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Effect of Waxing with Paraffin and Modified Atmosphere Packaging on the Storage of Cavendish Banana (*Musa cavendishii* L var. Montel)

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Keywords: Postharvest treatments, physico-chemical changes, storage life, 'Montel' banana

ABSTRAK

Kajian perlakuan lepastuai dengan cecair parafin, 'clingwrap', polietilena berdensiti rendah (LDPE) dengan dan tanpa kalium permanganat ($KMnO_4$) telah dijalankan untuk memanjangkan hayat simpanan buah pisang 'Montel' (Musa cavendishii *L.) di bawah suhu penyimpanan 15±1°C dan juga suhu ambien (27±1°C). Buah-buah yang dibungkus di dalam LDPE dengan $KMnO_4$ telah masak dalam jangkamasa 60 hari selepas dituai pada minggu 12 dari mulanya pengeluaran jantung. Ini diikuti oleh perlakuan dengan 'clingwrap' (42 hari), cecair parafin (36 hari), kawalan pada suhu 15±1°C (24 hari) dan kawalan pada suhu ambien (27±1°C; 18 hari). Peratus kehilangan berat buah, warna kulit dan isi buah telah meningkat dengan sangat bererti (P<0.01) bagi semua perlakuan di sepanjang masa penyimpanan. Selain daripada itu, nilai tekstur dan kandungan tanin buah pula menurun dengan sangat bererti (P<0.01). Peningkatan jumlah pepejal larut (TSS) dan gula adalah perlahan pada mulanya tetapi beransur-ansur meningkat di akhir masa penyimpanan. Walau bagaimanapun, nilai pH, kandungan-kandungan asid tertitrat (TA), asid askorbik (AA) dan kanji bagi buah-buah dari semua perlakuan didapati tidak seragam semasa penyimpanan. Terdapat perbezaan yang sangat bererti (P<0.01) dalam pengeluaran etilena (C_2H_4) dan karbon dioksida (CO_2) bagi buah-buah dari semua perlakuan semasa penyimpanan. Oleh yang demikian, didapati bahawa buah yang dibungkus dalam polietilena berdensiti rendah (LDPE) dengan $KMnO_4$ merupakan perlakuan yang terbaik untuk memanjangkan hayat simpanan buah pisang 'Montel'.*

ABSTRACT

Postharvest treatment with liquid paraffin, clingwrap, low density polyethylene (LDPE) with and without potassium permanganate ($KMnO_4$) was studied to extend the shelf life of 'Montel' banana (Musa cavendishii *L), under refrigeration (15±1°C) and at ambient temperature (27±1°C). The fruits packed in LDPE with $KMnO_4$ ripened within 60 days after harvesting at week 12 from flower emergence. This was followed by treatments with clingwrap (42 days), liquid paraffin (36 days), control at 15±1°C (24 days) and control at ambient temperature (18 days). The percentage weight loss, peel and pulp colours of fruits increased significantly (P<0.01) for all treatments during the storage period. On the other hand, the texture values and tannin content of the fruit decreased significantly (P<0.01). The rise in total soluble solids (TSS) and sugar was slow initially but gradually increased at the end of the storage period. However, pH, titratable acidity (TA), ascorbic acid (AA) and starch contents of fruits from all treatments were found to be inconsistent during storage. There is a highly significant (P<0.01) difference in the production of ethylene (C_2H_4) and carbon dioxide (CO_2) found from fruits of all treatments during storage. Fruits packed in low density polyethylene (LDPE) with $KMnO_4$ was found to be the best treatment to extend the storage life of 'Montel' banana.*

INTRODUCTION

Storage of dessert bananas at temperatures below 13°C result in chilling injury symptoms such as retarded development of yellow colour (Kim and Lee 1962; Olorunda *et al.* 1978) and failure to ripen (Haard and Timbie 1976), whereas

storage at 13°C is limited to two weeks (Salunkhe and Desai 1984). Modified atmosphere (MA) storage is a method which can be used in conjuction with refrigeration to enhance storage life of some fruits and vegetables (Kader *et al.* 1989). Bananas can be successfully stored in sealed polyethylene bags (Scott and Roberts 1966). As a result of respiration, an atmosphere high in CO_2 and low in O_2 will ensue with time. There are many factors affecting the respiration rate such as temperature, stage of development, fruit injury and many others (Phan *et al.* 1975). Ethylene is of great importance in postharvest physiology because it is intimately involved in the ripening of fruits and is sometimes called the ripening gas (Ryall and Lipton 1979). Refrigeration at 10-14°C alone is only sufficient to preserve the greenness of the banana for 10-34 days (Abdullah *et al.* 1990). Effect of surface treatment of 'Montel' banana with paraffin and various techniques of modified atmosphere have never been reported. Therefore, the objective of this work was to study the effect of these treatments on the physico-chemical characteristics of 'Montel' banana harvested at week 12 after flower emergence during storage.

MATERIALS AND METHODS

Fruit Source

Banana bunches were harvested at week 12 after flower emergence. The harvested fruits were immediately transported from UPM experimental plot to the laboratory of the Faculty of Food Science and Biotechnology, UPM for further evaluation. Three hands (2nd, 3rd and 4th hands from the top) were taken from each bunch. Only fruits that were free from mechanical injury were used in this study. Changes in texture and pulp colour were obtained from 8 individual fruits from each hand at each observation. The same individual fruit samples were used as composite samples for the determination of all the other chemical characteristics. Continuous assessment of percentage weight loss and change in peel colour were obtained from three other (2nd, 3rd and 4th) hands. Observations were made at every 6 day intervals and experiments were done in triplicates.

Postharvest Treatments

Banana hands were randomly selected from the bunches and subjected to different postharvest treatments as below:

- control at optimum temperature (15±1°C; 85-90% RH),
- control at ambient temperature (27±1°C; 55-85% RH),
- fruits dipped for 1 min in liquid paraffin and followed by air drying,
- fruits wrapped in commercial clingwrap of 0.01 mm thickness,
- fruits packed and sealed in low density polyethylene (LDPE) bag of 0.05 mm thickness, and
- fruits packed and sealed in LDPE bag with an inclusion of a cement block impregenated with saturated $KMnO_4$ solution.

All treated fruit hands except the control at ambient temperature were stored at 15±1°C; 85-90% RH.

Physical Analysis

The following parameters were determined: fruit weight, peel and pulp colours and hardness. Fruit weight was determined using an electronic top pan balance (model 4000C Precissa). Fruit peel and pulp colour measurements were taken at four different positions of the fruit using Hunter Lab (model Minolta CR-300). The firmness measurements were performed on 3 different places of the fruit using an Instron Universal Testing Machine Model 1140 with an 8 mm diameter plunger and a drive speed of 100 mm min^{-1}. The load cell force range used was 0-50 kg.

Chemical Analysis

Analyses of the chemical parameters were carried out on the following day after the fruit pulp were blended and kept in the freezer (-20°C). The sample extraction and preparation methods for sugar analysis were carried out according to Wills *et al.* (1980). Sugar was analysed by HPLC using method of Hunt *et al.* (1977). Titratable acidity (TA) and ascorbic acid (AA) were analysed according to the methods of Ranganna (1977). Samples prepared for TA were also used to determine pH (AOAC 1980). For the estimation of titratable acidity, 10 g of homogenised fruit pulp samples were heated to boiling in a beaker with distilled water for 30 min. The volume was made up to 100 ml and a 5 ml aliquot was titrated against 0.1 M NaOH, with phenolphthalein as indicator. The results were expressed as percentage malic acid. The puree extracted was taken to measure the solu-

ble solids content of the fruit using an Otago hand refractometer (0-32°Brix).

The amount of starch was determined by the method of SIRIM (1992) with some modification. For starch extraction, the weighed sample (2.5 g dried sample) of banana pulp was heated with 150 ml of 80% ethanol for 1 hr. The solution was then centrifuged for 10 min at 3000 rpm and the residue was washed with 50 ml distilled water. The pellet was considered to be starch. It was then heated in the oven for 3 hrs to remove the ethanol and to gelatinise the starch. The gelatinised starch was then hydrolysed with 2.5% hydrochloric acid for 2 hrs. The prepared solution was titrated using Fehling solution A and B and starch was calculated based on the reducing sugar released from the hydrolysis.

Sugars were determined by HPLC using a Waters 600 Controller liquid chromatograph with a Model 410 RI detector, a Hibar prepacked stainless steel column (300 X 3.9 mm i.d.) packed with 10 µm µBondapak-NH_2, and with acetonitrile and distilled water (80/20; v/v) as eluent. The solvent mixture was degassed for 20 min under sonicator. Fructose, glucose, sucrose and maltose (1-5%; w/v) were used as calibration standards. Ten grams of the fruit material were heated with 60 ml of 85% methanol on a steam bath for 30 min, filtered through Whatman No. 1 filter paper into a round bottom flask and the residue was similarly re-extracted twice with 60 ml portions of methanol. The filtrate was evaporated to less than 10 ml under vacuum at 50°C in a rotary evaporator and made up to 10 ml in a volumetric flask. The solution was then filtered through a Sep-Pak C_{18} cartridge and a 0.45 µm membrane filter, using a syringe. The injection volume was 10 µl.

Detection of Ethylene and Carbon Dioxide

One hand from each postharvest treatments was used for this continuous assessment. After measuring the volumes of the fruits, they were sealed in 4900 ml dessicator with inlet and outlet septa for 4 hrs. Amount of ethylene produced were detected for each postharvest treatment during storage time by injecting 1000 µl of the gas sample (under a static system) into a 5890 Hewlett Packard gas chromatograph fitted with flame ionization detector (FID) and a stainless steel column (254 mm X 3.175 mm OD) packed with 80-100 mesh Haye Sep D. Simultaneously, carbon dioxide was detected using a different detector (thermal conductivity detector; TCD), although the same column was used. The flow rate of the purified helium gas was 30 ml min^{-1} and the oven temperature was 40°C. Experiments were done in triplicates.

Statistical Analysis

For data analyses, the SAS (Statistical Analysis System) programme was used (SAS INSTITUTE 1985). The values obtained were subjected to analyses of variance and tested using the Duncan's Multiple Range Test (DUNCAN).

RESULTS AND DISCUSSION

Bananas packed in LDPE with $KMnO_4$ ripened within 60 days after harvesting at week 12 from flower emergence. This was followed by treatments with clingwrap (42 days), liquid paraffin (36 days), control at 15°C (24 days) and control at ambient (18 days). However, bananas packed in LDPE (without $KMnO_4$) gave the same storage life as with LDPE (with $KMnO_4$). Bananas stored in sealed polyethylene (PE) bags are known to have a longer storage life than those stored without bags (Scott and Roberts 1966; Chiang 1967; Smock 1967; Woodruff 1969; Liu 1970). Polyethylene bags delayed ripening and the use of potassium permanganate ($KMnO_4$) further extended the storage life of bananas by about two weeks, by reducing deterioration of the fruit due to softening during storage (Scott *et al.* 1970; Wills *et al.* 1981).

Fig. 1-3 show that the peel colour of bananas from different postharvest treatments differ very significantly ($P<0.01$). The peel colour of fruits was dark green at the early and then started to change during storage time until day 60 where the peel colour change to bright yellowish. Maximum colour development can be attained by rapid ripening at temperatures up to 24°C at high humidity, although this would reduce shelf life (Marriott 1980). There was also a highly significant ($P<0.01$) difference in pulp colour of fruits from different postharvest treatments during storage *(Fig. 4-6)*. The pulp colour of fruits was white creamy at the early storage and then started to change during storage time until day 60 where the pulp colour change to slightly bright yellowish as similarly reported by Wainwright and Hughes (1989). Bananas that were not sealed in polyethylene bags became ripe after seven days of storage, while all fruits

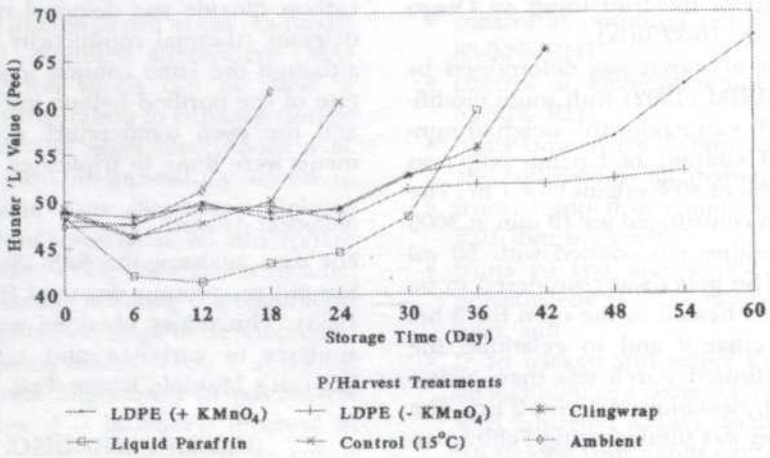

Fig. 1. Effect of postharvest treatments on Hunter 'L' value of 'Montel' banana peel during storage

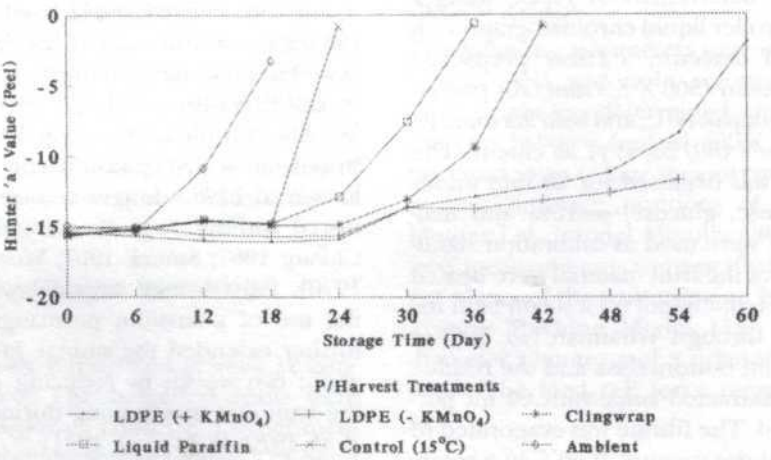

Fig. 2. Effect of postharvest treatments on Hunter 'a' value of 'Montel' banana peel during storage

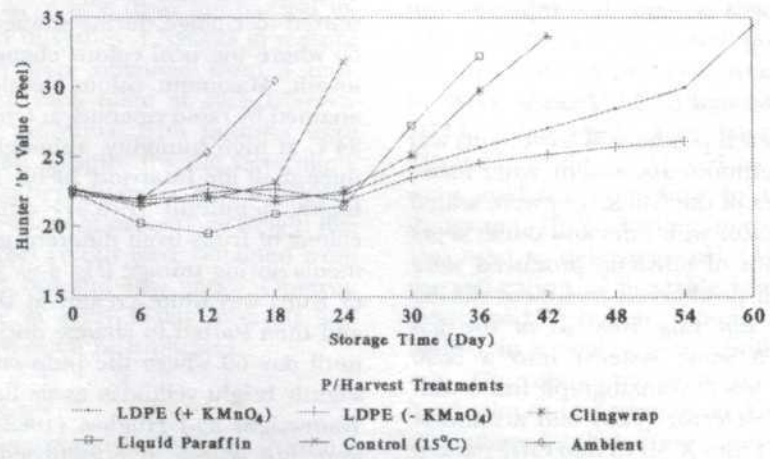

Fig. 3. Effect of postharvest treatments on Hunter 'b' value of 'Montel' banana peel during storage

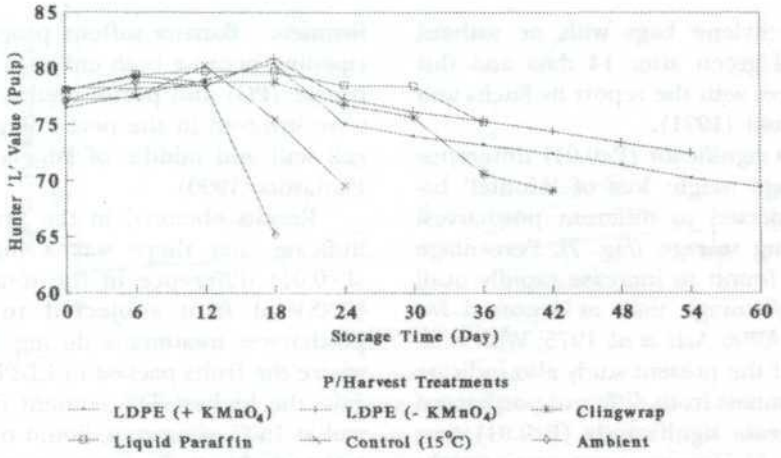

Fig. 4. Effect of postharvest treatments on Hunter 'L' value of 'Montel' banana pulp during storage

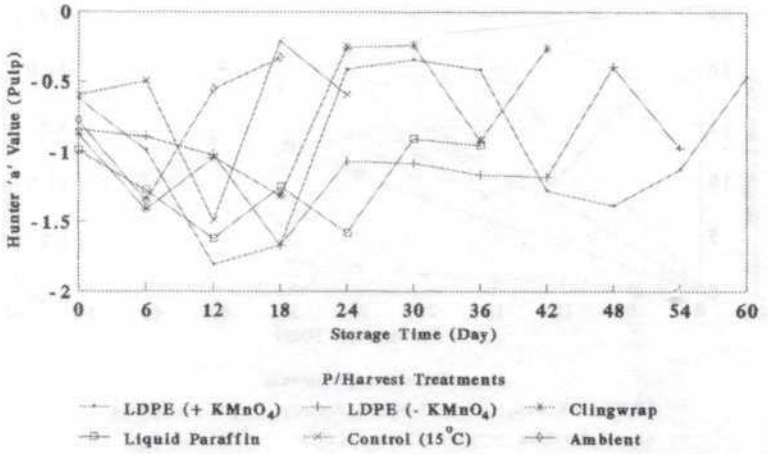

Fig. 5. Effect of postharvest treatments on Hunter 'a' value of 'Montel' banana pulp during storage

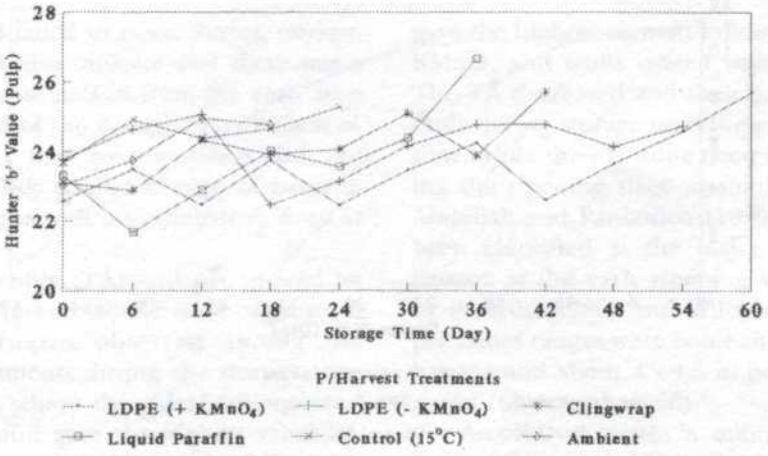

Fig. 6. Effect of postharvest treatments on Hunter 'b' value of 'Montel' banana pulp during storage

sealed in polyethylene bags with or without $KMnO_4$ was still green after 14 days and this observation agrees with the report by Fuchs and Temkin-Gorodeiski (1971).

There was a significant ($P<0.01$) difference in the percentage weight loss of 'Montel' banana when subjected to different postharvest treatments during storage *(Fig. 7)*. Percentage weight loss was found to increase rapidly until the end of the storage time as reported for other bananas (Abou Aziz *et al.* 1975; Wills *et al.* 1981). Results of the present study also indicate that the fruit firmness from different postharvest treatments decrease significantly ($P<0.01$) during storage *(Fig. 8)*. However percentage weight loss was negatively correlated ($r^2 > 0.5$) with firmness. Banana softens progressively during ripening because both enzymes the polygalacturonase (PG) and pectin methyl esterase (PME) were involved in the pectin degradation in the cell wall and middle of lamela (Abdullah and Pantastico 1990).

Results obtained in the present study also indicate that there was a highly significant ($P<0.01$) difference in the total soluble solids (TSS) of fruit subjected to the different postharvest treatments during storage *(Fig. 9)*, where the fruits packed in LDPE (with $KMnO_4$) gave the highest TSS content followed by control at 15°C, clingwrap, liquid paraffin and control at ambient. Fruits wrapped in LDPE (without $KMnO_4$) had the lowest TSS content and the

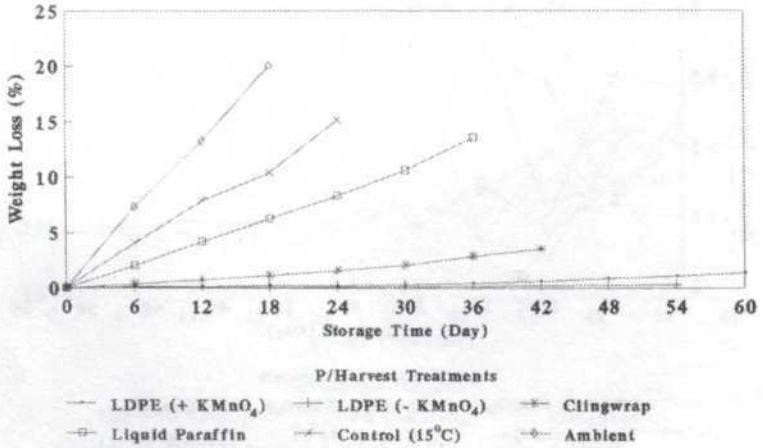

Fig. 7. Effect of postharvest treatments on percentage weight loss of 'Montel' banana during storage

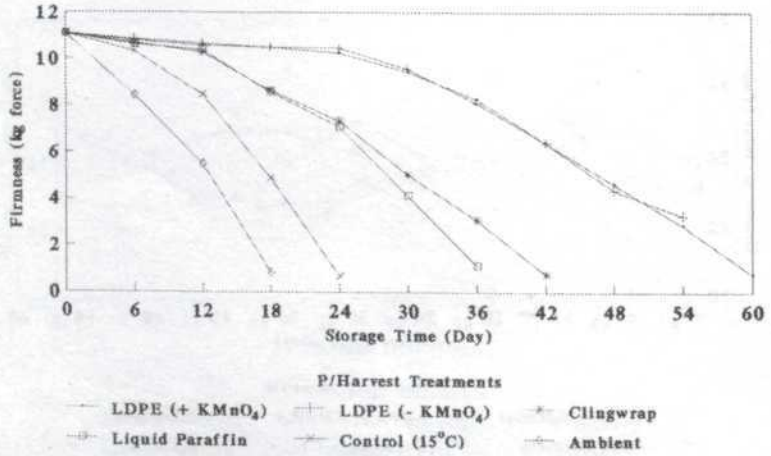

Fig. 8. Effect of postharvest treatments on firmness of 'Montel' banana during storage

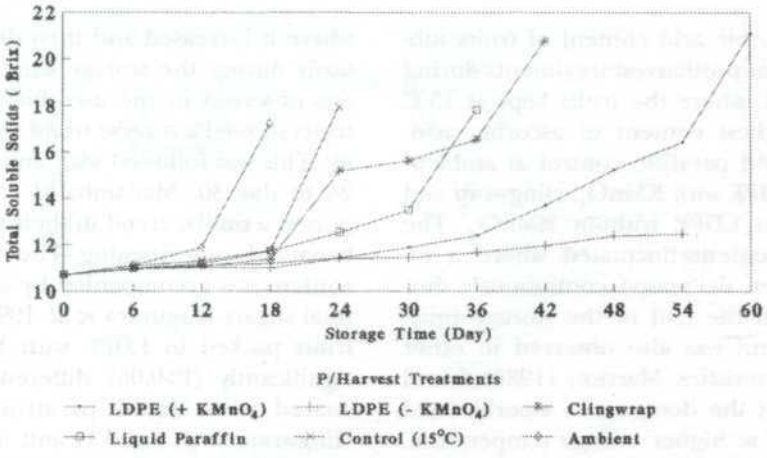

Fig. 9. Effect of postharvest treatments on total soluble solids of 'Montel' banana during storage

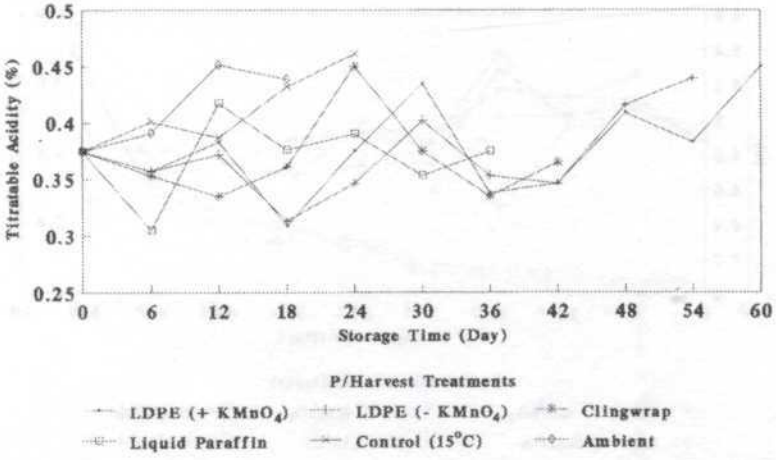

Fig. 10. Effect of postharvest treatments on titratable acidity of 'Montel' banana during storage

fruits rotted and failed to ripen during storage. Results obtained also indicate that there was a continuous increase in TSS from the early storage until the end of the storage time. Wills *et al.* (1983) reported that soluble solids did not change significantly during storage of bananas in fiber-board trays with a polyethylene wrap at 2-8°C.

Titratable acidity (TA) and pH showed an irregular pattern and there were significant ($P<0.01$) differences observed among the postharvest treatments during the storage time *(Fig. 10 and 11)*, where the pH of fruits coated with liquid paraffin gave the highest value followed by the control at 15°C and LDPE (with $KMnO_4$). However, the TA of fruits kept at 15°C gave the highest content followed by LDPE with $KMnO_4$ and fruits coated with liquid paraffin. The TA decreased and then increased continuously during storage until the end of the storage time, while the pH value decreased when reaching the ripening stage as similarly reported by Abdullah and Pantastico (1990). Malic acid has been identified as the major acid present in banana at the early ripening stage as reported by Forsyth (1980) and Wills *et al.* (1983). The pH values ranges were between 5.0-5.8 for green banana and about 4.2-4.8 in postclimacteric bananas (Simmonds 1966).

Ascorbic acid was a minor constituent of fruits (Wills *et al.* 1989). In the present study, there was a highly significant ($P<0.01$) differ-

ence in the ascorbic acid content of fruits subjected to different postharvest treatments during storage *(Fig. 12)*, where the fruits kept at 15°C showed the highest content of ascorbic acid, followed by liquid paraffin, control at ambient temperature, LDPE with $KMnO_4$, clingwrap and fruits packed in LDPE without $KMnO_4$. The ascorbic acid content fluctuated where it increased and then decreased continuously during storage until the end of the storage time. This similar trend was also observed in other chemical characteristics. Marriott (1980) found in his study that the decrease in ascorbic acid content is rapid at higher storage temperature.

From *Fig. 13*, it can be seen that the starch composition of the fruits was found to fluctuate where it increased and then decreased continuously during the storage time. A similar trend was observed in the ascorbic acid content but fruits showed a reverse trend with titratable acidity. This was followed with an increase from day 24 to day 30. Madamba *et al.* (1977) also reported a similar trend in their study of 'Lakatan' banana during ripening. The decrease in starch content was accompanied by an increase in the total sugars (Esguerra *et al.* 1992). However, the fruits packed in LDPE with $KMnO_4$ were not significantly ($P>0.05$) different with the fruits coated with liquid paraffin, wrapped with clingwrap, kept at 15°C and ambient temperature. A similar trend was reported by Esguerra *et al.* (1992).

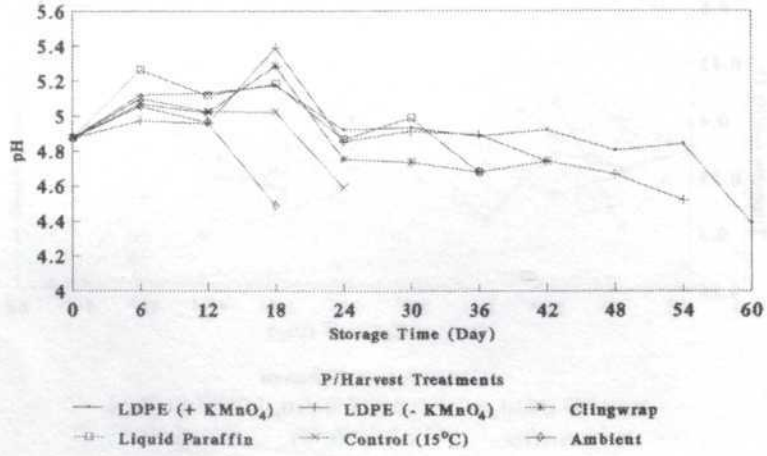

Fig. 11. Effect of postharvest treatments on pH of 'Montel' banana during storage

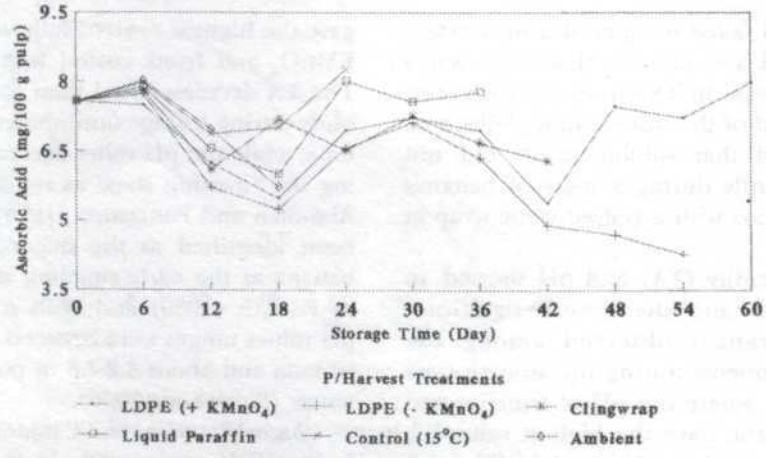

Fig. 12. Effect of postharvest treatments on ascorbic acid of 'Montel' banana during storage

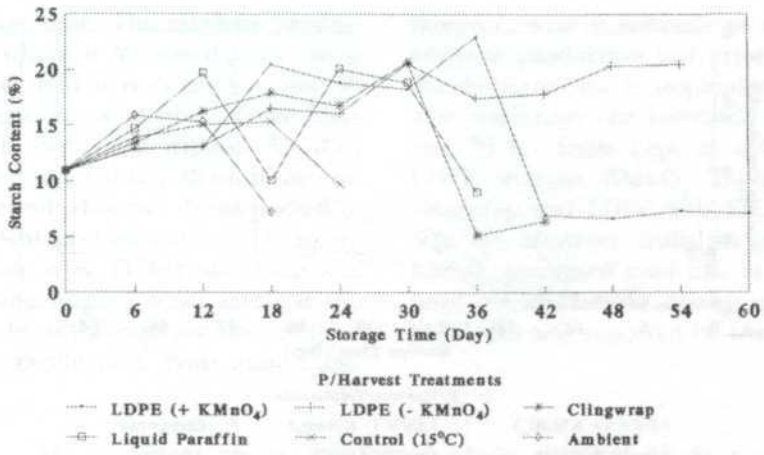

Fig. 13. Effect of postharvest treatments on starch content of 'Montel' banana during storage

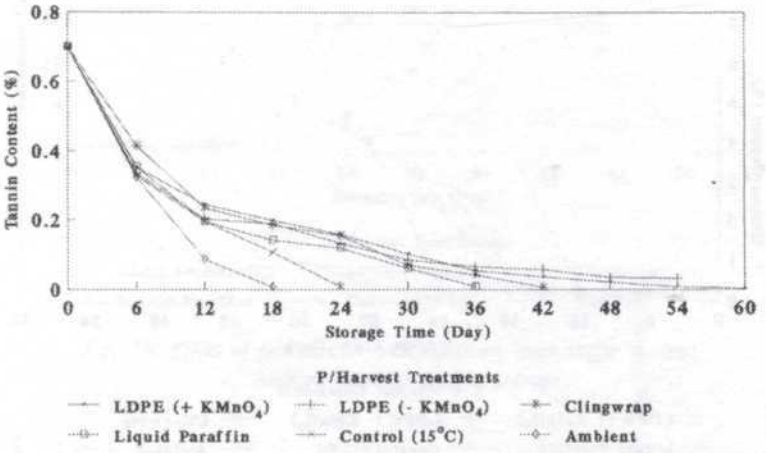

Fig. 14. Effect of postharvest treatments on tannin content of 'Montel' banana during storage

The tannin content of fruits from different postharvest treatments decreased drastically ($P<0.01$) during storage *(Fig. 14)* and a similar trend was also reported by Goldstein and Swain (1963). The other component that affects the taste of fruits is the sugar content. There is a highly significant ($P<0.01$) difference in the sugar content of 'Montel' banana from different postharvest treatments during storage *(Fig. 15-18)*. The total sugar increased from day 0 until the end of the storage time *(Fig. 18)* as similarly reported by Madamba *et al.* (1977) and Munasque and Mendoza (1990). There were an irregular increase in fructose *(Fig. 15)*, glucose *(Fig. 16)* and sucrose *(Fig. 17)* at the early storage but the increase was rapid with ripening. Broughton and Wu (1979) reported that there was no decrease in sugar with prolonged storage even after the "eating ripe" stage had been passed. The level of glucose increased with post-ripened storage of 'Embun' and 'Rastali' bananas (Broughton and Wu 1979), as it did in other bananas (Spencer 1966; Simmonds 1966). From the figures, it can be seen that the sucrose content was the highest individual sugar compared to the other sugar contents and a similar results was reported by Wills *et al.* (1983) who found that the sucrose content was always the major sugar present in 'Cavendish' banana.

There were significant ($P<0.05$) differences in ethylene *(Fig. 19)* and carbon dioxide *(Fig. 20)* production among the different postharvest treat-

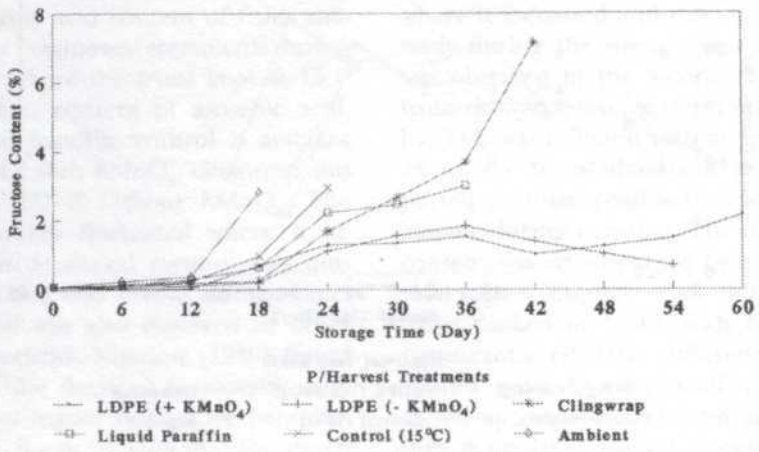

Fig. 15. Effect of postharvest treatments on fructose content of 'Montel' banana during storage

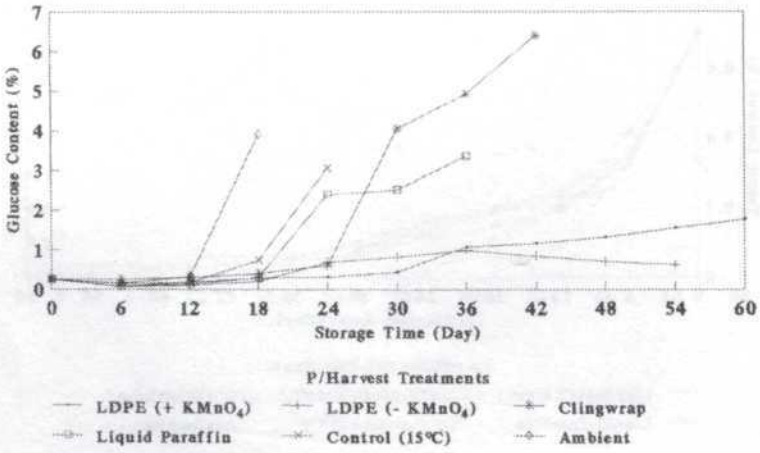

Fig. 16. Effect of postharvest treatments on glucose content of 'Montel' banana during storage

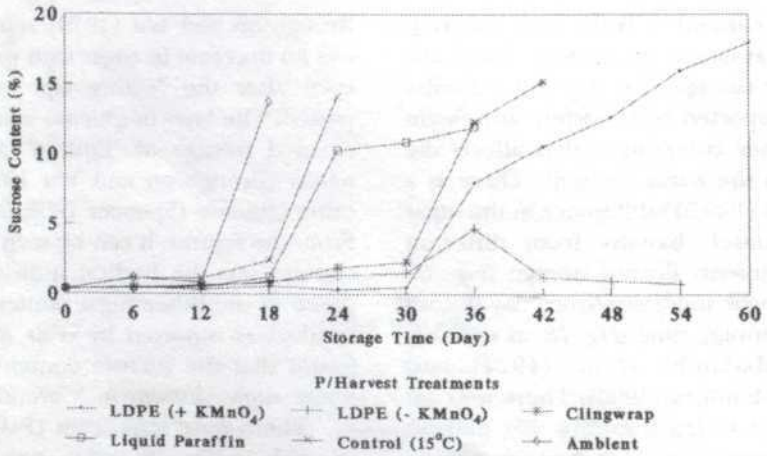

Fig. 17. Effect of postharvest treatments on sucrose content of 'Montel' banana during storage

ments with storage time. The ethylene production fluctuated, where it increased from day 0 until day 24 and then it decreased at day 36 (*Fig. 19*). The amount of ethylene from fruits packed in LDPE (with and without $KMnO_4$) started to increase again at day 42 until the end of the storage period. However, fruits packed in LDPE without $KMnO_4$ rotted and failed to ripen. According to Biale *et al.* (1954) and Burg and Burg (1962), some tropical fruits ripen at the climacteric without any abrupt increase in their rate of ethylene production. With most fruits, however, there is evidence of some increase in ethylene production just prior to the onset of the climacteric rise in respiration (Mapson 1969). The respiratory rate increased at day 18, 24, 30 and 36 for fruits kept at ambient, 15°C and LDPE without $KMnO_4$, liquid paraffin and clingwrap and LDPE with $KMnO_4$, respectively *(Fig. 20)*. However, fruits packed in LDPE with $KMnO_4$ increased gradually at day 48 onwards until the end of the storage period. A similar trend was also reported by Marriott (1980).

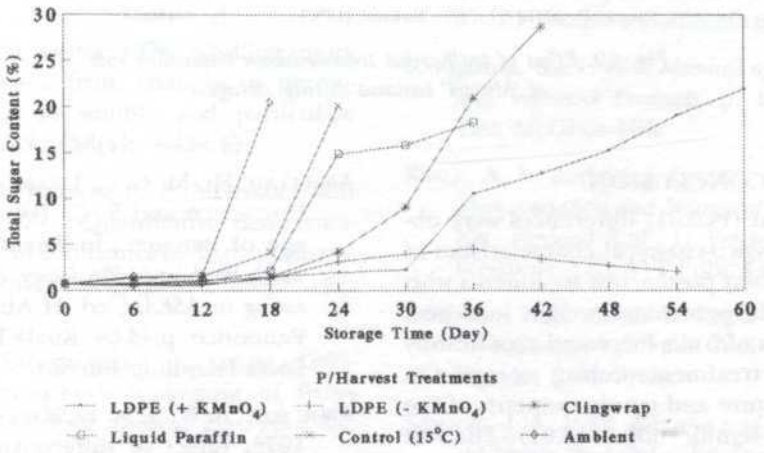

Fig. 18. Effect of postharvest treatments on total sugar content of 'Montel' banana during storage

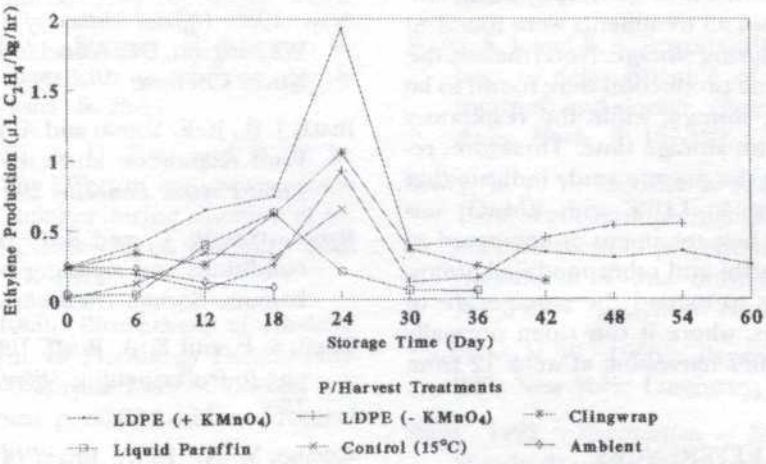

Fig. 19. Effect of postharvest treatments on ethylene production of 'Montel' banana during storage

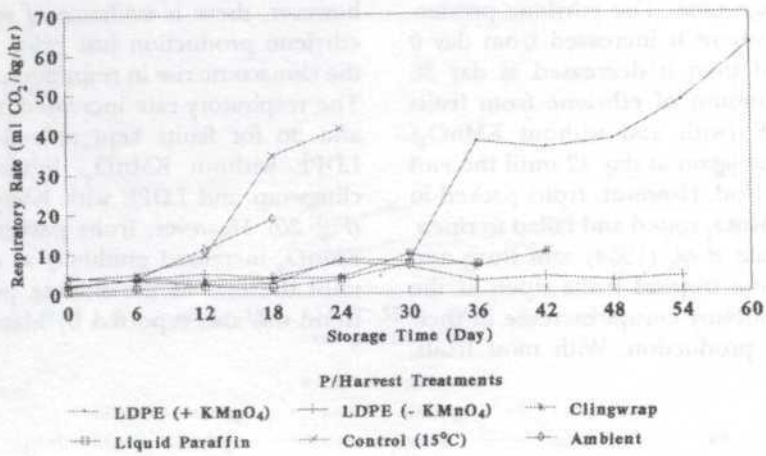

Fig. 20. Effect of postharvest treatments on respiratory rate of 'Montel' banana during storage

CONCLUSION

Highly significant ($P<0.01$) differences were observed in the physico-chemical characteristics of fruits from different postharvest treatments with storage time. The percentage weight loss, peel and pulp colours of fruits increased significantly ($P<0.01$) for all treatments during storage. On the contrary, texture and tannin contents of the fruits decreased significantly ($P<0.01$). The rise in total soluble solids (TSS) and sugar were slow initially but gradually increase rapidly at the end of the storage period. However, pH, titratable acidity (TA), ascorbic acid (AA) and starch contents of fruits from all treatments were found to be inconsistent during storage. Nevertheless, the pattern of ethylene production were found to be irregular during storage, while the respiratory rate increased with storage time. Therefore, results obtained in the present study indicate that the fruits packed in LDPE with $KMnO_4$ was found to be the best treatment as compared to waxing with paraffin and other modified atmosphere packaging to extend the storage life of 'Montel' bananas, where it can ripen normally within 60 days after harvesting at week 12 from flower emergence.

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Trace Element Concentration in Mango (*Mangifera indica L.*), Seedless Guava (*Psidium guajava L.*) and Papaya (*Carica papaya L.*) Grown on Agricultural and Ex-mining Lands of Bidor, Perak

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Keywords: trace element contamination, tin tailings, *Carica papaya, Mangifera indica, Psidium guajava*

ABSTRACT

Buah-buahan seperti mangga, betik dan jambu batu tanpa biji yang ditanam di atas tanah pertanian dan tanah bekas lombong di Bidor disampel untuk analisis pencemaran oleh logam berat. Kepekatan merkuri (Hg), plumbum (Pb), kuprum (Cu), zinkum (Zn), nikel (Ni), arsenik (As) dan kadmium (Cd) di dalam buah-buahan tersebut telah dianalisis. Keputusan menunjukkan bahawa buah-buahan yang ditanam di atas tanah pertanian mempunyai tahap logam berat yang lebih tinggi berbanding dengan buah-buahan yang ditanam di atas tanah bekas lombong, kecuali Hg dalam mangga dan Pb dalam jambu batu. Kepetakan logam berat dalam kesemua buah-buahan bagi kedua-dua jenis tanah mempunyai julat daripada 0.06 hingga 0.55 mg kg^{-1} untuk Pb, 5.20 hingga12.22 mg kg^{-1} untuk Zn dan 2.01 hingga 5.74 mg kg^{-1} untuk Cu. Kedua-dua kromium (Cr) dan Ni tidak dapat dikesan dalam betik yang ditanam di atas tanah bekas lombong manakala As tidak dapat dikesan dalam kesemua buah-buahan yang ditanam di atas kedua-dua jenis tanah. Keputusan ini mencadangkan bahawa kesemua buah-buahan mengandungi tahap Hg dan Pb yang sangat tinggi. Faktor-faktor yang mungkin menyebabkan pencemaran akan dibincangkan. Kajian lanjutan diperlukan untuk menentukan punca pencemaran oleh logam berat dalam kesemua buah-buahan tersebut.

ABSTRACT

Fruits namely mango, papaya, and seedless guava grown on agricultural and ex-mining lands in Bidor were sampled for analyse of heavy metal contamination. The concentration of mercury (Hg), lead (Pb), copper (Cu), zinc (Zn), nickel (Ni), arsenic (As) and cadmium (Cd) in the fruits were analysed. The results showed that, with the exception of Hg in mango and Pb in guava, fruits grown on agricultural land have higher levels of heavy metals than those grown on ex-mining land. The concentration of heavy metal in all fruits of both soil types ranged from 0.06 to 0.55 mg kg^{-1} for Cd, 0.02 to 0.78 mg kg^{-1} for Hg, 0.63 to 8.71 mg kg^{-1} for Pb, 5.20 to 12.22 mg kg^{-1} for Zn, and 2.01 to 5.74 mg kg^{-1} for Cu. Both Cr and Ni were not detected in papaya grown on mine spoils, whilst As was not detected in all fruits grown on both types of soils. The findings indicate that all fruits contained unacceptably high levels of Hg and Pb. The probable causes of contamination are discussed. Further studies are required to investigate the cause of heavy metal contamination in these fruits.

INTRODUCTION

Heavy metals are components of normal soils, and they are absorbed by plants only in ionic forms. In some disturbed sites such as ex-mining land; it has been reported that some areas have a large amount of heavy metals (Malm *et. al.*, 1995). The formation of heavy metal ions can be due to the lowering of soil pH, and/or, an excessive introduction of free heavy metal ions from contaminated sources such as sewage, polluted water and fertiliser (Davis 1984; Smith 1996).

Heavy metal contamination in agricultural products is known to cause health hazards. The uncontrolled uses of fertilisers and agro-chemicals which are environmentally unsound and health unfriendly have raised public concerns over the contamination of food and fruits with

heavy metal residues. Of the numerous trace elements that are present in contaminated soils, Cd, Pb, Hg, As, Se, Zn, Cu and Ni have been identified as elements of primary concern because of their potential hazard to man (Chaney 1983).

Reports on the heavy metal levels found in the temperate fruits such as strawberries, raspberries, blackcurrant, and food crops namely asparagus, peanuts, tomatoes, paprika, cauliflower, cucumber, leek, Chinese cabbage, lettuces, potatoes, sweet corn, and wheat had been reported (Chlopecka 1995; Tahvonen and Kumpulainen 1995; Wojciechowska-Mazurek *et al.* 1995). A severe heavy-metal contamination of agricultural produces has also been noted in waste disposal sites (Brandt and Rickard 1996). However, little is known about the heavy metal content of tropical fruits such as mango, seedless guava and papaya which are amongst the favourite fruits consumed widely in the tropics. In Malaysia, these fruits are mainly produced locally. They are normally grown in good agricultural soils, while in some cases, they are also cultivated on ex-mining land. Fruit orchards are a classical example of intensive farming where fertilisers, herbicide, and insecticide applications are extensively employed by farmers. The application of these chemicals may result in undesirable heavy metal contamination of soils, plants and their produces (Smith 1996). In addition, mining spoils resulted from tin mining activities have also been reported to have heavy metal contamination (Ang and Ang 1997). The fruits produced from these orchards have not been assessed for their heavy metal concentrations. Hence, the objectives of this study were to examine and to compare the concentrations of heavy metals in mango, guava and papaya produced from the agricultural land and mining spoils respectively.

Study Site

Samples of mango, seedless guava and papaya were collected from orchards located in Bidor which is about 128 km north of Kuala Lumpur. Bidor is located at 4°06'N latitude and 101° 16'E longitude, and had been a famous mining town during the 1940's. Presently, Bidor is popular for fruit productions especially the mango and seedless guava. Fruit orchards established along the main access road from Bidor to Teluk Intan comprise agricultural land and mining spoils. The agricultural land under fruit production in Bidor, is mainly characterised by Orthic Ferralsols and Orthic Luvisols that originated from riverine alluvium (Panton 1995), mostly with an average fertility. Whereas, the type of ex-mining land where the fruit orchards are established belong to sandy tin tailings that comprises > 99% sand. Generally, it is very infertile and requires intensive nutrient inputs and a good watering system if required for agricultural production. The chemical and physical properties of sandy tin tailings were well documented (Ang 1994; Ang & Ang 1997) *e.g.* the concentration of trace elements of ex-mining land in Bidor is given in the following table:

Some potentially toxic trace elements of tin tailings at 0-20 cm soil depth (Adapted from Ang & Ang 1997)

Trace element	sample size (n)	sand (mg kg^{-1})	slime (mg kg^{-1})
As	4	0.02-4.48	0.03-3.18
Cd	9	0.02-0.36	0.03-0.58
Cu	9	3.36-9.47	3.96-17.01
Cr	9	0.09-4.66	0.30-17.01
Hg	4	0.03-0.07	0.08-0.64
Ni	9	0.29-8.78	3.64-29.31
Zn	9	2.85-55.0	0.1-30.43

Three fruit orchards established on agricultural land and three on mining spoils were selected as the sampling sites.

METHODS

Sampling

Fruits comprising of mango (*Mangifera indica L*), seedless guava (*Psidium guajava L.*), and papaya (*Carica papaya L.*) grown on agricultural land and mining spoils were selected for this study. Only ripe fruits were harvested for determination of their heavy metal concentration. About 10 to 15 fruits of each species grown on each soil type were randomly collected. The fruit samples were then properly labelled and kept separately in a plastic bag.

Sample Preparation

The fruits were processed on the same day after collection. They were rinsed with distilled water before peeling, and the edible portion of the fruits were then sliced and oven-dried at 65°C

till constant weight. After drying, the slices of dried fruit were ground to powder using an electrical blender and stored in air-tight plastic bags until taken for analysis.

Fruit samples from both soil types were analysed for moisture and heavy metal contents based on AOAC Methods (1980). The wet extraction technique was employed for the digestion of the fruit samples. Total lead (Pb), copper (Cu), zinc (Zn), nikel (Ni), arsenic (As), and cadmium (Cd) were determined using an atomic absorption spectrophotometer. Total mercury (Hg) concentration was analysed using automated cold vapour, EPA stannous chloride method (Dorminski 1985). All samples were analysed in quadruplicate.

RESULTS AND DISCUSSION

Moisture Content

The moisture content of fruits varied from 83 to 90% (Table 1). Mango and seedless guava grown in mine spoils had significantly lower water content than those grown on agricultural soils. The lower moisture content of these fruits could be due to the interaction of environmental factors and their physiological characteristics. One of the possible reasons could be the harsh environment of mine spoils which are characterised by high air temperature and low water availability (Ang 1994), resulting in drier fruit tissues of the two woody species. However, the moisture content of papaya grown on ex-mining land was greater than those planted in agricultural land. This suggests that papaya, which has succulent property may have a different strategy to overcome drought through conserving tissue water, *e.g.* cactus.

TABLE 1
The moisture content (%) of fruits

Fruit	Sample size (n)	Mean moisture content of fresh fruit ± SEM (100 x g g-1)
Mango		
(a) Agricultural land	4	86.4 ± 0.5a
(b) Ex-mining land	4	82.6 ± 0.3b
Guava		
(a) Agricultural land	4	89.6± 0.2a
(b) Ex-mining land	4	82.6± 0.2b
Papaya		
(a) Agricultural land	4	88.1 ± 0.0a
(b) Ex-mining land	4	88.9 ± 0.1b

Note: For same species only, alphabetical letters indicate significant differences by SEM between two values in the same column.

Chemical Content

The heavy metal content of the edible portion of fruits are presented in Table 2. Generally, fruits grown on agricultural land have greater mean levels of heavy metals than those grown on mine spoils, except for Hg in mango, and Pb and Ni in guava.

Mango, papaya and guava grown on agricultural land contained a greater concentration of Cd, Cr, Cu and Zn than those planted on ex-mining land. The concentrations of Cd and Cu present in all fruits ranged from 0.06 to 0.55 mg kg^{-1} and 2.01 to 5.74 mg kg^{-1}, respectively. The concentration of Cd was below the permissible limit of 0.5 mg kg^{-1} independent of soil types *(Fig. 1a)*.

For fruits grown on ex-mining land, mango had a significantly higher level of Cu than guava and papaya (Table 2). The mean Cr level of all fruits was below 0.7 mg kg^{-1} and was below the level of concern (USFDA, 1993a), regardless of species and soil types. Interestingly, Cr was not detected in papaya grown on ex-mining land. The mean level of Zn in all fruits ranged from 5.20 to 12.22 mg kg^{-1}, and with the highest level was found in mango. The level of As was not detected in all fruits. Mango grown on ex-mining land had the highest mean concentration of Ni (Table 2).

The sources of contamination remain unclear in this study, however, the findings have indicated that several important heavy metals were found in the fruits grown in Bidor. Such metals can be easily absorbed into the human body through dietary intake. These potentially harmful elements can cause undesirable health problems such as kidney failure, cancer, liver failure and other illness (USEPA 1979; Logan & Chaney 1983; Smith 1996).

TABLE 2
Heavy metal concentration in dry weight basis of fruits grown on agricultural and ex-mining lands

Element		Mango		Guava		Papaya	
	Permissible level (mg kg^{-1})	Agricultural land (mg kg^{-1})	Ex-mining land (mg kg^{-1})	Agricultural land (mg kg^{-1})	Ex-mining land (mg kg^{-1})	Agricultural land (mg kg^{-1})	Ex-mining land (mg kg^{-1})
Cadmium (Cd)	1.0	0.12 ± 0.07	0.06 ± 0.06	0.24 ± 0.00	0.12 ± 0.07	0.55 ± 0.32	0.34 ± 0.20
Mercury (Hg)	0.05	0.16 ± 0.15	0.26 ± 0.15	0.78 ± 0.75	0.02 ± 0.02	0.70 ± 0.44	0.08 ± 0.05
Lead (Pd)	0.5	1.64 ± 0.77	0.63 ± 0.44	2.44 ± 0.72	8.71 ± 0.71	1.38 ± 0.83	1.89 ± 0.67
Arsenic (As)	0.1	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.
Zinc (Zn)	5	12.22 ± 3.20	7.63 ± 0.15	11.62 ± 1.22	8.74 ± 0.71	8.13 ± 0.93	5.20 ± 0.54
Copper (Cu)	10.0	5.74 ± 0.70	3.90 ± 0.17	4.61 ± 0.26	3.74 ± 0.36	3.48 ± 0.19	2.01 ± 0.49
+ Chromium (Cr)	4.86	0.66 ± 0.12	0.52 ± 0.52	0.58 ± 0.34	0.39 ± 0.39	0.58 ± 0.33	N.D.
+ Nickel	29.3	0.06 ± 0.06	1.07 ± 0.68	0.18 ± 0.18	0.55 ± 0.55	0.65 ± 0.49	N.D.

Notes: Permissible limit is after Food Act 1983 and Food Act Regulations 1985 (MDC, 1996) except for the contents of Cr and Ni which are calculated after USFDA (1993a, b) and known as levels of concern. N.D. denotes below detection limit and (denotes SEM.
+ denote level of concern of Cr and Ni in dry fruit :
- Average daily consumption rate of fruits per person (fresh weight) = 300 g
- Average daily consumption rate of fruits per person (dry weight) = 300 x 0.137 g =41.1 g
- Level of concern for Cr uptake through eating shellfish = 0.2 mg per person per day, if the Cr uptake comes only from fruits alone = (200 /41.1) = 4.86 mg kg^{-1}.
- Level of concern for Ni uptake through eating shellfish = 1.2 mg per person per day. If the Ni uptake comes only from fruits alone = (1200 /41.1) = 29.3 mg kg^{-1}

Mercury

Guava and papaya grown on agricultural land, were found to have a higher Hg content than those grown on ex-mining land (Table 2). With the exception of guava grown on ex-mining land, the mean Hg level of all fruits exceeded the acceptable limit of 0.05 mg kg^{-1} *(Fig. 1c).* Several studies showed that high Hg concentration was found in sites of previous gold mining and smelting factories (Malm *et al.* 1995), farmland through the application of organomercurial fungicides (Smith 1996), and sludge-treated soil through sludge application as a fertiliser (Estes *et al.* 1973). Smelting of gold involving

the use of pure mercury (Chan 1983; Malm *et al.* 1995). Release of Hg in the environment occurs basically in two ways: [a] sublimation of Hg from amalgam during melting and purification processes which involves burning, and [b] direct release to aquatic systems or to mine tailings. The record of gold mining and extraction in Bidor shows that the production of gold varied between 2.70 to 6.98 kg month^{-1}, with a maximum average of 56.50 kg month^{-1} for the period from June 1981 to May 1982 (Chan 1983). Hence it is not surprising to discover Hg content in the fruits grown in Bidor. The uptake of Hg in edible part of fruits may also come from the applications of agro-chemicals, organic fertiliser, fertiliser through foliar application, irrigation using contaminated water from the mining pond, and/or, from the contaminated soils. Interestingly, fruits grown on agricultural land contain a significantly higher mercury concentration than those grown in mine spoils *(Fig. 1c)*. A higher Hg uptake in plant is normally possible through direct aerial contact rather than from contaminated soils (Bache *et al.* 1973; Vigerut and Selmer Olsen 1986; Smith *et al.* 1992). However, aerial contact may also come from the volatilisation from soils and absorption by the aerial parts of plants, and could result in an increase of the Hg concentration in plant tissue (Lindberg *et al.* 1979). The soils of fruit orchards that are in the vicinity of a former gold smelting factory in Bidor may receive the Hg deposits from the contaminated source from the last decade. However, further analyses on the content of Hg of the agro-chemicals, water, fertilisers, and agricultural soils, are needed to further determine their roles in heavy metal contamination of fruits.

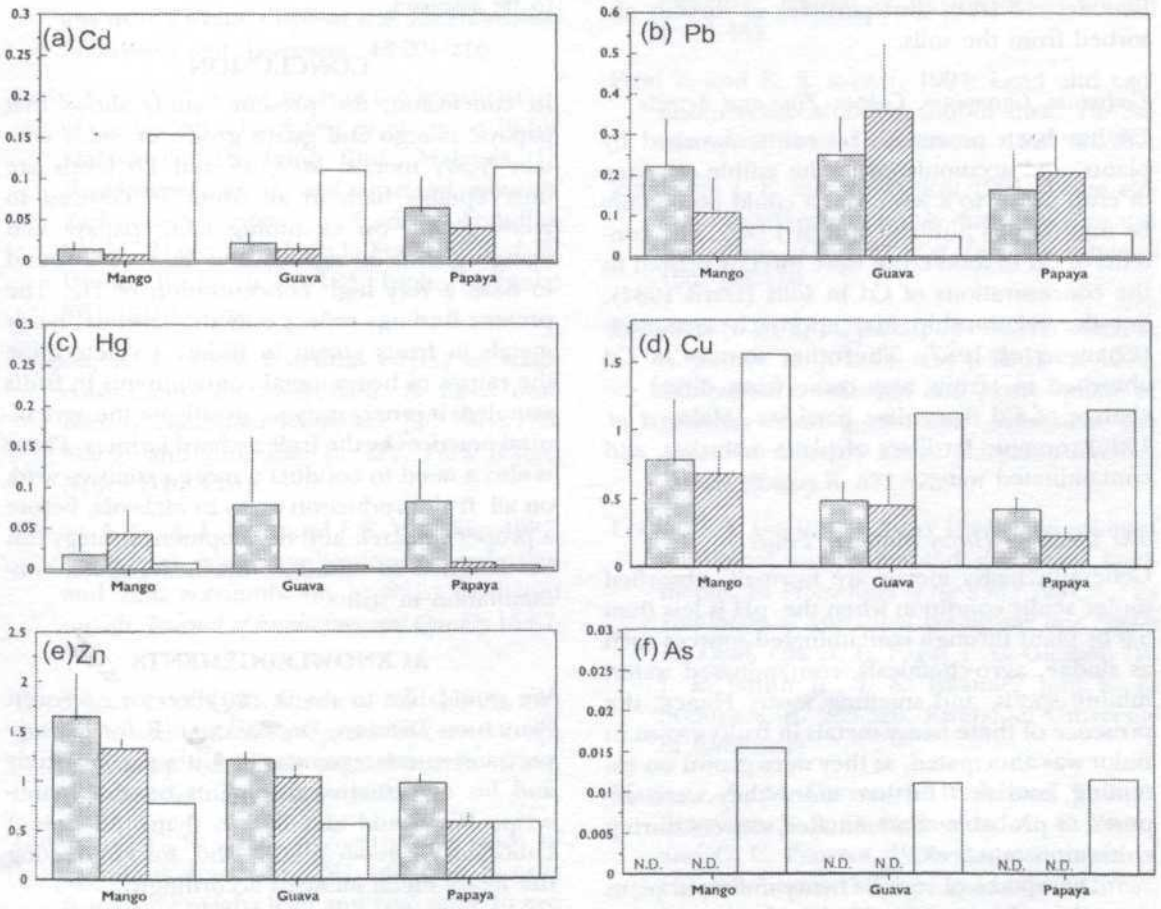

Fig. 1. Some heavy metal contents of fruits grown in Bidor. [a] Cd, [b] Pb, [c] Hg, [d] Cu, [e] Zn and [f] As. The concentrations of heavy metal in fresh weight basis with SEM in fruits grown in ex-mining land (▨), agricultural land (▩), and the permissible limit (□) calculated according to fresh weight basis based on the standards stated in MDC (1996). N.D. denote below detection limit.

Lead

The level of Pb in fruits ranged from 0.63 to 8.71 mg kg^{-1} (Table 2). This value was not significantly different between fruits *(Fig. 1b)*. The Pb content of guava collected from ex-mining land was shown to have about 17 folds greater than the permissible limit (Table 2). The unacceptable concentration of Pb in the fruits could have resulted from direct contacts with contaminated sources such as the dust particles (Fergusson & Kim 1991; Feng and Barrat 1994), and in an extreme acidic soil conditions with < pH 3.0 (Smith 1996). Certainly, the Pb contamination is unlikely to come from direct contacts with fruits during the sample preparation because the fruits were rinsed with distilled water, and the outer coat of the fruits were peeled off. Hence, the Pb contamination of fruits grown in Bidor is probably in the form of exchangeable ions derived from dust particles or directly absorbed from the soils.

Cadmium, Chromium, Copper, Zinc and Arsenic

Cd has been proven to be easily absorbed by plants, and accumulated in the edible portions of crop plants to a level which could potentially be injurious to humans (Smith 1996). The contents of Cd in food crops were directly related to the concentrations of Cd in soils (Davis 1984), but the relationship may approach asymptote (Chang *et al.* 1987). The other sources of Cd absorbed in fruits may come from direct exchange of Cd from dust particles (Malm *et al.* 1995), organic fertiliser of plant materials, and contaminated water.

The Uptake of Heavy Metals in Fruits

Generally, heavy metals are normally absorbed under acidic condition when the pH is less than 5.0 by plant through contaminated sources such as sludge, agro-chemicals, contaminated water, mining spoils, and smelting waste. Hence, the presence of these heavy metals in fruits grown in Bidor was anticipated, as they were grown on ex-mining land and further more they were exposed to probable contaminated sources during cultivation practices.

The uptake of various heavy metals in plant tissues varies according to species preference (Wolnik *et al.* 1983; Eriksson 1989; Chukwuma 1995). The preference of heavy metal uptake is also observed in cultivars (Wolnik *et al.* 1983; Clopecka 1995). Mango grown on ex-mining land accumulated a higher concentration of Hg than papaya and guava. This could be due to its predilection to the metal. Similarly, the preference of guava to Pb uptake was observed. Guava had a greater Pb content per kg fresh weight than mango and papaya irrespective of planting sites *(Fig. 1b)*.

The species preference of fruit trees for certain heavy metal uptake needs further confirmation in control environments. In addition, further investigations on site toxicity and cultivation practices of fruit tree orchards in Bidor are needed. The suitability of ex-mining land in Bidor for food production has to be ascertained due to the presence of heavy metals. In view of these findings, the chemical properties of the ex-mining land nation wide need to be systematically characterised and investigated. The site toxicity and suitability for food production need to be assessed.

CONCLUSION

In conclusion, the present study shows that papaya, mango and guava grown in Bidor contain heavy metals. Mercury and Pb levels are unacceptably high in all fruits. In contrast to guava grown on ex-mining land, papaya and mango grown on agricultural land were found to have a very high concentration of Hg. The present findings reflect only the level of heavy metals in fruits grown in Bidor. To determine the causes of heavy metal contaminants in fruits sampled, it is necessary to investigate the agricultural practices by the fruit orchard farmers. There is also a need to conduct a more extensive work on all fruit production areas in Malaysia, before a proper research and development strategy can be designed to minimise the heavy metal contamination in fruits.

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Pathogenicity and Characteristics of *Spodoptera litura* Nucleopolyhedrovirus from Peninsular Malaysia

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ABSTRAK

Satu kajian keatas ciri-ciri Sopdoptera litura *nukleopolihedrovirus (SpltNPV) telah dilakukan. Hasil kajian mendapati bahawa virus tersebut sangat patogenik dan spesifik terhadap hosnya, larva* S. litura. *Ia menyebabkan 95% kematian larva dalam masa 10 hari dengan dos 6 x 10^8PIBs/larva dan tidak menjangkiti larva lepidoptera* Spirama retorta *Clerck (Noctuidae) dan ulat bungkus,* Pteroma pendulla *Joannis (Psychidae). Polihedra mempunyai berbagai bentuk dan mengandungi banyak virion dengan lebih daripada satu nukleopkapsid setiap virion.*

ABSTRACT

A study on the characteristics of the Spodoptera litura *nucleopolyhredovirus (SpltNPV) was carried out. The result shows that the virus was pathogenic and specific to its host,* S. litura *larva. It caused 96% larval mortality within a period of 10 days at a dosage of 6 x 10^8 PIBs/larva and did not affect lepidopteran larvae,* Spirama retorta *Clerck (Noctuidae) and bagworm,* Pteroma pendula *Joannis (Psychidae). The polyhedra, varied in their shape, contained many virions with more than one nucleocapsid in a virion.*

INTRODUCTION

Spodoptera litura (Fabricius) is a cosmopolitan polyphagous insect pest of many food crops and tobacco. As in most other parts of the tropic, these armyworms are subjected to a disease caused by a *Spodoptera litura* nucleopolyhedrovirus (SpltNPV) infection in the field (Kalshoven 1981). A similar phenomenon occurred in Malaysia, but heretofore, no record of such an infection had been reported. Recently, an epizootic of NPV disease occurred in *S. litura* population attacking a tobacco crop in Kelantan, Malaysia. This incidence occurred when the population of the pest was high particularly in farms where chemical insecticides were not applied. Though the impact of the disease on the *S. litura* population in the field has yet to be known, NPV has the potential to be used as a control agent to be incorporated in management of *S. litura* in Malaysia. Many field trials carried out elsewhere had shown that NPV used either alone or in combination with chemical insecticides could bring the population of *S. litura* to below an economic level (Su 1992). The use of such viruses would not only reduce the dependency on chemical insecticides but also conserve the natural enemies.

Before field trials could be initiated, we examined the characteristics of this SpltNPV. Thus the purpose of this study was to determine (a) the relative virulence to its host, *S. litura* larvae and to non-host larvae of *Spirama retorta* Clerck (Lepidoptera: Noctuidae) and bagworm *Pteroma pendula* Joannis (Lepidoptera: Psychidae) and (b) the characteristics the of SlptNPV by restriction endonuclease analysis.

MATERIALS AND METHODS

Host Insect

Spodoptera litura pupae were collected from a tobacco field at Malaysian Agricultural Research and Development Institute (MARDI) Research Station in Kelantan, Malaysia. The pupae were surface-sterilized with 1% sodium hypochlorite and rinsed with distilled water. The pupae were sexed and five pairs were placed in an oviposition cage consisting of cylindrical wire mess, 15 x 30 cm, lined with a paper towel. Egg masses were collected, surface-sterilized with 1% sodium hypochlorite. The larvae were reared in a plastic container, 30 x 20 x 10 cm, lined at the bottom with a paper towel. Fresh castor leaves, *Ricinus communis,* were provided as their food. The leaves and the paper changed on alternate days.

Propagation of Virus

The SpltNPV was originally isolated from naturally field-infected *S. litura* larvae collected from the same field in 1995. The virus was mass-propagated in the laboratory by feeding the third instar on castor leaves dipped in NPV suspension containing 11 x 10^{10} PIBs/ml. Twenty-four hours after exposure, the larvae were then transferred to 9 cm Petri dishes in a batch of five individuals and maintained on fresh uncontaminated leaves. Diseased larvae were collected and kept in a freezer at - 20°C till further use.

Bioassay

Virus suspension was prepared from heavily viral-infected fifth instars. The larvae were homogenized in a Waring^{R} blender and filtered through double layers of muslin cloth. The homogenate was then sonicated and refiltered. Second instars (3-day old) *S. litura* were used in the bioassay. Circular castor leaf discs measuring 1.5 cm in diameter were contaminated with serial concentrations of SpltNPV. Eight concentrations of NPV ranging from 1 x 10^{1} to 1 x 10^{8} PIBs/ml were used in the study.

Sixty µl of each viral concentration was pipetted onto each leaf disc. The leaf discs were air-dried and then placed individually in a 25, 2 x 2 cm, compartmentalized plastic plate containing 2% agar. The agar maintained the humidity and thus reduced leaf desiccation. One larva was then placed in each compartment. A total of 25 larvae were used for each concentration. Larvae, having eaten the entire leaf disc, were transferred to 9 cm plastic petri dishes and fed fresh uncontaminated castor leaves. Mortality was recorded daily until pupation. The experiment was replicated four times.

For the host range, infectivity of the SpltNPV was tested against two other insect species, *Spirama retorta* Clerck (Noctuidae) and *Pteroma pendula* Joannis (Psychidae) using the same procedure prescribed for *S. litura* larvae.

The mortality data of the three insects used in the bioassay were corrected using Abbott's formula. Probit analysis of Finney (1971) was performed on the data using the generalized linear inodel of the SAS (SAS 1985) package at MARDI computer centre. The lethal doses; LD_{50} and LD_{90} and lethal times LT_{50} and LT_{90} were calculated from the regression equations.

Purification of Viral PIBs and DNA Extraction

Two batches of SpltNPV isolated separately in 1995 and 1997 were prepared for this study. These isolates were propagated in the laboratory.

The purification and DNA extraction procedure of the SpltNPV was adopted from that of Gzrywacz (pers. com.). Diseased larvae were macerated and homogenized individually in 1 ml of distilled water. The homogenate was centrifuged at 13000 *g* for 5 min and pelleted PIBs were obtained. The PIBs were resuspended in 750 µl distilled water and recentrifuged at 13000 *g* for 15 min. The PIBs were then diluted in 120 µl of distilled water to which 25 µl of 500 mM EDTA and 3 µl of 20 mg/ml protease K were added. The mixture was incubated for 1.5 h at 37°C after which 1/2 volume of 1 M Na_2CO_3 was added. The mixture was again incubated at 37°C until the mixture turned translucent. At this stage, 25 µl of 10% SDS was added and the mixture was reincubated for 30 min at 37°C and recentrifuged at 13000 *g* for 60 min. The supernatant containing DNA was extracted with an equal volume of Tris-phenol (pH 7.6), followed by Tris-phenol:chloroform: isoamyl alcohol (25:24:1, v/v/v) and chloroform:isoamyl alcohol (24:1, v/v). The DNA was precipitated twice with 1/5 volume of 3 M sodium acetate and 3 volumes of absolute ethanol at -20°C. The mixture was then centrifuged at 13000 *g* for 5 min. The pellet was washed gently in 500 µl of chilled 70% ethanol, centrifuged and dried with Speed Vac Concentrator Savant. The purified

DNA was dissolved in 20 µl of Te buffer and stored at -20°C.

Restriction Endonuclease Digestion and Agarose Gel Electrophoresis

DNA was digested with *Eco*RI, *Bam*HI and *Hind*III and *Pst*I restriction endonucleases in their respective buffers as recommended by the supplier (GIBCO BRL) for 3 h at 37°C. The DNA restriction endonucleases fragments were separated by electrophoresis in 0.6% agarose gel in 1X TAE buffer. Lambda DNA marker (GIBCO BRL) and High Molecular Weight DNA marker (GIBCO BRL) were used as standards for the determination of fragments sizes.

Electron Microscopy

Two samples were prepared for transmission electron microscopy. The pelleted polydedra and pieces of infected fat tissues were fixed in 4% glutaraldehyde in 0.1 M sodium cacodilate buffer pH 7.4, and postfixed in 1% osmium tetraoxide. The samples were dehydrated through a graded series of acetone, embedded in Epon-Araldite and sectioned using a glass knife. The thin sections were stained with uranyl acetate and lead citrate. The stained sections were examined and photographed in a Hitachi® 600 electron microscope.

RESULTS AND DISCUSSION

Bioassay

The corrected mortality percentages for the three insect pest species are given in Table 1. At the highest dosage (6 x 10^8 PIBs/larva), the NPV could cause about 96% mortality to its original host, *S. litura* larvae within a period of 10 days *(Fig. 1)*. Larval mortality rates within the same period for the doses of 6 x 10^7, 6 x 10^6 and 6 x 10^5 PIBs/larva were 80, 74 and 55%, respectively. At the lowest dosage (60 x 10^1 PIBs/larva), larval mortality was only 18% after 13 days of infection. The corrected larval mortalities for *S. retorta* and *P. pendula* from all viral concentrations were less than 1%.

The results of probit analysis showed that the values of LD_{50} and LD_{90} and for the NPV were 5.50 x 10^4 and 5.26 x 10^8. PIBs/larva, respectively. The regression equation was Y = 3.47 + 0.32X. The lethal time of the NPV at LD_{50} and LD_{90} were eight and six days, respectively. The regression equation had a value of Y = 13.59 - 9.41X.

SlptNPV evidently is very specific and pathogenic to its host, *S.litura* larvae. The NPV bioassayed against *S. retorta* and *P. pendula* showed no specific trend of mortality. However, ingestion of this NPV continuously throughout the life span of an assassin bug, *Sycanus leucomesus* Walk. did, to acertain extent impair the development of the predator (Sajap *et al.* 1999). The infection in *S. litura* and the subsequent larval mortality period were dependent on the dosage of the virus being ingested. Viral concentration and time taken to kill the larvae increased with an increase in larval age.

Restriction Endonuclease Analysis

The fragmentation profiles resulting from the digestion of the SpltNPV DNA with *BAM*HI, *ECO*RI and *Hind*III and *Pst*I, are shown in *Fig. 2.* The enzymes, *Bam*HI, *ECO*RI and *Hind*III and *Pst*I cleaved the genomes from the first isolate of SpltNPV into 9, 15, 24 and 16 but cleaved the second isolate into 10, 16, 24 and 17 fragments, respectively. Even though there were minor submolar were detected when two isolates digested DNA when they were coelectophoresed in 0.6% agarose gel. Similarities in the fragment patterns were also observed from both viral DNAs

TABLE 1
Mortality of *S. litura*, *S. retorta* and *P. pendulla* treated with SpltNPV ten days after treatment

(PIBs/ larva	Number treated	% Corrected mortality		
		S. litura	*S. retorta*	*P. pendulla*
$6X10^8$	100	95.95	0	0
$6X10^7$	100	80.81	0	0
$6X10^6$	100	69.70	0	0
$6X10^5$	100	54.55	0	0
$6X10^4$	100	49.49	0	0
$6X10^3$	100	38.38	0	0
$6X10^2$	100	28.28	0	0
$6X10^1$	100	17.17	0	1.00

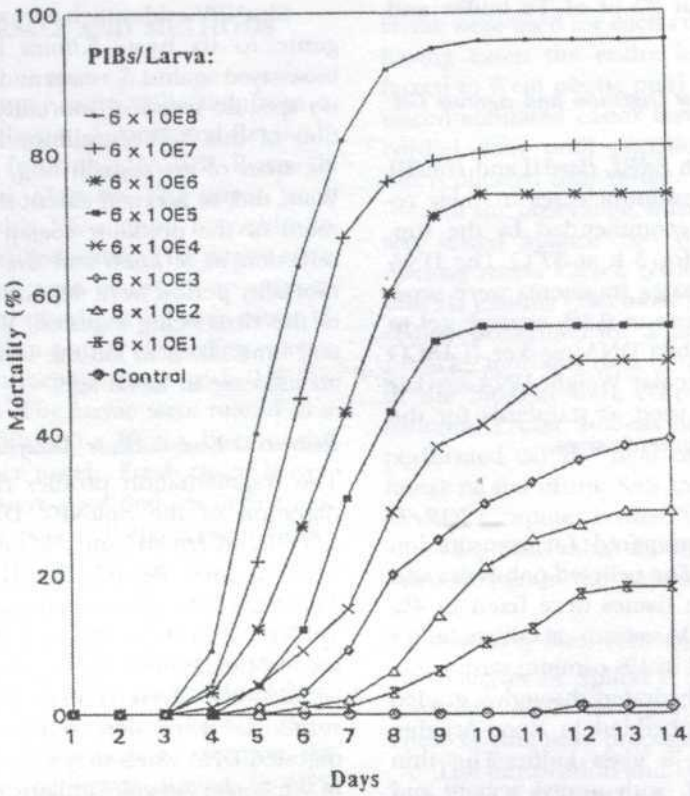

Fig. 1. Cumulative percentage mortality of S. litura larvae infected with SpltNPV

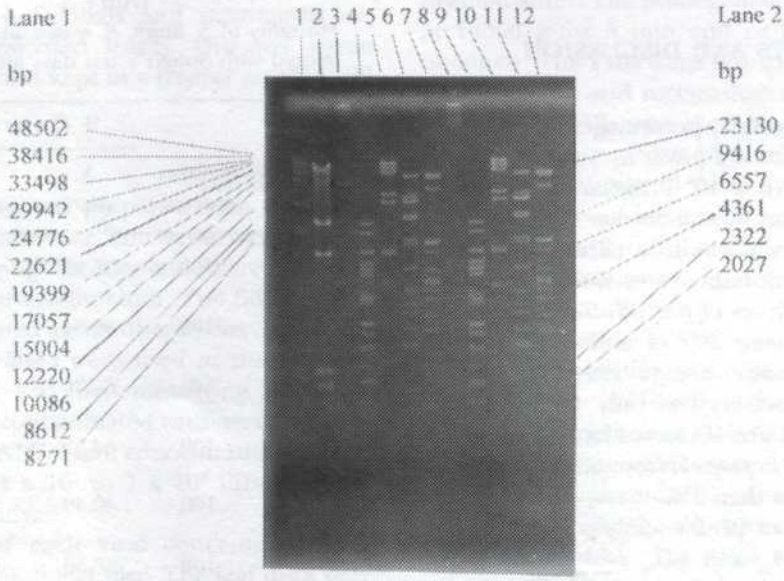

Fig. 2. Restriction endonuclease digestion of DNA fron SpltNPV

Note: lane 1, high molecular weight marker; lane 2, λDNA *Hind*III, lane 3. Undigested SpltDNA (second isolate); lane 4, *Hind*III; (second isolate);lane 5, *Bam*III (second isolate); lane 6, PstI (second isolate), lane 7, *Eco*RI (second isolate); lane 8, Undigested SpltDNA (first isolate); lane 9, *Hind*III (first isolate); lane 10, *Bam*III (first isolate); lane 11, *Pst*I (first isolate), lane 12, *Eco*RI (first isolate)

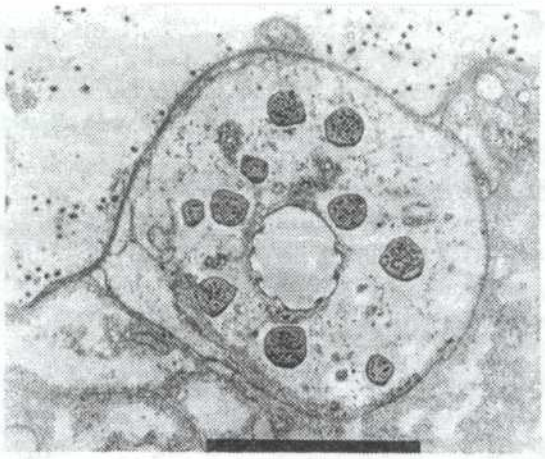

Fig. 3. SpltNPV polyhedra in the nucleus of a fat bodycell. Bar=9.3μm

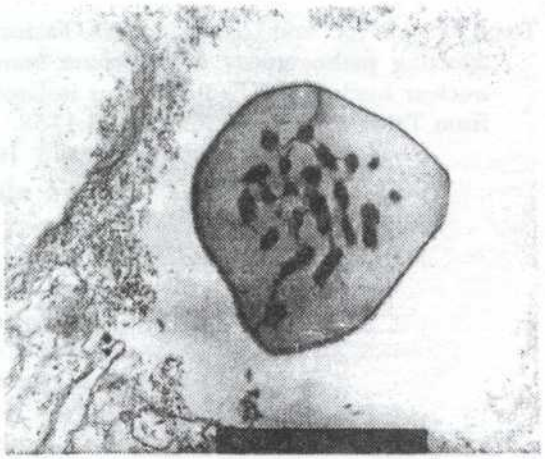

Fig. 4. A polyhedron containing a number of virions. Bar = 1.8μm

digested by *Bam*HI and *Eco*RI. Minor submolar bands, however, occurred when the two isolates were digested with *Pst*I. Such variants commonly occurred in many field-collected NPVs (Wang and McCarthy 1993). Although there was a minor difference in the restriction fragment profiles, in general both isolates exhibited similar mobility and fragmentation patterns.

Electron microscopy

The SpltNPV multiplied in the nucleus of the fat body. *Fig. 3* shows a section through an infected nucleus three days after post-infection of the larva. The polyhedra occurred in various shapes and sizes. They were round, spherical and oval, and the size ranged from 1.9 to 2.8 μm in diameter. These features resembled the polyhedra of NPVs from *S. litura* from Taiwan (Tuan *et al.* 1995), *S. exigua* (Kondo et al. 1994) and *S. exempta* NPV (Kelly 1985).

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Medicinal Properties of *Plantago major* : Hypoglycaemic and Male Fertility Studies

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ABSTRAK

Kajian ini dijalankan untuk menentukan keberkesanan ekstrak berakua Plantago major untuk merawat diabetes mellitus dan meningkatkan kesuburan lelaki. Ciri-ciri hipoglisemia ekstrak daun P. major *ditentukan dengan memberikan empat dos rawatan secara oral (100, 200, 400 dan 600 mg/kg berat badan). Salin dan glibenklamida disediakan sebagai kawalan. Ujian Toleransi Glukosa dilakukan pada masa -10, 0, 5, 15, 30, 60, 120 dan 180 minit lalu kepekatan glukosa plasma darah ditentukan dengan ujian oksidase glukosa. Keputusan kajian menunjukkan bahawa dos rawatan 600 mg/kg berupaya merendahkan aras glukosa darah tikus diabetik. Walau bagaimanapun, kesan ekstrak kurang ketara berbanding dengan glibenklamida. Dalam kajian kesuburan, ekstrak berakua biji* P. major *pada dos rawatan berbeza (30, 60, 100 dan 200 mg/kg berat badan) diberikan secara oral kepada tikus. Kesan setiap dos ke atas kepekatan sperma vas deferens selepas 120 hari ditentukan. Keputusan kajian menunjukkan bahawa dos-dos 60, 100 dan 200 mg/kg meningkatkan kepekatan sperma. Walau bagaimanapun, peningkatan aras testosteron adalah tidak ketara pada hari ke-8 dan ke-14 untuk dos 60 dan 200 mg/kg. Faktor lain yang mungkin mempengaruhi keputusan ini ialah ciri antiestrogen bijinya yang mempunyai kesan spermatigenik. Keputusan kajian ini menunjukkan bahawa ekstrak berakua* P. major *mungkin mengandungi bahan kimia untuk merawat diabetes mellitus dan masalah ketidaksuburan lelaki.*

ABSTRACT

Plantago major *extract has been traditionally used for treating diabetes and to increase male fertility. This study was conducted to verify its efficacy. The hypoglycaemic property of* P. major *aqueous leaf extract was determined by oral administration of four treatment doses (100, 200, 400 and 600 mg/kg body weight). Saline and glibenclamide were used as controls. Glucose Tolerance Test was done at –10, 0, 5, 15, 30, 60, 90, 120 and 180 minutes and the plasma glucose concentration was determined by the glucose oxidase assay. The study showed that only the 600 mg/kg dose had a significant effect in reducing blood glucose level in diabetic rats. However, the effect of the aqueous extracts was less pronounced compared to glibenclamide. In the fertility study, an aqueous extract from* P. major *seeds was given orally to rats at 30, 60, 100 and 200 mg/kg body weight respectively. The effect of each dose on vas deferens sperm concentrations after 20 days of treatment was determined. Analysis of the data showed significant increases in sperm concentrations in the 60, 100 and 200 mg/kg body weight groups. However, the trend in increased testosterone levels from day 8 to 14 in the 60 and 200 mg/kg groups was insignificant, suggestive of other factors, possibly antiestrogens in the seed extract contributing to the spermatogenic effect. The studies suggest that aqueous extract from* P. major *could contain chemicals for treating diabetes mellitus and male infertility problems.*

INTRODUCTION

In Malaysia, *Plantago major* ('Ekor Anjing' in Malay) has been used by the Chinese and Malays as a diuretic, tonic (Hyatt 1978) and cough mixture (Muhammad and Mustafa 1994). It is also a folk remedy for the blacks in South Africa (Veale *et al.* 1992), Spanish and Mexican (Conway and Slocumb 1979) and the natives in Brazil (Franca *et al.* 1996). Recent research on *P. major* has touched on anti-cancer (Lithander 1992), anti-inflammation (Nunez-Guillen *et al.* 1997), anti-oedema (Than *et al.* 1996) and anti-ulcerogenic properties (Yesilada *et al.* 1993) to name but a few. Other uses of the plant for treatment of various diseases are summarized in Table 1.

TABLE 1
Other medicinal uses of *P.major*

	Usage	References
1	Treatment for panaritium	Dharma 1987
2	To induce abortion	Conway and Slocumb 1979
3	Promote blood coagulation	Hornok 1992
4	Treatment against Leishmanial ulcers on skin	Franca *et al.* 1996
5	Remedy for gall and renal stones	Riawan and Sangat-Roemantyo 1992
6	Chronic bronchitis	Matev *et al.* 1982
7	Anti-parasitic properties against *G.duodenalis*	Ponce *et al.* 1994
10	Stimulate mucus and proteolytic activity of gastric juice	Vymyatnina 1997
11	Anti-bacterial activity	Ravn and Brimer 1988
12	Anti-nematodal	Insunza and Valenzuela 1995
13	Treatment against *Amanita sp. poisoning*	Perez-Silva and Herrera 1992
14	Treatment of mastitis	Deryabin 1991
15	Promote wound healing	Salas-Auvert *et al.* 1985

The prevalence of diabetes mellitus in the general population is on the upswing while over the past 40 years, male fertility has declined alarmingly. In traditional Malay medicine, *Plantago major* is used for treating diabetes mellitus (Muhammad and Mustafa 1994). Hypoglycaemic studies had been done locally on plant extracts such as *Akar Seruntun (Tinospora crispa)* (Noor and Ashcroft 1998), *Petai Papan (Parkia speciosa)* (Fathaiya *et al.* 1995) and fenugreek seeds *(Trigonella foenum-graecum)* (Mariam *et al.* 1995). Modern treatments such as with oral hypoglycaemic like glibenclamide and troglitazone unfortunately could cause hepatic dysfunction to the patient, which in several instances has been fatal (Watkin and Whitcomb 1998). Therefore Ajgoankar (1979) had suggested alternative treatment of diabetes mellitus, using oral administration of plant extracts based on traditional medicine.

Meanwhile, in traditional Chinese medicine, *P. major* is said to be able to increase sperm and fertility (Hyatt 1978). Historically, a number of plants have been used as sex hormones in native medicine (Farnsworth *et al.* 1975). Plants that have been used in fertility enhancement include *Panax schinseng, Trigonella foenum-graecum* (Lucas 1978) and *Epimedium brevicornum* Maxim. (Lu 1994).

The objective of this study is to verify the hypoglycaemic and fertility enhancing properties of *Plantago major* extract.

MATERIALS AND METHODS

Preparation of Extracts

Plantago major seeds were obtained from Sibu, Sarawak and were planted at UPM Biology Department's nursery for three months. The leaves were dried and boiled in 0.5 liter of water for 5 minutes. In the male fertility study, the aqueous seed extract of *Plantago major* was used. The aqueous extract was prepared using the Soxhlet extraction apparatus (Soxhlet Electrothermal, England). Both extracts were then concentrated and later freeze dried at -70°C to obtain the powder form.

Experimental Procedure

The experimental animals used were white albino rats (250-350g) of the Sprague Dawley strain. They were given food pellets (Gold Coin (M) Bhd. Pelabuhan Kelang, Selangor) and drinking water *ad libitum.*

Hypoglycaemic Study

The rats were intravenously injected with 40 mg/kg body weight of alloxan monohydrate to induce diabetes. Only rats with blood glucose concentration of 250 - 400 mg/dl were selected for the experiment.

The diabetic rats were divided into 6 groups (5 rats each). The first two groups were treated with saline 0.95% (5ml/kg) and glibenclamide (10mg/kg) as controls. The other four groups were treated with water extracts of various doses (100, 200, 400 and 600 mg/kg). Saline, glibenclamide and the water extracts were administered through oral catheter. The rats were anaesthetized with Zoletil 50 and blood samples were obtained from the tip of the tail (Noor and Ashcroft 1989).

For the Oral Glucose Tolerance Test, the rats were fasted overnight before the test. The first sampling was obtained at time -10 minute and another sampling at time 0 minute, followed by treatment administration. After 15 minutes, a glucose load of 1.5g/kg rat body weight was orally administered. Consecutive blood samples were taken at 5, 15, 30, 60, 90, 120 and 180 minutes after the oral glucose load. The blood glucose concentrations in the samples (mg% or mg/100ml) were analyzed by Glucose Oxidase method (GOD-Perid Method, Boehringer Mannheim).

Areas under the blood glucose concentration curves (AUC) were calculated by trapezoidal integration method (Campbell and Madden, 1990) whereby

Area under curve (AUC)

$$= \sum_{1}^{n-1} [(Y_i + Y_{i+1})/2][t_{i+1} - t_i]$$

In which

n	is the number of replicates
$(Y_i + Y_{i+1})/2$	is the height of the rectangle (estimated as the midpoint between Y_i and Y_{i+1}) in mg%
$t_{i+1} - t_i$	is the width of the rectangle in minutes

Male Fertility Study

The seed extract was given orally to four groups of rats with doses of 30, 60, 100, and 200 mg/kg body weight. 0.95% saline solution was used for control purpose. Treatment was given every day immediately after blood sampling using an oral catheter that was connected to a syringe. Blood samples were taken from the tail tip at days 0, 8, 14 and 20. The blood and later the serum were both centrifuged at 6500 rpm for 15 minutes and stored at –20°C before Testosterone RIA analysis (Gamma-B Testosterone Kit, IDS Ltd.).

Sperm concentrations were determined on the last day of treatment (day 20). The rats were killed and the vas deferens fluid was taken immediately using a fine needle syringe, which was inserted into the vas deferens. A 10(l aliquot of vas deferens fluid was aspirated into the syringe and then diluted to 2 ml in 0.95% saline solution and examined under light microscope. The sperm concentration was determined using a haemocytometer (Hafez 1987).

RESULTS AND DISCUSSION

Hypoglycaemic Study

The basal plasma glucose levels in the experimental animals (230-300 mg%) were higher compared to normal animals which have basal levels in the range of 80 mg% to 100 mg% (Noor and Ashcroft 1989 and Abdel-Barry *et al.* 1997). The high basal values confirmed the diabetic condition in the experimental animals. Furthermore, there is no significant difference in the readings at -10 minutes and 0 minute for all treatment groups (Figure 1). This shows that the diabetic condition in the animals was stable. After the rats were given a glucose load of 1.5g/kg body weight at time 0 minute, the plasma glucose level of the rat group treated with saline reached a peak at 30 minutes (388.38 mg%) and very gradually became lower until it reached the basal diabetic level after 120 minutes (Figure 1). Although Alloxan is believed to specifically destroy the β-cells (Chattopadhyay *et al.* 1997) there might be a chance of recovery from the drug and the surviving β-cells could still produce insulin (Chattopadhyay *et al.* 1997). This explains the gradual lowering of the blood glucose level from the peak to the basal diabetic level. In the glibenclamide-administered group, the peak blood glucose level was significantly lower (ANOVA, $p < 0.05$, $n = 5$) (349.56 mg%) compared to the saline control (388.38 mg%). This shows that the drug (glibenclamide) was effective in slowing the rate of increase in blood glucose level after a glucose load. The decrease in the blood glucose level might be due to the possibility of stimulation of the (cells to release insulin (Ligtenberg *et al.* 1997).

Since the treatment of saline and glibenclamide showed the ability of the blood glucose level to reach basal diabetic level, the results could indicate that the rats are from the Type II diabetes mellitus (Non-Insulin Dependent Diabetes Mellitus) categorised by insulin

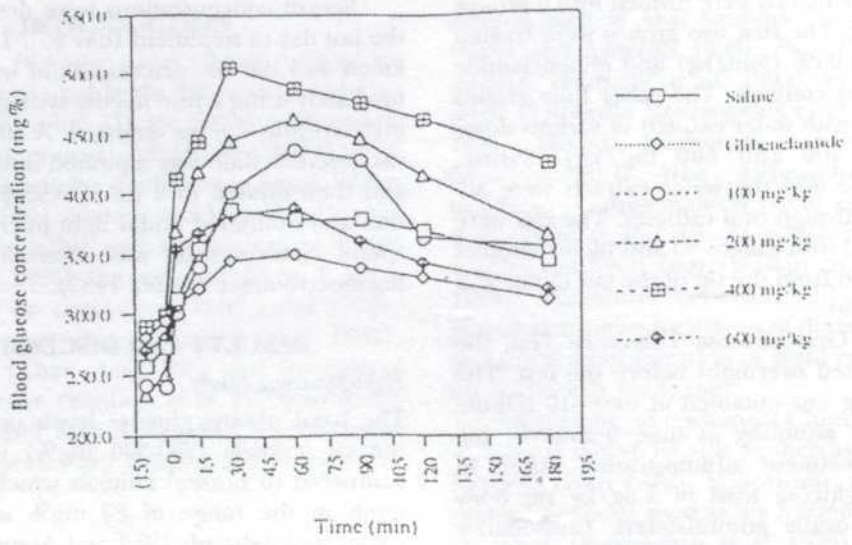

Fig. 1. Blood glucose concentration (mg%) at different time intervals (min) in diabetic rats

deficiency or insulin resistance (Ferrannini 1998). Invariably, the time taken for the high blood glucose level in saline and glibenclamide groups to reach basal diabetic level is longer (120 and 180 minutes respectively) compared to normal rats where the blood glucose returned to basal within 90 minutes to 120 minutes (Trejo-González *et al.* 1996 and Peungvicha *et al.* 1998)

The area under the graphs has been used to indicate the amount of blood glucose level due to the glucose load and also to see the effect of the extracts (Leatherdale *et al.* 1981). The area from the time of glucose load (0 minute) until the end of the experiment (180 minutes) was calculated for each treatment (Table 2). The area under the glibenclamide graph (7.4358 × 10^3 mg%minute) is significantly lower than the rest (ANOVA, $p < 0.05$, $n = 5$) (Figure 1). This could be due to the increase in insulin release and/or increase in glucose uptake by the peripheral cells stimulated by glibenclamide (Grodsky *et al.* 1963).

TABLE 2
Area under graph indicating blood glucose concentration for each treatment

Treatment	Area under graph (× 10^3 mg%minute)
Glibenclamide	7.4358 [a]
Saline	16.7434 [b]
100 mg/kg	25.9535 [c]
200 mg/kg	30.4419 [c]
400 mg/kg	29.4215 [c]
600 mg/kg	13.5818 [d]

Note : Groups labeled with different letters are significantly different compared to saline control, P<0.05

The extract of 600 mg/kg dose has an area of 13.5818 × 10^3 mg%minute, which is significantly less than that of the saline control (16.7434 × 10^3 mg%minute) but greater compared to glibenclamide control graph (Figure 1). The results showed that the extract might contain some unidentified substances that can lower the blood glucose level. However, three other lower doses of the extracts (100 mg/kg, 200 mg/kg and 400 mg/kg) did not show any hypoglycaemic properties or dose dependent effect as statistical data analysis (ANOVA, $p < 0.05$, $n = 5$) did not show any significant increase in blood glucose level compared to saline at all time intervals.

Male Fertility Study

After 20 days of treatment, the extract doses of 60, 100 and 200 mg/kg body weight significantly increased the sperm concentrations (Table 3) by an average of 18% as compared to the control group. The 30 mg/kg group failed to show any significant increase in sperm concentration compared to the control. There were also no significant differences in sperm concentrations between the 60, 100 and 200 mg groups. The

TABLE 3
Sperm concentrations of treatment groups after 20 days of treatment

Treatment	Sperm concentrations (× 107 cells per ml)
Control	123 ± 6.60 a
30 mg/kg	127 ± 6.16 a
60 mg/kg	144 ± 10.05 b
100 mg/kg	146 ± 7.04 b
200 mg/kg	144 ± 11.23 b

Note : Groups labeled with different letters are significantly different, P<0.05.
Data presented as mean ± s.d., n=4.

testosterone levels (Table 4) show a trend increase for the 60 and 200 mg/kg groups for days 8 and 14 but it is not statistically significant due to high standard error and a limited sample. The sperm characteristics on all treatment groups did not show any signs of abnormality.

The results failed to show concrete evidence to suggest testosterone's direct role in increasing sperm concentration. Other factors may have contributed to the extract's spermatogenic action. One possible explanation is that the seed extract has anti-estrogenic properties. No studies has yet been done on *P. major's* anti estrogenic properties but other plants have been known to possess this property (Kallela 1974). Anti estrogens act on the pituitary gland to stimulate the production of FSH (Teoh 1987). FSH increases the availability of germ cells at certain steps of development during spermatogenesis for entry into androgen dependant stages which included stage 19 where spermatids are released (Sharpe 1994). This coupled with the trend increase in testosterone levels may have helped to significantly increase the sperm concentrations.

CONCLUSION

Results from the hypoglycaemic studies showed that only the 600mg/kg *P. major* water extract demonstrated hypoglycaemic effect on blood glucose level in diabetic rats. The effect is, however, less pronounced compared to the established hypoglycaemic agent, glibenclamide. The use of the plant as a drug in treatment of diabetes will depend on the proper processing and dosages of the extracts in order to obtain the desired hypoglycaemic effect. In the male fertility study, the doses of 60, 100 and 200 mg/kg were able to significantly increase sperm concentrations. There was also a trend in increased testosterone levels from day 8 to 14 in the 60 and 200 mg/kg groups but it was insignificant and suggested other factors, possibly antiestrogens in the seed extract contributing to the spermatogenic effect. All together, the studies suggest that aqueous extract from *P. major* could contain chemicals for treating diabetes mellitus and male fertility problems. Further studies should be carried out to verify its hypoglycaemic and male antifertility effects.

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TABLE 4
Testosterone levels of serum (ng/ml) during the course of treatment
Data presented as mean ± s.e.m.

Treatment	Days				
	0	4	8	14	20
Control	1.21 ± 0.49	0.72 ± 0.31	1.07 ± 0.66	0.68 ± 0.27	1.16 ± 0.741
60mg/kg	1.28 ± 0.99	0.728 ± 0.44	1.53 ± 0.58	1.67 ± 0.72	0.44 ± 0.21
200mg/kg	0.83 ± 0.51	0.76 ± 0.14	1.42 ± 0.56	1.55 ± 0.74	0.35 ± 0.58

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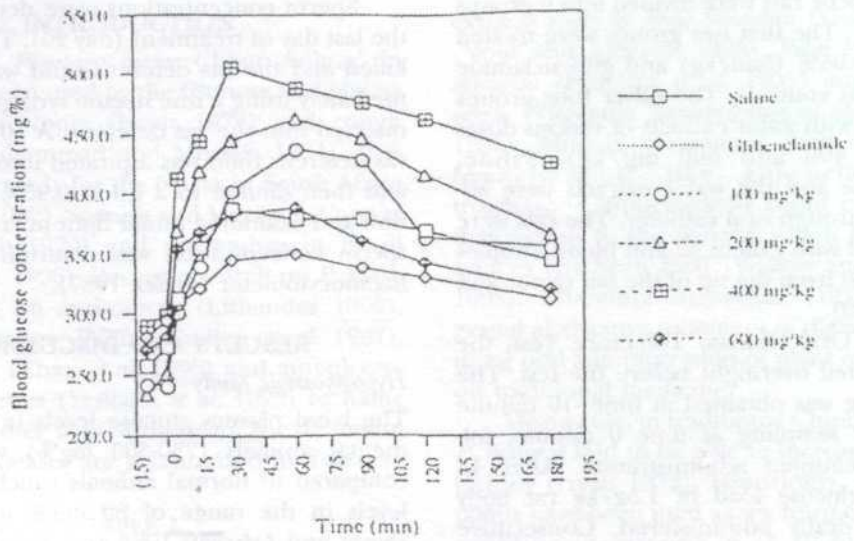

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The results failed to show concrete evidence to suggest testosterone's direct role in increasing sperm concentration. Other factors may have contributed to the extract's spermatogenic action. One possible explanation is that the seed extract has anti-estrogenic properties. No studies has yet been done on *P. major's* anti estrogenic properties but other plants have been known to possess this property (Kallela 1974). Anti estrogens act on the pituitary gland to stimulate the production of FSH (Teoh 1987). FSH increases the availability of germ cells at certain steps of development during spermatogenesis for entry into androgen dependant stages which included stage 19 where spermatids are released (Sharpe 1994). This coupled with the trend increase in testosterone levels may have helped to significantly increase the sperm concentrations.

CONCLUSION

Results from the hypoglycaemic studies showed that only the 600mg/kg *P. major* water extract demonstrated hypoglycaemic effect on blood glucose level in diabetic rats. The effect is, however, less pronounced compared to the established hypoglycaemic agent, glibenclamide. The use of the plant as a drug in treatment of diabetes will depend on the proper processing and dosages of the extracts in order to obtain the desired hypoglycaemic effect. In the male fertility study, the doses of 60, 100 and 200 mg/kg were able to significantly increase sperm concentrations. There was also a trend in increased testosterone levels from day 8 to 14 in the 60 and 200 mg/kg groups but it was insignificant and suggested other factors, possibly antiestrogens in the seed extract contributing to the spermatogenic effect. All together, the studies suggest that aqueous extract from *P. major* could contain chemicals for treating diabetes mellitus and male fertility problems. Further studies should be carried out to verify its hypoglycaemic and male antifertility effects.

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TABLE 4
Testosterone levels of serum (ng/ml) during the course of treatment
Data presented as mean ± s.e.m.

Treatment	Days				
	0	4	8	14	20
Control	1.21 ± 0.49	0.72 ± 0.31	1.07 ± 0.66	0.68 ± 0.27	1.16 ± 0.741
60mg/kg	1.28 ± 0.99	0.728 ± 0.44	1.53 ± 0.58	1.67 ± 0.72	0.44 ± 0.21
200mg/kg	0.83 ± 0.51	0.76 ± 0.14	1.42 ± 0.56	1.55 ± 0.74	0.35 ± 0.58

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Niacin Requirement of Broilers Fed on a Diet Based on Maize-Palm Kernel Meal

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ABSTRAK

Tujuh kandang yang sama dipenuhi dengan anak-anak ayam komersil berusia 25 hari diberi makanan bersalai berasaskan makanan sampingan kernel-jagung palma dengan paras niacin yang berubah-ubah supaya catuan mempunyai 15.0, 22.5, 30.0, 37.5, 45.0, 52.5 dan 60.0 mg niacin per kg. Perawatan berjalan selama 42 hari. Keputusannya bergantung pada pertambahan berat, pengambilan makanan, penggunaan nitrogen yang ketara, tenaga metabolisme, kalsium dan posporus serta ciri-ciri karkas. insiden dermatitis dan kecacatan kaki menunjukkan bahawa paras diet niacin 37.5 mg/kg mencukupi untuk menghasilkan penggunaan nutrien yang baik, mengoptimum prestasi pengeluaran dan untuk penjagaan kesihatan.

ABSTRACT

Seven duplicate floor pens with 25 day-old commercial chicks were fed a practical type of broiler diet based on maize-palm kernel meal supplemented with varying levels of niacin so that the rations had 15.0, 22.5, 30.0, 37.5, 45.0, 52.5 and 60.0mg of niacin per kg of feed. The treatments were maintained for a period of 42 days. The results based on liveweight gain, feed intake, apparent utilisation of nitrogen, metabolizable energy, calcium and phosphorus and carcass characteristics, and the incidence of dermatitis and leg deformities showed that dietary niacin level of 37.5 mg/kg feed was adequate for achieving good nutrient utilisation, optimising productive performance and for maintaining good health.

INTRODUCTION

In the Nigerian feed industry, palm kernel meal (PKM) is now widely being used *in lieu* of groundnut cake (GNC) due to the high cost and inadequate supply of the latter. The use of PKM as a substitute for GNC, causes problems on micronutrient requirement of birds due to its characteristically high fibre content and relatively poorer amino acids bioavailability (Nwokolo *et al.* 1977; Oloyo 1991; Best 1993). In this regard, niacin requirement of birds present a rather special problem when fed on PKM-based rations, especially since it has been established that the vitamin requirement is a function of composition and bioavailability of amino acids of the diet (Leeson 1988). Since maize/PKM play an important role in poultry feed formulation in Nigeria, it was felt necessary to estimate the niacin required for optimum productive performance of broilers fed on rations having these ingredients.

MATERIALS AND METHODS

A practical ration based on maize-PKM was formulated (Table 1), analysed for niacin content by the method of Association of Vitamin Chemists (1966) and then supplemented with feed-grade niacin (LONZA, Switzerland) such that the experimental diets contained 15.0, 22.5, 30.0, 37.5, 45.0, 52.5 and 60.0 mg of the vitamin per kg of ration. Each diet was given to a duplicate group of 25 day-old commercial broiler chicks (i.e., 50 birds per treatment) for a period of 42 days. Experimental chicks were housed in 14 deep litter pens, each of 5.5 m2 floor area covered with dry-wood shavings litter. Feed and water were provided in a trough feeder and two 4-litre drinkers and brooding was carried out

using 200-W Tungsten filament lamp. Throughout the period of experimentation, feed and fresh clean water were offered at all times, and routine vaccinations were administered.

TABLE 1
Composition of a practical maize-palm kernel meal based diet

Constituent	%
Yellow maize	52.0
Palm kernel meal	20.2
Blood meal	8.2
Fish meal	5.2
Wheat offal	10.3
Oyster shell	1.0
Bone meal	2.0
Vitamin/mineral premix*	0.1
Salt (NaCl)	0.2
Palm kernel oil	1.0
Total	100.0
Analysed Chemical composition	
Crude protein (%)	21.5
Gross energy (Kcal/kg)	3120.4
Calcium (%)	1.21
Phosphorus (%)	0.79
Niacin (mg/kg)	15.0
Tryptophan, calculated (%)	0.25

*Vitamin/mineral premix supplied the following vitamins and mineral elements per kg of feed: Vit. A, 1200 IU; Vit. D, 2500 IU; Vit. E, 10 IU; Menadione sodium bisulphite (Vit. K), 1.5mg; Vit. B1, 2.5mg; Vit. B2, 5mg; Choline chloride, 500mg; Calcium D-pantothenate, 10mg; Vit. B6, 4mg; Vit. B12, 0.02mg; Biotin, 0.2mg; Iron, 50mg; Manganese, 150mg; Copper, 2.5mg; Zinc, 45mg; Cobalt, 0.2mg; Selenium, 0.08mg; Iodine, 1.4mg.

The chicks were weighed individually at the commencement of the experiment (i.e. at day-old) and weekly thereafter until they were 6 weeks when the trial was terminated. Weekly records were kept of feed intake, weight gain, feed efficiency (gain/feed intake) and per cent incidence of dermatitis and of leg bone deformities.

In the last three weeks of experimentation, metabolic studies were conducted on 4 replicate samples of experimental chicks randomly selected from each group (i.e. 2 birds/replicate/treatment) at the beginning of the fourth week. In accordance with the total collection procedure, excreta were collected daily from each treatment group on 14 successive days during the fifth and sixth week for nutrient analysis. Feed and excreta were analysed for nitrogen and phosphorus (AOAC, 1980) and calcium (Perkin-Elmer Inc., 1973). Gross energy values were determined with a ballistic bomb calorimeter and apparent metabolizable energy values of diets were calculated. Apparent retention of nitrogen, phosphorus and calcium was calculated based on the difference between the amount of the constituent in the diets and excreta samples collected.

At the end of the experimentation, 4 replicate samples of birds were selected from each treatment group (i.e. 2 birds/replicate/treatment) in the floor pens. They fasted for 6 hours, after which they were weighed, slaughtered and dressed for carcass characteristic evaluation. The carcasses were weighed and dressing percentage calculated. The abdominal fat pad was carefully excised and weighed. Bones in the carcasses were carefully removed and the edible meat was separated. Care was taken to prevent loss of meat to the bones. Every effort was made to dress all the carcasses as identically as possible. Total edible meat and total bone from each of the carcasses were weighed and the meat to bone ratio was calculated. Meat and bones were also expressed as percentages of carcass weights.

All data obtained in the study were subjected to analysis of variance (Steel and Torrie, 1960) and significantly different treatment means were compared using the multiple range test of Duncan (1955).

RESULTS AND DISCUSSION

Establishment of niacin requirement of broiler chicken could be undertaken using synthetic diet, but the use of experimental ration formulated with actual feed ingredients would be beneficial for practical application. Studies by Childs *et al.* 1952; Fisher *et al.* 1955; Patterson *et al.* 1956 and NRC 1984 revealed that 18-33 mg niacin/kg of purified diet containing adequate amounts of tryptophan (0.14-0.24%) was required by chicks for normal growth and prevention of deficiency symptoms, whereas higher amounts of the vitamin (30-100 mg/kg feed) were needed by the chicks when fed practical rations (Fisher *et al.* 1955; Czarnecki *et al.* 1983 and Waldroup *et al.* 1985). Variation in the vitamin requirement of birds/and or supplementation levels was due to the types of ingredients used in the diets, niacin

bioavailability in diets and tryptophan content of diets. Practical diet based on maize/PKM and containing 0.25% tryptophan (Table 1) was supplemented with varying levels of niacin and then constituted the test diets for this study.

Data on feed utilisation for growth and health performance of broiler chicks shown in Table 2 indicated that those on up to 30 mg niacin/kg consumed significantly less feed, had poorer weight gain and weighed less at 42 days than those given 37.5-60 mg niacin/kg. Reduced feed consumption by birds given 15-22.5 mg niacin/kg might be due to the high incidence in the groups of leg bone deformities (bowing, twisting, and crippling) and development of dermal lesions in the feet, which resulted in reduction in free movement of the birds. Types of skin lesions and leg deformities that developed in these groups of birds were characteristic of niacin deficiency in young chickens (Gries and Scott 1972; Summers *et al.* 1984; Cook *et al.* 1984 and Leeson 1988) and that these symptoms appeared within the first 2 weeks of the feeding trial. In affected birds, vision was impaired because the edges of the eyelids became granular and contracted. Skin lesions of varying degree appeared around the corners of the mouth and nostrils. Dermal lesions occurred on the feet and toes with pronounced haemorrhagic fissures on the bottom of the feet to the extent that the affected birds had locomotory problem. The results indicated that while a minimum of 37.5mg niacin/kg was needed for promotion of better feed utilisation for growth, a lower level of 30 mg niacin/kg was required for the prevention of occurrence of dermatitis and for normal leg bone development.

Results in Table 2 showed significant treatment effect on apparent nutrient utilisation in broiler chicks, where higher dietary niacin levels resulted in remarkable improvement in the utilisation of nitrogen, metabolizable energy, calcium and phosphorus. This observation is in agreement with earlier report on the physiological function of niacin and its effect on the utilisation of metabolizable energy and dietary nitrogen (Lockhart *et al.* 1966). LONZA (1984) reported poor utilisation of calcium and phosphorus, which are required for normal bone formation in niacin-deficient chickens. The results of the present study indicated that while dietary level of 30mg niacin/kg seemed adequate for better utilisation of nitrogen, metabolizable energy and calcium, it was inadequate for phosphorus utilisation.

TABLE 2
Performance, deficiency symptoms and apparent nutrient utilisation of broilers fed varying levels of niacin

Parameter	Dietary niacin levels (mg/kg)							
	15.0	22.5	30.0	37.5	45.0	52.5	60.0	±SEM**
Performance:								
Body weight at 42 days (g)	1099.2c*	1204.1b	1279.5b	1590.4a	1552.8a	1569.6a	1578.0a	96.85
Body weight gain (g/bird/day)	25.1b	27.6b	29.4b	36.8a	35.9a	36.3a	36.5a	2.31
Feed intake (g/bird/day)	86.4b	88.0b	91.2b	117.0a	112.6a	113.8a	116.4a	6.60
Feed efficiency (g gain/g feed)	0.29	0.31	0.32	0.31	0.32	0.32	0.31	4.949E-03
Deficiency symptoms:								
Incidence of dermatitis (%)	10a	4b	0c	0c	0c	0c	0c	1.77
Incidence of leg deformities (%)	6a	2b	0c	0c	0c	0c	0c	1.05
Nutrient Utilisation:								
Nitrogen retention (%)	53.7b	54.8b	65.6a	67.8a	66.2a	66.7a	65.5a	2.76
Metabolizable energy (Kcal/kg)	2750.0b	2774.2b	2884.6a	2865.5a	2890.4a	2876.0a	2882.8a	27.04
Calcium retention (%)	65.4b	67.4b	72.9a	72.6a	74.8a	73.2a	73.7a	1.65
Phosphorus retention (%)	55.2c	58.6c	64.8b	74.1a	72.5a	72.6a	73.4a	3.62

*Mean values in a row bearing different subscripts differ significantly at P<0.05
**SEM, standard error of the mean

TABLE 3
Carcass characteristics of broilers fed varying levels of niacin

Parameter	Dietary niacin levels (mg/kg)							
	15.0	22.5	30.0	37.5	45.0	52.5	60.0	±SEM**
Carcass weight (g)	687.0b*	759.8b	835.5b	1094.2a	1073.0a	1087.7a	1084.1a	82.64
Dressing percentage	62.5b	63.1b	65.3ab	68.8a	69.1a	69.3a	68.7a	1.38
Total edible meat (g)	434.2c	484.0bc	566.5b	745.2a	732.9a	735.3a	737.2a	62.69
Meat (% carcass weight)	63.2b	63.7b	67.8a	68.1a	68.3a	67.6a	68.0a	1.03
Total bone (g)	224.6b	246.9b	243.1b	326.0a	317.6a	330.6a	323.0a	21.62
Bone (% carcass weight)	32.7a	32.5a	29.1b	29.8b	29.6b	30.4b	29.8b	0.67
Meat : bone ratio	1.93b	1.96b	2.33a	2.29a	2.31a	2.22a	2.28a	0.08
Abdominal fat pad (g)	28.2a	28.9a	25.9ab	23.0b	22.5b	21.8b	23.9b	1.31
Abdominal fat (% carcass weight)	4.1a	3.8ab	3.1b	2.1c	2.1c	2.0c	2.2c	0.41

* Mean values in a row bearing different subscripts differ significantly at P<0.05
** SEM, standard error of the mean

The significance of producing carcass of desirable quality for a profitable broiler production enterprise emphasized the importance of evaluation of carcass characteristics of the experimental chicken. With the exception of fat deposition in the abdominal region of chickens, information is lacking on the niacin requirement for all the parameters of carcass quality examined in this study. A higher niacin level is required for desirable carcass characteristics as evident from the significant dietary treatment effect on the parameters (Table 3). A level of 30 mg niacin/kg appeared to be adequate for both meat and bone expressed as per cent of carcass weight and meat to bone ratio. It was only marginal for dressing percentage and abdominal fat pad and seemed inadequate for carcass weight, total edible meat and bone weights.

The significant depression in abdominal fat deposition in chickens at higher dietary levels of niacin is in agreement with earlier report (Waldroup *et al.* 1984) and it is a desirable feature. Excessive abdominal fat in broiler carcasses from the consumerís view point can be objectionable and undesirable because it resulted in reduced processing yield and greater cooking losses, and from the production economicsí view point, it is wasteful as it is an uneconomical conversion of dietary energy (Bartov *et al.* 1974). The problem associated with disposal of fat contaminated water at poultry processing plants (Kubena *et al.* 1972, 1974) adds further to the undesirability of the presence of excessive abdominal fat in broiler chickens.

From the foregoing results, therefore, it may be concluded that a dietary niacin level of 37.5 mg/kg feed in a maize-PKM based diet was adequate for optimum feed and nutrient utilisation, prevention of dermatitis and leg deformities, and production of desirable carcass quality in chicken.

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A Study on Surface Wash and Runoff Using Open System Erosion Plots

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ABSTRAK

Kajian tentang hakisan dan aliran permukaan ini yang menggunakan plot hakisan sistem terbuka dijalankan di hutan simpanan Tekala, Hulu Langat, Selangor. Tujuannya ialah untuk mengukur satu proses geomorfologi yang penting tentang hakisan dan aliran permukaan. Kadar variasi bagi kedua-dua hakisan dan aliran permukaan dianalisis dengan merujuk kepada sifat tanih kawasan kajian dan 14 parameter hujan. Hasil kajian menunjukkan bahawa kadar hakisan berada di antara 49.5 g m^{-2} yr^{-1} ke 137 g m^{-2} yr^{-1} dengan purata sebanyak 85.04 g m^{-2} yr^{-1}. Sedimen ampaian merupakan lebih 80% daripada jumlah sedimen yang diangkut oleh hakisan permukaan. Kadar purata tentang jumlah aliran permukaan ialah 133.8 lm^{-2} yt^{-1}. Hasil kajian juga menunjukkan bahawa korelasi antara hakisan permukaan, aliran permukaan dan sifat tanih adalah berbeza-beza. Bagaimanapun, korelasi antara aliran permukaan dengan hakisan permukaan didapati sangat signifikan dengan kebanyakan parameter hujan. Jumlah keseluruhan hujan adalah parameter hujan yang terbaik untuk meramal kedua-dua hakisan dan aliran permukaan.

ABSTRACT

This study on surface wash and runoff using open system erosion plots was carried out in Tekala forest reserve, Hulu Langat, Selangor. Variations in the rates of surface wash and runoff were analysed with reference to soil characteristics of the site and 14 rainfall parameters. The results showed that the rate of surface wash ranged from 49.5 g m^{-2} yr^{-1} to 137 g m^{-2} yr^{-1} with an average of 85.04 g m^{-2} yr^{-1}. Suspended sediment constituted approximately 80% of the total sediment transported by surface wash. The average rate of total surface runoff was 133.8 lm^{-2} yt^{-1}. The results also showed that correlations between surface wash, surface runoff and soil characteristics varied. However, the correlation between surface runoff and surface wash was found to be highly significant with most rainfall parameters. The total amount of rainfall was most suitable rainfall parameter to predict both surface wash and runoff.

INTRODUCTION

Soil erosion resulting from deforestation and agricultural practice is a serious problem in Malaysia and the tropics in general. A recent study in the Sungai Tekala catchment, east of Selangor, indicated that soil loss under forests on moderate to steep hills can range from 0.5 t ha^{-1} yr^{-1} to 1.38 t ha^{-1} yr^{-1} (Sabry 1997) while Hatch (1981), in the Semangkok Forest Reserve, reported soil loss of 0.15 t ha^{-1} yr^{-1}. The erosion is caused primarily by the process of rainsplash erosion and surface wash. Under tropical climate, the processes of erosion are accelerated due to the fast pace of land use change such as the replacement of natural forest by agricultural land or from agricultural land to urban development. Soil loss estimates during land use conversion from forest to oil palm range from 220-250 t km^{-2} yr^{-1} while that in construction sites was reported to be phenomenal, from 40,000 – 50,000 t ha^{-1} yr^{-1} (JAS 1996).

The low rate of soil loss in forest area is attributed to the function of the forest itself. Forest protects the underlying soil from direct

effect of rainfall. The rain forest vegetation intercepts between 21.8 % and 26.0 % of annual rainfall (Nik Mohamad et.al 1979). Runoff is therefore greatly reduced. Tree roots bind soil, and under undisturbed conditions, provide relatively high infiltrability rate. The litter layer in the forest also protects the ground from rainsplash erosion. This paper reports on a soil erosion study conducted in the Sg.Tekala forest catchment using an open system erosion plot.

METHODOLOGY

Study Area

The 9.8 km^2 Sungai Tekala catchment is located in the Hulu Langat, Selangor, at 3^0 3' 12" and 3^0 5' 34" N and 101^0 50' 18" and 101^0 52' 32" W (Fig. 1). Situated about 40 km east of Kuala Lumpur, Sg. Tekala is a tributary of Sg. Langat, which drains the western flank of the Main Range.

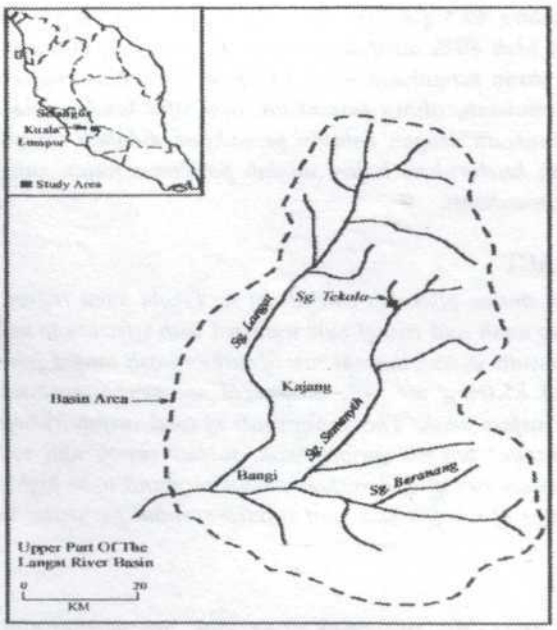

Fig. 1. Location of study area

Wash Trap Design

The wash trap used in this study was similar to that used by Gerlach (1967) but with some modification. The traps, made from sheets of zinc tin has a collection tank which measures 100 cm × 40 cm × 50 cm. The trap lip measured 100 cm × 25 cm and the cover was 100 cm × 60 cm (Fig.2). A cover was used to prevent direct rainfall entering the trap and subsequent evaporation of collected water. A divisor was fixed at the highest position so as to channel the overflow discharge to another lower collection bin as shown in Figure 2.

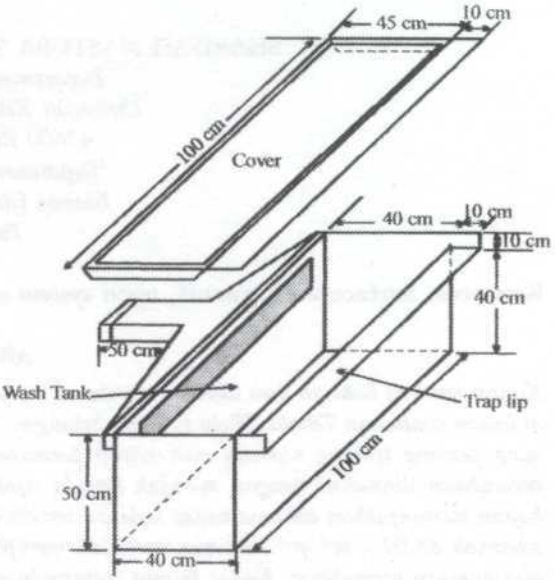

Fig. 2. Wash trap design

Location of Trap Sites

Four wash traps were installed on each of the three slope profiles, A, B, and C selected for study. Four wash traps were located on each profile. At profile A, wash traps were labeled as A1, A2, A3, and A4 and were installed on four slope segments of 8^0, 14.4^0, 23.4^0 and 40.9^0 respectively (Fig.3). At profile B, four wash traps namely B1, B2, B3 and B4 were also installed on slope segments of 70^0, 16^0, 24^0 and 39.7^0 respectively. Profile C had four wash traps namely C1, C2, C3 and C4, located at 6.5^0, 17.4^0, 20^0 and 37.2^0 respectively. The wash traps were located at convex points of the slope except for wash trap C3, which was located at concave points, while two other plots (A1 and A3) were located on an exposed ground area without any vegetative cover except for litter.

Sampling Procedure

Surface wash samples were collected from the traps after individual rainfall events over a year from 7th August 1994 to 17th August 1995. Ninety-nine samples were collected during the study period. Prior to sample collection, the water and sediment in the collecting tank were mixed so

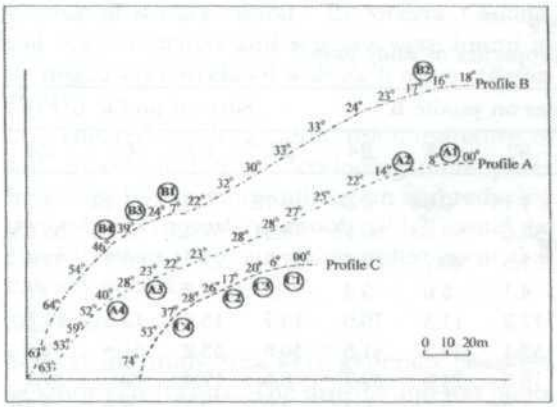

Fig. 3. Location of the open system plots at Tekala Catchment

that the samples, then collected using a one litre polyethylene bottle, represented the surface wash in the tank. The samples were then taken to the laboratory for analysis of suspended and dissolved sediment and concentration.

Rainfall Data Collection and Indices

Two rainfall stations were established in the catchment. A 16 cm rim diameter automatic reading Wilh. Lambrecht gauge was placed in the middle of profile A. A storage gauge with a 12.7 cm diameter was located nearby. The second station was sited in the Forest Department at Sg. Tekala catchment using one automatic rain gauge recorder. Both the stations were checked daily after each event during the first year. The first station recorded rainfall data from August 17, 1994 to August 17, 1995 while the second station recorded rainfall data from September to November 1996. Fourteen rainfall and erosivity indices used were based on the rainfall data collected at 15 minutes interval (Table 1).

TABLE 1
Rainfall and erosivity indices

Symbol	Description
Rainfall Indices	
AM	The amount of rainfall for each event in mm
MI	The mean intensity of each event. AM/duration (mm h^{-1})
AI_{15}	The kinetic energy (joules m^{-2} mm). Calculation on 15 min interval from KE = 29.8 – 127.5/I; I is rainfall intensity.
TKE	The total kinetic energy for each storm which was used to determine rainfall erosivity for all events together (J m^{-2})
I_{15}	Rainfall intensity index for 15 minutes.
I_{30}	Rainfall intensity index for 30 minutes.
I_{45}	Rainfall intensity index for 45 minutes.
I_{60}	Rainfall intensity index for 60 minutes.
Erosivity indices	
I_{15}	TKE the maximum sustained intensity for 15 minutes (Jeje & Agu 1990)
I_{30}	TKE the maximum sustained intensity for 30 minutes (Wischmeier & Smith 1958)
I_{45}	TKE the maximum sustained intensity for 45 minutes
I_{60}	TKE the maximum sustained intensity for 60 minutes
Evd	Daily erosivity = 16.64 Rd-173.82 where Rd is the daily rainfall (Morgan 1974)
API	Antecedent precipitation index. API = pt. 1/t or pt.kt Where pt is precipitation for a given day; t is time (number of days hours) since last rainfall; k is recession factor that is less than one but ranges from 0.85 to 0.98 (Gregory and Walling 1973)

TABLE 2
Physical and chemical soil properties of study plots

Characteristic of the Plot Site	Sites on profile A				Sites on profile B				Sites on profile C			
	A1	A2	A3	A4	B1	B2	B3	B4	C1	C2	C3	C4
Bulk density	1.12	0.78	0.96	1.08	1.02	1.15	0.96	0.88	0.78	0.82	1.20	1.17
Pore space %	57.42	70.33	63.73	59.22	61.33	56.6	63.67	66.71	70.21	67.45	54.56	55.63
Clay %	30.2	46.0	43.0	44.9	33.6	31.5	36.7	42.2	37.5	27.7	30.5	31.4
Silt %	4.4	5.0	6.0	3.3	4.4	4.1	5.6	5.4	5.3	5.2	4.2	4.5
Fine Sand	11.2	10.0	9.0	8.8	11.0	12.2	11.5	10.9	10.7	13.6	13.5	11.5
Coarse sand %	54.2	39.0	42.0	43.5	51.0	52.1	46.2	41.5	46.5	53.2	51.8	52.6
Slope Degree	8	14.4	23.4	40.9	7.0	16.0	24.0	39.7	6.5	17.4	20	37.2
Organic matter	2.71	7.06	5.45	2.45	4.09	1.76	3.36	6.44	4.97	2.11	2.8	3.19

RESULTS

Soil Characteristics and their Relationship with the Rate of Surface Wash

Bulk Density

The average bulk density in the study area ranged from 0.79 to 1.20 (g cm^{-3}) with an average of 0.99 g cm^{-3} (Table 2). Compared to other studies in Malaysia, the values were comparable at between 0.94 g cm^{-3} and 0.93 g cm^{-3} for Bukit Lagong and Bukit Mersawa respectively (Peh 1978). About 0.97 g cm^{-3} was reported for the undisturbed soils at Tekam Forest Reserve (Kamaruzaman 1987) while 1.13 g cm^{-3} was measured at the undisturbed forests of the Jengka Experimental Basin in Pahang (Baharuddin et al. 1995).

High bulk density indicates a more mineralised, compact and coarse-textured soil and therefore led to lower infiltration properties. Insignificant correlation was found between bulk density and both the rate of surface wash and the value of runoff at the study area (Table 3).

TABLE 3
Correlation coefficient between sites characteristics with both surface wash and surface runoff

Characteristics of the Plot Site	Rate of Surface Wash	Rate of Surface Runoff
Bulk density	0.31	0.20
Pore space %	-0.29	-0.20
Clay %	0.37	0.10
Silt %	0.14	0.27
Coarse Sand %	-0.32	-0.17
Fine Sand %	-0.36	0.10
Slope Degree	0.92**	0.97**
Organic matter	0.04	0.04

** Significant at the 0.001 level
* Significant at the 0.05 level

Total Porosity

The average porosity in the study area was 62.2 per cent with a range of 54.5 % to 70.3 % at C3 and A2 respectively (Table 2). High values of pore space leads to higher infiltration rates and reduced runoff. This value was found to be close to the value reported at Bukit Lagong and Bukit Mersawa which were 61.6 % and 61.1% respectively (Peh 1978). Porosity in this study were much higher than that found at Jengka Experimental Basin which was only 29% (Baharuddin *et al.* 1995). Correlation analysis showed that the total porosity was significantly and positively correlated with the amount of organic matter but insignificant and negatively correlated when calculated with the rate of surface wash and volume of surface runoff (Table 3). At the study area, it was expected that the rate of infiltration would be very high and hence led to very low surface runoff and may therefore be potentially less erodible.

Soil Organic Matter

The average organic matter was low (3.8 %) when compared to Bukit Lagong (9.8 %) and Bukit Mersawa (10.6 %) (Peh 1978). However, organic content was comparable with soils at the Jengka Experimental Basins which was 2.8 % (Baharuddin 1995).

Correlation coefficient showed that a significant relationship was found between soil organic matter and both bulk density (0.65) and pore space (0.69), but insignificantly correlated with both the rate of surface wash and the

volume of surface runoff. In Alberta, Canada, soil organic matter and soil loss were found to be negatively correlated with each other (Bryan 1974).

This reflects, in general, the importance of soil organic matter in developing more pores hence leading to more infiltration and reducing the volume of surface runoff which could be considered as the main geomorphic agent that has led to surface erosion.

Soil Texture

Soils at the study area were generally classified as sandy clay texture. The distribution was bimodal with coarse sand and clay being the two predominant textural classes (Table 4). This was probably due to the removal of fine silt and fine sand by surface wash. Two textural classes, sandy clay and sandy clay loam were identified in the study area. The former was more prevalent at profiles A and B while the latter was more prominent at profile C.

Correlation analysis on soil texture showed that clay and silt fractions did not have significant relationship with the rate of surface wash and the amount of surface runoff (Table 3), a finding similarly reported elsewhere (e.g. Bryan 1974 and Mutter and Burnham 1990). In contrast however, Epstein and Grant (1967) reported that soil loss increased in soils with high clay content. But in another study, it has been found that soil loss increased with an increase in the clay fraction up to its maximum at 19 %. Insignificant and negative correlations were found between coarse sand fraction with both the rate of surface wash and the volume of surface runoff (Table 3). In Africa, it was reported that there was a negative correlation between sandy soil and surface runoff and soil loss (Obi et al. 1989).

Particles Size Distribution of Suspended Sediment

The eroded soil contained a higher proportion of silt and fine sand compared to the insitu soil. Presumably the fine fraction (fine sand and silt) was more susceptible to erosion and transportation by surface wash than coarse sand or clay (Table 4). The findings were similar to that found in Hulu Langat, Malaysia (Sharifah Mastura, 1988) and Nigeria (Lal, 1988). It may also be attributable to the clay fraction which was found to be less erodable than sand if only the factor of rainfall was taken into consideration (Le Roux and Roos 1988).

Surface Wash and Influence of Rainfall

The Rate of Surface Wash

Surface wash ranged from 20.3 g m^{-2} yr^{-1} for plot C1 to 137.7 g m^{-2} yr^{-1} for plot A 4 (Table 5). The average rate was 85.04 g m^{-2} yr^{-1} (0.85t ha-1 yr-1). There was considerable variations in the extent of surface wash due largely to differences in slope angles and soil characteristics.

In all plots, surface wash increased directly with slope angles. The plots A 4, B 4 and C 4 with slope angles of 37^0 - 40^0 showed rate of surface wash between 130-137 gm^{-2} yr^{-2}. Surface wash was highest in March 1995 and second highest in October 1994. Lower rates occurred

TABLE 4
Suspended sediment texture of plot sites at Sg. Tekala (OSP)

Plot Site	Texture(%)							
	Clay		Silt		Fine Sand		Coarse Sand	
A1	22	*30.2*	11	*4.4*	23	*11.2*	44	*54.2*
A2	29	*46*	11	*5.0*	27	*10*	33	*39.0*
A3	29	*43*	13	*6.0*	23	*9.0*	35	*42.0*
A4	25	*44.9*	9	*3.3*	30	*8.8*	36	*43.5*
B1	17	*33.6*	11	*4.4*	30	*11*	42	*51.0*
B2	17	*31.5*	11	*4.1*	29	*12.2*	43	*52.1*
B3	16	*36.7*	12	*5.6*	34	*11.5*	38	*46.2*
B4	21	*42.2*	12	*6.4*	33	*10.9*	34	*41.5*
C1	17	*37.5*	12	*5.3*	32	*10.7*	39	*46.5*
C2	17	*27.7*	12	*5.2*	27	*13.6*	44	*53.2*
C3	18	*30.5*	9	*4.2*	33	*13.5*	40	*51.8*
C4	18	*31.4*	10	*4.5*	30	*11.5*	42	*52.6*

Data in italic indicate before erosion.

TABLE 5
Rate of surface wash and surface runoff at plot sites

Site	Slope (degree)	Rate of Surface wash (g m^2 yr^{-1})	Rate of Surface Runoff (1m2 yr-1)	Rate of Runoff/Rainfall (Q/P)
A1	8°	60.98	80.17	3.36
A2	14.4°	71.31	143.97	6.02
A3	23.4°	96.032	123.7	5.18
A4	40.9°	137.74	137.5	5.76
B1	7°	56.46	85.5	3.78
B2	16°	77.66	160.4	6.71
B3	24°	113.31	145.45	6.09
B4	39.7°	135.67	189.43	7.92
C1	6.5°	20.32	69.6	2.91
C2	17.4°	49.54	130.8	85.48
C3	20°	70.96	145.6	6.09
C4	37.2°	130.51	187.71	7.86

in February and July 1995. The monthly surface wash distribution suggest bimodal with maximum peaks occurring in March and October and two minima occurring in February and July. This was similar to the monthly rainfall distribution recorded at the study area.

In Malaysia, similar studies under forest cover land use have been carried out which used erosion plot techniques. Peh (1978) studied at Bukit Lagong and Bukit Mersawa and found that the rates of sediment loss were 0.30 and 0.31 cm^3 cm^{-1} yr^{-1} respectively. In his study at the Pasoh Forest Reserve, Negeri Sembilan, Leigh (1982) reported that the average suspended sediment transport was 0.29 cm^3 cm^{-1} yr^{-1}. Other studies on the rate of soil loss in forest area is given in Table 6.

Correlation between Surface Wash and Rainfall parameters.

The correlation coefficients were generally significant and positive for all the rainfall parameters except for mean intensity (MI), where the correlates were low but significant (Table 7). The total amount of rainfall (AM) total kinetic

TABLE 6
The rate of soil loss in forest areas

Study Area	Period of Study	Slope Angle	Plot Size	No. of Plots	Soil Loss	Author
Mesilau, Kinabalu Park Sabah	4 months	36-38%	25m x 6m	1	0.408 t ha^{-1} yr^{-1}	George (1987)
Mendolong, Sabah	10 months	19.6-42%	Open Plot	7	38 kg ha^{-1} yr^{-1}	Malmer (1996)
Semongkok F.R	5 months	25°-30°	10m x 4m	3	0.3553 t ha^{-1} yr^{-1}	Hatch (1978)
Semongkok F.R (Primary Forest)	One year	25°	10m x 4m	3	0.1491 t ha^{-1} yr^{-1}	Hatch (1981)
Semongkok F.R (Secondary Forest)	One year	25°	10m x 4m	3	0.0573 t ha^{-1} yr^{-1}	Hatch (1981)
Southwestern Nigeria (Secondary Forest)	2 years	10%	25m x 4m	1	78.9 kg ha^{-1} yr^{-1}	Jeje (1987)
Usambara Mts Tanzania	2 years	10°-15° 20°-25°	12m x 2m 12m x 2m	1	14.2 g m^{-2} yr^{-1} 10.1 g m^{-2} yr^{-1}	Lundgren (1980)
Central Himalaya India	8 months	5°-25°	20m x 20m	6	15.3-57.2 g ha^{-1} yr^{-1}	Pathak *et al* (1984)

TABLE 7
Correlation coefficients between surface wash and rainfall parameters

Rainfall Parameter	Sites on Profile A				Sites on Profile B				Sites on Profile C				Average	n
	A1	A2	A3	A4	B1	B2	B3	B4	C1	C2	C3	C4		
AM	**0.83	**0.87	**0.82	**0.86	**0.75	**0.80	**0.82	**0.79	**0.88	**0.86	**0.85	**0.73	**0.82	77
MI	**0.39	**0.49	**0.38	**0.46	**0.37	**0.41	**0.40	**0.35	**0.51	**0.43	**0.47	**0.50	**0.43	77
AI_{15}	**0.74	**0.74	**0.78	**0.85	**0.65	**0.68	**0.76	**0.74	**0.77	**0.75	**0.66	**0.79	**0.74	77
TKE	**0.81	**0.86	**0.82	**0.86	**0.73	**0.78	**0.80	**0.77	**0.85	**0.83	**0.83	**0.73	**0.80	77
EI_{15}	**0.73	**0.73	**0.79	**0.84	**0.64	**0.67	**0.75	**0.72	**0.76	**0.74	**0.65	**0.77	**0.73	77
EI_{30}	**0.72	**0.74	**0.78	**0.85	**0.63	**0.67	**0.74	**0.72	**0.75	**0.73	**0.64	**0.73	**0.72	56
EI_{45}	**0.73	**0.74	**0.79	**0.86	**0.60	**0.63	**0.75	**0.72	**0.74	**0.73	**0.62	**0.73	**0.72	45
EI_{60}	**0.74	**0.75	**0.80	**0.86	**0.60	**0.64	**0.76	**0.72	**0.74	**0.74	**0.62	**0.73	**0.73	37
I_{15}	**0.68	**0.70	**0.64	**0.69	**0.66	**0.64	**0.72	**0.68	**0.73	**0.70	**0.72	**0.76	**0.69	77
I_{30}	**0.73	**0.80	**0.72	0.77	**0.69	**0.72	**0.74	**0.72	**0.81	**0.76	**0.78	**0.76	**0.75	56
I_{45}	**0.77	**0.84	**0.78	**0.84	**0.68	**0.74	**0.78	**0.75	**0.82	**0.79	**0.78	**0.73	**0.77	45
I_{60}	**0.78	**0.86	**0.79	**0.85	**0.68	**0.74	**0.78	**0.74	**0.81	**0.80	**0.79	**0.72	**0.78	37
EVD	**0.79	**0.84	**0.80	**0.86	**0.69	**0.76	**0.78	**0.76	**0.82	**0.82	**0.81	**0.66	**0.78	44
API	**0.80	**0.81	**0.78	**0.83	**0.69	**0.65	**0.80	**0.80	**0.69	**0.82	**0.77	**0.72	**0.76	75

** Significant at 0.001 level
* Significant at 0.05 level

energy (TKE), storm erosivity (EVd) and maximum intensity for 60 minutes (I_{60}) were the highest correlated rainfall parameters of surface wash. The EI_{60} index was therefore highly correlated with the rate of surface wash as compared to EI_{15}, EI_{30} and EI_{45}. The correlation coefficients between the rate of surface wash with maximum rainfall intensity for 15, 30, 45 and 60 minutes were higher than the rainfall parameter that contained the interaction of energy and intensity (EI_{15}, EI_{30}, EI_{45} and EI_{60}). Other studies reported positive correlation between one or more rainfall parameters and the rate of surface wash for different land use such as forest and agriculture (Lal 1976, Sharifah Mastura 1988, and Armstrong 1990.

Wischmeier and Smith (1958) showed that the product of the kinetic energy of the storm and the maximum EI_{30} was most significantly correlated with soil loss. The study in Rhodesia reported that in a bare area within agricultural use, the use of EI_{30} was a better predicator for soil loss than EI_{15}. In Malaysia, Peh (1978) found that EI_{30} had the highest correlation with surface wash at Bukit Lagong. Hudson (1981) however, found that total kinetic energy of rainfall intensities which was greater than 25 mm hr^{-1} to be a more significant predictor of soil loss than the EI_{30} index. Mutter and Burnhan (1990) reported that kinetic energy was the most successful rainfall parameter related to soil erosion. At Pasoh, Malaysia, Peh (1978) found that total kinetic energy, EI_{15}, EI_{30} EI_{45} and EI_{60} were significantly correlated with sediment transport but the correlation coefficients were lower than that between the amount of rainfall and sediment transport. At Bukit Mersawa, the same author found that the total kinetic energy index and the maximum intensity for EI_{15} had the highest correlation with sediment transport.

Pinczes (1982) and Ruangpainit (1985) found positive correlation between soil loss and both amount of rainfall and intensity. In South Africa, Garland (1987) found that the best relationship was between sediment transport and I_{60}. But in America, Wischmeier and Smith (1958) found that the correlation between soil loss and the amount of rainfall as well as the maximum intensity for 5, 15, or 30 minutes intervals was poor. This result was supported by Armstrong (1990) who found that the correlation between various erosivity indices and soil loss was poor.

Highly significant and positive correlation was found between surface wash and antecedent precipitation index. Chang and Ting (1986) also reported a significant positive correlation between sediment transport and the antecedent precipitation index while Jeje (1987) found no significant correlation between antecedent precipitation index and soil loss in Nigeria.

Overall other results did not show consistency in identifying the best rainfall and erosivity indices. This is probably because the experiments have been carried out in different climatic regions with different techniques applied to it. This is also an indication of the complexities involved in erosion studies and more research is required in the future.

Relationship between Surface Runoff and Surface Wash

Rate of Surface Runoff

The rate of annual surface runoff for all erosion plots at the study area is shown in Table 5. The average rate of total surface runoff at the Sg. Telaka was 133.8 lm^{-2}, with a range from 69.6 at plot CI to 189.4 lm^{-2} at plot B4. The low surface runoff at the study area was probably due to the vegetative cover that intercepted a certain amount of total rainfall that subsequently evaporated to the atmosphere. It was found that in Peninsular Malaysia the amount of rainfall intercepted ranged from 20% to 25% (Ffolliott 1990) and 27% according to Nik Muhammad and Shaharuddin (1979). The presence of canopy provide the soil with more time to receive the rainfall and thus allowing time for more infiltration before surface runoff starts. Soil characteristics, such as low bulk density, high porosity and high percentage of sand, also led to a higher rate of infiltration, hence reducing the amount of surface runoff.

In order to compare with other studies, the rate of surface runoff was converted to rainfall (P)/ runoff (Q) coefficient or Q/P. The average percentage of Q/P for all plots at Sg. Tekala was 5.6 %, with a range of 2.91 to 7.92 %. In other areas, the Q/P coefficient is given in Table 8. The results varied from 2.3% at Jengka Pahang to as high as 6.8 % in Thailand.

As discussed above different percentages of Q/P have been reported for different rain forest areas. This showed that the tropical forest was far from being uniform, as there existed differ-

TABLE 8
The value of rainfall/Runoff coefficients

Study Area	P/Q Coefficients (%)	Author
Jengka, Pahang	2.3	Baharuddin *et al.* (1995)
Sabah	2.9	Malmer (1996)
Sabah	4	George (1987)
Nigeria	3.2	Jeje and Agu (1990)
Thailand	6.8	Thongmee and Vannapraset (1990)

ences in canopy structure, in the composition of the ground layer and in the rate of litter fall. Differences in the experimental sites and differences in plot sizes used have also caused different results. In addition, other climatic factors also contributed to the differences in the Q/P coefficient, such as the total amount of rainfall and its annual pattern of distribution.

Analysis of the monthly surface runoff was bimodal with two maximum results registered in March and October. The highest maximum value occurred in March. The two minima occurred in February and July. Overall the rate of surface runoff distribution was highly linked to the rainfall distribution of the study area.

Correlation between Surface Runoff and Surface Wash

A significant and positive correlation was found between the rate of surface wash (WS) and surface runoff (VW) at all the 12 erosion plots in the study area as shown in Table 9. Another study showed similar results (Mutter and Burnham, 1990). The strongest correlation was registered at plots C 3 anf C 4 with r = 0.96 and highly significant at 0.001 level. A lower correlation was registered at plot A 3 with r = 0.68 but the correlation was also highly significant at 0.001 level.

CONCLUSION

Soil loss from the Sg. Tekala catchment is low. Changes to its forest cover would result in considerable increase in erosion rates. The relationships between both surface runoff and surface wash with soil characteristics and 14 selected rainfall parameters were analysed. Out of the fourteen used rainfall amount, total kinetic energy, hjjstorm erosivity and maximum intensity 60 minutes indices were found to be highly correlated with the rate of surface wash. These indices, therefore, can be recommended as predictor indices for erosion studies in tropical region. Comparisons of results made with previous studies carried out in Malaysia and elsewhere showed that the results differed in some because of differences in land cover, plot size and methods adopted.

The relationship between surface wash and runoff was significantly correlated, hence the close link between the rate of surface runoff and rainfall distribution in the study area. Although the relationships between surface wash, surface runoff with soil characteristics were not clear except for slope angle, further research in this area is suggested to establish a better understanding between these parameters.

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TABLE 9
Correlation coefficient between rate of surface wash and surface runoff.

Study Plot	A1	A2	A3	A4	B1	B2	B3	B4	C1	C2	C3	C4	All
Correlation coefficient	**0.92	**0.93	**0.68	**0.79	**0.89	**0.83	**0.76	**0.81	**0.95	**0.92	**0.96	**0.78	**0.75

**Significant at 0.001 level

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Some Physical Characteristics of Sambar Deer (*Cervus unicolor*)

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ABSTRAK

*Berat badan dan ukuran badan bagi 115 rusa sambar (*Cervus unicolor*) daripada tiga negeri di Malaysia telah dianalisis. Rusa-rusa tersebut mempunyai umur di antara tiga minggu dan tujuh tahun. Ternakan ini dibahagi kepada beberapa kumpulan dan dibiarkan meragut di dalam petak-petak ragutan dipagar. Rusa-rusa yang dipelihara dalam persekitaran sama seperti kawasan semula jadi mereka mempunyai berat badan yang tertinggi (100.18 kg). Terdapat kesan lokasi, tinggi badan, panjang badan dan lilitan dada ke atas berat badan yang sangat bererti (p<.001). Koefisien regresi bagi tinggi badan, panjang badan dan lilitan dada ialah 0.91, 0.84 dan 1.00.*

ABSTRACT

*Weights and body measurements of 115 sambar deer (*Cervus unicolor*) from three states of Malaysia were analysed. The deer range in age from three weeks to seven years old. They were divided into groups and allowed to graze the fenced up paddocks. The deer which were raised in an environment similar to their natural habitat had the heaviest body weight (100.18 kg). The effects of location and partial regression of body height, body length and heart girth, had highly significant (p<.001) effects on body weight. The partial regression coefficients for body weight, body length and heart girth were 0.91, 0.84 and 1.00, respectively.*

INTRODUCTION

There are some 20 species of large herbivores in the world which have been domesticated for meat production (Clutton-Brock 1981). The concept of sustainable use of wildlife has become policy to some world bodies which reassess the true potential of the wild species as meat producers (Kyle 1994). The deer family has been domesticated in large numbers, principally in the developed world. Several Asiatic forms of deer were introduced into Britain and Europe at the end of the nineteenth century and the beginning of the twentieth century (Banwell 1993).

Recently, Malaysian importation of venison has increased rapidly, and with the present interest and awareness for deer meat, deer farming in the country is increasing in popularity (Vidyadaran 1993). Rusa deer *(Cervus timorensis)* accounts for 25.6% of the total deer population in Malaysia. The sambar deer *(Cervus unicolor)* is indigenous to Malaysia. However, the wildlife ordinance of the country prohibits the farming of this species of deer. Thus this species of deer is confined to the wildlife sanctuaries, zoos and parks.

The most extensive studies conducted to date with tropical species have been with rusa deer *(Cervus timorensis)* and chital deer *(Axis axis)*. There is a lack of biological data on sambar deer *(Cervus unicolor)*(Semiadi et al. 1994). The objective of this study was to compare some of the physical characteristics of the sambar deer in three different locations of Malaysia.

MATERIALS AND METHODS

This study was conducted on deer in three states of Malaysia, namely Perak in Peninsular Malaysia, and Sabah and Sarawak in East Malaysia. In Perak, the study was carried out in the National Park and Wildlife Department deer farm in Sungkai. In Sabah it was done in the Livestock Breeding Station, Department of Veterinary Services and Animal Industry, Sebrang, Keningau, while in Sarawak it was carried out at the Sabal Agroforestry Centre of the Sarawak Forestry Department.

A total of 115 animals were utilized in the three locations, with 44 *Cervus unicolor equinus* in Perak, while 25 animals in Sabah and 46 animals in Sarawak were of the *Cervus unicolor brookei*. The animals ranged in age from three weeks to about seven years old. The animals were divided into groups and put into different paddocks. Data gathering was carried out in 1997 and 1998.

Sungkai Deer Farm, Perak

The deer were divided randomly into four groups and put into different paddocks of varying sizes, ranging from two to six hectares. Each group comprised different numbers of stags, hinds and fawns, in accordance to the size of the paddocks they were put in. The stags within each group were at different antler development stage, thus indicating that they were at different sexual dominance level. The paddocks were generally fenced up jungle areas with some cleared parts cultivated with improved grasses, such as guinea *(Panicum maximum)* and setaria *(Setaria kazungula)*. A flowing stream meanders through the paddocks providing fresh water for the animals to drink.

The animals were allowed to graze the grass and shrubs freely. Cut leaves of some jungle trees *(Trema spp. and Sapium spp.)* were provided daily. Supplementary feeding of cattle pellets at the rate of 1.0 kg per head per day was given. Mineral blocks were given free choice.

Sebrang Livestock Breeding Station, Sabah

The deer were allowed to graze in fenced up grass paddocks which comprised mainly guinea grass. Very few shade trees were available to the animals during the hot days. However, in almost every paddock there were puddles of water where the animals wallow and cool themselves during the day. Supplemental feeding of cattle pellets was given at the rate of 1.0 kg per head per day. Mineral blocks were given free choice.

The drought from February to June 1998 had affected the growth of grass very severely. Almost the entire pasture areas were devoid of grass during that time. This has led to all the deer losing their weights and body conditions.

Sabal Agroforestry Centre, Sarawak

The deer were kept in semi-extensive system similar to their natural habitat, that is, within the secondary forest. The animals browsed on a wide range of feeding stuff, as forest shrubs and undergrowth. However, due to the deer's intense grazing habit, the paddocks within the fenced up areas were depleted of undergrowth. Freshly cut green herbage were given to the deer at the rate of 6-7 kg per head per day, besides the supplementation of 1.0 kg per head per day of cattle concentrate.

Data Collection

The measurements taken were body weight, height at withers, heart girth and body length. The body weights were taken either immediately after immobilization in the case of the Perak farm, or when they pass through the chute and the weighing scale, for the Sabah and Sarawak farms. Body height was taken as the vertical measurement from the ground to the shoulder. The heart girth was measured immediately behind the shoulder (Rabinowitz 1993 and Sharples and Domelow 1990), while the body length was measured horizontally, from the tip of the nose to the dock of the tail. These activities were done in the mornings in order to avoid the animals from being exhausted under the heat of the sun.

Yerex (1987) indicated that to get the best results out of the deer, it was important that they were not subjected to stress. The deer were very temperamental and easily excitable (Semiadi et al. 1994). Thus, at the Sungkai farm, measurements of the physical characteristics of the deer were taken after they were immobilized by using chemical restraints, with the aid of high velocity dart guns, administered by trained personnel from the National Park and Wildlife Department. A successful immobilization depended upon the right technique (Keep 1984). The target area for the dart was generally around the upper area of the hind leg, or over the shoulder and in the neck, as recommended by

English (1984). Excessive stress may result in the death of the deer.

Once the deer was immobilized, its body weight was immediately taken, using a mobile platform mechanical weighing scale that was moved as close as possible to the place where the deer fell. The animal height, was measured by using a cattle measuring tape. Subsequently the body length, and the heart girth, were also measured. After all the measurements were taken, the deer was given the antidote and it was standing again within a few minutes. The antidote was generally administered intravenously.

At the Sebrang Livestock Station and the Sabal Agroforestry Centre, the deer were herded through holding yards into the dark house where the measurements of the physical characteristics were taken while the animals were standing. This was possible due to the availability of chutes and squeezes which were used to restrain the animals. The body weights were taken using the mechanical or electronic weighing scale located within the holding yard. Although the deer in these two locations were considered docile, there were instances when they jumped and ran away once they were excited. Heart girth measurement was not recorded for the deer in Sarawak.

RESULTS AND DISCUSSION

The body weights and body dimensions were analysed using the General Linear Model procedure (SAS, 1994). The data were analysed across locations and within location, to determine the effects of various factors on live weight of the deer.

Table 1 shows the mean squares for body weight of deer adjusted for sex and location. There was no significant effect of sex on body weight although the males were slightly heavier than the females (86.59 kg vs. 81.83 kg). Locations had a very highly significant ($p<.001$) effect on body weight. The highly significant effect of location on weight could be due to different management systems of the deer. The deer at the Perak farm, with a mean height of 100.18 ± 1.64 cm and a mean weight of 97.39 ± 4.58 kg, had the largest body size compared to the deer from Sabah and Sarawak.

The deer from Sabah had a mean height of 87.00 ± 1.75 cm and a mean weight of 79.08 ± 5.17 kg, while the deer from Sarawak had a mean height of 82.33 ± 2.04 cm and a mean weight of 70.51 ± 5.29 kg. In the Perak farm, the paddocks had more natural shade, thus giving an environment similar to their natural forest habitat. In Sabah, the paddocks were more open and had very little shade for the deer. The presence of shrubs and undergrowth for browsing for the deer in Perak may be the cause of better body growth and weight gain, and this was supported by the report by Semiadi et al. (1995). The weight to height ratios of the deer in the three locations also suggested that the animals in Perak had the heaviest weight per unit height.

The partial regression of body height and body length had very highly significant ($p< .001$) effects on body weight of the deer in the three locations (Table 1). The partial regression coefficients for body height on body weight was 0.91 and for body length on body weight was 0.84.

TABLE 1
Mean squares from the analysis of covariance for body weight of deer from three locations

Source	df	MS
Sex	1	391.8889
Location	2	2619.2934***
β_{Height}	1	4664.2357***
β_{Length}	1	14528.3401***
Residual	101	244.3393

*** $p<.001$

The mean and standard error for various characteristics of the deer is presented in Table 2. The deer had an overall mean weight of 82.98 ± 3.14 kg, with a maximum weight of 164.0 kg. This mean value fell within the weight range for adult Australian Fallow deer (English 1984), even though they were much lighter than the weight of full grown wild Australian Sambar stag or hind (Slee 1984). Although the deer in Sabah were exposed to severe drought and lack of forage, they were able to maintain their body conditions (weight loss ± 10 kg). With the availability of good feed and conducive environment for the deer in Perak, they exhibited the heaviest mean weight, followed by the deer in Sabah, while the lightest were the deer in Sarawak. Under normal conditions, deer of similar age from Sabah and Sarawak will be slightly smaller than those from Peninsular Malaysia (Whitehead 1972). This was probably due to the subspecies difference that existed between them.

Table 2
Mean and Standard Errors for various characteristics of the deer

		Height (cm)	Weight (kg)	Length (cm)	H. girth (cm)
Overall	n	115	112	110	68
	Mean	90.17	82.98	133.40	108.65
	S.E.	1.33	3.14	2.57	2.05
Perak	n	44	44	44	44
	Mean	100.18	97.39	153.28	113.45
	S.E.	1.64	4.58	3.41	2.61
Sabah	n	25	25	25	24
	Mean	87.00	79.08	130.68	99.83
	S.E.	1.75	5.17	3.10	2.46
Sarawak	n	46	43	46	-
	Mean	82.33	70.51	118.02	-
	S.E.	2.04	5.29	3.79	-

The overall mean height for the deer was 90.17 ± 1.33 cm, with 100.18 ± 1.64 cm being the mean height for the deer in Perak. The tallest deer which came from the Perak herd had a height of 121.0 cm. This height was between that for the full grown wild sambar stag and hind (Slee 1984). The deer had an overall mean body length of 133.40 ± 2.57 cm, with those in Perak having the mean of 153.28 ± 3.41 cm. The larger size of the deer in Perak is attributed to the larger number of mature animals. The mean heart girth of the overall deer was 108.65 ± 2.05 cm. This measurement is indicative of the body condition of the deer. The deer in all locations, although were fed free choice, have quite limited natural resources. This, in part, was due to the limited areas available which encouraged the grazing pattern of the sambar deer to modify the forage nutritive value of the area, as was indicated by Semiadi et al. (1993). This was demonstrated by the rate of prehending biting of the sambar deer which was 35% faster than that of other species of deer.

The mean squares for body weight of deer from Perak and Sabah, adjusted for sex and location, is presented in Table 3. A separate analysis of covariance for the animals in these two locations was performed in order to include the effect of heart girth. Sex and animal height did not contribute significantly to the difference in body weight. Location had a significant ($p<.05$) effect on body weight of deer with those from Perak being 18.31 kg heavier than those from Sabah. Body length had a significant ($p<.05$) effect on body weight, with a regression coefficient of 0.43. This indicated that for the deer in the two locations, for every one cm increase in body length there was an increase of 0.43 kg of body weight. Heart girth had a very highly significant ($p<.001$) effect on body weight, with a regression coefficient of 1.00. This showed that for every one cm increase in heart girth there was an increase of 1.0 kg of body weight. This situation was very evident in the animals. The deer with a small frame would weigh very light as compared to those which were well built and had a large frame.

TABLE 3
Mean squares from the analysis of covariance for body weight of deer from two locations

Source	df	MS
Sex	1	235.7274
Location	1	935.2422*
β_{Height}	1	422.2526
β_{Length}	1	989.9446*
$\beta_{heart\ girth}$	1	3814.2381***
Residual	57	175.9373

* $p<.05$
*** $p<.001$

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Preparation of Manuscript

A full article should not exceed 10 printed pages (one printed page is roughly equivalent to 3 type-written pages) including figures and tables.

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Abstracts in Bahasa Melayu and English, each not exceeding 200 words, should follow immediately after the names and affiliation of author(s). Papers from outside of Malaysia may be submitted with an English abstract only.

Keywords
Up to a maximum of 10 keywords are required and they should be placed directly below the abstract.

Illustrations & Photographs
Illustrations including diagrams and graphs will be referred to as 'figures' and photographs as 'plates' in the text and numbered consecutively in Arabic numerals. All photographs (glossy black and white prints) should be supplied with appropriate scales.

Illustrations should be of print quality; outputs from dotmatrix printers are not acceptable, Illustrations should be on separate sheets, about twice the size finished size in print. all letters, numbers and legends must be included on the illustration with the author's name, short title of the paper, and figure number written on the verso. A list of captions should be provided on a separate sheet.

Tables
Tables should conform to page size. Vertical lines should be avoided.

Measurements
Metric units must be used for all measurements.

Equations and Formulae
These must be set up clearly and should be typed triplespaced. Numbers identifying equations should be in square brackets and placed on the right margin of the text.

Scientific Names
Scientific names should be given for all organisms.

Abbreviations
Standard abbreviations should be used.

Citations and References
Items in the reference list should be referred to in the text by inserting, in parentheses, the year of publication after the author's name. If there are more than two authors, the first author should be cited followed by '*et al*'. The names of all authors, however, will appear in the reference list.

In case of citing an author(s) who has published more than one paper in the same year, the papers should be distinguished by addition of a small letter, e.g. Choa (1979a); Choa (1979b); Choa (1979c).

References should be arranged alphabetically according to first author. Serial titles are to be given in full.

Examples of reference citations are provided:

Monographs
Turner, H.N. and S.S.Y. Yong. 1969. Quantitative Genetics in Sheep Breeding. Ithaca: Cornell University Press.

Serials
Ho, Y.W. and A. Nawawi. 1991. Effect of carbon and nitrogen sources on growth of *Ganoderma boninense* from oil palm. *Journal of Plant Protection in the Tropics* **8**: 37-43

Chapter in Edited Book
Roberts, D.W. 1980. Toxins of entomopathogenic fungi. In *Microbial Control of Pests and Plant Diseases*, ed. H.D. Burgess, p. 441-463. New York: Academic Press.

Proceedings
Hussein, M.Y. 1986. Biological control of aphids on potatoes by inundative releases of predators. In *Biological Control in the Tropics*, ed. M.Y. Hussein and A.G. Ibrahim, p. 137-147. Serdang: Universiti Pertanian Malaysia Press.

Unpublished Materials (e.g. theses, reports & documents)
Normah, M.N. 1987. Effects of temperature on rubber (*Hevea brasiliensis* Muell - Arg.) Seed storage. Ph.D. Thesis, 206p. Universiti Pertanian Malaysia.

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