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Utilisation of Different Protein Sources for Growing Rabbits

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Keywords: Protein sources, groundnut cake, local fish meal, protein utilisation

ABSTRAK

Kesan hampas kacang tanah dan serbuk ikan tempatan ke atas pertumbuhan dan penggunaan protein oleh arnab dikaji. Percubaan pemberian makanan selama lapan minggu melibatkan dua puluh empat arnab putih eksotik jantan dan betina berusia enam minggu seberat 550-610 g dalam satu pola rawak sepenuhnya telah diberi makan tiga diet: satu diet kawalan yang mengandungi kombinasi hampas kacang tanah dan serbuk ikan tempatan, dan diet eksperimen yang mengandungi sama ada hampas kacang tanah (GNC) dan serbuk ikan tempatan (LFM) adalah tinggi kandungan protein (masing-masing 48.13% dan 52.15%), serbuk ikan tempatan mengandungi tahap serat yang rendah (0.83%) manakala hampas kacang tanah rendah tahap abunya (5.22%). Kedua-duanya sumber protein membekalkan jumlah protein dan mineral yang ketara. Diet kawalan memberikan signifikan (P<0.01) tambahan berat yang lebih baik daripada hampas kacang tanah dan serbuk ikan tempatan. Nisbah keberkesanan protein juga secara signifikannya lebih baik (P<0.01) dengan diet kawalan, apabila dibandingkan dengan diet GNC dan LFM. Tiada perbezaan signifikan diperoleh pada tahap pemberian makanan: Tambahan berat daripada arnab yang diberi makan diet percubaan. Tiada insiden kematian yang berlaku di kalangan kumpulan rawatan.

ABSTRACT

The effects of groundnut cake and local fish meal on the growth and protein utilisation of rabbits were studied. The eight weeks feeding trial involving twenty-four, six-week-old male and female exotic white rabbits weighing 550-610 g in a completely randomised design were fed three diets: a control diet which had the combination of groundnut cake and local fish meal, and experimental diets that had either groundnut cake or local fish meal as the protein source. The groundnut cake (GNC) and the local fish meal (LFM) were high in protein (48.13% and 52.15% respectively), the local fish meal had a low fibre level (0.83%) while the groundnut cake was low in ash (5.22%) but high in fiber (5.52%). Both protein sources supplied appreciable amounts of protein and minerals. The control diets gave significantly (P<0.01) better weight gains than the groundnut cake and local fish meal diets respectively. Protein efficiency ratio was also significantly (P<0.01) better with control diets, when compared with the GNC and LFM diets. No significant differences were obtained in the level of feed: Gain from rabbits fed the trial diets. There was no incidence of enterities or mortality among the treatment groups.

INTRODUCTION

The world is faced with inflation and there is the problem of lack of food to sustain its ever-growing population. The demand for protein has risen greatly and the human dependence on animals for protein is increasing, especially since plant proteins are generally deficient in four essential amino i.e. acids lysine, methionine tryptopham and lencine. If meat consumption demands are to be met in Nigeria, encouragement for the production of short-cycle animals like poultry and especially rabbits should be given

great consideration by government and appropriate research institutes.

Rabbits possess attributes that make them advantageous over other livestock species. Rabbits can be produced on forage alone, although production can be improved by addition of other feed supplements. Rabbits are highly prolific and have a short gestation period (28-32 days). They are also good converters, easy to care for and they require low capital investment in rearing.

In biological value, it is comparable to chicken (Biobaku and Oguntona 1997). The meat is white, highly favoured nutrition and appetizing (Biobaku 1998). There is no religious prohibition against the consumption of rabbit meat in most countries (Biobaku 1998).

The objective of this study is to examine the effect of protein concentrates sourced from plant and animal products on the growth performance of weaner rabbits.

MATERIALS AND METHODS

Three diets termed A,B, and C were prepared. Diet A contained the groundnut cake and local fish meal. Diet B had only the groundnut cake while Diet C had only the fish meal as shown in Table 1. The diets were all pelleted and were calculated to contain 20.42-20.89% crude protein and 9.48-10.42KJ/gM.E.

Twenty four six week old New Zealand white male and female rabbits weighing 550-610 g were obtained from University Teaching and Research Farm. The animals were divided into three groups of eight rabbits per group with an average weight of 579 g in each groups. Each group was further sub-divided into two, such that duplicate groups of four rabbits were obtained for each sub-group with two rabbits per cage. The rabbits were fed the pelleted diet daily at 8:30 am. Water was provided *ad libitum.*

Feed refusals were weighed during the last fourteen days of each collection period in order to calculate the feed intake in g/d over the fourteen-day period.

Digestibility of the diet was carried out in the sixth week of the experiment and lasted for seven days. The first four days were for adaptation of the animals to the new environment and the last three days were used for collection. Two rabbits were used per replicate. Records of the daily feed intake and the daily faeces voided were kept. Faeces collected were dried in an oven at about 125°C for 24-48 h.

Records of weekly body weight gain, feed intake faeces voided, feed efficiency ratio, protein efficiency ratio and digestibility of protein were calculated for eight weeks. These calculations were done on dry matter basis.

TABLE 1
Percentage composition of experiment diets

Components	Control Diet	Groundnut cake Diet (GNC Diet)	Local fish meal (LFM Diet)
Yellow	52.97	47.85	54.11
Groundnut cake	6.36	18.61	-
Local fish meal	7.17	-	12.39
Brewer's Grains	30.00	30.00	30.00
Oyster shell	2.00	2.00	2.00
Bone meal	1.00	1.00	1.00
Vit./Mineral Premix	0.25	0.25	0.25
Salt	0.25	0.25	0.25
Total	100.00	100.00	100.00
Calculated Analyses			
Protein (%)	20.42	20.89	20.55
Energy (KJ/gm)	10.42	9.48	10.20
Calcium (%)	2.64	2.44	2.48
Methionine (%)	0.74	0.70	0.72
Lysine (%)	0.64	0.58	0.62

Feed efficiency ratio determination

Feed efficiency (per week)=

$$\frac{\text{Total body weight gain/week (kg)}}{\text{Total feed intake/week (kg)}}$$

Protein efficiency ratio determination

Protein efficiency ratio (per week)=

$$\frac{\text{Total body weight gain/week (kg)}}{\text{Total protein consumed/week (kg)}}$$

Analytical Techniques

Samples of the groundnut cake, local fish meal and the three ratios were analysed for proximate composition according to AOAC (1990).

Statistical Analysis

Performance records of the animals were subjected to one way analysis of variance by method of Snedecor and Cochran (1967).

Duncan's (1955) multiple range test method was used to determine significant differences between means.

RESULTS AND DISCUSSION

The groundnut cake used in this trial contained 48.13% protein which was significantly ($P<0.01$) lower than the protein in the local fish meal, which contained 52.15%.

The dry matter, fat and ash contents of local fish meal were significantly higher than those found in the groundnut cake (Table 2).

The levels of the crude fibre were (18.25%) for the control diet, groundnut cake (20.25%) and local fish meal (20.25%). Cheeke (1983) had reported that although rabbits digest fibre poorly, dietary fibre is useful in preventing entiritis and for chewing. A level of 15-18% dietary fibre is therefore suggested for optimum growth.

Beyond 18%, it may caecal impaction, but fibre again is indispensable for adequate filling of the digestion tract and maintenance of normal peristatic movements (Arveux 1980).

Although the crude fibre in their diets varied from 18.25-20.45%, there was no observation of caecal impaction, and this agrees with the findings of Ekpenyong and Biobaku (1986), that

TABLE 2
Chemical composition of groundnut cake, local fish meal and experimental diets (% dry matter basis)

Chemical Composition (% Dry matter)	Groundnut cake (GNC)	Local fish meal (LFM)	Control diet	Groundnut cake diet (GNC	Local fish meal diet LFM diets)
Moisture	7.48	4.81	11.50	12.00	17.00
Crude protein	48.13	52.15	20.15	18.85	19.25
Ether extract	6.05	7.76	2.20	2.25	1.50
Crude fibre	5.52	0.83	18.25	20.45	20.25
Nitrogen free extract	26.53	6.11	24.90	23.20	18.00
Ash	5.22	26.84	22.00	22.00	23.00
Minerals					
Potassium (%)	0.332	0.442	0.342	0.348	0.356
Phosphorus %	0.048	0.640	0.042	0.062	0.068
Calcium %	0.440	0.560	0.364	0.342	0.354
Magnesium Ppm	0.714	0.124	0.164	0.184	0.174
Managanese Ppm	226	268	224	218	216
Iron (ppm)	142	142	138	136	136

TABLE 3
Effects of diets on growth performance of rabbits

Performance Parameters	Control diet 1	Groundnut cake diet (GNC Diet)	Local fish meal diet (LFM Diet)	[1]SEM
Numbers of rabbits	579[a2]	579[a]	579[a]	6.49
Initial body weight (g)				
Final body weight (g)	1145[a]	1084[a]	1132[a]	16.42
Daily Wt gain (g/day)	10.11[a]	9.02[b]	9.88[a]	0.44
Daily feed intake (g/day)	90.37[a]	84.37[b]	86.41[ab]	3.42
Feed gain	8.94[a]	9.40[a]	8.75[a]	2.46
Feed efficiency	0.30[a]	0.21[b]	0.29[a]	0.80
Protein efficiency Ratio	7.2[a]	6.29[b]	7.01[a]	0.04
Protein digestibility	88.40[a]	85.98[c]	86.55[b]	6.42
Mortality	0	0	0	

1: Standard error of mean; values are the mean of eight analyses
2: Means along the same row with different superscripts are significantly different (P<0.05)

crude fibre in the diet of rabbits can be varied from 28-32% with no caecal impaction.

The effect of the trial diets on growth, feed efficiency, protein efficiency and protein digestibility is shown in Table 3. Rabbits fed control diets had the highest body weight gain, which is statistically significant (P<0.01) when compared with rabbits fed the groundnut cake. There is no significant difference between rabbits fed the control diet and those rabbits fed the local fish meal diet.

The rabbits on the control diet consumed the highest amount of the diet to produce unit weight gain though they had the best feed efficiency which is statistically not significant when compared with other two diets. However, this may be due to the fact that the control diet contained a balanced protein profile.

Table 3 also shows that the rabbits fed the fish meal diet consumed more feed with higher body weight gain when compared with rabbit-fed groundnut cake diet. This may be due to the fact that animal protein source is better in quality when compared with plant protein source, which is deficient in some essential amino acids and this view was supported by Tewe (1995).

The poor performance of rabbits fed the diet containing groundnut cake as protein source was supported by Omole (1982) who reported that the groundnut cake protein contained large amounts of nutritionally essential amino acids, particularly arginine but its nutritive value tends to be limited, particularly by its low content of lysine and methionine which are very essential for proper growth of animals.

Table 3 also shows the value for the protein efficiency ratio. The protein efficiency of rabbits fed the control diet is significantly (P<0.01) higher than those of rabbits fed the groundnut cake diet. However, there are no differences between rabbits fed the control diet and those rabbits fed the local fish meal diet.

The protein digestibility in rabbits fed the control diet was significantly (P<0.01) higher than those rabbits fed diets containing groundnut cake and fish meal as protein sources; this may be due to the levels of protein and crude fibre. The level of crude protein in the control diet was higher and level of crude fibre lower than in the other two diets and it had been demonstrated that feeding elevated levels of fibrous materials and lower protein levels, reduces the apparent digestion coefficient of the crude protein in rabbits (Besedina and Pereidik 1970).

Table 4 shows that the diet containing groundnut cake as the protein source reduced the cost of feed of the minimum level, but this has not led to improvement in body weight gain, feed consumed, feed efficiency, which shows that although it is possible to have the cheapest sources of feed, they do not have to produce the best results on rabbit production.

TABLE 4
The influence of experimental diets on the economy of rabbit production

Components	Control diet	Groundnut cake diet (GNC Diet)	Local fish meal diet (LFM Diet)
Total no of rabbits	8	8	8
Initial average wt (g)	579	579	579
Final average wt (g)	1145	1084	1132
Total body wt gain (kg)	0.566	0.505	0.553
Total feed consumed (kg)	5.06	4.75	4.86
Protein efficiency ratio	7.2	6.29	7.01
Total cost of feed Consumer (N)	61.080	44.89	52.776
Cost/kg feed (N)	12.071	9.450	10.859
Cost of feed/kg wt Gain (N/kg)	21.327	16.697	19.637

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Weathering Behaviour of a Basaltic Regolith from Pahang, Malaysia

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Keywords: Regolith, weathering, saprolite, elemental mobility

ABSTRAK

Ciri-ciri dan kadar luluhawa pada regolit saprolit dalam terluluhawa yang terbentuk daripada batuan basalt telah dikaji. Jujukan regolit tanah-saprolit-batu sedalam 15 m yang terletak di bahu jalan di Pahang, Malaysia, telah dipilih untuk tujuan ini. Kadar luluhawa pada regolit ini telah dinilai dengan beberapa indisis luluhawa, perubahan pada sifat kimia-fizik tanah, mineralogi lempung dan ciri-ciri mikrofabrik. Kesemua nilaian telah membuktikan bahawa kadar luluhawa adalah tinggi dan cepat, walaupun di peringkat pembentukan saprock. Kehilangan keterlaluan elemen utama seperti K, Na, Ca, dan Mg, dan penambahan jelas oleh Fe, Ti, Cu dan Nb berlaku semasa proses saprolitizasi, mungkin menjelaskan bentuk luluhawa yang tinggi pada regolit tanah ini.

ABSTRACT

The characteristics and degree of weathering in a deep saprolitic regolith developed on basalt were investigated. A 15 m deep regolith of soil-saprolite-rock sequence, located along a new road cut in Pahang, Malaysia, was selected. The intensity of weathering in this regolith was assessed by various weathering indices, as well as by the changes in the physico-chemical properties, clay mineralogy and the microfabric characteristics of the profile. All assessments gave strong evidences of intense weathering, even at the stage of saprock formation. Extreme depletion of major elements such as K, Na, Ca and Mg, and significant enrichment of Fe, Ti, Cu and Nb occurred during saprolitization process, and, these perhaps explain the extreme weathering pattern of this regolith.

INTRODUCTION

The extent to which particular elements are mobilized during weathering depends upon the control of solubility in aqueous solutions and their interaction between these solutions and the complex surfaces of primary and secondary minerals. Thornber (1992) reported that cations were more mobile at low pH while anions were mobile at higher pH.

Weathering and mineral transformation are important phenomena in the arena of the Malaysian soils. Roe (1951) first reported the observations of weathering profiles in Peninsular Malaysia. An intensive study on soil formation and its variation with altitude throughout the peninsula was investigated by Burnham (1978). Eswaran and Wong (1978) and Yeow (1975), made some detailed studies on the chemical, mineralogical and micromorphological changes related to the transformation of parent rocks to soils in granite. Study on metamorphic rocks was conducted by Stoops *et al.* (1990), and Zauyah (1986). The genesis of basaltic soils was reported by Ives (1966), and West and Dumbleton (1970), who concluded that the basaltic rocks were formed probably during the middle Eocene age.

The present basalt regolith studied was composed of the soil-transition zone-saprolite-rock sequence. The saprolite, that is the weathered rock, comprised the largest portion of the regolith, and is increasingly important to the Malaysian upland plantation industry. The unavoidable terracing during crop planting and road construction activities, landslides and erosion had exposed these materials to or near the surface and become accessible to roots. This

paper describes the degree of weathering and changes in the regolith characteristics under the humid tropical, well drained conditions of Peninsular Malaysia.

MATERIALS AND METHODS

Location and Geological Setting

The selected regolith developed on basalt is located in Pahang state on the West coast of Peninsular Malaysia. The geological study by Hutchison (1973), suggested that the extrusion of basaltic lava occurred during the Quaternary age and it overlies and surrounds the granitic hills to the north and northwest of Kuantan town. The rock is compact, microcrystalline, and greenish black vesicular olivine basalt. The climate is equatorial with an annual precipitation of 3660 mm and potential evapotranspiration of 1,130 mm. The soil moisture regime is udic and the soil temperature regime is isohyperthermic with the mean annual soil temperature of 28.7 °C. The landscape is gently undulating and the profile was sampled on a new road along the road from Kuantan to Sungai Lembing, about 8 km from Kuantan town. A profile of about 15 m deep (*Fig. 1*) was horizonated and morphologically described following the criteria of the USDA (1981). The profile is classified as fine, clayey, kaolinitic, isohyperthermic, typic Acrudox (Soil Taxonomy 1996). Bulk samples and undisturbed samples of soil, saprolite and rock were taken for various analysis.

Laboratory Analysis

The bulk samples were air-dried and were passed through a 2 mm sieve. The undisturbed core samples were taken for the bulk density determination. The pipette method was employed to estimate the soil texture and the water dispersible clay (WDC) was determined by successive sedimentation method (Tessens 1984). Soil pH was measured in suspension of 1:2.5 soil:solution ratio, while pH in sodium fluoride was estimated after stirring vigorously for two minutes (Rayment and Higginson 1992). The method proposed by Ferrari and Megaldi (1983) was used to determine the soil abrasion pH. All pH readings were recorded using a glass electrode pH meter. Soil organic carbon was determined by the Walkley-Black dichromate titration method and nitrogen by macro-kjedahl digestion procedure (Bremner and Mullraney

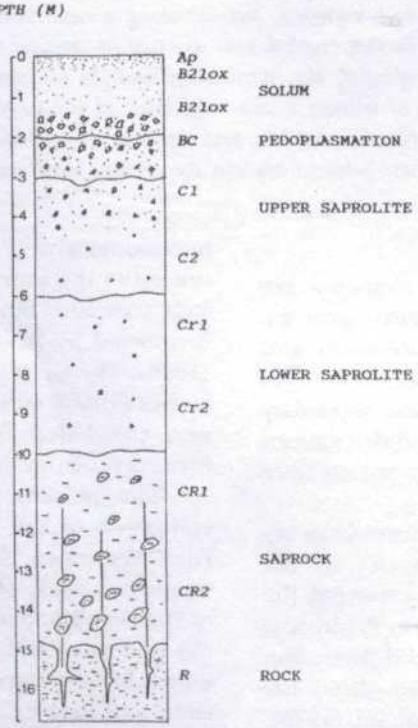


Fig. 1: A schematic sketch of a basalt profile

1982). The free Fe content was extracted by dithionite-citrate-bicarbonate method of Mehra and Jackson (1960). For the CEC and exchangeable bases determination, the leaching method with 1M ammonium acetate buffered at pH 7 was used. The determination of aluminium and P sorption index were done following the aluminon method (Hsu 1963) and Bache and Williams (1971), respectively. The bulk mineralogy of the clay fraction of the soils and saprolites were analyzed by x-ray diffraction. Soil charge characterization was determined by potentiometric titrations and ion retention methods (Uehara and Gillman 1981).

Undisturbed samples from major horizons were collected in Kubiena boxes of 55 x 75 x 50 mm size. These samples were air-dried and impregnated with resin (araldite). Thin sections were prepared and described using the terminology outlined by Bullock *et al.*(1986). The major elemental chemical content of basalt rock and its weathering product were analyzed with the Inductively Coupled Plasma (ICP). An isovolumetric calculation was performed to determine the intensity of weathering through elemental mobility, converting the percentage weight chemical analysis to volume-based concentration by multiplying with the bulk density of the respective sample. Using the unweathered bedrock as the reference, absolute percentage gains and losses of each element were then calculated. The weathering indices of Parker (1970), Rock Alteration Index by Eswaran *et al.* (1973), and the Molar ratio and abrasion pH by Ferrari and Megaldi (1983), were adopted to evaluate the degree of weathering of this profile.

RESULTS AND DISCUSSION

Morphological Descriptions

A summary of the profile morphological descriptions is presented in Table 1. The profile is prominently characterized by clayey yellowish brown solum. The surface layers have a strong crumb that changes gradually to weak subangular blocky structure in the subsoil horizons. The consistency is friable and fluffy but becomes firm towards the upper saprolite layer. Drainage of the soil profile is somewhat excessive. The saprolitic layers are well weathered, yet massive, clayey, slightly friable but increase in firmness with depth, and become more coherent towards the rock. The upper saprolites are variegated in colors of mostly dark brown with reddish and grayish patches, but becomes more gray and greenish with depth towards the saprock. The drainage of the saprolite layers is somewhat imperfect.

Physico-Chemical Characterization

The selected physico-chemical properties of the profile are presented in Table 2. The profile is physically characterized by the high clay content throughout the regolith, with the content in the solum of > 70% while the saprolite of > 60%. The high clay content did not coincide with the very friable and fluffy consistency of the solum layers observed in the field, and this would suggest strong aggregation of clay by iron to form pseudosands and clayballs (Paramananthan 1977). Log clay percent (*Fig. 2*) roughly follows a straight line pattern in the saprolitic layers, suggesting that the presence of clay is most probably due to weathering (Eswaran and Wong 1978). This assumption is supported by the low

TABLE 1
Morphological characteristics of a basalt regolith

Horizon	Depth (m)	Texture	Color		Consistency	Structure
			Matrix	Mottles		
Soil	0 - 2	Clay	10YR4/4	nil	Friable-Fluffy	Crumb-SAB
Transition	2 - 3	Clay	10YR3/4	nil	Friable	SAB
Upper saprolite	3 - 6	Clay	2.5YR3/4	10YR4/1	Friable	Massive
Middle saprolite	6 - 10	Clay	2.5YR3/4	10YR4/1	Firm	Massive
Lower saprolite	10 - 15	Silty clay	2.5YR3/4	nil	Hard	Massive

TABLE 2
Selected physico-chemical characteristics of a basalt regolith

Horizon	Clay %	Log Clay %	B.D (g/cm³)	WDC %	CEC	Exchangeable Bases (cmol (+)/kg soil)				PC (cmol(+)/kg clay)	PDC (cmol(+)/kg clay)	%O.C	%N	Fe_d %	P Sorption Index	Al	Soil pH		
						Ca	Mg	K	Na								pH_w	pH_{KCl}	ΔpH
Ap	78	1.9	0.98	33	6.2	0.87	0.59	0.28	0.11	2.5	23.0	2.60	0.27	12.4	78	0.01	5.34	4.79	-0.55
B2ox	80	1.9	1.09	0.4	2.2	0.30	0.05	0.09	0.05	1.0	20.1	0.86	0.13	12.5	82	0.01	4.81	4.52	-0.32
BC	66	1.8	1.37	0.4	1.1	0.26	0.05	0.05	0.05	0.8	23.1	0.06	0.02	14.9	82	0.01	5.14	5.44	0.30
Upper saprolite	64	1.8	1.17	0.3	2.9	0.29	0.04	0.09	0.03	1.3	22.8	0.02	0.01	12.5	82	0.04	4.84	4.47	-0.37
Middle saprolite	57	1.8	1.07	0.1	3.0	0.28	0.06	0.06	0.02	3.9	22.5	tr	tr	12.3	78	1.97	4.73	4.11	-0.62
Lower saprolite	30	1.5	1.17	0.1	5.7	0.27	0.09	0.06	0.02	15.7	45.4	tr	tr	12.5	71	3.64	4.61	3.92	-0.69
Rock	nd	nd	2.41	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd

Note: B.D - Bulk density, WDC - Water Dispersible Clay, CEC - Cation Exchange Capacity, PC - Permanent Charge, PDC - pH Dependent Charge, O.C - Organic carbon, N - Nitrogen, Fe_d - Dithionite extractable ferum, Al - Aluminium.

WDC values in these layers which range from 0.1 to 0.5%. The silt to clay ratio (*Fig. 2*) shows a curvilinear pattern with depth, being maximum at the lower saprolitic layer, and decreasing towards the solum. The decrease from the saprolitic layer towards the surface horizons points to the increase in weathering and soil formation (Van Wambeke 1962). The two phase curvilinear patterns as observed by Eswaran and Wong (1978) on a deep granitic profile were not detected in this profile, suggesting that this profile may experience extreme stage of weathering. This is supported by a drastic change in bulk density that occurs between the unweathered rock (2.41 g/cm^3) and the saprock layer (1.17 g/cm^3).

Intense rainfall and high temperature along the east coastal region of the peninsula resulted in a higher rate of organic matter decomposition and mineralization. The plot of log organic carbon shows a curvilinear distribution. The nick point appearing at 2.5 m corresponds to the separation of the pedological from saprolite layers (Singh and Singh 1987). The organic content is very low in the saprolite and could be attributed to its migration along fissures and remnants of cracks in the saprolite (Stone and Comerford 1994). The soil pH shows a decrease with depth, and could be attributed to the effect of organic matter content and degree of weathering. This is also supported by the drastic change in the abrasion pH values in Table 6. The DpH data indicates a positive value at the transition zone, suggesting the dominance of positive charge. Further study on the surface charge properties (Table 3) shows that values of the point of zero net charge (PZNC) are higher than the zero point of charge (ZPC or pH_0) values throughout the profiles, except for the surface horizon where the organic matter content

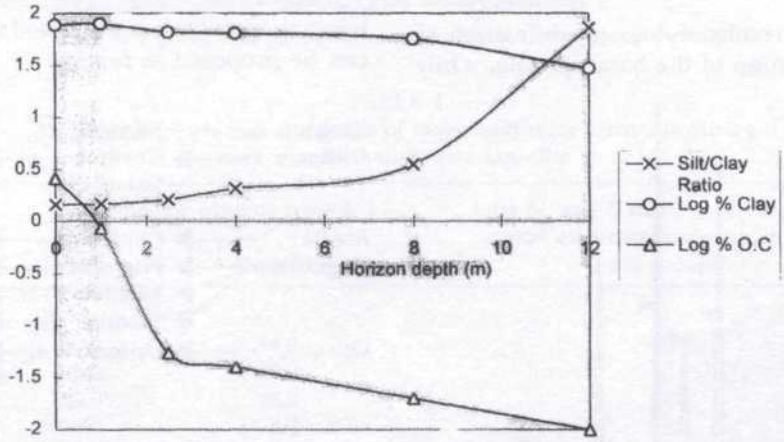


Fig. 2: Depth function or related soil properties

TABLE 3
Surface charge characteristics of a basalt regolith

Horizon	PZNC	ZPC (pH_0)	Negative Charge	Positive Charge	Net Charge
			(μmol(+)/g soil)		
Ap	3.71	4.17	-20.01	15.46	-4.54
B2ox	5.29	4.35	-13.57	15.71	2.14
Upper saprolite	5.85	3.91	-14.01	16.01	2.01
Middle saprolite	5.69	3.85	-13.14	16.45	3.31
Lower saprolite	3.74	3.65	-17.88	18.13	0.25

Note: PZNC - Point of zero net charge, PZC - Point of zero charge.

is higher. Similarly, the positive charge values were also found to be higher than the negative charge values at these horizons. The presence of positive charge indicates that the soil experiences extreme weathering stage (Tessens and Zauyah 1982; Van Wambeke 1992).

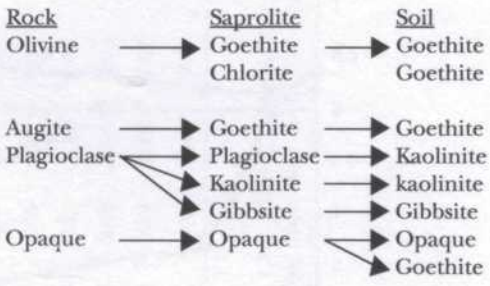
The PC and PDC values do not demonstrate increase with depth. The almost constant values may suggest little change in mineralogy as a result of intense weathering even at the lower saprolite layer. The CEC values are extremely low, except for the surface horizon where organic matter is abundant. The exchangeable bases for the whole regolith also point to a similar distribution pattern. In the east coastal areas of Peninsular Malaysia where rainfall and temperatures are high, weathering may reach great depth. Most of these bases may have already leached away even at the initial stage of saprolite formation (Burnham 1989).

Clay Mineralogy

Fig. 3 shows the results of the x-ray diffraction of the clay size fraction of the basalt profile. Only traces of primary minerals such as feldspar and olivine is detected in the saprolite layers. In the solum layers, strong peak for goethite and gibbsite are observed. The peak for gibbsite is weak in the saprolite layers, suggesting that most aluminium is well crystallized in soil materials. The low aluminium content in the soil as determined by aluminon method, may also point to a similar conclusion. Kaolinite peak is distinctively high in all horizons, suggesting that most weatherable minerals such as feldspar and olivine are well weathered into clay minerals, even at the lower saprolite layer.

The diffraction pattern of the profiles shows that the main bulk of the soil mineralogy is not detected. It is suspected that a reasonable amount of x-ray amorphous materials are present in the profile but is not detected by the diffractogram technique. The assumption is supported by the relatively high values of soil pH in 1N NaF solution, particularly in the saprolite.

From the study, the transformation of minerals from rock-saprolite-soil for basalt profile, can be proposed as follows:



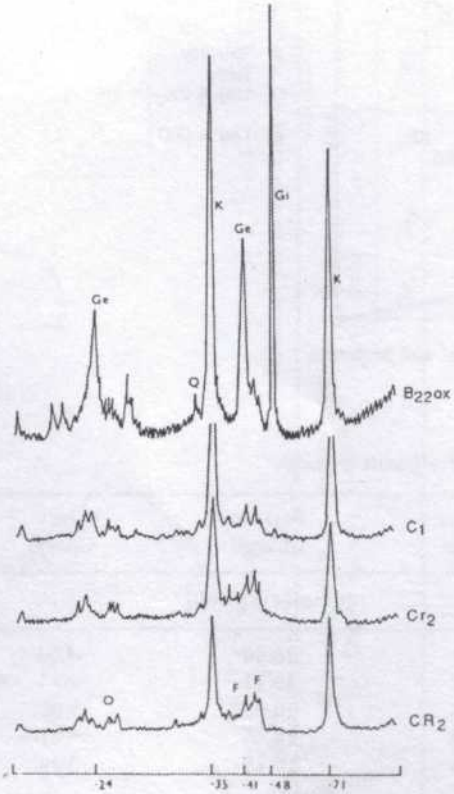


Fig. 3: The x-ray diffraction pattern of the Mg saturated clay size fraction of a basalt profile (Note: M-mica, Gi-gibbsite, Q-quartz, K-kaolinite, Ge-goethite)

Microfabric Observation

The study of the profile shows drastic changes in the microfabric of the rock-saprock-saprolite-soil sequence. The fine-grained basalt rock is composed of microphenocrysts of plagioclase, olivine and pyroxene. The groundmass is composed of the same three minerals together with roughly rectangular magnetite minerals. At the saprock zone, the olivine and pyroxene minerals are moderate to well altered. The matrix has an undifferentiated b-fabric and an open porphyric c/f related distribution pattern (RDP), suggesting that a certain amount of clay formation is already occurring at this layer. The massive saprock gradually developed into vughy microstructure, with the void size < 1 mm, and increased in porosity from 8% to 15%. Most

primary minerals in these saprolite zones are well weathered into dark brown clayey matrix. Pseudomorphs of olivine and pyroxene are rare. The total porosity increases to approximately 50% in the soil layers, comprising dominantly packing voids. Abundant of opaque minerals and some quartz are observed. Only very few subhedral olivine and pyroxene are seen coated by iron oxides that may have protected them from weathering. The b-fabric and RDP remain the same throughout the profile.

Elemental Mobility

A study of elemental mobility during saprolite formation was conducted using isovolumetric method. The results are presented in *Fig. 4* and Table 4.

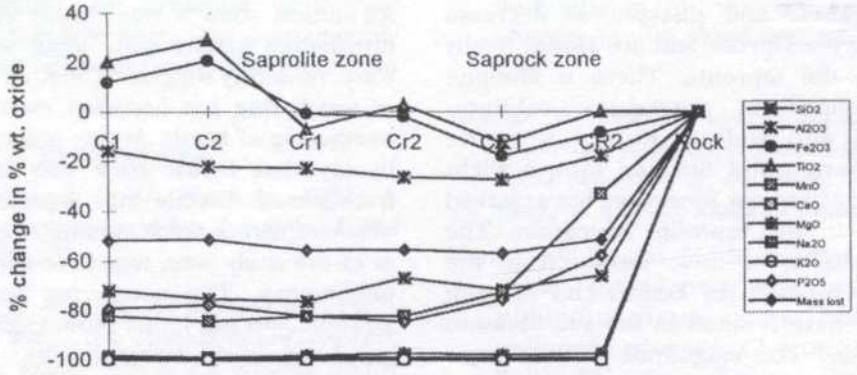


Fig. 4: The loss and gain of elements during the formation of saprolite

TABLE 4
The isovolumetric loss and gains of major and trace elements during rock transformation into saprolite

Element	Elemental content (cg cm^{-1})		Loss (-) and Gains (+) of elements (% total elemental content in rock)
	Rock	Saprolite	Rock – Saprolite
Major element			
SiO_2	19.0	34.0	-72
Al_2O_3	39.0	33.0	-16
Fe_2O_3	29.0	33.0	12
TiO_2	4.3	5.2	21
MnO	0.5	0.1	-82
CaO	21.0	0.2	-98
MgO	15.2	0.3	-98
Na_2O	6.2	0.1	-99
K_2O	3.3	0.0	-100
P_2O_5	1.3	0.3	-79
Trace element			
Ba	925	51	-95
Cr	1920	448	-77
Cu	77	182	136
Nb	36	75	108
Ni	241	212	-11
Sr	1356	24	-98
V	438	338	-23
Y	84	8	-90
Zn	342	149	-57
Zr	361	309	-14
Bulk Density (cg/cm^3)	241	117	

i. Mobility of Major Elements

The transformation of rock to saprolite has resulted in a total mass loss of 57.8%. During basalt weathering, most of the rock constituents are depleted significantly even at the initial stage of weathering, i.e. saprock formation. Silica depletes abruptly from 66% to 76% during the saprock and saprolite formation, respectively, and this accounts for 62% of the total mass loss. Aluminium demonstrates a gradual loss of 18 to 26%. Manganese and phosphorus decrease drastically in the saprock and are almost totally depleted in the saprolite. There is absolute impoverishment of potassium, calcium, magnesium and sodium during saprolite formation. Ferum and titanium show a slight decrease during saprock formation, but a marked enrichment in the saprolite formation. The marked depletion of these cations from the saprolite, as reported by Ferrari and Megaldi (1983), may have resulted in low soil abrasion pH (Table 5). The magnitude of the major element depletion in this profile, with decreasing order, is: K, Na, Ca , Mg > Mn , P > Si > Al > Fe, Ti.

ii. Mobility of Trace Elements

Among the trace elements studied, strontium, barium and yttrium are most mobile, in which > 90% are depleted from the saprolite. The sequence of trace element mobility, with decreasing order, is: Sr > Ba > Y > Cr > Zn > V > Zr > Ni > Cu, Nb. Copper and nobium indicate enrichment in the saprolite.

From the study, the mobility of the various elements in basaltic profile can be summarized as follows:

Most mobile : K_2O, Na_2O, CaO, MgO, Sr, Ba, and Y
Intermediate : MnO, P_2O_5, SiO, Cr, and Zn
Least Mobile : Al_2O_3, V, Zr, and Ni
Enrichment : TiO_2, Fe_2O_3, Cu, and Nb

Weathering Pattern

Various weathering indices were analyzed and assessed for their applicability to determine the degree of weathering in basalt profile (Table 5). All indices show a very drastic change in the distribution pattern with depth at the saprock zone, obviously suggesting that an intense stage of weathering has occurred even at the early weathering of basalt. At this point, it is possible to say that basalt rock may have actually transformed directly into saprolite. The high weatherability of basalt in humid tropical climate, as in the study area, may have resulted in such phenomena. The weathering pattern in the saprolite and soil layers show a gradual change, yet already at the extreme stage.

CONCLUSIONS

The study suggests that weathering in this profile is intense and rapid, even at the lower saprolite layers. This is indicated by the changes in the physico-chemical properties, charge characterization, and drastic transformation of weatherable minerals into secondary forms. The regolith is characterized by drastic change in bulk density and soil abrasion pH values, high free iron content and P sorption index, as well as low aluminium content. The low CEC and base saturation, even at the deepest horizons, suggest that extensive leaching has taken place (Burnham 1989; Hamdan 1995), attributed to

TABLE 5
The degree of weathering as determined by various weathering indices

Horizon	Molar Ratio SiO_2/Al_2O_3	Silt/Clay Ratio	WIP	Abrasion pH	RAI
Ap	1.14	0.16	0.17	4.20	3.50
B2ox	1.11	0.16	0.90	4.50	3.40
BC	1.29	0.21	1.30	4.80	3.20
Upper saprolite	1.66	0.32	1.40	4.80	2.10
Middle saprolite	1.81	0.35	1.50	4.80	1.60
Lower saprolite	2.18	1.86	2.00	4.95	1.53
Rock	5.22	nd	104.4	8.90	0.44

Note: WIP - Weathering Index of Parker
RAI - Rock Alteration Index

the significant depletion of bases during saprolitization. This may explain why soils in the humid tropics are relatively low in fertility (Hamdan and Burnham 1996). In this case, the contribution of nutrients from basalt weathering seems insignificant. The atmospheric inputs and the recycling of organic inputs are therefore important to sustain minimum level of soil fertility in the tropics. The mineralogy of the parent rock as well as climatic influence, perhaps attribute markedly to accelerate weathering processes in this soil type, that consequently does not reflect its age of deposition (Hutchison 1973).

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Pressure Treatment of Fresh and Ponded *Heritiera minor* (Roxb.) Logs with Chromated Copper Arsenate (CCA)

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Keywords: Preservative, pressure treatment, logs, chromated copper arsenate

ABSTRAK

Sundri (Heritiera minor *Roxb.*) *adalah spesies kayu keras yang mempamerkan kualiti kekuatan yang baik. Ia mempunyai potensi sebagai tiang elektrik, tetapi jangka hayat khidmatnya yang pendek menimbulkan masalah. Kajian ini menguji sama ada rawatan dengan pengawet Kuprum Arsenat berkromat, atau 'Chromated Copper Arsenate' (CCA) melalui cara tekanan penuh-sel pada 15.40 – 16.10 kg/cm² selama 8 jam, boleh memanjangkan jangka hayat khidmatnya. Sampel ujian adalah kayu segar dan kayu rendaman, kira-kira 1 m panjang dengan lilitan jejari 0.25 m. Keputusan menunjukkan nilai purata kemasukan ialah 23.84% lilitan jejari untuk spesimen segar dan 32.29% untuk rendaman. Walau bagaimanapun, kedua-dua sampel masih tidak melepasi nilai piawai kemasukan, iaitu 44% lilitan jejari kayu. Hanya nilai kesimpanan pengawet untuk kayu rendaman sahaja dengan nilai dalam lingkungan 20 kg/cm², yang mencapai nilai piawai berasaskan bahan oksida kering. Dengan itu, rawatan melalui tekanan sel-penuh mampu memanjangkan jangka hayat khidmat* H. minor *pada kadar yang terhad iaitu kayu rendaman memberi rangsang balas yang lebih memberangsangkan untuk rawatan dengan pengawet.*

ABSTRACT

Sundri (Heritiera minor *Roxb.*) *is a hardwood species which exhibits good strength qualities. They have potential for use as electric poles but their short service life posed a problem. This study examined whether treatment with the preservative Chromated Copper Arsenate (CCA) by full cell pressure method at 15.40 – 16.10 kg/cm² for 8 hours could extend their service life. The samples tested were processed fresh and ponded* H. minor *logs of approximately 1 m length and 0.25 m diameter. Results showed that that the preservative treatment gave a mean penetration value of 23.84% log radius for fresh specimens and 32.29% for ponded logs, which were short of the standard requirement of 44% log radius penetration. The preservative retention of ponded logs was within the acceptable standard values of 20 kg/cm² of dry oxide basis, but that of fresh logs did not meet the standard requirement. Thus, the full pressure treatment can extend the service life of* H. minor *to a certain extent in which ponded logs gave encouraging response to the preservative treatment.*

INTRODUCTION

The application of chemical preservatives to wooden electric poles is a routine exercise in countries such as USA, Finland, Philippines, Norway, Canada and Bangladesh. The advantage of using wooden poles for electrification is its low cost. In his studies, Finntrepo (1987) showed that the cost of one wooden pole is 2 to 5 times lower than that of a traditional steel or concrete pole. The availability of competitively priced wooden poles would also mean that more countries, particularly the developing nations, can now afford to supply vast areas with power sources. Apart from its low cost, wooden poles are easier to handle in terms of transportation as well as for erecting and climbing purposes. They are non-conductive and can easily be re-cut for conversion if the need arises.

As with most developing countries, electrical supply in Bangladesh is mainly confined to the urban and semi-urban areas. To supply electricity to the rural areas, which makes up a major portion of the country, the importation of wooden poles for rural electrification purposes was endorsed. However, this was later found to be ineffective in the long run because improperly-treated poles generally deteriorated within a few years' installation, resulting in a high replacement frequency. Thus, research on wood preservation is very essential as properly-treated poles can extend the service life to at least 40 more years (Hunt and Garratt 1953). Presently, 65 to 75 % of the total local demand for poles was met as imported commodities. It is thus timely that the use of properly-treated woods be sourced from within the country as this could prove to be a more cost-effective move besides creating employment for the local community.

Sundri (*Heritiera minor* Roxb.) is a mangrove species, abundantly found in the Sundarban forests and coastal areas of Bangladesh. It constitutes about 74.2 % of the total mangrove population in the Sundarbans (Satter and Bhattacharjee 1987). It is a moderate to large-sized evergreen tree, attaining an average girth of 60 to 120 cm and a height of 12 to 15 m. Currently, they are mainly used as anchor and stabilizer logs. According to estimates, about 50,000 sundri poles could be extracted annually from the Sundarban forests (Latif 1965). Sundri woods exhibit very good strength properties but when untreated, woods are prone to decay in a very short period. Latif *et al.* (1989) found that the average service life of fresh, untreated sundri wood in graveyard tests was only 18 months.

As a hardwood species, sundri logs are also very difficult to impregnate with preservatives. Nevertheless, several options are still available to the researcher when experimenting with chemical preservatives, based on the Book of Standards (1986) of the American Wood Preserver's Association (AWPA). Logs to be used as electric poles fall under Commodity Standards C1-86 of the AWPA (1986). Under this category, several types of preservatives were recommended, one of which was of 'waterborne preservatives' (Standard P5) which has given excellent service with products that are clean and paintable. Of the preservatives listed, chromated copper arsenate (CCA Type C oxide formulations) was selected for use in this study. Thus, all procedures undertaken were in accordance with American Wood Preservers Association (AWPA) standard P5–86, sections 6 and 9.

The present investigation undertakes to determine whether CCA could be impregnated in sundri logs using 5.5% CCA by full cell pressure method. The efficacy is measured based on the ability of the preservative's penetration and retention within the samples. For effective penetration, the standard requirement is a minimum of 44% penetration of the log radius and 100% of sapwood. For effective retention, the standard requirement is that the pressure in the specified assay zones should not be less than 20 kg/cm^2 for logs with sapwood thickness of between 0.01 to 1.3 cm (Anon 1992).

MATERIALS AND METHODS

Source of Log Samples

Two types of samples, namely fresh and ponded logs were used in this study. Fresh logs for the production of anchor and stabilizer logs were collected from the Sundarban mangrove forests by the Bangladesh Forest Industries Development Corporation (BFIDC) at Khulna. Ponded samples consisted of logs that had been submerged for at least 3 years for the local use of the Cabinet Manufacturing Plant (CMP). A total of 20 pieces each of fresh and ponded logs were collected and cut to sizes ranging from 1.0–1.20 m in length and 0.2 to 0.3 m in diameter with each being conditioned prior to treatment. Conditioning was done by drying the logs to 13–25% moisture content at 3.80 cm depth from the log surface, in a steam-heated kiln.

Source of Preservative

The preservative selected was 5.5% Chromated Copper Arsenate, a waterborne preservative, whose preparation follows Standard P5 as stipulated by the American Wood Preservers' Association (AWPA). The active ingredients of each of the CCA component were 47.5% chromium oxide (CrO_3), 18.5% cupric oxide (CuO) and 34% arsenic oxide (As_2O_5) respectively.

Full-cell Pressure Treatment

Based on previous undertakings, the protocol selected for this study was by the Bethell method or full-cell pressure treatment, which recommended a 5.0 to 5.5% concentration of

CCA applied at 14.0 to16.15 kg/cm^2 of pressure, for 6 to 8 hours. Thus, after conditioning, both log types were treated with 5.5% CCA at a pressure of 15.44 to 16.14 kg/cm^2 for 8 hours.

The treatment cylinder into which the preservative was filled measured 2.0 m in diameter and 11.5 m in length. It was equipped with an initial and final vacuum pump. Impregnation pressure was applied by a pressing pump for liquid pressure.

Measurement of Preservative Penetration and Retention

After treatment, a total of 40 bore samples of 0.2 m diameter were extracted up to a depth of 5.0 m from both types of logs. The preservative retention of each of the CCA components was determined as kg/cm^2 of CrO_3, CuO and As_2O_5 respectively, of samples taken from treatment zones 0.0 to 1.3 cm, 1.3 cm to 2.5 cm, 2.5 cm to 3.8 and 3.8 to 5.0 cm of the outermost to the inner depths of samples.

Penetration of CCA was measured by using chromazurol solution (a Copper indicator) as described in AWPA-A3-84 Book of Standards (AWPA 1984). CCA retention was measured by using X-ray spectroscopy (Asoma Instrument) as indicated in AWPA-A9-86 Standard (AWPA 1986).

RESULTS

CCA Penetration

The overall CCA penetration was found to be 2.10 (± 1.34) cm or 23.84% radius for fresh logs and 2.80 (± 0.68) cm or 32.29% radius for ponded logs. Although both samples showed that chemical penetration was higher for ponded logs, neither sample type actually met the standard requirement, which was a minimum of 44% or 3.8 cm penetration of log radii. The readings obtained were lower than the standard requirement by 20.16% and 11.71% for fresh and ponded logs, respectively (Table 1). In addition, 25% of the fresh logs showed irregular chemical penetration in the outer zone. This irregularity was absent in ponded logs (Table 2).

CCA Retention

The mean preservative retention of fresh *Heritiera* logs was 8.68 kg/cm^2, which was well below the

TABLE 1
CCA penetration in treated *H. minor* logs

	Fresh Logs	Ponded Logs
Mean radius of logs (cm)	8.78 ± 0.63	8.68 ± 0.50
Mean penetration (cm)	2.10 ± 1.34	2.80 ± 0.68
percentage of radius	23.84 %	32.29 %
No. of irregular penetration in bore samples	10/40	None
Remarks on chemical penetration:	20.16% lower than standard requirement.	11.71% lower than standard requirement.

* ± denotes standard deviation.

TABLE 2
CCA retention (kg/cm^2) in *H. minor* logs

Depth of zone (cm)	Fresh Logs				Ponded Logs			
	CrO_3	CuO	As_2O_5	Total	CrO_3	CuO	As_2O_5	Total
0.00 – 1.3	9.63	3.00	4.89	17.53	16.38	5.39	8.81	30.59
1.3 – 2.5	4.68	1.65	2.06	8.36	11.05	4.13	5.58	20.75
2.5 – 3.8	3.99	1.15	1.42	6.32	7.47	2.80	3.40	13.69
3.8 – 5.0	1.34	0.49	0.67	2.51	4.38	1.80	2.00	8.19

standard requirement of 20 kg/cm². The retention readings too could not meet the 10 % contingency range of 18.0 kg/cm² up to 21.6 kg/cm² of the standard requirement limits allowable during the time of inspection of the material.

For ponded *Heritiera* logs, the retention values of the 2 outermost sampling zones was within the standard requirements. The assay zones of 0.00-1.3 cm and 1.3-2.5 cm gave retention values of 30.59 kg/cm² and 20.75 kg/cm² respectively. This was in excess of the standard requirement by 10.59 kg/cm² and 0.75 kg/cm² respectively. The overall mean retention value of 18.31 kg/cm² was also within the standard requirement, exceeding slightly the 18.0 kg/cm² contingency limit of 10% allowed at time of inspection.

DISCUSSION AND CONCLUSION

Studies have shown that wooden poles are more cost-effective than concrete poles for electrification. Although wooden poles are used in Bangladesh, they are all resourced as imported materials. *Heriteria minor* or 'sundri' is a local hardwood species found in abundance and they offer a good alternative for electric poles. Its only drawback is that it is prone to decay. Thus, studies on proper preservative treatment are necessary in order to extend its service life. Latif *et al.* (1982) used oilborne preservatives on sundri poles and found them to be ineffective. Subsequent studies by Ilias and Kabir (1994) also showed poor preservative penetration and retention by sundri, resulting in very poor service life after treatment. This study used CCA as a waterborne preservative and found that a treatment schedule of 15.40 – 16.14 kg/cm² for 8 hours may extend the service life to a certain extent compared to non-treated poles.

The preservative penetration readings were better for ponded logs compared to fresh ones, but neither sample actually met the standard requirements set by the AWPA. The preservative retention of fresh logs too could not be accepted, but the mean retention value of 18.31 kg/cm² for ponded logs was within the accepted range of the standard requirement. This study shows that unlike the fresh samples, ponded logs gave regular chemical penetration and good retention properties. Further studies should be carried out to upgrade the penetrability of the preservative before ponded logs can be recommended as electric poles. The results obtained may give direction towards better choice of chemicals or its mode of treatment. Thus, more studies should be carried out in order to find the most effective method in the treatment of sundri poles.

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Utilisation of Blood, Chicken Offal and Fish Meal as Cockerels' Dietary Supplements

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Keywords: Blood meal, chicken offal meal, fish meal, supplementation, methione, lysine

ABSTRAK

Satu eksperimen dilakukan untuk mengkaji prestasi ayam jantan berusia 16-20 minggu yang diberi makan berasaskan tiga hampas biji benih berminyak iaitu hampas kacang tanah (GNC), GNC/bungkil isirung sawit, GNC/hampas biji kapas ditambahkan dengan empat sumber metionina dan lisina iaitu sumber minyak sintetik, serbuk darah (baja), serbuk ikan atau serbuk isi ayam dalam rawatan berfaktor 3 x 4. Hasil keputusan menunjukkan bahawa tambahan berat, makanan kepada nisbah pertambahan, nitrogen hati penahanan nitrogen dan lemak hati sama seperti SGPT tidak secara signifikannya (P<0.05) berbeza sama ada disebabkan oleh sumber protein tumbuhan atau bentuk metionina dan suplementasi metionina dan suplemantasi liasinina atau kedua-duanya. Walau bagaimanapun, nilai protein keseluruhan serum, SGOT, menunjukkan peratus dan lemak abdomen secara signifikannya (P>0.05) mendapat kesan daripada pengehadan makanan.

ABSTRACT

An experiment was undertaken to investigate the performance of 16-20 week-old cockerels fed diets based on three oil seed cakes viz. groundnut cake (GNC), GNC/Palm kernel cake, GNC/cotton seed cake supplemented with four sources of methionine and lysine viz. synthetic sources, blood meal, fish meal or chicken offal meal in 3 x 4 factorial treatment. The results indicated that weight gain, feed to gain ratio, nitrogen retention, liver nitrogen and liver fat as well as SGPT were not significantly (P<0.05) different either due to plant protein sources or methionine and lysine supplementation forms or both. However, the values for the serum total protein, SGOT, dressing percentage and abdominal fat were significantly (P>0.05) affected by dietary treatments.

INTRODUCTION

Although synthetic amino acids are expensive, results of a previous study (Nwokoro 1993) indicated that accurate supplementing of cockerel starter diets with crystalline methionine and lysine gave better performance and economy of feed conversion than blood meal, fish meal or chicken offal meal supplements. Another study (Nwokoro 1992) showed that supplementing diets of 8-16 weeks cockerels with any of the supplemental sources sustained optimal performance and economic feed conversion. The lysine levels, which were optimum for cockerels during 8-16 weeks of age, were found to be deficient during 16-20 weeks of age (Nwokoro 1998; Nwokoro and Bamgbose 1995). Thus, this experiment was initiated to complement the results of the earlier studies and to test the effect of supplementation of oil seed cake based diets with different sources of methionine and lysine on performance and serum metabolites of 16-20 week old cockerels.

MATERIALS AND METHODS

Experimental Birds and Management

A total of 600-day barred harco cockerels were used for the experiment. The birds were reared together on the same diet for seven weeks (diet A) and diet B for 8-15 weeks (Table 1).

At the end of the 15th week, 540 birds were selected and randomly distributed into 12 groups such that each group was replicated thrice. The twelve groups were fed to twelve diets (Table 2) which were formulated in a 3 x 4 factorial form such that three combinations of plant protein sources (GNC only, GNC/Palm kernel cake

TABLE 1
Composition of diets fed to cockerels aged 0-8 Weeks (Diet A) and 8-16 weeks (Diet B)

	Diets	
Ingredients	A	B
Maize	44.41	29.20
Maize offals	22.46	52.70
Groundnut cake	29.13	14.15
Bone meal	2.00	2.00
Oyster shell	1.00	1.00
Premix	0.25	0.25
Salt	0.25	0.25
DL- methionime	0.15	0.15
L-Lysine	0.35	0.35
Composition (on-as-fed basis)		
ME (Kcal/kg)	2650	2250
CP (%)	21.00	16.00
Methinime + Cystine	0.73	0.63
Lysine (%)	0.98	0.82

(expeller pressed) or GNC/cotton seed cake) were each supplemented with four major sources of M + L (synthetic, blood meal, fish meal or chicken offal meal) to bring dietary levels to the requirements as established previously (Nwokoro 1991). The PKC was expeller pressed type and gossypol levels were calculated as in Ikurior (1982) and Ikurior and Fetuga (1984). The birds were fed and housed in deep litter system partitioned with wire netting into pens (110 cm x 280 cm). Brooding was carried out in the bird's first 4 weeks of life. Vitamin-mineral supplements (anti-stress) were administered for the first four days of the chicks' arrival. In addition, the cockerels were vaccinated with New Castle Disease Vaccine (i/o) in the second day, Gumboro (second week) and Lasota (sixth week) in drinking water. Coccidiostats were administered between the 4th and 5th week, while drowning was carried out in the 8th week. In each of the Vaccines medication in drinking water, chickens are usually starved of water overnight before drugs were administered. The antistress drug was administered subsequently for 3 days. Feeders and drinkers were cleaned daily, and the experiment was terminated at the end of the 20th week.

In the 20th week, blood samples were collected and pooled on replicate bases. To obtain the serum, the bloods after 24 hours were centrifuged at 480xg to obtain the serum. Samples were labelled and preserved at -10°C prior to analysis and subsequent thawing for analysis was done at room temperature. The serum total protein, SGOT and SGPT were analysed using Gelson and Ackerman (1975) procedures.

A nitrogen balance trial was carried out in the 20th week in which two chickens from each replicate were placed for compartment (36 cm x 36 cm) in Metabolism cages. Then Experimental diets were offered *ad libitum* for one week duration: the first four days for adjustments and the remaining 3 days for daily collection of records of feed intake and droppings. The latter was collected in metal trays fitted under each tier, which were initially cleaned, covered with aluminium foil and sprayed with 1% boric acid solution. The faecal samples were oven-dried for 72 hours at 50°C before analysis. The proximate composition of test ingredients and feed, faecal and liver samples were analyzed using the A.O.A.C (1980) method.

Carcass Analysis

Two cockerels per replicate in the 20th week were selected, wet plucked, eviscerated and dressing percentage computed. The liver with gall bladder removed was sampled, oven dried at 55°C for 3 days and at 105°C for 24 hours before analysis for nitrogen and fat. Dressed

TABLE 2
Gross composition of experimental diets

Protein Supplement	Groundnut Cake (GNC) Based Diets				GNC/Palm Kernel Cake Based Diets				GNC/Cotton Seed Cake (CSC) Based Diets			
Diets	1	2	3	4	5	6	7	8	9	10	11	12
Amino Acid Supplement Ingredients	M+L	BM+M	FM	COM	M+L	BM+M	FM	COM	M+L	BM+M	FM	COM
Maize	22.59	22.59	22.59	22.59	22.59	22.59	22.59	22.59	22.59	22.59	22.59	22.59
Maize Offals	58.25	56.98	56.47	55.54	47.09	45.45	44.73	43.53	55.57	55.54	55.15	54.41
Groundnut Cake (GNC)	15.40	15.40	15.40	15.40	8.75	8.75	8.75	8.75	5.42	5.42	5.42	5.42
Palm Kernel Cake (PKC)	-	-	-	-	17.78	17.78	17.78	17.78	-	-	-	-
Cotton Seed Cake (CSC)	-	-	-	-	-	-	-	-	11.71	11.71	11.71	11.71
Bone Meal	2.00	2.00	2.00	2.00	2.00	2.00	2.00	2.00	2.00	2.00	2.00	2.00
Oyster Shell	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
Premix (Growers)	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25
Salt (NaCl)	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.05	0.25	0.25	0.25	0.13
DL-Methionine (M)	0.13	0.11	-	-	0.13	0.09	-	-	0.11	0.11	-	-
L-Lysine (L)	0.13	-	-	-	0.16	-	-	-	0.10	-	-	-
Blood Meal (BM)	-	1.42	-	-	-	1.84	-	-	-	1.14	-	
Fish Meal (FM)	-	-	2.04	-	-	-	2.65	-	-	-	1.63	-
Chicken Offal Meal (COM)	-	-	-	3.12	-	-	-	4.05	-	-	-	2.49
CALCULATED COMPOSITION												
Crude Protein (%)	16.00	17.57	17.69	18.12	16.00	16.40	16.50	17.09	16.00	17.18	17.28	17.62
Metabolisme Energy (kcl/g)	2.25	2.25	2.27	2.27	2.25	2.26	2.28	2.27	2.25	2.31	2.32	2.32
DL-Methionine + Cystine (%)	0.63	0.63	0.63	0.63	0.63	0.63	0.63	0.63	0.63	0.63	0.63	0.63
L-Lysine (%)	0.77	0.77	0.74	0.72	0.77	0.77	0.74	0.69	0.77	0.77	0.74	0.72
Cost/kg Diet (N)	1.84	1.55	1.57	1.61	1.61	1.44	1.69	1.41	1.49	1.91	1.41	1.38
TOTAL GOSSYPOL (%)	-	-	-	-	-	-	-	-	0.085	0.085	0.085	0.085
FREE GOSSYPOL (%)	-	-	-	-	-	-	-	-	0.0046	0.0046	0.0046	0.0046
DETERMINED COMPOSITION												
Ether extract (%)	3.41	3.59	3.89	5.55	4.22	4.46	4.60	4.99	3.81	3.94	9.96	4.80

carcasses were frozen for one week, thawed, manually deboned and ratio of flesh to bone was determined.

Statistical Analysis

The data obtained from the experiment were subjected to analysis of variance and significance of difference of means was determined (Steel and Torrie 1980).

RESULTS

Data on performance characteristics of cockerels reared on various dietary regimes are presented in Tables 3 and 4. These indices were not significantly (P>0.05) affected by dietary treatments.

Cockerels on diet 1 (M + L) gained at a lower rate than those on others. Those on diets 3 (FM) and 12 (COM) recorded the best gains followed by those on diets 9, 8 and 6 (BM + M). These differences were however not consistent with dietary regimes.

Results of the factor effect of plant protein sources (PPS) (that is ignoring methionine and lysine supplemental sources) and methionine and lysine supplemental forms (MLSF) and ignoring PPS on performance characteristics are shown in Table 4. The parameters were not significantly (P>0.05) affected by dietary treatments. The PPS indicated that GNC/CSC based diets gave the best weight gain, feed conversion ratio, nitrogen retention and lowest feed consumption. Those for other groups were similar and MLSF show that birds on COM based diets recorded the best gains. Feed consumption was highest in birds on BM + M, M + L and PM while the lowest intake was recorded in the COM group where the best feed conversion ratio was obtained.

Although nitrogen retention was efficient in all the dietary groups, the highest retention was obtained in M + L group followed by those on FM. The experiment had no effect on mortality.

Table 5 shows the effects of dietary treatments on some serum metabolites, carcass characteristics, liver nitrogen and fat content of cockerels. The factor effects of PPS and MLSF on these parameters are presented in Table 6. Apart from serum total protein, SGOT, dressing percentage and abdominal fat, other indices were not significantly (P>0.05) affected by dietary treatments. The least SGOT activity was obtained in diet 2 where the highest concentration of serum total protein and optimal dressing percentage were recorded.

Parameters for the PPS (Table 6) were not significantly affected by dietary treatment. The effect of MLSF (ignoring PPS) revealed that with the exception of the abdominal fat, all other parameters were not significantly (P>0.05) different.

DISCUSSION

The weight gains were generally high irrespective of the dietary treatments. This might not be unexpected as supplementation was to meet requirement level. In addition, it may also be due to the high fat accretion as indicated by abdominal fat recorded.

In the diets where animal protein was used as supplements, where dietary proteins were more than the recommended 16% (Okosun 1987), the bird performed optimally. This might be an indication that at the age range of experimental birds, they were able to adjust to the disproportionate amount of other amino acids. This is contrary to that reported previously (Nwokoro 1993) in starter cockerels.

The results of the feed consumption show that birds on COM supplemented group consumed least feed, which was also the group where maximum weight gain and abdominal fat were recorded. The higher dietary fat may have contributed to lower feed intake leading to better efficiency of meeting energy requirement. Similarly, this higher crude fat in diet may have contributed to higher abdominal fat for the COM diets, as a previous report (Olomu and Baracos 1990) show that the dietary level of lipids has a direct relationship with body lipid accretion. That weight gains, feed intake including feed per gain ratio of cottonseed cake (CSC) based diets (Tables 3 and 4) were similar to others without CSC is an indicative of sub lethal level of the free gossypol in the diets (9, 10. 11, 12). Also, the dietary level of 0.0046% is less than the tolerance level (0.01% or 100%) recommended by Ikurior and Fetuga (1984).

The carcass dressing percentage and flesh to bone ratio appear to support the view that diets were adequate irrespective of dietary treatments as the values recorded are within the range reported (Okosun and Tewe 1987; Nwokoro and Bamgbose 1995; Nwokoro and Tewe 1997).

TABLE 3
Performance characteristics of 16-20 week old cockerels fed varying forms of methionine and lysine supplemented diets

	Diet (amino acids supplemental form- %Diet)											
	1 (M+L)	2 (BM+M)	3 (FM)	4 (COM)	5 (M+L)	6 (BM+M)	7 (FM)	8 (COM)	9 (M+L)	10 (BM+M)	11 (FM)	12 (COM)
Daily Weight Gain (g)	17.98 (1.252)	19.98 (2.301)	21.11 (1.792)	19.68 (2.011)	19.42 (2.661)	20.12 (3.550)	18.54 (0.572)	20.68 (2.092)	20.72 (2.222)	18.49 (2.901)	19.22 (0.552)	21.81 (0.550)
Daily feed consumption (g/bird)	102.52 (5.112)	102.11 (14.400)	100.01 (1.040)	101.82 (3.521)	99.78 (3.092)	102.38 (11.121)	100.19 (4.811)	100.96 (5.000)	98.92 (4.820)	101.33 (1.293)	100.18 (12.001)	93.64 (3.178)
Feed/gain ratio	5.70	5.11	4.74	5.17	5.14	5.09	5.40	4.88	4.77	5.48	5.21	4.29
Nitrogen retention	69.97 (2.431)	72.85 (0.221)	71.73 (2.468)	67.98 (1.928)	73.00 (2.861)	68.51 (2.861)	67.51 (3.010)	72.34 (8.420)	72.11 (7.681)	69.23 (0.679)	73.11 (4.972)	70.92 (102.10)
Total Mortality	3.33	0	0	0	0	0	0	0	0	0	0	3.33

( ) ± Standard error or mean

TABLE 4
Performance characteristics of 16-20 week old cockerels fed varying forms of methionine and lysine supplemental or plant protein diets

Parameters	Plant Protein Sources				M And L Supplemental Forms (% Of Diet)				
	GNC Only	GNC/PKC	GNC/CSC	S.E. of X (±)	M+L	BM+M	FM	COM	S.C. of X (±)
Daily Weight Gain (g)	19.688	19.690	20.060	0.2142	19.373	19.530	19.623	20.723	0.6159
Daily feed consumption (g/bird)	101.615	100.828	98.518	1.6097	100.407	101.940	100.127	98.807	1.2876
Feed/gain ratio	5.180	5.128	4.938	0.1274	5.203	5.227	4.117	4.780	0.2066
Nitrogen retention	70.633	70.340	71.343	0.5158	71.693	70.197	70.783	70.413	0.6603
Total Mortality	3.33	0	3.33	0	3.33	0	0	3.33	-

TABLE 5
Serum metabolics, carcass characteristics and liver nitrogen and liver fat of 16-20 week old cockerels fed varying forms of methionine and lysine supplemental diets

	Diet (amino acids supplemental form- %Diet)											
	1 (M+L)	2 (BM+M)	3 (FM)	4 (COM)	5 (M+L)	6 (BM+M)	7 (FM)	8 (COM)	9 (M+L)	10 (BM+M)	11 (FM)	12 (COM)
Serum Total Protein	6.24^{a} (0.021)	8.24^{b} (0.112)	6.11^{a} (0.032)	5.76^{a} (0.066)	5.76^{a} (0.111)	6.81^{ab} (0.012)	5.92^{a} (0.211)	5.82^{ab} (0.006)	6.82^{ab} (0.040)	4.92^{c} (0.041)	5.98^{a} (0.080)	6.22^{a} (0.000)
SGOT (SF Unit/ML)	96.10^{ab} (10.010)	94.00^{b} (4.222)	97.50^{a} (3.021)	98.50^{a} (0.982)	97.92^{a} (0.001)	100.01^{a} (8.421)	97.00^{a} (2.222)	98.38^{a} (3.222)	98.32^{a} (3.221)	101.62^{a} (1.282)	95.92^{ab} (2.000)	97.38^{a} (1.892)
SGPT (SG Unit/ML)	39.84 (1.520)	38.11 (3.021)	40.21 (2.928)	40.71 (3.011)	40.11 (1.001)	37.35 (0.098)	38.75 (0.062)	39.75 (2.970)	38.92 (2.222)	39.82 (2.221)	40.21 (0.111)	37.92 (3.212)
Dressing Percentage (%)	70.52^{a}	71.22^{a}	68.52^{b}	72.11^{a}	70.92^{a}	67.98^{b}	66.97^{b}	69.88^{ab}	70.82^{a}	71.22^{a}	69.32^{b}	70.34^{a}
Abdominal Fat (g)	2.80^{a}	6.70^{b}	9.30^{b}	10.30^{b}	3.34^{a}	6.92^{b}	6.78^{b}	8.88^{b}	5.20^{a}	5.60^{ab}	6.21^{b}	9.32^{b}
Flesh to bone ratio	4.01	3.87	3.89	4.19	4.09	3.21	3.61	3.98	4.11	3.45	4.01	
Liver Nitrogen (%)	9.98	9.22	9.72	9.34	10.01	9.45	9.82	9.86	9.68	9.63	9.59	
Liver fat (%)	9.50	11.18	12.60	13.42	15.52	13.14	11.68	11.86	9.78	11.86	12.10	10.38

abc means on the same row with same superior superscript or without superscript are not significantly (P>0.05) different.

TABLE 6
Serum metabolites, carcass characteristics liver nitrogen and liver fat of the cockerels fed various forms of diets abc within M and L supplemental forms, means on the same row with same superscript or no superscript are not significantly (P>0.05) different

Parameters	Plant Protein Sources				M And L Supplemental Forms (% Of Diet)				
	GNC Only	GNC/PKC	GNC/CSC	S.E. of X (±)	M+L	BM+M	FM	COM	S.C. of X (±)
Serum Total Protein	6.633	6.078	5.985	0.3504	6.273	6.717	6.003	5.933	0.3553
SGOT (SF Unit/ML)	96.525	98.328	98.310	1.0352	97.447	98.543	96.807	98.087	0.7572
SGPT (SG Unit/ML)	39.718	38.990	39.218	0.3724	39.623	38.427	39.723	39.460	0.5974
Dressing Percentage (%)	70.593	68.938	70.425	0.9109	70.753	70.140	68.270	70.0777	1.1807
Abdominal Fat (g)	7.275	6.480	6.583	0.4323	3.780[a]	6.407[b]	7.430[b]	9.500[c]	2.3777
Flesh to bone ratio	3.935	3.775	3.888	0.0822	4.047	4.070	3.510	3.837	0.2595
Liver Nitrogen (%)	9.705	9.655	9.690	0.0257	9.700	9.890	9.433	9.710	0.1883
Liver fat (%)	11.675	12.300	11.030	0.6350	10.600	12.060	12.127	11.887	0.7195

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Efficacy of Entomopathogenic Fungi, *Paecilomyces fumosoroseus*, *Beauveria bassiana* and *Metarhizium anisopliae* var. *majus* Against *Crocidolomia binotalis* (Lepidoptera; Pyralidae)

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Keywords: ***Beauveria bassiana*, bioassay, *Crocidolomia binotalis*, *Metarhizium anisopliae* var. *majus*, *Paecilomyces fumosoroseus***

ABSTRAK

Bioasai di makmal tiga pencilan tempatan entomopatogen hyphomycete ke atas ulat hati kubis, Crocidolomia binotalis *Zeller (Lepidoptera: Pyralidae) dan efikasi lapangan beberapa persediaan formulasi* Paecilomyces fumosoroseus *(Wise) Brown & Smith telah dinilai. Bioasai dos-kematian menunjukkan kesemua pencilan berupaya menyebabkan maut ke atas larva instar kedua. Kebanyakan larva menjadi moribund dalam masa dua hari selepas rawatan. Pendedahan kepada dos mulai 2 x 10^1 konidia mL^{-1} hingga 2 x 10^7 konidia mL^{-1} mengakibatkan purata kematian larva dari 10.3 hingga 100%. Pada kepekatan melebihi 2 x 10^6 konidia ml^{-1} kematian larva adalah melebihi 80%, dan 100% kematian telah didapati pada 2 x 10^7 konidia mL^{-1} bagi ketiga-tiga spesies kulat. Perhubungan signifikan ($P<0.005$) telah diperolehi di antara log dos dan kematian probit bagi kesemua pencilan. Nilai EC_{50} bagi* P. fumosoroseus *telah dianggarkan pada 1.926 x 10^3 konidia mL^{-1}, dan didapati lebih rendah dan signifikan daripada* Beauveria bassiana *(Bals) Vuill pada 5.038 x 10^3 konidia mL^{-1}. Nilai EC_{50} bagi* Metarhizium anisopliae *var.* majus *(Metsch) Sorokin adalah yang tertinggi dan signifikan pada 2.0 x 10^4 konidia mL^{-1}. Pencilan yang terbaik sekali ialah* P. fumosoroseus *dengan LT_{50} hampir setengah hari lebih singkat pada dos setanding 2 x 10^7 konidia mL^{-1}, dengan nilai kecerunan 4.656 berbanding 4.356 bagi* B. bassiana *dan 4.193 bagi* M. anisopliae *var.* majus. *Pada rawatan lapangan, tanaman telah disembur dengan ampaian mengandungi 10^7 konidia mL^{-1}. Purata peratus kematian bagi kesemua rawatan didapati melebihi 70% dan lebih signifikan ($P<0.05$) daripada kawalan. Konidia yang diampai dalam minyak kelapa sawit Vesawit® telah memberi keputusan yang paling menggalakkan ke atas ulat hati kubis.*

ABSTRACT

Laboratory bioassays of three local isolates of entomopathogenic hypomycetes against the cabbage-heart caterpillar, Crocidolomia binotalis *Zeller (Lepidoptera: Pyralidae) and field efficacy of several formulations of* Paecilomyces fumosoroseus *(Wise) Brown and Smith were evaluated. Dosage-mortality bioassays revealed that all the isolates were able to cause mortality to second instar larvae. Majority of the larvae became moribund within two days after treatment. Exposures to doses varying from 2 x 10^1 conidia mL^{-1} to 2 x 10^7 conidia mL^{-1} resulted in mean larval mortalities from 10.3 to 100%. At concentrations exceeding 2 x 10^6 conidia mL^{-1} larval mortality was in excess of 80% and 100% mortality was observed at 2 x 10^7 conidia mL^{-1} for all three fungal species. Significant relationships ($P<0.05$) were obtained between log dosage and probit mortality for all the isolates. The EC_{50} for* P. fumosoroseus *was estimated at 1.926 x 10^3 conidia mL^{-1}, and was significantly lower than that of* Beauveria bassiana *(Bals) Vuill at 5.038 x 10^3 conidia mL^{-1}. The EC_{50} for* Metarhizium anisopliae *var* majus *(Metsch) Sorokin was significantly the highest, at 2.0 x 10^4 conidia mL^{-1}. The best isolate was* P.fumosoroseus *which had LT_{50} almost half a day lower at a comparable dosage of 2 x10^7 conidia mL^{-1}, with a gradient of 4.656 as compared to 4.356 for* B. bassiana *and 4.193 for* M. anisopliae *var* majus. *In field treatments, plants were sprayed with suspensions containing 10^7 conidia mL^{-1}. Mean percent mortality for all the treatments were in excess of 70% and significantly higher ($P<0.05$) than the control. Conidia in palm oil Vesawit® gave the most promising result against the cabbage-heart caterpillar.*

INTRODUCTION

Cruciferous vegetables are economically important throughout the world. However, their production has been seriously affected by a steady increase in insect pest damage. The cabbage-heart caterpillar (CHC), *Crocidolomia binotalis* Zeller, is currently considered the second most important insect pest of cabbage in the Cameron Highlands of Malaysia (Ooi and Kelderman 1979). It is almost exclusively found in hot humid highland tropics, and constitutes a more serious pest problem during the drier months. Even a single mature larva is capable of causing economic loss to head cabbage, *Brassica oleracea* L. (Peter *et al.* 1988). This pest is not reported in Europe and the Americas (Waterhouse and Norris 1987). The larvae live gregariously feeding at first on the underside of cabbage leaves which may eventually be eaten completely. Damage to the heart at preheading leads to abortion or production of multiple heads which then are unmarketable. By and large, farmers use large quantities of insecticides, often spraying tank mixes of several chemicals to control the pest. As reported by Ooi and Sudderuddin (1978) and Fauziah *et al.* (1992), these practices have resulted in many problems such as development of insecticide resistance, pest resurgence, excessive chemical residues and environmental contamination.

The concept of integrated pest management (IPM) in vegetables is beginning to be accepted by farmers, but so far no parasitoids of CHC have been reported in Malaysia. Consequently, microbial control could play an important role in the IPM strategy. The role of fungal pathogens as natural enemies for cruciferous insect pests has recently been explored and several isolates of hyphomycetous fungi have been identified. Besides being infective against the bagworms, the cocoa mirids and cocoa podborer (Lim *et al.* 1988), *Paecilomyces fumosoroseus* (Wise) Brown and Smith and *Beauveria bassiana* (Bals.) Vuill have been reported effective against the cosmopolitan diamondback moth, *Plutella xylostella* L (Lepidoptera: Yponomeutidae) (Ibrahim and Low 1993; Ibrahim and Hashim 1998), while *Metarhizium anisopliae* var. *majus* (Metsch) Sorokin has been shown to be infective against the diamondback moth (Hashim *et al.* 1999).

Against this background, studies were undertaken to evaluate the IPM potential of microbial control of CHC. Three endemic fungal species, *P. fumosoroseus, B. bassiana,* and *M. anisopliae* var. *majus* were compared in bioassays against the CHC. The efficacy of several formulations of the best fungal species was tested in a field trial.

MATERIAL AND METHODS

Insect Culture

Cultures of CHC were maintained in the insectary under a controlled environment of 27±2°C, 80±10% RH and with 12:12 day:night photoperiod on hybrid cabbage leaves, *Brassica oleracea* var. *capitata,* obtained from greenhouse-grown plants. Distilled water and 5% (w/v) honey were provided separately as food for the moths. To obtain larvae of standardised age, plants with 1-day old eggs from oviposition cages were transferred daily into new cages. Only second instar larvae were used in the study.

Fungal Culture

For the purpose of this study, fungal isolates originated from heterologous hosts (Table 1) were passaged through *P. xylostella* larvae and then re-isolated as single spore isolates by means of a micromanipulator under a microscope, cultured and maintained at an ambient environment of 27±2°C on sterilised potato dextrose agar (PDA) as well as on sterilised rice flour medium maintained in autoclaved polybags.

TABLE 1
Origin of fungal isolates

Species	Insect host	Plant host	Location
Paecilomyces fumosoroseus	*Pteroma pendula* (Psychidae, larva & pupa)	*Acacia mangium*	Selangor
Beauveria bassiana	*Glenia celia* (Cerambycidae, pupa)	*Theobroma cacao*	Sabah
Metarhizium anisopliae var *majus*	*Oryctes rhinoceros* (Scarabaeidae, larva)	*Elaeis guineensis*	Selangor

To prepare fungal inocula, conidia from 3-week old PDA cultures were scraped from the surface of the plates with a sterile scalpel and suspended in 0.05% Tween 80 in sterile distilled water. A Neubauer haemocytometer was used to estimate the conidial concentration and subsequent appropriate serial dilutions were prepared from 2×10^7 to 2×10^1 conidia mL^{-1}. A Sigma hand altomiser was used to deliver approximately 2 mL of each treatment on a cabbage leaf preparation containing the test larvae. The leaf preparation consists of Chinese cabbage leaf, *Brassica juncea*, of ca. 120 cm^2 kept fresh with its stalk wrapped in wet cotton enveloped in aluminium foil. In this way the leaf freshness could be maintained for about 5-7 days. Each treatment was also sprayed on clear bacteriological agar (Bacto®) plate for CFU (germinated conidia) counts under a microscope 24 h after treatment.

Dosage-Mortality Bioassay

Ten second instar larvae of CHC were transferred onto each cabbage leaf preparation and left to acclimatise for about 10 min. Six such leaf preparations (replicates) were assigned randomly to each dosage-response assay. The assay included seven inoculum dosages of each fungal species plus a sterile control which consisted of 0.05% aqueous Tween 80. It was conducted in an ambient environment of 27 ± 2°C, 80 ± 10% RH and with 12:12 day:night photoperiod. Immediately after inoculation with the Sigma hand atomiser, the treated leaf preparations were each placed in an unsealed plastic container (15.5 x 8.5 x 9 cm) which was covered with a clear plastic sheet to maintain 100% RH for 24 h. Thereafter, the sheet was removed and water trays were used to maintain high air humidity in the containers.

Larval mortality, including moribund individuals, was recorded daily for 12 days. The EC_{50s} and LT_{50s} from the regression line with 95% fiducial limits were obtained through a probit programme (S103, Statistical Research Service, Canada DOA, unpublished) based on probit analysis (Finney 1971).

Field Trial with P. fumosoroseus

Five-week-old cabbages grown in polybags in the glasshouse were placed in an open field spaced 0.5 m within and 0.5 m between rows. There were four treatments and an untreated control, each consisted of six plants arranged in a row. Only the middle four plants were used for the purpose of evaluation. The plants were arranged in a randomised completed block design with five replications. Ten second instar CHC larvae were transferred to each cabbage plant, and to prevent ants from predating on the larvae, gamma-HCH (Lindacide®) powder was scattered over the area. Four formulations of *P. fumosoroseus* were prepared with conidia from PDA in oil palm cooking oil (40% mono-unsaturated, Vesawit®), conidia from PDA in kaolin (fine dust), conidia from rice flour culture (powder) and conidia from PDA in sterile distilled water containing 0.05% Tween 80. Sterile distilled water containing 0.05% Tween 80 was the spray carrier which also served as the control. A concentration of 2×10^7 conidia mL^{-1} was used in this spray programme. Oil and kaolin formulations were prepared from conidia (0.03 g) from 3-week old cultures scraped from the surface of the plates with a sterile scalpel mixed with either 30 mL Vesawit® or 30 g kaolin. Conidia from rice flour medium were collected from 3-week old cultures suspended in sterile distilled water containing 0.05% Tween 80. This was then shaken vigorously and the conidia were sieved using muslin cloth in order to separate the rice flour. Sterile distilled water containing 0.05% Tween 80 was used to dilute the conidial suspension which was standardised to 2×10^7 conidia mL^{-1}.

Four plants in the centre of each treatment row were designated as the experimental units. Four mL of each treatment were sprayed using a Sigma hand atomoser on each cabbage plant infested with 10 second instar CHC larvae. All treatments were sprayed late in the evening. Twenty-four hours after treatment, each group of 10 larvae from the four plants were transferred to a Petri dish with fresh cabbage leaves which were replaced after five days. Larval mortality was recorded daily for 12 days to determine the cumulative percent mortality. All dead and moribund larvae suspected of being were individually surface-sterilised in 0.5% sodium hypochlorite for three minutes, rinsed in sterilised distilled water and then placed on PDA tro ascertain the presence of *P. fumosoroseus.*

Data for ANOVA were transformed by Arc Sine $\sqrt{x}$ to stabilise the variance prior to the analysis. Treatment means were subjected to two-way ANOVA and subsequently compared using LSD at 5% level of probability.

RESULTS AND DISCUSSION

Dosage-Mortality Biasssay

All the fungal isolates were pathogenic for second instar larvae of *C. binotalis* (Table 2). The majority of the larvae became moribund within two days after treatment. All the fungal species were observed to sporulate on the surface of cadavers which were all mummified. Hashim *et al.* (1999) had previously observed *P. fumosoroseus* sporulation on the surface of dead diamondback moth larvae. The cadavers infected with *M. anisopliae* var. *majus* were not completely overgrown with the fungal mycelium unlike those larvae infected with *P. fumosoroseus* or *B. bassiana.*

Larval mortality was positively correlated with dose rate. Exposures to conidial doses varying from 2 x 10^1 conidia mL^{-1} to 2 x 10^7 conidia mL^{-1} resulted in larval mortality from 10.3 to 100%. At concentrations exceeding 2 x10^6 conidia mL^{-1}, larval mortality after 12 days was in excess of 80%, and a 100% mortality was observed at 2 x 10^7 conidia mL^{-1} for all the three species. The isolates of *P. fumosoroseus* and *B. bassiana* have also been found to be pathogenic against diamondback moth (DBM) larvae (Ibrahim and Hashim 1998). Tulloch (1976) had previously reported that *M. anisopliae* var. *majus* appeared to be restricted to the rhinoceros beetle *Oryctes* spp. However, Hashim *et al.* (1999) found that this isolate of *M. anisopliae* var *majus* caused over 80% mortality on DBM larvae when exposed to 2 x 10^6 conidia mL^{-1}.

Results of probit analyses indicated that there was significant relationship ($P< 0.05$) between log-dosage and probit mortality for the three fungal species (Table 3). Estimates of the median effective concentration (EC_{50}) computed for *P. fumosoroseus* were 1.926 x 10^3 conidia mL^{-1} with 95% fiducial limits between 7.0 x 10^2 – 4.58 x 10^3 conidia mL^{-1}. This was significantly lower than that of *B. bassiana* which was 5.038 x 10^3 conidia mL^{-1} with 95% fiducial limit between 1.907 x 10^3 – 1.198 x 10^4 conidial mL^{-1}. The EC_{50} for *M. anisopliae* var *majus* was much higher than that of the earlier mentioned fungal species. Suffice to say that *P. fumosoroseus* was the most virulent against the CHC, being ca. 2.5 times more virulent than *B. bassiana* and ca. 10 times more virulent than *M. anisopliae* var. *majus.*

The virulence by these three fungal species, as displayed by the decreasing LT_{50} values, demonstrated a common trend of generally increasing potency (i.e. the rate and speed of mortality) with increasing concentration (Table

TABLE 2
Mean percent mortality of second instar larvae of *Crocidolomia binotalis* after 12 days exposure to *Paecilomyces fumosoroseus, Beauveria bassiana* and *Metarhizium anisopliae* var *majus*

Dosage (Conidia mL^{-1})	*Paecilomyces fumosoroseus*	*Beauveria bassiana*	*Metarhizium anisopliae var majus*
2 x 10^7	100.0 (1248.09)[a]	100.0 (44.750)	100.0 (318.13)
2 x 10^6	89.5 (295.84)	83.9 (125.84)	81.0 (133.32)
2 x 10^5	70.2 (79.52)	62.5 (33.43)	56.9 (17.29)
2 x 10^4	57.9 (9.10)	53.6 (11.19)	44.8 (4.44)
2 x 10^3	47.4 (1.33)	42.9 (10.15)	29.3 (0.78)
2 x 10^2	38.6 (1.25)	28.6 (7.24)	25.9 (0.34)
2 x 10^1	28.1 (0.73)	26.8 (4.62)	10.3 (0.25)

Control = zero mortality
[a] CFU / mm^2 equivalent

TABLE 3
Effect of *Paecilomyces fumosoroseus* (Pf), *Beauveria bassiana* (Bb) and *Metarhizium anisopliae* var *majus* (Mam) on second instar larvae of *Crocodolomia binotalis*

Species	a (intercept)	b ± SE (slope)	ED_{50} (conidia mL^{-1})	95% fiducial limit
Pf	3.735	0.3852±0.0404	1926	700.2 – 4580
Bb	3.592	0.3838±0.0407	5038	1907 – 11980
Mam	3.021	0.4602±0.0430	20000	9443 – 41530

4). The most virulent isolate was *P. fumosoroseus*. This is shown by the lower LT_{50} for *P. fumosoroseus*, which was almost half day lower than that of the other two species.

Field Trial with P. fumosoroseus

Mean percent mortalities for all the treatments were in excess of 75% except for the control (Table 5). Conidia from PDA in palm oil Vesawit® significantly resulted in the highest larval mortality. The prevailing high humidity of the surrounding environment, typical of the weather conditions in Malaysia throughout the year, could have positively influenced the infectivity. Conidia from rice flour culture achieved 76.0% mortality, which did not differ significantly from conidia in kaolin. However, this formulation caused significantly lesser mortality than the conidia in palm oil formulation. Oil may have helped in spreading the conidia on the surface of a hydrophobic surface such as insect cuticle (Inglis *et al.* 1996). Ibrahim and Low (1993) reported *P. fumosoroseus* to be highly efficacious in the cabbage field against the diamondback moth when applied at the rate of 10^8 conidia mL^{-1} in 375 L water ha^{-1}, however, further effort is necessary in order to develop management strategies for CHC in the highlands.

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TABLE 4
Median lethal time for varying dosages of *Paecilomyces fumosoroseus* (Pf), *Beauveria bassiana* (Bb) and *Metarhizium anisopliae* var. *majus* (Mam) on second instar larvae of *Crocidolomia binotalis*

Dosage (conidia mL^{-1})	Pf				Bb				Mam			
	a	b±SE (slope)	LT_{50} (days)	95% FL	a	b±SE (slope)	LT_{50} (days)	95% FL	a	b±SE (slope)	LT_{50} (days)	95% FL
2×10^7	3.93	4.656± 0.402	1.70	1.518-1.876	3.57	4.356± 0.330	2.13	0.415-3.006	3.58	4.193± 0.310	2.18	1.952-2.388
2×10^6	4.92	2.229± 0.336	2.29	1.440-3.037	3.65	2.427± 0.187	3.61	2.356-3.737	2.61	3.368± 0.241	5.11	4.716-5.505
2×10^5	3.52	2.164± 0.185	4.83	4.308-5.365	3.54	1.904± 0.182	5.87	3.267-5.191	3.06	2.310± 0.294	6.93	5.887-8.299
2×10^4	3.60	1.700± 0.179	6.62	5.814-7.631	3.32	1.943± 0.193	7.34	7.926-11.49			na	

na LT_{50} is not available since mean % mortality was below 50%

TABLE 5
Mean percent mortality for varying treatments of *Paecilomyces fumosoroseus* on second instar larvae of *Crocidolomia binotalis*

Treatments [a]	Mean % mortality
1. Conidia from PDA in Vesawit® oil	88.5 a
2. Conidia from PDA only	78.0 ab
3. Conidia from rice flour	76.0 b
4. Conidia from PDA in kaolin	75.0 b
5. Control (Tween 80)	15.5 c
LSD	13.48
MSE	403.5
CV	21.97

Means followed by the same letter are not significantly different at P=0.05 as determined by 2-way ANOVA and LSD.
Analysis was performed on Arc Sine $\sqrt{x}$ values.
[a] sterile aqueous Tween 80 (0.05%) was the spray carrier.

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The Influence of Seasonal Variations on Yield Components of Sunflower

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Keywords: ***Helianthus annus*, head diameter, yield components, seasonal variation, non-conventional**

ABSTRAK

Kelebihan upaya penyesuaian tumbuhan dan kepelbagaian cuaca di Pakistan akan memungkinkan pertumbuhan dua jenis bunga matahari dalam setahun. Kajian lapangan pada musim bunga dan musim luruh dijalankan di University of Arid Agriculture, Rawalpindi, Pakistan untuk menilai pengaruh variasi bermusim ke atas hasil bunga matahari dan komponennya. Empat kacukan pokok bunga matahari ditanam dalam blok rawak dengan tiga sampel. Dua barisan tengah sampel tersebut dipetik untuk mengukur hasil serta komponennya. Ia menunjukkan saiz pucuk pada musim bunga lebih lebar daripada tanaman pada musim luruh dan dianggap sebagai keputusan keseluruhan bagi struktur tumbuhan, kepanjangan kitaran hayat tumbuhan, pertumbuhan perlahan dalam darjah hari-hari terkumpul yang lebih baik. Tidak seperti pucuk tersebut, ribuan benih tanaman yang ditanam pada musim luruh lebih banyak ditemui berbanding tanaman pada musim bunga. Kekurangan benih tanaman pada musim bunga mungkin disebabkan oleh persaingan dalam penyerapan yang mengakibatkan banyak benih tidak mendapat cukup khasiat yang disebabkan oleh pengenapan awal. Walau bagaimanapun, hasil tanaman pada musim bunga melebihi dari hasil tanaman musim luruh. Ini menyebabkan penanaman pada musim bunga adalah lebih baik. Walau bagaimanapun, penanaman pada musim luruh boleh memberi penambahan dalam pengeluaran minyak bijirin.

ABSTRACT

The wider adaptability of the crop and wide range of climatic conditions of Pakistan make it possible to have two crops of sunflower in a year. Field experiments, one in spring and one in autumn were conducted at the University of Arid Agriculture, Rawalpindi, Pakistan to evaluate the influence of seasonal variation on yield and yield components of sunflower. Four sunflower hybrids were planted in randomized complete block design with three replications. Two central rows were harvested for the measurement of yield and yield components. It was observed that head size of spring crop was larger than autumn crop which was considered to be the result of overall better plant structure, length of crop life cycle, slow and gradual rise in cumulative degree days. Contrary to head size, thousand seed weight of autumn crop was found to be more than that of spring crop. Lesser seed weight of spring crop may be the result of competition for assimilates which left many seeds malnourished as larger head might have encouraged the initial setting of seeds. However, final yield of spring crop was greater than that of autumn crop. It led to the conclusion that having spring crop is the best option, however, autumn crop could be supplementary one to increase the production of oilseeds.

INTRODUCTION

The major sources of edible oil production in Pakistan are the conventional oilseed crops (cottonseed, rapeseed, mustard and sesame) and non-conventional oilseed crops (sunflower and soybean). Except for cotton, the rest of the traditional oilseeds are grown on marginal lands which is why the gap between consumption and local production is widening every year. Among the non-conventional oilseed crops, sunflower has the potential to narrow the existing gap between production and consumption of edible oil. Sunflower grown in the country has the potential to yield up to 3,000 kg ha^{-1}, however, average yield in Pakistan is 1400 kg ha^{-1} (Govt. of Pakistan 2001).

Though sunflower is a temperate zone crop, it can perform well under various climatic and soil conditions. The wider adaptability of the crop and wide range of climatic conditions of

Pakistan make it possible to have two crops of sunflower in a year. Amir and Khalifa (1991) concluded that sunflower can germinate and grow successfully across the wide range of climatic environments including hot tropical climates. Similarly, Khalifa *et al.* (2000) concluded that wide geographic, morphological and habitat wise diversity of sunflower extending from very hot to very cold areas might have developed the unique characteristics of sunflower tolerance to both low and high temperature and accounted for wide adaptation of the crop.

Experimental and farm research trials have indicated that sunflower can successfully be grown in two seasons (spring and autumn) in Pakistan due to its wide range of adaptability (Rana *et al.* 1991). In spring, sunflower is sown under the low temperatures of January and February. It grows vegetatively under the range of low to medium temperatures of February and March, before entering into the reproductive stage. The reproductive stage develops under the high temperature of May while it matures and is harvested under the high temperature of June/July. Contrary to spring, the autumn crop is sown at high temperatures and high humidity conditions of July-August. It germinates and grows vegetatively during the high to medium temperatures of August and September before entering into the reproductive stage. The reproductive phase of the autumn crop takes off at the medium temperature of October. It matures and is harvested under the low temperatures of November. So the two opposite sets of environmental conditions prevail from germination to maturity of the sunflower when it is grown in two seasons i.e. spring and autumn. The overall length of crop life cycle is affected accordingly. The germination and vegetative stage of spring crops takes a relatively longer time due to lower temperature as compared to autumn crops where germination and vegetative growth take place under high temperatures taking less time and completing their cycle very shortly.

Being grown in opposite environmental conditions, all phases are affected accordingly. The present study was contemplated to evaluate the seasonal variation effects on yield and yield components of sunflower hybrids.

MATERIALS AND METHODS

Field experiments were conducted at the University of Arid Agriculture, Rawalpindi during spring and autumn 2000 to quantify the effects of seasonal variations on yield and yield components of sunflower. The spring crop was sown on 23rd February while the autumn crop was sown on 18th August. Five sunflower hybrids viz. Parsun-1, Suncross-42, SMH-9706, SMH-9707 and XF-263, were sown in randomized complete block design with three replications. There were 4 rows of 5 m-length, 75 cm apart in each plot making a plot size of 5 m x 3 m. Plant to plant distance was maintained at 25 cm. A uniform dose of fertilizer @ 120 kg N and 60 kg P_2O_5 per hectare was applied in the form of Urea and DAP and mixed with soil during land preparation. Planting was done by dibbler placing 3-4 achenes per hill was maintained by manual thinning. Weeding and earthing up was done manually when needed.

Cumulative growing degree days was calculated by the equation of Dwyer and Stewart (1986).

$$CGDD = \sum_{t_1}^{t_2} [(T_{Max} + T_{Min}) / 2 - 10]$$

where $[(T_{Max} + T_{Min})/2 - 10] \geq 0$

$T_{Max} + T_{Min}$ are daily maximum and minimum air temperatures in degrees centigrade and t_1 and t_2 are the time intervals. The base temperature for sunflower was 8°C (Sadras and Hali 1988).

Two central rows from each crop were harvested on 16th June and 11th November, 2000 of spring and autumn, respectively. Ten heads were randomly selected for the measurement of head diameter. Head diameter was measured with measuring tape (Sublime Sports Ltd. Sialkot, Pakistan) and the average was calculated. All the heads were thrashed manually. Three lots of 1000 seeds were weighed with an analytical balance (Technio Instrument Ltd. UK) separately and yield was calculated on hectare basis. The data collected were subjected to statistical analysis appropriate to randomized complete block design by using microcomputer MSTAT separately for both the seasons (Freed and Eisensmith 1986). Duncan's Multiple Range Test (Duncan 1995) was used for separation of treatment means.

RESULTS AND DISCUSSION

All the hybrids produced the heads of different sizes in spring. Hybrid SMH-9707 produced the largest (17.63 cm) head while XF-263 produced the smallest (13.43cm) head (Table 1). Hybrid SMH-9707 was significantly different (p=0.5) from Parsun-1 and XF-263 while it was at par with the rest of the hybrids i.e. SMH-9706 and Suncross-42.

Head diameter of all the hybrids decreased in autumn as compared to the spring. However, variations in head diameter of the autumn crop were narrow. The largest head (15.58 cm) was produced by Suncross-42 while XF-263 again produced the smallest (10.37 cm) one. All the hybrids were significantly different from XF-263 while those were at par with each other.

Reduction in head size of the autumn crop varied from 3 to 22%. The minimum (3%) reduction was observed in suncross-42 while the maximum (22%) in XF-263. The next to minimum (9%) was recorded in SMH-9607, while it was 13% and 15% in SMH-9606 and Parsun-1, rrespectively.

The reduction of head diameter in all the hybrids may be the combined function of LAI, plant structure (plant height & dry matter), and environmental factors. The better plant structure (leaf area index, plant height & dry matter), of the spring crop might have encouraged the development of large sized heads. Ujjinaiah *et al.* (1987) found smaller heads from that of the autumn crop than that of the spring crop, while Ahmad (2001) reported the significant relationship of plant height and head diameter in spring (r2=0.62) and autumn (r2=0.90) respectively. Longer crop life cycle of the spring crop with more cumulative degree days might also have contributed into the development of larger heads. However, the autumn crop got a short period of time in the field and accumulated less number of degree days, so it developed the smaller heads.

Thousand seed weight (TSW) of all the hybrids varied in the spring crop. Hybrid Suncross-42 produced the maximum (36.43 g) TSW, which was significantly (p=0.05) different from XF-263 while it was at par with rest of the hybrids. Hybrid XF-263 produced the minimum (21.27 g) TSW (Table 1).

Thousand seed weight of the autumn crop increased as compared to that of the spring crop in all the hybrids. Contrary to other parameters, those showed a decline. Hybrid Suncross-42 produced the maximum (45.08 g) 1000-seed weight, which was significantly different from XF-263 while, it was at par with others. Hybrid XF-263 produced the minimum (27.81 g) TSW.

Increase in TSW of the autumn crop ranged from 8 to 31 percent. The minimum (8%) increase was recorded in Parsun-1 while maximum (31%) in XF-263. The increase of TSW in the rest of the hybrids was 17.9, 18.19 and 23.16% in SMH-9606, SMH-9607 and Suncross-42 respectively.

Relatively large heads of the spring crop encouraged the setting of more numbers of seeds per head and those required greater amount of assimilates which was not possible for the plant to supply, creating a competition for assimilates. The competition for assimilates and rapid rise in cumulative degree days (*Fig. 4*) at the time of seed development and maturation might have left many seeds malnourished resulting in lesser thousand seeds weight from that of the spring crop. Small heads of the

TABLE 1
Influence of seasonal variation on yield and yield components of sunflower

Hybrids	Parameters					
	Head diameter (cm)		TSW (g)		Seed yield (kg ha-1)	
	Seasons		Seasons		Seasons	
	Spring	Autumn	Spring	Autumn	Spring	Autumn
PARSUN-1	15.83 b	13.42 a	35.58 a	38.53 a	1757 b	1628 b
SMH-9706	16.10 ab	14.64 a	35.73 a	42.33 a	2122 a	1631 b
SMH-9707	17.63 a	15.26 a	34.63 a	40.85 a	1738 b	1353 c
SUNCROSS-42	16.13 ab	15.58 a	36.43 a	45.08 a	2175 a	1827 a
XF-263	13.43 c	10.37 b	21.27 b	27.81 b	940 c	768 d

Treatment means followed by the same letter are not significantly different at P=0.05 (Duncan's Multiple Range Test).

autumn crop would have allowed less number of seeds to be produced in the limited space. Less number of seeds would have the adequate amount of assimilates for proper development and maturity. Teklewold *et al.* (2000) concluded that increase in head size simultaneously increases the husk percentage and incidence of empty seed increases, reducing the TSW. The significant linear relationship between head diameter and thousand seed weight (*Fig. 1*) support the view that thousand seed weight is directly dependent upon the head size.

The seed yield of sunflower sown in spring showed variation in all the hybrids. Hybrid Suncross-42 produced the highest (2175 kg/ha) seed yield, which was significantly (p=0.5) different from all other hybrids except SMH-9706. Hybrid XF-263 produced the lowest (940.30 kg/ha) yield (Table 1).

The seed yield of all the hybrids decreased in autumn as compared to spring. Hybrid Suncross-42 produced the maximum (1827 kg/ha) yield, while XF-263 gave the minimum (740 kg/ha) yield. The hybrid Suncross-42 was found to be significantly different from all the hybrids in contrast to the spring but it was at par with SMH-9706.

The overall reduction in yield ranged between 7 to 23%. Minimum (7%) reduction was recorded in Parsun-1 while maximum (23%) in SMH-9607. In other hybrids it was 22, 16 and 18% in SMH-9606, Suncross-42 and XF-263, respectively.

Seed yield is the combined function of different components. The comparison of both the parameters i.e. head size and thousand seed weight revealed that final yield dependence is more inclined towards thousands seed weight as compared to that of head size. Chaudhary and Anand (1993) observed the high positive direct influence of head diameter on seed yield. However, Patil *et al.* (1996) reported low positive direct effect for head diameter on seed yield. The significant linear relationship between thousand seed weight and final yield (*Fig. 2*) contradicts the earlier hypothesis. The non-significant relationship between head diameter and final seed yield (*Fig. 3*) support the earlier findings of Patil *et al.* (1996). The higher yield obtained from the spring crop confirms the earlier results of Habibullah *et al.* (1983), those reported that spring crop have the overall advantage of better plant structure, better environment conditions during crop growth

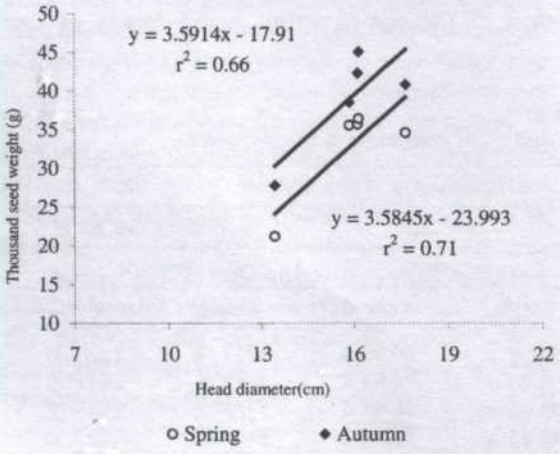

Fig. 1: Relationship between head diameter and thousand seed weight

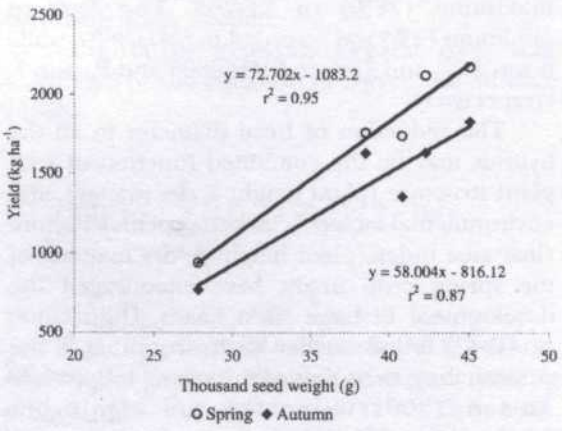

Fig. 2: Relationship between thousand seed weight and seed yield

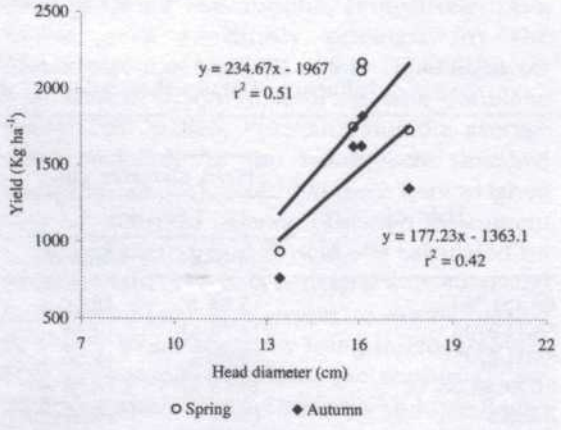

Fig. 3: Relationship between head diameter and seed yield

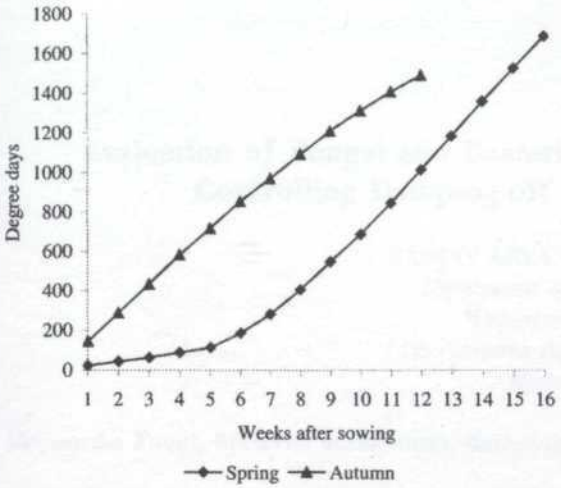

Fig. 4: Degree days accumulated during crop life cycle

period and maturity over the autumn crop. Better environmental conditions of the spring crop are the slow and gradual rise in cumulative growing degree days. Degree days accumulated during crop life cycle are presented in *Fig. 4*.

It can be concluded from the above findings that spring crops have the superiority over autumn in terms of yield. However, autumn crops could be fitted well in the present cropping system of Pakistan to oversoe the deficiency of edible oils.

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Evaluation of Fungal and Bacterial Antagonists' Seed Treatment for Controlling Damping-off Disease in Forest Nurseries

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ABSTRAK

Potensi kawalan biologi enam agen kawalan bio yang dilaporkan, Trichoderma viride, T. harzianum, Gliocladium virens, Bacillus sp., B. subtilis *dan* Pseudomonas fluorescens *ke atas* Rhizoctonia solani, R. bataticola, Fusarium oxysporum, F. moniliformae, F. solani *dan* Pythium aphanidermatum *menyebabkan lecuh pangkal dalam tapak semaian hutan dikaji secara in vitro dan disyaratkan di bawah rumah penyaring. Penilaian in vitro agen kawalan bio oleh kaedah penginokulatan duaan mendedahkan bahawa* P. fluorescens, Bacillus sp. *dan* T. viride *secara signifikannya menyekat pertumbuhan miselium kulat lecuh pangkal. Dalam percubaan berpasu, rawatan biji benih* T. viride *dan* P. fluorescens *membuktikan lebih kebaikan kepada agen kawalan bio kulat dan bakteria lain dalam mengurangkan insiden lecuh pangkal (sebelum dan selepas kemunculan) berbanding kawalan yang tidak dirawat*

ABSTRACT

Biological control potential of six well reported biocontrol agents, Trichomerma viride, T. harzianum, Gliocladium virens, Bacillus *sp.,* B. subtilis *and* Pseudomonas fluorescens *against* Rhizoctonia solani, R. bataticola, Fusarium oxysporum, F. moniliformae, F. solani *and* Phythium aphanidermatum *causing damping-off in forest nurseries was studied in vitro and under screen house conditions.* In vitro *evaluation of biocontrol agents by dual inoculation method revealed that* P. fluorescens, Bacillus *sp. and* T. viride *significantly inhibited mycelial growth of the damping-off fungi. In pot experiments, seed treatment of* T. viride *and* P. fluorescens *proved superior to other fungal and bacterial biocontrol agents in reducing damping-off (pre and post emergence) incidence compared to untreated controls.*

INTRODUCTION

Damping-off disease in forest nurseries is one of the economically most important diseases causing heavy losses in different parts of the world. Besides inflicting significant economical losses, the disease might disturb the whole forthcoming planting program. The disease complex is caused by species of *Pythium, Phytophthora, Rhizoctonia* and *Fusarium.* Many rhizospheric miroorganisms are known to be equipped with antagonistic potential against soil borne pathogens (Cook and Baker 1983; Elad *et al.* 1986). The damping-off fungal pathogens being predominantly soil and seed-borne, seed treatment with bio control agents can protect the seeds from such seed and soil borne damping-off pathogens. Therefore, the present investigations were carried out to explore the biocontrol potential of six well reported rhizospheric microorganisms, viz. *Trichoderma viride, T. harzium, Gliocladium virens, Bacillus subtilis, Bacillus* sp. and *Pseudomonas fluorescens* against the damping-off pathogens of forest nurseries under *in vitro* and *in vivo* conditions.

MATERIALS AND METHODS

The rhizospheric antagonistic microorganisms (fungi and bacteria) were isolated from nursery soil by dilution plate method (Johnson 1957) and identified using standard phytopathological techniques. The fungal bio control agents viz., *Trichoderma viride, T. harzium* and *Gliocladium*

virens were maintained on potato dextrose agar (PDA) medium while bacterial antagonists, *Bacillus subtilis, Bacillus* sp. and *P. fluorescens* were maintained on yeast peptone glucose agar (YPGA) medium and King's B medium, respectively.

For testing the bio control efficiency of the isolated fungal and bacterial antagonists, the dual inoculation method was followed. Four milimeter (diameter) round bits from actively growing cultures of antagonistic fungus/bacteria were inoculated on one side of Petri plate having PDA/YPGA media (King's B medium for *P. fluorescens)* and on the other side, an equal sized mycelium of the pathogenic fungus was inoculated. A control having only pathogenic fungal culture was paced on one edge of the Petri plate for comparison purposes. Radial growth of the pathogenic fungi was measured after seven days of incubation at 25±1°C and expressed as per cent inhibition after comparison with control following Vincent's (1947) formula-

$$1=100\left(\frac{C-T}{C}\right)$$

Where 1 = per cent inhibition
C = growth of pathogen in control
T = growth of pathogen in treatment

The experiment was run in triplicate and each replication had five Petri plates. The plates were arranged in completely randomized design.

For *in vivo* studies, the experiment was conducted in earthen pots (20 cm diameter) arranged in completely randomized design in a screen house. The experiment was triplicated and each replication had five pots with each pot having two plants. Sandy loam soil collected from the field was sterilized at 15 p.s.i. pressure for two hours and filled in pots. The damping-off pathogens, *Pythium aphanidermatum, Rhizoctonia solani, Rhizoctonia bataticola, Fusarium oxysporum* and *F. moniliformae* cultured on sand maize medium (Muthusamy 1972), were added to soil @1:20 (W/V) ration of pathogen and soil one week before sowing. The pots having seeds of *A. nilotica, A. lebbeck, D. sissoo & P. juliflora* sown in pathogen-infested soil and without any antogonist treatment served as control.

The antagonistic fungi, *Trichoderma viride, T. harzianum* and *Gliocladium virens* were cultured on 20 mL potato dextrose broth in 100 mL Erlenmeyer flasks maintained at 25±1°C for seven days. The resultant fungal mycelial mat and metabolites were mixed with talc powder @ 1:2 (v/w) of fungal mycelia and talc using a mixer. After shade drying, carboxymethyl cellulose was added @10 g per kg of talc powder formulation. These talc-based formulations of *Trichoderma* spp. & G. *virens* were then used for treating the seeds @ 4 g/kg seeds and yielded 1 x 10^7 and 1.4 x 10^7 cfu/g of talc on serial dilution.

The bacterial biocontrol agents, *Bacillus* sp. *B. sublitis* and *Pseudomonas fluorescens* were grown on nutrient agar and King's B medium broths respectively at 25±1°C for 72 hours in rotary incubator shaker. The resultant bacterial cultures were diluted with sterile distilled water to obtain a final concentration of 1 x 108 cfu/mL. The seeds of *acacia nilotica, albizia lebbeck, Dalbergia sissoo & Prosopis juliflora* were soaked in the bacterial suspension for 4 h and were sown immediately after in artificially-infested soil.

The data on pre and post emergence damping-off incidence was recorded 10 and 30 days after seed germintaion. The resultant data was analyzed by employing the Analysis of Variance (ANOVA) method.

RESULTS AND DISCUSSION

In vitro biological studies by dual inoculation method revealed significant inhibition in the mycelial growth of the damping-off fungi by all the fungal and bacterial antagonists. *Pseudomonas fluorescens* inhibited growth of *Rhozoctonia solani, R. bataticola, F. oxysporum, F. solani* and *Pythium apahaniermatum* compared significantly to controls (Table 1). Among the fungal antagonists *T. viride* exhibited the maximum antagonistic activity (75.2% mycelial inhibition) against *R. solani* and *P. aphanidermatum* (75.8% mycelial inhibition) while it was statistically at par with *G. virens* and *T. harzianum* against *F. oxysporum* and *Fsolani,* respectively. However, *T. harzianum* inhibited mycelial growth of *F. moniliformae* to the maximum extent (80.1% inhibition). Hadar *et al.* (1979), Chet and Baker (1981), Kim and Roh (1987) and Mathew and Gupta (1998) also reported antagonistic activity of *T. harzianum, T. viride* and *G. virens* against *R. solani.*

Results of the pot culture exeriment revealed that fungal antagonists *t. viride, T. harzianum* and *G. virens* when applied as seed coating on seeds of *A. nilotica, A. lebeck, D. sissoo* and *P. juliflora* significantly reduced pre and post emergence damping-off of seedlings in *R. solani*

TABLE 1
In vitro evaluation of fungal an bacterial antagonists against the damping-off fungi

Sr. No.	Antagonist	Mycelial growth inhibition (%)					
		Rhizoctonia solani	*Rhizoctonia bataticola*	*Fusarium oxysporum*	*Fusariam moniliformae*	*Fusariam solani*	*Pythium aphanidermatum*
1	*Trichoderma viride*	75.2	70.6	74.7	73.7	77.2	75.8
2	*Trichoderma harzianum*	72.4	76.3	65.9	80.1	76.7	55.7
3	*Gliocladium virens*	66.5	68.7	71.3	74.5	70.2	64.1
4	*Bacillus sp.*	71.2	70.2	65.7	82.8	74.6	73.6
5	*Bacillus subtilis*	75.6	84.6	80.9	76.6	73.9	63.8
6	*Pseudomonas*	80.0	85.2	83.4	75.4	88.2	75.6
	C. D. (P=0.05	**2.3**	**2.6**	**3.5**	**2.8**	**3.1**	**2.9**

TABLE 2
Evaluation of fungal antagonists against damping-off forest nurseries caused by *Rhizoctonia solani*

Sr. No.	Treatment	Pre-emergence Damping-off(%)	Post-emergence damping-off(%)
	Trichoderma viride		
1	*Acacia nilotica*	2.8	7.8
2	*Albizia lebbeck*	2.4	7.1
3	*Dalbergia sissoo*	3.3	6.5
4	*Prosopis juliflora*3.4	6.2	
	Trichoderma harzianum		
5	*Acacia nilotica*	3.2	10.1
6	*Albizia lebbeck*	4.2	8.2
7	*Dalbergia sissoo*	2.1	7.2
8	*Prosopis juliflora*2.2	6.7	
	Gliocladium virens		
9	*Acacia nilotica*	6.2	12.6
10	*Albizia lebbeck*	11.9	18.9
11	*Dalbergia sissoo*	12.0	14.0
12	*Prosopis juliflora*	12.6	14.6
13	*Acacia nilotica* (control)	14.3	13.6
14	*Albizia lebbeck* (control)	15.2	18.2
15	*Dalbergia sissoo* (control)	17.1	19.7
16	*Prosopis juliflora* (control)	13.2	22.4
	C. D. (P=0.05)	**1.4**	**0.6**

inoculated pot soil (Table 2). *D. sissoo* (control) plants inoculated with *R. solani* alone recorded as high as 17.1% pre-emergence and 19.7% post-emergence damping-off incidence.

A. lebbeck seeds treated with *T. viride* registered the lease (6.8%) pre emergence damping-off while seed treatment with *T. harzianum* recorded the lease (6.4%) post-emergence damping-off (Table 3) in *F. oxysporum* infested pots. Control pots of *A. nilotica, A. lebbeck D. sissoo* and *Juliflora* inoculated with *F. oxysporum* recorded the highest pre and post emergence damping-off incidence.

In pots inoculated with *P. aphanidermatum* and *R. bataticola* together, marked decrease in pre and post emergence damping-off over control was observed in *A. nilotica* seeds treated with *T. viride* (Table 4).

As low as 6.2% pre-emergence damping-off was observed in *P.juliflora* seeds treated with *T. viride* followed by *A. nilotica* (7.2%) and *a. lebbeck* (8.1%) (Table 5) in pots inoculated with *F. moniliformae* and *F.solani*. Seed treatment of *A. nilotica* and *P. juliflora* with *T. harzianum* recorded the minimum (7.8% & 8.5%, respectively) pre-emergence damping-off. *P. juliflora* seeds treated

TABLE 3
Evaluation of fungal antagonists against damping-off forest nurseries caused by *Fusarium oxysporum*

Sr. No.	Treatment	Pre-emergence Damping-off(%)	Post-emergence damping-off(%)
	Trichoderma viride		
1	*Acacia nilotica*	7.6	10.2
2	*Albizia lebbeck*	6.8	8.6
3	*Dalbergia sissoo*	8.9	8.5
4	*Prosopis juliflora*8.1	11.4	
	Trichoderma harzianum		
5	*Acacia nilotica*	7.7	8.3
6	*Albizia lebbeck*	7.5	6.4
7	*Dalbergia sissoo*	10.2	14.6
8	*Prosopis juliflora*7.5	10.2	
	Gliocladium virens		
9	*Acacia nilotica*	10.5	17.5
10	*Albizia lebbeck*	12.3	20.2
11	*Dalbergia sissoo*	14.5	13.3
12	*Prosopis juliflora*	13.2	12.2
13	*Acacia nilotica* (control)	21.8	26.6
14	*Albizia lebbeck* (control)	25.7	24.7
15	*Dalbergia sissoo* (control)	19.0	22.0
16	*Prosopis juliflora* (control)	27.6	41.3
	C. D. (P=0.05)	**1.6**	**1.1**

TABLE 4
Evaluation of fungal antagonists against damping-off forest nurseries caused by *Pythium aphanidermatum & Rhizoctonia bataticola*

Sr. No.	Treatment	Pre-emergence Damping-off(%)	Post-emergence damping-off(%)
	Trichoderma viride		
1	*Acacia nilotica*	3.2	9.3
2	*Albizia lebbeck*	8.8	10.2
3	*Dalbergia sissoo*	10.1	14.6
4	*Prosopis juliflora*	6.7	8.1
	Trichoderma harzianum		
5	*Acacia nilotica*	12.2	16.2
6	*Albizia lebbeck*	15.5	15.7
7	*Dalbergia sissoo*	11.6	18.8
8	*Prosopis juliflora*	10.2	10.9
	Gliocladium virens		
9	*Acacia nilotica*	4.8	8.6
10	*Albizia lebbeck*	8.2	10.4
11	*Dalbergia sissoo*	10.1	15.6
12	*Prosopis juliflora*	13.4	20.7
13	*Acacia nilotica* (control)	25.3	30.3
14	*Albizia lebbeck* (control)	20.8	25.6
15	*Dalbergia sissoo* (control)	41.2	38.5
16	*Prosopis juliflora* (control)	33.2	36.4
	C. D. (P=0.05)	**2.5**	**1.7**

with *G. virens* exhibited minimum (5.2%) damping-off incidence. The least post-emergence damping-off (7.7%) was registered in *A. nilotica* seeds treated with *T. viride.*

Seed bacterization with bacterial antagonists also led to a significant reduction in pre and post-emergence damping-off over *F.oxysporum* and *R. solani* inoculated controls (Table 6). Seed

TABLE 5
Evaluation of fungal antagonists against damping-off forest nurseries caused by *Fusarium moniliformae* and *Fusarium solani*

Sr. No.	Treatment	Pre-emergence Damping-off(%)	Post-emergence damping-off(%)
	Trichoderma viride		
1	*Acacia nilotica*	7.2	7.7
2	*Albizia lebbeck*	8.1	10.2
3	*Dalbergia sissoo*	9.1	12.2
4	*Prosopis juliflora*	6.2	15.3
	Trichoderma harzianum		
5	*Acacia nilotica*	7.8	14.6
6	*Albizia lebbeck*	10.1	18.2
7	*Dalbergia sissoo*	11.3	17.6
8	*Prosopis juliflora*	8.5	13.7
	Gliocladium virens		
9	*Acacia nilotica*	7.4	10.1
10	*Albizia lebbeck*	7.3	12.7
11	*Dalbergia sissoo*	8.2	13.5
12	*Prosopis juliflora*	5.2	19.6
13	*Acacia nilotica* (control)	18.2	25.7
14	*Albizia lebbeck* (control)	21.6	33.3
15	*Dalbergia sissoo* (control)	30.0	36.2
16	*Prosopis juliflora* (control)	41.3	48.7
	C. D. (P=0.05)	**0.9**	**1.3**

TABLE 6
Evaluation of bacterial antogonists against damping-off forest nurseries caused by *Fusarium moniliformae* and *Fusarium solani*

Sr. No.	Treatment	Pre-emergence damping-off(%)	Post-emergence damping-off(%)
	Trichoderma viride		
1	*Acacia nilotica*	10.2	13.3
2	*Albizia lebbeck*	8.9	17.8
3	*Dalbergia sissoo*	8.2	17.6
4	*Prosopis juliflora*	7.1	10.2
	Trichoderma harzianum		
5	*Acacia nilotica*	6.8	12.2
6	*Albizia lebbeck*	7.5	12.8
7	*Dalbergia sissoo*	13.1	18.5
8	*Prosopis juliflora*	6.2	13.3
	Gliocladium virens		
9	*Acacia nilotica*	10.2	14.6
10	*Albizia lebbeck*	4.2	12.5
11	*Dalbergia sissoo*	11.7	16.6
12	*Prosopis juliflora*	8.2	15.5
13	*Acacia nilotica* (control)	34.2	40.6
14	*Albizia lebbeck* (control)	40.3	46.2
15	*Dalbergia sissoo* (control)	42.6	47.1
16	*Prosopis juliflora* (control)	40.1	50.1
	C. D. (P=0.05)	**1.8**	**1.5**

bacterization of *A. lebbeck* with *P. fluorescens* cell suspension recorded the least (4.2%) pre-emergence damping-off followed by bacterization of *P. juliflora* and *A. nilotica* with *B. sublitis* (6.2 and 6.8%, respectively). *Bacillus* sp. seed bacterization of *P. juliflora* recorded the least post-emergence damping-off incidence.

Both *Trichoderma* spp.and *Gliocladium virens* are known to be potential antagonists of fungal plant pathogens (Papavizas 1985). Biological seed treatment has been found to be an attractive as well as an efficient method for introducing the antagonists into the soil-plant environment. Chao *et al.* (1986) and Dutta and Das (1999) reported significant decrease in stem rot of soybean by seed pelleting with spore suspension of *T. harzianum* along with methyl cellulose. Papavizas (1985) reported detailed account of biocontrol potential of *Trichoderma* and *Gliocladium* spp. Lumsden and Locke (1989) reported biological control of damping-off caused by *P. ultimum* and *R. solani* in soil-less mix. Effectiveness of seed coating with *Trichoderma* spp. spores for the control of *R. solani* in cotton has been reported by Elad *et al.* (1982). A similar observation was made by Clique and Scheffer (1996).

The use of bacterial antagonists in disease management has been well reported (Hubbard *et al.* 1983; Westeijin 1990; Merriman *et al.* 1974; Rao *et al.* 1999). Hamed (1999) reported antagonistic potential of *B. sublitis* against *P. ultimum* and *F. oxysporum* f. sp. *cucumerinum*. Manoranjitham *et al.* (2000) also confirmed the biocontrol efficiency of *T. viride* and *P. fluorescens* in controlling pre and post-emergence damping-off of tomato caused by *P. aphanidermatum* under pot culture experiments.

The present investigation has shown encouraging results in use of fungal bioconrol agents, *T. viride, T. harzianum* and *G. virens* and bacterial antagonists *Bacillus* sp., *B subtilis* and *P. fluorescens* as seed pelleting agent for the successful control of damping–off of forest nurseries and may be exploited for evolving eco-friendly disease management strategies.

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Variation of Cultivated Mungbean and Wild *Vigna* as Revealed by Random Amplified Polymorphic DNA Markers

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ABSTRAK

Variasi genetik sembilan varieti tamanan kacang hijau (Vigna radiata) *dan tiga populasi liar tempatan* Vigna (V. trinervia) *telah diselidik dalam kajian ini dengan menggunakan penanda RAPD. Sejumlah 65 fragmen DNA dengan saiz menjulat antara 173-1,500 bp telah dihasilkan daripada amplifikasi PCR menggunakan lima primer RAPD, di mana 95.38% fragmen adalah polimorfik. Analisis kelompok menunjukkan terdapat dua kumpulan utama, di mana kumpulan pertama terdiri daripada sembilan varieti* V. radiata *manakala kumpulan kedua terdiri daripada tiga populasi* V. trinervia. *Maklumat ini penting bagi pembiak baka tumbuhan agar dapat membuat keputusan yang tepat dalam usaha merangka program pembiakan atau kacukan bagi meningkatkan tanaman ini.*

ABSTRACT

The genetic variation of nine varieties of cultivated mungbean (Vigna radiata) *and three local populations of wild* Vigna (V. trinervia) *were evaluated in this study using RAPD markers. A total of 65 scorable DNA fragments ranging in size from 173–1,500 bp were obtained from the PCR amplification using five RAPD primers of which 95.38% were polymorphic. Cluster analysis revealed two major groups in which the first group consists of the nine varieties of* V. radiata, *while the second group includes the three populations of* V. trinervia. *This information is useful for plant breeders to make informed decisions in an effort to devise breeding or crossbreeding programmes for the development of the crop.*

INTRODUCTION

The Asian *Ceratotropis* is the most important subgenus under the genus *Vigna* and it comprises five major domesticated crops, the mungbean (*V. radiata* (L.) Wilczek), black gram (*V. mungo* (L.) Hepper), azuki bean (*V. angularis* (Willd.) Ohwi and Ohashi), rice bean (*V. umbellata* (Thunb.) Ohwi and Ohashi) and moth bean (*V. aconitifolia* (Jacq.) Maréchal (Baudoin and Maréchal 1988). Mungbean, in particular, is especially important as the major food crop under the subgenus *Ceratotropis* in developing countries in South and Southeast Asia where 80% of the world's mungbean are grown. It is rich in plant protein and is highly digestible, thus providing an alternative and inexpensive source of vegetable dietary protein. Since mungbean is a short duration legume (55 to 70 days) it is grown during the the inter-cropping season contributing to the farmers' income (Fernandez and Shanmugasundaram 1988).

However, mungbean is usually cultivated under low input conditions, thus improvement by breeding is important to increase yield. Breeding programs for mungbean improvements are specifically targeted at developing stable high yielding mungbean lines with resistance to disease and pest, uniform maturity, large seeded cultivars

and improved quality suitable for growth in the tropics and subtropics. As a result of breeding programs, hundreds of commercial varieties have been introduced with varying degrees of success (Shanmugasundaram 1988). To achieve varietal improvement effectively, it is important to incorporate wild stock into cultivated forms of mungbean to increase genetic variability in order to avoid any inbreeding depression and genetic bottlenecks in the future. Wild species generally exhibit a wide range of genetic diversity in terms of agronomic characteristics involving pest and disease resistance, maturity span, environmental adaptations and yield potential. Acknowledging the importance of wild plant stocks, several field surveys were conducted to collect wild samples of mungbean from various locations in Peninsular Malaysia, which resulted in *V. trinervia* being identified as the most dominant form of the local wild mungbean (Bujang *et al.* 1994).

Reports on the characterization of the mungbean genome using molecular markers are few. Recently Lakhanpaul *et al.* (2000) used Random Amplified Polymorphic DNA (RAPD) markers to characterize Indian mungbean varieties while Lambrides *et al.* (2000) used RAPD and Restriction Fragment Length Polymorphism (RFLP) markers to construct linkage maps for mungbean. RAPD markers have also been successfully used to characterize azuki bean (Yee *et al.* 1999; Xu *et al.* 2000) while linkage maps for crosses between the azuki bean and rice bean using RAPD and AFLP markers are now available (Kaga *et al.* 2000). In view of the success of using RAPD markers to characterize mungbean in other countries, we have used them to characterize the genetic relationships among several accessions of cultivated mungbean as well as local populations of wild *V. trinervia* from Peninsular Malaysia.

MATERIALS AND METHODS

Plant Material and Isolation of DNA

Seven varieties (V 1104, V 2273, V 2273-S, V 2773, V 3912, V 4717 and V 5973) and two varietal crosses (VC 3031A and VC 1131A) of domesticated *Vigna radiata* were obtained from the Asian Vegetable and Plant Research Center (AVRDC) while three populations of local wild mungbean *V. trinervia* designated as Bentong B13, Bentong B16 and 18 Bentong Std. respectively, were collected from Bentong district (Pahang state, Malaysia). Genomic DNA from seeds (30 seeds per variety/population) was extracted using a plant DNA extraction kit (Clontech Laboratories, USA). The extraction protocol followed that of the manufacturer's with minor modifications.

RAPD-PCR Amplification

Five arbitrary 10-mer primers OPA-01, OPA-02, OPA-3, OPA-05 and OPA-07 (Operon Technologies, USA), with 60-70% GC content, were used in this study (Table 1). The PCR reactions were carried out in a total volume of 10 µl containing 30 ng of genomic DNA, 2.5-4.5 mM $MgCl_2$, 10 mM Tris-HCl, 50 mM KCl, 0.1% Triton-X 100, 0.5 mM each of dATP, dCTP, dGTP and dTTP, 10 pmol of primer and 2.5-5 U of *Taq* DNA polymerase (Promega, USA). PCR amplifications were performed in a PTC-150 Thermal Cycler (MJ Research Inc., USA) with the following temperatures: a predenaturation for 3 min at 96 °C, followed by 40 cycles of 10 s denaturation at 96 °C, 10 s annealing at 36 °C, 30 s extension at 72 °C and concluded with a 5 min final extension at 72 °C. The PCR products were separated according to size on a 2% agarose gel and visualized over ultraviolet light after ethidium bromide staining.

TABLE 1
Primers used in RAPD analysis and the magnesium chloride and *Taq* DNA polymerase concentrations for PCR amplification

Primer	Sequence 5' to 3'	Magnesium Chloride Concentration	*Taq* DNA Polymerase Concentration
OPA-1	CAGGCCCTTC	3.5 mM	2.5 U
OPA-2	TGCCGAGCTG	3.0 mM	2.5 U
OPA-3	AGTCAGCCAC	2.5 mM	2.5 U
OPA-5	AGGGGTCTTG	4.5 mM	5.0 U
OPA-7	GAAACGGGTG	4.0 mM	5.0 U

RAPD Data Analysis

Only reproducible RAPD markers were included in the analysis. The DNA bands produced were scored as 1 for presence and 0 for absence. The data matrix was then analyzed using the RAPDistance Package (Armstrong *et al.* 1994). Pairwise genetic similarity (S) was calculated using Nei and Li's (1979) similarity index. The unweighted pair group method with arithmetic averaging clustering (UPGMA; Sneath and Sokal 1973) was then performed based on genetic distance (D=1-S) using the Numerical Taxonomy and Multivariate Analysis System (NTSYS-PC, Version 1.3) computer software program (Rohlf 1989).

RESULTS AND DISCUSSION

Identification of RAPD Bands

The five primers tested produced a total of 65 reproducible bands with a range in size of 173 to 1,500 bp. The number of amplified bands per primer varied from 10 to 18 (*Fig. 1*). The total number of polymorphic bands was 62 while the overall level of polymorphism calculated was 95.38% (Table 2). Only three bands, OPA1.7 (755 bp), OPA1.10 (445 bp) and OPA5.9 (390 bp) were monomorphic for all samples of the 12 accessions. There was however, one band, OPA3.4 (960 bp), which was shared exclusively by all the individual samples of *V. radiata* but was absent in all samples of *V. trinervia*. This band may prove useful as a diagnostic marker for differentiating between *V. radiata* and *V. trinervia*. It was found that variations within varieties were present but this was small and not reported here. However, the levels of variation within the wild *Vigna* populations were higher than the cultivated *Vigna*.

Genetic Distance and Cluster Analysis

Genetic distance between varieties ranged from 0.1425 to 0.4133. The highest genetic distance was observed between accessions Bentong B13 and V2273-S, while the lowest genetic distance was found between accessions VC 1131A and VC 3031A (Table 3). Cluster analysis based on UPGMA grouped the 12 accessions into two major clusters according to species. The first cluster consists of the nine varieties of *V. radiata* while the second cluster consists of the three populations of *V. trinervia* (*Fig. 2*).

In this study, the RAPD technique was found to be useful for identifying genetic variation in mungbean. The DNA fingerprinting pattern produced using five different RAPD primers enabled the twelve accessions to be distinguished from one another. The taxa studied show a high level of band sharing in the cultivated seeds. Any

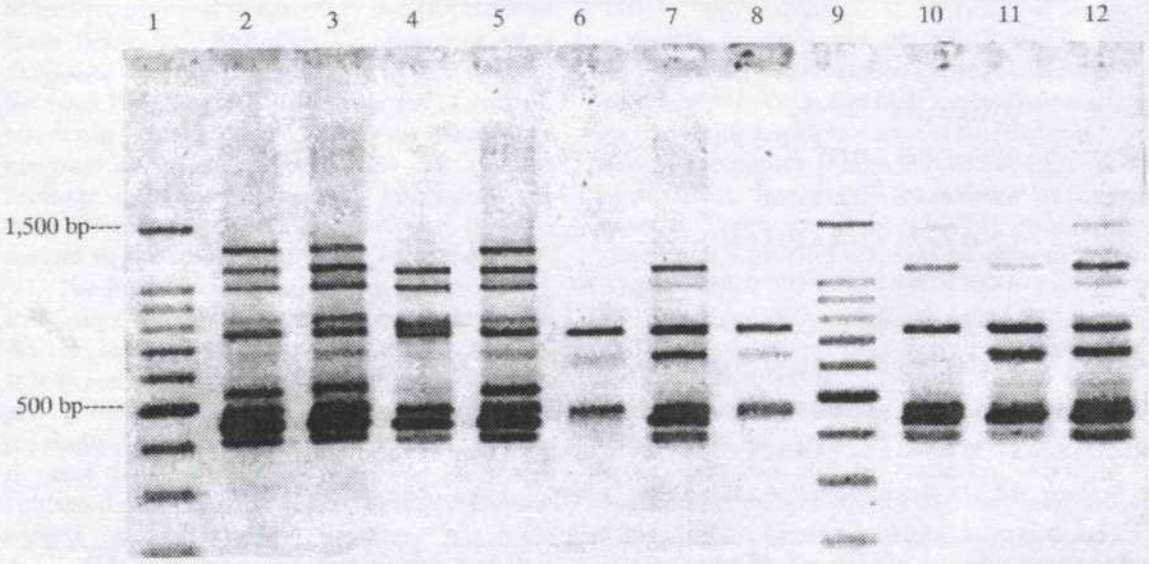

Fig. 1: PCR product generated by primer OPA-01 for the different varieties and populations. Lanes 1 and 9: 100 bp ladder. Lanes 2-5: Population 18 Bentong Std. Lanes 6-8: Varietal cross VC 1131A. Lane 10: Varietal cross VC3031A. Lanes 11 and 12: Variety V 3912

TABLE 2
RAPD profiles obtained from the five primers as observed in the *Vigna* accessions

	V2773	V2273-S	V2273	V1104	V3912	V4717	V5973	VC3031A	VC1131A	Bentong B13	Bentong B16	18 Bentong Std.
Number of Bands	52	53	51	56	51	54	55	56	49	51	51	52
Number of Polymorphic Bands	36	24	31	38	29	41	36	27	21	41	31	27
% of Polymorphic Bands	69.23	45.28	60.78	67.86	56.86	75.93	65.45	48.21	42.86	80.39	60.78	51.92
Total Number of Bands			65									
Total Number of Polymorphic			62									
Bands Total % Polymorphism			95.38									

TABLE 3
The pairwise genetic distance among the 12 accessions of *Vigna*

	V2773	V2273-S	V2273	V1104	V3912	V4717	V5973	VC3031A	VC1131A	Bentong B13	Bentong B16	18 Bentong Std.
V2773	—											
V2273-S	0.2132	—										
V2273	0.1923	0.1773	—									
V1104	0.2470	0.2163	0.2174	—								
V3912	0.2473	0.1854	0.2176	0.2365	—							
V4717	0.2741	0.2498	0.2474	0.2583	0.2561	—						
V5973	0.2398	0.1916	0.1986	0.2197	0.2069	0.2219	—					
VC3031A	0.2025	0.1586	0.1855	0.2130	0.1983	0.2440	0.1714	—				
VC1131A	0.2137	0.1617	0.1926	0.2029	0.1885	0.2522	0.1868	0.1425	—			
Bentong 13	0.3633	0.4133	0.3796	0.3811	0.3744	0.3950	0.3770	0.3645	0.3695	—		
Bentong B16	0.3392	0.4005	0.3632	0.3639	0.3538	0.3760	0.3699	0.3447	0.3551	0.2005	—	
18 Bentong Std.	0.3291	0.3792	0.3552	0.3539	0.3335	0.3622	0.3497	0.3269	0.3256	0.1914	0.1488	—

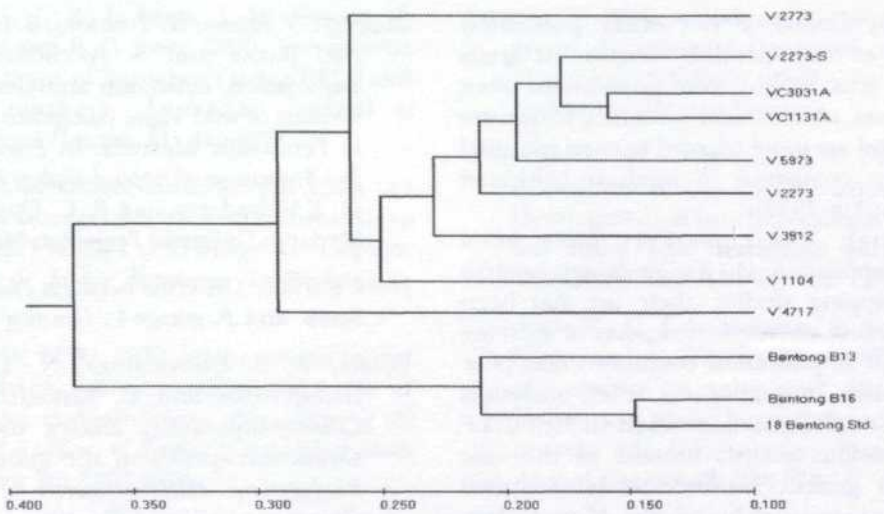

Fig. 2: UPGMA dendrogram showing the relationship among the 12 Vigna *accessions*

pair of individuals examined shared more than 70% of all the bands. Overall, the study showed that a high percentage of the RAPD markers were polymorphic when compared among the accessions. Based on the genetic distance values, the wild populations exhibited higher genetic distances compared to the domesticated varieties. This indicates a larger gene pool and higher levels of diversity in the wild populations. The Bentong B13 in particular, reveals higher genetic diversity compared to the other two populations from Bentong. Although the presence of a diagnostic marker that was able to differentiate between *V. radiata* and *V. trinervia* was identified, screening populations of *V. trinervia* from other geographical areas are needed to confirm this finding. Diagnostic markers will be useful since it is difficult to differentiate between the two species in the absence of seeds and flowers.

The RAPD-based dendrogram obtained from the cluster analysis is important because it reveals the relationships among the different accessions. It is expected that crosses between varieties that are genetically distant would demonstrate maximum heterosis or hybrid vigour. Despite the fact that there are numerous accessions of cultivated mungbean available commercially, the success rate of breeding programs has been poor. This may be partly due to the fact that different environmental conditions can affect the potential exploitation of the bean. To overcome this problem, local wild accessions are often selected and crossed with potential cultivar lines to confer some protection against pests.

A lot of focus has been given to *V. radiata* var. *sublobata* as a gene source for mungbean improvement since it is suspected to be the wild ancestor and putative progenitor of mungbean (Lukoki *et al.* 1980). The wild species are especially important since desirable genes from it could be incorporated into cultivated forms to enhance its economic value (Tomooka *et al.* 1992a). For example, *V. r. sublobata* showed complete resistance to the azuki weevil (Fujii and Miyazaki 1987). It also exhibits tolerance to Yellow Mosaic Virus, has high methionine content in the seeds, high photosynthetic efficiency and drought tolerance (Singh and Ahuja 1977; Babu *et al.* 1988). By using *V. r. sublobata* as a gene source, Tomooka *et al.* (1992a) successfully developed a bruchid resistant mungbean line in Thailand.

V. trinervia was initially classified and treated as *V. r. sublobata* until 1985 when it was recognized as a distinct species (Tateishi 1985). Crosses between *V. r. sublobata* and *V. trinervia* were successfully developed by Egawa *et al.* (1996). *V. r. sublobata* can also be easily crossed with *V. radiata* and no genetic barrier has been found (Dana 1966). Crosses between *V. radiata* and *V. trinervia* can be carried out and this may confer the same bruchid resistance to domesticated varieties. Other wild *Vigna* such as *V. minima* and *V. reflexo-pilosa*, which are also found in Malaysia,

may confer similar if not other potentially economic or commercially important traits (Tomooka *et al.* 1992b). Wild populations often contain genes, which confer resistance to diseases and pests and are more adapted to environmental stress when compared to modern cultivated species (Harlan 1976).

Although RAPD markers have been frequently applied in population genetic analysis and in mapping studies, their use has been limited because of frequent reports of extreme sensitivity to amplification conditions and poor reproducibility. In spite of this, RAPD analysis is favored over other genetic markers such as RFLP or microsatellite analysis because of the ease with which genetic information is identified without prior sequence knowledge. Nevertheless, if reproducibility of the markers is assured, the RAPD markers could then be further exploited to identify loci controlling disease resistance and traits of economic importance. This knowledge is imperative in order to improve breeding strategies in mungbean breeding programs.

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Infrared Absorption Spectra of a Series of 2, 6-diamino and 2-amino-6hydroxy-8-choroalkyl Purines

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Keywords: Infrared analysis, purines, synthesize, melting point, absorption spectra

ABSTRAK

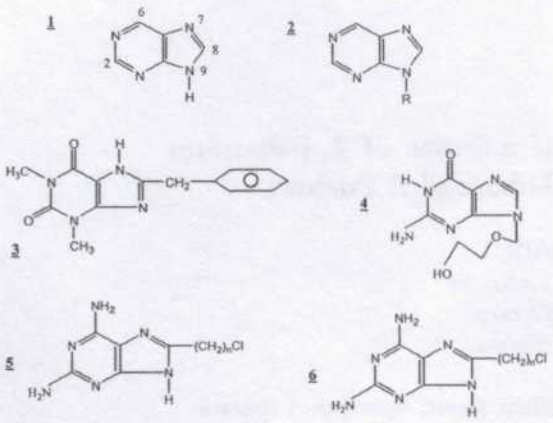
8 purina haloalki disintesiskan dan diuji dengan menggunakan analisis inframerah (IR). Sintesis dan analisis inframerah tersebut dicatatkan. Kajian ke atas purina tersebut merangkumi (i) 2,6-diamino-8-purina-Klorometil, (ii) 2,6-diamino-8-purina kloretil, (iii) 2,6-diamino-8-purina kloroprofil, (iv) 2-amino-6-hidroksil-8-purina korotil, (v) purina 2-amino-6-hidroksil 8 purina (3-kloroprofil). Penyerapan Imax ultra ungu terhadap purina tersebut diambil pada $_{P}^{H11}$ dan keputusannya dibentangkan. Tahap kecairan dan elemen analisisnya turut dibentangkan. Spektra inframerah (inframerah dalam KBr) C-8 diganti 2, 6-diamino; 5 or 2-amoni 6-purina hidroksi, 6 dibandingkan dengan 7 inframerah pteridin; 8; benzimidazola, 9; pirrols, 10; indole, 11 (Jadual I, II, III dan rajah 1, 2, 3, 4, 5 dan 6). Rajah 4 dibentangkan untuk dikaitkan purina baru tersebut dengan (i) hati semula jadi L. faktor casei, (A) (ii) hati sintetik L. faktor casei (asid Rasemik pteroylgutamik (C) (v) hati sintetik rasemik L. faktor casei, asid petrylgutamik rasemik (D) (iv) Hati rasemik semula jadi L. casei faktor asid pteroik (E).

ABSTRACT

8-haloalky purines were synthesized and infrared analysis (IR) carried out on them. The syntheses as well as the IR analyses are reported. The purines synthesized and studied include (i) 2,6-diamino-8-Chloromethyl purines, (ii) 2,6-diamino-8-chlorethyl purine, (iii) 2,6-diamino-8-chloropropyl purine, (iv) 2-amino-6-hydroxyl-8-chloroethyl purine, (v) 2-amino-6-hydroxyl 8-(3-chloropropyl) purine. The ultra violet UV lmax absorption of these purines were taken at $_{P}^{H11}$ and results are presented. The melting points and their elemental analyses are also presented. The IR (infrared in KBr) spectra of C-8 substituted 2, 6-diamino; 5 or 2-amino –6hydroxy purines, 6 are compared to the IR spectra of pteridines 7; 8; benzimidazole, 9; Pyrroles, 10; indole, 11 (Tables I, II, III and figures 1, 2, 3, 5 *and* 6*). Figure 4 is presented to relate these novel purines to (i) natural liver L. casei factor (A) (ii) synthetic liver L. cacei factor (Racemic pteroylgutamic acid (C) (v) Synthetic racemic liver L. casei factor, racemic pteroylglutamic acid (D) (iv) Natural racemic liver L. casei factor pteroic acid (E).*

INTRODUCTION

Purines 1 and derivatives 2, 3, and 4 are in use in medicine as drugs (Daly 1985). Adenosine 2 (a purine nucleoside) is known to demonstrate cardiovascular, nervous and endocrine activities. 8-benzyl theopylluine 3 has vassopresor actvity (Chemical and Eng. News 1986); Brigden *et al.* (1981), Maylor *et al.* (1961). Acycloguanosine, 4 (a purine nucleocide (Martins *et al.* 1985). 2, 6-diamino-8-chloroalkyl purines, 5 and 2-amino-6-hydroxy-8-chloroalkyl purines, 6 are a new series of purines related to the adenosines 2 and unlike adenosines have received limited investigations as therapeutic agents (Ejimadu 1988). It is expected that these new purines will physiologically mimic adenosines or analogs on account of their structural (or 2-amino –6) hydroxy groups of drugs. C_8 – haloakyl substituted 2, 6 –diamino (or 2-amino – 6 hydroxy) purines are good alkylating agents for N-nuceophiles (functional group modifying agents, (Ejimadu 1992) and may become good antineoplastiv agents, just like many anticancer alkylators e.g. mitomycins. Their N_9 – if



SYNTHESIS

14 n=2

15 n=3

5a: R1 = R2 = NH2 (n=1) **5b:** R1 = R2 = NH2 = (n =2)

5c: R1 = R2 = NH2 (n=3)

6a: R1 = OH; R2 = NH2 (n=2) **6b:** R1 = OH; R2 = NH2 (n=2)

12: R1 = R2 = NH2 **13:** R1 = OH; R2 = NH2

14: n = 2 **15:** n = 3

glycosylated with N_9 sugar analogs 4 may confer anti viral activity on these new purines (Martins 1985; Watt 1990; Schaeffer *et al.* 1978).

It is therefore significant to present the Infrared spectra of these new purines 5. and 6 which are lacking in the literature.

MATERIALS AND METHODS

The melting points were taken on a melting point apparatus and were uncorrected. The ultra violet lmax values were obtained on Beckman DB-9 and infrared (IR) analyses of products were taken on a Nicolet model 700 FTIR interferometer and absorption frequencies reported in cm-1. Elemental analysis (C, H, N) was done by Atlantic Micro Laboratories Inc. Atlanta Georgia, USA.

The following reagents used for reactions were purchased from Aldrich Chemical Company.

a) 2, 6-triamino-4-hydroxy pyrimidine sulphate salt.
b) 2, 4, 5, 6-tretraamino pyrimidine sulphate salt.
c) 4-chlorobutyryl chloride
d) 3-chloropropionyl chloride

2, 6-diamino-8-chloromethyl purine 5, (a=1)

2, 4, 5, 6-tetra-amino pyrimidine sulphate salt 92. 1g,; 0.01 mole) solid was thoroughly mixed in a mortar with chloroacetic acid (4.7 g; 0.05 mole). The mix was taken in a 250 mL round bottom flask. A water aspirator was then attached to this flask (an arrangement to evacuate water produced by the reaction), so as to subject the reaction to some vacuum. The flask with its contents was heated for 2 hrs and allowed to cool. The reaction mixture was washed with diethyl ether (930 mL) for three consecutive times to remove excess (unreacted) chloroacetic acid. The residue was taken in 50 mL of water and filtered (while hot). Fine crystals were obtained on cooling the filtrate. The crystals weighed 0.72 g (32.2% yield).

UV λmax 290 nm at P^H11.

IR (Kbr) cm-1 3415, 3269, 3154, (-NH2, -NH) 3020, 2971, 2809 (-CH2-C1)

Elemental analysis

Calculated C, 30.71 H, 4.72 N, 35.81 C1, 15.11

Found C, 30.99 H, 4.92 N, 36.68 C1, 15.42

Molecular formula: $C_6H_7N_6C1.2H_2O$

2, 6-dimino-8-chloroethyl purines 5, (n=2)

2, 4, 5, 6-tetraamino Pyrimidine sulphate- (7 g; 0.08 mole) was dissolved in 2 M NaOH (100 mL) in a 250 mL round bottom flask and an undertermined quantity of ice chips added to the solution. The outside of the reaction flask was also surrounded with ice blocks.

3-chloropropionyl chloride (7 mL; 0.08 mole; 2-equivalents) was added to the flask through an injection needle in two disproportionate batches (4 mL followed by 3 mL later) and vigorously stirred (magnetic stirrer). The flask was stoppered and stirring continued until it became difficult to continue the stirring (because the reaction mixture was very syrupy). The reaction lasted for 20 min and was worked up by filtering with abuchner funnel attached to a powerful water aspirator (to provide sufficient suction pressure). The residue was dissolved in ammonium hydroxide and filtered (hot). A dry weight of crystal of 2.06 g, 20.0% yield. (oven dried at 100°C) was obtained. UV lmax 300nm at P^H.11.

IR KBr cm-1 3400, 3344, 3203, 3014 (-NH_2; - NH) 2956, 2886, 2745 (-CH_2- C1)
Elemental analysis:

Calculated	C, 33.81 H, 5.23 N, 33.70 C1, 14.28
Found	C, 33.59 H, 5.31 N, 33.61 C1, 14.20
Molecular formular	$C_7H_9N_6C1.2H_2O$

2,6-diamino-8-chloropropyl purine, 5, (n=3)

This compound was made in the same way as for 2, 6-diamino-8-chloro ethyl purines 5, 9n=2) using 2, 4, 5, 6-tetraamino pyrimidine sulphate salt. 96g; 0.023 mol) and 4-chlorobutyryl chloride (0.05 mole; 2 equivalents). A dry weight of 1.66g (31.3% yield) of expected product was obtained after crystallation (hot water).
UV lmax 292nm at pH11.

IR KBr cm-1 3492, 3386, 3344, 3154 (-NH2;-NH), 2985, 2942, 2816 (-CH2C1), Elemental analysis:

Calculated	C, 39.26 H, 5.32 N, 34.35 C1, 14.15 (a)
Found	C, 39.15 H, 5.36 n, 34.98 C1,. 1291 (b)

$\frac{C}{N}$ *ratio(a)* = 1.14

$\frac{C}{N}$ *ratio* (b) = 1.12

Molecular formula: $C_8H_{11}N_6C1.H_2O$

2-amino-6-hydroxyl-8-chloro ethyl purine 6, (n=2)

2, 5, 6-triamino-4-hydroxy pyrimidine sulphate salt **13** (6g, 0.025 mole) was dissolved in 2M HaOH (50 mL) in 100 mL round bottom flask (with some chips of ice inside and outside the flask as in the case for 2, 6-diamino-8-chloroethyl purine **5** (n=2)). 2-chloro proponyl chloride 14 (6.24 ml, 0.05 mole, 2-equivalents) was introduced into the flask and stirred. A similar work up procedure was followed as for 5b (n=2). The product obtained weighed 4.07 g (65.02% yield) after hot water recrystallisation **10**
UVlmax 292nm at pH11.

IR KBr cm^{-1} 3400, 3337, (-NH_2;-NH) 2858, 2745 (-CH_2-) 744 (-CH_2-C1) Elemental analysis:

Calculated	C, 33.67 H, 4.84 N, 28.05 C1, 14.23 9a0
Found	C, 33.34 H, 4.79 N, 27.66 C1, 13.66 (b)

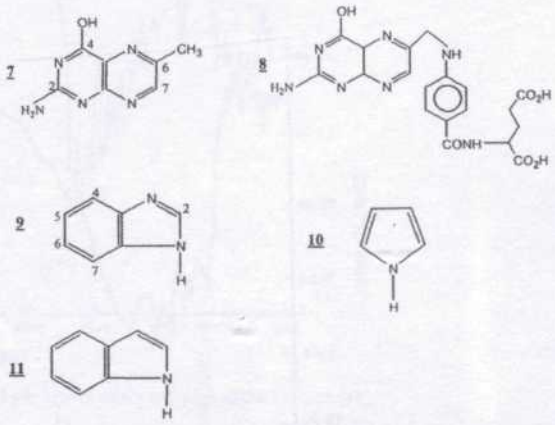
$\frac{C}{N}$ *ratio (a)* = 1.20

$\frac{C}{N}$ *ratio (b)* = 1.20

Molecular formula: $C_8H_{10}ON_5C1.2\ 1/2\ H_2O$

RESULTS AND DISCUSSION

The infrared absorption spectra of these new agents (*Figs. 2, 3, 5* and *6*) present definite regional difference with those of related benzimdazoles **9** but maintain some semblance with pteridine derivatives (Mowat *et al.* 1947; Waller 1948; Taylor and Dumas 1982) e.g. 2 – amino – 4 – hydroxy – 6 – methyl pteridines, 7, and folic acid, **8** (*Figs. 7* and *4*). Hitchings *et al.* (1949).



Hydrogen bonding between N_7 and N_9 positions of neighbouring purines has been put forward to rationalize the high melting point of purines (213°C) lin, T *et al.* (1984). The purines were therefore thought to exist as chains of molecules (in the solid phase) because of the extensive hydrogen bonding. Hydrogen bonding of the kind N-H ...N has been used to explain the absence of absorption in the normal stretching region (i.e. 3400 cm-1) in benzimdazoles, **9** (close relatives of the purines) in solid specimens (Morgan 1961) (*Fig. 8*). Hydrogen bonding apparently is not operative for these purines in KBr (Potassium bromide).

DISCUSSION

The infrared spectra and bands of these novel compounds (Tables 1, 2, 3 and *Figs. 1, 2, 3, 5* and *6*) present clear pictures of the N-H stretching region and other regions of the

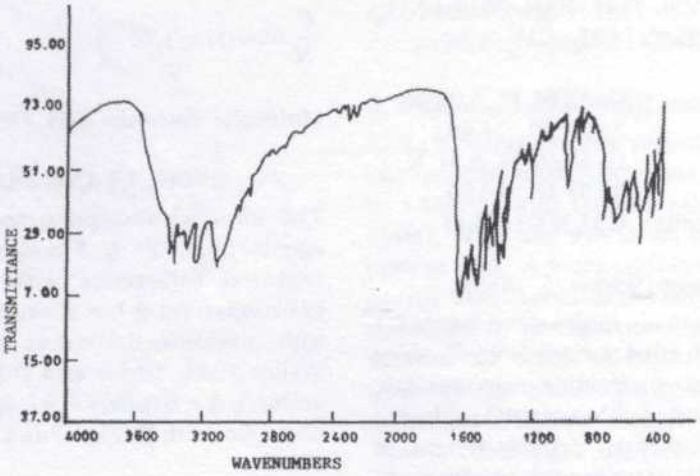


Fig. 1: 1R-absorption spectra of 2,6 – diamino–8–chloromethyl purine

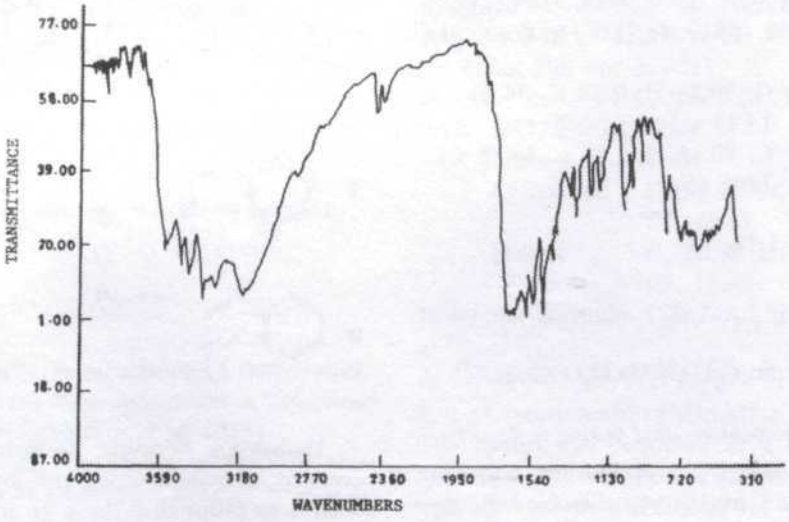


Fig. 2: 1R-absorption spectra of 2,6 – diamino–8–chloroethyl purine

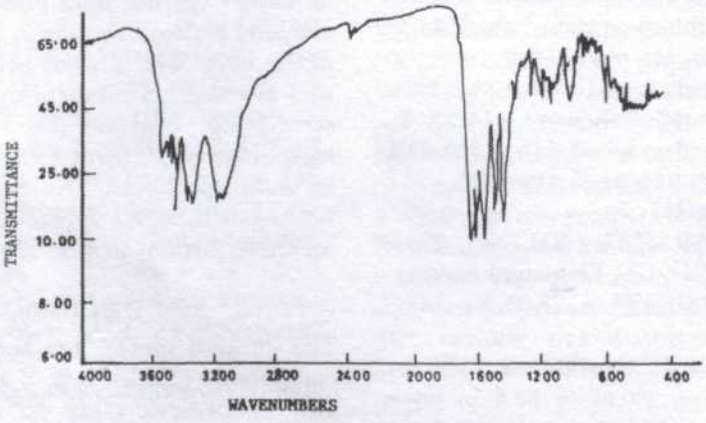


Fig. 3: 1R-absorption spectra of 2,6 – diamino–8–chloropropyl purine

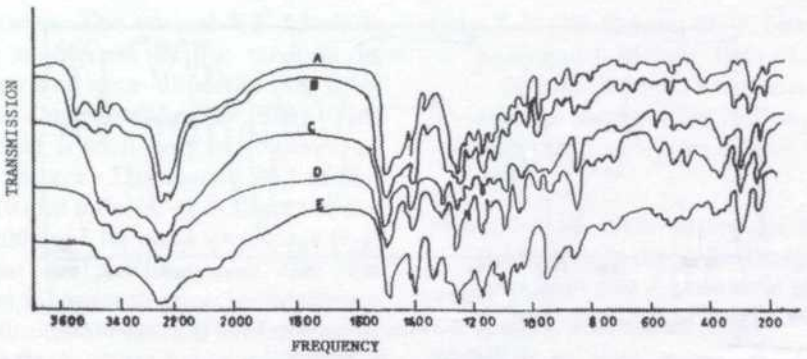


Fig. 4: Infrared absorption Spectra A natural Liver L. Casei factor B. Synthetic Liver L. Casei factor or (pteroyglutamic acid) C. Synthetic racemic Liver L. Casei factor (racemic pteroylglutamic acid); D. natural racemic Liver L. Casei factor E. pteroic acid. (II)

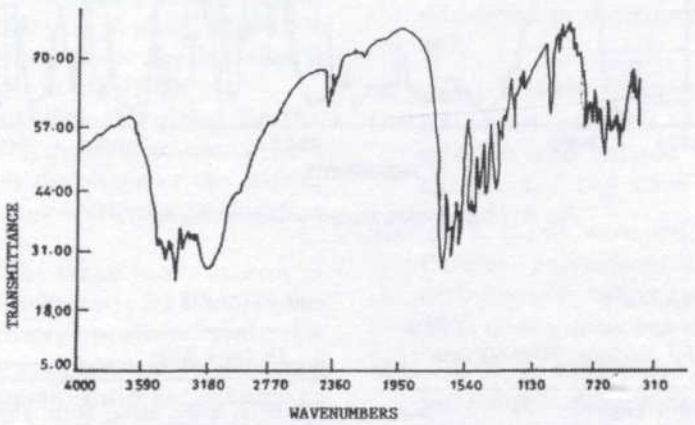


Fig. 5: IR-absorption spectra of 2–amino–6–hydroxy–8–chloroethyl purine (Guanine)

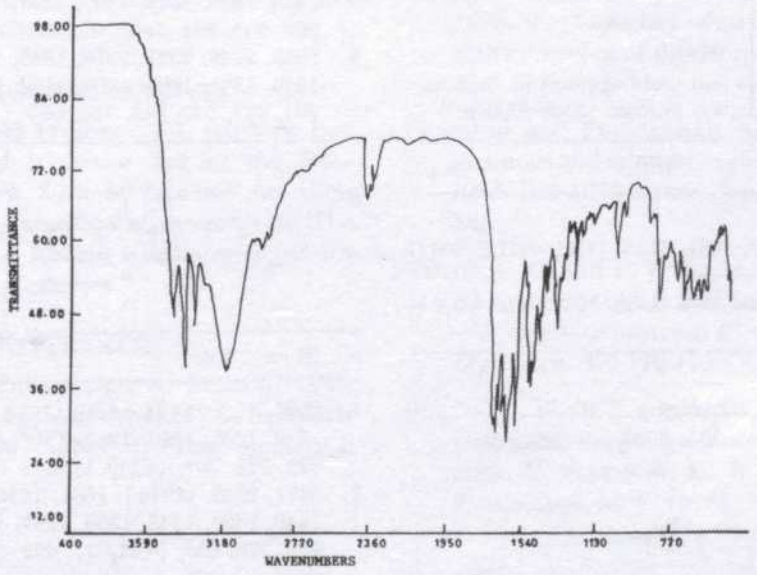


Fig. 6: IR-absorption spectra of 2–amino–6–hydroxy–8–chloroprophyl purine (Guanine)

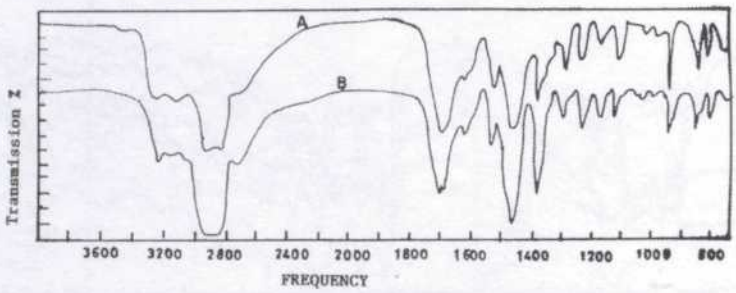


Fig. 7: IR-absorption spectra of 2–amino–4–hydroxy–6–methyl pteridine (10)
A: Natural B: Synthetic

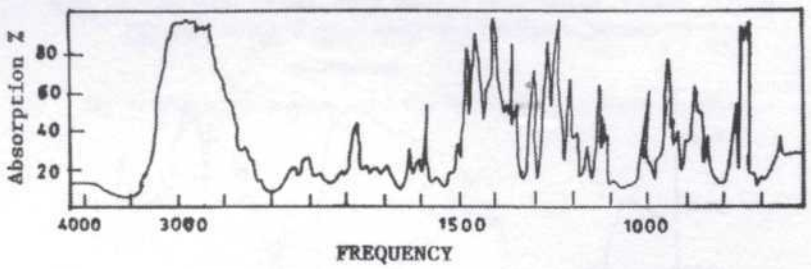


Fig. 8: IR-absorption spectra of Benzimidazole (7)

TABLE 1
IR absorption bands and melting point of 2, 6-diamino-8-chloroalkyl purines (3000-4000cm^{-1})

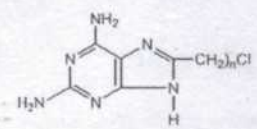
n	IR cm-1 (KBr)	m.p
1	3415, 3337, 3269, 3154, 3020 – NH2,-NH	300^{0}C
2	3400, 3344, 3202, 3147 – NH2,-NH	300^{0}C

TABLE 2
IR absorption bands and melting point of 2-amino-6-hydroxy-8-chloroalkys purines (3000-4000cm^{-1})

n	IR cm –1 (KBr)	m.p
1	- - -	- - -
2	3464, 3450,3339, 3281, 3125, - OH, -NH2, - NH	300 C
3	3450, 3393, 3380, 3374, 3168, 3006 -OH, -NH2, -NH	300 C

TABLE 3
Other bands (3000 – 600 cm-1) diagram

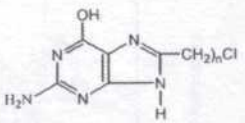
A



cont. TABLE 3

n	IR cm-1 KBr
1	2971, 2809, 1647, 1618, 1590, 1569, 1520, 1492, 1449, 1435, 1400, 1315, 1259, 1238, 175, 1020, 981, 963, 780, 710, 639
2	2956, 2886, 2745, 2661, 1660, 1618, 1561, 1498, 1449, 1399, 1378, 1308, 1272, 1202, 1160, 1019, 984, 934, 892, 786, 709, 666
3	2985, 2942, 2921, 2816, 1646, 1611, 1561, 1498, 1449, 1371, 1285, 1209, 1174, 1146, 1089, 1005, 984, 927, 899, 843, 786, 666

B



n	IR cm-1 KBr
1	2971, 2928, (-CH_2-),1681, 1632, 1575, 1484, 1449, 1406, 1315, 1237, 1216, 1153, 1012, 927, 892, 772, 702, (-CH_2Cl), 652
2	2971, 2935, (CH2-), 1681, 1632, 1575, 1484, 1449, 1408, 1348, 1300, 1237, 1209, 1106, 1012, 989, 778, 695, (-CH_2Cl), 652

different molecules. The normal N-H bands at 3400 cm^{-1} are unaffected by the medium in which these purines were dispersed for their infrared spectral determinations (i.e. **KBr**). This band (3400 cm-1) is seen only in solutions of benzimidazole spectra. The spectra 90.1 mole/ L) 9, pyrroles 10 and indoles, 11 as simple in the region 2400-3200 cm-1 for solid specimens (e.g. *Fig. 8*) (Morgan 1961). The use of KBr (for benzimidazoles) is known to cause no significant change in benzimidazole spectra. The spectra of these purines 5 9a, b, c) are being reported for the first time. It is interesting to note that the region of the spectra 3000-4000 cm-1 are similar in both the purines (e.g. 2 – amino-6 – hydroxy derivatives, *Figs. 5, 6*) and in the pteridine seriesw 9pteroyl glutamic acid – *Fig. 4*, and 2-amino – 4 – hydroxy-6 –methyl pteridine- *Fig. 7*; Waller *et al.* (1948); Wein Stock *et al.* (1970).

The replacement of 6- Oh group (in the pteridines) with – NH_2 group does not create a marked difference in the shape of the spectra (even though – OH and – NH_2 groups absorb at different frequencies).

The contrast in the shape of the spectra in this region (3200 to 4000 cm-1) for the diamino or amino – hydroxy purines and the benzimidazole is a consequence of replacement of pyrimidine component (fused to imidazole in benzimidazole – *Figs. 1, 2, 3, 5, 6* compared with *Fig. 8*).

The melting point for purines 5 and 6 are high and range from 259-300°C. The purines melt lower (140°C).

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Species Diversity of Macrobenthic Invertebrates in the Semenyih River, Selangor, Peninsular Malaysia

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ABSTRAK

Kajian ini dijalankan di sebuah sungai di Semenanjung Malaysia, Sungai Semenyih. Sungai ini menyokong pelbagai makrobentik invertebrata di mana stesyen yang berada di hulu sungai didominasi oleh Crustacea, Ephemeroptera, Odonata, Gastropoda, Trichoptera, Coleoptera dan Diptera sementara Hirudinea and Oligocheata adalah organisma-organisma bentik yang mendominasi stesyen di hilir sungai ini. Species-species 'caddisfly' seperti Microstenum similior *dan* Amphipsyche meridiana *didapati berpotensi menjadi penunjuk biologi kepada ekosistem yang bersih. Cacing yang berketahanan tinggi,* Limnodrilus hoffmeisteri, *ditemui sebagai species yang berdominasi di bahagian hilir sungai ini dan dianggap sebagai penunjuk biologi kepada ekosistem yang tercemar. Spesies diversiti yang rendah dan kewujudan spesies yang berketahanan tinggi menunjukkan bahawa hilir sungai ini telah tercemar disebabkan oleh degradasi kualiti air. Dengan pertambahan penduduk yang pesat, perindustrian and aktiviti pertanian di Malaysia telah menyebabkan masalah serius kepada kualiti air di kebanyakan sungai, makrobentik invertebrata yang ditemui di sepanjang sungai-sungai boleh digunakan sebagai penunjuk biologi kepada pengaruh ekotosikologi oleh pencemaran sungai di Malaysia.*

ABSTRACT

This study was carried out on one of the rivers in Peninsular Malaysia, the Semenyih River. The river supported diverse macrobenthic invertebrates in which the upstream sampling stations were dominated by Crustacea, Ephemeroptera, Odonata, Gastropoda, Trichoptera, Coleoptera and Diptera while Hirudinea and Oligocheata were the benthic organisms found predominant at downstream stations. Some caddisfly species such as Microstenum similior *and* Amphipsyche meridiana *were found to be potential bioindicators for a clean ecosystem. The resistant worm,* Limnodrilus hoffmeisteri, *was found to be the most dominant species at the downstream of the river and is considered a potential bioindicator for a polluted ecosystem. Low species diversity and occurrence of resistant worm species indicated that the downstream of the river deteriorated due to water quality degradation. As rapid increases in population growth, industrialization and agricultural activities in Malaysia have caused serious problems to the water quality of many rivers, the macrobenthic invertebrates found along the rivers can be used as bioindicators of the ecotoxicological effects of river pollution in Malaysia.*

INTRODUCTION

In recent years, numerous publications have critically reviewed the use of macrobenthic invertebrates as bioindicators as well as the appropriateness and shortcomings of certain indices. Macrobenthic invertebrates have been much used for biological monitoring of environmental quality in aquatic ecosystems in Europe and North America (Hellawell 1986; Metcalfe 1989; Rosenberg and Resh 1993; Pinel-Alloul *et al.* 1996). They have sedentary lifestyles that reflect local sediment conditions, life spans that integrate contaminant impacts over time, they live in the sediment and water interface where contaminants accumulate, and most importantly they show differential levels of tolerance to contaminants (Dauer 1993). Since changes in taxonomic richness and composition of macrobenthic invertebrates were considered sensitive tools for detecting alterations in aquatic ecosystems (Pinder *et al.* 1987), methods based on indicator species have been developed. Most

of the indices based on the presence-absence of pollution scored taxa focussed on the detection of organic pollution (Hilsenhoff 1987, 1988). Later, assessment of macrobenthic community structure was used as a measure of contamination by organic matter and pollutants (Jones *et al.* 1981; Johnson and Wiederholm 1989).

In Malaysia, the use of macrobenthic invertebrates in the study of river pollution has not been practised because the Department of the Environment (DOE 2001) has not included this methodology for the river pollution studies. For the year 2000 (DOE 2001), there were 34 river basins (28.3%) found to be clean, 71 (61.7%) were slightly polluted and 12 (10%) were polluted. These classifications were based on a river water quality index (WQI) which focussed on six parameters (ammoniacal nitrogen (NH_3-N), biochemical oxygen demand (BOD), chemical oxygen demand (COD), dissolved oxygen (DO), pH and suspended solids). This paper provides a list of macrobenthic invertebrates found in Semenyih River in Peninsular Malaysia which could be used to assess water quality.

MATERIALS AND METHODS

Study Area and Sampling

This study, which was carried out in June 1997 during the dry season, covered the riverine system of the Semenyih River in the district of Ulu Langat, Selangor, Peninsular Malaysia (*Figure 1*). The depth of the river ranged from 0.15 to 0.33 m. The Semenyih River (2° 54' N to 3° N and 101° 48'E to 101° 53'E) is a tributary of the Langat River. The Semenyih Dam, across the Semenyih River, is one of the major sources of water supply for the densely populated Klang Valley. Seven sampling stations were established (*Fig. 1*). The substrata of stations 1 to 3 consisted mostly of cobbles or pebbles and a modified

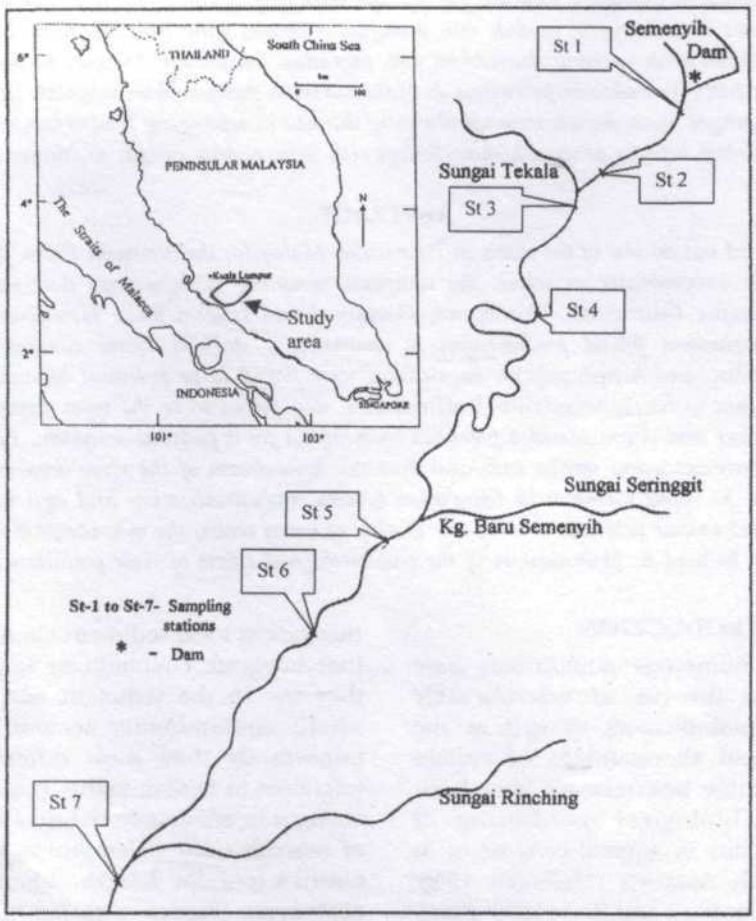

Fig. 1: Sampling stations of macrobenthic invertebrates from Semenyih River

Surber sampler (1256.8 cm^2) was used at these two sites. The bottom of Station 4 consisted mainly of sand while Stations 5 to 7 were characterized by silt or muddy sand, where a PVC tube (10.19 cm^2) was used for sampling in these stations. Three sites were located at each sampling station. At each sampling site, three replicates were sampled. Thus, a total of 63 samples were collected for this study.

Storage and Identification of Macrobenthic Invertebrates

The samples collected were sieved using an 80 mm mesh. After field collection, the samples were put into polyethylene bottles and preserved with 80% alcohol. In the laboratory, the macrobenthic invertebrates were sorted, enumerated and identified to the lowest possible taxon. The keys used were Gloyd and Wright (1966), Brinkhurst and Jamieson (1971), Merritt and Cummins (1978), Pennak (1978), McCafferty (1981), Scott (1983) and Ismail (1993). Data on individuals per taxon were used to calculate various biological parameters.

A biotic index, the Biological Monitoring Working Party (BMWP) system (Armitage *et al.* 1983) which depends on family level identification, was employed for pollution assessment. By using this system, families of macrobenthic invertebrates were allocated scores in the range of 1 (tolerant) to 10 (intolerant) according to their tolerance to organic pollution and the scores of all the families present were then summed up to obtain the BMWP score (Wright *et al.* 1993).

Statistical Analysis

Simple statistical analyses were done by using the Statistical Analysis System Version 6.0 (SAS 1987). At each station, parameters such as density (numbers/m^2), number of species (R0), Margalef's species richness index (R1), Menhinick's species richness index (R2), Shannon's diversity index (H'), Hill's diversity index (N1) and Hill's evenness index (E4) and Alatalo's evenness index (E5) (Ludwig and Reynolds 1988), were calculated using a statistical software that had been exclusively programmed in SAS by Rashid *et al.* (1998) to use count data (Ludwig and Reynolds 1988), that is, the number of individuals (N) of each species and the total number of individuals sampled.

RESULTS AND DISCUSSION

Density and Distribution of Macrobenthic Invertebrates

The densities of macrobenthos are shown in Table 1. High diversities were observed at upstream stations (Stations 1 to 3), but at downstream stations (Stations 4 to 7) the populations were characterized by the sand-bottomed substratum (Station 4) and the anoxic conditions of silt or mud (Stations 6 and 7) and only one or two species could survive (Thorne and Williams 1997). Station 7 was found to have only one single species, namely *Limnodrilus hoffmeisteri* (Oligochaeta) and this worm species is known to be able to tolerate unfavourable conditions such as low dissolved oxygen and high pollutant concentrations (Brinkhurst and Kennedy 1965; Brinkhurst 1967). For example, a high density of oligochaetes is a good indication of organic pollution (Slepukhina 1984; Lang 1985). Therefore, *L. hoffmeisteri* is a potential bioindicator for the polluted ecosystem of the river. On the other hand, clean areas with cobbles and pebbles were the major substrata composition at the upstream sampling stations (Stations 1 to 3) and Station 2 was recorded to have the highest number of species (R0= 18). Among the caddisfly species found, *Microstenum similior* and *Amphipsyche meridiana* were found to be potential bioindicators for the clean ecosystem since they could be found at the clean upstream stations (Stations 1 to 3) of Semenyih River. At the sandy substratum of Station 4, it was only dominated by a single bivalve species, *Corbicula javanica.*

Of all the taxa recorded, Hirudinea and Oligocheata were the organisms found predominately at downstream stations (Stations 5 to 7) with *L. hoffmeisteri* being the most abundant species. On the other hand, the other taxa (Crustacea, Ephemeroptera, Odonata, Gastropoda, Trichoptera, Coleoptera and Diptera) were recorded mainly at upstream sampling stations with Baetidae and *Filopaludina martensi martensi* showing the least number of individuals at Station 1. These species, although low in density, were still of importance in contributing toward the species richness. The taxa Leptophlebiidae, *Hydropsyche annulata, Polymorphanisus* species and Tipulidae were only found at Station 3. This occurrence might be due to drift from the Tekala River (*Fig. 1*) which

TABLE 1
Densities (numbers/m^2 ± standard error; N= 3) of macrobenthos in the 7 stations of the Semenyih River, Selangor, Peninsular Malaysia. St: Sampling station; NF= Not found

Taxa	St-1	St-2	St-3	St-4	St-5	St-6	St-7
Hirudinea							
Piscicola sp.	NF	15.91 ± 7.96	NF	NF	654 ± 327	NF	NF
Batrachobdella sp.	NF	5.30 ± 2.65	NF	NF	3598 ± 865	3598 ± 865	NF
Oligochaeta							
Limnodrilus hoffmeisteri	NF	NF	NF	NF	13413 ± 865	70985 ± 5675.3	30095 ± 3980
L. hoffmeisteri (Juvenile)	NF	NF	NF	NF	9814 ± 1499	109910 ± 4840.9	21263 ± 2852
Crustacea							
Penaeus sp.	5.30 ± 2.65	NF	NF	NF	NF	NF	NF
Ephemeroptera							
Caenidae	15.9 ± 4.60	5.30 ± 2.65	47.7 ± 9.19	NF	NF	NF	NF
Baetidae	2.65 ± 2.65	NF	61.0 ± 16.1	NF	NF	NF	NF
Heptageniidae	15.9 ± 4.60	5.30 ± 2.65	39.8 ± 4.59	NF	NF	NF	NF
Leptophlebiidae	NF	NF	5.30 ± 2.65	NF	NF	NF	NF
Odonata							
Leucorrhinia sp.	5.30 ± 2.65	13.3 ± 2.65	2.65 ± 2.65	NF	NF	NF	NF
Ophiogomphus sp.	NF	5.30 ± 2.65	5.30 ± 2.65	NF	NF	NF	NF
Gastropoda							
Filopaludina martensi martensi	2.65 ± 2.65	5.30 ± 2.65	NF	NF	NF	NF	NF
Melanoides turberculata	NF	5.30 ± 2.65	NF	NF	NF	NF	NF
Bivalvia							
Corbicula javanica	42.4 ± 7.02	21.2 ± 7.02	NF	1636 ± 327	NF	NF	NF
Trichoptera							
Macrostemum similior	1172 ± 375	504 ± 44.1	66.3 ± 11.6	NF	NF	NF	NF
Amphipsyche meridiana	76.9 ± 29.9	7.96 ± 4.59	NF	NF	NF	NF	NF
Hydropsyche annulata	NF	NF	10.6 ± 2.65	NF	NF	NF	NF
Polymorphanisus sp.	NF	NF	101 ± 37.1	NF	NF	NF	NF
Beraeidae	66.3 ± 7.02	39.8 ± 4.59	NF	NF	NF	NF	NF
Polycentropodidae	82.2 ± 29.9	7.96 ± 4.59	5.30 ± 2.65	NF	NF	NF	NF
Coleoptera							
Limnebiidae	5.30 ± 2.65	NF	NF	NF	NF	NF	NF
Diptera							
Tipulidae	NF	NF	34.5 ± 7.02	NF	NF	NF	NF
Simuliidae	39.8 ± 12.2	7.96 ± 4.59	NF	NF	NF	NF	NF
Chironomidae							
Pentaneura sp.	519.8 ± 78.3	286 ± 41.3	196 ± 45.3	NF	NF	NF	NF
Parachironomus sp.	3846 ± 987	1578 ± 636	321 ± 38.5	NF	NF	NF	NF
Ceratopogonidae	13.3 ± 5.30	13.3 ± 2.65	18.6 ± 7.02	NF	NF	NF	NF
Empididae	5.30 ± 2.65	5.30 ± 2.65	NF	NF	NF	NF	NF

was believed to have very high species richness due to its pristine condition.

Diversity Indices

The results of species richness, diversity and evenness indices of the macrobenthic invertebrates found in the Semenyih River are shown in Table 2. Diversity indices give better information about the environmental conditions under which the organisms live (Gaufin 1973; Hawkes 1979; Teles 1994) than a consideration of individual taxa alone.

A detailed analysis of the species richness in the macrobenthic communities at several sampling stations along the Semenyih River has led us to consider the factors responsible for the establishment and maintenance of the macrobenthic community structure in the river.

TABLE 2
Species richness, diversity and evenness indices for macrobenthic invertebrates found in the Semenyih River. St: Sampling station

Indices	St-1	St-2	St-3	St-4	St-5	St-6	St-7	Overall
Number of individuals (N)	17,848	7,640	2,760	4,905	82,404	544,455	154,017	814,029
Number of species (R0)	17	18	14	1	4	3	2	27
Richness								
Margalef (R1)	1.63	1.90	1.64	0.00	0.27	0.15	0.08	1.91
Menhinick (R2)	0.13	0.21	0.27	0.014	0.014	0.004	0.005	0.03
Diversity								
Shannon (H')	1.13	1.20	1.96	0.00	1.07	0.69	0.68	0.98
Hill (N1)	3.09	3.32	7.12	1.00	2.92	1.99	1.97	2.66
Evenness								
Hill (E4)	0.69	0.68	0.71	1.00	0.90	0.96	0.99	0.84
Alatalo (E5)	0.54	0.55	0.67	1	0.84	0.93	0.97	0.74
Biotic								
BMWP	72	75	66	0	5	5	1	
Status	(good)	(good)	(good)	(poor)	(poor)	(poor)	(poor)	

Types of substrata and pollution levels are two important factors that determine the distribution of macrobenthic invertebrates found in Semenyih River.

The richness indices (R1 and R2) varied differently with numbers of species and numbers of individuals. The Margalef's species richness index (R1) values were higher at the upstream (Stations 1 to 3) (R1= 1.63-1.90) than those (R1= 0.00-0.27) at the downstream (Stations 4 to 7). Similarly, Menhinick's species richness indices (R2) show higher values (R2= 0.13-0.27) at the upstream (Stations 1 to 3) than those (R2= 0.004-0.014) at the downstream (Stations 4 to 7). Although these richness values computed here are for illustrative purposes (Ludwig and Reynolds 1988) we can conclude that species richness generally declined from the upstream to the downstream stations.

The diversity indices are suitable for addressing any question that a heterogeneity index can answer (Peet 1974). These indices showed higher values of Shannon's diversity index (H'= 1.13-1.96) and Hill's diversity index (N1= 3.09-7.12) at the upstream (Stations 1 to 3) than those (H'= 0-1.07; N1= 1.00-2.92) at the downstream (Stations 4 to 7). These numbers indicate an increase in dominance of fewer species especially at the downstream stations.

For the evenness indices, the highest values were found at Station 4 since there was only one species found at all three sites of this station. The lowest values are computed for the upstream stations. For instance, lower values of Hill's evenness index (E4= 0.68-0.71) and Alatalo's evenness index (E5= 0.54-0.67) were found at upstream (Stations 1 to 3) than those (E4= 0.96-1.00; E5= 0.84-1.00) at the downstream (Stations 4 to 7). This indicates the evenness indices (E4 and E5) increased from the upstream to the downstream stations. The lower values of E4 and E5 at the upstream Station 1 seemed to be related to the co-dominance by two species out of the 17 present and their numbers of individuals were very high (1,172 and 3,846) when compared to the remaining 15 species (Tables 1 and 2). As for Station 7, E4 and E5 values were 0.99 and 0.97, respectively. At this station, only one species of Oligochaeta was found. As both the juvenile and adult worms were considered as *L. hoffmeisteri*, the sampling site thus was dominated by a single worm species. When all species in a sample were equally abundant, it seems reasonable that an evenness index should be maximum and this value decreases toward zero as the relative density of the species diverges away from evenness (Ludwig and Reynolds 1988). In addition, conditions of unstable substrata at downstream stations could be a contributing factor to the low species richness of the river ecosystem.

Another important characteristic of ecological communities is the spatial heterogeneity (Allen 1984) of macrobenthic invertebrates-namely random, clumped and uniform (Rosenberg and Resh 1993). The preferences of macrobenthic invertebrates to aggregate in the more favourable parts of the

river substrata had caused their distributions to be clumped. Furthermore, nature is multifactorial (Quinn and Dunham 1983) in which many interacting processes, both biotic and abiotic, may contribute to the existence of the pattern. Hewitt *et al.* (1997) revealed that macrobenthic invertebrates in a dynamic estuarine system exhibited stability of spatial pattern, even when they were undergoing movement and changes in mean density. The distribution of the macrobenthic invertebrates in the Semenyih River could possibly remain in the clumped pattern for a period of time unless the river is disturbed by natural and anthropogenic activities.

Based on the scores of BMWP, the three stations located at the upstream (Stations 1 to 3) were found to be 'good' status (66-75) while the stations found located at the downstream (Stations 4 to 7) had 'poor' status (0-5), as shown in Table 2. This is in agreement with the report by DOE (2001) that rivers in Malaysia were generally clean at the upstream while those located at the downstream were either slightly polluted or polluted due to urban wastes and agricultural activities. This has strengthened our ecotoxicological point of view that pollution level rather than types of substrate contributed to the distribution of macrobenthic invertebrates in Semenyih River.

CONCLUSION

The present study established a list of macrobenthic invertebrates in a typical river in Malaysia. The collection for certain macrobenthic species present particularly in polluted and non-polluted parts of a river indicated that they could be used as good bioindicators for river pollution studies. Since information on the life histories of macrobenthic invertebrates from Malaysia is lacking, our list of macrobenthic invertebrates found in the Semenyih River is useful and more taxonomic work should be done for the identification of the organisms at the generic level. Future studies should also include the physico-chemical parameters of rivers and relate them to the macrobenthic invertebrate data.

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Unpublished Materials (e.g. theses, reports & documents)
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