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Contents

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Effects of Spice Dust on Lung Functions and Respiratory Symptoms in Spice Factory Workers in Selangor

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Keywords: fine dust (PM10), lung function, vital capacity (VC), forced vital capacity (FVC)

ABSTRAK

Pendedahan kepada habuk rempah telah lama dikaitkan dengan penurunan fungsi paru-paru dan peningkatan simtom-simtom respirasi manusia. Kajian ini telah dijalankan untuk mengkaji kesan pendedahan tersebut ke atas fungsi paru-paru dan simtom respirasi di kalangan pekerja-pekerja tiga kilang rempah di Selangor. Pengukuran spirometri (VC, vital capacity; FVC, forced vital capacity; FEV_1, forced expiratory volume in 1 second) telah dilakukan ke atas 56 pekerja (39 lelaki, 17 wanita), yang terdedah kepada habuk halus, PM_{10} sebanyak 2496μg/m³. Subjek kajian juga mengisi satu set borang soal selidik kesihatan (soal selidik ATS yang diubahsuai) yang merangkumi simtom-simtom respirasi. 61 subjek dari UPM dipilih sebagai kawalan (36 lelaki, 25 wanita), dengan aras dedahan hanya 101μg/m³. Kajian mendapati perbezaan yang signifikan bagi VC, FVC dan FEV_1 antara subjek kajian dan kawalan bagi kumpulan lelaki dan wanita. Di samping penurunan nilai-nilai spirometri, lebih ramai subjek daripada kumpulan pekerja melaporkan kejadian simtom-simtom respirasi berbanding kawalan. Oleh itu, kajian ini mencadangkan bahawa pendedahan kepada habuk rempah di kilang-kilang berkenaan membawa kepada pertambahan kejadian simtom-simtom respirasi dan penurunan fungsi paru-paru di kalangan pekerja-pekerjanya.

ABSTRACT

Exposure to spice dust has long been associated with increased prevalence of respiratory symptoms and reduced lung function in man. This study was carried out to investigate the effect of such exposure on the workersí lung function and respiratory symptoms in three spice-processing factories in Selangor. Spirometry measurements (VC,vital capacity; FVC, forced vital capacity; FEV_1, forced expiratory volume in 1 second) were performed on 56 workers (39 males, 17 females) who were occupationally exposed to 2496μg/m³ respirable fine dust, PM_{10}. The subjects also completed a set of standard respiratory questionnaires (modified ATS questionnaires). 61 persons from Universiti Putra Malaysia (36 males, 25 females) served as controls. The PM_{10} measurement in UPM was only 101μg/m³. Significant differences in VC, FVC and FEV_1 were observed between the two groups for both the male and the female. In addition to the decrease in spirometric values, the workers also reported higher prevalence of respiratory symptoms compared to controls. Therefore, the study suggests that exposure to spice dust in the spice factories leads to an increased prevalence of respiratory symptoms and impaired lung function.

INTRODUCTION

As a multi-racial country, Malaysians enjoy a variety of dishes; many are hot and spicy. One of the major food ingredients is spice; dried parts of various plants cultivated for their aromatic and pungent components. The spice includes chili pepper, cinnamon, coriander, ginger, garlic etc (Zuskin *et al.* 1988). Because of the high demand, spice-processing factories become one of the major food-processing industries in Malaysia, involving many labourers.

Since the process of spice preparation involves grinding, the labourers are constantly exposed to spice dust. The health of workers exposed to highly dusty environment (especially particles less than 10μm) is of serious concern because it has been implied that chronic pulmonary problems afflict one of every five persons

exposed to dust. Such problems include reductions in spirometry values, increases in chest tightness, and also wheezing (U.S. National Research Council 1989).

Occupational exposure to spice dust has been reported to cause allergic reactions manifested by dermatological, gastrointestinal or neurological symptoms (Zuskin *et al.* 1988). Adverse effect of the exposure on the respiratory system has been widely reported elsewhere. Brooks (1985) reported an association between numerous spices and occupational asthma. The spices includes garlic dust (Felleroni *et al.* 1981), cinnamon (Uragoda 1984), coriander, mace, ginger and paprika (Toorenenbergen and Dieges 1985), and buckwheat aerosols (Gohte *et al.* 1983). Fuller *et al.* (1985) reported irritation of the airways in relation to inhaled capsicum aerosols. In terms of other respiratory symptoms, Uragoda (1966) observed a very high incidence of sneezing, runny nose and cough among workers occupationally exposed to chili peppers. Blanc *et al.* (1991) confirmed the association between the exposure with complaints of cough. A high percentage of upper respiratory tract infections (URTI) symptoms including sneezing and runny nose was also observed in spice grinders in Singapore (49.2%) as reported by Chan *et al.* (1990).

Despite the above-mentioned evidence, no study on this occupational hazard has yet been reported in Malaysia although there are a large number of spice-processing factories in this country. This study was carried out to investigate the effect of exposure to respirable spice dust (PM_{10}) to the lung function and respiratory symptoms of workers employed in three spice factories in Selangor.

MATERIALS AND METHODS

This study involved a total of 117 participants and the usage of health questionnaires (for respiratory symptoms survey), a spirometer (lung function test) and a diaphragm pump (dust measurement). All instruments were calibrated prior to every session of test in every study location.

Subjects and Locations

Three similar spice factories located in Selayang, Puchong and Rawang were randomly selected as the study locations to represent spice factories in Selangor and Universiti Putra Malaysia (UPM) for the control.

Since smoking and asthma are known to be among the dominant confounders in spirometry studies, only those who were non-smokers and non-asthmatics were randomly selected to perform the spirometry test. The selected 56 workers (39 males, 17 females) from the 3 factories were constantly exposed to mixed spice dust including coriander, turmeric, chili, pepper, cardamom and cloves during the work-shifts. Almost all subjects did not wear masks to protect against dust inhalation. Such exposure was not experienced by the 61 controls (36 males, 25 females).

All parameters known to be major confounders in spirometry studies (sex, age, height, race) were taken into account in the analysis (Table 1).

TABLE 1
Comparison of lung function measurements between study groups

	Male (mean ± SD)			Female (mean ± SD)		
	Controls(36)	Workers(39)	P value	Controls(25)	Workers(17)	P value
Age (years)	35.24 ± 62.46	37.05 ± 59.27	0.3934	29.96 ± 38.40	28.80 ± 33.43	0.5941
Height (cm)	163.49 ± 34.80	164.83 ± 34.53	0.2693	158.88 ± 25.65	156.25 ± 17.77	0.0479*
Weight (kg)	65.78 ± 72.36	67.78 ± 55.08	0.3431	54.00 ± 51.15	53.78 ± 20.16	0.9076
VC (L)	3.08 ± 3.24	2.65 ± 3.62	0.0005*	2.4 ± 1.45	1.75 ± 2.39	0.0000*
FVC (L)	3.14 ± 4.02	2.39 ± 3.56	0.0000*	2.46 ± 2.05	1.59 ± 2.89	0.0000*
FEV_1 (L)	2.26 ± 3.24	1.81 ± 3.18	0.0001*	1.89 ± 1.95	1.24 ± 2.72	0.0001*
FEV_1/FVC%	78.37 ± 3.05	71.36 ± 4.86	0.0311*	71.22 ± 3.42	68.74 ± 16.41	0.8472
$FEF_{25-75\%}$	1.93 ± 4.20	2.08 ± 7.37	0.4864	1.90 ± 2.90	1.38 ± 3.05	0.0065*
FMFT (s)	0.99 ± 3.36	0.86 ± 3.56	0.2564	0.84 ± 1.65	0.70 ± 0.91	0.0886

*significant difference (t-test, $p<0.05$)

Dust Measurement

Physiologically, only particles less than 10μm or PM_{10} (also termed as respirable dust) is known to be inhaled into the inner respiratory system, affecting the ventilatory lung function and also responsible for the prevalence of respiratory symptoms (Brewis 1985). Therefore, only PM_{10} was measured instead of total dust in the working areas. The PM10 concentration was determined using a diaphragm pump (Kimoto MP-1) that trapped particles less than 10μm on a 37mm diameter, 0.8μ pore size cellulose acetate filter paper. The PM_{10} concentration in $\mu g/m^3$ was calculated using the formula below:-

$$PM_{10}\ (\mu g/m3) = \frac{W(g) \times 10^9}{F(L/min) \times 10^{-3} \times T(min)}$$

W = weight of particles trapped on filter paper in gram
F = flow rate of air drawn into the sampling device (2L/min)
T = duration of sampling

The aerial sampling in both UPM and the factories was done continuously from 9.00 am-5.00 pm (working hours). The machine was placed as close to the workers as possible without disturbing them.

Lung Function Tests

Lung function tests were performed by the subjects during working hours using a spirometer (Vitalograph, England; ATS standards) with standard techniques (American Thoracic Society 1979). Each subject performed at least three attempts of VC (vital capacity) and FVC (forced vital capacity) with a gap of at least a minute between attempts. From the best curve, FEV_1 (forced expiratory volume in 1 second) was determined, and other parameters including $FEF_{25-75\%}$ (mid-expiratory flow volume) and FMFT (forced mid-expiratory flow time) were calculated. The measurements were then converted into BTPS unit. Height was also measured.

Respiratory Symptoms

Structured questionnaires based on the American Thoracic Society (1979) were distributed to each subject prior to lung function test. All of the participants were required to answer the questions in detail with regard to their personal and medical background, respiratory symptoms and history, smoking habit and occupational history.

RESULTS

Dust Measurement

Fig. 1 shows the values of PM_{10} concentration measured in UPM and the spice factories. In UPM, the dust concentration was only 101μg/m^3. The mean concentration in the factories was 2496μg/m^3, which was more than 20-fold higher than the control area. However, the level is far below the OSHA standards of 5000 for respirable dust for 8-hour exposure for workers. The high concentration measured in Factory 3 might be due to the non-stop working hours (2 shifts) and the fact that it was the largest operating spice factory compared to the other two. Despite the high concentration, the majority of the workers did not wear any mask.

Subjects

The subjects and controls were 16 to 59 years of age. Table 1 shows the physical background of the respondents. There is no significant difference in the physical parameters among the male

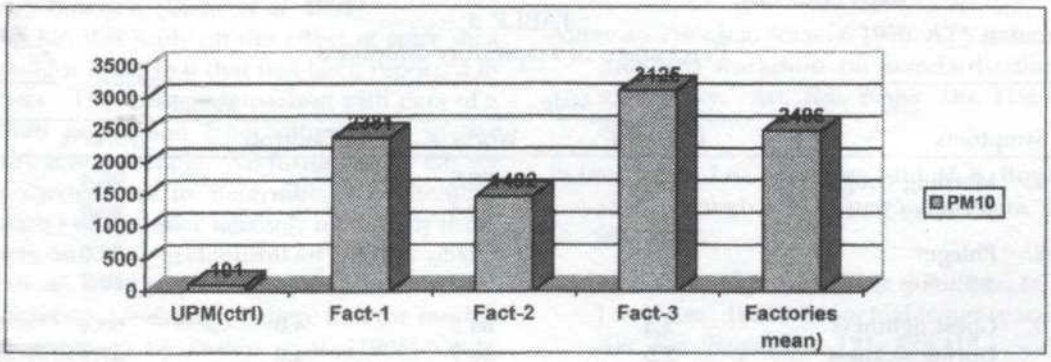

Fig. 1. Mean PM10 concentration in the control and study areas

subjects, while for the females, a significant difference was observed in height (t-test, $p<0.05$).

Lung Function Tests

Table 1 also shows the spirometry values of the subjects. The workers performed significantly lower VC, FVC and FEV_1 compared to controls (t-test, $p<0.05$) for both the male and female groups respectively. The male workers also exhibited lower $FEV_1/FVC\%$ compared to controls, suggesting a possible obstructive problem in their lungs. Since other confounders such as age and height between the male workers and controls did not show any significant difference, a reduction in their lung functions could possibly be associated with exposure to spice dust. In the female groups, the reductions in lung functions of the workers were expected due to the significantly lower values of height compared to controls. However, the reductions might also be attributed to the additive effect of exposure to high concentration of spice dust during working hours.

Table 2 shows the spirometry values of the male workers according to period of employment. The male workers (no difference in other physical characteristics) who had worked more than 5 years showed significantly lower mean values of VC and FVC compared to those with less duration of service. These statistical results suggest that lung function might worsen if the workers are constantly exposed to spice dust over a long period of time.

Respiratory Symptoms

Table 3 compares the prevalence of chronic respiratory symptoms in the workers and control subjects. The most frequently reported symptoms was morning coughs, experienced by more than 80% male workers compared to none for the controls; followed by chest tightness, experienced by most of the workers especially during work-shifts. The female workers showed a higher percentage of respiratory symptoms compared to controls and the male groups.

TABLE 2
Spirometry values of male spice workers according to period of employment in the spice factories

	Period of employment		
	Less than 5 years	More than 5 years	P-value
Number of subjects	15	24	
Age (years)	33.12 ±11.58	37.48 ± 8.71	0.1359
Height (cm)	164.89 ± 5.69	162.04 ± 5.66	0.0798
Weight (kg)	64.85 ± 9.39	66.76 ±14.45	0.5761
VC (L)	2.85 ± 0.44	2.44 ± 0.64	0.0105*
FVC (L)	2.58 ± 0.32	2.22 ± 0.72	0.0250*
FEV1 (L)	1.85 ± 0.35	1.77 ± 0.40	0.5694
FEF25-75%	1.95 ± 0.70	2.21 ± 1.54	0.4359
FMFT (s)	0.94 ± 0.59	0.78 ± 0.54	0.3191

*significant difference (t-test, $p<0.05$)

TABLE 3
Percentage of respiratory symptoms

	Male		Female	
Symptoms	Controls	Workers	Controls	Workers
1. Morning cough	–	86.3	–	96.4
4-5 times a week	–	29.4	–	25.0
2. Phlegm	2.5	49.0	4.0	50.0
4-5 times a week	–	9.8	–	10.7
3. Chest tightness	2.5	84.3	4.0	96.4
During sickness	2.5	62.7	4.0	57.1
During workshift	–	76.5	–	75

DISCUSSION

Our study suggests that constant exposure to high levels of spice dust in spice factories (even below the OSHA standards) might have possible adverse effects on the lung functions of the workers. Studies done on Yugoslavian spice workers showed similar findings even though the workers were exposed to a much lower dust concentration (Zuskin *et al.* 1988).

Despite the homogeneity in age, height and weight (determinants of lung capacity) between the participants, the workers showed significantly lower values of VC, FVC and FEV_1 compared to controls. Therefore, the reduction might be attributed to the difference in exposure level between the study groups.

The effect of spice dust on the workers was further evidenced by the significantly lower spirometry values shown by workers who have worked for more than 5 years, despite the insignificant difference in other confounders compared to those with less period of service. This observation strengthens our hypothesis that reduction in lung function is strongly associated with the constant exposure to spice dust over a long period of time.

Besides this chronic decrease in lung function, Zuskin *et al.* (1988) also reported acute reductions in lung functions after a work-shift in spice factory workers. Other researchers had also observed similar trend in workers exposed to tea and coffee dust (Jayawardana and Udupihille 1997; Zuskin *et al.* 1979; Zuskin and Skuric 1984).

As expected, higher prevalence of respiratory symptoms was reported by the workers. This phenomenon is in perfect agreement with other studies elsewhere, some of which had reported a higher incidence of respiratory symptoms even without significant reduction in pulmonary function (Blanc *et al.* 1991).

So far, this study on the effect of spice dust in Selangor is the first that had been reported in Malaysia. Therefore, comparison with data of a matched population from other parts of the country is not possible. No further study has yet been carried out to determine the chemical properties of the spice aerosols inhaled by these workers and the mechanisms of toxicity of the aerosol on their respiratory system.

However, we strongly believe that the mechanisms proposed by Zuskin *et al.* (1988) could play an important role in reducing lung function and increasing the prevalence of respiratory symptoms in the spice workers. The mechanisms include hyperreactivity due to increased permeability of the airway mucosa to irritants, resulting in the direct effect on the airway muscle damage to the airway mucosa, as represented by the presence of cough in most of the workers (Nadel *et al.* 1954; Boushey *et al.* 1980;) repeated damage of the airway epithelium (Widdicombe 1954); and development of inflammation in the airways causing airway responsiveness (Cooper *et al.* 1986).

Zuskin *et al.* (1988) also suggested that the adverse effects of spice dust might be due to the release of mediators in the airway that might constrict airway smooth muscle directly or by reflex. Using disodium cromoglycate (DSC), they reported that spice dust affects airway cells causing the release of these mediators and therefore concluded that food spice has a bronchoconstrictor potential, resulting in reduced lung function and increased prevalence in respiratory symptoms, as observed in this study.

CONCLUSION

Observation from this study suggests that exposure to high PM_{10} of spice dust in spice-producing factories in Selangor leads to increased prevalence of respiratory symptoms in the workers. The decrease in lung function among the workers also suggests that they might be facing other acute and chronic lung diseases. In addition, the high concentration of dust measured in the factories suggests that there is a need to improve the ventilation in these factories, and introduce personal protective equipment such as mask, in order to safeguard the respiratory system of workers.

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Leaf Growth and Stomatal Sensitivity after Water Stress Relief and its Relation to Xylem Sap Absicisic Acid

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Keywords: pepper (Capsium annuum); after effect: water stress: water relations: stomatal conductance: absicisic acid

ABSTRAK

*Pengaruh tanaman cili (*Capsicum annuum *L. var Bell Boy) yang di beri pemulihan tegasan air yang di berikan secara perlahan telah di kaji. Pokok di beri tegasan air selama 5 dan 10 hari kemudian di beri pengairan semula. Pertumbuhan daun, katian air, konduksi stomata dan kandungan asid absisik (ABA) di dalam xilem di bandingkan di antara pokok yang didedahkan kepada tegasan air dan tanpa tegasan air. Potensi air daun telah pulih dengan cepat apabila pokok yang didedahkan kepada tegasan air di beri pengairan semula tetapi konduksi stomata tidak pulih sehingga 48 jam selepas di beri pengairan. Kepekatan ABA di dalam xilem menyamai kepekatan ABA pada pokok yang disiram air berterusan selepas 6 jam di beri pengairan. Selepas diberi pengairan, pertumbuhan pokok diberi rawatan tegasan air selama 11 hari adalah cepat dan melebihi pertumbuhan pokok yang didedahkan kepada pengairan berterusan. Pokok yang berada pada tegasan air berterusan menunjukkan pengurangan pertumbuhan daun, konduksi stomata dan pertambahan kepekatan ABA didalam xilem.*

ABSTRACT

Responses of pepper seedlings to rewatering after being subjected to 5 and 11 days of gradual water stress were investigated. Leaf growth, water relations, stomata conductance and xylem sap ABA in these plants were compared with plants grown under continuous well watered and stressed conditions. Leaf water potential returned to the control values immediately after rewatering but the stomatal conductance of stressed plants did not recover until 48h after rewatering. The concentration of ABA in the xylem sap in the pre-treated stress plants returned to similar values to the control 6h after rewatering. After rewatering, leaf growth of plants pre-treated with 11d of water stress was rapid and exceeded growth of continuously well watered plants. The plants grown under continuous water deficit show reductions in leaf growth, stomata conductance and increase in xylem sap ABA.

INTRODUCTION

When plants were exposed to drought, their growth rate is impaired due to several physiological and biochemical processes being disturbed. Over the past decade, the role of ABA induced leaf growth and stomatal closure has been investigated and reviewed extensively (Passioura *et al.* 1993; Schulze 1994). Researches have been concentrated on the responses of duration of water deficit on leaf growth and stomatal closure in relation to ABA, but little information is available on the ability of plants to recover upon stress relief. Under field conditions in the tropics, plants were normally subjected to a period of gradual water stress and on several occasions, this period of water stress is followed by period of rainfall. Gates (1955) indicated that plants which were exposed to a brief period of water stress and then rewatered, showed a better stomatal regulation, and hence

productivity. This supported the earlier findings by Moroton and Watson (1948) who found renewed growth and development upon rewatering in sugar beet, which often proceeded at a more rapid rate than in the continuously watered plants. There were reports on the stomata responses that upon rewatering are associated with ABA metabolism. Doerffling *et al.* (1977) reported that a delayed recovery in stomatal was due to elevated ABA persisting several days after relief of stress. Similarly, Correria and Pereira (1994) showed a 100-fold increase in apoplastic ABA concentration with soil drying but did not return to pre-stress values immediately following rewatering. In contrast, several other investigators reported that the delay in stomatal recovery is not associated with the residual ABA following rewatering (Loveys and Kreidemann, 1973; Cornish and Zeevart, 1985). The discrepancy could be contributed to the experimental procedures, genotypic differences or plants parts in which ABA have been detected. In pepper plants, Aloni *et al.* (1991) indicated that leaf water potential returned to the control values 24 h after rewatering but photosynthetic rate of stressed plants did not recover and was dependent on the extent to which the water potential had decreased during stress. To our knowledge, the roles of ABA influencing leaf growth and stomata closure upon stress relief in pepper plants has not yet been investigated. In this paper, we report the plant responses upon stress relief and examine the role of ABA influencing leaf growth and stomatal response.

MATERIAL AND METHODS

Experimental Procedures

Seed of pepper (*Capsicum annuum* cv Bell Boy) were germinated in seed trays under greenhouse conditions. After 7 d, seedlings were transferred to pots with a diameter of 90mm containing a mixture of John Innes compost No 2 and grit. At the 5-6 leaf stage seedlings were transferred to the polyvinyl-chloride (PVC) tubes which were filled with a similar compost mixture. The PVC tubes of 105mm inside diameter and 300mm length accommodated 3.2 litres of compost mixture. The plants in the column were grown in the controlled environment cabinet with temperature at 22-25° C (day) or 18° C (night), relative humidity of 48%-56% and photoperiod of 14h with PFD average of 320 umol m^{-2} s^{-1}. The plants grown with daily watering in the growth cabinet for acclimation. The plants were divided into four groups in which two groups were watered daily or left unwatered. The other two groups consisted of plants that were left unwatered for 5 and 11 days, and then rewatered. The plants randomly arranged in the growth cabinet in four replicates. Leaves were tagged prior to treatments for determination of leaf length increment. Water relations, stomatal conductance and leaf growth were measured at 3, 6, 24, 48, 72, and 96 h after rewatering. The pre-stress values were also recorded prior rewatering. Xylem sap was collected from each plant by extracting extrudate from cut stem protions. A pressure of 0.3 – 0.5 Mβa above the balance pressure was applied for the collection of extrudate. The first 5mm of extrudate were excluded from the samples. The measurements of leaf water potential and stomatal conductance were carried out on the same leaf. Xylem sap ABA was determined from the extrudate from the cut stem portion of the same plant.

The mean volumetric soil water content was measured in the soil column prior to watering the plants. Three random samples were taken for each treatment and after oven-drying at 80°C for 48h, soil water content was calculated. Leaf water potential was measured on the youngest fully expanded leaf using a pressure chamber on each sampling day. These leaves were then inserted into the 10ml syringe and immediately placed in liquid nitrogen before storage in a deep freezer at –20°C for 5 d before determination of osmotic potential. Two fractions of leaf extrudates were collected from each leaf and osmotic potential of extrudates was determined using a vapor pressure osmometer. Turgor potential was calculated from the difference between water potential and solute potential.

Measurements of stomatal conductance were made on the abaxial surface of the youngest fully expanded leaves with a diffusion porometer (AP-4, Delta-T Devices Ltd, Cambridge).

Leaf length and breadth increments were determined on the leaves that were tagged before treatments began. The leaf length and breadth increments were calculated at each sampling date by measuring the differences between the length on the sampling date and the initial measurement.

Concentration of xylem sap ABA was determined using a radioimmuoassay (RIA) (Quarrie *et at.* 1988).

RESULTS

Mean moisture content from different portion of soil column supporting plants was 0.34g cm^{-3}, 0.25 g cm^{-3} and 0.18g cm^{-3} under well watered, 5 and 11 d of water stress, respectively. This reduction in soil moisture content had reduced leaf length by 2 and 4.4 cm in plants which were subjected to 5 and 11 of water stress, respectively, compared to the well watered conditions (Fig. 1). Restoration of water to the plants that were pre-stressed resulted in a resumption of leaf growth. The recovery seemed dependent on the duration of plants being left unwatered. These was an over recovery in leaf growth on the plants that were subjected to 11 d on water stress 24 h after rewatering (Fig. 2).

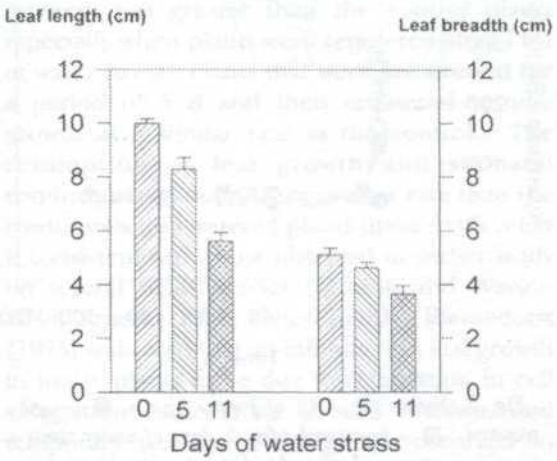

Fig. 1. Leaf length and breadth of pepper plants exposed to duration of water stress. Bars represent S.E. Each point represent means of replicates.

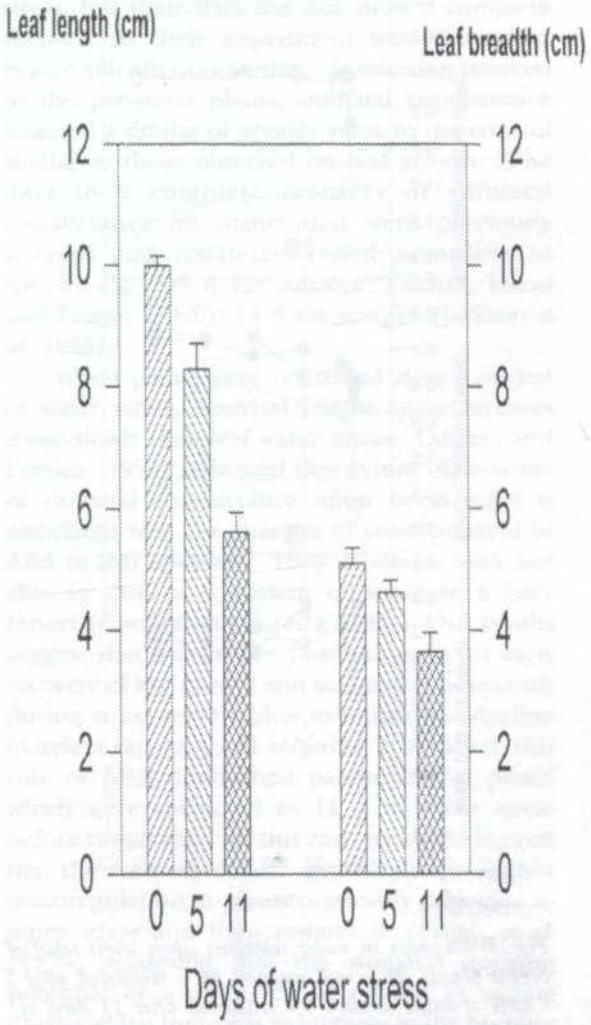

Fig. 2. Cumulative leaf and breadth of pepper plants after rewatering. ● = well watered, ■ = water stress for 5 days and rewatered: □ = water stress for 11 days and rewatered: ○ = continuous water stress. Bars represent S.E. of 6 replicates.

Fig. 3 shows the change in water relations as water was restored to the soil column of water stressed plants. Leaf water potential had already declined to –0.65 Mβa and –1.09 Mβa when plants were left unwatered for 5 and 11, respectively. Upon restoration of water, leaf water potential in plants that were subjected to pre-stress treatments, increase immediately and maintained at a similar value to the control throughout the experimental period. Osmotic potential of plants subjected to water stress for 11 d, maintained a lower osmotic potential but increased progressively after rewatering. As expected, plants that were subjected to continuous water stress maintained a continuous lower osmotic potential. These changes in leaf water potential and osmotic potential in plants subjected to various treatments resulted in changes in turgor potential in which plants left unwatered for 11 d and rewatered, maintained higher turgor than the other treatments.

Stomatal conductance declined progressively with the cessation of watering to the plants, reaching to less than 100mm0l $m^{-2}s^{-1}$ after 4 – 5 d of water stress. Once plants were rewatered, stomatal conductance began to recover, but not until 48h to reach a similar values to the control. Similar to the effects on leaf length increment,

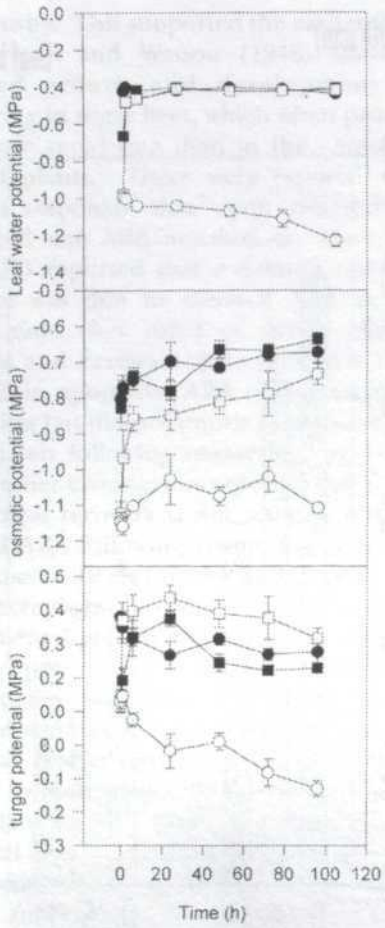

Fig. 3. Changes in water relations upon stress relief of pepper plants. ● = well watered, ■ = rewatered after 5 days of water stress; □ = rewatered after 11 days of water stress; ○ = continuous water stress. Bars represent ± SE of 4 replicates.

there were was a tendency that the plants stressed for 11 d to recover at a greater rate than control plants 96h after rewatering (Fig. 4).

Xylem sap ABA in pepper plants when subjected to water stress for 5 and 11 d, increased from less than 40umol m^{-3} to over 160umol m^{-3}. Xylem sap ABA decreased rapidly in these plants after restoration of water to a similar values to the control 6h after rewatering. Xylem sap ABA in continuous water stress plants increased as water stress proceeded. (Fig. 5).

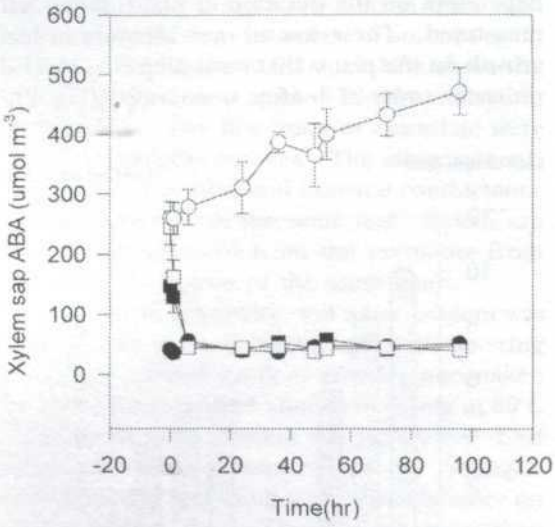

Fig. 5. Xylem sap ABA of pepper plants. ● = well watered : ■ = rewatered after 5 days of water stress : □ = rewatered after 11 days of water stress : ○ = continuous water stress. Bars represent ± SE of 4 replicates.

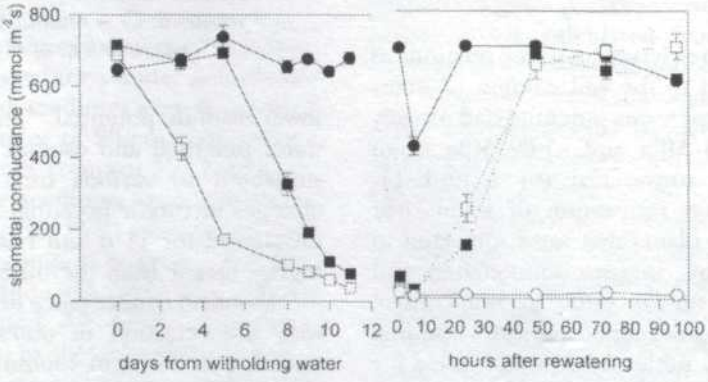

Fig. 4. Stomatal conductance of pepper plants exposed to duration of water stress and the effect on rewatering (●) well watered : (■) rewatered after 5 days of water stress: (□) rewatered after 11 days of water stress; (○) continuously stress. Bars represent S. E. Each point represent means of 6 replicates. Measurements after rewatering at 6 hours were carried out at 6pm where plants had already being exposed to 12 hours of light period and relative humidity had declined to 39% RH.

DISCUSSION

The reduction in leaf growth is considered as the most sensitive response to water stress in most plant species (Hsiao, 1973; Dale, 1988). The causes of this reduction could be either hydraulic or non hydraulic. Under progressive development of water deficit, the reduction in leaf growth has been correlated with high levels of ABA in leaves or xylem sap (Zhang and Davies, 1990; Passioura, 1988). In our earlier investigation, we showed that early reduction in leaf growth of pepper species occurred without any detectable changes in leaf water potential but coincided with a slight increase in xylem sap ABA (Mohd Razi and Davies 1997). In this experiment, we show a recovery of leaf growth and stomata conductance upon stress relief. The recovery was greater than the control plants especially when plants were rewatered after 11 d of water stress. Plants that were pre-stressed for a period of 5 d and then rewatered resume growth at a similar rate as the control. The resumption of leaf growth and stomatal conductance at a similar of greater rate than the continuous well watered plants upon stress relief is consistent with those observed in earlier study on several plant species (Morton and Watson 1948; Kramer 1950; Gates 1955). Kleinedorst (1975) indicated that an inhibition in leaf growth in maize plants to be due to retardation in cell elongation rather than in cell division, and temporary accelerated leaf growth occurred on discontinuing the stress condition. This supports earlier suggestions by Hsiao and Acevedo (1974) who indicated that the reduction in growth during a mild and short water stress could be offset completely by a rapid transitory phase of growth following release of stress. Aloni, Daie and Karni (1991) investigated the recovery of young transplant pepper plants grown in a rapid soil drying, however, showed a contradictory results on the leaf growth response upon stress relief. They reported that plants which were stressed for 24 and 48h and then rewatered, resume leaf elongation but did not attain similar values as the control even after 10 d and of rewatering due to the disturbance of assimilate partitioning.

The stomatal conductance did not attain a similar values to the well watered plants until 48h after rewatering. This is consistent with observation by Correia and Perreira (1994) upon rewatering of lupins plants exposed to 5 d of stress, but their data did not show a complete recovery as their experiment was terminated before 48h after rewatering. As watering proceed to the pre-stress plants, stomatal conductance attained a similar of greater value to the control similar to those observed on leaf growth. The days to a complete recovery of stomatal conductance on plants that were previously stressed and rewatered varied according to species e.g: 205 d for tobacco; Fischer, Hsiao and Hagan (1970); 14 d for conifer (Jackson *et al.* 1995).

When plants were rewatered after a period of water stress, stomatal conductance recovers more slowly than leaf water status. Correia and Pereira (1994) indicated that extent of recovery of stomatal conductance upon stress relief is associated with the changes in concentration of ABA in leaf apoplast. They, however, were not able to show a consistent response in a later report (Correia and Pereira 1995). Our results suggest that one of the possibilities in an early recovery of leaf growth and stomatal conductance during stress relief is due to immediate decline in xylem sap ABA. As watering progressed, this role of ABA diminished particularly in plants which were subjected to 11 d of water stress before rewatering. In this case, we could suggest that the recovery of leaf growth due to higher osmoregulation in plants previously subjected to water stress and then rewatered. Fisher *et al.* (1970) suggested that the stomatal opening potential of a given leaf is related to ageing during which there was as increase in the number of leaves separating the leaf from the apex. Since post-stress leaves were closer to the apex representing a physiological younger state contribute to the better stomatal opening compared to the control. Cornish and Zeevart (1985) rewatered the wilted *Xanthium* plants and found that the stomatal reopening did not coincide with the decline of bulk leaf ABA but as a result of elevated levels of ABA remained in the apoplast after the bulk leaf contents had returned to their pre-stress values. We, however, would not be able to suggest the role of ABA in rewatered plants since stomatal conductance was at least 60% lower than the continuous well watered plant 24 h after rewatering when ABA has already declined to a similar values to the control. We could argue that with a gradual development of soil water deficit, it is possible that when plants were watered, ABA could be

more diluted and the concentration would be similar to the well watered plants. There are many reports suggesting that under condition of slight decline in soil moisture content, stomatal closure precedes any detectable increase in xylem sap ABA (Trejo and Davies, 1991; Jackson *et al* 1995). It has been suggested ABA does not impinge directly on stomata immediately after roots were subjected to soil drying. This has been suggested earlier b Weyers and Hillman (1979) indicating that although stomatal reopening occurred when net efflux of ABA was allowed, it is not possible to suggest that uptake of ABA as a single factor was directly related to the events of stomatal closure. The events leading to abscicic acid-evoked stomata closure has been discussed by Blatt and Thiel (1993) who indicated that the progression of events from ABA stimulus is not linear and least pass through Ca and pH intermediate.

Our attempt to explain the recovery of stomata conductance and leaf the basis of the decline in ABA after restoration of water to drying soil, was inconclusive. Relief from water stress results in a decline in ABA concentration to a pre-stress values within a few hours. The stomata, however, require at least 48h to achieve values similar as the continuous well watered plants. We speculated that there may be also involvement of hydraulic factors which regulate cell metabolism in the process of recovery to water stress. The over recovery of leaf growth and stomata response upon restoration of water to the drying soil presumably is associated with the physiological 'younger' condition following turgor recovery.

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CERES-Maize Simulation Model: Establishment of Planting Windows for Grains Maize under Rainfed Conditions

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Keywords: crop model, planting window, ecological zone

ABSTRAK

Konsep jangkamasa pertumbuhan atau peluang menaman merupakan satu pendekatan yang berguna dalam mengenalpasti masa menanam yang sesuai terutama di bawah pengurusan tanaman tanpa pengairan. Ia dapat membantu petani dalam menentukan kejayaan tanaman yang diusahakan. Model simulasi tanaman (model CERES-Maize) telah digunakan untuk mengenalpasti masa yang sesuai untuk menanam dan jagung digunakan sebagai tanaman penunjuk. Keupayaan model dalam membuat ramalan juga turut ditentukan berdasarkan keputusan-keputusan simulasi yang telah direkodkan. Model menunjukkan berkeupayaannya dalam meramal pengeluaran hasil jagung sebenar di peringkat percubaan tetapi selalu melebihi jangkaan bagi ramalan di peringkat ladang. Dalam persekitaran setempat dengan keadaan tanah dan cuaca yang sesuai, potensi hasil jagung dapat dijangkakan melebihi 7 t/ha. Potensi hasil untuk kebanyakan zon adalah rendah pada penghujung tahun disebabkan keadaan kemarau yang dialami oleh tanaman sepanjang musim. Berdasarkan bentuk potensi hasil, ia selaras dengan bentuk taburan hujan. Dengan gabungan had limit hasil 5 t/ha, ia menunjukkan kebanyakan dari zon-zon tersebut mempunyai dua musim menanam kecuali dua zon (1 dan 26) yang hanya mempunyai satu musim menanam sahaja. Di samping itu masa yang paling sesuai dalam musim menaman bagi zon 9 dan zon 10 turut juga dikenalpasti. Maklumat sebeginilah yang petani-petani perlukan dalam membantu mereka merancang aktiviti di ladang ke arah mencapai operasi yang cekap.

ABSTRACT

Growing period or planting windows concepts is a useful approach in identifying suitable planting time for crop under rainfed management. It will help farmers to ensure the crop success. A crop simulation model (CERES-Maize model) was used to identify the suitable planting time and maize was used as an indicator crop. The model was validated using compiled data to ensure its fitness within the setup acceptable limit. The model was capable to predict maize yield potential close to the actual yield at the experimental trails but always over estimated at the farm production levels. Under local conditions with favourable soil and climate, the yield potential of maize could be expected greater than 7 t/ha. The yield potentials for most of the zones are relatively low towards the end of year due to a dry period, experienced in most of the crop growing cycle. Based on the yield potential trends, it corresponds to rainfall pattern. In combination with a cut off point at 5 t/ha, its shows most of the zones have double planting windows except for two zones (1 and 26), which have a single planting window. In addition, as example the most suitable planting time within the planting window for zone 9 and 10 were also identified. This information can help farmers in planning their farm in order to have the most efficient operation.

INTRODUCTION

Rainfed farming is widely accepted in Malaysia, especially by small-scale farmers. Under rainfed condition, moisture adequacy is an important factor to ensure the successfulness of crop production. A suitable planting time with sufficient

soil moisture during a planting season should be available before planting. Planting window concept has been proven to be a very useful approach to ensure sufficient moisture availability in the soil. This concept sometimes called the growing period was firstly introduced by Cocheme and Franquin (1967). The concept was defined as the period in a year where agriculture can be produced due to adequate soil moisture and absense of temperature limitations. The Penman Open-water Evaporation (Eo) method was used in the calculation with precipitation as input data.

The concept was then modified by FAO and defined, as a continuous period in one year where precipitation is greater than half-potential evaporation with a number of days required for evaporation (Kowal, 1978). The Penmans method was used in the calculation and with the assumption that soil can only store up to 100 mm of rainwater at each precipitation event.

In view of that, an exercise was conducted to identify the planting windows for maize on various ecological zones. The CERES-Maize model (Kiniry and Jones, 1986) was used in this study instead of Eo and FAO methods. It was seen to be more reliable whereby actual crop growth was taken into account during the simulation process. Although only a single crop at one time can be handled by the model, the selected crop can probably show the actual scenario of the planting window for some of the other crops. In addition, validity of the model was also taken into consideration in this study.

MATERIALS AND METHODS

CERES-Maize Model Validation

Before embarking the planting window identifications, the CERES-Maize model needs to be validated. The validation process is necessary because it ensures the model works properly and the simulation result of the model is within the acceptable limit such as Root Means Square Error (RMSE) is less than 1 t/ha or statistically significant at least at 5 % confidence. For this reason, twenty sets of simulated and measured grain maize yield data were compiled. The data was from experimental trails and farm productions, which were carried out elsewhere. A statistical methodology i.e. RMSE was used to analyze the data, in order to evaluate the fitness of the model. The RMSE value should be as small as possible to indicate the best fit between simulation output of the model and actual result from the field.

Planting Windows Identification

Simulation Exercise

The CERES-Maize model was used to simulate potential yield using the weather, soil and crop data. Simulations were considered on every ten days, throughout the year of eleven years (1985-1995). Only predicted yield potential at maturity stage of the simulation output was considered. Simulated yield outputs of the same planting date from eleven years were singled out using Y80 (80% probability) calculation technique. Finally, each zone has 37 simulated yield data representing a ten-day interval of planting dates throughout the year.

Two assumptions were made during the simulation exercises. First, no pest and disease infestations occurred that could cause crop damage. Second, free from weed competition for available resources such as fertilizer, light, water, etc., which would affect maize crop performance and therefore reduce yield production.

Weather Data Used

Sets of long term weather data, representing eight ecological zones of the northern parts of Peninsular (Nieuwolt *et al.*, 1982) were used in the study. The weather stations where data were obtained are Kota Bharu representing Zone 26, Kuala Krai for Zone 25, Kuala Terengganu for Zone 22, Kuantan for Zone 21, Melaka for Zone 12, Petaling Jaya for Zone 10, Sitiawan for Zone 9, Chuping for Zone 2 and Alor Star for Zone 1. The data consists of daily solar radiation (MJ/m^2/day), maximum and minimum temperature (°C) and precipitation (mm/day) from January 1985 to December 1995. Each data set was reformatted as required by the model for simulation purposes.

Soil Data Used

A set of soil data, extracted from soil profile descriptions was used in this study. The soil physical property inputs were in the form of thickness of the layer, the lower limit of plant-extractable water, the drained upper limit, water content at saturation, a weighting factor for rooting, and initial soil water content as required by the model. The soil chemical properties include organic carbon, pH, initial soil ammonium, and soil nitrate content.

Crop and Management Data Used.

In this exercise, maize cultivar Suwan 1 (*Zea mays* L var. Suwan 1) that was used by most farmers for grain production was selected. Crop management practices were treated as under experimental condition. The rate of fertilizer used was 120N, $60P_2O_5$ and $40K_2O$ kg/ha. Nitrogen fertilizer was divided into two applications where half of them were applied together with other fertilizers during the planting as a basal. Another half was applied after the crop age of 35 days on the field.

Planting Window Identification.

The calculated yield data of each zone based on Y80 was plotted against planting date. A high yield cut off point i.e. 5.0 t/ha (Abd. Razak), which correspond to actual farm production of about 4.0 t/ha, after 10% less due to rats, diseases and bores, and 5% less due to mechanical harvesting loses, was setup. Above the cut off point is considered as suitable planting time or within a planting window whereby lower is considered outside the planting window.

RESULTS

Model Validation

Validity of the CERES-Maize model was evaluated based on 20 sets of simulated and measured grain maize yield data. The simulated and measured yield under experimental trials ranges from 2.7 to 13.6 t/ha and from 2.7 to 14.7 t/ha, respectively. Analysis result shows the differences between simulated and measured yields are relatively small where the RMSE is only 586 kg/ha (Table 1) as compared to the acceptable limit, which is 1000 kg/ha.

Under large-scale farm management the simulated and measured yield on the first season, ranges from 2.3 to 4.7 t/ha and from 5.5 to 5.8 t/ha, respectively. In the second season, it ranges from 3.5 to 5.5 t/ha and from 5.9 to 6.1 t/ha. The RMSE value for the first and the second season is 1878 and 1825 kg/ha, respectively, higher than the experimental trial (Table 2).

TABLE 1
Validations of CERES-Maize model under experimental condition

Year	Location	Experimental status	Observed (kg/ha)	Simulated (kg/ha)
1982	Urbana Illinois, USA	High fertilizer	14700	13600
1989	Mead NE, USA	High fertilizer	10800	10700
1990	Ibadan, Nigeria	Low fertilizer	4904	4909
1991	Ibadan, Nigeria	Low fertilizer	4403	4760
1985	Hawaii	High fertilizer, elevation 77m	11533	12339
	Hawaii	High fertilizer, elevation 340m	11600	12234
1988	MARDI Serdang, Malaysia	Low fertilizer	2184	2717
	MARDI Serdang, Malaysia	Low fertilizer	2984	3058
1989	UPM Serdang, Malaysia	Low fertilizer	3825	3870
	UPM Serdang, Malaysia	Low fertilizer	2739	3607
	RMSE			586
	BIAS			212

TABLE 2
Validations of CERES-Maize model under large scale farm management

Block name	First season 1991		Second season 1991	
	Observed (kg/ha)	Simulated (kg/ha)	Observed (kg/ha)	Simulated (kg/ha)
20 AF	3970	5791	4090	6053
20 BD	4740	5678	5010	5936
21 AG	2250	5463	3690	6020
22 BK	4660	5686	3490	5993
23 BE	4290	5725	5500	6009
RMSE		1878		1825
BIAS		1686		1646

Estimated Yields on Various Locations

The results of simulated yield potential in each ecological zone indicate variability at different planting time as well as location of the area. It ranges between less than 1 t/ha and more than 7 t/ha (Figure 1). In the Northern west and Central east of Peninsular such as Alor Star, Chuping, and Kuantan, maximum yield at the best planting time is about 6 t/ha, which is relatively low compared to the other areas. The highest yield, which was above 7 t/ha, is in Melaka and followed by Kuala Terengganu, Sitiawan, Kuala Krai and Kota Bharu at the middle range. The simulated yields are relatively low towards the end of the year, except for Sitiawan.

Planting Windows on Various Zones

The model shows the yield potential of grain maize under local environment is about 7 t/ha. However, annual yield pattern is fluctuated depending on the weather condition where precipitation, plays a major role. Figure 1 shows the yield variability in eight climatic zones, representing parts of Peninsular Malaysia. High yield potential could be expected when planting during the wet season while poor yields in the dry season. Based on the set up yield potential above 5 t/ha, planting windows for each location or zone were identified (Table 3). Most zones in this study have double planting windows except Zones 1 and 24. Zone 10 has the longest planting window (10 months) while Zone 24 is the shortest (5.5 months).

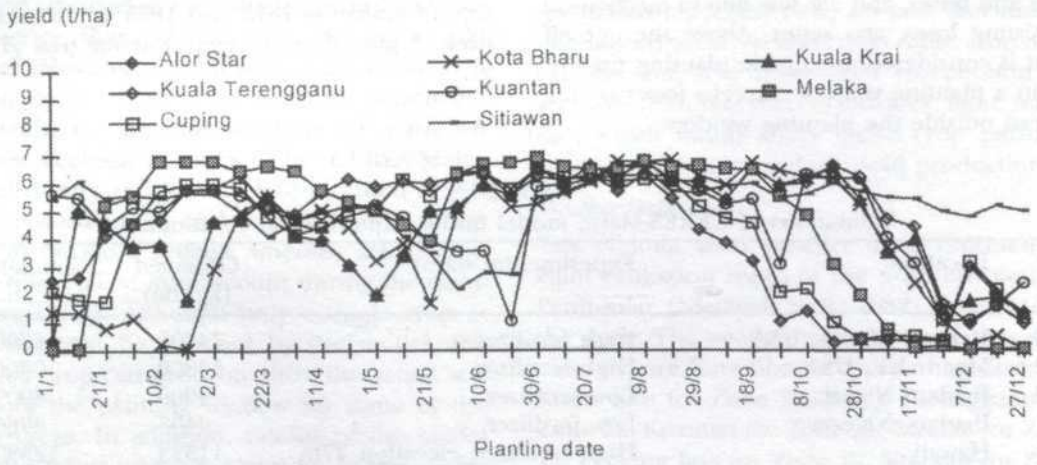

Fig. 1. Simulated yield potential of grain maize on various climatic zones of Peninsular Malaysia

TABLE 3
Planting windows for grain maize in various climatic zones

Ecological zone	Representative weather station	Main season	Off-season
26	Kota Bharu, Kelantan	June - October	Mid March - Middle April
24	Kuala Krai, Kelantan	Mid May - October	-
22	Kuala Terengganu, Terengganu	Mid May - Early October	March
21	Kuantan, Pahang	July - October	January - Mid May
12	Melaka	June - Early October	Late January - Mid May
10*	Petaling Jaya, Selangor	Mid June - December	January - Mid April
09	Sitiawan, Perak	July - December	January - Mid March
01	Alor Star, Kedah	February - Mid August	-
02	Chuping, Perlis	Late April - Mid September	February - March

* Source : Ismail et al., 1990

DISCUSSION

CERES-Maize Model Performance at Experimental Level

Maize is extremely endurable crop, which can be cultivated in many conditions and ways. Agronomic practice is one of the main factors affecting the production level. Favourable climatic and soil conditions with excellent management practices will produce higher yields. In the temperate region for example, grain yields of more than 15 t/ha have been reported (Vander Meer 1982) while in the lowland tropics, yields may range from 5 to 8 t/ha with good management. Adequate nitrogen and potassium with excess phosphorus fertilizer will cause a major increase in maize yield (Miller *et al.* 1987). In addition, high yielding variety could also play a major role for high production. This explains why there is great variability of the yields from local and oversea trails (Table 1).

Under such great yield variability, the model is still capable of predicting reasonable yield output. Statistical analysis shows the overall performance of the model for predicting yield potential at experimental level is highly significant. It was also confirmed by this study, where the Root Mean Square Error (RMSE) of simulated and measured yield was about 586 kg/ha. A smaller RMSE value (less than 1 t/ha) indicates the simulated values are closer to the actual values. Kiniry and Jones (1986) found the relationship between simulated and measured yields was highly significant ($p = 0.0001$) and was within the 95 % confidence band for the 1:1 regression line. Therefore, the model is considered reliable and can confidently be used for simulating grain maize yield under experimental condition.

Model Performance at Production Farm Level

Under large-scale farm management, the simulated yield is always about 10-40 % overestimated (Abd. Razak 1995). The RMSE value at this level is 1850 kg/ha, which is slightly higher than the RMSE at the experimental level. It is due to the insufficient soil characteristic data used in simulation. The soil data from one point of a big block was applied to represent the whole block of farm production, which is variable and less homogeneous. Extra points of detail soil characteristic data from a big block are probably required in order to have a better simulation result. In addition, other two main factors which probably caused the errors in simulated grain yields are that the soils were not well characterized and the inaccurate estimates of water status and the depth of the soil. In this respect, model shows its stability when dealing with the homogeneous soil data although under different climatic condition. A small difference of RMSE between the first and the second season of yield potential on the same soil (about 50 kg/ha) from this study confirmed the model stability. The yield potential difference occurs mainly due to the variability of weather pattern such as precipitation, radiation and air temperature, between seasons. However, the simulation yield trends and the over estimate simulated yield value which were produced by the model are still within the acceptable limit. Therefore, the model could be used to simulate the potential yield under farm production, provided specific measures be considered to suit the local conditions.

Estimated Yields on Various Zones

The model shows potential grain maize yield under local environment is greater than 7 t/ha. However, the yield potential is variable throughout a year depending on the planting date which influence by weather patterns especially annual precipitation. The amount, distribution and intensity of precipitation are very important factors that affect the production of maize especially under rainfed management. Huda et al. (1976) found that the variation in precipitation during different growth stages had different effects on yield. High yield potential could be expected when planting in the wet season while poor yield potential during the dry seasons. However, excessive precipitation and poor soil condition will also decrease yield potential (Shaw 1977; Young 1980).

Besides precipitation, solar radiation status is another factor in determining the maximum yield. During simulation, the model assumes 5.0 g of dry biomass is produced per MJ of intercepted photosynthetically active radiation under non-stressed condition (Jones *et al.* 1986). Areas with high level of radiation during the wet season would produce a higher yield compared to the zones with cloudy wet seasons. Pendleton and Egli (1969) proved maize yield was higher with increasing radiation interception. It explains the zones such as Kuantan and Petaling Jaya, with much cloudy condition during a rainy sea-

son have low yield potential (about 6 t/ha) compared to other zones.

In addition, the level of crops management with phosphorus and potassium fertilizers application, pest and disease management and weed control, which is not taken into consideration by the model, is also important contribution towards a better crop performance and high yield production.

Planting Windows on Various Zones

In tropical and subtropical areas, where soil moisture adequacy is a major constraint to crop production, the planting windows or growing period concept has proven to be very useful (Sys *et al.* 1991). Inaccurate planting time causes crop failure and very low yield. Generally, towards the end of the year, chances to have a successful crop are very minimal, except for Sitiawan zone because a rainy season of the zone started towards end of the year. For other climatic zones, such as Kota Bharu, Kuala Krai and Kuantan, during the first half of the year are considered not suitable for planting due to dry period. The planting time of July to mid September is considered the best time to have a successful crop for all climatic zones.

A combination of total ten days precipitation pattern and yield potential, the most suitable planting time for grain maize within the identified planting window could be worked out (Abd. Razak 1995). Based on this approach, some zones can have double plantings while the other zones only have a single planting per year. The most suitable planting times for Zone 9 are 10 May and 20 September of the year. For Zone 10, the most suitable planting times are 14 February and 7 September of the year (Ismail *et al.* 1990).

CONCLUSION

The CERES-Maize model could be used as a tool to estimate yield potential of grain maize throughout the country. A combination of the model output with ten days precipitation pattern could be also used to identify the planting windows and the most suitable planting time for high yield production. With reliable information, it can help the operator to daily manage the maize farm efficiently. High yield production with minimal risk of crop failure could be expected. Farmers cum entrepreneurs most likely are more confident to invest in the maize cultivation with such information. Entrepreneurs could also plan their farms at different locations and planting time in the country, targeted for continuous productions throughout the year with a high return.

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Effect of *Dactylaria higginsii* on Purple Nutsedge (*Cyperus rotundus*) Interference with Pepper (*Capsicum annuum* L)

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ABSTRAK

Satu kajian rumah hijau telah dijalankan untuk menilai kesan patogen berkulat, Dactylaria higginsii *pada nutsedge ungu yang dicampur dengan lada hitam 'Capistrano'* (Capsicum annuum). *Tanaman nutsedge ungu yang tumbuh daripada umbi pada peringkat awalnya ditanam dengan kepadatan 40, 80, 160 dan 320 tanaman m bersama-sama lada hitam di dalam pot komersial sederhana bergaris pusat 35-cm, dengan keadaan pengairan dan pembajaan yang tidak terhad. Tiga hingga empat-peringkat-daun nutsedge ungu dan empat-peringkat-daun tanaman lada hitam telah disiram dengan* D. hingginsii *dalam 0.5% Metamucil, suatu pembawa: rawatannya cuma pembawa sahaja, 10^4 conidia ml^{-1} + pembawa, atau 10^6 conidia ml^{-1} + pembawa. Secara signifikan, nutsedge ungu pada kesemua umbi yang padat mengurangkan hasil lada hitam tanpa kehadiran* D. hingginsii. *Peratus hasil lada hitam menyusut lebih tinggi dalam rawatan bersama dengan 10^4 conidia ml^{-1}. Walau bagaimanapun, peratus kesusutan hasil lada hitam adalah sangat kecil jika dirawat dengan* D. higginsii *pada 10^6 conidia ml^{-1} berbanding kawalan tanpa tumbuhan berumput. Secara signifikan, kadar perkembangan penyakit dalam rawatan bersama 10^6 conidia ml^{-1} (r^G = 0.113 0.123) lebih cepat berbanding yang dirawat bersama 10^4 conidia ml^{-1} (r^G = 0.049 0.050). Pada 10^6 conidia ml^{-1},* D. higginsii *mengurangkan pencampuran nutsedge, memberi kawalan yang lebih tinggi pada nutsedge, dan meningkatkan hasil lada hitam berbanding kawalan berumput.*

ABSTRACT

Greenhouse studies were conducted to evaluate the effect of the fungal pathogen, Dactylaria higginsii, *on purple nutsedge interference with 'Capistrano' pepper* (Capsicum annuum). *Purple nutsedge plants established from tubers were planted at initial densities of 40, 80,160, and 320 plants m^2 with pepper in 35-cm diam pots with a commercial potting medium, under nonlimiting fertilization and irrigation conditions. Three to four-leaf-stage purple nutsedge and four-leaf-stage pepper plants were inoculated by spraying* D. higginsii *in 0.5% Metamucil, a carrier; the treatments were carrier only, 10^4 conidia ml^{-1} + carrier, or 10^6 conidia conidia ml^{-1} + carrier. Purple nutsedge at all tuber densities significantly reduced pepper yield in the absence of* D. higginsii. *Percentage yield loss of pepper was greater in treatment with 10^4 conidia ml^{-1}. However, percentage yield loss of pepper was negligible in treatments with* D. higginsii *at 10^6 conidia ml^{-1} when compared to the non-weedy control. The disease progress rate was significantly faster in treatments with 10^6 conidia ml^{-1} (r_G = 0.113 - 0.123) compared to 10^4 conidia ml^{-1} (r_G = 0.049 - 0.050). At 10^6 conidia ml^{-1},* D. higginsii *reduced nutsedge interference, provided greater nutsedge control, and increase pepper yield compared to weedy checks.*

INTRODUCTION

Purple nutsedge is rated as one of the world's worst weed and has been reported in more than 70 countries. It competes and interferes with crops particularly early in the growing season and heavy infestation of purple nutsedge can cause high yield loss in vegetable crops. Although (chemical) herbicides inhibit the growth

of the weed, adverse environmental factors and plant-growth stages at the time of application act against the effect of the herbicide (Gricher *et al.* 1992). Several other nonchemical methods have been used, but none have provided acceptable control. Long-term, sustained control of purple nutsedge has been difficult to achieve.

Research has recently commenced into the use of a bioherbicide to reduce interference by purple nutsedge in cropping systems. *Dactylaria higginsii*, a fungal pathogen of purple nutsedge has been reported to be capable of controlling this weed (Kadir and Charudattan 1996, Kadir *et al.* 1997a; 1997b). However, its potential to reduce nutsedge interference in cropping system has not been studied. Therefore, the objectives of this research are: 1) to determine the effective inoculum concentration needed to reduce interference from the purple nutsedge and 2) to determine the effect of *D. higginsii* on the interference of purple nutsedge on pepper.

MATERIALS AND METHODS

Experimental Method

The experimental method used in this study was the additive series approach. In this method, the density of one species (usually, called the indicator crop) is held constant and the density of the other species (the weed) is varied. Since the latter is added into the first of this bipartite series, this approach is called the additive series. This system uses the response of the first species in fixed density as an indicator of the relative aggressiveness or competitive ability of the second species to the first. This system is applicable in cropping systems with encroaching weeds and in intercropping systems (Cousens 1990; Nickel *et al.* 1990).

Purple Nutsedge Interference

The experiment was carried out in a greenhouse in spring 1996 and repeated in autumn 1996, using transplants pepper cv 'Capistrano' as the indicator crop. A mixture of pepper and purple nutsedge were grown in 30 cm (diam) × 10 cm (height) pots filled with 0.07 m^3 of commercial potting medium (Metro Mix 220, Scott-Sierra Horticultural Product Co., Maryville, OH.) consisting of horticultural vermiculite, Canadian spaghnum peat, and horticultural perlite. Each pot contained one transplant of pepper and one of the following purple nutsedge densities: 0, 40, 80, 160, and 320 tubers per m^2. Plants in pots were watered by drip irrigation three times daily to stimulate soil moisture in the field. Soil fertility was maintained by adding water-soluble Peters Professional All Purpose Plant Food (20:20:20 + Trace Elements, Spectrum Group, Div. of United Industries Corp., St Louis, MO) at the recommended rate of 3.785 liters of solution (9.5 g/3.785 liters water) for 0.09 $meter^2$ bed, every two weeks.

Fungal Inoculation

Inoculum used in this experiment was produced in trays on a thin layer of PDA (Kadir 1997). Three inoculum concentrations were used: 0 [0.5% Metamucil (w/v), used as a humectant; as a control]; 10^4 conidia/ml with 0.5% Metamucil (w/v); and 10^6 conidia/ml with 0.5% Metamucil (w/v). One hour before the plants were inoculated, they were misted for 5 min to wet the leaf surfaces. The 4-leaf old pepper plants and 3-4-leaf old purple nutsedge were inoculated by spraying the conidial suspension with an aerosol sprayer until the excess fluid dripped off the foliage. Starting 6h. after inoculation, the greenhouse misters were turned on for 5 min at every 6h. interval for the first 24h. to maintain leaf wetness. This was to ensure that *D. higginsii*, which requires a dew-duration period of at least 12h. for disease development, would be able to infect purple nutsedge under greenhouse conditions.

Data Collection

Disease severity was assessed every five days using the Horsefall Barratt scale (Horsefall and Barratt 1945), modified by Kadir (Kadir 1997). The values of the total portion of disease were transformed by using the Gompertz model transformation (Berger, 1981) of the form:

$$\text{Gompit } y = -\ln(-\ln(y))$$

to linearize the disease progress curve. The area under the disease curve (AUDPC) was calculated from this linearized curve using the equation (Campbell and Madden, 1990):

$$\text{AUDPC} = \sum_{i}^{n+1}\left(\frac{y_i + y_{i+1}}{2}\right)\left(t_{i+1} - t_i\right).$$

Pepper was harvested at 50 and 65 days after transplantation and the yield was recorded as fruit weight (in gram) per plant. Pepper fruits were harvested twice, since the yields during the first harvest did not show any expected trend. The data from the first and second harvest were

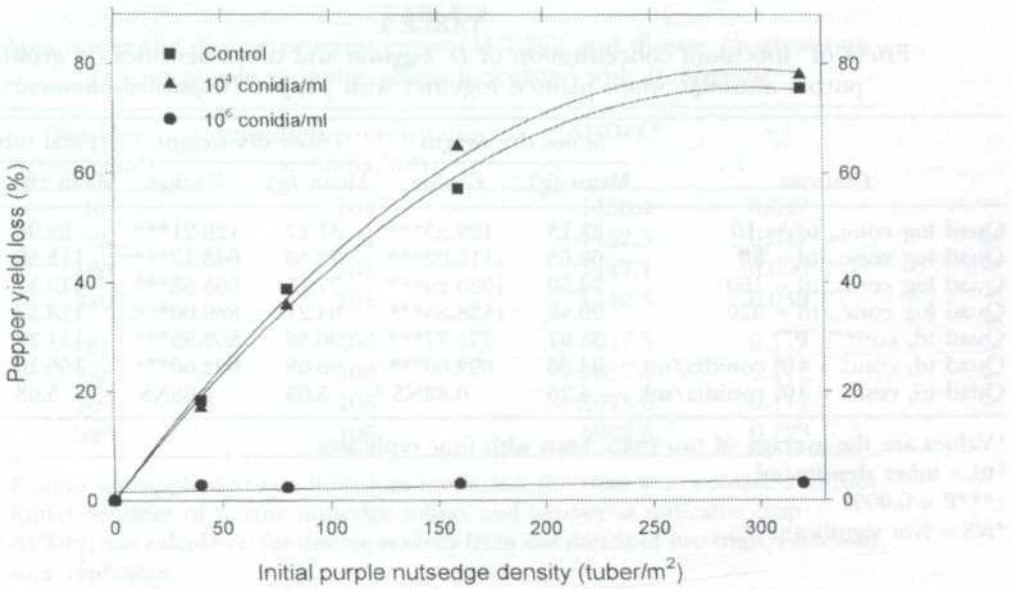

Fig. 1. Effect of inoculation of purple nutsedge with D. higginsii *on the percentage yield loss of* C. annuum. *Each data point represents the mean value from two trials each with four replicates. Control = noninoculated control; 10^4 conidia ml^{-1} = inoculated with 10^4 conidia ml^{-1} at the rate of 90 ml m^{-2}; and 10^6 conidia ml^{-1} = inoculated with 10^6 conidia ml^{-1} at 90 ml m^{-2}.*

pooled and recorded as the total yield per plant. Final tuber numbers of purple nutsedge were recorded at the final harvest time (65 days after transplantation), from each pot. The tubers and the bulbs were separated after washing the soil from the roots and rhizomes. Both were recorded as tubers. The shoot plus tuber biomass was determined at harvest time by weighing the shoots and tubers after they were dried at 75°C for 5 days. These parameters represent weed-growth components.

Statistical Analysis

The study was a factorial experiment with two factors (tuber densities as the main factor and inoculum concentration as the sub-factor). The experiment had a randomized complete block design with four replications. Mean values of four replications were used for statistical analysis. Orthogonal contrasts of the log inoculum concentration and tuber densities, and of the slopes of the linear regression models, was performed to determine the individual effect of tuber density and inoculum concentration and their interactions on weed-growth components and crop yield. Linear regression of AUDPC against yield was done to determine their relationship.

RESULTS

Homogeneity of variance among treatments were noted in the levels of control of the weed-growth components and disease severity of Dactylaria leaf blight on inoculated purple nutsedge from both trials. The data on weed growth components and disease severity, the latter expressed as the AUDPC, were therefore combined and averaged over both trial dates.

Effect of D. higginsii *on weed-growth components and pepper yield*

The initial planting density of tubers had a significant effect on shoot and tuber dry weight of purple nutsedge in noninoculated control and in treatments where plants were inoculated with 10^4 conidia/ml (Table 1). The final shoot and tuber dry weight of purple nutsedge increased with increasing purple nutsedge tuber density. Exception was the treatment in which the purple nutsedge plants were inoculated with *D. higginsii* at 10^6 conidia/ml. The final shoot and root dry weight were significantly reduced in these treatments regardless of initial planting densities of tuber compared to the non-inoculated weedy control and treatments where purple nutsedge plants were inoculated with 10^4 conidia/ml.

TABLE 1
Effect of inoculum concentration of *D. higginsii* and tuber densities on growth of purple nutsedge when planted together with pepper (*Capsicum. annuum*)[a].

Contrast	Shoot dry weight		Tuber dry weight		Final tuber number	
	Mean (g)	F-value	Mean (g)	F-value	Mean (no.)	F-value
Quad log conc., td [b]= 40	23.13	129.25***[c]	31.27	128.21***	52.96	4.38***
Quad log conc., td = 80	66.63	1115.02***	72.38	643.17***	115.50	288.95***
Quad log conc., td = 160	74.60	1080.20***	77.15	665.58***	119.17	308.47***
Quad log conc., td = 320	90.42	1538.85***	84.21	889.00***	128.37	432.06***
Quad td, conc. = 0	91.97	775.77***	96.42	803.95***	151.28	386.25***
Quad td, conc. = 10^4 conidia/ml.	94.66	692.60***	96.68	692.60***	155.10	297.62***
Quad td, conc. = 10^6 conidia/ml.	4.26	0.82NS [d]	5.63	1.38NS	5.63	0.18NS

[a]Values are the average of two trials, each with four replicates
[b]td = tuber density/m^2
[c]***P < 0.0001
[d]NS = Not significant

The slope comparison for relationship of the weed-growth components of purple nutsedge with initial planting densities of tubers is shown in Table 2. The slopes of the non-inoculated control and treatments where purple nutsedge plants were inoculated with 10^4 conidia/ml were comparably similar, but were significantly lower in treatment where purple nutsedge plants were inoculated with 10^6 conidia/ml.

The percentage of yield loss of pepper was significantly high even at 40 tubers/m^2 (19.07% for the control and 15.42% for 10^4 conidia/ml. The application of 10^4 conidia/ml of *D. higginsii* did not have any significant effect in reducing the yield loss of pepper. The percentage yield loss of pepper was significantly reduced irrespective of tuber densities, when purple nutsedge were inoculated with 10^6 conidia/ml. This could be explained by the reduction in weed growth components (explained earlier).

Effect of D. higginsii *on AUDPC*

Purple nutsedge plants inoculated with 10^4 conidia/ml developed low levels of disease compared to plants inoculated with 10^6 conidia/ml. Almost all of the plants in the 10^6 conidia/ml treatments died. Secondary spread of *D. higginsii* from the previously diseased leaves caused subsequent infection on the regrowth, thus very little or no regrowth were observed.

The disease severity of the inoculated plants was expressed as the AUDPC (Table 3). The

TABLE 2
Slope values and comparisons of slopes from linear regression of growth components of purple nutsedge and the initial tuber densities of purple nutsedge in pepper recorded 65 days after inoculation with *D. higginsii*

Treatments	Slope values		
	Shoot dry weight (g)	Tuber dry weight(g)	Final tuber numbers
Control	1.02	1.20	2.71
10^4 conidia/ml	1.11	1.14	2.52
10^6 conidia/ml	0.05	0.06	0.05
	Contrasts of slope values		
Control vs 10^4	NS [a]	NS	NS
Control vs 10^6	***[b]	***	***
10^4 vs 10^6	***	***	***

[a]NS = Not significant.
[b]*** = P< 0.001.

TABLE 3
Area under the disease progress curve (AUDPC) and disease progress rate (r_G) on purple nutsedge plants inoculated with *D. higginsii*.[a]

Densities (tuber/m²)	Inoculum concentration (conidia/ml)	AUDPC[b]	r_G[c]
40	104	1456.4	0.049
80	104	1452.5	0.047
160	104	1447.1	0.050
320	104	1442.3	0.049
40	106	5647.5	0.113
80	106	5887.5	0.112
160	106	5975.0	0.117
320	106	5962.5	0.123

[a] Fungus was applied at two inoculum levels and the treatment consisted of four initial densities of purple nutsedge tubers and pepper as indicator crop.
[b] AUDPC was calculated for disease severity from the means of two trials, each with four replicates.
[c] Disease progress rate was calculated by using the Gompertz model (Berger, 1981).

AUPDC values of treatment where purple nutsedge plants were inoculated with 10^4 conidia/ml were lower compared to AUDPC values of treatment where purple nutsedge plants were inoculated with 10^6 conidia/ml. The disease progress rates (r_G) of the treatment where purple nutsedge plants were inoculated with 10^4 conidia/ml (r_G = 0.047 - 0.050) was slower compared to the apparent infection rates (r_G = 0.112 - 0.123, Table 3) of the experiment where purple nutsedge plants were inoculated with 10^6 conidia/ml.

TABLE 4
Slope values and comparisons of slopes from linear regression of percentages of yield loss of *C. annuum* on initial tuber densities of purple nutsedge recorded 65 days after inoculation with *D. higginsii*.

Treatment	Slope values
Control	0.75
10^4 conidia/ml	0.74
10^6 conidia/ml	0.06
	Contrasts of slope values
Control vs 10^4	NS[a]
Control vs 10^6	***[b]
10^4 vs 10^6	***

[a] NS = not significant
[b] *** = $P < 0.001$

DISCUSSION

D. higginsii did not infect pepper. This was expected as this fungus had been previously determined to be host specific to *Cyperus* spp. (Kadir and Charudattan 1999). Infection was observed on purple nutsedge in the control, due to cross-contamination but the level of infection was below 5% severity. This low level would not account for any significant effect on the yield of pepper or the final weed-growth components of purple nutsedge. The final weed-growth components of purple nutsedge were influenced by the initial planting density of tubers, however, these components were significantly reduced in treatments where nutsedge plants were inoculated with 10^6 conidia/ml of *D. higginsii*. The nutsedge plants in these treatments were severely diseased. Tubers from the diseased plant resprouted, but the growth was suppressed. This finding is contradictory to the report by Marambe (1996) who found that purple nutsedge, even when completely defoliated, tends to increase shoot and tuber numbers. However, his study was done in the absence of a crop, unlike our study which was carried out in the presence of pepper plant. The shading provided by the vigorously growing pepper plant probably helped to maintain humid conditions and promote disease development with severe secondary infection.

The faster disease progress rate (r_c) in treatments inoculated with 10^6 conidia/ml could be explained by the higher inoculum level, combined with the presence of pepper that shaded purple nutsedge plants, predisposing them to infection by *D. higginsii*. The high humidity under the crop canopy provided a conducive environment for disease development. Moreover, shading appeared to have caused purple nutsedge to produce weaker plants, which were less competitive and more prone to infection by *D. higginsii*. Bantillan et al. (1974), Patterson (1982), Santos et al. (1997), and William and Warren (1975) found that shading reduced light available to the parent purple nutsedge plants. Thus, thinner and weaker shoots were produced that were less competitive.

D. higginsii has the potential to reduce the interference of purple nutsedge in a pepper cropping system when applied at 10^6 conidia/ml. However, the field efficacy of this fungus under different cropping systems needs to be studied further.

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Macronutrients Distribution and Cycling of Pineapple Planted on Tropical Peat

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ABSTRAK

Kajian ini mengkuantitikan kemasukan, kehilangan, penahanan (tanah) dan pengambilan serta pengembalian P, K, Ca, dan Mg untuk pengurusan sisa nenas yang dibakar dan tidak dibakar. Rawatan yang digunakan adalah: sisa daun dikeluarkan dan tiada pembajaan (LRRNF), sisa daun dibakar dengan tiada pembajaan (LRBNF), sisa daun dikeluarkan dan diikut pembajaan (LRRF) dan sisa daun dibakar dan pembajaan dilakukan (LRBF). Nitrogen, P dan K dibekalkan dalam bentuk urea (46.00% N), batuan fosfat China (CPR 14.00% P) dan muriate of potash (MOP 49.80% K) pada kadar 701.04, 35.56 dan 556.56 kg N, P dan K per hektar mengikut turutan. Simulator hujan digunakan untuk menghitung larian permukaan pada plot sisa dibakar sebelum penanaman. Sampel tanah pula diambil pada kedalaman 0-5, 5-25 dan . 25 sm sebelum, semasa dan selepas peringkat-peringkat pembajaan. P, K, Ca dan Mg yang tersedia diestrak menggunakan kaedah dwiasid. Kaedah subtraksi pula digunakan untuk menganggar P, K, Ca dan Mg yang dilarut lesap (kg per ha). Pada peringkat matang, sampel tumbuhan diambil pada setiap rawatan dan dibahagikan kepada akar, batang, daun, buah, jambul dan tangkai serta berat kering dan kandungan P, K, Ca dan Mg di tentukan. Kandungan P, K, Ca dan Mg pada tanah, bahagian tumbuhan dan air hujan ditentukan melalui kaedah 'molybdate blue' dan spektrofotometer penyerapan atom (AAS). Penambahan P, K, Ca dan Mg daripada baja, abu dan presipitasi bagi LRBF dianggarkan pada 54.25, 816.68, 103.31 dan 23.54 kg per ha. Rawatan LRRF (pembajaan dan presipitasi) pula dianggarkan pada 35.56 P, 576.05 K, 100.17 Ca dan 4.93 Mg kg per ha. Anggaran kehilangan P, K, Ca dan Mg pada rawatan LRBF adalah pada 18.44, 300.45, 66.06 dan 8.63 kg per ha manakala pada rawatan LRRF, nilai-nilai adalah pada 23.19, 244.88, 45.79 dan 5.49 kg per ha. Larut lesap merupakan punca utama kehilangan P, K, Ca dan Mg bagi kedua-dua jenis pengurusan dan ini dapat dirujukkan dengan frekuensi pembajaan yang kurang sesuai. Satu keseimbangan positif P, K, Ca dan Mg dapat direkodkan untuk LRBF, 46.00% P, 28.005 K, 20.00% Ca dan 27.00% Mg dapat dikitarsemula selepas penanaman. Bagi rawatan LRRF pula, keseimbangan positif P, K, Ca dan Mg diperhatikan. Lebih kurang 60.00% P, 20.00% K, 13.00% Ca dan 36.47% Mg digunasemula untuk rawatan ini.

ABSTRACT

This research quantifies P, K, Ca and Mg inputs, losses, retention in soil, and uptake and returns for burnt and unburnt pineapple residue in management practices. Treatments used were: leaves residue removed and no fertilization (LRRNF), leaves residue burnt and no fertilization (LRBNF), leaves residue removed and fertilization (LRRF), and leaves residue burnt and fertilization (the usual practice) (LRBF). Nitrogen, P and K were applied in the forms of urea (46.00% N), China phosphate rock (CPR 14.00% P) and muriate of potash (MOP 49.80% K) at the rates of 701.04, 35.56, and 556.56 kg N, K, and P per ha respectively. Rainfall simulator was used for surface runoff measurement on burnt plots before planting. Soil sampling at the depths of 0-5, 5-25 and . 25 cm were done before planting, during and after fertilization stages. Extractable P, K, Ca and Mg were extracted using the double-acid method. The subtraction method was used to estimate P, K, Ca and Mg leached (kg per ha). At maturity, plants were sampled from each treatment and partitioned into roots, stem, leaves, fruit, crown and peduncle, and the dry weights, of P, K, Ca and Mg contents determined. Molybdate blue method and atomic absorption spectrophotometer were used in determining P, K, Ca and Mg in soil, plant parts and

rainwater, K, Ca and Mg additions from fertilizer, ash and precipitation for LRBF were estimated at 54.25, 816.68, 103.31 and 23.54 kg per ha, and those of LRRF (fertilizer and precipitation) were 35.56, 576.05, 100.17, and 4.93 kg P, K, Ca, and Mg per ha, respectively. The estimated amounts of P, K, Ca and Mg lost under LRBF were 18.44, 300.45, 66.06 and 8.63 kg per ha and in the case of LRRF, the losses were 23.19, 244.88, 45.79 and 5.49 kg per ha. Leaching was the major source of P, K, Ca and Mg loss for both practices and this was attributed to inappropriate fertilization frequency. A positive balance of P, K, Ca and Mg was recorded for LRBF, 46.00% P, 28.00% K, 20.00% Ca and 27.00% Mg which could be recycled after cropping. In the case of LRRF, a positive balance of P, K and Ca was observed. About 60.00% P, 20.00% K, 13.00% Ca and 36.47% Mg get recycled for LRRF.

INTRODUCTION

Cultivation of pineapple (*Ananas comosus*) on peat in Malaysia has been in existence for about a century (Selamat and Ramlah 1993). The cultivation currently practiced on large-scale initially started on small-scale basis with no fertilization. After the extensive and comprehensive survey of the pineapple cultivation (Dunsmore 1957), the need to apply balanced fertilizers for the growth and production of pineapple surfaced. Consequently, various fertilizer recommendations (Tay 1972; Tay 1973) for varieties like Singapore Spanish, Masmerah and Johorel were put forward. The recommendations were nutrient response oriented and as such none of the studies attempted or gave due cognizance of nutrient losses through leaching and runoff, nutrient retention after cropping not to mention inputs from ash and precipitation.

Perhaps when it became obvious that the previous recommendations have outlived their usefulness, a study was initiated to determine the right requirement of N, P, and K of these varieties on peat (Selamat and Ramlah 1993). Again, physical and physiological parameters were the focus of the study. Even though the study was conducted at a time when nutrients addition through burning crop residue, precipitation, leaching, and surface runoff losses and the mechanism of nutrient retention in organic soils had advanced, a holistic approach like nutrient balance was not considered.

With the ever increasing cost of fertilizers, plus the ever increasing awareness of the polluting effects that excess fertilizer applications have on the environment, there is a need to quantify the movement of nutrients into, within and without pineapple cultivation system. A broad based approach of this kind referred to as nutrient budget can be used as a tool in estimating the nutrient requirements of pineapple and hence help in the reduction of polluting effects that excess fertilizer applications may have on the environment. The study was conducted to quantify P, K, Ca and Mg inputs, losses, retention (soil), and returns for burnt and unburnt pineapple residue management practice.

MATERIALS AND METHODS

The experiment was carried out at Simpang Rengam Pineapple Estate, Johor, Malaysia. This place is a representative area for pineapple cultivation on peat in Malaysia. Treatments used were: (i) leaves residue removed and no fertilization (LRRNF), (ii) leaves residue burnt and no fertilization (LRBNF), (iii) leaves residue removed and fertilization (LRRF) and, (iv) leaves residue burnt and fertilization (the usual practice) (LRBF). The experimental unit was individual plants planted in 4 m x 12 m plot. Altogether 300 pineapple plants were planted in each plot having a randomized complete block design (RCBD) with 4 replications. The 1997 haze incident in South-East Asia has drawn public attention and concern to the polluting effect of open burning of crop residue on the environment, hence the inclusion of treatments i and iii.

Nitrogen, P and K were applied in the forms of urea (46.00% N) China phosphate rock (CPR-14.00% P) and muriate of potash (MOP-49.80% K) at the rates of 701.04, 35.56, and 556.56 kg of N, K, and P per ha, respectively. These rates are the rates used by the pineapple estate.

Prior to the start of the experiment, infiltration rate was measured in all the burnt plots using the double ring infiltrometer. Before first fertilization, water samples from surface runoff on the burnt plots using rainfall simulator (Kamphorst 1987) were collected, filtered and analyzed for P, K, Ca and Mg. Throughout the study, P was analyzed using molybdate blue method (Murphy and Riley 1962) and K, Ca, and Mg were analyzed using the atomic absorption spectrophotometer.

K, Ca, and Mg concentrations were multiplied by volume of runoff collected within 3 minutes per 0.0625 m^2 (area covered by the base of the rainfall simulator). Simple proportion was then used to convert the values to kg per ha basis. Kilogram per ha P, K, Ca and Mg multiplied by the number of runoff days gave the total amounts of P, K, Ca and Mg (kg per ha) lost through surface runoff between the period at which the experiment was started and the first fertilizer application. Surface runoff days refer to the rainy days on which runoff occurred and these days were selected based on the assumption that surface runoff occurs when rainfall intensity is higher than infiltration rate (Jackson 1989).

Peat core samplers of diameter 7.50 cm were used to collect peat samples at the depths of 0-5, 5-25 and .25 cm for bulk density determination. These sampling depths were also used to monitor leaching loss. Soil samples were taken before planting, fertilization and after fertilization stages. At the fertilization phase, samples were taken 3 weeks after fertilization so as to ensure uniform distribution and dissolution of the fertilizers. Subsequent post fertilization samples were taken bimonthly until the end of the experiment. Rainwater was collected from three rain gauges located at three different places in the estate at every sampling period stated and analyzed for P, K, Ca, and Mg.

Some plants of the different treatments were randomly selected and tagged when visual differences between the treatments began showing at the third month. Nutrients accumulation in leaves with time was monitored right from the third month of planting until plants were harvested by taking D-leaf and the contents of P, K, Ca, and Mg determined. D-leaf is the longest and easily identifiable leaf that provides a reliable and sensitive indication of pineapple nutritional status (Py *et al.* 1987). At maturity, plants from each treatment were randomly sampled and partitioned into roots, stem, leaves, peduncle, fruit and crown. These parts were oven dried at 60°C and their dry weights taken. Dry ashing (single dry ashing) was adopted for the extraction of P, K, Ca, and Mg., K, Ca, and Mg distribution were determined by multiplying the weight of plant parts by their respective concentrations. The products of P, K, Ca and Mg distribution per plant and plant density gave the total P, K, Ca, and Mg kg per ha in the distinct parts of the pineapple.

K, Ca, and Mg in soil were extracted using the double acid method (0.05*M* HCl: 0.025*M* H_2SO_4) with soil to solution ratio of 1:10 (modified from Van Lierop *et al.* 1980). The reason behind the modification of the extraction method were: (i) A dilute soil extractant helps in eliminating the possibility of the neutralization of the extracting solution through reaction with the soil and possibly reaction of Ca and Mg coming from the burnt crop residue plus artifacts in the soil, and (ii) Prolonged extraction time plus wider extraction ratio helps in minimizing the effects of rewetting time variability of dry peat (Van Lierop *et al.* 1980).

Weight of soil per ha at .25 cm (i.e. approximately 25-50 cm) multiplied by the respective concentrations of P, K, Ca and Mg gave the total amounts of kg P, K, Ca, and Mg per ha at that depth (leaching zone). Weight of soil per ha was estimated by multiplying a hectare volume of soil at .25 cm with the corresponding bulk density. The amounts of P, K, Ca, and Mg leached per ha at each sampling period were calculated using the subtraction method shown: TNL = NAF - NAU; where TNL = Total nutrient leached, NAF = Nutrient accumulated at .25 cm in fertilized plots, and NAU = Nutrient accumulated at .25cm in unfertilized plots (modified after Pomares-Gracia and Pratt 1978). For the burnt practice, leaching loss at sampling periods started from the first sampling after burning until the end of the cropping period and that of the unburnt started from the first sampling after first fertilization till the end of the cropping period. The depth of .25 cm was chosen as leaching zone because it was assumed that nutrients at this zone are beyond the reach of pineapple roots as roots grow laterally (Py *et al.* 1987).

RESULTS AND DISCUSSION

General Information

The bulk density at the depths of 0-5, 5-25, and .25 cm were 0.16, 0.23 and 0.13 g per cm^3 respectively. The infiltration rate of 0.2 cm per minute was obtained. The initial status of the extractable P, K, Ca, and Mg were relatively high (Table 1). Since the land has been under cultivation for the past 30 years, there is the tendency that residual accumulation might have taken place. Insignificant difference in P, K, Ca, and Mg contents in the soil were observed for the plots assigned the intended treatments:

LRRNF, LRBNF, LRRF and, LRBF before the start of the experiment (Table 1).

The general accumulation of the extractable P, K, Ca, and Mg for LRRF and LRBF at 0-5 cm (Table 2) may be due to inappropriate fertilizer application frequency. Besides the less mobile nature of P, the high accumulation of Ca might have also contributed to the P accumulation at 0-5cm (Cogger and Duxbury 1984).

Nutrients Addition

The sources of P, K, Ca, and Mg additions were fertilizer, ash, and precipitation with fertilizer contributing the highest amounts of P, K, and Ca for LRBF and LRRF (Table 3). For LRBF, ash contributed the highest amount of Mg with its P and K contribution second to fertilizer. The Ca from ash was relatively low as compared to those of fertilizer and precipitation (Table 3). Precipitation contributed no P, however, its contribution of K, Ca, and Mg was relatively high (Table 3) compared to the findings of Mohammad (1981) and Veneklass (1990). Possibly, the distance of the experimental site from the sea (about 20 km), the haze problem (during the experiment) in South-East Asia and the possibility of some ash suspension during and after burning may account for the difference. The total amounts of P, K, Ca, and Mg from fertilizer, ash, and precipitation addition to the pineapple nutrient cycle were 54.25, 816.48, 103.31 and 23.54 kg per ha for LRBF and 35.56, 576.05 100.17 and 4.93 kg per ha for LRRF (Table 3).

Nutrient Losses

Leaching was the major source of P, K, Ca, and Mg loss under LRBF and LRRF. In the case of LRBF, second to leaching loss was through fruit harvest (Table 3). Apart from Mg where the loss through fruit harvest was third to leaching, P, K, and Ca loss through residue removal was second to leaching followed by fruit harvest. Phosphorus, Ca, and Mg loss through surface runoff was relatively low. The total amounts of P, K, Ca, and Mg lost under LRBF were 18.44, 300.45, 66.06 and 8.63 kg per ha and those of LRRF were 23.19, 244.88, 45.79 and 5.49 kg per ha (Table 3).

Except for Mg where leaching occurred after fertilization, leaching loss of P, K, and Ca occurred before, during and after fertilization. Generally, the bulk of K, Ca, and Mg got leached at the post fertilization stage. The reason being that, more of P, K, Ca, and Mg got accumulated at the fertilization phase especially during the last fertilization (263rd day after planting) – a period where the plants were nine months old. At that stage, nutrient requirement of pineapple is generally lower than during or early growth

TABLE 1
The initial status of extractable P, K, Ca and Mg at three different depths before experimentation

Treatments	Depth (cm)	P	K	Ca	Mg
			mg/kg		
LRRNF	0 – 5	48	540	1397	90
	5 – 25	20	446	835	60
	. 25	20	383	940	85
LRBNF	0 – 5	40	503	1418	88
	5 – 25	20	400	920	60
	. 25	20	382	865	58
LRRF	0 – 5	53	472	1347	83
	5 – 25	20	460	800	58
	. 25	25	423	850	63
LRBF	0 – 5	43	535	1370	107
	5 – 25	20	520	775	70
	. 25	20	427	928	73

Note: Insignificant difference between treatments at same depths using LSD ≤ 0.05 was observed.

TABLE 2
Extractable P, K, Ca and Mg at various sampling stages

Sampling days	Extractable nutrients															
	P				K				Ca				Mg			
	LRNF	LRBNF	LRRF	LRBF	LRNF	LRBNF	LRRF	LRBF	LRBF	LRBNF	LRRF	LRBF	LRBF	LRBNF	LRRF	LRBF
	kg ha-I															
48	88	115	113	200.00	555	1395	563	1758	2718	4163	2198	4446	195	383	183	348
144	40	48	110	193	520	448	885	1040	1628	1865	2130	2143	130	198	180	220
263	53	45	2700	2733	320	373	3300	3093	1945	2265	7175	7843	253	283	635	655
365	33	33	1450	1207	135	182	574	443	1222	2205	4810	4873	78	235	233	181
417	18	13	1145	590	260	228	265	368	1105	1925	4838	4528	78	175	120	123
466	23	25	793	500	245	266	290	270	1127	1900	1970	3536	77	93	95	95

TABLE 3
P, K, Ca and Mg inputs, losses, returns and retention (soil) for burnt and unburnt practices

	LRBF (leaves burnt)				LRRF (leaves removed)			
	P	K	Ca	Mg	P	K	Ca	Mg
	kg/ha							
Inputs								
Fertilizer	35.56	557.09	62.48	0.41	35.56	557.09	62.48	0.41
Ash	18.69	240.43	3.14	18.60	0.00	0.00	0.00	0.00
Precipitation	0.00	18.96	37.69	4.53	0.00	18.96	37.69	4.53
Total (a)	54.25	816.48	103.31	23.54	35.56	576.05	100.17	4.93
Losses (b)								
Fruit	5.72	71.61	2.26	2.03	4.97	50.32	2.61	1.62
Leaves residue	0.00	0.00	0.00	0.00	6.52	51.16	2.66	1.27
Leaching	11.57	183.69	59.15	5.20	11.70	143.40	40.52	2.60
Runoff (ash)	1.15	45.15	4.65	1.40	0.00	0.00	0.00	0.00
Total (b)	18.44	300.45	66.06	8.63	23.19	244.88	45.79	5.49
Input-Loss (c)	35.81	516.03	37.25	14.91	12.37	331.17	54.38	-0.56
Uptake (d)	16.10	142.94	7.39	3.96	7.43	65.00	6.14	2.83
Amount Retained in soil = c – d	19.71	373.09	29.86	10.95	4.94	266.17	48.24	-3.39

stage (Py et al. 1987) therefore, with annual rainfall of about 1917 mm coupled with the very low clay (Stevenson 1994), the potential for P, K, Ca, and Mg lost through leaching was high.

Nutrients Removal and Returns

Under LRBF, the total amounts of P, K, Ca, and Mg taken up (roots, stem, leaves, peduncle and crown) from fertilizer, ash and precipitation that can be recycled were 16.10, 142.94, 7.39, and 3.96 kg per ha. Those of LRRF (roots and stem) were 7.43, 65.00, 6.14, and 2.83 (Table 3). It must be noted that, since leaves, peduncle and crown for LRRF are not recycled, their nutrients removal was considered loss and were excluded in this section.

Nutrients Retention after Cropping

After taking into account nutrients returns and losses, the amounts (kg per ha) of P, K, Ca, and Mg from inputs (fertilizer, ash, and precipitation retained) in the soil (at 0-5 and 5-25cm) after cropping were 19.71, 373.09, 29.86 and 10.95 for LRBF and 4.94, 266.17, 48.24 and 2.27 kg per ha for LRRF (Table 3).

Unutilized P, K, Ca, and Mg under LRBF were relatively high. K and Ca retained under LRRF were also high but with low P. This low P may partly be due to residue removal as it contributed about 28% (second to leaching loss) of the total P lost. It is therefore anticipated that if this practice goes on for some time, more P will be depleted and hence rescheduling of fertilizer program will be inevitable. The negative balance of Mg (Table 3) recorded for LRRF may be attributed to the unaccountability of Mg addition through stem and roots decomposition during the cropping period as these parts under LRRF are normally not removed from the field. However, for the sake of accountability if it is considered or assumed that the amount of Mg taken from the inputs (fertilizer and rainfall) by these parts is proportional to the amount returned through decomposition, about 2.83 kg Mg per ha is recycled and hence the 2.83 kg Mg per ha balances the deficit.

CONCLUSIONS

The existing fertilization regime particularly for K in pineapple cultivation on peat for the burnt and unburnt pineapple residue management

practices is inappropriate. There is therefore the need to reschedule the present fertilizer regime. Perhaps the last fertilization needs to be stopped.

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Effects of Food Plants on Development of *Spirama retorta* (Lepidorptera: Noctuidae)

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Key-words: ***Spirama retorta, Acacia mangium, Acacia auriculiformis, Acacia crassicarpa, Paraserianthes falcataria***

ABSTRAK

Pembesaran Spirama retorta *(Lepidoptera: Noctuidae) larva yang diberi makan daun tiga spesies* Acacia, *iaitu* A. mangium, A. auriculiformis *dan* A. crassicarpa, *dan* Paraserianthes falcataria *telah dinilai di makmal. Larva yang didedahkan kepada daun* A. crassicarpa *dan* P. falcataria *kesemuanya mati pada instar pertama. Lebih daripada 64% larva yang memakan daun* A. mangium *dan* A. auriculiformis *mencapai peringkat pupa. Jangkamasa larva pada daun* A. auriculiformis *ialah 22.10 hari manakala pada* A. mangium *ialah 24.83 hari. Jangkamasa pupa dari* A. auriculiformis *ialah 10.51 hari dan dari* A. mangium *ialah 11.32 hari. Ini menghasilkan rama-rama dewasa yang hidup selama 36.51 dan 37.94 hari. Walaupun pada keseluruhannya, angkaubah pembesaran tidak signifikan, rama-rama betina yang pada peringkat larva memakan daun* A. auriculiformis *menghasilkan lebih banyak telur daripada yang memakan* A. mangium *iaitu 412 telur// dari* A. auriculiformis *dan 255 telur// dari* A. mangium. *Kajian ini menunjukkan daun* A. auriculiformis *dan* A. mangium *sesuai untuk pemakanan larva* S. retorta. *Oleh itu, spesies ini boleh menjadi sumber alternatif makanan penting untuk dinamik populasi rama-rama ini jika ketiadaan tumbuhan hos asli.*

ABSTRACT

Development of Spirama retorta *(Lepidoptera: Noctuidae) larvae fed on foliage of three* Acacia *spp., namely* A. mangium, A. auriculiformis *and* A. crassicarpa, *and* Paraserianthes falcataria *was assessed in the laboratory. The larvae did not survive when fed on either* A. crassicarpa *or* P. falcataria. *More than 64% reached pupal stage when fed on* A. auriculiformis *and* A. mangium. *The larval period was completed in 22.10 and 24.83 days when the larvae fed on* A. auriculiformis *and* A. mangium *foliage, respectively. The average pupal period was 10.51 and 11.32 and, the resulting adults lived for 36.51 and 37.94 days on* A. auriculiformis *and* A. mangium, *respectively. Even though the overall development variables were not significantly different, females from larvae fed* A. auriculiformis *had a significantly higher fecundity than those females from* A. mangium. *A total of 412 eggs// was recorded from those fed* A. auriculiformis *as compared to 255 eggs// on* A. mangium. *This study thus shows that foliage of* A. auriculiformis *and* A. mangium *provided a suitable diet for* S. retorta *larvae. As such, these species may serve as alternative food resources important in the population dynamics of the moth in the absence of indigenous host plants.*

INTRODUCTION

The declining supply of timber from natural forests has led many tropical countries including Malaysia replenish their timber resource by adopting a reforestation program involving planting of fast-growing exotic species. In Malaysia, about 500,000 ha of unproductive forest has been alienated for establishment of forest plantations. To date, approximately 100,000 ha of forest plantations have been established. Ninety percent of this was planted with *Acacia mangium.* While *Gmelina arborea* and *Paraserianthes falcataria* were planted on a smaller scale.

A. mangium is a fast growing leguminous tree indigenous to Northern Australia, Papua New Guinea and Irian Jaya (Anon. 1983). The

tree can grow up to 30 m high with a straight bole measuring 40 cm in diameter at breast height. It can be harvested for pulpwood in five to seven years or sawlog production in 12 to 15 years. In addition to *A. mangium*, *A. auriculiformis* and *A. crassicarpa* have also been the subject of many researches in Malaysia. Results from provenance trial plots indicated that these trees have the potential to be grown for commercial plantations (Nor Aini *et al.* 1994; Kamis *et al.* 1995).

Even though trees like *Acacia* often perform very well when grown as exotics, they are, however, prone to attack by diseases and insects. To date, many indigenous insects have been reported to be associated with these *Acacia* and some could pose serious threats to the plantations (Abe 1983; Hutacharern 1993; Chey 1996, Sajap *et al.* 1997). One of these insects was a rare moth, *Spirama retorta* (Lepidoptera: Noctuidae). The larvae of this insect were found in an outbreak where they defoliated a one-year old *A. mangium* stand in an area of 800 ha at Gunung Besaut Forest Plantation, Sungkai, Perak. The biology of this insect was described by Sajap *et al.* 1996. Apart from *A. mangium*, no other host plant has yet to be associated with this insect in Malaysia. *Albizzia lebbek* was the only recorded host plant elsewhere (Beeson 1961).

In this study, we examined the suitability of three *Acacia* spp. and *P. falcataria* for the development of *S. retorta*. *Paraserianthes falcataria* was included as it was related to the reported host plant, *A. lebbek*. This information is pertinent in determining the host range of the insect in view of its becoming a potential pest of Acacia spp.

MATERIALS AND METHODS

Insect Rearing

A colony of *S. retorta* was established from larvae collected from *A. mangium* plantation at Gunung Besaut, Perak. The larvae were kept in 11 × 15 × 25 cm plastic boxes provided with fresh *A. mangium* foliage. The foliage was changed everyday. When the larvae reached the fifth instar, vermiculite which acted as a substrate for pupation, was added into the box. The pupae were collected, sexed and surface-sterilized with 1% sodium hypochlorite. Five pairs of male and female pupae from the same cohort were held in a cage for adult emergence, subsequent mating and oviposition. The oviposition cage consisted of a cylindrical wire mesh, 9 × 12 cm, internally lined with a netting cloth which was used oviposition site. Ten percent honey solution in a cotton-plugged vial and a slice of very ripe papaya were placed in the cage and served as food sources. Eggs collected from the colony were kept in 9 cm petri dishes for hatching.

Feeding experiment

Test Plants

A. mangium, *A. auriculiformis* and *A. crassicarpa*, and *P. falcataria* plant materials were obtained from provenance trial plots located at Universiti Putra Malaysia, Serdang.

Experimental Procedure

Fresh foliage was placed in 9 cm petri dishes lined with two pieces of moistened filter papers. One neonate was introduced into each petri dish. The foliage and the filter papers were changed daily and weekly, respectively. Vermiculite was added into the petri dishes when the larvae reached the end of the fifth instar. Growth and development parameters: mortality, molting period, pupal weight and size of the head capsule, were recorded. Faeces defaecated throughout the larval stages were collected daily, oven-dried at 80°C for 24 h, cooled and weighed. Newly emerged adults were sexed and paired. Each pair was placed in a cage for oviposition. Eggs were collected and counted daily. A total of 100 larvae per treatment in four replicates, 25 per replicate, were used in this study. All experiments were carried out in a room at 27 - 32°C and 70 - 80% RH.

Data Analysis

Statistical analysis of all the developmental variables was conducted by t-test ($\alpha = 0.05$)

RESULTS

Larval Mortality

The result from this feeding study shows that *S. retorta* could feed and develop on foliage of *A. mangium* and *A. auriculiformis*. They failed to feed on *A. crassicarpa* and *P. falcataria* and died in the first stadium. The total mortality for larvae fed on *A. mangium* and *A. auriculiformis* were 36% and 31%, respectively, with more than 22% dead before reaching the third instar. All the larvae that pupated emerged into adults (Table 1).

TABLE 1
Developmental time (days) and percent mortality of *S. retorta* larvae fed on *A. auriculiformis* and *A. mangium* foliage

Stage	*A. auriculiformis* days *	%	*A. mangium* days *	%
I	3.07 ± 0.26a	13.00	3.11 ± 0.31a	15.00
II	3.12 ± 0.32a	9.18	3.36 ± 0.55a	10.00
III	3.46 ± 0.53a	2.23	3.64 ± 0.57a	3.00
IV	3.48 ± 0.53a	2.59	3.61 ± 0.58a	3.00
V	3.91 ± 0.61a	2.66	4.06 ± 0.94a	3.00
VI	6.39 ± 1.90a	5.48	5.19 ± 1.41a	4.00
VII	7.52 ± 0.72a	1.00	7.81 ± 0.82a	1.00
Pupa	10.51 ± 1.85a	0	11.32 ± 1.24a	0
Adult /	10.11 ± 0.90a	-	9.60 ± 0.51a	-
?	9.11 ± 0.51a	-	7.70 ± 0.68a	-
Egg	4.00 ± 0.87a		4.20 ± 0.84a	

* Means in the same row followed by the same letter are not significantly different (α =0.05)

Developmental Period

The development time of *S. retorta* larvae fed on *A. mangium* and *A. auriculiformis* is shown in Table 1. The larval period was completed in 22.10 ± 2.42 days and 24.83 ± 2.71 days when the larvae fed on *A. auriculiformis* and *A. mangium* foliage, respectively. The larvae went through either six or seven instar before pupation. On *A. mangium*, 22% and 78% attained their pupal stage after the sixth and seventh instars, respectively. On *A. auriculiformis*, 56% attained pupal stage after the sixth instar and 44% after the seventh instar. The average pupal period was 10.51 ± 1.85 days and 11.32 ± 1.24 days when the preceeding larvae were fed on *A. auriculiformis* and *A. mangium*, respectively. The longevity of the adults emerging from larvae previously fed on *A. auriculiformis* stages was relatively shorter than those fed on *A. mangium*. With *A. auriculiformis* their longevities were 10.11 ± 0.90 days and 9.11 ± 0.51 days for the females and the males, respectively. With *A. mangium* the longevities were 9.60 ± 0.51 days and 7.70 ± 0.68 days for the females and the males, respectively.

Head Capsule Size and Pupal Weight

Head capsule size and pupal weight were not significantly different for individuals fed on either foliage. The width of head capsule increased from 0.30 mm in the first instar to about 3.00 mm in the seventh instar (Table 2). The resultant pupae also had similar sizes and weights. The pupal wieght and length from larvae fed on *A. auriculiformis* were 0.90 ± 0.11 g and 2.65 ± 0.10 mm, respectively and those fed on *A. mangium* attained pupal weight and length of 0.89 ± 0.10 g and 2.66 ± 0.13 mm, respectively.

TABLE 2
Means of head capsule width (mm) of *S. retorta* larvae fed on A. mangium and *A. auriculiformis* foliage

Instar	*A. mangium* (mm)*	*A. auriculiformis* (mm)*
I	0.30 ± 0.00a	0.30 ± 0.00a
II	0.54 ± 0.05a	0.55 ± 0.50a
III	0.86 ± 0.10a	0.85 ± 0.06a
IV	1.40 ± 0.18a	1.40 ± 0.12a
V	2.07 ± 0.21a	2.08 ± 0.14a
VI	2.69 ± 0.23a	2.75 ± 0.24a
VII	2.92 ± 0.16a	2.94 ± 0.17a

*Means in the same row followed by the same letter are not significantly different (α =0.05)

Faeces Production

S. retorta consumed about the same amount of either *A. auriculiformis* or *A. mangium* foliage except in the seventh instar (Table 3). This was shown by the amount of faeces defaecated throughout the larval period. The weight of faeces obtained from the larvae in the seventh stadium feeding on *A. auriculiformis* was significantly higher than those larvae feeding on *A. mangium* foliage. The total amount faeces defecated by a larva fed on *A. auriculiformis* and *A. mangium* was 773.49 and 724.71 mg, respectively.

Fecundity

The number of eggs laid by females previously fed on *A. auriculiformis* (412//) was almost double those females previously fed on *A. mangium* (255//) foliage during their larval stages. The daily oviposition rates of the moths is shown in Figure 1. Moths emerging from *A. auriculiformis*-reared larvae laid an average number of eggs varied from 50 on the first day, reached its

TABLE 3
Means of faecal weight (mg) produced by *S. retorta* larvae fed on *A. mangium* and *A. auriculiformis* foliage

Instar	*A. mangium* (mg)*	*A. auriculiformis* (mg)*
I	8.98 ± 0.98a	8.58 ± 0.90a
II	10.93 ± 1.00a	11.02 ± 1.10a
III	24.21 ± 5.72a	24.19 ± 5.92a
IV	50.88 ± 9.59a	48.28 ± 10.43a
V	137.41 ± 59.88a	137.23 ± 58.45a
VI	296.79 ± 92.66a	305.22 ± 94.31a
VII	195.52 ± 70.51a	238.97 ± 83.55b

* Means in the same row followed by the same letter are not significantly different (α =0.05)

maximum of 115 on the second day and dropped to 38 eggs on the seventh day. Although a similar trend of oviposiotion pattern was also observed on moths emerging from *A. mangium*-reared larvae but the average number of eggs laid daily was lower. On the first day, 43 eggs were laid. The number increased to 90 on the third day and dropped to 22 on the fifth day. All eggs hatched in three days with hatching rates exceeding 90% for both batches.

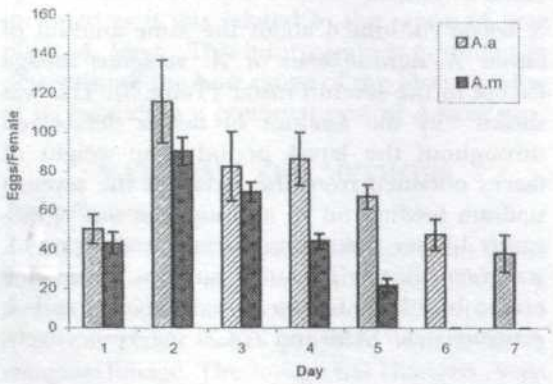

Fig. 1. Fecundity of S. retorta *moths previously fed on* A. mangium *and* A. auriculiformis *foliage during the larval stage*

DISCUSSION

Host plants play an important role in growth and development of an insect. The resultant impact of the nutritive value of the plant consumed by the insect could be reflected in the larval development rate, pupal weight, female fecundity, survivorship and behaviour of the insect (Beck and Reese 1976; Slansky 1982; Hagen *et al.* 1984.). In this study, even though the larvae initiated feeding, indicated by the many biting marks on *P. falcataria* foliage, they, however, failed to continue feeding and died in the first stadium. The rejection of the foliage by the larvae could be due to the absence of token stimuli or the presence of deterrent chemicals that inhibit them from further feeding on the foliage despite the very tender foliage texture (Ehrlich and Raven 1964). This phenomenon commonly occurred in insects that were exposed to non-host plants (Hough and Pimentel 1978). When offered foliage of *A. crassicarpa*, *S. retorta* larvae did not initiate feeding and left no biting marks on the foliage. This could be attributed to the toughness of the foliage that led to a 100% mortality at the early stage.

Even though, there were no significant differences in the overall developmental period and growth parameters between individuals fed on *A. auriculiformis* and *A. mangium*, larvae fed on *A. auriculiformis* apparently developed relatively faster than on *A. mangium*. The larvae that developed through seventh instar defaecated more faeces when fed on *A. auriculiformis* than on *A. mangium*. Consequently, the adults had a relatively longer reproductive period and laid a higher number eggs than those previously reared on *A. mangium* foliage. This result suggests that *A. auriculiformis* presumably has a superior nutritive value and thereby served a better host plant than *A. mangium*.

In summary, the foliage of *A. auriculiformis* and *A. mangium* provided a suitable diet for *S. retorta* larvae. As such, these tree species may serve as food resources important in the population dynamics of the moth in the absence of the indigenous host plants.

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Quality Assessment of Local and Franchise Beef and Chicken Burgers

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ABSTRAK

Enam jenama burger lembu dan ayam, tiga jenama burger lembu francais dan dua jenama burger ayam francais telah dinilai dari segi kandungan proksimat, kandungan daging dan mioglobin, warna (nilai L, a, b) dan kandungan mikrobiologi iaitu Kiraan Jumlah Plat (CFU/gm), Kiraan Koliform dan Escherichia coli *(MPN/gm), Kiraan* Staphylococus aureus *(CFU/gm) dan kehadiran* Salmonella *sp. Kesemua burger lembu francais mempunyai kandungan protein dan kelembapan yang lebih tinggi (kecuali burger C) dan kandungan karbohidrat yang lebih rendah dari jenama tempatan. Didapati tiada perbezaan yang nyata ($p > 0.05$) dalam kandungan abu, lemak dan serabut kasar antara burger lembu francais dan jenama tempatan. Kebanyakan burger ayam jenama tempatan mempunyai kandungan protein dan kelembapan yang lebih rendah dan kandungan lemak, serabut dan karbohidrat yang lebih tinggi berbanding dengan burger ayam francais. Didapati tiada perbezaan yang nyata ($p > 0.05$) dalam kandungan abu antara burger ayam jenama tempatan dan francais. Kesemua burger lembu mempunyai kandungan mioglobin dan daging yang rendah (< 65%) kecuali burger A1, F1 dan G1. Burger ayam E1, F1dan burger francais B mempunyai kandungan daging yang lebih tinggi (> 65%) berbanding dengan yang lain. Kesemua burger ayam dan lembu mempunyai nilai 'L' yang lebih tinggi iaitu antara 45.13% hingga 53.68% dan 62.75% hingga 72.48% masing-masing kecuali F1 yang lebih gelap. Burger lembu jenama tempatan mempunyai nilai 'a' lebih tinggi berbanding dengan francais dan kesemua burger ayam mempunyai nilai 'a' yang rendah kecuali F1 yang lebih merah. Kiraan Jumlah Plat, Kiraan Koliform dan* E. coli *yang rendah didapati di dalam semua sampel burger. Kiraan* S. aureus *dalam sampel burger lembu dan ayam berjenama tempatan adalah lebih tinggi daripada francais iaitu antara 2 hingga 11 CFU/gm sampel dan antara 6 hingga 22 CFU/gm sampel masing-masing. Tiada kehadiran* Salmonella *sp dapat dikesan dalam semua sampel burger.*

ABSTRACT

Six brands of local beef and chicken burgers, three brands of franchise beef and two brands of franchise chicken burgers were evaluated for proximate composition, myoglobin and meat content, colour (L, a, b values) and microbiology composition i.e. Total Plate Count (CFU/gm), Coliform and Escherichia coli *Counts (MPN/gm),* Staphylococus aureus *Count (CFU/gm) and presence of* Salmonella *sp. All franchise beef burgers had higher protein and moisture contents (except burger C) and lower carbohydrate content than the local brands. No significant differences ($p > 0.05$) in fat, ash and crude fibre contents were observed between local brands and franchise beef burgers. Most local brands of chicken burgers had lower levels of protein and moisture and higher levels of fat, fibre and carbohydrate than the franchises. No significant differences ($p > 0.05$) in ash content was observed between the local brands and franchise chicken burgers. All beef burgers had low myoglobin and meat contents (<65%) with the exception of A1, F1 and G1 burgers. Chicken burgers, E1, F1 and franchise burger B had higher meat content (>65%) than the others. All beef and chicken burgers had higher 'L' values which ranged between 45.13% to 53.68% and 62.75% to 72.48% respectively except F1 which was darker. Local brands of beef burgers had a higher 'a' value compared to the franchises and all chicken burgers had a low 'a" value except F1 which was redder. Low Total Plate Count, Coliform and* E. coli *counts were detected in all burger samples.* S. aureus *counts in most local brands of beef and chicken burger samples were higher than the franchises which ranged from 2 to 11 CFU/gm sample and 6 to 22 CFU/gm sample respectively.* Salmonella *sp was not present in all burger samples.*

INTRODUCTION

An increase in the demand for fast food in Malaysia is due to the changing habits of the consumers in the 90's; it is convenient, easy to serve and eat, and suitable for those always 'on the run'. The western type of meat products which are currently adopted and manufactured in Malaysia are mostly beef and chicken burgers and frankfurters. Burgers have became one of the most popular fast food in Malaysia and there has been a rapid growth in local production of burgers in the past few years. In 1985, the giant foreign franchises were MacDonald's, Wendy's and the A&W chain of restaurants (Babji and Letchumanan 1989). This trend was followed by local producers and many franchise companies were formed such as Ramly, Yeo Hiap Seng, Purnama and Saudi. However, there are major differences between local burgers and those franchised. Differences include organoleptic properties, chemical composition, formulations, nutritional composition and overall acceptance of these burgers by consumers.

The inherent high price for premium quality animal protein have induced local producers to manufacture meat products of a lower quality for the mass consumption by the local population. Various unconventional raw materials and non-meat ingredients were utilized for further processing with only a low percentage of meat as the raw material being blended into the formulation. In processed meat production, premium meat cuts are seldom used. The utilization of unconventional raw materials and plant protein in meat products affects the chemical and nutritional composition and also the microbiological quality of the products. Under the Food Regulation of Malaysia 1985, burgers are classified as manufactured meat which must contain not less than 65% meat, 1.7% nitrogen and not more than 30% fat in organic combination. Babji et al (1984, 1985) and Babji (1988) have reported various aspects of nutritional composition, use of food binders and additives, and the processing and quality control standards in the manufacturing of local beef burgers in Malaysia. Many of the local manufacturers paid little attention to the nutritional as well as quality aspects of the products. Quality control in the processed meat industry is still unsatisfactory. There are also problems encountered in the establishment of minimum standards and specifications for such new products (Babji 1988). Information on the raw material composition, microbiological status and quality control aspects, particularly more so on the non-conventional raw materials from the livestock industry is poorly documented. The quality of locally produced and franchise burgers should be monitored from time to time to ensure that the products the minimum requirements of the standards and specifications, and are of acceptable quality to the consumers.

This study was carried out to observe the quality of the local and franchise beef and chicken burgers by determining the proximate composition, myoglobin and meat content and microbiological aspects. It is necessary to ascertain the quality of products consumed by the consumer. This study also provides information to satisfy the needs of consumers who demand meat products that are nutritions, well-balanced and safe from toxic and microbial contaminations.

MATERIALS

The analyses were carried out on six local brands of beef burgers i.e. Angus, Biffi, Fika, Ramly, Purnama and Saudi, and six local brand chicken burgers i.e. Ramly, Ayamas, Ayam Dinding, Fika, Purnama and Saudi. Most of which are available in the local supermarkets. The three types of franchise beef burgers were obtained from Mac Food Services, A&W and MBF Food Division and two types of franchise chicken burgers were obtained from Mac Food Services and MBF Food Division. The franchise burgers (beef and chicken) were labelled A, B and C and local burgers (beef and chicken) were labelled A1, B1, C1, D1, E1, F1, G1, H1 to fulfill the companies requirement for product anonymity.

The burger samples were analysed (duplicate) for proximate composition, colour in terms of lightness ('L'), redness ('a') and yellowness ('b'), myoglobin and meat content. The microbiology quality of the burger were tested by determinating Total Plate Count (CFU/gm), Coliform and *E. coli* counts (MPN/gm) *S. aureus* count (CFU/gm) and the presence / absence of *Salmonella* sp.

METHODS

Proximate Analysis

Proximate analyses were carried out using AOAC (1984) methods which included protein determination using Kjeldahl method, fat extraction via Soxhlet method, crude fibre determination using digestion with sulphuric acid, moisture determination by drying the sample for 16 - 18

hours at 100 - 102°C in oven and the ash by ashing the sample at 550°C for 9 hours in furnace oven. Carbohydrate content was determined by substracting the value from the total (100%) minus the percentages of other contents.

Physico-chemical Analysis

The lightness ('L'), redness ('a') and yellowness ('b') values for colour determination were measured using a chromameter (Minolta Chromameter Model CR - A70).

The myoglobin content was determined by using Poel Cyano Method (Topel, 1949). A 10 g sample was homogenized for 2 minutes in cold water mixed with X ml 1N H_2SO_4 in a waring blender. (X = (pH sample - 5) × 0.35). The pH of meat samples was determined using the AOAC method (1980). The homogenate was centrifuged at 3000 rpm for 2 minutes in a polyethylene tube (50 ml) using the MSE Desk centrifuge. The supernatant obtained was transferred to a 50 ml tube and heated slowly to reach a temperature of 54°C after which it was soaked in a water bath to reach 25°C. The homogenate was placed in a 100 ml beaker and the pH brought to 7.2 using Na_2CO_3. The homogenate was transferred back to a 50 ml tube and centrifuged for 10 minutes at 2500 rpm. The supernatant was filtered into a 50 ml Erlenmeyer flask and 2-3 small crystals of potassium ferricyanide was added. Absorbance was read at 540 nm using the Spectronic 20. Calculation of myoglobin (Mb) was derived by Poel-Cyano (Topel, 1949):

mg Mb / g wet tissue = absorbance × 7.50

Meat Content

The meat content for the burger samples was determined by using the myoglobin contents obtained earlier using the Poel Cyano Method (1949). A standard curve was constructed using myoglobin content of beef : soy protein or chicken : soy protein mixtures with standardized percentage of meat i.e. beef / chicken : soy ; 100/0, 80/20, 60/40, 40/60, 20/80, 0/100. The beef used in the mixtures was Indian beef as it is commonly used in the burger industry. The chicken meat used in chicken soy protein mixture was from the defatted breast meat. The soy protein used was soy protein isolate 500 E obtained from local suppliers. The meat content of the burger samples were obtained from these standard curves using their myoglobin contents which had been determined earlier.

Microbiological analysis

The following analyses were carried out using procedures described by Oxoid (1979); Total Plate Count (TPC), Coliform count (MPN), *E. coli* (MPN), *S. aureus* count and presence/absence of *Salmonella* sp. A 10 gm sample of each material (frozen) was homogenized aseptically in a stomacher bag with 90 ml sterile Ringer solution using a stomacher (Model Seward BA 7021). The homogenous sample solution was used for the determination of Total Plate Count, Coliform, *E. coli* and *S. aureus* count. Total Plate Count was carried out using the pour plate technique, with Plate Count Agar (PCA, Oxoid) and incubated at 37°C for 48 hours. For the Coliform count, MacConkey broth media containing Neutral Red as an indicator was used. The number of presumptive positive tubes (5 tubes) were counted and refered to the MPN Table. For *E. coli* count (MPN), positive tubes from Coliform count were tested in pairs, using Eijkman test (Mac Conkey broth) and Indole test (Tryptone water). Only tubes showing positive results for both tests are considered presumptive positive for *E. coli*. For *S. aureus* count, Baird Parker Agar (Oxoid) was used which was enriched with Egg Yolk Tellurite Emulsion (Oxoid). The innoculum was spread on the surface of the agar and incubated at 37°C for 24 - 48 hours. *Salmonella* sp (25 gram sample) was isolated using pre-enriched buffered peptone water, followed by selective enrichment in Selenite Cystine Broth (SCB, Oxoid) and Tetrathionate Broth (TTB, Merck) and finally selective agar medium, Brilliant Green Agar (BGA, Oxoid) and Bismuth Sulphite Agar (BSA, Difco). The presence of *Salmonella* sp was confirmed with Triple Sugar Iron Agar (TSI, Oxoid) and Lysine Iron Agar (LIA, Oxoid).

RESULTS AND DISCUSSION

Table 1 showed the proximate composition of local brands and franchise beef burgers. From the statistical analysis, there were significant differences ($p < 0.05$) in protein, moisture and carbohydrate contents between the local brands and franchise beef burgers. However, there were no significant difference ($p > 0.05$) in fat, ash, and crude fibre contents. From Table 2, there were significant differences ($p < 0.05$) between the local brands and franchise chicken burgers in moisture, fat, protein, carbohydrate and crude fibre contents except in the ash.

TABLE 1
Proximate composition of local and franchise beef burgers

Samples *	Percentage (%)					
	Protein	Fat	Moisture	CHO	Ash	Fiber
Local :						
A1	15.51 ± 0.96	12.36 ± 1.15	57.41 ± 0.82	12.76 ± 0.03	1.63 ± 0.29	0.33 ± 0.01
B1	10.00 ± 0.71	25.74 ± 0.62	45.26 ± 0.97	16.45 ± 0.25	2.02 ± 0.12	0.53 ± 0.02
C1	11.71 ± 0.67	21.83 ± 0.84	47.16 ± 1.34	16.54 ± 0.62	2.18 ± 0.15	0.58 ± 0.02
F1	15.26 ± 2.41	17.47 ± 0.99	49.10 ± 0.72	14.78 ± 0.51	2.85 ± 0.03	0.54 ± 0.01
G1	12.70 ± 0.65	19.05 ± 0.08	55.06 ± 0.73	10.80 ± 0.63	2.08 ± 0.13	0.31 ± 0.01
H1	14.42 ± 0.84	23.38 ± 0.19	45.32 ± 0.38	14.16 ± 0.13	2.18 ± 0.17	0.54 ± 0.02
Mean	13.27 ± 1.04	19.97 ± 0.65	49.89 ± 0.69	14.25 ± 0.36	2.16 ± 0.15	0.47 ± 0.01
Franchise :						
A	18.07 ± 0.61	15.23 ± 0.36	61.61 ± 0.32	2.77 ± 0.02	1.53 ± 0.16	0.79 ± 0.02
B	21.26 ± 0.16	19.27 ± 0.42	56.89 ± 0.49	1.05 ± 0.03	0.83 ± 0.13	0.70 ± 0.02
C	20.76 ± 0.34	20.19 ± 0.17	56.42 ± 0.44	0.11 ± 0.02	2.07 ± 0.08	0.45 ± 0.01
Mean	20.03 ± 0.37	18.23 ± 0.32	58.31 ± 0.42	1.31 ± 0.02	1.48 ± 0.12	0.65 ± 0.02

* Mean of two samples/treatment

TABLE 2
Proximate composition of local and franchise chicken burgers

Samples *	Percentage (%)					
	Protein	Fat	Moisture	CHO	Ash	Fiber
Local :						
A1	12.67 ± 0.69	23.05 ± 0.66	50.81 ± 0.25	10.16 ± 0.88	1.54 ± 0.03	1.77 ± 0.09
D1	13.96 ± 1.53	12.72 ± 0.54	68.00 ± 0.34	2.06 ± 0.05	1.52 ± 0.19	1.74 ± 0.18
E1	14.38 ± 0.66	15.26 ± 0.71	64.89 ± 0.46	1.97 ± 0.32	1.94 ± 0.17	1.56 ± 0.03
F1	15.66 ± 1.25	21.02 ± 0.94	48.01 ± 1.35	11.50 ± 0.61	2.04 ± 0.05	1.77 ± 0.09
G1	13.33 ± 1.54	16.27 ± 1.00	57.82 ± 1.54	9.09 ± 0.63	1.85 ± 0.11	1.64 ± 0.06
H1	15.54 ± 0.55	23.55 ± 0.41	44.57 ± 0.61	12.53 ± 0.13	2.08 ± 0.09	1.73 ± 0.04
Mean	14.26 ± 1.04	18.65 ± 0.71	55.68 ± 0.76	7.89 ± 0.44	1.83 ± 0.11	1.70 ± 0.08
Franchise :						
A	22.74 ± 0.88	5.86 ± 0.25	68.40 ± 0.43	1.27 ± 0.03	1.32 ± 0.28	0.41 ± 0.02
B	18.20 ± 0.32	7.63 ± 0.63	66.44 ± 1.08	5.69 ± 0.03	1.69 ± 0.15	0.35 ± 0.02
Mean	20.47 ± 0.60	6.75 ± 0.44	67.42 ± 0.76	3.48 ± 0.03	1.51 ± 0.22	0.38 ± 0.02

* Mean of two samples/treatment

Franchise beef burgers had higher protein content, ranging between 18.07% to 21.26%, compared to local brands which ranged from 10.00% to 15.51%. For chicken burgers, the protein level in franchise burgers was higher than the local brands which were 22.74% and 18.20% for franchise burger A and B respectively. Some local beef burgers were found to contain high fat, (more than 21%) and carbohydrate (more than 14%) contents but lower in protein content. In local burgers with protein contents ranging from 11% to 16%, it is obvious that some of the meat protein have been replaced by binders and fillers such as rusk, breadcrumbs, cereal, legumes and soy protein. This is reflected in the higher carbohydrate contents in local burgers. This was similar with the H1, A1 and F1 chicken burgers where the fat and carbohydrate contents were more than 21% and 10% respectively and low in protein content. Babji et al. (1989) reported that manufacturers in their efforts to cut cost, often used meat substitutes such as cereals, soy proteins, ground nuts and lately mechanically deboned meat to formulate hamburgers.

For G1 beef burgers and D1 and G1 chicken burgers, although they have low protein content, their fat content was not as high as in the others. However they had a higher moisture content which were 55.06%, 68.00% and 57.82% respectively. More water could be added to the burgers with the assistance of binders and fillers such as rusk, cereals, breadcrumbs, textured vegetable protein and soy protein. The use of carbohydrate fillers add to the volume of the product since it absorbs water and binds well with the meat (Smith 1979). Carbohydrate and soy protein also aid in increasing water holding capacity of the meat product (Wilner 1979). Soy protein is popular because of its high water holding capacity, good texture and bulkiness in weight when hydrated (Babji *et al.* 1989). Although the use of soy protein in meat and meat products is strictly regulated overseas, in Malaysia, there is currently no specific regulation concerning its use in local meat products (Babji et al. 1984). Nevertheless, although the moisture level was high in franchise beef and chicken burgers including D1 and E1 burgers, the carbohydrate contents were low ranging from 0.11% to 5.69%.

Although there were no significant differences in ash and crude fibre contents in beef burgers, the local brand had a higher ash content but lower in crude fibre content when compared with franchise beef burgers A and B. This was also the same for chicken burgers, where the level of ash and crude fibre were lower in some local brands. The presence of spices for seasoning, high fibre carbohydrate, starches, cereals, legumes and soy protein could increase the ash and fibre contents in the burgers. The incorporation of mechanically deboned chicken meat in burgers also could increase the ash content due to the presence of bone particles and high calcium content. Method using myoglobin content can be used to quantitate the meat content in meat products. Its inherent variability in meat tissue is well-defined but its conversion to cyanometmyoglobin from this procedure reduces its heterogenous variability in comminuted meat samples (Babji *et al.* 1989). Figures 1 and 2 showed the standard curves plotted from the myoglobin content in the beef : soy protein, and chicken : soy protein mixtures which have standardized percentages of meat. From these curves, the meat content for all burger samples were calculated based on the

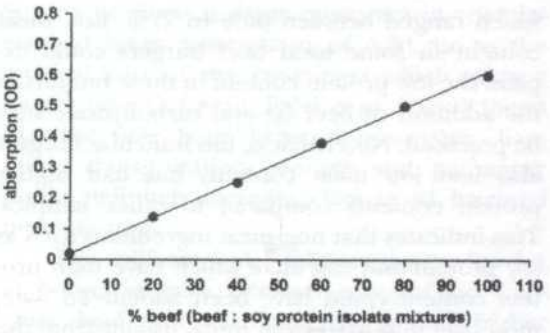

Fig. 1. Standard curve for beef burgers using beef : soy protein isolate mixture

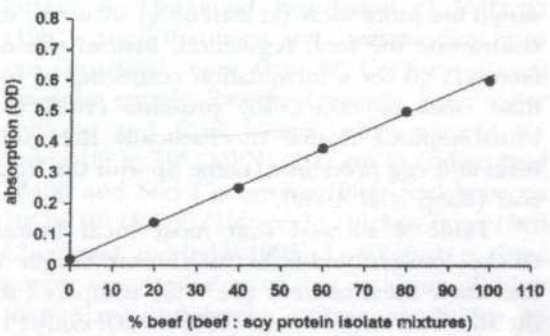

Fig. 2. Standard curve for chicken burgers using chicken meat : soy protein isolate mixture

myoglobin content. All franchise beef burgers and some local brands had lower meat contents than standard requirement (65% meat) for it ranged from 59% to 63% (Table 3). Only A1, F1 and G1 beef burgers had higher meat contents

TABLE 3
Myoglobin and meat content in local and franchise beef burgers.

Samples *	Myoglobin Content (mg/g sample)	Meat Content (%)
Local :		
A1	3.32 ± 0.19	77.25 ± 4.90
B1	2.64 ± 0.30	61.00 ± 7.27
C1	2.55 ± 0.14	59.00 ± 2.75
F1	2.86 ± 0.23	66.00 ± 5.45
G1	3.08 ± 0.20	71.38 ± 4.31
H1	2.70 ± 0.07	62.50 ± 2.20
Franchise :		
A	2.71 ± 0.36	62.13 ± 2.32
B	2.71 ± 0.04	62.13 ± 1.04
C	2.70 ± 0.05	61.98 ± 1.14

* Mean of two samples/treatment

which ranged between 66% to 77%. Low meat content in some local beef burgers could explain the low protein content in these burgers as the addition of beef fat and carbohydrate may be practised. Nevertheless, the franchise burgers also had low meat contents but had higher protein contents compared to other samples. This indicates that non-meat ingredients such as soy protein and caseinate which have high protein content could have been added. To date, most beef imported from India, usually from the fore quarters. It is even cheaper than the imported soy protein isolate and concentrate, which would lead one to believe that manufacturers would use more meat (at least 65%) so as not to contravene the food regulation. Instead manufacturers go for a formulation consisting of Indian Beef (40-60%), soy proteins (10-30%), wheat/tapioca flours, mechanically deboned meat and egg proteins to come up with the least cost (Babji *et al.* 1989).

Table 4 showed that most local brand chicken burgers and franchise chicken burger A had lower meat content (64.50%) compared to the standard requirement (65% meat). Only F1, franchise chicken burger B and E1 had high meat contents and these burgers also had high protein contents (Table 2). High protein content and low meat content in H1 and franchise chicken burger A indicated that there were addition of high protein, non-meat ingredients in the formulations.

Because of the lower amount of meat used in some local products, producers probably have to complement it with beef flavourings and colours to resemble meat. The use of food colourings in the manufacture of beef burgers is mainly to camouflage the use of fillers such as soy proteins and carbohydrates. Fresh meat colour is related to the total heme myoglobin pigment and biochemical condition (Desrosier 1977). From Table 5, no significant differences were observed ($p > 0.05$) for 'L' values but significant differences occurs for 'a' values between the local brand and franchise beef burgers. A1 and F1 beef burgers had a darker colour (high 'a' value); similar to the high myoglobin and meat contents. For 'b' (yellowness) value, there were significant differences ($p < 0.05$) between the local brands and franchised beef burgers. This high value for yellowness could be due to high fat content in the burgers.

TABLE 4
Myoglobin and meat content in local and franchise chicken burgers.

Samples *	Myoglobin content (mg/g sample)	Chicken meat (%)
Local :		
A1	2.45 ± 0.04	62.50 ± 0.09
D1	2.55 ± 0.34	64.50 ± 3.04
E1	2.65 ± 0.02	67.00 ± 0.77
F1	3.16 ± 0.15	80.63 ± 3.84
G1	2.42 ± 0.04	60.50 ± 0.48
H1	2.32 ± 0.05	58.00 ± 1.01
Franchise :		
A	2.61 ± 0.07	64.50 ± 0.02
B	2.88 ± 0.03	77.50 ± 0.95

* Mean of two samples/treatment

TABLE 5
The colour values (L-lightness, a-redness, b-yellowness) for local and franchise beef burgers

Samples *	Beef burgers		
	L	a	b
Local :			
A1	47.89 ± 0.03	+20.45 ± 0.29	+15.86 ± 0.01
B1	53.68 ± 2.53	+21.89 ± 0.56	+19.47 ± 0.33
C1	48.14 ± 1.55	+25.32 ± 1.44	+11.43 ± 0.79
F1	43.39 ± 1.20	+31.78 ± 1.37	+17.43 ± 1.48
G1	52.75 ± 0.53	+23.37 ± 0.98	
H1	51.92 ± 1.77	+23.64 ± 1.44	+15.55 ± 0.92
Franchise :			
A	52.76 ± 0.80	+6.38 ± 0.35	+16.38 ± 0.57
B	52.02 ± 1.20	+15.20 ± 1.36	+13.24 ± 0.55
C	45.13 ± 0.20	+5.38 ± 0.18	+12.57 ± 0.25

* Mean of two samples/treatment

Most of the chicken burgers had high 'L' values which ranged between 62.75 to 72.48 except for F1 chicken burgers (Table 6). F1 chicken burger was observed to have a redder colour (a higher 'a' value) compared to the other burgers. Generally there was no significant differences ($p > 0.05$) between the local brands and franchise chicken burgers for 'L', 'a' and 'b' values. Chicken meat is lighter and less red in colour than beef or Indian beef especially the breast meat. The thigh meat is redder and darker because of the muscles and high content of myoglobin.

Tables 7 and 8 showed the Total Plate Count (TPC), Coliform, *E.coli* and *S. aureus* counts for all burger samples. TPC showed that the burger samples meet the standards stipulated by the Food Regulation of Malaysia (1985) which stated that the number of microorganisms in meat and meat products must not exceed 10^6 per gram sample. TPC for local brand beef burgers was very low, in the range of 1×10^1 to 8×10^1 per gm sample. Higher TPC was found for franchise beef burgers ranging 2×10^2 to 2×10^3 per gm sample. Similarly with the chicken burgers, the franchise burgers had higher counts (9×10^2 to 2×10^3 per gm sample) than the local brands (1×10^1 to 4×10^1 per gm sample). Higher counts in the beef and chicken franchise burgers could be due to packaging and storage condition. The local brand burgers were packed in small quantities i.e. 8 to 10 pieces per pack and stored frozen. In the case of franchise burgers, large quantities were packed in a container, stored frozen and sent to the outlets or restaurants. Storing products in large quantities in containers had lower penetration of cold air to the internal part of the containers which takes a longer time to freeze. Babji *et al.* (1983) stated that the time lapse between processing, handling, transportation, storage and packaging would definitely increase chances of bacterial multiplication.

The coliform and *E.coli* counts were low for all burger samples. However, some of the franchise beef and chicken burgers had higher counts for coliform, which were 17 (MPN)/gm sample for franchise beef burgers B and C, and 27 (MPN)/gm sample for franchise chicken burger B. The Food Regulation of Malaysia (1985) stated that meat and meat product must not contained more than 50 Coliform counts per gram sample. Raw meat usually had higher coliform and *E.coli* counts, which ranged between 10^3 to 10^4 (MPN)/100 gm in Indian beef (Babji and Seri Chempaka 1994) and between 10^2 to 10^3 (MPN)/100 gm in chicken meat (Seri Chempaka and Babji 1995). Low counts in these burger samples indicated that inclusion of other ingredients and frozen storage conditions reduced the number of bacteria and retarded their growth. Chuah and Yeoh (1984) stated that *E.coli* is quite sensitive to low temperature, and freezing reduced the *E. coli* present. The growth of mesophilic bacteria like *E. coli* is retarded at low temperatures, and no growth was observed below 5°C (Barnes 1976). Mandokhot and Garg (1985) informed that coliform index has found wide use in assessing the sanitary quality of food including meats. Presence of *E.*

TABLE 6
The colour values (L-lightness, a-redness, b-yellowness) for local and franchise chicken burgers

Samples *	Chicken Burger		
	L	a	b
Local :			
A1	62.75 ± 0.76	+5.17 ± 0.35	+17.13 ± 0.82
D1	72.48 ± 0.47	+2.40 ± 0.25	+12.29 ± 0.41
E1	63.83 ± 1.21	+5.62 ± 0.35	+13.73 ± 0.82
F1	43.78 ± 1.54	+35.41 ± 0.76	+20.79 ± 0.58
G1	68.61 ± 1.20	+4.52 ± 0.28	+16.96 ± 0.23
H1	65.35 ± 1.11	+2.88 ± 0.35	+17.65 ± 0.26
Franchise :			
A	72.47 ± 0.77	+2.03 ± 0.25	+13.94 ± 0.39
B	69.53 ± 0.47	+5.55 ± 0.25	+14.13 ± 0.41

* Mean of two samples/treatment

TABLE 7
Total plate count (TPC), coliform, *E. coli* (MPN/g sample) and *S. aureus* (CFU/g sample) counts on local and franchise beef burgers.

Samples *	Total plate count CFU/g sample	Coliform count MPN/g sample (*E.coli*)	*S. aureus* count CFU/g sample
Local :			
A1	3×10^1	1(<1)	11
B1	8×10^1	1(<1)	7
C1	4×10^1	2(<1)	6
F1	1×10^1	1(<1)	2
G1	3×10^1	3(<1)	6
H1	3×10^1	1(0)	3
Franchise :			
A	2×10^3	1(0)	2
B	2×10^2	17(0)	–
C	2×10^2	17(<1)	2

* Mean of two samples/treatment

TABLE 8
Total plate count (TPC), coliform, *E. coli* (MPN/g sample) and *S. aureus* (CFU/g sample) counts on local and franchise chicken burgers

Samples *	Total plate count CFU/g sample	Coliform count MPN/g sample (*E.coli*)	*S. aureus* count CFU/g sample
Local :			
A1	4×10^1	8 (<1)	13
D1	3×10^1	2 (<1)	9
E1	1×10^1	0 (0)	6
F1	3×10^1	1 (0)	9
G1	4×10^1	7 (<1)	6
H1	3×10^1	12 (<1)	22
Franchise :			
A	2×10^3	1 (0)	-
B	9×10^2	27 (<1)	28

* Mean of two samples/treatment

coli (enterococci) is employed as an indicator of faecal pollution in food.

S. aureus counts in most local brand beef and chicken burgers were varied and higher than the franchise burgers which ranged berween 2 to 11 CFU/ gm sample and 6 to 22 CFU/gram sample respectively. DHSS United Kingdom (1989) stated that *S. aureus* in food should not exceed 10^2 per sample respectively. Fennema, Powrie and Marth (1973) reported that although freezing killed some microorganisms in food, many survived the freezing process and microorganisms that survived will grow and cause undesirable changes when the thawed food reaches a suitable temperature. The processed meat product producers must be aware of the critical control points during processing and maintain low temperature and clean sanitation during manual handling by the workers especially during processing, forming and packing the burgers. Most *S. aureus* biotype from human could produce enterotoxin (Brown 1982). *S. aureus* is a good hygienic indicator of meat base food and its presence is linked and heavy use of equipment and food handling (Shelton *et al.* 1962). In humans, the main reservoir for *S. aureus* is the nose cavity and it spreads to the skin or wound directly or indirectly (Jay 1986).

The results obtained in the study showed that there was no Salmonella sp present in all burger samples this meets the standards stipulated by the United Kingdom DHSS (1989) i.e. no *Salmonella* must be detected in 25 gram samples. Low temperature at 5°C (Alcock 1987) or lower at 4.4°C (Nickerson and Ronsivalli, 1980) could retart the growth of *Salmonella*. Principal sources of *Salmonella* are dust, food handlers, pets, insects, rodents, birds, live-haul trucks and the air. In the processing area, dust should be eliminated from the environment and equipment kept clean during the processing day. Clean-up procedures should include a sanitation programme aimed towards eliminating *Salmonella*, and should include spot bacterial checks prior to start up (Wabeck 1987). Microorganisms may pass from one raw food to another and from raw to cooked or processed foods by means of equipment, cloths and surfaces and also via people handling raw and cooked food together without realising the fact and significance of contaminated raw materials. There is much emphasis on the spread of infection from human faecal excreters to foods but little attention has been paid to the human hands passing *Salmonella* from one food to another (Hobbs and Gilbert 1970).

CONCLUSION

Results showed that there were some differences in proximate and microbiology composition between the local brand and the franchise burgers. Most franchise burgers had lower fat and carbohydrate contents and higher protein and moisture contents compared to the local brands. High fat and carbohydrate contents and low protein and meat contents in the local brand burgera showed the utilization of carbohydrate fillers/binders and addition of fat. Thus affected the colour of the product. In some local brands and franchise burgers, the utilization of non-meat protein ingredients and addition of high amount of water may have occured based the analysis of which showed high protein and moisture contents but low in fat, carbohydrate and meat contents. Absence of *Salmonella* sp, low Total Plate Count (TPC), Coliform, *E. coli* and *S. aureus* counts in the burger samples showed that the manufacturers had paid due attention to quality especially in microbiology composition by maintaining the sanitation and cleanliness of the equipment, storage facilities and workers in the processing plant. Such a study should be carried out from time to time to monitor the quality of the products in terms of nutritional value and microbiological safety.

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Contents

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