correlations among these characters. From the selection response, simple recurrent selection based on fresh largest ear weight per plant was

found to be more effective in increasing population yield than in the full-sib reciprocal recurrent selection, and the response was higher on Bakti-1 than in Manis Madu, although differences were significant only in one environment (Field 2). Previous researchers have also reported a higher response of selection based on $S_1$ progeny evaluation in bulked inbred or random-mated populations compared to full-sib reciprocal recurrent selection to increase yield in corn. (West *et al.* 1980; Odhiambo and Compton 1989). Genter (1971), Jinahyon and Moore (1973) and Hable (1985) also reported that simple recurrent selection showed significant effects in improving genetic composition of the traits used as the criteria of selection.

The Bakti-1 population showed a higher response to both selection procedures compared to Manis Madu in improving fresh ear yield, fresh dehusked ear yield and number of ears per hectare.

Further selection using both procedures in the succeeding cycles should produce higher responses in the population improvement.

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# Morphological, Chemical and Mineralogical Properties of the Soils of Abugi, Nigeria, and their Agricultural Potential

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**Keywords: Abugi, flood plain soils, Nigeria, Humaquept, Tropaquept**

## ABSTRAK

*Kajian telah dilakukan ke atas tiga profil tanah di Abugi - dalam dataran banjir Sungai Niger, Negeri Kogi di Nigeria. Tanah di permukaan horizon didapati berasid (pH 4.7 - 6.0). Nilai kation tukargantinya rendah kecuali kandungan kalsiumnya (Ca) yang sederhana dan merupakan kation tukarganti yang dominan dengan nilai dalam julat 0.50 - 7.00 cmol(+)/kg tanah. Mineral lempung yang dominan ialah koalinit, mika terurai dan kuarza, sementara sedikit smektit dan feldspar dikesani. Profil Satu Abugi telah dikelaskan sebagai Eutric Planasol (FAO 1988) atau Fluvaquentic Humaquept (Soil Survey Staff 1992). Profil Dua dan Tiga telah dikelaskan sebagai Eutric Gleysol (FAO 1988) atau Aeric Tropaquept (Soil Survey Staff 1992). Tanah di kawasan Abugi mempunyai potensi baik untuk penghasilan padi dan tebu sekiranya pengawalan banjir dan peningkatan asid dapat dikawal dengan baik.*

## ABSTRACT

*Three soil profiles at Abugi in the flood plains of River Niger, Kogi State, Nigeria were studied. The soils are moderately acidic (pH 4.7 to 6.0) in the surface horizons. Exchangeable cations are low with the exception of calcium which is moderate and is the dominant exchangeable cation with values ranging from 0.50 to 7.00 cmol(+)/kg of soil. The dominant clay minerals are kaolinite, degraded-mica and quartz, while traces of smectite and feldspar were also detected. Abugi profile one was classified as Eutric Planosol (FAO 1988) or Fluvaquentic Humaquept (Soil Survey Staff 1992) while profiles two and three were both classified as Eutric Gleysol (FAO 1988) or Aeric Tropaquept (Soil Survey Staff 1992). The soils of Abugi area offer great potential for rice and sugarcane production if excessive flooding and increasing acidity can be controlled.*

## INTRODUCTION

Abugi is situated in Kogi Local Government Area of Kogi State, Nigeria. It is the main settlement within the studied area which falls between longitudes 6°10′ and 6°20′ East and latitudes 8°35′ and 8°45′ North (*Fig. 1*). The climate of the area is tropical with pronounced wet and dry seasons and steady high temperatures; it receives a mean annual rainfall of 1,160 mm. The area is a part of the nuclei of rice production centres within the middle belt region of Nigeria south of River Niger. The alluvial soil which covers an exclusive area has not been thoroughly studied previously. The objectives of this study are to describe the morphology of the soils, determine their physical, chemical and mineralogical properties, classify them and make an appraisal of their agricultural potential.

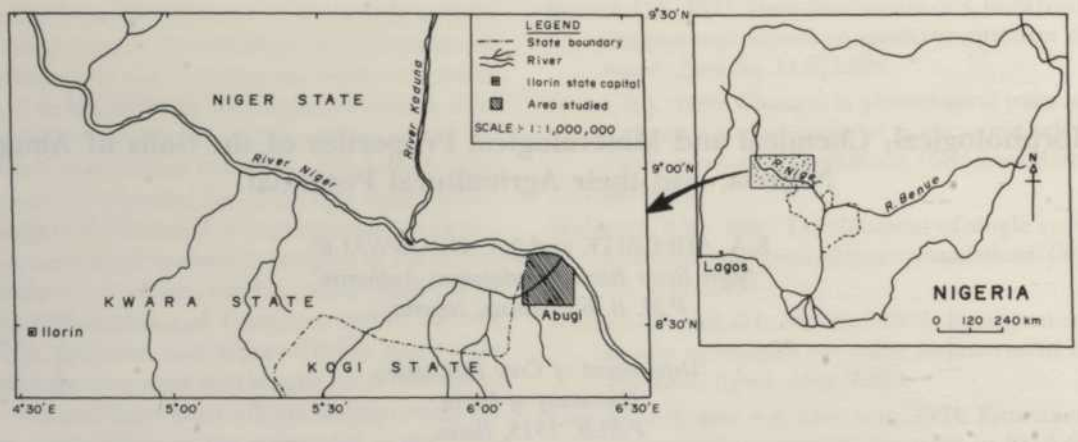


*Fig. 1 : Map of parts of Kwara and Kogi States of Nigeria showing the location of Abugi*

## MATERIALS AND METHODS

Three profile pits sunk in the studied area were morphologically described according to the guidelines for soil description (FAO 1977). The soil samples collected from the genetic horizons of the soils were air-dried, ground and sieved to pass through a 2 mm sieve. These samples were used for chemical and particle size analyses. The particle size analysis was determined by the hydrometer method (Buoyoucos 1962). Exchangeable calcium, magnesium, potassium and sodium were extracted with neutral normal ammonium acetate. Calcium and magnesium in the $NH_4OAc$ solution were determined by atomic absorption spectrophotometry, while K and Na were determined by flame photometry. Available phosphorus (Bray 1) was determined using Murphy and Riley (1962) reagent. Organic matter was determined by Walkey and Black method (Jackson 1958). Effective CEC was the summation of $NH_4OAc$ bases and KCl exchangeable Al and H (Juo *et al.* 1976). The pH was determined with the glass electrode pH meter in soil: water and soil: KCl media, each of ratio 1:2.

Clay fractionation of the soil samples from profiles one and three and preparation of slides for X-ray analysis were carried out as outlined by Jackson (1969), after the hydrogen peroxide pretreatment method of Brewer (1964). X-ray diffraction pattern was obtained for Mg-saturated and glycerol-solvated clay samples. A similar diffraction pattern was obtained for the K-saturated and glycerol-solvated sample after heating to 550°C for 3 h. The samples were run using Cu-K $\propto$ radiation, with goniometer run from 2° 2θ to 40° 2θ at a speed of 2° per minute and 1000 counts per second for each of the slides.

## RESULTS AND DISCUSSION

*Morphology of the Soils*

Buntley and Westin (1965) showed that colour gradation is a good criterion for interpreting drainage conditions among soils. The colour of Abugi soils is black on the surface, changing to different shades of brown in the subsurface horizons. The taints of grey in the subsurface horizons with hues of 7.5 YR or 10 YR, and values less than 4 (Table 1) confirm the imperfect drainage conditions in these soils. The structure of the soils range from structureless to moderate medium sized with granular, subangular blocky or columnar shapes.

The texture of the soils range from loamy sand to sandy loam to sandy clay to clay loam. The three soils exhibited abrupt textural changes with profile depth (Table 1), and this is indicative of different depositional era. The silt/silt + clay weathering indicies of Stewart *et al.* (1970) are very low (< 0.7) (Table 1) in all the horizons of the soils, and they generally decreased with profile depth. These low values of the weathering index indicate that a large proportion of the silt had weathered to clay, either at depositional or at a pre-depositional era.

TABLE 1
Physical and morphological characteristics of the soils of Abugi, Nigeria

| Horizon depth (cm) | sand | silt | clay | texture.+ | silt / silt + clay | colour * | structure ** |
|---|---|---|---|---|---|---|---|
| | | % | | | | | |
| *Abugi P 1* | | | | | | | |
| 0 - 16 | 66.80 | 20.88 | 12.32 | SL | 0.63 | BL (7.5YR 1.7/1) | 2 SAB |
| 16 - 30 | 44.80 | 30.88 | 24.32 | L | 0.56 | Br (7.5YR 4/3) (Mott) | 3 AB |
| 30 - 52 | 22.80 | 38.88 | 38.32 | CL | 0.50 | Gr Y Br (10YR 4/2) (Mott) | 3 C |
| 52 - 94 | 72.80 | 12.88 | 14.32 | SL | 0.47 | Br (10YR 4/4) (Mott) | 1 AB |
| 94 - 118 | 48.80 | 16.88 | 34.32 | SC | 0.33 | Br Gr (7.5YR 4/1) (Mott) | 3 C |
| 118 - 125 | 89.68 | 2.00 | 8.32 | LS | 0.19 | Br Bl (7.5YR 2/2) | 0 G |
| *Abugi P 2* | | | | | | | |
| 0 - 4 | 47.68 | 26.00 | 26.32 | SCL | 0.50 | Bl (7.5YR 1.7/1) (Mott) | 3 SAB |
| 4 - 13 | 45.68 | 22.00 | 32.32 | SCL | 0.41 | Gr Br (7.5YR 4/2) (Mott) | 3 SAB |
| 13 - 42 | 43.68 | 22.00 | 34.32 | CL | 0.39 | Br Gr (7.5YR 4/1) (Mott) | 3 SAB |
| 42 - 76 | 61.68 | 14.00 | 24.32 | SCL | 0.37 | Gr Br (7.5YR 6/2) (Mott) | 3 SAB |
| 76 - 148 | 45.68 | 8.00 | 46.32 | SC | 0.15 | Br Gr (7.5YR 5/1) (Mott) | 3 C |
| 148 - 165 | 79.68 | 2.00 | 18.32 | SL | 0.10 | Gr Y Br (10YR 6/2) (Mott) | 1 SAB |
| *Abugi P 3* | | | | | | | |
| 0 - 3 | 67.68 | 13.00 | 19.32 | SCL | 0.40 | Br Bl (7.5YR 2/2) (Mott) | 2 SAB |
| 3 - 22 | 71.68 | 16.00 | 12.32 | SL | 0.56 | Br (7.5YR 4/3) (Mott) | 2 SAB |
| 22 - 55 | 79.68 | 9.00 | 11.32 | SL | 0.44 | D T Or (10YR 7/3) (Mott) | 1 SAB |
| 55 - 112 | 61.68 | 8.00 | 30.32 | SCL | 0.21 | L Gr (10YR 7/1) (Mott) | 3 SAB |
| 112 - 125 | 53.68 | 7.00 | 39.32 | SC | 0.15 | Br Gr (10YR 6/1) (Mott) | 3 SAB |

+ S = Sand, L = Loam, C = Clay
* Bl = Black, Br = Brown, Y = Yellow, L = Light, Gr = Grey, Or = Orange, D = Dark
** AB = Angular Blocky, SAB = Sub angular Blocky, C = Columnar, G = Granular

*Chemical Properties*

The pH values vary from 4.70 to 6.00 in the surface horizons and from 4.10 to 6.70 in the subsurface horizons (Table 2). Abugi soils are moderately acidic. This could in part be due to the regular flooding of the soils (Young 1976) and the regular and uncontrolled use of ammonium sulphate, urea and other nitrogenous fertilizers for rice production in the area (IADP 1982).

The exchangeable calcium for the surface horizon ranges from 1.20 to 6.10 cmol(+)/kg of soil, and from 0.50 to 7.00 cmol(+)/kg of soil in the subsurface horizons. Magnesium contents vary from 0.70 to 5.95 cmol(+)/kg of soil for the surface horizons and from 0.40 to 7.25 cmol(+)/kg of soil for the subsurface horizons. The potassium values are higher in the surface horizons than in the subsurface horizons and are generally fairly adequate (0.3 to 0.6 cmol(+)/kg of soil) in the surface horizons (Table 2). Sodium values are high for these sedimentary soils deposited by inland river probably because the primary parent material that formed the deposited alluvium was initially high in sodium. These sodium values are higher than the 0.01 - 0.03 cmol(+)/kg of soil recorded for sandstone-derived soils at Iperu, South Western Nigeria (Ogunwale and Ashaye 1975). Exchangeable calcium is the dominant cation and this is followed by magnesium. The effective cation exchange capacity (ECEC) ranging from 2.22 to 12.38 cmol(+)/kg of soil in the surface horizon and from 1.35 to 10.87 cmol(+)/kg of soil in the subsurface horizons indicate that the soils vary in their clay mineral suite.

The organic matter contents vary from 0.45% to 5.60% in the surface horizons and from 0.07% to 0.98% in the subsurface horizons, thus revealing moderate levels for the surface horizons and very low levels for the subsurface horizons. This shows that there is little or no translocation of organic matter within the profiles.

TABLE 2
Chemical characteristics of the soils of Abugi, Nigeria

| Horizon Depth (cm) | pH $H_2O$(1:2) | pH KCl(1:2) | CA | Mg | Exch K | Exch Na | Acidity | ECEC | OC | Avail P | Total N | BSP |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | cmol(+)/kg | | | | | | % | mg/kg | % | |
| *Abugi P1* | | | | | | | | | | | | |
| 0 - 16 | 6.00 | 5.10 | 5.55 | 5.59 | 0.51 | 0.17 | 0.20 | 2.38 | 1.95 | 1.52 | 0.15 | 98.38 |
| 16 - 30 | 5.35 | 4.50 | 2.90 | 2.10 | 0.28 | 0.18 | 0.20 | 5.66 | 0.46 | 0.00 | 0.03 | 96.47 |
| 30 - 52 | 5.20 | 4.50 | 7.00 | 2.20 | 0.32 | 0.17 | 0.16 | 15.51 | 0.57 | 0.00 | 0.03 | 98.97 |
| 52 - 94 | 5.20 | 4.90 | 2.10 | 1.10 | 0.15 | 0.13 | 0.20 | 3.68 | 0.11 | 0.00 | 0.03 | 94.57 |
| 94 - 115 | 5.40 | 4.50 | 4.15 | 3.85 | 0.26 | 0.19 | 0.12 | 8.57 | 0.30 | 0.70 | 0.02 | 98.60 |
| 115 - 125 | 5.75 | 5.50 | 0.75 | 7.25 | 0.09 | 0.10 | 0.12 | 8.31 | 0.07 | 0.00 | 0.03 | 98.56 |
| *Abugi P2* | | | | | | | | | | | | |
| 0 - 4 | 4.70 | 4.00 | 5.90 | 1.50 | 0.77 | 0.25 | 0.48 | 8.90 | 3.28 | 0.70 | 0.25 | 94.61 |
| 4 - 13 | 5.10 | 4.20 | 6.10 | 2.10 | 0.34 | 0.22 | 0.26 | 9.02 | 1.18 | 0.00 | 0.02 | 97.12 |
| 13 - 42 | 5.70 | 5.10 | 3.20 | 6.90 | 0.41 | 0.22 | 0.14 | 10.87 | 0.41 | 0.00 | 0.02 | 98.71 |
| 42 - 76 | 6.25 | 5.50 | 2.40 | 6.40 | 0.15 | 0.23 | 0.06 | 9.24 | 0.15 | 0.00 | 0.04 | 99.35 |
| 76 - 148 | 6.70 | 5.80 | 5.60 | 2.20 | 0.44 | 0.28 | 0.12 | 8.64 | 0.04 | 7.00 | 0.09 | 97.45 |
| 148 - 165 | 4.80 | 4.00 | 1.80 | 1.00 | 0.21 | 0.11 | 0.36 | 3.48 | 0.12 | 0.00 | 0.05 | 89.66 |
| *Abugi P3* | | | | | | | | | | | | |
| 0 - 3 | 5.40 | 5.20 | 1.30 | 0.70 | 0.22 | 0.14 | 0.12 | 2.48 | 0.83 | 8.40 | 0.12 | 95.16 |
| 3 - 22 | 5.40 | 4.90 | 1.20 | 0.70 | 0.13 | 0.11 | 0.08 | 2.22 | 0.24 | 5.60 | 0.05 | 96.40 |
| 22 - 55 | 5.10 | 4.35 | 0.50 | 0.40 | 0.14 | 0.11 | 0.20 | 1.35 | 0.04 | 7.00 | 0.04 | 85.19 |
| 55 - 112 | 4.60 | 3.90 | 1.80 | 0.90 | 0.24 | 0.16 | 0.80 | 3.90 | 0.04 | 10.30 | 0.07 | 79.49 |
| 112 - 125 | 4.50 | 4.05 | 2.60 | 3.30 | 0.35 | 0.19 | 0.52 | 6.96 | 0.20 | 4.20 | 0.05 | 92.53 |

*Clay Mineralogy of the Soils*

X-ray diffraction studies showed that the clay fractions were dominated by kaolinite, degraded-mica and quartz (*Fig. 2*). Traces of smectite and feldspar were also detected.

The potassium saturated and glycerol solvated clay sample indicated kaolinite (0.719 nm and 0.357 nm) and was confirmed on heating to 550°C with the disappearance of the peaks. The potassium saturated and glycerol solvated sample gave low intense mica peaks (0.101 nm and 0.499 nm) which became persistent and enhanced on heating the sample to 550°C. These mica peaks were also prominent in the magnesium saturated and glycerol solvated samples. Quartz was indicated in both the potassium and magnesium saturated and glycerol solvated samples with two peaks (0.426 nm and 0.334 nm). Although the 0.334 nm was over two times as intense as the 0.426 nm peak, the latter peak confirmed the presence of quartz in the samples. A trace of smectite (1.82 nm) was observed in the magnesium saturated and glycerol-solvated sample of Abugi profile one. Traces of feldspar (0.324 nm) were also indicated in both the potassium and magnesium saturated and glycerol-solvated samples (*Fig. 2*).

The high kaolinite and quartz contents of the clay fractions of Abugi soils could be attributed to material which is sedimentary in nature, and highly weathered. The presence of quartz in clay fraction is associated with intense degree of weathering. Thus, the alluvium must have been derived from primary basement complex rock probably of the Precambrian age. The presence of micaceous mineral is typical of younger parent material in a tropical environment (Okusami *et al.* 1985) and this is the younger secondary parent material (alluvium) derived from the older primary parent material. The presence of smectite could be adduced to the degradation of part of the micaceous minerals (Ogunwale 1984) or it could have been hydrothermally deposited with the alluvium.

*Soil Classification*

Profile one at Abugi was classified, using organic carbon and clay distributions with percent base saturation as: order-Inceptisol; sub-order-

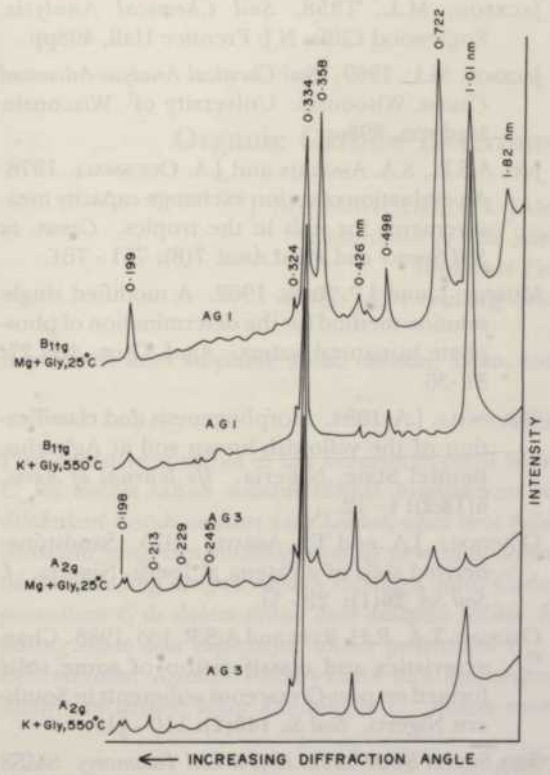


*Fig. 2: X-Ray diffractograms of clay fractions in Abugi soils*

Aquepts; great group-Humaquepts and sub-group-Fluvaquentic Humaquepts of the Soil Survey Staff (1992). Profiles two and three were also classified as: order- Inceptisol; suborder- Aquepts; great group-Tropaquepts and sub-group Aeric Tropaquepts. According to the FAO/UNESCO (1988) mapping legend, profile one falls within the group Eutric Planosol while profiles two and three fall within the group Eutric Gleysol.

*Agricultural Production Potential of Abugi Soils*

Young (1976) noted that soils derived from alluvium occupy a distinctive and important place in tropical agricultural while at the same time he observed that the produvtivity of alluvial soils in Africa is more often potential than actual. The flat nature of alluvial soils is one of the factors that confers on it production potential especially in terms of irrigation. Other factors include the suitable textural classes (medium-heavy textured) and its moderate organic matter content. However, the greatest constraint to crop production on alluvial soils is the poor drainage occasioned by a high water table during the rainy season and occasional flood problems. Therefore, in order to tap the immense benefits offered by alluvial soils, a concerted effort towards providing a suitable drainage and flood control system must be attempted.

In Abugi, rice (*Oryza sativa*) is the dominant crop produced since it tolerates water logging. Local varieties are popular, but yields are quite low varying from about 500 kg to 1000 kg per hectare, the average being about 750 kg per hectare. However, increasing usage of improved varieties and other modern agricultural input such as herbicides and fertilizers by a number of farmers have resulted in considerable yield increase from 1,500 to 2,500 kg per ha and more in some cases. Sugarcane (*Saccharum officinarum*) is produced on a very small scale (plots averaging less than 0.1 hectare) in Abugi mainly for home consumption. The varieties are entirely local, yielding less than 50 t/ha cane in the first year. Few efforts are made either at local or national level to encourage or/and improve sugarcane production in Abugi. The reason may be partly due to the great distance and the remoteness of the area from the nearest sugar company in Nigeria. Nonetheless, in the face of the ever increasing cost of sugar in Nigeria, the area offers very great, and realisable financial returns for estate agriculture involving cultivation of mainly rice and sugarcane.

Other crops produced on a small scale in the area include Guinea corn (*Sorghum* spp.) maize (*Zea mays*), cassava (*Manihot* spp.), cowpea (*Vigna* spp.) and yams (*Dioscorea* spp.). Special cultivation practices have to be employed to ensure that these crops do not suffer from the effects of the high water table usually associated with these soils during the rainy season. Such practices include land preparation and/or planting towards the end of the rainy season when the high water table is beginning to recede; making extra-large ridges to keep the roots of crops above waterlogged zones; and provision of crude but functional surface drainage facilities. The high labour and drudgery involved in the provision of these facilities have largely led to fragmentation and much reduced sizes of plots for these crops. Among all these crops, maize is the most important with yields averaging about 1 to 1.5 tonnes per hectare.

## CONCLUSION

The physical and chemical characteristics and the location of Abugi soils make them suitable for rice and sugarcare production. Such soils, however, need good drainage if other arable crops such as maize, yam and guinea corn are to be grown. Granular urea should be used on the soils as nitrogen source instead of sulphate of ammonia which has the potential of increasing soil acidity. Moderate applications of inorganic phosphorus and organic manure would boost yields of most crops grown on these soils.

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# Organic Carbon Determination in Acid Sulphate Soils

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**Keywords: acid sulphate soils, carbon, Leco, total oxidation**

## ABSTRAK

*Pirit adakalanya wujud di zon penurunan profil tanah asid sulfat. Pirit ini dipercayai mengganggu penentuan $C_{org}$ di dalam tanah melalui kaedah pembakaran basah dengan menggunakan $Cr_2O_7^{2-}$. Satu kajian telah dilakukan untuk mencari satu kaedah tepat serta boleh dipercayai untuk taksir $C_{org}$ dalam tanah asid sulfat di Malaysia yang mengandungi jumlah pirit yang berbeza. Beberapa kaedah penentuan karbon (C) yang mantap telah dibanding dengan kaedah standard, iaitu Carbon Determinator (Leco IR-312). Gangguan pirit di dalam penentuan C di dalam tanah asid didapati sedikit. Kaedah pengoksidaan total didapati sebagai kaedah yang paling tepat dan dipercayai untuk penentuan $C_{org}$ di dalam tanah asid sulfat tanpa kehadiran Carbon Determinator. Kaedah Walkley-Black dan kehilangan melalui pembakaran masing-masing didapati kurang taksir dan terlebih taksir kandungan $C_{org}$ dalam tanah.*

## ABSTRACT

*Pyrite is almost exclusively present in the reduced zone of acid sulphate soil profiles. This pyrite is believed to interfere with determination of $C_{org}$ in the soils by the wet combustion method using $Cr_2O_7^{2-}$. A study was conducted to determine an accurate and reliable method for estimation of $C_{org}$ in acid sulphate soils in Malaysia containing variable amounts of pyrite. Some of the established methods of carbon (C) determination were tested against Carbon Determinator (Leco IR-312) which was regarded as the standard method. It was found that the interference of pyrite in the determination of C in acid sulphate soils by wet combustion was minimal. The total oxidation procedure was found to be the most reliable and accurate method for determination of $C_{org}$ in acid sulphate soils in the absence of Carbon Determinator. The Walkley-Black method underestimated, while the loss on ignition method overestimated the $C_{org}$ content in the soils.*

## INTRODUCTION

Carbon (C) is an important chemical constituent of organic matter in a soil. Organic matter has the ability to reduce Al toxicity in the soil via chelation mechanism. It has to be estimated accurately in order to assess its contribution to the reduction of Al toxicity. However, C in the soil can exist as a chemical component of organic matter or as impurities in inorganic compounds. In most soils of the humid tropics, $C_{org}$ is the dominant form. On the other hand, $C_{org}$ is found almost exclusively in association with calcite and/or dolomite in the soils of the arid and semi-arid regions (Nelson and Sommers 1982; Yeomans and Bremner 1991).

Soils in the aquic moisture regime, where acid sulphate soils occur, contain variable amounts of organic matter. These acid sulphate soils are often characterized by the presence of pyrite in the reduced zone of the profiles. Carbonate is absent in the active acid sulphate soils of Malaysia (Shamshuddin and Auxtero 1991; Auxtero *et al.* 1991), suggesting that C in the soils is present exclusively as a component of organic compounds. Hence, $C_{org}$ in the soils can be approximated from determination of total C. Organic matter can then be calculated from the total C.

Total C in highly weathered soils under well drained conditions can be determined accurately by dry combustion using Leco Carbon

Determinator (Yeomans and Bremner 1991). Currently, $C_{org}$ in such soils is determined by reduction of $Cr_2O_7^{2-}$ by organic C compounds and subsequent determination of unreduced $Cr_2O_7^{2-}$ by oxido-reduction titration with $Fe^{2+}$ (Nelson and Sommers 1982). However, this procedure may not be accurate for determination of C in the soils containing substantial amounts of pyrite ($FeS_2$). This is because $Cr_2O_7^{2-}$ reacts with pyrite (Dent 1986), leading to an overestimation of the value of C. Willet and Beech (1987) found that overestimation of $C_{org}$ occurred when pyrite content exceeded 0.29%. Acid sulphate soils in Malaysia (Shamshuddin and Auxtero 1991) and elsewhere in the world (Dent 1986) contain appreciable amounts of pyrite. Thus, it appears impractical to determine C in acid sulphate soils by the wet combustion method using $Cr_2O_7^{2-}$. The objective of this paper was to determine the accuracy of the wet combustion method using $Cr_2O_7^{2-}$ or any other available methods in the estimation of $C_{org}$ in acid sulphate soils.

## MATERIALS AND METHODS

*Theoretical Consideration*

Organic matter in soils (Schollenburger 1927) may be oxidized by treatment with a hot mixture of $Cr_2O_7^{2-}$ and $H_2SO_4$ according to the equation:

$$2Cr_2O_7^{2-} + 3C^{o} + 16H^{+} = 4Cr_2^{3+} + 3CO_2 + 8H_2O$$

After reaction, the excess $Cr_2O_7^{2-}$ is titrated with $Fe(NH_4)_2(SO_4)_2\,6H_2O$, and $Cr_2O_7^{2-}$ reduced during the reaction with soil is assumed to be equivalent to the organic C in the sample (Nelson and Sommers 1982).

Oxidation of pyrite (Retsema and Groenenberg 1993) in acid sulphate soils resulting from exposure to the atmosphere through drainage or otherwise can be visualized as follows:

$$4FeS_{2(s)} + 15O_{2(g)} + 14H_2O \rightarrow 4Fe(OH)_{3(s)} + 16H^{+}_{(aq)} + 8SO_4^{2-}{}_{(aq)}$$

This pyrite can be similarly oxidized if it comes into contact with $Cr_2O_7^{2-}$. Hence, $Cr_2O_7^{2-}$ does not only oxidize $C_{org}$ but also pyrite in the acid sulphate soils. The assumption that the excess $Cr_2O_7^{2-}$ titrated with $Fe(NH_4)_2(SO_4)_2.6H_2O$ is equivalent to the $C_{org}$ is no longer valid.

*The Soils*

Soils for the study were acid sulphate soils sampled in Muar, Pontian and Serkat (Johor), Kuantan (Pahang) and Kuala Linggi (Melaka); these are important acid sulphate areas in Peninsular Malaysia. Appropriate samples were stored at 4°C to be used for incubation study and for determination of pyrite content. Other samples were air-dried and ground to pass through a 2-mm sieve. The pH, carbon and pyrite content are given in Table 1.

TABLE 1.
The pH, C and pyrite content in the Cg horizons of Sedu, Linau, IPRS and Jawa soils

| Soils | Location | pH ($H_2O$, 1:1) | Carbon (%) | Pyrite (%) |
|---|---|---|---|---|
| Sedu | Kuala Linggi | 1.9 | 7.49 | 0.60 |
| Linau | Kuantan | 3.1 | 5.87 | 0.02 |
| IPRS | Pontian | 2.2 | 4.19 | 0.12 |
| Jawa | Serkat | 2.4 | 4.33 | 0.26 |

*Methods*

a) Determination of Pyrite

Pyrite in the soils was determined according to a procedure proposed by Haering *et al.* (1989). Samples for this analysis were first dried under vacuum to avoid oxidation of pyrite before the determination.

b) Incubation Experiment

The samples from the Cg horizon of the Jawa and IPRS soils were incubated for 180 d. The samples were kept under moist conditions at room temperature by spraying with distilled water at appropriate times. Carbon in the soils was determined every 30 d by wet combustion using $Cr_2O_7^{2-}$ (total oxidation).

c) Carbon Analysis

Leco IR - 312. Carbon in the soils was first determined by the Leco IR - 312 Carbon Determinator. In this analysis, a sample was weighed in a crucible on a pan balance built into the measurement unit. About 0.1 g sample was needed for each determination. The crucible was transferred to the induction furnace, and the sample was burnt

off by $O_2$ to produce $CO_2$ at temperature around 2000°C. The C was detected by infrared. This represents total C in the soils. This method is regarded as the standard method for determining C in this study, and is referred to as Leco.

Wet combustion using $Cr_2O_7^{2-}$. $C_{org}$ was also determined by total oxidation using $Cr_2O_7^{2-}$ by a modified Mebius procedure and by Walkley-Black method (Nelson and Sommers 1982). These are referred to as TO and WB, respectively.

A modified version of the wet oxidation method followed by colorimetric determination of $C_{org}$ in acid sulphate soils was adopted. This method made use of $Al_2O_3$ equal in weight to the soil used. The $Al_2O_3$ was expected to reduce the oxidation of pyrite by dichromate ion (Frendorf *et al.* 1993). Determination of $C_{org}$ was carried out with $Al_2O_3$ (+) or without $Al_2O_3$ (-).

Loss on Ignition. Carbon in the soils was finally determined by the method of loss on ignition. A 10 g oven dry soil sample was placed in a muffle furnace for 16 h at 375°C. The loss in weight was determined, and was attributable to the oxidation of organic matter (Dent 1986). $C_{org}$ in the soil sample was estimated by dividing the organic matter so obtained by 1.724. Note that pyrite in the soil did not interfere with the determination as it only undergoes endothermic reaction at 450 - 650°C (De Coninck 1978). This method is refered to as LI.

## RESULTS AND DISCUSSION

The appearance of yellowish mottles after 30 d of incubation showed that the soils taken from the Cg horizon of acid soil profiles contained pyrite. The yellowish mottles were jarosite formed by the oxidation of pyrite. The pyrite content of the study soils ranged from 0.02 to 0.60% (Table 1).

The C content in the IPRS and Jawa soils was 4.19 and 4.33%, respectively (Table 1). The C content in both soils decreased linearly as the time of incubation increased (*Fig. 1*). The decrease in the C content was presumably due to the loss of organic matter resulting from its mineralization during the 180 d incubation. Some pyrite could have been oxidized to jarosite during the oxidation period. The decrease in C value with time of incubation could also be due to the smaller contribution from the oxidation of pyrite. It appeared that pyrite did interfere with the determination of C by wet combustion using $Cr_2O_7^{2-}$.

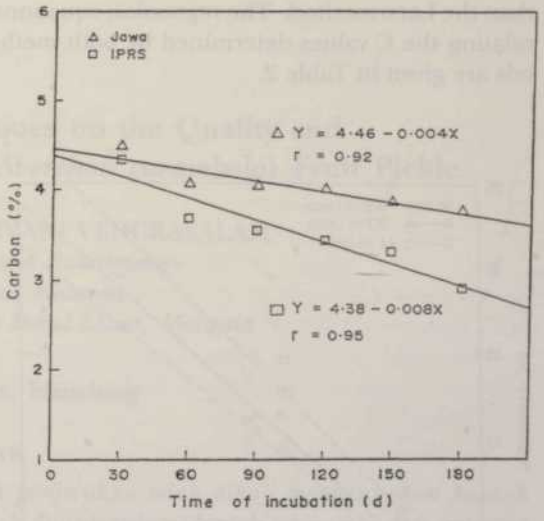


*Fig 1: Reduction of C values in relation to time of incubation*

The extent to which pyrite interfered with the determination of C by wet combustion using $Cr_2O_7^{2-}$ is illustrated in *Fig. 2.* The total oxidation Mebius procedure gave C values closest to the values determined by the standard method (Leco), giving a linear regression line of:

$$Y = 0.106 + 0.961X, \qquad r = 0.994, \qquad P < 0.01$$

There was a good agreement between C determined by total oxidation and that by Leco. Thus, the total oxidation Mebius method can be used accurately for determination of C in acid sulfate soils containing pyrite. The contribution of pyrite oxidation to the overall result of C determination by wet combustion appeared to be small.

The Walkley-Black method estimated lower values of C than the Leco method. On the other hand, the loss on ignition method recorded higher values than the Leco method. The higher values recorded by the loss on ignition method could be due to dehydration and/or dehydroxylation of minerals in the soil when it was heated to 375°C.

The C content determined by the loss on ignition was higher than that determined by the total oxidation or Walkley-Black method (Table 2). As expected, the C content determined by the total oxidation was higher than that determined by the Walkley-Black method. The modified Walkley-Black method through digestion with or without $Al_2O_3$ gave consistently lower C values

than the Leco method. The regression equations relating the C values determined by both methods are given in Table 2.

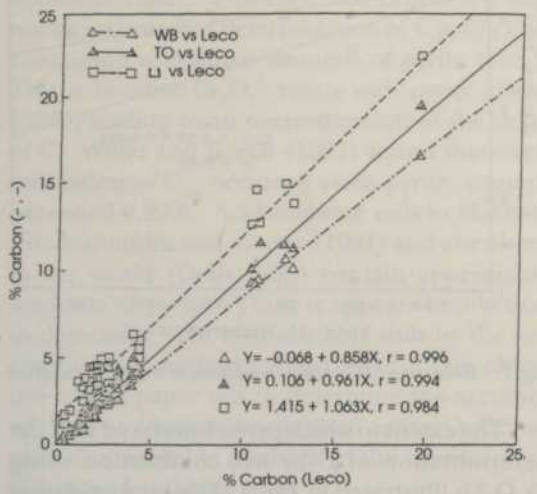


*Fig 2: Carbon content determined by normal methods as compared to that by the standard method*

TABLE 2
Linear relationship between $C_{org}$ determined by total oxidation (TO), loss on ignition (LI), Walkley-Black (WB), Walkley-Black through digestion with $Al_2O_3$(+) and without $Al_2O_3$(-)

| Parameter | Equation | r(P<0.01) |
|---|---|---|
| LI vs TO | Y = 1.39 + 1.09X | 0.975 |
| LI vs WB | Y = 1.56 + 1.23X | 0.979 |
| TO vs WB | Y = 0.19 + 1.12X | 0.994 |
| $Al_2O_3$(-) vs Leco | Y = -0.19 + 0.93X | 0.997 |
| $Al_2O_3$(+) vs Leco | Y = -0.28 + 0.91X | 0.994 |

## CONCLUSION

Pyrite appears to interfere with determination of C in acid sulphate soils by wet combustion using $Cr_2O_7^{2-}$, but its overall contribution is minimal. The total oxidation method gives the closest estimate to the actual C content in the soils. Total oxidation Mebius method is, therefore, suitable for C determination in acid sulphate soils containing pyrite in the absence of Carbon Determinator.

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# Effect of Processing Techniques on the Quality and Acceptability of Young Carambola (*Averrhoa carambola*) Fruit Pickle

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**Keywords: carambola, pickle, conventional salt-stock, blanching**

## ABSTRAK

*Potensi pengunaan buah-buah belimbing muda untuk penjerukan telah dikaji menggunakan kaedah konvensional rendaman garam dan penceluran. Buah-buah dijeruk dalam bentuk sebiji-sebiji dan terpotong tebal dan nipis. Kesesuaian setiap teknik telah dinilai berdasarkan kualiti dan penerimaan produk akhir. Sampel untuk analisis telah diambil pada peringkat-peringkat pemprosesan yang berbeza dan dikaji tentang kandungan asid askorbik, keasidan tertitrat dan tekstur; dan produk akhir turut didedah kepada analisis deria untuk dinilai tentang kerangupan, rasa, citarasa dan saiz yang disukai.*

*Tiada terdapat perbezaan bererti di dalam kualiti fisikal dan kimia bagi produk yang diperolehi dari kedua-dua kaedah; tetapi penilaian deria menunjukkan jeruk yang dipotong tebal dan disediakan secara penceluran telah digemari kerana ia lebih rangup. Walau bagaimanapun kajian penyimpanan menunjukkan jeruk tersebut menjadi lembik selepas satu bulan. Nilai keaktifan air jeruk ialah 0.94.*

## ABSTRACT

*The potential use of young carambola fruits for pickle production was studied using the conventional salt-stock and the blanching methods. Fruits pickled were in the form of whole fruits and thick and thin slices. The suitability of each technique was graded based on the quality and acceptance of the final product. Samples for analysis were taken at different stages of processing and were analysed for ascorbic acid content, titratable acidity, and texture; and the final product was subjected to sensory analysis for evaluation on crunchiness, taste, flavour and size preference.*

*There was no significant difference in the physical and chemical quality of products obtained from both methods but sensory evaluation showed that the blanched, thickly sliced pickle was preferred for its crunchiness. However, storage study showed that the blanched pickle became soft after 1 month. The water activity of the pickle was 0.94.*

## INTRODUCTION

Malaysia now grows carambola for export. The total area grown in 1990 was about 973 hectares and the total production in 1990 was estimated to be about 51,000 metric tons (FAMA report 1989). It is a common practice during maintenance pruning of the tree to wrap only 30% of the fruit. The remaining fruits are discarded. Maintenance pruning is neccessary to remove unhealthy plant parts, reduce fruit numbers, balance growth and improve light penetration.

The discard normally consists of small, improperly shaped or shrivelled fruits; and since they are young fruits they are not suitable for processing into any other value-added products (Mohd Som and Adinan 1989). In the pickle industry, pickling has normally been carried out using the salt-stock method. The purpose of this study was to compare the quality and acceptance of products obtained using two different methods of pickle processing, namely a blanching method with the conventional salt-stock method. The blanching method has advantages in reducing the pickling time. However, the quality and acceptance of the product using this technique is still not known. The second objective was to determine the storage life of the product using the preferred or chosen technique.

## MATERIALS AND METHODS

Young carambola *Averrhoa carambola* fruits were obtained from a farm in Cheras. Fruits chosen were 3.0 to 5.0 cm long and free from blemishes, insect bites or scab rot. After trimming and washing, the fruits were sliced vertically into 0.25 cm and 1.0 cm thicknesses. Some fruits were used as whole fruits.

For the coventional pickling method, prepared fruits were soaked in 15% brine solution and the concentration of the salt solution was monitored daily using a salometer. When the brining process was complete as indicated by the stabilisation of the salt concentration, the fruits were desalted with three changes of water. They were then soaked in water containing 0.4% alum (Etchells and Bell 1972) for 24 h. After the water had been drained, the fruits were arranged in bottles and pickling solution (syrup with 45% sugar, 0.2% alum and vinegar, pH 2.5) was added.

For the Quick Blanching method, the prepared fruits were blanched in water (70-80°C) for 5 min and cooled immediately. They were then soaked in water containing 0.4% alum overnight. Drained fruits were then placed in the pickling solution of a similar composition as in the conventional method except that it contained 0.4% salt.

Samples were taken at different stages of both treatments and analysed for vitamin C content using the dye titration method, titratable acidity (Ranggana 1977), texture using an Instron machine and colour using a Hunterlab colorimeter Model D25L. The final product was subject to sensory analysis for evaluation on crunchiness, taste, flavour and size preference (Larmond 1977).

Upon completion of the above experiment, it was found that the blanched, thick pickle was most acceptable. The 1-cm sliced pickles were again prepared using the blanching technique. They were stored at room temperature (28°C) and repeated observations were made on the colour, texture, titratable acidity and pH along with its general appearance for a period of one month. The water activity of the product was determined using the graphical interpolation method (Landrock and Proctor1951).

## RESULTS AND DISCUSSION

*Processing of Pickle*

The daily changes in brine concentration were as recorded in *Fig. 1* and are typical of a conventional brining process where at the initial stage, the salt concentration would drop due to the dilution effect of the water extracted from the fruits. Enough salt had to be added every day to maintain the salt concentration at 15%. The amount of salt added was found to decrease day by day. The brining process was complete when the brine concentration reached equilibrium.

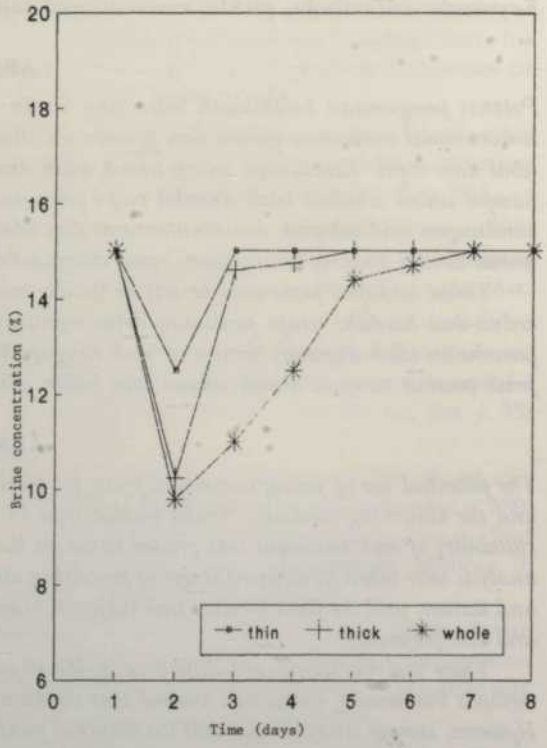


*Fig.1: Changes in salt concentration during fermentation*

The rate of brining was fastest in thin sliced fruit (3 d), slightly slow in thick sliced fruit (5 d) and slowest in whole fruit (7 d). The difference in the rate was due to the difference in total surface area exposed to the medium which influenced the osmotic rate. This means that thin-sliced, thick-sliced and whole fruit required in total 4, 6 and 8 d respectively to be ready for the next phase, i.e. soaking in pickling solution. On the other hand, fruits prepared by the blanching method were ready for soaking on the same day.

*Ascorbic Acid and Titratable Acidity*

The results of ascorbic acid analysis are shown in Table 1. At the initial stage, the different techniques of processing seemed to influence the ascorbic acid retention to different extents. The loss of vitamin C by blanching was less than the loss during brining in the conventional method. Blanching retained about 77% of the ascorbic acid in the whole fruit while in the brined fruit the amount retained was only 30%. Fellers

TABLE 1
Changes in ascorbic acid content and titratable acidity at different steps of processing

| fruit size | fresh fruit | blanching 1 | blanching 2 | blanching 3 | conventional method 4 | conventional method 5 | conventional method 6 |
|---|---|---|---|---|---|---|---|
| ascorbic acid (mg/100 g fruit) | | | | | | | |
| whole | $5.17^{ax}$ | $4.0^{bx}$ | $1.33^{dx}$ | $0^{dx}$ | $1.5^{cx}$ | $0^{dx}$ | $0^{dx}$ |
| thick | $5.17^{ax}$ | $2.17^{by}$ | $0^{dy}$ | $0^{dx}$ | $0.75^{cy}$ | $0^{dx}$ | $0^{dx}$ |
| thin | $5.17^{ax}$ | $0.50^{bz}$ | $0^{cy}$ | $0^{dx}$ | $0^{cz}$ | $0^{dy}$ | $0^{dx}$ |
| titratable acidity (% oxalic acid) | | | | | | | |
| whole | $0.22^{ax}$ | $0.21^{bx}$ | $0.21^{bx}$ | $0.22^{ax}$ | $0.18^{bx}$ | $0.18^{bx}$ | $0.18^{bx}$ |
| thick | $0.22^{ax}$ | $0.17^{by}$ | $0.13^{by}$ | $0.13^{by}$ | $0.14^{by}$ | $0.13^{bx}$ | $0.15^{by}$ |
| thin | $0.22^{ax}$ | $0.06^{bz}$ | $0.06^{bz}$ | $0.06^{bz}$ | $0.06^{bz}$ | $0.06^{by}$ | $0.06^{by}$ |

Values are means of six determinations
Means followed by the same letter in the same column and row are not significantly different at $P < 0.05$

Note :
1 = after blanching
2 and 5 = after soaking in alum
3 and 6 = finished product
4 = after brining

TABLE 2
Changes in texture of fruit at different stages of processing

| Fruit | texture (kg) | | | | | | |
|---|---|---|---|---|---|---|---|
| | fresh fruit | blanching | | | conventional method | | |
| | | 1 | 2 | 3 | 4 | 5 | 6 |
| Whole | $6.76^{a}$ (0.42) | $4.97^{c}$ (0.59) | $6.26^{b}$ (0.80) | $5.8^{b}$ (0.63) | $6.53^{b}$ (0.40) | $6.7^{a}$ (0.10) | $6.7^{b}$ (0.34) |
| Thick | $4.26^{b}$ (0.43) | 2.67c (0.66) | $4.0^{b}$ (0.23) | $3.80^{b}$ (0.26) | $4.10^{b}$ (0.68) | $4.5^{a}$ (0.40) | 4.40b (0.63) |
| Thin | $28.0^{a}$ (0.94) | $16.77^{b}$ (0.74) | $26.4^{a}$ (2.01) | $24.8^{a}$ (2.27) | $16.06^{b}$ (2.73) | $24.0^{a}$ (2.36) | $23.1^{a}$ (0.43) |

Values are means of six determinations
Means followed by the same letter in the same column and row are not significantly different at $p < 0.05$.
(The numbers in parentheses are the standard deviations)

Note :
1 = after blanching
2 and 5 = after soaking in alum
3 and 6 = finished product
4 = after brining

(1960) reported 86% loss of vitamin C in desalted cucumber. According to Jones (1975), severe loss of nutrients occurred during brining due to leaching of the material into the brine.

Even though blanching could better retain vitamin C, this value was significantly less than the amount originally present in fresh fruit. In other words, blanching also causes reduction in the vitamin C content of foods. The extent of loss usually depends on the blanching method and on the way the product is prepared. The sliced fruits lost a higher amount of vitamin C than the whole fruits as sliced fruits had higher surface areas exposed to the blanching medium. However, both techniques of pickling finally resulted in an equal 100% loss of ascorbic acid in the finished products.

The acidity in carambola fruit was expressed as a percentage of oxalic acid. There was significant reduction in the acid content of sliced fruits after blanching and brining. The amount reduced in whole fruit was slight and insignificant, again as a result of leaching and the total surface area exposed.

Further processing (freshening and acidification) did not change the acidity of the fruit significantly. Despite the addition of vinegar in the acidification step, the acidity of the final products was low in both blanched and fermented sliced pickles. This could be possibly due to the acetic acid present in the pickle which was not detected using this titration method. Acetic acid is a volatile acid and it could have been volatilized during sample preparation.

*Texture*

The changes in texture during processing are shown in Table 2. Blanching and brining caused significant reduction in the firmness of prepared fruits. Reduction of firmness after blanching and brining could be related to ultrastructural changes as has been reported by earlier workers. Jewell (1979), in a light microscopy study of blanching effects on carrots, observed that blanched carrots had suffered some cell collapse and wall rupture when compared with the fresh tissue. At the ultrastructural level water blanching caused a loss of fine structural detail and pronounced separa-

TABLE 3
Changes in green (-a value) and yellow (b-value) colour at different steps of processing

| fruit | fresh | blanching | | | conventional method size | | |
|---|---|---|---|---|---|---|---|
| | fruit | 1 | 2 | 3 | 4 | 5 | 6 |
| a-value | | | | | | | |
| Whole | $-11.1^{ax}$ | $-2.1^{bx}$ | $-2.0^{cx}$ | $-2.0^{c}$ | $-1.7^{ex}$ | $-2.0^{cx}$ | $-1.9^{dx}$ |
| Thick | $-11.1^{ax}$ | $-1.9^{by}$ | $-2.0^{bx}$ | $-1.9^{cx}$ | $-2.0^{by}$ | $-2.0^{by}$ | $-1.8^{dy}$ |
| Thin | $-11.1^{ax}$ | $-2.0^{bz}$ | $-1.9^{cy}$ | $-1.9^{cy}$ | $-1.9^{cz}$ | $-2.0^{bx}$ | $-1.7^{dy}$ |
| b-value | | | | | | | |
| Whole | $18.0^{gx}$ | $19.5^{fy}$ | $20.1^{ey}$ | $20.5^{dx}$ | $22.8^{bx}$ | $23.4^{ax}$ | $20.6^{bz}$ |
| Thick | $18.0^{fx}$ | $19.5^{ey}$ | $19.5^{ey}$ | $21.8^{a}$ | $21.6^{by}$ | $21.4^{dy}$ | $21.5^{cy}$ |
| Thin | $18.0^{gx}$ | $20.5^{ex}$ | $20.2^{fx}$ | $21.7^{bx}$ | $20.8^{dz}$ | $21.5^{cy}$ | $22.3^{ax}$ |

Values are means of six determinations
Means followed by the same letter in the same column and row are not significantly different at $p < 0.05$.

Note :
1 = after blanching
2 and 5 = after soaking in alum
3 and 6 = finished product
4 = after brining

tion of cell walls in the region of middle lamella. Studies on the changes produced during pickling of cauliflower (Saxton and Jewell 1969) and onions (Jewell 1972) showed that brining (in 16% NaCl) produced a gradual degradation of the fine structural organisation within the cells which eventually left just a network of cells. According to Matz (1962), fermentation processes generally left the principal structural element intact but the semi-permeability of the cell membrane was destroyed, resulting in loss of cell turgor and crunchiness. These changes in structure might have resulted in the differences in firmness readings obtained.

Soaking of fruit in water containing alum or freshening of samples generally caused a marked increase in firmness of all samples. This increase in firmness may be due to the action of the trivalent aluminium ion which forms a complex with the pectic substances, particularly those in the middle lamella (Matz 1962).

The firmness of the finished product (after 1 week) was slightly lower than after freshening. This occurs similarly for both methods but varies with different sizes of fruit. This means that acidification of fruit might have resulted in the degradation of cell wall structures.

*Colour*

Colour is one of the important characteristics which adds to the aesthetic value of a product. Francis and Clysdale (1975) suggested that application of heat causes considerable loss of chlorophyll content. The major reason for this change has been attributed to the formation of phaeophytin from chlorophyll. The effects of processing on greenness or 'a-value'of pickle is shown in Table 3. There is significant reduction in 'a-value' of processed fruit compared with the fresh sample. Both the conventional and the blanched samples had the same effect but the blanched sample had slightly higher values than the fermented ones. This shows that blanching retains more chlorophyll than fermentation. Complementary to the loss in green colour, the 'b-value' or yellowness of sample showed significant overall increase throughout both processes, indicating that the colour of the fruit was becoming yellow.

*Sensory Evaluation*

Results of the sensory evaluation of pickles on flavour, colour, taste, crunchiness and size are shown in Table 4. The unique flavour of fermented pickle is usually attributed to lactic

TABLE 4
Sensory evaluation on flavour, colour, taste, crunchiness and size

| sample | flavour | colour | taste | crunchiness | size |
|---|---|---|---|---|---|
| 1 | 6.70[a] | 5.81[a] | 6.16[a] | 6.40[b] | 6.28[b] |
| | (1.60) | (1.70) | (2.18) | (1.42) | (1.79) |
| 2 | 6.43[a] | 5.72[a] | 6.23[a] | 6.46[a] | 5.88[b] |
| | (1.23) | (1.44) | (2.00) | (1.18) | (1.62) |
| 3 | 7.27[a] | 5.66[a] | 6.86[a] | 7.64[a] | 7.10[a] |
| | (1.26) | (1.53) | (1.87) | (1.27) | (1.75) |
| 4 | 6.71[a] | 5.61[a] | 7.17[a] | 6.22[b] | 7.08[a] |
| | (1.86) | (1.26) | (1.64) | (1.64) | (1.72) |

Means followed by the same letter are not significantly different at $p < 0.05$.
The numbers in parentheses are the standard deviations

Note: Sample 1 = thin slices (blanched)
2 = thin slices (conventional method)
3 = thick slices (blanched)
4 = thick slices (conventional method)

acid present after fermentation. In this case, the panelists were not able to detect any significant difference in flavour among the four samples. Perhaps the amount of fermentable sugar was low in the fresh fruit, resulting in low production of lactic acid; or most of the sugars have been washed off during desalting. The fact that the blanched thick pickle was rated slightly higher than the fermented pickle (even though not significantly different) showed that the panelists approved the flavour of this type of pickle.

In terms of colour, all the samples were rated quite low, indicating poor preference for the colour. The colour of thin slices were rated higher than that of the thick ones. There was no significant difference in the taste of all four samples. Both types of pickles had equally high ratings, showing, that they were both equally well accepted.

Blanched thick sliced pickle showed a significantly higher rating for crunchiness ($p < 0.05$) compared with other types of pickles. This response is in contrast to the result obtained from texture analysis (by Instron Universal Testing Machine) for firmness. This indicates that firm pickles may not neccessarily be crunchy. For size, the thick slices were preferred over the thin ones. The blanched thick-sliced pickle received the highest score even though the score was not significantly different.

*Storage Study*

Sensory data indicated that the blanched pickle was more acceptable. The results of storage study of the pickle conducted at room temperature (28°C) are shown in Table 5. Even though observations on general appearance were made every two to three d and data on the acidity and pH of pickling solution as well as colour and texture of product were recorded every week, only data obtained after 24 h and one month storage are presented to indicate real difference. The results showed that there were significant changes in texture and colour. The titratable acidity of the pickling solution decreased with a consequent increase in pH. The pickles and the pickling solution turned darker in colour. This was apparent from the lower L-value (lightness), greater a-value (greenness) and lower b-value compared with the freshly produced pickles. The decrease in the L-value followed by increase in the a-value after a one-month storage was due not to an increase in intensity of greenness by chlorophyll content but to an increase in darkness intensity.

TABLE 5
Changes in titratable acidity and pH of pickling solution and colour and texture of pickle during storage

| | after 24 hours | | after 1 month | |
|---|---|---|---|---|
| | 1 | 2 | 1 | 2 |
| Pickling solution | | | | |
| titratable acidity (% acetic acid) | 0.92[a] | 0.95[a] | 0.87[b] | 0.89[b] |
| pH | 2.58[a] | 2.61[a] | 3.0[b] | 3.35[b] |
| pickle (Fruit) | | | | |
| | -a b- | -a b- | -a b- | -a b- |
| colour | -2.7 21.4 | -2.9 20.6 | -4.8 16.5 | -4.6 14.8 |
| texture | 4.1[a] | 4.17[a] | 2.5[b] | 2.3[b] |

Values are means of six determinations
Means followed by the same letter in the same column are not significantly different at $p < 0.05$.

Note: 1 = without preservative
2 = with preservative (sodium benzoate)

The effect was the same for pickles with or without preservative. According to Bhasin and Bhatia (1981), darkening of pickle products is directly related to the amount of iron and tannin in the vinegar. Black pickles may owe their colour to the formation of hydrogen sulphide by bacteria and combination with iron in the water to yield black ferrous sulphide (Frazier and Westhoff 1978).

It was also observed that the pickles became significantly soft after a one-month storage. It is unlikely that the softening was due to microbial activity since there were no apparent microbial changes in the product. Pederson (1979) stated that softening in pickle is usually associated with an enzymatic action and the source of the enzyme is usually from the fruit. Enzymes are more acid-tolerant than organisms. It is possible that blanching did not inactivate all the pectolytic enzymes, thus allowing it to act on the pectic materials of fruit tissues during storage.

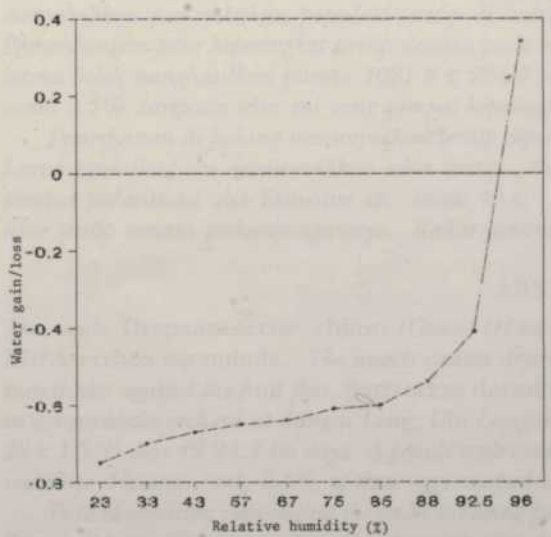


*Fig. 2. Water gain/loss of sample after equilibration*

*Water Activity*

The water activity value of product was about 0.94 (*Fig. 2*). The sugar in the pickling solution was not able to reduce the water activity of the pickle to a low value. Moulds were found on the surface of products that were placed under conditions of high humidity (92.5 and 96% relative humidity). The water activity value is important when one is considering the form and nature of packaging material for the product. Pickle cannot be packed dry. It has to be submerged in the pickling medium all the time to enhance its flavour.

## CONCLUSIONS

This study shows that young carambola fruits which are normally discarded away can be made into a pickle. There was no significant difference in the organoleptic attributes in products produced by the two techniques. However, the blanching method seemed to produce pickle of higher crunchiness than the coventional method. The blanching method shortened the time of processing which was the main advantage of the process. Results of the storage study on the blanched pickles showed that the product became soft and the colour turned adversely darker upon storage. The water activity was high. Further studies need to be carried out with a view of improving the texture of the product.

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# The Biology and Natural Control of the Scale *Drepanococcus chiton* (Green) (Homoptera : Coccidae), a Minor Pest of Carambola in Malaysia

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**Keywords:** ***Drepanococcus chiton*, nympal development,field, parasitisation, *Eunotus* sp., carambola orchard**

## ABSTRAK

*Teritip,* Drepanococcus chiton *(Green) (Homoptera : Coccidae) mempunyai potensi sebagai perosak pada tanaman belimbing besi, varieti B10* Averrhoa carambola. *Serangga ini menyebabkan kematian pada pucuk dan dahan bunga. Semburan racun secara jadual ke atas lalat buah* Bactrocera dorsalis *kemungkinan menyebabkan pertambahan populasi teritip di ladang carambola di Sungai Long Ulu Langat, Selangor. Perkembangan telur keperingkat teritip dewasa pada suhu 28°C ± 1.5°C ialah 49.9 ± 1.06 hari. Seekor teritip betina boleh menghasilkan purata 1081.9 ± 256.0 telur dengan 97.9% penetasan. Walau bagaimanapun, cuma 2.5% daripada telur ini yang sampai keperingkat dewasa di ladang.*

*Pemerhatian di ladang menunjukkan teritip diparasitkan oleh* Eunotus *sp. (Hymenoptera : Pteromalidae). Larva parasitoid ini memusnahkan telur teritip. Purata bulanan dari Ogos 1992 sehingga February 1993 peratus parasitisasi oleh* Eunotus *sp. ialah 40.6. Seekor larva parasitoid berupaya memusnahkan 93.2% telur teritip semasa perkembangannya. Kadar jantina parasitoid jantan dengan betina ialah 1 : 1.*

## ABSTRACT

*The scale,* Drepanococcus chiton *(Green) (Homoptera: Coccidae) is a potential pest of carambola, variety B10* Averrhoa carambola. *The insects caused drying of shoots and flower stalks. The regimental spraying of insecticides against the fruit flies,* Bactrocera dorsalis, *possibly gave rise to a build-up of the scale populations in a carambola orchard at Sungai Long, Ulu Langat, Selangor. The development of eggs to adult maturity at 28 ± 1.5 °C was 49.9 ± 1.06 days. A female scale could produce an average of 1081.9 ± 256.0 eggs with 97.9% viability. However, only 2.5% of these eggs reached adult maturity in the field.*

*Field observation showed that the scales are being parasitised by an egg-parasitoid,* Eunotus *sp. (Hymenoptera: Pteromalidae). The average monthly parasitisation by* Eunotus *sp. from August 1992 to February 1993 was 40.6%. The parasitic larva was capable of destroying 93.2% of the scale's eggs during its larval development. The sex ratio of male to female parasitoid was 1 : 1.*

## INTRODUCTION

The scale, *Drepanococcus chiton* (Green) is a polyphagous insect (Williams and Watson 1990). It was first reported in Sri Lanka by Green in 1909 and has since been recorded in Papua New Guinea and the Solomon Islands. The insect has a waxy secretion to protect itself in the course of feeding (Ebeling 1959). This waxy layer adheres the scale to the plant tissues (Metcalf and Flint 1962). In addition to its feeding action, the scale also encourages the growth of sooty-moulds which adversely affect the growth of the plants (Peairs and Davidson 1966).

Although the scales have been reported on cocoa and several ornamental plants, in Malaysia the scales infest the carambola, *Averrhoa carambola.* The use of pesticides to control the major pests of carambola particularly the fruit-flies, *Bactrocera dorsalis* complex (Hendel), was suspected in the build-up of the scale population in the orchard. This was particularly so during the dry season. This work investigates the biology of the scale, with the aim of identifying an effective way of controlling the scale in the carambola orhard.

## MATERIALS AND METHODS

The biological studies were conducted under laboratory conditions of 28°C ± 1.5°C and 75% R.H. at the Department of Plant Protection, Universiti Pertanian Malaysia. The field trial was carried out in a carambola orchard of 100 ha at Sungai Long, District of Hulu Langat, Selangor.

### (A) *Laboratory Study*

For the life-cycle study, 500 freshly laid eggs of *D. chiton* were incubated in 10 separate petri dishes lined with moist filter-paper. The length of 50 eggs and their development were recorded. For nymphal development, 3000 crawlers were transferred using a fine brush onto five separate carambola seedlings which were individually placed inside a cylindrical cage measuring 60 cm high and 27 cm in diameter. Every day 20 nymphs were sampled at random from the seedlings for determining the nymphal development. Nymphs and adults for morphological examination were fixed overnight in KAAD alcohol and then prepared by the method used by Peterson (1943).

Another 40 gravid females from the field were sampled at random for determining the relationship between body length and the total numbers of eggs. Eggs from selected specimens were evenly scattered over a petri dish and counted using a graph paper grid fastened underneath the dish.

Selected specimens such as eggs, nymphs and adult scales were then measured optically using a binocular microscope at X15 magnification. The use of transmitted light greatly facilitated the measurement of the specimens.

### (B) *Field Study*

For studying the development of *D. chiton*, 25 flower stalks each accommodating a gravid scale were selected. Immature scales were removed from the selected stalks. Each stalk was individually labelled on 21st December 1992. The presence of a trace of what appeared to be a waxy secretion around the base of the scale served as a useful indication of a gravid female. When this secretion, which seemed to act as a seal or cement was broken or discontinuous, crawlers were suspected as having emerged.

For studying the parasitisation of the scale, four samples were taken at two-monthly intervals commencing in the first week of August 1992 until February 1993. During the sampling period, young shoots accommodating 1000 matured scales were kept in 100 aerated test-tubes of three cm diameter. The scales were incubated at room temperature of 28°C ± 1.5°C. The number of parasitoids and their sexes were recorded.

## RESULTS AND DISCUSSION

### (A) *Laboratory Study*

Table 1 gives the average developmental time of different stages in *D. chiton*. The total duration from egg to adult emergence was 49.9 + 1.06 days.

#### (i) *Eggs:*

The egg is oval in shape with a broader anterior. Initially, it was pale white but later changed to a reddish colour. When hatching was about to occur, the eyes of the embryo appeared as a red-brown stage on both sides of the anterior portion of the egg. A similar condition occurs in the hemispherical scales, *Saissetia* spp. (Argyrio 1963; Barber 1980). The incubation period of the egg was 6.30 ± 0.58 days (Table 1).

#### (ii) *Nymphal Development*

There were three nymphal instars as indicated by the width of the head capsule and body length. The first stage i.e. the crawler is an active stage measuring 0.36 ± 0.02 mm. It lasted for one week before the nymphs settled on the softer tissues of the plant (Peairs and Davidson 1966). The subsequent instars were sessile, adhering closely to the plant tissues (Ebeling 1959; Metcalf and Flint 1962). The nymph significantly increased in size during the third instar. The waxy substance surrounding the scale became obvious and by now the nymph measured 2.30 ± 0.17 mm. The total duration of the 3rd nymphal stage was 22.30 ± 1.15 days.

Attempts were made to obtain the growth ratio of different instars by measuring the body-length and the width of the head capsule. The average ratio of body-length and head capsule width of the different instars were 2.5 and 1.9 respectively. Neither ratio agreed with Dyars Law of 1.4 (Wigglesworth 1965). The size of the nymph could possibly be influenced by the surface of plant tissues. It was observed that nymphs on flower stalk were more oblong than rounded. The scale became an adult female after 49.90 ± 1.06 days. The dorsum was usually membraneous

TABLE 1
The developmental period and growth ratios of the scale, *D. chiton* from carambola orchard

| Stage | N | duration (in days) X ± S.E. | body-length (mm) X ± S.E. | growth ratio | width of head-capsule(mm) X ± S.E. | growth ratio |
|---|---|---|---|---|---|---|
| Eggs | 50 | 6.30 + 0.58 | 0.30 + 0.01 | | | |
| Nymph I | 20 | 6.00 + 1.00 | 0.36 + 0.02 | | 0.09 + 0.002 | |
| | | | | 2.70 | | 2.00 |
| Nymph II | 20 | 15.30 + 1.53 | 0.98 + 0.26 | | 0.19 + 0.004 | |
| | | | | 2.30 | | 1.80 |
| Nymph III | 20 | 22.30 + 1.15 | 2.30 + 0.17 | | 0.32 + 0.004 | |
| Adult | 40 | | 3.78 + 0.48 | | 0.62 + 0.061 | |

with irregular rows of minute pores and cornical seta. The body-length and width of head capsule were 3.78 ± 0.48 mm and 0.62 ± 0.061 mm respectively. A gravid female could produce an average of 1081.9 ± 256 eggs. This egg number is comparable to that of *S. coffeae* in the glasshouses (Barber 1980). The percentage of egg viability was 97.9. The body length of gravid scale on average measured 3.78 ± 0.48 mm. There was a weak relationship ($r^2 = 0.44$) between the body-length of adult females and the number of eggs.

(B) *Field Study*

The field observation showed that a gravid scale could produce an average of 25 ± 5.0 matured scales during the period of seven weeks from December 1992. Although a gravid scale can produce an average of 1081.9 ± 256 eggs with 97.9% viability only 2.5% of these eggs reached maturity. Crawler mortality could be due to both abiotic and biotic factors with natural activity of enemies being expected to contribute significantly to this (Miller and Kosztarab 1979).

Although, reproduction of *D. chiton* is by parthenogenesis, occasionally the males appeared in the field. During the sampling period males of the scale were only found in February 1993. This suggests that males are only present at specific periods. The males are very delicate insects measuring 2.30 ± 0.03 mm in length. The body is brownish with one pair of wings. The posterior of the abdomen is equipped with genital seta and style. The role of the male is for periodic sexual reproduction (Mckenzie 1967).

Sampling of adult scales from August 1992 to February 1993 showed that the scales were parasitised by *Eunotus* sp. (Hymenoptera: Pteromalidae). The female parasitoid oviposits in gravid scales and the parasitic larva prey on the eggs within the scales (Kirkpatrick 1962; Graham 1992). Normally one parasitic larva was found in a single scale and over 93.2% of the eggs were consumed by the larva. A small proportion of eggs gave rise to crawlers which settled on the younger parts of the plants.

The degree of parasitisation varied with the time of the year. There was a significant difference ($p < 0.05$) between the percentage of parasitisation in October 1992 compared with other sampling months (*Fig. 1*). The dry weather in October 1992 when the total rainfall was only 72 mm most likely brought about the build-up of scales on the flower stalks and shoots, which in turn, resulted in a higher percentage of

parasitisation. On the other hand, in December 1992, the amount of rainfall rose to 338 mm. This resulted in a significant reduction of scales population which correspondingly brought about a drop in percentage of parasitisation. Schultz (1984) observed a similar fluctuation of levels of parasitisation of scales in relation to weather.

The sole emergence of *Eunotus* sp. suggests that they were the dominant parasitoids of the scales in the field. This genus, *Eunotus*, has been successfully used in the biological control of the scales, *Eriopeltis signoret*, in the United Kingdom (Manawadu 1984). Another related species, *Eunotus lividus* has been used in the biological control of oak lecanium (Schultz 1990). Hence there is a potential for the use *Eunotus* sp in the biological control of the scale *Drepanococus chiton* in carambola orchards in Malaysia.

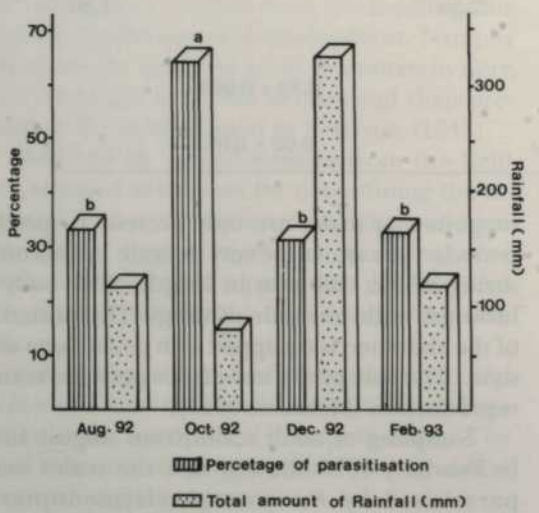


*Fig. 1 : Percentage of parasitisation of* D.chiton *by* Eunotus *sp. in a carambola orchard. Columns with the same letters are not significantly different at the 5% level*

## ACKNOWLEDGEMENT

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# Kajian Awal terhadap Sistem Pemilihan Somaklon Cili Yang Resistans kepada *Colletotrichum capsici* (Syd.) Butler & Bisby

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**Keywords: somaklon, cili, *Colletotrichum capsici***

## ABSTRAK

*Perlakuan toksin daripada patogen antraknos,* Colletotrichum capsici *ke atas kultur tisu cili telah digunakan sebagai satu kaedah pemilihan varian yang resistan terhadap patogen tersebut. Sediaan toksin kasar itu disterilkan dan kemudian dicampurkan ke dalam medium MS pada kepekatan 0, 10, 20, 30 atau 40% (v/v). Kepekatan toksin yang melebihi 20% telah menghalang pembentukan kalus daripada eksplan hipokotil, manakala pada kepekatan 10% dan 20%, pembentukan kalus adalah sangat ditekan. Kalus yang bermandiri daripada perlakuan toksin tidak berupaya untuk meregenerasi. Sebaliknya, kalus yang dikulturkan pada medium MS yang ditambah dengan jus V8 dan medium kultur kawalan MS, masing-masing menghasilkan regenerasi sebanyak 37.6% dan 62.7%. Penginduksian varian yang resistans juga dilakukan melalui penginokulatan kalus dengan konidium patogen antraknos. Antibiotik yang diekstrak daripada kulat antagonis,* Chaetomium trilaterale, *telah digunakan untuk menghadkan perkembangan patogen pada medium daripada kalus cili. Daripada pengasaian antibiotik, perkembangan patogen adalah bergantung kepada dos antibiotik yang digunakan. Kepekatan minimum antibiotik yang berjaya menghalang pertumbuhan patogen sepenuhnya adalah 100 mg/ml. Pada kepekatan antibiotik yang digunakan itu pertumbuhan kulat patogen dicerap pada kalus tetapi tidak pada medium. Bagaimanapun, kalus tersebut mengalami pemerangan selepas 2 minggu penginokulatan. Di samping itu, kalus yang bermandiri menjadi padat dan tidak berjaya diregenerasikan.*

## ABSTRACT

*Toxin treatment from the anthracnose pathogen,* Colletotrichum capsici *on chilli tissue culture was utilised as the selection mechanism for variants which are resistant to this pathogen. The crude toxin preparation was sterilised and then added to the MS medium at the concentration of 0, 10, 20, 30 or 40% (v/v). At concentrations higher than 20%, the toxin prevented the formation of calli from hypocotyl explants, and at 10% and 20%, the formation of calli was strongly inhibited. The calli which survived the toxin treatment did not regenerate. Conversely, calli which were cultured on MS medium suplemented with V8 juice or on the control MS medium, were able to regenerate at 37.6% and 62.7%, respectively. Induction of resistant variants was attempted by inoculating the calli with the konidia from the anthracnose pathogen. The antibiotic which had been extracted from an antagonistic fungus,* Chaetomium trilaterale, *was added to the MS medium to limit the spread of the pathogen to the medium from the chilli calli. From the antibiotic assay, the developement of the pathogen was dependent on the antibiotic concentration used in the media. The minimum antibiotic concentration which successfully prevented the growth of the fungal pathogen was 100 mg/ml. At that concentration, the fungus was only observed on the calli but not on the medium. However, the calli turned brown after 2 weeks of inoculation. In addition, the calli that survived became compacted and failed to regenerate.*

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## PENDAHULUAN

Perlakuan pada kultur tisu dengan patogen atau toksin merupakan kaedah pemilihan yang selalu digunakan untuk mendapatkan varian tumbuhan yang resistans kepada penyakit (Daub 1986). Sel-sel yang berjaya melintasi kesan tekanan pemilihan yang diberikan oleh patogen atau toksin dianggap berjaya mengatasi infeksi oleh agen-agen penyakit yang berkenaan (Ingram 1977; Sacristan 1979; Sacristan & Hoffman 1979; Ostry & Skilling 1988). Untuk menghalang perkembangan kulat patogen, Lepoivre *et al.* (1986) telah memasukkan bahan antikulat seperti Benlate(R) dan Mycostatin ke dalam medium, untuk mengawal perkembangan kulat patogen yang digunakan sebagai agen tekanan pemilihan. Kaedah ini dilaporkan mudah dan berkesan untuk memilih somaklon yang resistans terhadap sesuatu patogen (Brettell & Ingram 1979). Menurut Daub (1986) kerintangan yang ditunjukkan pada planlet adalah berkorelasi dengan kerintangan penyakit pada tumbuhan lengkap.

Satu kajian awalan telah dilakukan untuk menguji keberkesanan kaedah ini untuk memilih secara *in vitro* kultur tisu cili yang resistans terhadap *Colletotrichum capsici* (Syd.) Butler & Bisby. Kulat ini dipilih kerana, semasa kewabakan, penyakit antraknos yang disebabkannya boleh mengurangkan hasil cili sehingga 60% (Kang 1983). Dalam kajian ini agen tekanan pemilihan yang digunakan ialah ekstrak kasar toksin dan konidium patogen yang tersebut di atas. Kaedah *in vitro* ini diharapkan dapat membantu mempercepatkan usaha untuk mendapatkan tumbuhan yang resistans terhadap patogen tersebut.

Untuk menghadkan pertumbuhan patogen pada kultur sel, ekstrak antibiotik dari kulat *Chaetomium trilaterale* Chivers (Ahmad *et al.* 1989) telah digunakan. Dua pencilan kulat antagonis, iaitu AW dan M telah digunakan. Penggunaan ekstrak antibiotik dari *C. trilaterale* ini adalah berdasarkan kajian yang menunjukkan kulat ini mempunyai ciri antagonis yang baik terhadap beberapa kulat patogen (Ahmad *et al.* 1990).

## BAHAN & KAEDAH

*Penghasilan Kalus Dan Planlet Daripada Hipokotil*

Penghasilan kalus dan planlet melalui pengkulturan hipokotil cili, koleksi CDI (Sudarisman 1991) adalah berdasarkan teknik yang telah dilaporkan oleh Nurina *et al.* (1992). Medium regenerasi planlet adalah medium pepejal MS (Murashige dan Skoog 1962) dengan kombinasi hormon pertumbuhan auksin dan sitokinin, iaitu IAA (asid indolasetik) pada kepekatan 1.0 mg/l dan BA (benziladenina) pada kepekatan 2.5 mg/l. Eksplan hipokotil dikulturkan pada medium regenerasi sebanyak lima eksplan bagi setiap piring Petri. Kultur seterusnya dieramkan pada suhu 26-28°C dalam keadaan bercahaya yang berterusan.

*Penyediaan Ekstrak Toksin C. capsici*

Turasan kasar toksin *C. capsici* disediakan dengan mengkulturkan patogen tersebut dalam medium cecair (Brettell & Ingram 1979) yang terdiri daripada 10% (v/v) jus V8 dalam air suling. Kultur dieramkan di atas penggoncang mendatar (Labline Shaker Model 3594) dengan kelajuan 200 rpm, pada suhu 30°C selama 20 hari. Seterusnya, kultur itu dituras dengan menggunakan empat lapis kain kasa untuk memisahkan miselium.

Hasil turasan kemudiannya diempar (Sorvall, RC 5B) dengan kelajuan 17,000 rpm, pada suhu 4°C selama 15 minit. Supernatan yang terbentuk adalah larutan bebas sel yang mengandungi toksin *C. capsici*. Hasil turasan tersebut ditetapkan kepada pH 5.8 dan seterusnya disterilkan dengan penuras membran (saiz liang 0.2 µm). Ketoksikan turasan kasar toksin ini diuji, dengan menunjukkan terdapatnya nekrosis pada cakera (6 mm) daun cili, sebelum ianya digunakan sebagai agen tekanan pemilihan dalam medium kultur tisu.

*Penyediaan Medium Bertoksin*

Kepekatan ekstrak toksin dalam medium MS untuk pemilihan ialah 10%, 20%, 30% atau 40% (v/v). Untuk penyediaan ini, 10, 20, 30 atau 40 ml ekstrak kasar toksin steril yang disediakan seperti di atas dicampurkan kepada 50 ml medium MS (kepekatan dua kali ganda) yang telah disterilkan secara autoklaf. Isipadu air suling tertentu ditambah supaya isipadu menjadi 100 ml. Sebelum percampuran toksin dan medium MS dilakukan, suhu medium diturunkan terlebih dahulu kepada 45°C - 60°C untuk mengelakkan penyahaktifan toksin. Campuran medium MS dan toksin kemudiannya dihomogenkan secukupnya sebelum diagihkan ke dalam piring Petri steril.

JADUAL 1
Pembentukan kalus dan regenerasi planlet daripada eksplanc cili yang dikulturkan pada medium pemilih yang mengandungi ekstrak toksin daripada *Colletotrichum capsici*

| Kepekatan toksin/jus | % Pembentukan Kalus[a] | | | % Regenerasi [b] | | |
|---|---|---|---|---|---|---|
| | MS | MS + Toksin | MS + Jus V8 | MS | MS + Toksin | MS + V8 Jus |
| 0 | 98.9 | - | - | 74.6 | - | - |
| 10 | - | 36.7 | 83.3[c] | - | 0 | 60.0 |
| 20 | - | 12.2 | 81.1[c] | - | 0 | 55.6 |
| 30 | - | 0 | 75.6 | - | 0 | 48.9 |
| 40 | - | 0 | 70.0 | - | 0 | 43.3 |

[a]Daripada 90 eksplan dalam 3 replikasi.

[b]Daripada jumlah eksplan.

[c]Perbezaan bererti (t=0.05) antara % pembentukan kalus dalam medium mengandungi jus V8 dan medium mengandungi ekstrak toksin.

Sebagai kawalan, pengkulturan juga dilakukan dengan menggunakan medium MS sahaja dan medium MS yang dicampurkan dengan medium cecair jus V8 dalam isipadu yang sama dengan toksin (Jadual 1). Kesemua bahan ditetapkan pHnya kepada 5.8 sebelum ditambah kepada medium. Sebanyak 30 eksplan digunakan bagi setiap perlakuan dan peratus pembentukan kalus dalam medium-medium tersebut dicerap. Kalus yang terbentuk disubkulturkan dalam medium MS tanpa toksin untuk diregenerasikan.

*Penyediaan Antibiotik daripada C. trilaterale*

Pengekstrakan antibiotik daripada pencilan kulat *C. trilaterale* AW dan M adalah ubahsuaian daripada kaedah yang digunakan oleh Geiger *et al.* (1944). Kulat dikulturkan pada medium PDA dan dieram selama 15-20 hari pada suhu bilik. Pengekstrakan dilakukan sebanyak tiga kali dengan mencampurkan aseton kepada miselium serta medium kultur, dan disimpan semalaman pada suhu 4°C. Hasil ekstrak seterusnya dituras melalui kain kasa dan tiga kali lagi melalui kertas turas Whatman No 1.

Hasil turasan dipekatkan dengan alat pengempar berputar (Tokyo, Rikakikai) pada suhu 55°C selama 2 jam. Seterusnya, pengekstrakan antibiotik dengan etil asetat dilakukan sebanyak dua kali. Lapisan nyahaktif (bahagian bawah) dibuang dan lapisan aktif (bahagian atas) dipekatkan dengan alat pengewapan berputar pada suhu 60°C, untuk selama 3 jam. Ekstrak yang diberi nama antibiotik AW dan M ini adalah dalam bentuk serbuk.

*Pengasaian Keamatan Kesan Antibiotik Terhadap Patogen*

Larutan stok berantibiotik (pH 5.8) daripada kulat *C. trilaterale* AW dan M disediakan. Medium regenerasi disterilkan dengan menggunakan penuras membran (saiz liang 0.22 µm). Selepas pengautoklafan pada suhu 121°C selama 20 minit dengan tekanan 25 kg/m$^3$, medium regenerasi disejukkan ke suhu 45-60°C. Kepada medium ini dicampurkan larutan stok berantibiotik untuk mendapatkan kepekatan akhir antibiotik seperti berikut: 0, 1.0, 3.0, 5.0, 10.0, 30.0, 50.0, 100.0 dan 200.0 mg/l. Campuran tersebut dihomogenkan dengan sempurna sebelum diagihkan ke dalam piring Petri steril.

Pengasaian dilakukan untuk menentukan kepekatan optimum antibiotik bagi perencatan sepenuhnya patogen, *C. capsici* pada medium regenerasi. Cakera kulat berdiameter 0.5 cm dikulturkan di tengah piring Petri, dan dieram selama seminggu, di bawah cahaya yang berterusan pada suhu bilik.

*Kesan Patogen Terhadap Pembentukan Planlet*

Eksplan hipokotil dikultur pada medium regenerasi. Selepas hari ke-10, kalus yang mempunyai saiz seragam disubkulturkan ke medium berantibiotik, AW dan M dengan kepekatan 100 mg/l. Kemudian ampaian konidium patogen yang berkepekatan $10^4$ konidium 1ml diinokulatkan pada kalus tersebut. Kultur seterusnya dieramkan pada suhu 26-28°C, di bawah cahaya yang berterusan. Pencerapan dilakukan berdasarkan kesan patogen terhadap kemandirian kalus (peratus yang tidak menjadi perang) dan pembentukan planlet.

## HASIL

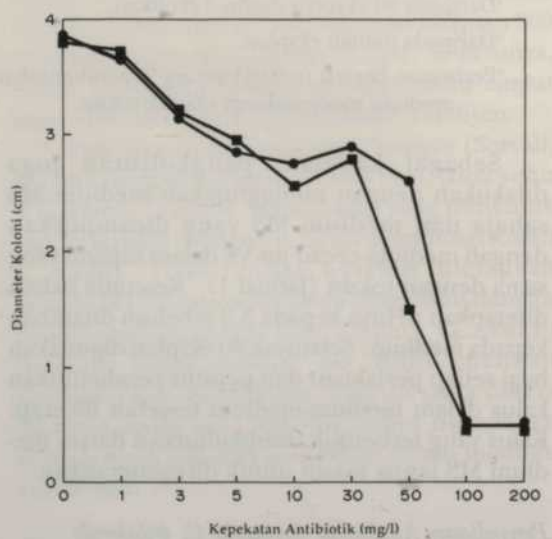
*Kesan Toksin Terhadap Pembentukan Kalus*

Selepas 2 minggu pengkulturan, cerapan diambil berdasarkan pembentukan kalus dan kematian yang dialaminya. Berdasarkan kepada ANOVA, penggunaan toksin dan jus V8 telah memberikan kesan yang bererti ($kb < 0.05$) terhadap pembentukan kalus berbanding dengan kawalan (Medium MS). Eksplan hipokotil yang dikultur pada medium toksin hanya berjaya membentuk kalus sehingga kepekatan 20% sahaja. Pada kepekatan yang lebih tinggi, tiada kalus yang berjaya terbentuk (Jadual 1). Pengurangan peratus regenerasi ini juga disebabkan oleh kehadiran medium jus V8, penggunaan Jus V8 telah menurunkan keupayaan pembentukan kalus berbanding dengan penggunaan medium MS tanpa sebarang tambahan. Peratus pembentukan kalus adalah 36.7% untuk 10% toksin dan 12.2% untuk 20% toksin. Keupayaan pembentukan kalus juga didapati menurun dengan peningkatan kepekatan jus V8 yang digunakan di dalam medium MS.

Kesemua kalus yang terbentuk pada medium toksin tidak berjaya diaruh bagi pembentukan planlet (Jadual 1). Kalus mula menjadi perang pada bahagian yang bersentuhan dengan medium dan seterusnya diikuti tisu yang bersebelahannya. Kalus yang terbentuk pada medium jus V8 telah diregenerasikan, walaupun kalus yang diperolehi pada kepekatan 30 atau 40% menunjukkan peratus regenarasi yang rendah (Jadual 1). Sementara itu, bagi kalus yang dijanakan pada medium MS kawalan, regenerasi telah dapat dihasilkan.

*Pengasaian Antibiotik Terhadap Patogen*

Diameter cakera miselium kulat *C. capsici* yang berkembang diukur selepas pengeraman selama 1 minggu. Peningkatan kepekatan antibiotik didapati mengurangkan diameter koloni kulat yang terbentuk. Sehingga akhirnya, pada kepekatan 100 mg/l, patogen tidak lagi berupaya untuk tumbuh (Rajah 1). Fenomena perencatan perkembangan miselium patogen tersebut berlaku untuk antibiotik daripada kedua-dua pencilan kulat AW dan M. Perbandingan keberkesanan perencatan antara keduanya menunjukkan bahawa pada kepekatan yang sama, antibiotik daripada pencilan M merencat patogen dengan lebih berkesan. Berdasarkan analisis ANOVA, perbezaan keberkesanan perencatan secara keseluruhan antara antibiotik pencilan M dan pencilan AW adalah sangat bererti ($kb < 0.01$).




*Rajah 1 : Pengasaian aktiviti ekstrak antibiotik daripada* Chaetomium trilaterale *pencilan AW (●) Dan Pencilan M ( ■ ) Terhadap pertumbuhan* C. capsici, *agen penyakit antraknos*

*Kesan Patogen Terhadap Pembentukan Planlet*

Patogen yang diinokulatkan pada kalus di atas medium regenerasi berantibiotik telah menginfeksi kalus tersebut tanpa membentuk pertumbuhan yang meluas pada permukaan medium regenerasi. Walau bagaimanapun kalus tersebut tidak berjaya menghasilkan planlet

kerana nekrosis yang berlaku pada sebahagian kalus didapati menjangkiti bahagian yang lain dan seterusnya mengakibatkan kematian kalus. Sebaliknya, kalus yang dikulturkan pada medium regenerasi berantibiotik tanpa penginokulatan patogen (iaitu kawalan) didapati berupaya untuk membentuk planlet.

## PERBINCANGAN

*Kesan Toksin C. capsici Terhadap Pembentukan Kalus*

Penambahan toksin *C. capsici* ke dalam medium regenerasi berjaya menekan kecekapan pembentukan kalus daripada eksplan hipokotil. Fenomena ini berlaku kerana kewujudan toksin yang telah mengganggu sistem perkembangan sel dan seterusnya menekan tumbesarannya (Ingram 1977). Kegagalan membentuk planlet oleh kalus yang berupaya bermandiri berlaku, mungkin disebabkan oleh perubahan dalam sistem perkembangan sel. Perubahan keperluan nutrien dan kepekatan hormon tumbesaran berlaku akibat perubahan persekitaran (medium) dan tekanan oleh tindakan toksin (Daub 1986). Kegagalan ini juga mungkin boleh diterangkan oleh keadaan fisiologi kalus yang padat. Sel-sel yang telah mati dan mengalami nekrosis akibat tindakan toksin turut mengeluarkan bahan toksik yang boleh merebak ke sel-sel bersebelahan yang bermandiri.

Ujian perbandingan antara medium cecair Jus V8 dan toksin dilakukan untuk menentukan kesan latar belakang medium penghasil toksin terhadap kemandirian sel hakis cili. Penurunan peratus pembentukan kalus yang bererti oleh medium Jus V8 menunjukkan bahawa komposisi medium tersebut mempengaruhi keupayaan pembentukan kalus pada sel-sel cili. Oleh sebab penggunaan jus V8 masih membenarkan berlakunya regenerasi, maka andaian dibuat bahawa medium tersebut boleh digunakan sebagai medium penghasilan toksin untuk ujian pemilihan kalus yang rintang terhadap toksin patogen.

*Kesan Antibiotik Terhadap Perkembangan Patogen*

Penggunaan kedua-dua antibiotik berjaya merencat sepenuhnya pertumbuhan patogen pada medium regenerasi. Ini membuktikan bahawa antibiotik daripada kedua-dua pencilan kulat mempunyai potensi untuk digunakan bagi menghalang perkembangan patogen apabila digunakan secara langsung sebagai agen pemilih. Antibiotik daripada pencilan M memperlihatkan keberkesanan perencatan yang lebih tinggi berbanding dengan antibiotik daripada pencilan AW.

*Kesan Patogen Terhadap Pembentukan Planlet*

Kegagalan kalus membentuk planlet mungkin disebabkan oleh morfologi kalus yang padat, yang memudahkan berlakunya jangkitan atau nekrosis dari sel ke sel. Masalah ini boleh diatasi dengan terbentuknya kalus rapuh yang embrionik supaya sel-sel rintang terhadap patogen boleh bermandiri tanpa dipengaruhi oleh faktor fisiologi tumbuhan seperti yang dialami oleh sel-sel rentan yang berhampiran.

Penggunaan kalus yang padat (walaupun terdapat sel atau tisu yang resistans), menimbulkan masalah semasa memisahkan tisu daripada bahagian yang terinfeksi. Masalah ini mungkin boleh diatasi dengan menggunakan kalus yang longgar atau ampaian sel yang embronik. Hasil daripada kejayaan tersebut boleh membuka jalan dalam penyelidikan bagi mengatasi masalah penyakit pada cili (Fari 1986).

Antibiotik yang dihasilkan oleh *C. trilaterale* boleh dijadikan agen perencatan patogen yang baik di dalam medium pemilihan kerana ia didapati tidak memberi sebarang kesan terhadap penghasilan planlet. Fenomena ini perlu diberi perhatian kerana antibiotik boleh didapati dengan mudah daripada kulat antagonis tersebut. Di samping itu, kejayaan mendapatkan sesuatu kaedah bagi merencat perkembangan patogen di dalam medium akan membuka jalan kepada pemilihan variasi sel yang rintang secara infeksi terus daripada patogen (Sacristan & Hoffman 1979). Mengikut Lepoivre *et al.* (1986) kaedah yang menggunakan patogen sebagai agen tekanan pemilihan dianggap lebih baik daripada kaedah menggunakan toksin. Ini adalah kerana kaedah penggunaan toksin bergantung kepada sama ada toksin adalah faktor virulen sebenar bagi penyakit yang disebabkan oleh patogen tersebut. Bagaimanapun, kaedah menggunakan patogen seperti yang telah dilakukan dalam kajian ini perlu diperbaiki supaya keadaan optimum diperolehi, dan seterusnya dapat digunakan bagi penghasilan tumbuhan yang resistans terhadap penyakit yang disebabkan oleh patogen yang dikaji.

## PENGHARGAAN

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# Antimicrobial Activity of some Tropical Fruit Wastes (Guava, Starfruit, Banana, Papaya, Passionfruit, Langsat, Duku, Rambutan and Rambai)

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**Keywords:** ***Psidium guajava, Averrhoa carambola, Musa sapientum, Carica papaya, Passiflora edulis* F. Flavicarpa, *Lansium domesticum, Nephelium lappaceum, Baccaurea motleyana*, antimicrobial activity**

## ABSTRAK

*Ekstrak buah jambu batu muda, ranum dan daun jambu batu (*Psidium guajava*); buah belimbing bintang (*Averrhoa carambola*) muda, ranum dan daun belimbing bintang; buah pisang (*Musa sapientum *variety Montel) muda dan ranum; buah betik (*Carica papaya*) muda dan ranum; kulit buah markisa (*Passiflora edulis *F. Flavicarpa); kulit buah duku dan langsat (*Lansium domesticum*); kulit rambutan (*Nephelium lappaceum*) dan kulit rambai (*Baccaurea motleyana*) diuji keaktifannya menentang bakteria gram positif, gram negatif, yis dan kulat* (Staphylococcus aureus, Bacillus subtilis, Bacillus cereus, Lactobacillus bulgaricus; E. coli, Proteus vulgaricus, Pseudomonas aeruginosa, Salmonelli typhi; Saccharomyces cerevisiae, Candida lypolytica; Rhizopus *spp.*, Aspergillus niger, *dan* Chlamydomucor *spp.*). *Keaktifan anti mikrob telah diuji menggunakan kedua-dua kaedah, iaitu resapan cakera kertas turas dan pencairan dalam tabung uji. Ekstrak dari belimbing bintang ranum, daun jambu batu dan kulit rambai menunjukkan kebolehan menentang semua bakteria yang diuji, dengan kekuatan yang lebih daripada 50ug streptomycin. Kulit buah markisa, buah jambu batu muda dan ranum, mempunyai kebolehan menentang kesemua bakteria melainkan* E. coli. *Kulit rambutan juga menunjukkan kebolehan menentang semua bakteria yang diuji melainkan* Pseudomonas aeruginosa. *Kebanyakan hasil buangan buah-buahan di atas menunjukkan kebolehan melawan bakteria tetapi tidak yis atau kulat. Ekstrak kulit pisang, betik, duku, langsat dan rambutan menunjukkan aktiviti ke atas* Candida lypolytica *dan ekstrak jambu batu menunjukkan aktiviti yang kuat melawan* Saccharomyces cerevisiae. *Selain dari jambu batu, buah belimbing ranum, kulit rambai dan rambutan menunjukkan potensi digunakan untuk menentang bakteria.*

## ABSTRACT

*Extracts of ripe, unripe and leaves of guava (*Psidium guajava*); ripe, unripe and leaves of starfruit (*Averrhoa carambola*); ripe and unripe banana (*Musa sapientum *variety Montel); ripe and unripe papaya (*Carica papaya*); passionfruit (*Passiflora edulis *F. Flavicarpa) peel; two varieties of* Lansium domesticum *peel (langsat and duku); rambutan (*Nephelium lappaceum*) peel and rambai (*Baccaurea motleyana*) peel were evaluated for antimicrobial activity against gram positive bacteria, gram negative bacteria, yeast and fungi* (Staphylococcus aureus, Bacillus subtilis, Bacillus cereus, Lactobacillus bulgaricus; E. coli, Proteus vulgaricus, Pseudomonas aeruginosa, Salmonelli typhi; Saccharomyces cerevisiae, Candida lypolytica; Rhizopus *spp.*, Aspergillus niger, *and* Chlamydomucor *spp*). *The antimicrobial activities were tested using both the filter paper disc diffusion and tube dilution assays. Extracts from ripe starfruit, guava leaves and rambai peel showed strong activity against all the bacteria tested, in most cases with activity stronger than 50ug streptomycin. Passionfruit peel, ripe and unripe guava showed activity against all the bacteria tested except* E. coli. *Rambutan peel too showed activity against all the bacteria tested except towards* Pseudomonas aeruginosa. *Most of the fruit wastes showed some activity towards bacteria but poor activity against yeast or fungi. Extracts from bananas, papayas, passionfruit peel,* Lansium domesticum *peels and rambutan peels showed activity against* Candida lypolytica *while extracts from guava showed strong activity against* Saccharomyces cerevisiae. *Other than guava, ripe starfruit, rambai peel and rambutan peel showed potential for use against bacteria.*

## INTRODUCTION

Various fruits (peel, flesh or seed) have been used in traditional medicine for stomach ache, sore eyes, fever, etc. Papaya has been shown to contain sulphydroxyl protease which can inhibit virus or microbial infection (Rajashekhara *et al.* 1990). Jensen *et al.* (1990) found passionfruit effective against *Bacillus subtilis* and some yeasts. The astringent compound in *Lansium domesticum* skin has been used in traditional medicine against hernia. Rambutan roots and leaves have been used by the Malays for fever, and the bark for tongue infection. Other fruits that have been shown to have some antimicrobial activity include mangosteen *Garcinia mangostana* (Sundram *et al.* 1983); cranberry (Senchyuk and Demkevich 1974); *Glaucium flavum* (Cabo *et al.* 1988); bedara *Ziziphus spinachristi* (Shah *et al.* 1986) and *Annona montana* (Wu *et al.* 1987).

Guava leaves are used in the Malay archipelago for diarrhoea, stomach ache, ermifuge, leucorrhoea, lotion for skin complaints and in childbirth to expel the placenta, while extracts have been on the market in Europe for diarrhoea or gastroenteritis (Burkill 1966). Starfruit flowers which are pleasantly acid are used in salads and are considered to have a vermifuge action; and applied topically for skin irritation or prickly heat. Crushed starfruit leaves and shoots are used for chicken-pox, ringworm and headache; and a decoction of the leaves and fruits is given to arrest vomiting. The fruit juice is also given as a corrective after a drinking-bout, biliousness, diarrhoea and as a cooling drink in fevers (Burkill 1966). Rambai skin is used for sore eyes, taken internally after childbirth and currently in the Malaysian and Indonesian traditional cosmetic industries, in medicated face powder preparation for treatment of acne and general skin complaints; and is also eaten as a vegetable.

The present work attempts to investigate if wastes from the fruit industry can be used for antimicrobial activity in cosmetics, food, etc. This work will also inform us whether some fruits are beneficial for consumption during sickness and help recovery as an alternative to taking antibiotics.

## MATERIALS AND METHODS

### *Plant Material*

Ripe, unripe and leaves of guava (*Psidium guajava*); ripe, unripe and leaves of starfruit (*Averrhoa carambola*); ripe and unripe banana (*Musa sapientum* variety Montel); ripe and unripe papaya (*Carica papaya*); passionfruit (*Passiflora edulis* F. Flavicarpa) peel; two varieties of *Lansium domesticum* peel (langsat and duku); rambutan (*Nephelium lappaceum*) peel and rambai (*Baccaurea motleyana*) peel were obtained from the UPM orchard or the evening market. The plant materials were chopped and dried at room temperature or 37°C oven. They were then ground to a fine powder in a cyclone mill and stored at room temperature for extraction.

### *Chemicals*

Petroleum ether (B.P. 40-60), chloroform, and ethanol were obtained from BDH, Poole, England. Bacterial nutrient agar (NA), Malt extract agar (MEA) for yeast and fungi, peptone water and triptone soya broth (TSB) were obtained from Oxoid (Basingstoke, Hants, England, UK). Miconozole (Johnson & Johnson Malaysia Ltd.) and streptomycin sulphate (Becton Dickson & Co., Cockeysville, D 21030, USA) were used as standards.

### *Microorganisms*

The test microorganisms include gram positive bacteria (*Staphylococcus aureus* ATCC 12598, *Bacillus subtilis* ATCC 6051, *Bacillus cereus* IFO 3457, *Lactobacillus bulgaricus*); gram negative bacteria (*E. coli* ATCC 25922, *Proteus vulgaricus* ATCC 13315, *Pseudomonas aeruginosa* IFO 3445, and *Salmonelli typhi* IFO 12529); yeasts (*Saccharomyces cerevisiae* ATCC 7754, *Candida lypolytica* ATCC 8661); and fungi (*Rhizopus* spp. ATCC 4270, *Aspergillus niger* ATCC 1015, and *Chlamydomucor* spp.) were obtained from the culture collection of the Department of Food Science, Universiti Pertanian Malaysia (ATCC = American Type Culture Collection; IFO = Institute of Fermentation, Osaka). The stock cultures were grown on TSB for 24 h at 30°C. The cells were diluted to give a final concentration of $10^5$-$10^6$ CFU/ml (Colony Forming Unite) determined by using a haemocytometer.

### *Plant Extracts*

The dried, powdered samples were weighed and successively extracted with petroleum ether, chloroform, and ethanol. A general extraction procedure was followed for each solvent by soaking the powdered seeds overnight and the solution filtered. The extraction was repeated

three times, each using a fresh solvent. The combined extracts of each solvent were filtered and the filtrates were evaporated under reduced pressure at temperatures below 50°C. The dark brown viscous residues were weighed, then reconstituted in 5 ml ethanol and the sterile filter paper discs (6 mm diameter) were impregnated with each plant extract solution and left to dry at room temperature.

*Agar Diffusion Technique* (Nazrul *et al.* 1984)

For antibacterial activity, nutrient agar (NA), pH 7 - 7.2, was sterilized for 15 min at 110°C. Twenty ml of NA were added to each 100 mm sterile Petri dish and kept at 30°C for 24 h to confirm sterility. All tests were done by placing the dried discs impregnated with plant extract on the agar surface previously inoculated with a suspension of each microorganism ($10^5$ - $10^6$ CFU/ml). The antifungal and anticandidal activities were similarly determined using malt extract agar.

The growth and the purity of each suspension was verified by using a Gram stain. Standard discs of Miconozole (1 µg /disc) and streptomycin sulphate (50 µg / disc) were used as positive controls. The plates were incubated at 30°C for 24 h and the antimicrobial activity was recorded as the width, in mm, of the clear inhibition zones surrounding the discs. Each test was repeated at least 3 times.

*Minimum Inhibition Concentration* (Tanaguchi and Satumura 1972; and Kubo *et al.* 1992)

0.5 ml of triptone soya broth containing $10^7$ test organisms $ml^{-1}$ was mixed with 100 - 2500 µg /ml extracts in two-fold dilution assay and incubated for 24 h at 30°C. Growth was measured by the optical density at 660 nm and the viability of the cultures was confirmed by incubation of the broth on agar plate (Oxoid, Basingstoke, Hants, England). Miconozole and streptomycin sulphate were used as standard antibiotics for comparison with the activities of the plant extracts against microbial species. The concentration of the tube of the highest dilution that was free from growth was recorded as the minimum inhibitory concentration (MIC, µg/ml).

## RESULTS AND DISCUSSION

All the microorganisms responded differently to the various plant extracts and all the plant extracts tested showed some antimicrobial activity (Table 1). Most of the fruit plants showed good activity against bacteria but poor activity against yeasts and fungi; thus this is the reason why spoilage of fruits is often caused by fungal and yeast infection (Table 2). Ripe starfruit, guava leaves and rambai peel showed antibacterial activity against all the bacteria tested. Rambai peel and guava leaves showed activity above that of 50 µg streptomycin and appeared to be as a good antimicrobial agent. Ripe and unripe guava and passionfruit peel showed activity against all the bacteria except towards *E. coli.* Unripe banana showed activity against all the bacteria except towards *P. vulgaricus.* On the other hand rambutan peel was active against all the bacteria except *Pseudomonas aeruginosa.* Quite a number of the fruits and especially rambutan peel showed good activity against *Candida lipolytica* while extracts from guava plants showed strong activity against *Saccharomyces cerevisiae* with an inhibition zone double that of the standard.

The most active extracts appeared to be from ripe starfruits, immature guava, rambai peel, and rambutan peel.

Guava extracts of all polarity were found to be active against bacteria and yeast tested, indicating that more than one component may be responsible for the observed antimicrobial activity. Past research findings indicate the presence of polyphenolic compounds in guava, quercetin, avicularin and guaijaverin (Seshadri and Vasista 1964) being the active antimicrobial components in guava leaf.

In general, the more polar extracts showed stronger antimicrobial activity. It is interesting to note that most gram-negative bacteria: *Pseudomonas, Proteus vulgaricus, Salmonelli typhi* and *E. coli* were inhibited by rambai skin extracts, ripe starfruit, guava, immature papaya and rambutan peel.

Bioassay guided isolation of the active antimicrobial components of starfruit, rambai skin and rambutan skin extracts will be carried out to identify the compounds responsible for these activities.

TABLE 1a
Antibacterial activity of the tested fruits and fruit leaves extracts on gram positive and gram negative bacteria after 24 h incubation at 30°C

| Plant / Bacteria | Average Diameter of Inhibition (mm) | | | | | | |
|---|---|---|---|---|---|---|---|
| | SA | BS | BC | LB | EC | PV | PA |
| **Ripe starfruit** | | | | | | | |
| Pet ether | - | - | - | - | - | - | - |
| $CHCl_3$ | **15** | 10 | 7 | 7 | - | - | - |
| Ethanol | **18** | 15 | **25** | 8 | 10 | 20 | 15 |
| **Immature starfruit** | | | | | | | |
| Pet ether | - | 10 | **18** | - | - | 20 | - |
| $CHCl_3$ | - | 10 | **18** | - | - | 20 | - |
| Ethanol | - | 20 | **30** | - | - | 15 | - |
| **Starfruit leaves** | | | | | | | |
| Pet ether | - | 10 | 12 | n.d. | 7 | - | - |
| $CHCl_3$ | - | 7 | - | n.d. | - | - | - |
| Ethanol | **10** | 18 | **18** | n.d. | - | 20 | - |
| **Ripe guava** | | | | | | | |
| Pet ether | **15** | 13 | 10 | 10 | - | - | - |
| $CHCl_3$ | **18** | 13 | 10 | 10 | - | - | - |
| Ethanol | **19** | 20 | 10 | 12 | - | 20 | 10 |
| **Immature guava** | | | | | | | |
| Pet ether | **25** | 18 | 15 | 15 | - | 25 | - |
| $CHCl_3$ | **25** | 20 | **20** | 10 | - | 20 | 10 |
| Ethanol | **20** | **25** | **35** | 15 | - | 28 | 10 |
| **Guava leaves** | | | | | | | |
| Pet ether | **20** | 20 | 15 | 15 | - | 20 | 15 |
| $CHCl_3$ | **20** | **25** | **20** | **18** | - | 20 | 17 |
| Ethanol | **19** | **25** | **30** | 15 | 10 | **30** | 15 |
| **Rambai** (*Baccaurea motleyana*) **peel** | | | | | | | |
| Pet ether | **12** | 15 | **18** | n.d. | 10 | 10 | - |
| $CHCl_3$ | **10** | 15 | 14 | n.d. | 9 | 7 | - |
| Ethanol | **20** | **25** | **35** | n.d. | **12** | **35** | 20 |
| **Standard** (streptomycin) | 10 | 25 | 18 | 18 | 12 | 30 | n.d. |

SA: *Staphylococcus aureus*
BS: *Bacillus subtilis*
BC: *Bacillus cereus*
LB: *Lactobacillus bulgaricus*
EC: *E. coli*
PV: *Proteus vulgaricus*
PA: *Pseudomonas aeruginosa*

TABLE 1b
Antibacterial activity of the tested fruits and fruit leaves extracts on gram positive and gram negative bacteria after 24 h incubation at 30°C

| Plant / Bacteria | Average Diameter of Inhibition (mm) | | | | | | |
|---|---|---|---|---|---|---|---|
| | SA | BS | BC | EC | PV | PA | ST |
| **Ripe Cavendish banana (Montel variety)** | | | | | | | |
| Pet ether | - | - | - | - | - | - | - |
| $CHCl_3$ | - | - | - | - | - | - | - |
| Ethanol | **15** | **27** | - | - | - | 26 | - |
| **Immature Cavendish banana (Montel variety)** | | | | | | | |
| Pet ether | - | - | - | - | - | - | - |
| $CHCl_3$ | - | - | - | - | - | - | - |
| Ethanol | 8 | 7 | 8 | 7 | - | 26 | 8 |
| **Ripe papaya** | | | | | | | |
| Pet ether | - | - | - | - | - | - | - |
| $CHCl_3$ | - | - | - | - | - | - | - |
| Ethanol | - | **28** | 13 | 10 | **21** | - | - |
| **Immature papaya** | | | | | | | |
| Pet ether | - | - | - | - | 7 | 7 | 7 |
| $CHCl_3$ | - | - | - | - | - | - | - |
| Ethanol | - | - | 7 | 8 | **24** | 26 | **26** |
| **Passionfruit peel** | | | | | | | |
| Pet ether | - | - | - | - | - | - | - |
| $CHCl_3$ | - | - | - | - | - | - | - |
| Ethanol | 8 | 12 | 14 | - | 8 | 8 | 7 |
| **Langsat (*Lansium domesticum*) peel** | | | | | | | |
| Pet ether | - | - | - | - | - | - | - |
| $CHCl_3$ | - | - | - | - | - | - | - |
| Ethanol | - | 7 | 7 | - | - | - | - |
| **Duku (*Lansium domesticum*) peel** | | | | | | | |
| Pet ether | - | - | - | - | - | - | - |
| $CHCl_3$ | 7 | 8 | 8 | 7 | - | - | - |
| Ethanol | - | - | 8 | 8 | - | - | - |
| **Rambutan (*Nephelium lappaceum*) peel** | | | | | | | |
| Pet ether | **11** | - | - | - | - | - | - |
| $CHCl_3$ | 8 | - | 7 | - | 8 | - | - |
| Ethanol | **27** | 14 | 15 | 15 | **15** | - | **26** |
| **Standard (streptomycin)** | **10** | **25** | **18** | **18** | **12** | **30** | **9** |

SA: *Staphylococcus aureus*
BS: *Bacillus subtilis*
BC: *Bacillus cereus*
EC: *E. coli*
PV: *Proteus vulgaricus*
PA: *Pseudomonas aeruginosa*
ST: *Salmonelli typhi*

TABLE 2a
Antimicrobial activity of the tested fruits and fruit leaves extracts on yeast and fungi after 24 h incubation at 30°C

| Plant / Bacteria | Average Diameter of Inhibition (mm) | | | | |
|---|---|---|---|---|---|
| | SC | CL | RS | AN | CS |
| **Ripe starfruit** | | | | | |
| Pet ether | - | - | - | - | - |
| $CHCl_3$ | | - | - | - | - |
| Ethanol | - | - | - | - | - |
| **Immature starfruit** | | | | | |
| Pet ether | - | - | - | - | - |
| $CHCl_3$ | - | - | - | - | - |
| Ethanol | - | - | - | - | - |
| **Starfruit leaves** | | | | | |
| Pet ether | - | - | - | - | - |
| $CHCl_3$ | - | - | - | - | - |
| Ethanol | - | - | - | - | - |
| **Ripe guava** | | | | | |
| Pet ether | - | - | - | - | - |
| $CHCl_3$ | **20** | - | - | - | - |
| Ethanol | **20** | - | - | - | - |
| **Immature guava** | | | | | |
| Pet ether | - | - | - | - | - |
| $CHCl_3$ | - | - | - | - | - |
| Ethanol | **20** | - | - | - | - |
| **Guava leaves** | | | | | |
| Pet ether | - | - | - | - | - |
| $CHCl_3$ | **15** | - | - | - | - |
| Ethanol | **15** | - | - | - | - |
| **Rambai *(Baccaurea motleyana)* peel** | | | | | |
| Pet ether | - | - | - | - | - |
| $CHCl_3$ | - | - | - | - | - |
| Ethanol | - | - | - | - | - |
| **Standard** (1µg Miconozole) | **10** | **25** | **18** | **18** | **n.d.** |

SC: *Saccharomyces cerevisiae* CL: *Candida lypolytica*
RS: *Rhizopus* spp. AN: *Aspergillus niger*
CS: *Chlamydomucor* spp.

TABLE 2b
Antimicrobial activity of the tested fruits and fruit peels extracts on yeast and fungi after 24 h incubation at 30°C

| Plant / Bacteria | Average Diameter of Inhibition (mm) | | | |
|---|---|---|---|---|
| | SC | CL | RS | AN |
| **Ripe Cavendish banana (Montel variety)** | | | | |
| Pet ether | - | - | - | - |
| $CHCl_3$ | - | - | - | - |
| Ethanol | - | 7 | - | - |
| **Immature Cavendish banana (Montel variety)** | | | | |
| Pet ether | - | - | - | - |
| $CHCl_3$ | - | - | - | - |
| Ethanol | - | 12 | - | - |
| **Ripe papaya** | | | | |
| Pet ether | - | - | - | - |
| $CHCl_3$ | - | 10 | - | - |
| Ethanol | - | 21 | - | - |
| **Immature papaya** | | | | |
| Pet ether | - | - | - | - |
| $CHCl_3$ | - | 10 | - | - |
| Ethanol | - | 17 | - | - |
| **Passionfruit peel** | | | | |
| Pet ether | - | 9 | - | - |
| $CHCl_3$ | - | 12 | 8 | - |
| Ethanol | - | 21 | - | - |
| **Langsat (*Lansium domesticum*) peel** | | | | |
| Pet ether | - | 7 | - | 10 |
| $CHCl_3$ | - | 9 | - | 11 |
| Ethanol | - | 10 | - | 10 |
| **Duku (*Lansium domesticum*) peel** | | | | |
| Pet ether | - | 15 | - | - |
| $CHCl_3$ | - | 9 | - | - |
| Ethanol | - | 18 | - | - |
| **Rambutan (*Nephelium lappaceum*) peel** | | | | |
| Pet ether | - | 10 | - | 11 |
| $CHCl_3$ | - | 12 | - | 12 |
| Ethanol | - | **30** | - | - |
| Standard (1µg Miconozole) | 10 | 25 | 18 | 18 |

SC: *Saccharomyces cerevisiae*
CL: *Candida lypolytica*
RS: *Rhizopus* spp.
AN: *Aspergillus niger*

TABLE 3
Minimum inhibitory concentration of the tested fruits and fruit leaves crude extracts after 24 h incubation at 30°C

| Strain | Minimum Inhibitory concentration (mg/ml medium) | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | RB | UB | RP | UP | PP | LP | DP | RS |
| *Staphylococcus aureus* | 2.5 | 2.5 | - | - | 2.5 | - | - | 0.5 |
| *E. coli* | - | 2.5 | 2.5 | 2.5 | - | - | 2.5 | 1.0 |
| *Bacillus subtilis* | 2.5 | 2.5 | 0.5 | - | 1.0 | 2.5 | - | 1.0 |
| *Bacillus cereus* | - | 2.5 | 1.0 | 2.5 | 1.0 | 2.5 | 2.5 | 1.0 |
| *Proteus vulgaricus* | - | - | 0.5 | 0.5 | 2.5 | - | - | 1.0 |
| *Pseudomonas aeruginosa* | 2.5 | - | - | 0.5 | 2.5 | - | - | - |
| *Salmonelli typhi* | - | 2.5 | - | - | 2.5 | - | - | 0.5 |
| *Rhizopus* spp. | - | - | - | - | - | - | - | - |
| *Aspergillus niger* | - | - | - | - | - | 2.5 | - | - |
| *Saccharomyces cerevisiae* | - | - | - | - | - | - | - | - |
| *Candida lypolytica* | 2.5 | 2.5 | 0.5 | 1.0 | 1.0 | 2.5 | 2.5 | 0.5 |

| | (g crude* extract/100g dried sample) |
|---|---|
| RB: Ripe banana (Cavendish, Montel variety) | 61.54 |
| UB: Unripe banana | 15.22 |
| RP: Ripe papaya | 36.40 |
| UP: Unripe papaya | 55.39 |
| PP: Passionfruit peel | 15.66 |
| LP: Langsat peel | 23.57 |
| DP: Duku peel | 23.30 |
| RS: Rambutan skin/Peel | 39.51 |

* Crude extracts obtained by extraction with ethanol and evaporating off the solvent.

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# Feeding Time and Botanical Composition of Diets Selected by Indigenous Goats on Native Pastures in Malaysia

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**Keywords: goat, range grazing, feeding time, botanical composition, grazing behaviour, diet selectivity**

## ABSTRAK

*Masa makan, tabiat pemakanan dan komposisi botanikal diet yang dipilih oleh Kambing Kacang di padang rumput yang berbeza di Selangor, Malaysia Barat ditentukan melalui pengawasan terus. Di antara bulan Nov-Dis 1992, empat kelompok kambing dipilih mengikut kawasan mereka meragut (sawah yang terbiar, di tepi-tepi jalan, tempat pembuangan sampah dan dusun) dan diawasi. Bergantung kepada kelompok, kedudukan kawasan ragut dan kemudahan mendapat makanan, kambing-kambing bergerak sejauh 0.6 hingga 5.6km sehari. Masa yang dihabiskan untuk makan adalah antara 79.2 hingga 152.7 minit. Kualiti diet yang paling tinggi diperolehi dengan memilih bahagian pokok yang paling muda dahulu. Dari segi masa yang dihabiskan untuk setiap spesis, spesis utama yang dipilih ialah* Mimosa pudica, *diikuti dengan* Ottochloa nodosa, Asystasia intrusa, Mikania cordata, Paspalum conjugatum, Axonopus compressus *dan* Ischaemum timorense. *Komposisi tumbuhan di empat kawasan ragut dinilai dengan menggunakan plot-plot wakilan. Tumbuh-tumbuhan memenuhi 100% kawasan yang disampel kecuali dikawasan pembuangan sampah (51%). Daripada rekod pemilihan makanan dan kesediaan tumbuhan, keutamaan indeks dikira untuk tiap-tiap kelompok dan spesis tumbuhan. Tumbuhan herba adalah yang paling digemari dan* Mimosa pudica *menjadi pilihan utama diantara tumbuhan kekacang. Berdasarkan kepada masa meragut, kambing-kambing ternyata tidak dapat menampung keperluan bahan kering mereka. Strategi tambahan berdasarkan pemilihan diet dan sumber makanan yang ada harus dibuat untuk meningkatkan produktiviti mereka.*

## ABSTRACT

*The feeding time, grazing behaviour and botanical composition of diets selected by the indigenous Kambing Kacang on different native pastures of Selangor State, West Malaysia, were determined by direct observation during Nov - Dec 1993. Four herds were selected according to their grazing area (abandoned ricefield, roadside verges, waste ground and orchard) and monitored. Grazing animals travelled from 0.6 to 5.6 km daily. Depending on the locality of the grazing area and the forage available, time spent feeding ranged from 79.2 to 152.7 min. The herds were observed to first select the most juvenile plant parts which provided the highest quality of diet. In terms of time spent on each species, the main species selected in descending order of importance were:* Mimosa pudica, Ottochloa nodosa, Asystasia intrusa, Mikania cordata, Paspalum conjugatum, Axonopus compressus *and* Ischaemum timorense. *The vegetation composition of the four grazing areas was assessed by means of representative plots. Vegetation covered 100% of the total sample area except on the waste ground (51%). From records of feed selection and vegetation available, preference indices were calculated for groups and*

*individual plant species. Herbs appeared to be very palatable, with* Mimosa pudica *among legumes by far the most palatable. Judging by the feeding time, it would appear that the goats could not adequately meet their dry matter demands. Supplementation strategies based on diet selectivity and available resources may have to be developed to increase their productivity.*

## INTRODUCTION

In Peninsular Malaysia the majority of ruminants rely on natural vegetation. Presently there are about 288,500 goats including the indigenous Kambing Kacang mostly reared by smallholder farmers. Despite some increase in productivity, goat and sheep production cannot keep up with the increasing local demand for mutton. Production efficiency of goats under extensive and semi-extensive management systems could be improved with better knowledge of their grazing behaviour.

The quantity of forage ingested by range grazing animals is controlled by animal and plant factors, their interaction, and management strategies. Important factors are duration of grazing, rate of biting, bite size, herbage availability and quality, and ease of prehension and removal of herbage from the plant. To what extent goats exploit available resources is important since the production and yield of these animals depend on their voluntary intake of digestible dry matter (Humphreys 1991). Different methods have been used to measure feed intake. Most common techniques include the use of markers, fistulated animals, faecal index techniques and the direct observation technique (Malechek and Provenza 1983; Meuret *et al.* 1985). The last mentioned technique has the following advantages: it can be used on heterogeneous pastures; measurements are easy and fast; and it does not require laboratory analyses. The botanical composition of the diet can be studied through direct observation by measuring the time spent feeding on each species. This indicates the proportion of species in bites and time, but not in DM.

The purpose of the present study was to assess the feeding time, grazing behaviour and dietary preferences of the indigenous Kambing Kacang on various types of native pastures.

## MATERIALS AND METHODS

### *Site of Investigation*

Four representative smallholdings were selected according to the vegetation types in the grazing area. These farms were located in Beranang (3) and Ulu Langat (1), Selangor State. Different vegetation types occurred: (i) The abandoned ricefield consisted mainly of grasses, cyperus and legumes. This grazing area was very rich in plant species. (ii) The roadside under study consisted mainly of grasses and shrubs, the former being mown frequently. (iii) The waste ground was an old tin mining area characterised by a large variety of vegetation types: vast scarcely covered sandplains, grassland, brushwood, pondside vegetation, etc. and (iv) The grazing area in Ulu Langat was a traditional Malay orchard, consisting mainly of *Musa* cv., *Artocarpus heterophyllus, Artocarpus integer, Nephelium lappaceum* and *Durio zibethinus.* The vegetation was homogeneous and disturbed. Relatively smaller groups of plants but dominating communities were found under such multi-strata canopies. Herbaceous weeds such as the shade-tolerant invasive *Asystasia intrusa* was one of the typical examples of native forage under orchard.

Selangor State, Malaysia, is located in the humid tropics in the agro-ecological zone with a 1-month or no prominent dry season (Nieuwolt *et al.* 1982). The mean temperature during the observation period was 27° C with a mean daily sunshine duration of 5 h. During November and December 273 mm and 348 mm of rain were recorded respectively.

### *Animals*

Herds under study comprised between 30 and 50 goats and were kept under semi-extensive management. The goats were of the local Kambing Kacang type and crossbreds. In each farm, five animals were selected for growth and grazing observation monitoring, and identified by ear tags. Young goats, 4 - 6 months old, were chosen to prevent interference of reproductive status with growth, and to avoid loss of information if the animals were sold or slaughtered during the period of observations. The goats had an average initial weight of 8.2 kg. They were weighed fortnightly over a period of 141 days to assess the growth rate. To minimise the effect of gastro-intestinal parasitism on their health status, body condition, and grazing behaviour, all ani-

mals were given systematic anthelmintic treatments (1 ml Supaverm/kg BW) two weeks before the beginning of the experiment and three months later.

Attention had to be paid to the observations made at the roadside, because the young goats tended to stay close to the shed and did not graze together with the adult ones.

In accordance with the management practice goats were allowed to graze in the late afternoon for about 2 - 4 h. Grazing without supervision was most common. In all herds kitchen salt was provided in the shed. Cut-and-carry was provided especially for kids and sick animals, after the herd had been taken to the grazing area. This consisted mainly of *Mikania cordata, Panicum amplexicaule, Nephrolepis biserrata* and *Manihot esculenta* leaves (abandoned ricefield), and of *Elaeis guineensis* leaves (waste ground). The herd grazing under orchard was irregularly supplemented with old banana peelings (*Musa* cv.). at irregular intervals.

*Experimental Procedures*

The study was carried out from November 1993 to April 1994. Sites where the farmers frequently take their goats were selected. A preliminary study of the pasture was carried out by inventorying the plant species. For easy identification, a reference collection of herbarium specimens was made before the experiment started.

After the observation period (Nov - Dec 1993) vegetation was assessed at the four grazing areas. Each grazing area was studied by dividing it into different vegetation types and measuring their respective proportions within the grazing area. Each vegetation type was analysed by means of representative plots (Knapp 1984). Vegetation composition was assessed by recording the cover using the decimal scale of Londo (1984). Vegetation at the waste ground characterised by sandy soils, was changing fast during the drier month (Jan 1994) following the observation period. Therefore, information on plant availability had to be gathered as fast as possible and the approach of Tansley (1939) was used in combination with the Londo-scale. At the other grazing areas changes due to the short drier period were not noticed.

TABLE 1
Cover of group of plants at different native pastures (%)

| Vegetation | Abandoned ricefield | Roadside | Waste ground | Orchard |
|---|---|---|---|---|
| Grasses | 76.7 | 91.4 | 16.3 | 20.6 |
| Legumes | 15.3 | 3.1 | 8.3 | 8.6 |
| Herbs | 10.9 | 6.2 | 9.8 | 46.3 |
| Others | 26.4 | 66.9 | 16.7 | 28.9 |

Feed preference studies were carried out using the direct observation technique. The same herd was observed for five consecutive days. Because herds split into different sub-flocks, it was not possible to observe all the animals. Hence one goat was marked and followed throughout its grazing journey across the rangeland. Successively, every other day, another animal was observed. Two observers recorded the grazing time (i.e. the total time spent in the range) and distance travelled (Lu 1988; Ricardi and Shimada 1992) by means of a pedometer. The plants and the time (rounded to 5 s) spent feeding on a particular plant species were recorded for each animal during its grazing journey. Total observation time was 54 h for the four herds.The information was tabulated and the plant species were divided into four groups: grasses, legumes, herbs and others. Data were subjected to analyses of variance using procedures of SAS (1987).

Even in entirely mixed pastures this method could be used because goats tend to take discernible individual bites and rarely go from one plant species to another within a few seconds. The closer the observers could get to the animal the better, enabling the animals to become accustomed to the observers (Meuret *et al.* 1985; Guerin 1988), which was achieved by the observer following the same animal throughout the day. During the experiment, animals could be observed from 1 to 2 m distance without influencing their grazing behaviour. The botanical composition of the diets needs to be related to available plant species in the pasture. From the records of feed selection and plant availability, preference indices were calculated for plant species and plant groups. The preference factor (PF) or index was calculated as:

$$PF = \frac{\text{plant consumed}}{\text{plant available}}$$

where plant consumed is the feeding time on respective vegetation expressed as a percentage of the total feeding time; and plant availability is the percentage cover of plant material not exceeding a height of 2 m (Becker and Lohrmann 1992). On the basis of preference indices plant species and groups were ranked in palatability classes (Table 2).

TABLE 2
Palatability based on the preference index

| Palatability | Preference Index |
|---|---|
| Highly palatable | > 5.00 |
| Very palatable | 1.51 - 5.00 |
| Palatable | 0.51 - 1.50 |
| Not palatable | 0.00 - 0.50 |

## RESULTS AND DISCUSSION

The average daily weight gain (ADWG) was calculated for each herd (Table 3). Animals grazing on the waste ground gained least weight (20.2 g/day) and those grazing on the abandoned ricefield the most (31.6 g/day). However, no statistical differences were found. Experiments with feeding Kambing Kacang goats under optimal conditions show an ADWG of 60 g/day for animals of the same age (4 - 6 months) weighed over the same period of time (M. P. Davis 1994, *Personal communication*).

The grazing time was different for the four herds (Table 4) depending on the management. Herds were brought to the grazing area in the late afternoon (14.30 h) or after heavy rains, even later. They were always brought back to the shed before darkness. The longer the period the goats were allowed to graze, the greater distance they travelled. Energy expended during grazing can account for a significant part of the total energy requirements of the goat. Huston (1978) suggested that goats travel greater distances than other ruminant species.

TABLE 3
Average daily weight gain (ADWG) and standard error (SE) of 4 - 6 months old animals weighed over a period of 141 days

| Vegetation | Abandoned ricefield | Roadside | Waste ground | Orchard | Average |
|---|---|---|---|---|---|
| ADWG | 31.5 | 27.3 | 20.2 | 24.6 | 25.6 |
| SE | 5.8 | 7.7 | 5.4 | 4.9 | 3.2 |

TABLE 4
Average and standard error of distance travelled (km per day), time grazing i.e. time spent in the range (min per day), feeding time on plants (min per day) and as percentage of the grazing time

| Vegetation | Abandoned ricefield | Roadside | Waste ground | Orchard |
|---|---|---|---|---|
| Distance (km) | 2.8 ± 0.3b | 0.6 ± 0.1c | 5.6 ± 0.6a | 1.6 ± 0.3c |
| Grazing time (min) | 190.0 ± 6.5b | 105.0 ± 5.0c | 245.0 ± 16.7a | 115.0 ± 8.7c |
| Feeding time (min) | 152.7 ± 3.8a | 79.2 ± 3.4c | 119.8 ± 7.5b | 92.0 ± 7.3c |
| Feeding time (%) | 80.4 | 75.4 | 48.9 | 80.0 |

Values within rows with similar superscripts are not stastistically different ($P < 0.05$).

Judging by the time spent on feeding, it would appear that the goats could not adequately meet their dry matter demands, which partially explains their rather low productivity. In all the pastures, except on the waste ground (48.9%), the goats spent 75.4 - 80.4% of their time feeding to ensure a sufficient forage intake. On waste ground vegetation was less abundant and the goats had to walk longer distances. This resulted in a relatively shorter feeding time. Animals were seldom seen to rest on the ground during the grazing period. Only in heavy rain did they seek shelter under trees or shrubs.

Vegetation covered 100% of the total sample area exept on the waste ground (51%). In the other cases the sum total of the cover of groups of plants exceeded 100%, which reflects the overlapping of the pasture (Table 1)

Different plant groups were selected according to the pasture vegetation (Table 5). This emphasizes the mixed nature of the feeding habits of goats.

The herd grazing under orchard had a significantly larger proportion of grazing time on dicotyledonous weeds (of higher nutrient content) than on grasses (of lower nutrient value) compared to the herd grazing along the roadside. However, no significant difference in growth between the herds was observed. Probably the overall low grazing time, which was less than half to two-thirds of normal grazing hours, imposed daily on the animals, was a critical constraint on their performance.

Dicotyledonous weeds were preferred to grasses in all cases except along the roadside, where palatability of plants in general was low (Table 6). On the waste ground, grasses had a very low preference index. Most of the grasses grew along well-established tracks. Here treading as well as excreta are important contributory factors to unpalatability. The general low palatability of the group 'others' is due to the occurrence of non-palatable species, comprising mainly *Eupatorium odoratum, Melastoma malabathricum* and *Lantana aculeata.* At the orchard, legumes had a low preference index. Seemingly glabrous leaves of other species were preferred to the hairy leaves of *Pueraria phaseoloides* and *Calopogonium mucunoides.*

TABLE 5
Average and standard error of time feeding on groups of plants at four different native pastures (min per day)

| Vegetation | Abandoned ricefield | Roadside | Waste ground | Orchard |
|---|---|---|---|---|
| Grasses | $69.5 \pm 2.6^a$ | $67.1 \pm 2.5^a$ | $2.0 \pm 0.4^c$ | $12.1 \pm 2.3^b$ |
| Legumes | $31.9 \pm 2.2^b$ | $0.0 \pm 0.0^c$ | $83.9 \pm 6.6^a$ | $3.0 \pm 0.3^c$ |
| Herbs | $40.5 \pm 1.8^a$ | $1.5 \pm 0.4^c$ | $32.6 \pm 3.0^b$ | $44.8 \pm 3.0^a$ |
| Others | $10.8 \pm 1.5^b$ | $10.6 \pm 1.7^b$ | $1.3 \pm 0.2^c$ | $32.1 \pm 3.5^a$ |

Values within rows with similar superscripts are not stastistically different ($P < 0.05$).

TABLE 6
Preference index and palatability of plant groups at four different native pastures

| Vegetation | Abandoned ricefield | Roadside | Waste ground | Orchard |
|---|---|---|---|---|
| Grasses | 0.59* | 0.93* | 0.10* | 0.55* |
| Legumes | 1.36* | — | 8.45*** | 0.40* |
| Herbs | 2.43** | 0.31* | 2.78** | 1.05* |
| Others | 0.27* | 0.20* | 0.06* | 1.21* |

Not palatable, * palatable, ** very palatable, *** highly palatable

Table 7 summarizes cover and relative feeding time on individual species. On the abandoned ricefield, roadside, waste ground and orchard there were 23, 11, 14 and 15 different species respectively grazed by the goats. Although they consumed a large variety of species, in terms of time spent, most of their diet consisted of just 8, 4, 2 and 6 species. These results are consistent with previous findings by Murken and Mukherjee (1988) and Peinado-Lucena *et al.* (1992) who stated that although goats may consume a large variety of species, most of their diet consists of 3 - 8 main species. *Mimosa pudica*, which contains the toxic agent mimosine (Hickey and King 1988), was highly selected by the goats. Species diversity of the pasture is an important factor as it allows grazing animals to select a greater variety of plant species. A mixed diet is not only better balanced but also prevents the animal's detoxification mechanism encountering a large dose of one single toxin (Moss 1991).

TABLE 7
Cover (C) and relative feeding time (FT) for different plant species

| Vegetation | Abandoned ricefield | | Roadside | | Waste ground | | Orchard | |
|---|---|---|---|---|---|---|---|---|
| | C (%) | FT (%) | C (%) | FT (%) | C (%) | FT (%) | C (%) | FT (%) |
| *Mimosa pudica* | 10.4 | 20.2 | — | — | 7.1 | 68.8 | — | — |
| *Ottochloa nodosa* | 2.6 | 1.6 | 38.0 | 34.7 | 1.2 | 1.1 | 13.1 | 9.3 |
| *Asystasia intrusa* | 0.3 | 0.2 | — | — | — | — | 36.6 | 43.3 |
| *Mikania cordata* | 4.7 | 13.4 | — | — | 4.9 | 21.5 | 1.9 | 5.2 |
| *Paspalum conjugatum* | 8.2 | 12.0 | 19.0 | 20.2 | — | — | 5.8 | 3.8 |
| *Axonopus compressus* | 8.2 | 3.5 | 22.9 | 24.4 | 2.2 | — | 1.9 | 1.7 |
| *Ischaemum timorense* | 23.2 | 16.4 | — | — | — | — | — | — |
| *Musa* cv. | — | 1.3 | — | — | — | — | 0.9 | 10.8 |
| *Heliconia hybrid* | — | — | — | — | — | — | 1.3 | 8.6 |
| *Borreria latifolia* | — | — | — | — | 1.4 | 4.0 | 5.7 | 5.5 |
| *Leersia hexandra* | 1.8 | 7.3 | — | — | — | — | — | — |
| *Ficus* spp. | — | 0.7 | 5.7 | 6.3 | — | — | — | — |
| *Imperata cylindrica* | 5.7 | 2.5 | 6.8 | 2.7 | 4.8 | 0.6 | — | — |
| *Cyperus* spp. | 5.2 | 2.5 | — | — | 0.9 | 0.4 | 4.8 | 2.5 |
| *Commelina nudiflora* | 1.8 | 5.2 | — | — | — | — | — | — |
| *Ipomoea aquatica* | — | 3.8 | — | — | — | — | — | — |
| *Pueraria phaseoloides* | — | — | — | — | — | — | 8.1 | 3.6 |
| *Caryota mitis* | — | — | 1.1 | 3.5 | — | — | — | — |
| *Aneilema nudiflorum* | 2.0 | 2.8 | — | — | — | — | — | — |
| *Saccharum arundinaceum* | — | — | 2.0 | 2.7 | — | — | — | — |
| Fern (undetermined) | — | 1.2 | — | 1.3 | — | — | — | — |
| *Merremia umbellata* | — | — | — | — | 0.6 | 1.3 | 2.2 | 1.0 |
| *Panicum amplexicaule* | 0.9 | 2.2 | — | — | — | — | — | — |
| *Melastoma malabathricum* | — | — | 3.0 | 1.0 | 3.5 | 0.2 | 4.2 | 1.0 |
| *Borreria laevicaulis* | — | — | 1.6 | 1.9 | — | — | — | — |
| *Nephelium lappaceum* | — | — | — | — | — | — | 0.8 | 1.8 |
| *Stachytarpheta jamaicensis* | — | — | 2.4 | 1.3 | — | — | — | — |
| *Limnocharis flava* | — | 1.1 | — | — | — | — | — | — |
| *Durio zibethinus* | — | — | — | — | — | — | — | 1.0 |
| *Lygodium circinnatum* | — | — | — | — | — | — | 0.8 | 1.0 |
| *Centrosema pubescens* | — | — | — | — | 0.6 | 0.8 | — | — |
| *Cassia tora* | — | 0.7 | — | — | — | — | — | — |
| *Manihot esculentum* | — | 0.7 | — | — | — | — | — | — |
| *Mimosa invisa* | — | — | — | — | 0.3 | 0.4 | — | — |
| *Lygodium flexuosum* | 0.1 | 0.2 | — | — | — | 0.1 | — | — |
| *Melochia corchorifolia* | — | — | — | — | — | 0.3 | — | — |
| *Lantana aculeata* | — | — | 10.0 | — | 1.6 | 0.3 | — | — |
| *Sida rhombifolia* | — | 0.3 | — | — | — | — | — | — |
| *Phyllanthus niruri* | — | — | — | — | — | 0.2 | — | — |
| *Urena lobata* | 0.7 | 0.2 | — | — | 0.5 | — | — | — |

– Not present or only found sporadically

TABLE 8
Preference index and palatability for individual plant species

| Vegetation | Abandoned ricefield | Roadside | Waste ground | Orchard |
|---|---|---|---|---|
| *Mimosa pudica* | 1.95** | — | 9.70*** | — |
| *Ottochloa nodosa* | 0.62* | 0.91* | 0.90* | 0.67* |
| *Asystasia intrusa* | 0.77* | — | — | 1.18* |
| *Mikania cordata* | 2.87** | — | 4.40** | 2.74** |
| *Paspalum conjugatum* | 1.46* | 1.06* | — | 0.66* |
| *Axonopus compressus* | 0.43* | 1.07* | — | 0.88* |
| *Ischaemum timorense* | 0.71* | — | — | — |
| *Musa* cv. | — | — | — | 12.1*** |
| *Heliconia hybrid* | — | — | — | 6.83*** |
| *Borreria latifolia* | — | — | 2.90** | 0.96* |
| *Leersia hexandra* | 3.96** | — | — | — |
| *Ficus* spp. | — | 1.11* | — | — |
| *Imperata cylindrica* | 0.44* | 0.39* | 0.13* | — |
| *Cyperus* spp. | 0.48* | — | 0.46* | 0.52* |
| *Commelina nudiflora* | 2.92** | — | — | — |
| *Pueraria phaseoloides* | — | — | — | 0.44* |
| *Caryota mitis* | — | 3.27*** | — | — |
| *Aneilema nudiflorum* | 1.41* | — | — | — |
| *Saccharum arundinaceum* | — | 1.35* | — | — |
| *Merremia umbellata* | — | — | 2.06** | 0.46* |
| *Panicum amplexicaule* | 2.39** | — | — | — |
| *Melastoma malabathricum* | — | 0.33* | 0.06* | 0.24* |
| *Borreria laevicaulis* | — | 1.17* | — | — |
| *Nephelium lappaceum* | — | — | — | 2.14** |
| *Stachytarpheta jamaicensis* | — | 0.54* | — | — |
| *Lygodium circinnatum* | — | — | — | 1.28* |
| *Centrosema pubescens* | — | — | 1.31* | — |
| *Mimosa invisa* | — | — | 1.25* | — |
| *Lygodium flexuosum* | 2.00** | — | — | — |
| *Lantana aculeata* | — | — | 0.18* | — |
| *Urena lobata* | 0.27* | — | — | — |

Not present or only found sporadically

* Not palatable, * palatable, ** very palatable, *** highly palatable

The main species selected were *Mimosa pudica, Ottochloa nodosa, Asystasia intrusa, Mikania cordata, Paspalum conjugatum, Axonopus compressus* and *Ischaemum timorense.*

Observations showed that the goats first selected the growing points which are the most juvenile and nutritious portion of the plant. Then they searched for the younger leaves, followed by more mature ones. In this way the highest quality diet could be selected from the pasture.

From the recordings of cover and relative feeding time, preference indices were calculated and species were ranked in palatability classes (Table 8). Banana leaves (*Musa cv.*), leaves from the Crab-claw (*Heliconia hybrid*) and leaves from the legume *Mimosa pudica* were highly palatable. The climber *Mikania cordata*, the herbs *Borreria latifolia* and *Commelina nudiflora*, leaves from the palm *Caryota mitis*, the rambutan tree (*Nephelium lappaceum*), and the fern *Lygodium flexuosum* were very palatable. The swamp grasses *Leersia hexandra* and *Panicum amplexicaule* had a higher palatability than the other grasses. Attention has to be paid in that palatability is also determined by the structure of the vegetation, i.e. accessability of plant species. The twining herb *Merremia umbellata* was very palatable at the waste ground, where vegetation was less dense, while it was found unpalatable at the orchard, where it was overgrown by other species. A study of the

nutrient content and voluntary intake, which is determined by feeding time, bite frequency and bite size, has been addressed in a related work in progress.

## CONCLUSIONS

In all the pastures, except on the waste ground (48.9%), the goats spent 75.4 - 80.4% of their time feeding to compensate for the short duration of grazing.

Results of this experiment have shown that goats consumed grasses readily only on pastures where their availability was high. Compared to grasses, herbs with a higher nutrient content were generally highly selected. The climbing herb *Mikania cordata* was very palatable. Among the legumes only *Mimosa pudica* was highly palatable. The leguminous cover crops *Pueraria phaseoloides* and *Centrosema pubescens* were less palatable. Banana leaves (*Musa* cv.) and leaves from the rambutan tree (*Nephelium lappaceum*) were highly to very palatable. Some species which were abundant in the pasture were not found in the diet, and the reverse situation occurred with some species of limited availability. The number of unpalatable species is a good indication of the pasture quality. This nature of selectivity in grazing by goats suggests that the number of plant species in pastures should be as high as possible so as offer to goats different levels of nutrition from herbage.

Preference indices calculated from records of feed selection and availability of vegetation were good indicators for palatability of plant species in general. However, in some cases pasture structure interfered in such a way that accessibility was more important than availability. This suggests that studies of comparitive palatability should be evaluated carefully when other vegetation types are assessed.

Poor animal performance indicates that the problem of increasing goat production could best be solved by increasing the grazing time and/or by supplementing the animals' diet especially in areas where goats depend mainly on grasses or where vegetation is less dense. Based on dietary selectivity and available resources, supplementation strategies on dietary needs could be developed to increase productivity.

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# Experimental Respiratory Infection of Goats with *Mycoplasma arginini* and *Pasteurella haemolytica* A2

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## ABSTRAK

*Dua puluh satu ekor kambing baka tempatan yang berumur kira-kira lapan bulan telah dibahagikan kepada empat kumpulan yang terdiri daripada enam ekor dalam setiap kumpulan 1, 2 dan 3, dan tiga ekor dalam kumpulan 4. Kambing dalam kumpulan 1 dan 2 telah dijangkiti dengan* Mycoplasma arginini *secara intra-trakea sebelum kambing dalam kumpulan 2 disuntik* Pasteurella haemolytica *A2 enam hari kemudian. Kambing dalam kumpulan 3 disuntik dengan* P. haemolytica *sahaja secara intra-trakea sementara kambing dalam kumpulan 4 disuntik dengan PBS. Kambing dibunuh pada hari ke 1, 3 dan 7 selepas suntikan* P. haemolytica. *Empat ekor kambing dalam kumpulan 1 dan tiga ekor kambing dalam kumpulan 3 menunjukkan tompok-tompok lesi pneumonia yang kurang teruk. Kambing dalam kumpulan 2 menunjukkan lesi pneumonia pasteurelosis yang teruk di bahagian antero-ventral peparu. Tidak seekor pun kambing dalam kumpulan 4 menunjukkan lesi pulmonari.* P. haemolytica *telah berjaya diasingkan daripada kesemua kambing dalam kumpulan 2 dan daripada tiga ekor kambing dalam kumpulan 3. Walau bagaimanapun,* M. arginini *gagal diasingkan.*

## ABSTRACT

*Twenty-one healthy local goats of about eight months old were divided into four groups consisting of six animals in groups 1, 2 and 3 and three animals in group 4. Goats in groups 1 and 2 were inoculated intratracheally with* Mycoplasma arginini. *Goats in group 2 were inoculated again with* Pasteurella haemolytica *A2 six days later. Goats in group 3 were inoculated intratracheally with* P. haemolytica *A2 alone while goats in group 4 received intratracheal inoculation of PBS. The goats were euthanised at day 1, 3 and 7 post inoculation with* P. haemolytica. *Four goats in group 1 and three goats in group 3 had small patches of mild pneumonic lesions. Goats in group 2 had severe lung lesions typical of pneumonic pasteurellosis at the anteroventral region of the lungs. None of the goats in group 4 had pulmonary lesions.* P. haemolytica *was reisolated from all goats in group 2 and from three goats in group 3 but* M. arginini *was not reisolated.*

## INTRODUCTION

Pneumonic pasteurellosis caused by *Pasteurella haemolytica* has been recognised as one of the most common diseases of sheep and goats (Gilmour 1980). The disease develops following various stresses on animals, and viral infection in the respiratory tract prior to the infection by *P. haemolytica* has been shown as one of the important causal factors (Davies *et al.* 1981; Buddle *et al.* 1990). While some species of *mycoplasma* have been recognised as primary pathogens of pneumonia in animals, the role of other species in causing lung disease of animals is still unknown (Jones 1983).

*Mycoplasma arginini* has been isolated from goats that died of pneumonic pasteurellosis in Malaysia, but the significance of this organism in this disease is uncertain (Sheikh-Omar and

Mutalib 1985). The present investigation was designed to study the role of *M. arginini* in the development of pneumonia either alone or in combination with *P. haemolytica* in healthy goats.

## MATERIALS AND METHODS

### *Animals*

Twenty-one clinically healthy local goats of about 8 months old were selected for the study. They were divided into four groups; six goats each in groups 1, 2 and 3 and three goats in group 4. The groups were kept in separate rooms and fed daily with cut grass. Drinking water was available *ad libitum*. All goats were healthy; neither *P. haemolytica* nor *M. arginini* were isolated from the nasal cavities for a period of 4 weeks prior to the experimental infection.

### *Inocula*

The *M. arginini* strain used was isolated earlier from the lungs of a goat that had died of severe fibrinous pneumonia (Sheikh-Omar and Mutalib 1985). The organism had been cloned five times. To prepare the inoculum for each goat, 10 colonies of *M. arginini* were cultured in 1 ml mycoplasma broth before they were incubated in carbon dioxide for 7 d.

The *P. haemolytica* used for the experimental inoculation in this study had been isolated from the pneumonic lung of a goat, and was identified as *P. haemolytica* serotype A2. Prior to the inoculation, the organism had been grown in infusion broth and later diluted to 108 colony forming unit (cfu) per ml.

### *Experimental Design*

All goats in groups 1 and 2 were inoculated with 1 ml of the prepared suspension of *M. arginini* intratracheally by inserting a hypodermic needle between the tracheal rings and injecting the inoculum with a syringe. Six days later, all six goats in group 2 were reinoculated intratracheally with 1 ml suspension of *P. haemolytica* A2 according to the method described earlier for *M. arginini*. The goats in group 3 were inoculated intratracheally with *P. haemolytica* alone while goats in group 4 were the control receiving phosphate-buffered saline (PBS).

The body temperature and clinical signs attributable to pneumonia were recorded daily. Two goats each from groups 1, 2 and 3 and one goat from group 4 were euthanised with saturated magnesium sulphate overdose at days 1, 3 and 7 post inoculation (p.i.) with *P. haemolytica*. Post mortem examination was carried out immediately with a detailed examination on the respiratory tract. Samples of the trachea and the lungs were collected for bacterial and mycoplasmal isolation and for histological examination.

### *Sample Processing*

Isolations of *P. haemolytica* from tissue samples were made by inoculation onto 5 per cent blood and McConkey agars, incubated at 37° C for 24 h before the colonies suspected of being *P. haemolytica* were reinoculated onto blood agar and later identified (Lenette *et al.* 1974).

Mycoplasmal isolations were performed essentially as described by Sheikh-Omar and Mutalib (1985). Samples were cultured on PPLO agar base plus mycoplasma supplement-G (Oxoid). The agar plates were incubated in carbon dioxide and examined daily for the typical 'fried egg' colonies. Samples for histological examination were fixed in 10% buffered formalin for at least 24 h embedded in paraffin wax, sectioned at between 4 and 6 um and stained with haematoxylin and eosin.

## RESULTS

### *Clinical Observations*

All goats were healthy at the time of inoculation, but six goats were coughing for a few seconds during the intratracheal administration of the inoculum. None of the goats in groups 1, 3 and 4 had high body temperature or clinical signs of respiratory tract infection. Four goats in group 2 were found to be depressed and inactive as early as 24 h following inoculation of *P. haemolytica* A2. After day 3 p.i., the goats were found to have mucoid nasal discharges. The body temperature of all goats, however, remained normal throughout the study period.

### *Pathology*

#### *Group 1*

The lungs of goats killed at day 1 p.i. were slightly congested and moderately oedematous. Four to five patches of dark red atelectatic and pneumonic areas of about 0.5 to 2 cm were observed particularly in the lung parenchyma near the terminal bronchi and major bronchioles. These lesions were observed mostly at the anteroventral region of the lungs. Histologically, there was moderate

bronchiolitis consisting of accumulation of a mixture of mononuclear cells and neutrophils in the subepithelial layer. The bronchiolar associated lymphoid tissue (BALT) was slightly hyperplastic and oedema fluid was found in most alveoli. The interalveolar septa were thickened due to hyperaemia and the presence of neutrophils and mononuclear cells.

Grossly, the distribution and size of pulmonary lesions observed in goats killed at day 3 p.i. were similar to those observed earlier at day 1 p.i. but the histological changes were less severe. There was bronchiolitis with marked BALT hyperplasia but the oedema fluid was not obvious and the interalveolar septa were not markedly thickened. Neutrophils were absent but alveolar macrophages were obvious in several areas.

At day 7 p.i. the gross pulmonary lesions were less extensive. Bronchiolitis and BALT hyperplasia were absent in most areas. Changes in the interalveolar septa were similar but milder than those observed at day 3 p.i.

*Group 2*

The lungs of goats killed at day 1 p.i. had lesions typical of pneumonic pasteurellosis, mostly involving similar lung areas as goats in group 1 (the anteroventral part of the right lung). The lesions that appeared dark red and firm were found to vary in size between 1 and 3 cm. The lungs were moderately oedematous and the distal trachea, bronchi and major bronchioles were markedly congested. Histologically, there was moderate bronchiolitis with mononuclear cells infiltration in the subepithelial layer. Interalveolar septa surrounding the affected bronchioles were slightly thickened due to congestion and presence of neutrophils and oedema fluid.

The ventral two-thirds of the right apical and intermediate lobes of the lungs of both goats killed at day 3 p.i. were dark red and firm. Mucoid exudate was observed on the cut surface of the affected lung parenchyma and from the lumen of bronchioles. The distal trachea, bronchi and major bronchioles were moderately congested. Most alveoli of the affected areas were congested and their spaces filled with oedema fluid and numerous neutrophils. Most bronchioles showed moderate bronchiolitis with similar neutrophilic exudate in the lumen.

The gross pulmonary lesions were most extensive in goats killed at day 7 p.i. The lesions involved the whole anterior one-third of the right lung and the ventral two-thirds of the apical lobes of the left lung; one goat had the right lung partially adhered to the thoracic wall. The cut surface of the lesions showed patches of pale necrotic areas of about 1 to 3 mm. Most affected alveoli were filled with a mixture of numerous alveolar macrophages, neutrophils and fibrin. Some interlobular septa were thickened due to the presence of the exudate. A similar exudate was observed in the lumen of many bronchioles.

*Group 3*

The lungs of goats in group 3 showed gross lesions similar to those of goats in group 1. There were patches of dark red discolouration in the anteroventral areas of the lungs. Histologically, these lesions consisted of slightly thickened inter-alveolar septa with moderate accumulations of neutrophils and macrophages in some alveoli.

*Group 4*

No obvious histological lesions were observed in the lungs of goats in this group except several foci of slightly congested areas.

*Microbiology*

*M. arginini* was not reisolated from the lungs of any of the goats. Reisolations of *P. haemolytica* were successfully made from the lungs of all goats in group 2 and of three goats in group 3.

## DISCUSSION

Most goats developed pneumonic lesions affecting the same lung area, but the extent of the lesions was different. Some of the goats infected with *M. arginini* alone and *P. haemolytica* alone had mild, localised pulmonary lesions which remained relatively similar in extent throughout the duration of the infection. Combined *M. arginini* and *P. haemolytica* infection, however, produced severe and extensive lesions typical of pneumonic pasteurellosis, and the extent of the lesions increased with time of infection. Similar synergestic effects had been described for viruses used in experimental pneumonic pasteurellosis of goats and sheep (Davies *et al.* 1981; Davies *et al.* 1982; Buddle *et al.* 1990).

This study also demonstrated that *M. arginini* can produce only mild lesions in the lung of goats, and thus this may not lead to a serious disease. The organism appeared to be eliminated early as

a result of the inflammatory response following the infection. A similar inflammatory response in pneumonic lungs was observed to eliminate caprine herpesvirus infection in experimental pneumonic pasteurellosis of goats (Buddle *et al.* 1990). Although the mycoplasma was eliminated early, the infection was thought to have induced initial lung lesions for later establishment of *P. haemolytica* in lungs. Most intratracheal infections in goats with *P. haemolytica* alone resulted in either mild pneumonia or the infection being eliminated quickly with no obvious pneumonia (Buddle *et al.* 1990).

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