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Mechanism of Paraquat Resistance in *Crassocephalum crepidioides* (Benth.) S. Moore During Immature Stage

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Keywords: Resistance, herbicide, cuticle, epicuticular wax, *Crassocephalum crepidioides*

ABSTRAK

Mekanisme kerintangan Crassocephalum crepidioides *terhadap parakuat pada peringkat 6 helai daun dikaji. Parakuat yang diekstrak daripada tisu daun biotip rintang dan rentan tidak mengalami metabolisme. Maka perbezaan metabolisme tidak memainkan peranan dalam mekanisme kerintangan. Biotip rentan menyerap lebih 44% ^{14}C-parakuat berbanding biotip rintang. Namun, lebih daripada 98% ^{14}C-parakuat yang diserap oleh biotip rintang dan rentan berada pada daun yang telah diberikan perlakuan. Perbezaan penyerapan adalah berkolerasi dengan kuantiti lilin epikutikular dan kuantiti membran kutikel pada permukaan daun. Hasil kajian ini mencadangkan bahawa perbezaan penyerapan mungkin merupakan satu faktor yang menyebabkan kerintangan spesies rumpai ini terhadap parakuat.*

ABSTRACT

The mechanism of paraquat resistance in Crassocephalum crepidioides *at the six-leaf stage was investigated. The extractable paraquat was not metabolized by the leaf tissue in the resistant (R) and susceptible (S) biotypes. Therefore, differential metabolism does not appear to play a role in the mechanism of resistance. The S biotype absorbed 44% more ^{14}C-paraquat than the R biotype. However, more than 98% of the absorbed ^{14}C-paraquat remained on the treated leaf of both biotypes. The difference in absorption had a negative correlation with the amount of epicuticular wax as well as the cuticle of leaf surfaces in both biotypes. The results of this study suggest that differential absorption may be a factor that accounts for resistance to paraquat at the six-leaf stage.*

INTRODUCTION

Paraquat is a fast acting, contact herbicide (McEwen and Stepheson 1979) with no activity in the soil due to strong adsorption to soil particles (Kokana and Aylmore 1993). Paraquat is used for controlling a wide range of broad-leaved weeds, sedges and grasses in the rice and vegetable agro-ecosystem for weed clearance before embarking on a new growing season. After the first case of a paraquat-resistant weed (*Conyza bonariensis* (L.) Cronq.) was reported in Egypt in 1979 (Summer 1980), resistance to the herbicide paraquat has been documented for 25 weed species worldwide due to extensive and consecutive use of the herbicide (Heap 1997). However, Chun and Kim have found that *Rehmannia glutinosa* (Gaertn.) Libosch. ex Fisch. & Mey., a perennial herb belonging to the family Scrophulariaceae, is tolerant to paraquat although this plant has never been sprayed with paraquat (Chun and Kim 1992).

Fuerst and Vaughn (1990) have suggested several hypotheses on the mechanism of paraquat resistance. These include reduced cuticular penetration of paraquat due to differential structure and/or properties of leaf surface, differential metabolism of paraquat, altered site of action, paraquat sequestration from the site of action and enzymatic detoxification of paraquat-generated radical oxygen species. Some of these mechanisms contribute to paraquat resistance in weeds such as *Hordeum glaucum* Steud.

(Bishop *et al.* 1987), *Conyza bonariensis* (L.) Cronq. (Norman *et al.* 1994) and *Erigeron canadensis* (L.) Cronq. (Tanaka *et al.* 1986).

A paraquat-resistant (R) biotype of the annual broadleaf weed *Crassocephalum crepidioides* was first reported in 1990 in tomato fields near Tanah Rata, Cameron Highlands, Malaysia (Itoh *et al.* 1990). *Crassocephalum crepidioides* is also known as redflower ragleaf, a common weed in tomato and potato fields in Cameron Highlands. Paraquat had been applied twice a year at a concentration of 1 kg a.i. ha^{-1} to these fields for ten years. Preliminary studies had shown that the level of resistance in the R biotype of *C. crepidioides* was 100-fold higher than the susceptible (S) biotype through leaf disc tests involving various concentrations of paraquat (Itoh *et al.* 1990). It has been reported that differential translocation was the factor contributing to the mechanism of resistance in mature plants of *C. crepidioides* (Ismail *et al.* 2001). The objective of this study was to compare the mechanism of resistance between the R and S biotypes of *C. crepidioides* at the six-leaf stage.

MATERIALS AND METHODS

Plant Materials

Seeds of the R and S biotypes were collected from the Research Station of the Malaysian Agricultural Research and Development Institute (MARDI) and from farmers' fields in Brinchang, Cameron Highlands, Pahang, Malaysia, respectively. The two populations had been characterized as R and S to paraquat. The seeds were used to establish the six -leaf stage seedlings for the experiments.

Seeds of both biotypes were germinated separately in 36 x 26 x 5 cm plastic trays containing moist sand. After two weeks, seedlings of each biotype were transplanted into 7 cm diameter pots containing sand and grown in the greenhouse at 29±4°C, with a 12-h photoperiod and a light intensity of 800 mE m^{-2} sec^{-1}. Plants in each pot were watered twice daily and fertilized with 5 ml of half strength Hoagland's nutrient solution three times weekly (Hoagland and Arnon 1950).

Herbicide

The herbicide used in the study was paraquat dichloride (Gramoxone) containing 200 g active ingredient (a.i) per litre aqueous solution. Radiolabelled paraquat dichloride ([^{14}C] methyl; specific activity 1.74 x 10^3 kBq mg^{-1}) was purchased from Sigma Chemical Co., USA.

Metabolism Study

Four plants of the R and S biotypes at the six-leaf stage were sprayed with nonlabelled paraquat dichloride at 0.50 kg a.i.ha^{-1} using a backpack sprayer that delivered 450 litre ha^{-1} spray volume at 100 kPa pressure. The plants were then treated with 10 µl (1.48 kBq) radiolabeled paraquat in the central portion of the adaxial surface of the third leaf from the plant base. Control vials contained ^{14}C-paraquat with no plant tissue. The treated plants and the control vials were placed in a growth chamber at 25°C in darkness at 65% relative humidity for 12 h. The treated leaf was then excised and the cut petiole end was submerged into a 20-ml vial containing distilled water and then placed in the growth chamber together with the control vial for 12 h at 30°C and light intensity of 50 µE m^{-2} sec^{-1}.

After incubation, the surface herbicide residue was removed by rinsing with 5 ml of distilled water. The radioactive content of the rinse was measured by liquid scintillation spectrometry (LSS) using 10 ml of the aqueous-based cocktail NBCS 104 (Amersham). The tissue was weighed and frozen at -30 °C until extraction. The frozen tissue was then pulverized with a mortar and pestle using liquid nitrogen. Radiolabelled paraquat was extracted from the tissue with 5 N H_2SO_4 using the fresh weight to volume ratio of 1:4 and centrifuged at 12,000 *g* for 5 min (Chun *et al.* 1997). The resulting supernatant was concentrated with a rotary evaporator at 30°C and analyzed for paraquat and potential metabolites by thin-layer chromatography (TLC) in 5 M NH_4Cl developing system. The TLC plates used were 20 x 20 cm, precoated with silica gel 60 F-254 plates (Merck). Following development, the TLC plate was air-dried at 25°C and placed on X-ray film (Kodak) at -30°C for one month (Craft and Yamaguchi 1964). After the autoradiography study, the TLC plate was separated into 1.5-cm segments from the original to the solvent front, scraped and the radioactivity in each segment was quantified by liquid scintillation spectrometry (LSS) using 1 ml of distilled water and 10 ml of the aqueous-based cocktail NBCS 104 (Amersham). The average of counting efficiency was more than 90%.

Uptake and Translocation Studies

Plants from the two biotypes were sprayed with non-labelled paraquat dichloride at 0.50 kg a.i.ha^{-1} and treated with 10 ml (1.48 kBq) ^{14}C- paraquat dichloride on the third leaf as described previously. The plants were then kept in a growth chamber at 25°C in darkness and 65% relative humidity. The treated leaf was excised at different intervals viz. 0.75, 1.5, 3, 6, 9 and 12 h after incubation. The surface herbicide residue was removed with a 5 ml rinse of distilled water and the radioactive content of the rinse was measured by LSS as described. The excised leaf was then cut into small segments (15-30 mg) and each piece of the segments placed into a pre-weighed 20-ml vial containing 250 µl distilled water, 1 ml NSCII tissue solubilizer (Amersham), 30 µl glacial acetic acid and 300 ml 30% H_2O_2 and incubated for 10 h in a water bath at 50°C (Bishop *et al.* 1987). The vial was cooled at room temperature and 10 ml of the cocktail NBCS 204 was added. It was then kept in the dark for three days before the radioactivity was assessed using LSS. The absorbed paraquat was caculated from the leaf digest and expressed as ng mg^{-1} fresh weight of leaf tissue.

For the translocation study, plants from both biotypes were sprayed with nonlabelled paraquat dichloride and treated with ^{14}C-labelled paraquat dichloride on the third leaf as previously described. Plants were then placed in the growth chamber at 25/30°C night/day temperature and 65% relative humidity. The photoperiod was set at 12 h with a light intensity of 50 µE m^{-2} sec^{-1}. After 24 h incubation, the surface herbicide residue of the treated leaf was removed and the radioactive content of the rinse was determined as before. The whole plant was then exposed to an X-ray film at –30°C for two weeks. After the autoradiography study, the plant was sectioned into three parts: treated leaf, shoot above treated leaf and shoot below treated leaf (including root). The leaf tissue and stem were cut into small segments (15-30 mg) and placed into a preweighed 20-ml vial containing 250 ml distilled water, 1 ml NSCII tissue solubilizer (Amersham), 30 ml glacial acetic acid and 300 ml 30% H_2O_2 and incubated for 10 h in a water bath at 50°C as above. The radioactive content for each part of the plant was quantified using LSS as described before. Translocation of the compound was expressed as a percentage of the total amount absorbed.

Leaf Surface Characteristics Studies

a) Cuticle assay

The third leaves were excised from plants of both biotypes without paraquat application and the total leaf area was measured using a leaf area meter. In order to remove epicuticular wax, the leaves of each biotype were individually submerged in 20 ml chloroform for 5 min. The chloroform was then filtered through Whatman No. 1 filter paper into a pre-weighed, acid-washed and oven-dried beaker. Following the evaporation of chloroform in a fume hood, the wax-containing beaker was allowed to equilibrate to room temperature in a desiccator before being weighed for determining the amount of epicuticular wax (µg cm^{-2}). After the surface wax removal, the cuticle was isolated by digestion of the remaining parts of the leaf tissues in a solution consisting of zinc chloride and hydrochloric acid in a ratio of 2:3 at room temperature for 24 h (Holloway and Baker 1968). Following digestion, the cuticle was extracted from the remaining tissues with 100 ml of methanol. The final methanol rinse (containing cuticular components) was decanted into a pre-weighed, acid-washed and oven-dried beaker. The beaker was dried in a fume hood at room temperature (27°C) and placed in a desiccator prior to being weighed for determination of cuticle (µg cm^{-2}).

b) Type of stomata, trichome and stomata density determination

The third leaves were excised from untreated plants of the R and S biotypes and fixed individually in a mixture of glacial acetic acid and 70% ethanol (1:3) for 48 h. From the central portion of each leaf blade, a small segment (1.5 x 1.5 cm) was cut, submerged in basic fuschin stain (containing 10% KOH) and placed in an oven at 60°C for one week. The leaf segments were stained in Alcian Green and dehydrated in an ethanol series (50-100%, at 5 min intervals). After dehydration, the specimens were mounted in Euparal. Observations were made on the type of stomata, trichome density (no. cm^{-2}) and stomatal density of the adaxial surface (no. mm^{-2}) using a light microscope (Leitz Diaplan) at 100x magnification.

c) Trichome and epicuticular wax structures

The third leaves without paraquat application were excised from five plants of the R and S biotypes. The central portion of the leaf blades

was cut into small segments (0.5 x 0.5 cm) before fixation in 2% glutaraldehyde for 48 h. Samples were dehydrated in an ethanol series (50-100%, at 5 min intervals), critical-point dried, mounted on aluminium stubs and coated with gold. The structure of trichomes and epicuticular wax of the adaxial surface was observed under a scanning electron microscope (Phillips XL 30).

Statistical Analysis

Each of the above experiments was performed using a randomized complete block design with four replications except for trichome and epicuticular wax structure studies that had three replications. All data were subjected to ANOVA and the means were compared with the t-test at 5% level of significance. A correlation test was undertaken to show the relationship between absorption and leaf surface characteristics in both biotypes.

RESULTS AND DISCUSSION

Metabolism Study

The extraction of paraquat residues from leaf tissues of both biotypes was limited; radioactivity recovered in H_2SO_4 extract accounted for approximately 50 % of the absorbed radioactivity by both *C. crepidioides* biotypes whilst the rest of ^{14}C was unextractable. It is possible that the unextractable radiolabel may have covalently bonded to the leaf tissue (Fuerst and Vaughn 1990). The average of total recovery of the radiolabel, however, was more than 90% of that applied.

Autoradiographs of the standard ^{14}C-paraquat, together with the R and S plant leaf extracts indicated a single spot of radioactivity (R_f = 0.00-0.20). However, radioactivity recovered from acid extracts was not only detected at R_f values of 0.00-0.20 (98.5-99%), but a small amount of radioactivity also appeared at R_f values of 0.20-0.30 and 0.70-0.90 after the TLC plate was quantified using LSS (Table 1). This could be due to the lower sensitivity of the X-ray film to radioactivity as compared to LSS. The large amount (98.5-99%) of radioactivity recovered with R_f values of 0.00-0.20 in the TLC plate corresponded to authentic paraquat while the rest of the radioactivity at R_f values of 0.20-0.30 and 0.70-0.90 were unidentified compounds (Slade 1966). These results suggested that extractable paraquat was not metabolized in the leaf tissues of both biotypes and the differential metabolism could not account for the resistance mechanism of *C. crepidioides* to paraquat as reported in paraquat-resistant weeds like *Conyza bonariensis* (L.) Cronq. (Norman *et al.* 1993) and cultivars like *Lolium perenne* L. (Harvey *et al.* 1978).

Uptake and Translocation Studies

Differential translocation may be due to the difference in degradation at the site of absorption yielding some metabolites that could be translocated and other metabolites that could not be translocated. However, the results from the metabolism study showed that 94-99% of the radioactivity recovered from treated leaf tissue corresponded to authentic paraquat (Table 1). Hence, radioactivity observed in translocation as well as absorption studies can be assumed as authentic paraquat.

Fig. 1 shows the absorbed ^{14}C-paraquat in treated leaves of both biotypes at the six-leaf stage. There was significant difference in its uptake by the R and S biotypes at each time interval. At first, the uptake rate of the S biotype was slower compared to the R biotype. However, the uptake of labelled paraquat by the S biotype increased 3 h after treatment (HAT) and overtook the R biotype approximately 4 HAT. Subsequently, the total uptake by both biotypes plateaued at 6 HAT, indicating that absorption had reached a maximum. There are several barriers to herbicide absorption into the leaf tissue. The first barrier is the trichome, followed by the epicuticular wax and the cuticle. The susceptible biotype absorbed less paraquat compared to the R biotype 3 HAT because it was inhibited by higher trichome density relative to the R biotype (Table 3). Once paraquat passed through the trichomes, the uptake in the S biotype increased and overtook the R biotype approximately at 4 HAT due to a lower amount of epicuticular wax as well as cuticle compared to the R biotype (Table 3). The S biotype absorbed 44% more ^{14}C-paraquat than the R biotype at 6 HAT, suggesting lower total uptake in the R biotype as a factor contributing to paraquat resistance. This result is in agreement with a previous report on *Erigeron philadelphicus* and *E. canadensis* (Tanaka *et al.* 1986).

However, differential translocation does not appear to play a role in the mechanism of resistance because most of the absorbed ^{14}C-

TABLE 1
TLC analyses of radioactivity in acid extracts from leaf tissue of resistant and susceptible biotypes treated with [^{14}C]-paraquat dichloride for 24 h at six-leaf stage

	Distribution (R_f) of radioactivity (% of recovery)		
R_f value	0.00-0.20	0.20-030	0.70-0.90
Standard ^{14}C-paraquat	94 (0.5)	6 (0.4)	*-
R	98.5 (0.4)	-	1.5 (0.1)
S	99 (0.3)	-	1 (0.1)

SE of means are given in parentheses.
* Not detected.

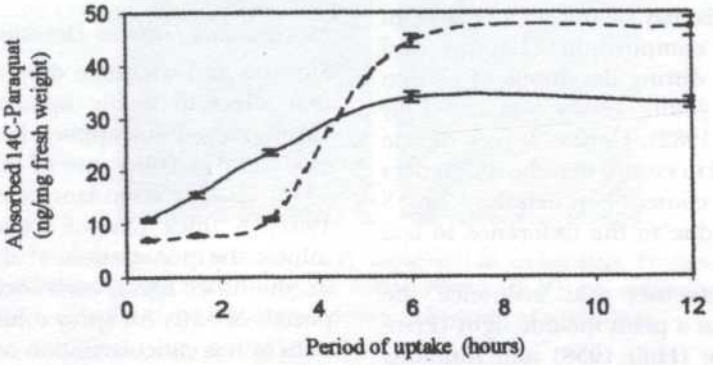

Fig.1: Absorption of ^{14}C-paraquat by leaves of resistant (_) and susceptible (....) biotypes at six leaf stage

TABLE 2
Distribution of total ^{14}C-paraquat recovered in plant leaf in resistant and susceptible biotypes 24 HAT at six-leaf stage

	Distribution of ^{14}C-paraquat (% of absorbed)		
^{14}C-Paraquat in plant sections	Treated leaf	Above treated leaf	Below treated leaf
R	98.99 a	0.78 a	0.23 a
S	98.72 a	1.00 a	0.27 a

Mean within columns with similar letters are not significant at the 5% level by T-test.

paraquat remained in the treated leaves of both biotypes 12 HAT, while less than 2% of the absorbed ^{14}C-paraquat was translocated out of the treated leaves at 24 HAT (Table 2). Qualitative detection of ^{14}C-paraquat distribution in both biotypes using autoradiography confirmed the quantitative results obtained with radioassays (*Fig. 1*). Autoradiographs showed that the absorbed ^{14}C-paraquat still remained in the treated leaf at the site of herbicide application and the adjacent cells in both biotypes.

Nevertheless, further studies are needed to examine the absorption as well as translocation patterns of the R and S biotypes at different temperature conditions and light intensities since the studies were carried out under a single

controlled environment only. Studies have shown that resistance to paraquat in *Hordeum glaucum* (Lasat *et al.* 1996) and *Hordeum leporinum* Link (Purba *et al.* 1995) is temperature-dependent.

Leaf Surface Characteristics Studies

Epicuticular wax and amount of cuticle, stomata and trichome densities in adaxial leaves of both biotypes were studied to observe the relationship between the leaf surface characteristics and paraquat uptake.

Cuticle Content

Several studies have shown that young leaves are more permeable than mature leaves (Leon and Bukovac 1978). This may be due to a change in epicuticular wax composition (Loomis and Schiefertein 1959) during development or that the cuticle from young leaves was not fully developed (Price 1982). Hence, leaves of the same age were used to ensure that the differences observed in cuticle content between the R and S biotypes were not due to the difference in leaf age.

Environment factors that influence the amount of cuticle in a plant include light (Price 1982), temperature (Hull 1958) and humidity (Daly 1964). Greenhouse-grown plants have been shown to have much thinner cuticle compared to field-grown plants (Hull 1958). However, the difference in cuticle content between the R and S biotypes in the present study was not due to environmental factors since both biotypes were planted under the same conditions.

Table 3 shows leaf surface characteristics of *C. crepidioides*. Epicuticular wax of the R biotype was approximately 150% more than the S biotype. The R biotype also had about 50% more cuticle than the S biotype. These results indicate that the third leaf was more difficult to penetrate as the R biotype absorbed 44% less ^{14}C-paraquat compared to the S biotype (*Fig. 1*). Correlation tests also showed that there is an inverse relationship in paraquat uptake with the content of epicuticular wax ($r = -0.95$) and the amount of cuticle ($r = -0.94$). The difference in absorption between the R and S biotypes is correlated to the amount of epicuticular wax as well as the cuticle of the leaf surface, which thereby played a role in the resistance of *C. crepidioides* to paraquat. Pereira *et al.* (1971) reported that nitrofen selectivity in cabbage was due to differences in the cuticular wax. The tolerant cultivar seemed to have thicker cuticle compared to the susceptible cultivar.

Stomata and Trichome Densities

Stomata and trichome densities are two factors that affect herbicide uptake. Guard cells can absorb more foliar-applied herbicides than other epidermal cells because of thinner cuticle (Hull 1970) and/or abundant ecotodesmata (Franke 1961). A thick mat of trichome is known to inhibit the penetration of the herbicide spray; on the other hand, each trichome may serve as portals of entry for spray solution due to thinner walls or less cuticularization near its base (Franke 1967). In this study, however, there was no correlation ($p>0.05$) between stomata, trichome density and paraquat absorption at 6 HAT although there was significant difference ($p>0.05$) in trichome density between the two biotypes (Table 3).

Stomata, Trichome and Epicuticular Wax Structures

Figs. 3, 4 and *5* show the structure of *stomata*, trichomes and epicuticular wax of *C. crepidioides* respectively. These leaf characteristics do not appear to play any role in the mechanism of paraquat resistance since both biotypes are

TABLE 3
Leaf surface characteristics of *C. crepidioides* at six-leaf stage

	R biotype	S biotype
Cuticle (mg cm^{-2})	27.8 a (0.4)	17.9 b (0.3)
Epicuticular wax (mg cm^{-2})	5.3 a (0.2)	2.1 b (0.1)
Stomata (no.mm^{-2})	13.6 a (0.7)	14.6 a (0.8)
Trichomes (no. cm^{-2})	22 a (5)	46 b (4)

Means within rows with similar letters are not significant at the 5% level by T-test. SE of means are given in parentheses.

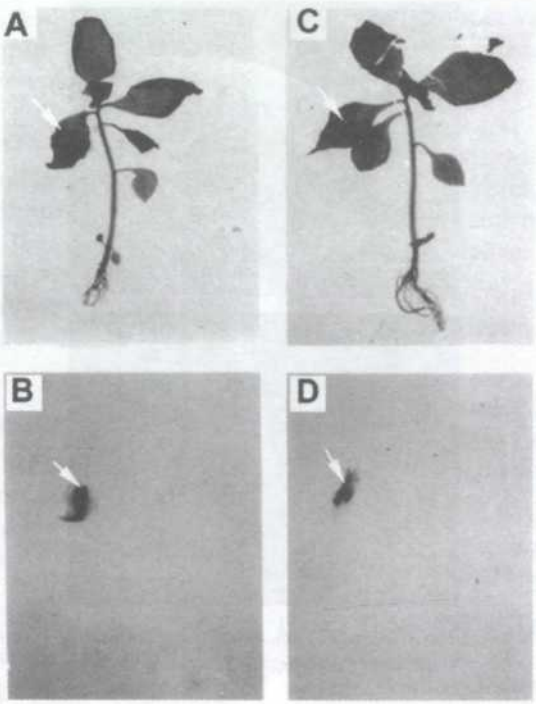

Fig. 2: Distribution of ^{14}C-paraquat in C. crepidioides at six-leaf stage. Photograph (A) and autoradiograph (B) of susceptible biotype; Photograph (C) and autoradiograph (D) of resistant biotype. Arrows indicate site of application

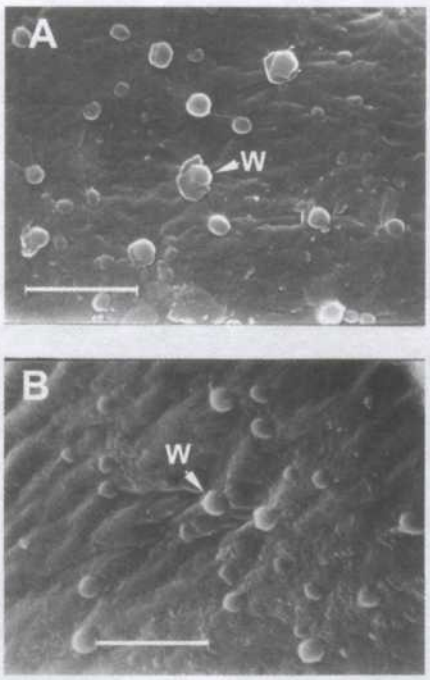

Fig. 3: Anomocytic stoma (S) on adaxial leaf surface of C. crepidioides A) Resistant biotype, B) Susceptible biotype

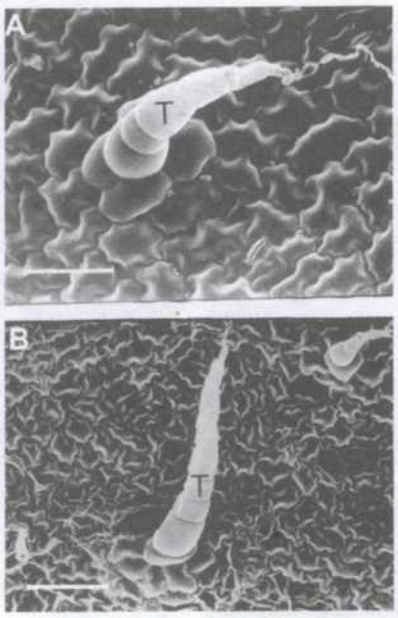

Fig. 4: Multicellular trichome (T) on adaxial leaf surface of C. crepidioides
A) Resistant biotype, B) Susceptible biotype

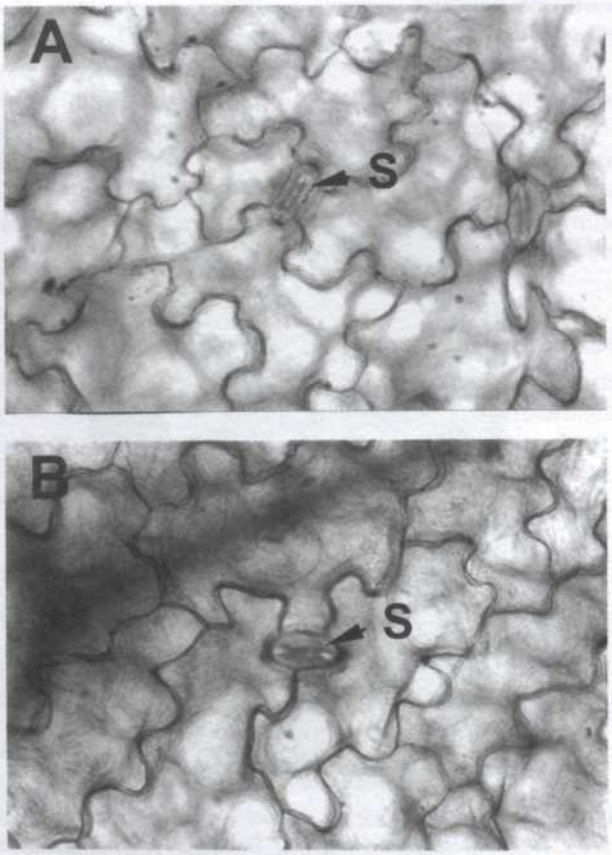

Fig. 5: Granular epicuticular wax (W) on adaxial leaf surface of C. crepidioides
A) Resistant biotype, B) Susceptible biotype

identical in these characters. Both biotypes have anomocytic stomata, simple mutlicellular trichomes as well as granular epicuticular wax.

CONCLUSION

It is suggested that in *C. crepidioides*, difference in absorption is a contributing factor for resistance to paraquat at the six-leaf stage. However, further studies are required to see whether resistance is still expressed in changing temperature conditions, light intensities and humidity.

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Intraspecific Polymorphism in *Mystus nemurus* (C&V) Detected by RAPD-PCR Fingerprinting

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Keywords: ***Mystus nemurus*, catfish, RAPD, PCR, Thailand**

ABSTRAK

Peringkat pembezaan sub-populasi genetik di kalangan sampel ikan baung, Mystus nemurus *(C&V) yang diperoleh daripada sebahagian kawasan, serta genetik populasi liar dan diternak dibandingkan. Aspek variasi genetik ikan yang dikumpul daripada lapan populasi di seluruh Thailand dan stok hatcheri ditentukan di peringkat molekul (DNA) dengan menggunakan teknik cap jari RAPD-PCR. Lima primer OPA-11, OPA-14, OPA-18, OPA-19 dan OPA-20 dipilih untuk mengamplifikasikan DNA. Ini menghasilkan 28 lokus polimorfik dalam 9 populasi yang dikaji. Jarak genetik (D) yang terbesar didapati antara populasi Chainat dan Suratthani dengan nilai 0.289, manakala jarak genetik terkecil didapati di antara pasangan populasi Songkhla dan stok hatcheri dengan nilai 0.087. Dendrogram menggambarkan perhubungan genetik di kalangan populasi M.* nemurus *yang digolongkan kepada empat kelompok mengikut kawasan asalnya.*

ABSTRACT

Yellow catfish, Mystus nemurus *(C&V), is becoming one of the major freshwater species farmed by aquaculturists in Southeast Asia. It was of interest to examine levels of genetic sub-population differentiation among samples of this species obtained from parts of its range, as well as to compare the genetics of wild and hatchery-bred fish. The genetic aspects of variation in the fish, which were collected from eight wild populations throughout Thailand and a hatchery stock, were determined at molecular (DNA) level using the technique of RAPD-PCR fingerprinting. Five arbitrary primers namely OPA-11, OPA-14, OPA-18, OPA-19 and OPA-20 were chosen to amplify products, which showed 28 polymorphic loci in 9 populations. The highest genetic distance (D) was found between Chainat and Suratthani populations with the value of 0.289, while the lowest was found in Songkhla population and hatchery stock with the value of 0.087. The dendrogram depicts the genetic relationship among populations of* M. nemurus, *which are grouped into four clusters according to their regional areas.*

INTRODUCTION

Genetic markers have been widely used for estimating genetic variation in breeding programs for many organisms. There are many methods of revealing genetic differentiation such as protein electrophoresis, DNA fingerprinting and others. Although protein electrophoresis has provided a wealth of genetic data to date, it has certain limitations. The resolution of protein electrophoresis is always inadequate for detecting differences between populations or individuals (Grant and Utter 1980). Thus, the potential amount of genetic variation detectable by DNA methods vastly exceeds the amount detectable by protein methods because DNA sequences are assayed more directly (Park and Moran 1994). There are various techniques used for the analysis of DNA level variation such as Random Amplified

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Polymorphic DNA (RAPD), Amplified Fragment Length Polymorphism (AFLP), Restriction Fragment Length Polymorphism (RFLP) and microsatellites. In 1990, a method of revealing DNA-based polymorphisms named Random Amplified Polymorphic DNA (RAPD) fingerprinting, which involves PCR amplification of genomic DNA using a single primer of arbitrary nucleotide sequence, was reported (Welsh and McClelland 1990; Williams *et al.* 1990). Numerous studies on genetic polymorphisms in various organisms using RAPD fingerprinting have now been documented. In botany, RAPD markers were used in strawberry and many plant species (Brown *et al.* 1993; Davis and Yu 1997; Schierenbeck *et al.* 1997). RAPD have been used in fishes for species identification in tilapia (Bardakci and Skibinski 1994), guppy (Foo *et al.* 1995), sea bass (Caccone *et al.* 1997), *Anguilla sp.* (Takagi and Taniguchi 1995) and *Liobagrus reini* (Na-nakorn *et al.* 1996). Several studies on RAPD markers in the yellow catfish, *Mystus nemurus*, have also been reported (Chong 1998; Foo 1998).

In this study, the genetic variations in yellow catfish, *Mystus nemurus* (*Fig. 1*), collected from eight wild populations throughout Thailand and a hatchery stock were determined at the molecular (DNA) level using the RAPD-PCR fingerprinting technique. The results provided, for the first time, data at the DNA level on the genetic structure of natural and hatchery populations of this species in Thailand. The information obtained will be useful for fish population identification, formulation of programs for breeding, and improvement for culture activities, and other aquacultural development programs in the future.

MATERIALS AND METHODS

Sample Collection

Thirty to fifty specimens of *M. nemurus* ranging from 10.5 to 41.0 cm in length and 7.0 to 781.3 g in weight were collected from 8 different locations in Thailand (*Fig. 2*). A hatchery stock was also obtained from the hatchery operated by the Suratthani Inland Fisheries Development Center (SIFDC), in southern Thailand. Live fish were transported from the local areas to the nearest fishery station for tissue collection. Flank muscle tissues of each sample were collected and then stored at – 80°C before being transported to the Genetics Laboratory, Department of Biology, Faculty of Science and Environmental Studies, Universiti Putra Malaysia using dry ice where laboratory experiments were conducted.

RAPD Procedure and Analysis

Extraction of genomic DNA: The flank muscle tissues from each individual were pulverized after thawing. The genomic DNA was extracted using the WIZARD™ a DNA purification kit, which was supplied by Promega Corporation. Six hundred microliters of Nuclei Lysis Solution were added to a 1.5 ml centrifuge tube and chilled on ice. Next, 10-20 mg ground tissue were transferred into the solution and gently mixed. The mixture was then incubated in a water bath at 65°C for 30 minutes. Three microlitres of RNaseA solution were added to the lysate, and then mixed by inverting the tube for 25 times before incubating in water bath at 37°C for 30 minutes. The mixture was then allowed to cool to room temperature for 5 minutes. Two hundred microlitres of Protein Precipitation Solution were added to RNaseA

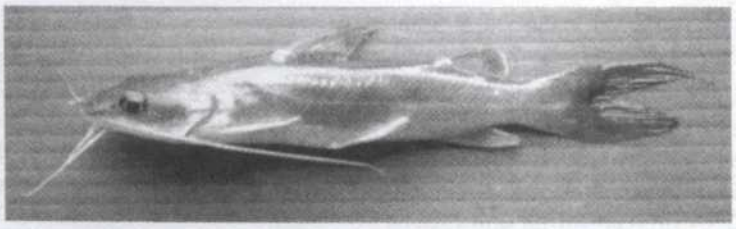

Fig. 1: Yellow catfish, Mystus nemurus (C&V)

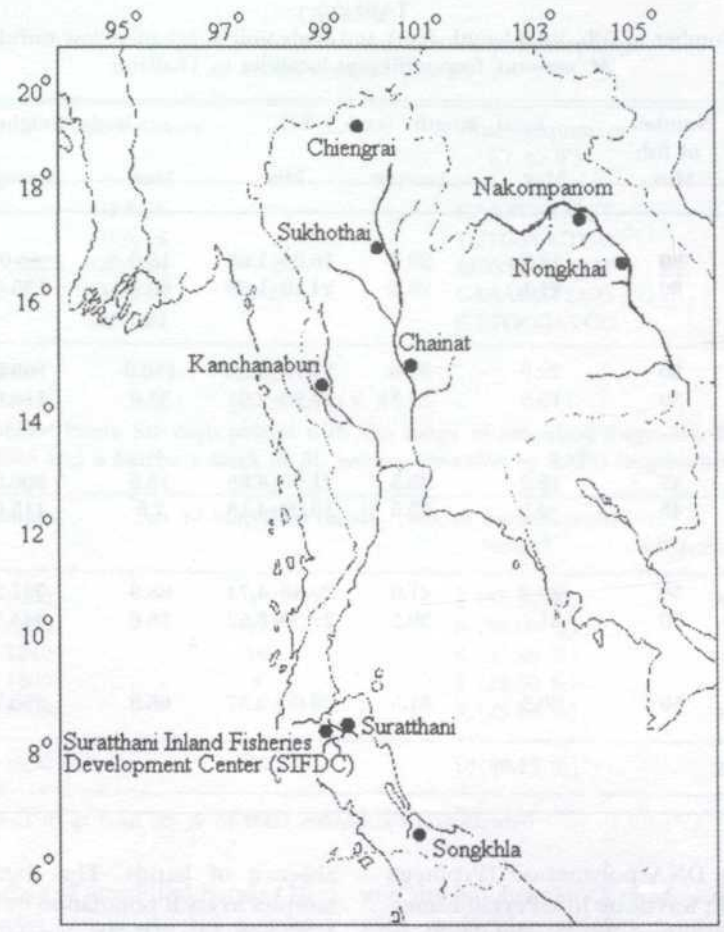

Fig. 2: Sampling locations of Mystus nemurus in Thailand

treated cells and vortexed vigorously at high speed for 20 seconds. The mixture was centrifuged for 3 minutes at 13,000-16,000 X g. After that, the supernatant containing the DNA was carefully removed (leave the residual liquid in the tube to avoid contaminating the DNA with the precipitated protein) and transferred to a clean 1.5 ml centrifuge tube containing 600 ml of room temperature isopropanol, then the solution was gently mixed by inversion until the white thread-like strands of DNA formed a visible mass. The solution then was centrifuged at 13,000-16,000X g for 1 minute. The DNA was visible as a small white pellet. The supernatant was then carefully decanted. The pelleted DNA obtained was then washed twice with 600 μl of 70% ethanol, and the tube inverted several times to clean up the DNA. The tube was then centrifuged at 13,000-16,000 X g for 1 minute. The solution was carefully poured off. The DNA pellet was dried at air temperature for 15 minutes. DNA Rehydration Solution (100 μl) was added to the DNA pellet and the solution was incubated overnight at room temperature. The DNA sample was then stored at 2-8°C until used. The extracted DNA was quantified by comparing its intensity to the intensities of a range of diluted DNA using 0.8 % horizontal agarose gel electrophoresis in 1X TBE buffer (0.045 M Tris-borate and 1 mM EDTA, pH 8.0) at 70 V for 1 hour.

Polymerase Chain Reaction: A total of 20 arbitrary primers from Kit A (Operon Technologies, USA) were screened. Only 5 arbitrary primers, which seemed to work well were selected for the study. The DNA amplification was performed following the procedure recommended by William *et al.* (1990) with slight modifications. The reactions were performed in a volume of 25 μl containing 10 mM Tris HCl; 50 mM KCl; 2.5 mM $MgCl_2$; 0.2 mM each of dATP, dGTP, dCTP and dTTP (Promega); 5 pmol of a single primer; 50 ng of genomic DNA;

TABLE 1
Number of fish, total length (cm) and body weight (g) of yellow catfish, *M. nemurus*, from different localities in Thailand

Sampling site in Thailand	Number of fish	Total length (cm) ± S.D.			body weight (g) ± S.D.		
	Min.	Max.	Average	Min.	Max.	Average	
North							
1. Chiengrai	30	13.2	20.7	16.00±1.68	15.0	55.0	25.20±8.39
2. Sukhothai	30	21.1	26.6	24.10±1.39	60.0	135.0	93.10±19.13
Central							
3. Kanchanaburi	30	22.8	39.0	27.70±3.36	150.0	365.0	230.20±60.72
4. Chainat	30	15.5	24.3	19.50±2.01	35.0	115.0	68.10±19.12
North-east							
5. Nongkhai	47	12.2	35.5	21.00±4.85	13.0	306.0	75.90±52.25
6. Nakornpanom	46	10.5	25.5	16.20±4.13	7.0	115.0	37.40±28.96
South							
7. Suratthani	50	20.2	41.0	29.40±4.74	68.9	781.3	214.20±132.92
8. Songkhla	50	21.1	39.5	27.20±3.52	58.6	444.5	140.40±64.69
Hatchery stock							
9. Suratthani Inland Fisheries Development Center (SIFDC)	50	20.5	31.5	26.60±2.57	66.5	250.7	144.30±37.31

and 2 unit of *Taq* DNA polymerase (Promega USA). Amplification was done in a Perkin-Elmer-Cetus model 2400 thermocycler. The amplification was performed as follows: predenaturation at 92°C for 2 min; followed by 40 cycles at 94°C for 30 sec, 40°C for 30 sec, 72°C for 1 min, and a final extension step at 72°C for 5 min (Chong 1998). The PCR products were kept at 4°C until subjected to electrophoretic analysis.

Agarose gel electrophoresis: The PCR products were separated by electrophoresis on a 1.8 % horizontal agarose gel. For each gel, 5 µl of a 100 bp DNA ladder (Promega USA) was used as a molecular weight standard. The gels were electrophoresed in TBE buffer at 90 V for 2 to 4 hours depending on the sizes of the amplified fragments for each primer. After electrophoresis, the gels were soaked in 1 µg/ml ethidium bromide in 1X TBE buffer for 10 to 15 minutes. After that, the gels were twice rinsed in distilled water and photographed on an ultraviolet transilluminator using polaroid film before data interpretation.

Data interpretation and analysis: The data scoring was based on observed presence or absence of bands. The data from individual samples in each population were used to calculate Nei and Li's (1979) similarity index and to produce an UPGMA dendrogram of genetic relationships based on genetic distances. These analyses were facilitated by using the "NTSYS-pc" (Version 1.8) computer program (Rohlf 1993).

RESULTS

As a preliminary step, a total of 20 primers, OPA-1–20, were screened for PCR amplification. Two primers (10 %), OPA-8 and OPA-12 failed to amplify products of sufficient quality for analysis. Only 18 RAPD primers (90 %) yielded good amplification products. Of these primers, OPA-11, OPA-14, OPA-18, OPA-19 and OPA-20 (Table 2) yielded PCR products which produced clear banding patterns and were selected for the detection of genetic variation in eight wild populations and a hatchery stock of *M. nemurus*.

The five selected primers produced a total of 46 scorable bands ranging in sizes from 380 to 1,550 bp. Each primer generated between 4-16 scorable bands. The complexity of the banding patterns varied among primers. Primer OPA-18

TABLE 2
Primer codes and sequences used to study polymorphism of *Mystus nemurus* (C& V)

Primer names	Primer sequences (5' to 3')
OPA-11	CAATCGCCGT
OPA-14	TCTGTGCTGG
OPA-18	AGGTGACCGT
OPA-19	CAAACGTCGG
OPA-20	GTTGCGATCC

TABLE 3
Total number of amplified bands for each primer with size range of amplified fragments (bp) in eight wild populations and a hatchery stock of *M. nemurus* revealed by RAPD fingerprinting

Primer No.	Size-range	No. of amplified bands	No. of monomorphic bands*	No. of polymorphic bands
OPA-11	750 – 1060	6	2 (33.33 %)	4 (66.67 %)
OPA-14	460 – 1550	12	6 (50.00 %)	6 (50.00 %)
OPA-18	380 – 1240	16	6 (37.50 %)	10 (62.50 %)
OPA-19	580 – 1300	8	1 (12.50 %)	7 (87.50 %)
OPA-20	440 – 1300	4	3 (75.00 %)	1 (25.00 %)
Total	380 – 1550	46	18(39.13 %)	28(60.87 %)

*These bands are present in at least 95 % of total individuals investigated

gave the highest number of amplified bands (16 bands) while the lowest was found for primer OPA-20. Eighteen of these bands (39.13 %) were monomorphic and was present in at least 95 % of all individuals. Twenty-eight bands (60.87 %) were polymorphic (present in some individuals, absent in others) (Table 3). The percentages of polymorphic bands generated by primers OPA-11, OPA-14, OPA-18, OPA-19 and OPA-20 were 66.67, 50.00, 62.50, 87.50 and 25.00 %, respectively.

For individual samples in each population, the fish which were collected from Chiengrai, Sukhothai, Kanchanaburi, Chainat, Nakornpanom, Nongkhai, Suratthani, Songkhla and the hatchery population generated 34, 32, 31, 34, 35, 36, 37, 31 and 34 bands with the percentage of polymorphic bands of 26.47, 18.75, 25.81, 23.53, 31.43, 33.33, 54.05, 25.81 and 23.53 % respectively (Table 4). The results showed that the population with the highest number of polymorphic bands was Suratthani (54.05 %), while the lowest was the Sukhothai population (18.75 %).

The primer OPA-11 generated 6 scorable bands in all individuals of the 8 wild populations and the hatchery stock of *M. nemurus* in Thailand with molecular weights ranging from 750 to 1060 bp. Four of these zones (66.67 %) were polymorphic. Each population generated 2 to 4 bands (Table 4). The RAPD patterns of the OPA-11 primer showed 2 polymorphic bands in the Chainat population, and a polymorphic band each in the population from Chiengrai, Sukhothai, and Songkhla.

Primer OPA-14 produced 12 scorable bands with molecular sizes ranging from 460 to 1,550 bp. Half of these bands (50 %) were polymorphic. Each population showed 7 to 12 bands (Table 4) showing 7 polymorphic bands in the Suratthani population; 3 polymorphic bands in the Chiengrai and Nongkhai populations; 2 polymorphic bands in the Chainat, Nakornpanom and hatchery populations; and only one polymorphic band each in the Sukhothai, Kanchanaburi, and Songkhla populations.

Primer OPA-18 generated 16 scorable zones with molecular sizes ranging from 380 to 1,240 bp (*Fig. 3*). Ten of these bands (62.50 %) were polymorphic. Each population generated 11 to 13 bands (Table 4). There were 8 polymorphic

TABLE 4
Total number of bands, percentage of monomorphic and polymorphic bands found in 8 wild populations and a hatchery stock of *M. nemurus* from Thailand, which were generated through RAPD fingerprinting

Populations	No. of bands	Primers					Total	%
		OPA11	OPA14	OPA18	OPA19	OPA20		
Chiengrai	Total	3	12	12	4	3	34	100.00
	Monomorphic	2	9	9	3	2	25	73.53
	Polymorphic	1	3	3	1	1	9	26.47
Sukhothai	Total	3	11	11	4	3	32	100.00
	Monomorphic	2	10	7	4	3	26	81.25
	Polymorphic	1	1	4	0	0	6	18.75
Kanchanaburi	Total	3	8	11	6	3	31	100.00
	Monomorphic	3	7	8	2	3	23	74.19
	Polymorphic	0	1	3	4	0	8	25.81
Chainat	Total	4	11	12	4	3	34	100.00
	Monomorphic	2	9	8	4	3	26	76.47
	Polymorphic	2	2	4	0	0	8	23.53
Nakornpanom	Total	2	11	12	6	4	35	100.00
	Monomorphic	2	9	8	2	3	24	68.57
	Polymorphic	0	2	4	4	1	11	31.43
Nongkhai	Total	2	11	13	6	4	36	100.00
	Monomorphic	2	8	8	3	3	24	66.67
	Polymorphic	0	3	5	3	1	12	33.33
Suratthani	Total	3	11	13	6	4	37	100.00
	Monomorphic	3	4	5	2	3	17	45.95
	Polymorphic	0	7	8	4	1	20	54.05
Songkhla	Total	4	7	11	6	3	31	100.00
	Monomorphic	3	6	8	3	3	23	74.19
	Polymorphic	1	1	3	3	0	8	25.81
Hatchery	Total	3	9	13	6	3	34	100.00
	Monomorphic	3	7	9	4	3	26	76.47
	Polymorphic	0	2	4	2	0	8	23.53

bands in the Suratthani population; 5 polymorphic bands in the Nongkhai population; 4 polymorphic bands in the Sukhothai, Chainat, Nakornpanom and hatchery populations; and 3 polymorphic bands in the Kanchanaburi and Songkhla populations, respectively.

The primer OPA-19 generated 8 scorable zones in all individuals of *M. nemurus* with molecular sizes ranging from 580 to 1,300 bp. Seven of these zones (87.50 %) were polymorphic. Each population generated 4 to 6 bands (Table 4). The patterns of bands produced by the primer OPA-19 showed 4 polymorphic bands in the Kanchanaburi, Nakornpanom, and Suratthani populations, 3 polymorphic bands in the Nongkhai and Songkhla populations, 2 polymorphic bands in the hatchery stock, and a polymorphic band in the Chiengrai population. The populations from Chainat and Sukhothai showed identical patterns for all individual fish.

Primer OPA-20 produced 4 scorable bands with molecular sizes ranging from 440 to 1,300 bp. Only one band (25.00 %) in the Chiengrai, Nongkhai, Nakornpanom and Suratthani populations was polymorphic. The other populations showed identical patterns.

The RAPD patterns of all individuals of *M. nemurus* were used to calculate the genetic similarity index within and between populations. The average genetic similarity within populations based on RAPD patterns with 5 primers ranged from 0.822 to 0.960 (Table 5). The highest average similarity within population was found in the Chainat population with a value of 0.960 ± 0.020, while the lowest was found in the Suratthani population with a value of 0.822 ± 0.064.

The similarity index among populations based on RAPD patterns produced by 5 primers ranged from 0.711 to 0.913. The lowest genetic similarity was found between the Chainat and Suratthani populations with a value of 0.711. The highest genetic similarity was found between the Songkhla population and the hatchery stock with a value of 0.913.

When the similarity indices among populations were converted to genetic distances (*D*), they ranged from 0.087 between Songkhla population and the hatchery stock, to 0.289 between the Chainat and Suratthani populations (Table 6). The genetic distance values among populations of *M. nemurus* were used to construct a dendrogram using the unweighted pair-group method of clustering (UPGMA). The dendrogram, which depicts the relationship among populations of *M. nemurus*, is shown in *Fig. 4.*

DISCUSSION

The 5 primer produced 46 bands, of which 28 (60.87 %) were polymorphic. The results suggested that the highest percentage of polymorphic bands was found in the population from Suratthani (54.04 %), while the lowest was found in the Sukhothai population (18.75 %).

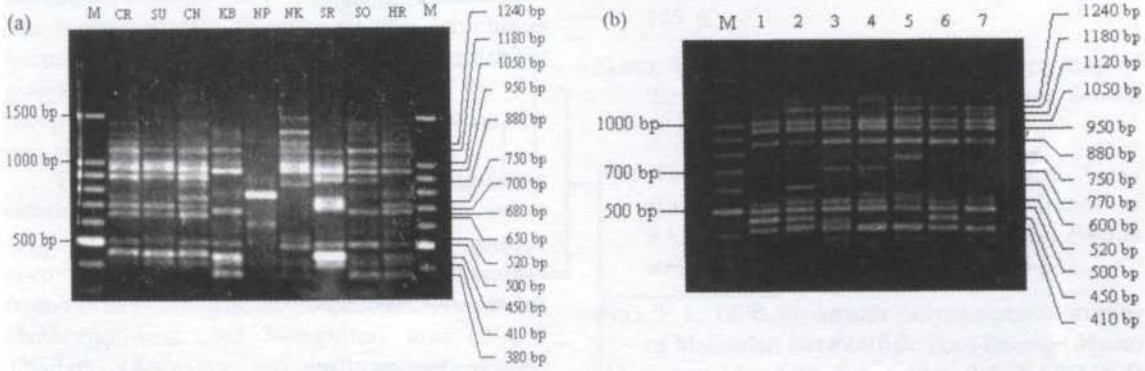

Fig. 3: RAPD patterns obtained from M. nemurus genotypes using primer OPA-18. Lane M: 100 bp DNA ladder. (a) The RAPD patterns were compared among different populations : CR=Chiengrai, SU=Sukhothai, CN=Chainat, KB=Kanchanaburi, NP=Nakornpanom, NK=Nongkhai, SR=Suratthani, SO=Songkhla, HR=Hatchery population. (b) Lanes 1-7: Individual samples collected from Nakornpanom (NP), north-eastern Thailand. Genetic distance (D)

TABLE 5
Estimated similarity index (*S*) within the eight wild populations and a hatchery stock of *M. nemurus* revealed by RAPD fingerprinting

Populations	Range of similarity index within population	Mean of similarity index within population
Chiengrai	0.868 – 1.000	0.948 ± 0.030
Sukhothai	0.818 – 1.000	0.929 ± 0.036
Kanchanaburi	0.857 – 1.000	0.946 ± 0.039
Chainat	0.909 – 1.000	0.960 ± 0.020
Nakornpanom	0.809 – 0.980	0.906 ± 0.036
Nongkhai	0.826 – 0.979	0.900 ± 0.030
Suratthani	0.634 – 0.978	0.822 ± 0.064
Songkhla	0.800 – 0.979	0.883 ± 0.037
Hatchery	0.800 – 0.981	0.898 ± 0.039

TABLE 6
Genetic distances among 8 wild populations and a hatchery stock based on Nei and Li (197) band sharing similarity index from RAPD markers

Population	CR	SU	KB	CN	NP	NK	SR	SO	HR
Chiengrai	****								
Sukhothai	0.091	****							
Kanchanaburi	0.139	0.139	****						
Chainat	0.139	0.145	0.092	****					
Nakornpanom	0.173	0.158	0.152	0.184	****				
Nongkhai	0.178	0.174	0.148	0.179	0.106	****			
Suratthani	0.266	0.283	0.252	0.289	0.230	0.255	****		
Songkhla	0.243	0.243	0.203	0.249	0.215	0.228	0.105	****	
Hatchery	0.258	0.249	0.188	0.236	0.228	0.229	0.140	0.087	****

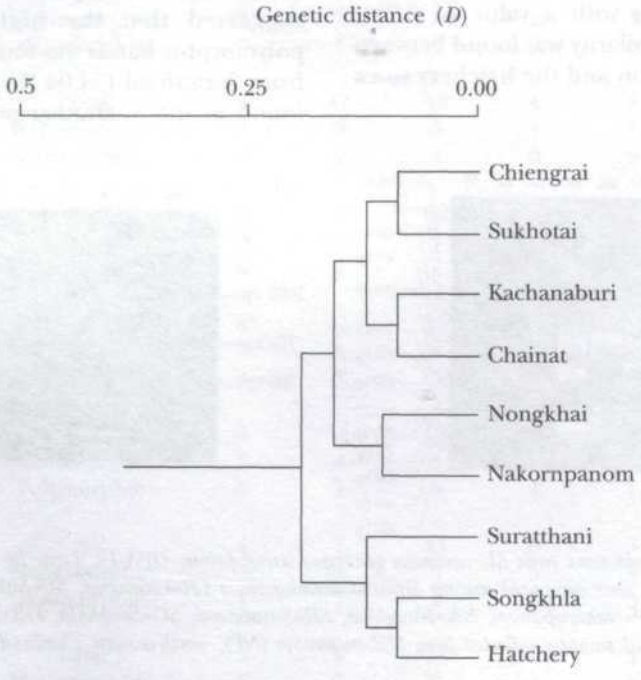

Fig. 4: UPGMA dendrogram constructed based on RAPD-PCR genetic distances among populations of M. nemurus in Thailand

The percentages of polymorphic loci revealed by RAPD fingerprinting were similar to the results obtained by Leesanga *et al.* 2000 using isozyme electrophoresis of 23 loci. They showed that the highest percentage of polymorphic loci was in the Suratthani population (43.48 %), and the lowest in the Sukhothai population with a value of 13.04 %. However, the percentage of polymorphic loci revealed by RAPD fingerprinting using 5 primers seemed higher than those revealed by isozyme analysis.

The estimated similarity index (*S*) within population ranged from 0.822 in the Suratthani population to 0.960 in the Chainat population. The values showed that there were a lot of genetic differences within populations from the south of Thailand (Suratthani, Songkhla and the hatchery stock) whereas less difference was found in the other populations. The results were in the same direction as the heterozygosity values revealed by isozyme electrophoresis of 23 loci (Leesanga *et al.* 2000).

The genetic similarities (*S*) among populations were converted to obtain the genetic distances (*D*), which ranged from 0.087 in the pair of Songkhla population and the hatchery

stock to 0.289 in the pair of Chainat and Suratthani populations (Table 6). The genetic distances among populations of *M. nemurus* in Thailand seemed lower than the genetic distances among populations of the fish reported in a neighboring country, Malaysia (Chong 1998; Foo 1998). However, the genetic distances in this study seemed in accordance with the results reported using isozyme electrophoresis of 23 loci (Leesanga *et al.* 2000). The genetic distance of *M. nemurus* from the Songkhla population to the hatchery stock located in Suratthani province is small (0.087). This may be because the fish from the hatchery population were transported to the Songkhla Inland Fisheries Station where they were used as broodstocks to produce fish fingerlings for release into natural water bodies or the reverse, that is the fish of the Songkhla population might have been transported and used as the broodstocks for the hatchery of the Suratthani Inland Fisheries Development Center. The highest genetic distance (0.289), which was found between the Chainat and Suratthani populations, should be supported by the morphometric data on characters between the two populations (Leesanga 2000).

The dendrogram depicts the genetic relationships among populations of *M. nemurus* (*Fig. 4*), which clustered into four groups according to their regions of origin. Samples from north (Chiengrai and Sukhothai), northeast (Nakornpanom and Nongkhai) and central Thailand (Kanchanaburi and Chainat) shared one major cluster with three subclasses whereas samples from south Thailand (Suratthani, Songkhla and the hatchery stock) were separated into another major cluster.

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Comparative Evaluation of Different Plant Residues on the Soil and Leaf Chemical Composition, Growth, and Seed Yield of Castor Bean (*Ricinus communis*)

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Keywords: Plant residue, castor bean, soil and leaf chemical composition

ABSTRAK

Penyelidikan kesan sisa sabut koko, sekam padi dan habuk kayu ke atas pertumbuhan, hasil benih, daun dan komposisi kimia tanah kacang kastor yang dijalankan pada tahun 1999 dan 2000 di zon hutan hujan Akure, Nigeria. Rawatan diguna pada 6t/ha, direplikakan sebanyak empat kali dan disusun secara rawak blok lengkap (RCB) dengan 300kg/ha baja NPK 15-15-15 juga rawatan kawalan (tanpa baja; tanpa sisa). Tanah dan kesan organik dianalisis secara kimia. Parameter yang direkodkan bagi pokok kacang kastor adalah ketinggian, kelebaran daun, ukur lilit batang, tanah dan N, P, K, Ca, Mg daun, nilai pH tanah, O.M dan hasil benih. Keputusan menunjukkan ada peningkatan signifikan ($p<0.5$) pada ketinggian, indeks kelebaran daun, ukur lilit batang, tanah dan daun N, P, K, Ca, Mg, nilai pH tanah, O.M dan hasil benih pokok kastor melalui rawatan kesan organik dan dibandingkan dengan rawatan kawalan. Rawatan menggunakan sabut koko meningkatkan berat benih sebanyak 55% dan 80% dalam dua tanaman kacang kastor berbanding rawatan sekam padi. Berbanding dengan sisa tumbuhan, rawatan NPK paling banyak menyumbang kepada peningkatan ketinggian, kelebaran daun, dan ukur lilit batang tetapi mengurangkan kesan benih.

ABSTRACT

This work investigated the effect of plant residues such as cocoahusk, ricebran and saw dust on the growth, seed yield, leaf and soil chemical composition of castor bean in 1999 and 2000 at Akure in the rainforest zone of Nigeria. The treatments were applied at 6t/ha, replicated four times and arranged in a randomized complete block (RCB) with NPK 15 -15 -15 fertilizer applied at 300 kg/ha and a control (no fertilizer; no residue). The soil and organic amendments were chemically analyzed. The parameters recorded for castor oil bean were plant height, leaf area, stem girth, soil and leaf N, P, K, Ca, Mg, Soil pH, O.M and seed yield. Results showed that there were significant increases ($p<0.05$) in plant height, leaf area index, stem girth, soil and leaf N, P, K, Ca, Mg, soil pH, O.M and seed yield of castor oil plant under different organic amendment treatments compared to the control. The cocoahusk treatments increased seed weight by 55% and 80% in the two crops of castor oil bean to the ricebran treatment. Compared to the plant residues, the NPK treatment resulted in the greatest increases in the plant height, leaf area and stem girth but reduced seed yield.

INTRODUCTION

Castor oil bean, which is considered to be an ordinary plant by farmers in tropical countries, has now become an important crop in the world market. Traditionally, they are used as fencing materials by farmers around homes and farms, however, the essential oil component of its seeds has not been exploited fully.

Essential oils have been used for thousands of years, not only in aromatherapy, but also in perfumes, pharmaceuticals and food flavoring, and as a more recent innovation in bio-pesticides. Spore CTA (2000) reported that the market for essential oils is well established, at an estimated $1.2 billion per year and with the growing interest in "healthy" lifestyles in Europe, for example, demand is rising steadily.

Between 1992 and 1997, millions of dollars were invested by local banks and companies: the World Bank, the United Nations Industrial

Development Organization (UNIDO) and the (ACP-EU) center for the development of essential oil industry. However, the investment failed because of lack of attention to consistent supply of plant materials, quality control and plant diseases.

Consequently, the production of castor oil beans in Nigeria and other tropical countries is still inadequate to support an essential industry. Some of the reasons that contribute to this situation are continued decline in soil fertility and lack of information on its production hamper increased production and yield. Efforts aimed at improving soil fertility using chemically prepared inorganic fertilizers may not be viable because of high cost of purchase, scarcity at the farmers' level and their negative effect on soil quality with continuous use.

Therefore, there is justification for alternative sources of fertilizers which are inexpensive, sustainable and environmentally sound.

Based on an extensive literature review, it is concluded that the research information on the use of plant residues such as sawdust, rice bran and cocoahusk for optimal castor oil bean is limited and conclusive. The objectives of this study are to compare the effects of these residues on soil fertility, growth, seed weight and leaf chemical composition of castor oil bean.

MATERIAL AND METHODS

Field Experiment

The experiments were conducted at Akure in the rainforest zone of Nigeria on a sandy loam soil, skeletal, kaolinitic, isohyperthemic oxic paleustalf (Alfisol) or Ferric Luvisol (FAO) with pH (H_20) of 5.2, organic matter 0.51%, 0.02%N, 4.6mg/kg Bray P1 extractable P, 0.05mmolkg^{-1} exchangeable K, 0.12mmolkg^{-1} exchangeable Ca and 1.12mmolkg^{-1} exchangeable Mg. The soil was under arable crops for 10 years and the field experiments were conducted in 1999 and 2000.

Three plant residue treatments (ricebran, sawdust, and cocoa pod husk) were individually applied at 6tha^{-1} to each crop castor oil bean. Additional treatments included inorganic fertilizer treatment (300 kgha^{-1} of NPK 15-15-15) and a complete control which did not receive fertilizer nor organic amendment. The 5 treatments were arranged in a randomized complete block design and replicated four times on each castor oil crop. The plots were 4m x 4m (16m^2) and the soils were ploughed and harrowed to maintain adequate tilth while the residue treatments were incorporated into the soil two weeks before planting to allow adequate decomposition.

Two dried seeds of castor oil bean were planted per hole of 2 cm deep hole on May 12, 1999 and April 30, 2000. The plant and row spacing were 100 cm and 100 cm respectively. The plots were manually weeded in the second, fifth and eighth weeks after planting respectively while the insects were controlled by spraying Karate at the rate of 1.8mL a_i. i L^{-1} of water at 2,4 and 6 weeks after planting.

Leaves were taken from the middle part of the stem at 18 weeks after planting and oven-dried for two days at 70°C. After grinding the sub-samples, 2 g each was dry-ashed using a muffle furnace at 450°C for 6 hours.

The nutrients in the ashed leaf of castor bean were extracted with water to measure the nutrient content of the leaf tissue. Percent N was determined by microjeldahl method (Jackson 1964) while the percent P was determined by using vanado-molybdate solution. Percent K and Ca were determined by flame photometer while Mg was determined on atomic absorption spectrophotometry for the plants of castor bean selected for each treatment plot.

In-addition, the six plants in each plot (24 plants per four replicates) were also selected for plant height, stem girth and leaf area measurements. These parameters were measured at 2 week intervals until 16 weeks after planting. The leaf area per plant was determined by measuring some representative leaves of the plant and this was used to calculate the total leaf area on castor bean plant. From the figures obtained, the leaf area index (m^2 of leaf area per m^2 of soil area) was calculated using the formulae LAI = LA/GA where LA = Leaf area and GA = Ground area or soil area.

Harvest or matured seeds capsule started at 22 weeks after planting and continued at four days interval until senescence using a total of 16 plants per plot (64 plants per four replicates)

Source and Preparation of Organic Materials

Cocoa pod husk and rice bran were obtained from the cocoa plantations and rice mill unit of the Federal College of Agriculture, Akure respectively while the sawdust was obtained from a local sawmill industry at Akure. The organic materials were air dried and processed to allow

partial decomposition and quick release of nutrients for crop use. The dried cocoa pod husk was ground using hammer mill while the ricebran and sawdusts were each partially composited separately for six weeks to reduce the C/N ratio.

During compositing of cocoa husk pod, sawdust and ricebran, some quantity of soil was added at a ratio of 1:2 of the proportion of the organic materials.

Chemical Analysis of Organic Materials

The processed forms of the three organic materials were analyzed for P, K, Ca, Mg, Na, and micronutrients content using wet digestion method with 25mL nitric acid, (HN03), 5ml sulphuric acid (H_2S0_4) and 5 ml of hyperchloric acid (HC104).

For P analysis, colour absorbance was determined using a spectronic 20 at 442 Um and a flame photometer was used for other nutrients except Mg, Zn, Fe, Cu and Mn concentrations which were determined using an atomic absorption spectrophotometer. Nitrogen was determined by microkjeldahl method (A.O.A.C 1970).

Soil Sampling and Analysis Before Planting

30 core soil samples were collected, mixed, air dried, sieved using a 2mm sieve prior to routine analysis. The processed soil samples were analyzed as follows:

(i) Soil pH (1:1 soil/water) and 1:2 soil/0.01M CaC_2 solution was determined using glass calomel electrode (Crockford and Nowell 1956).

(ii) Percent nitrogent was determined by using mirokjeldahl method (Assoc of Anal. Chem. 1970).

(iii) Available phosphorus was extracted from the soil by using Bray Pi extractant and determined using a spectronic 20 at 882 Um (Murphy and Riley 1962).

(iv) Exchangeable cations (K, Ca, Mg and Na) were extracted using 1m $NH_4OAcpH7$ solution and the concentrations were determined with a flame photometer (Jackson 1958) while Mg content was measured using an atomic absorption spectrophotometer.

(v) The exchangeable acidity (H+ abd $^{A13+}$) was measured from 0.01M HCL extracts by titrating with 0.1 NaOH (McLean 1965).

(vi) The micronutrients (Mn, Cu, Zn and Fe) were extracted with 0.1M MCl (Ogunwale and Udo, 1978) and measured with Perkin Elmer atomic absorption spectrophotometer.

Soil Analysis After the Experiment

At the end of each experiment, soil samples were taken from each plot per treatment, air-dried and passed through 2mm sieve for routine chemical analysis. The procedure for the analysis of soil pH, O.M, N, P, K, Ca, Mg and Na were as described above.

Statistical Analysis

Data were subjected to ANOVA F-test and means separation by Duncan Multiple Range Test (DMRT) at the 5% possibility.

RESULTS

Table 1 shows the chemical analysis of the soil before the experiment. The soil is highly acidic and very low in O.M and nitrogen. The available N, P, K, Ca, Mg and Na in the soil were less than the critical levels 0.15% N, 10mg/kg^{-1}P, 0.2 mmol Ca and Mg, and 0.30 mmolkg^{-1} K recommended for crop production in South West Virginia (Agboola and Corey 1973; Adeoye 1986, 1983; Folorunso *et al.* 2000). The poor chemical composition of the spoil implied that the castor bean would respond favorably to the application of organic materials for optimum production. The high value of bulk density 1.62Mgm^{3} would affect root development and nutrient uptake by the plant.

Organic Materials Analysis

Table 2 presents the chemical composition of the plant residues used for the cultivation of castor bean. Among the plant residues, the compost based cocoa husk had the highest nutrient content of C, N, K, Ca, Mg, Fe, Mn, Cu and Zn followed by compost based ricebran and the sawdust had the lowest nutrient contents. Ricebran and sawdust had the highest C/N ratio (1:23 and 1:19) respectively but these C/C ratio values were still relatively lower than their ordinary forms because they were composted.

Effect of Plant Residues on Castor Bean Leach Chemical Composition

Plant residues increased leaf tissue % N, P, K. Ca, and Mg of castor bean significantly ($p<0.05$)

TABLE 1
Soil chemical composition before planting castor oil plant

pH		Organic matter							Exchangable cations	
H_2O	$CaCl_2$	N	P	K	Ca	Mg	Na	Bulk	Density	Soil Type
%		mg/kg soil			mmol/kg soil				Mgm^{-3}	
5.20	4.80	0.51	0.02	4.60	0.05	0.12	0.12	0.43	1.62	Alfisol

+ Analysis based on dry sample basis

TABLE 2
Chemical analysis of the organic fertilizers

Organic Fertilizers	C	N	C/N	P	K	Ca	Mg	Na
	mg/kg		mg/L		%			
Sawdust-based Compost (SC)	8.00	0.42	18.96	10.00	5.12	0.10	1.30	4.39
Rice bran-based Composed (RC)	14.00	0.60	23.33	56.00	7.93	0.12	1.80	4.33
Cocoahusk-based Compost (CC)	16.00	1.44	11.11	100.00	20.59	9.34	7.10	4.41

+ The organic fertilizers were composted for six weeks and soil added before application, hence, low C/N values to the original forms.

TABLE 3

Treatments	Crop 1 (1999)					Crop 2 (1999)				
	N	P	K	Ca	Mg	N	P	K	Ca	Mg
			%					%		
Control (no fertilizers)	0.60d	0.15d	0.20c	0.16d	0.15d	0.50c	0.12d	0.15c	0.13c	0.12d
Sawdust-based Compost (Sc)	1.40c	0.34c	0.38b	0.30c	0.28c	1.80d	0.53c	0.45d	0.47b	0.35c
Ricebran-based Compost	1.80c	0.39c	0.40b	0.43	0.31b	2.10c	0.58c	0.52c	0.50b	0.40b
Cocoahusk-based Compost	2.40b	0.50b	0.64a	0.70a	0.42a	2.80b	0.73b	0.68b	1.10a	0.65a
NPK Fertilizer	3.20a	0.78b	0.64a	0.13d	0.12d	3.80a	0.92a	0.72a	0.10d	0.10e

Treatment means within each column followed by the same letter are not significantly different from each other using Duncan Multiple Range test at 5% level.

compared to the control (Table 3). Cocoa husk gave the highest values that leaf N, P, K, Ca and Mg followed by ricebran and sawdust respectively. The residues had higher values of Ca and Mg than NPK 15-15-15 treatment; however, the use of NPK fertilizer resulted in higher leaf N, P and K contents of castor bean compared to the use of plant residues.

Effect of Plant Residues on Growth and Seed Yield of Castor Bean

The plant residues and NPK fertilizer increased the seed yield (Table 4), plant height (Table 5), leaf area index (Table 6) and stem girth (Table 7) significantly ($p<0.05$) relative to the control treatment.

Plant height, leaf area index, stem girth and seed weight of castor bean were greatest in the cocoa husk plots followed by the rice bran and sawdust treated plots respectively.

Cocohusk ricebran and sawdust treatments increased mean plant height by 80%, 71% and 65% respectively compared to the control treatments. NPK increased plant height, leaf area and stem girth of castor bean more than the three residues cocohusk, ricebran and sawdust.

Cocohusk increased the seed weight of castor bean better than NPK and control treatments. For instance, cocoa husk increased the seed weight of castor oil plant by 55 and 80% in crops 1 and 2 compared to that of ricebran treatment.

Effect of Plant Residues on the Soil Chemical Composition After Harvest

Table 8 represents the soil chemical composition after the experiment on castor bean. The cocoa husk, rice bran and sawdust increased the soil N, P, K, Ca, Mg, Soil pH, O.M significantly ($p<0.05$) compared to the control treatment. The cocoa husk produced the highest values of soil N, P, K. Ca and Mg followed by rice bran and sawdust treatments respectively; however, soil N, P, K contents in NPK fertilizer treatment were higher than the organic residue treatment. The use of NPK also reduced the soil of pH and O.M compared to residue treatments.

DISCUSSION

The soils used for planting castor bean were generally low in pH. O.M, N.P, K, Ca and Mg and this could be responsible for the poor growth of castor oil plants as shown in the control treatment.

This observation is supported by Agoola and Corey (1973), Adeoye (1986) and Ayodele (1983) who had reported that poor growth of crops occurred in soils with less than 0.15%N, $10mg/kg^{-1}$-P and $0.2mmol\ kg^{-1}$ Ca and Mg critical levels considered for crop production in South-west Nigeria, therefore, it is expected that the application of cocoa husk, rice bran and sawdust would increase the growth responses and seed yield of castor bean.

The increase in plant height, leaf area and stem girth of castor bean grown with cocoa husk, rice bran and sawdust could be linked to their chemical composition. Among the residues, cocoa husk had the lowest C:N ratio which implies that it decomposes faster and makes its nutrients more easily available compared to rice bran and sawdust.

Cocoa husk had the highest available N, O, K, Ca, Mg, Fe, Mn, Cu and Zn content and this could be responsible for the best values of seed weight, plant height, leaf area index, stem girth, soil and leaf N, P, K, Ca, Mg, Soil pH and O.M. Rice bran and sawdust were the least efficient of the plant residue treatments in providing nutrients for castor bean. Accordingly, this might be responsible for the lowest values of leaf and soil N, P, K, Ca, Mg, soil pH and O.M, and measured growth parameters of castor bean in treated plots with sawdust and rice bran.

The fact that cocoa husk increased soil pH more than the other nutrients is consistent with previous findings that its ash contains high levels of K, Ca and Mg (Ojeniyi 1995) and the cocoa husk is an excellent source of K (Adu – Daaph *et al.* 1994).

The increase in soil pH as a result of cocoa residues application and superior seed yields provide evidence that this material is an excellent source of fertilizer for castor bean.

The finding that use of NPK fertilizer resulted in the greatest plant height, leaf area index and stem girth of castor bean compared to plant residues is also consistent with its relative higher N, P and K nutrient content than the residue. The N, P and K in the fertilizer are more readily available than those supplied by organic sources. However, it was observed that the NPK fertilizer encouraged the vegetation of castor bean but delayed the seed formation when compared to the residues.

TABLE 4
The effect of experimental treatments on seed weight of two crops of castor bean during the 1999 and 2000 growing seasons

Treatments	1999				2000				
	See + Capsule weight (Kg/16m²)	Seed Weight							
		Kg/16m²	Kg/ha	Metric Tonne	Seed + Capsule Kg/16m²	Kg/16m²	Kg/ha	Metric tonnes (MT)	Mean Seed Weight (MT)
(1) Control (no fertilizer)	46.80e	1.17e	731.30e	0.73e	46.70e	1.16e	729.70e	0.73e	0.73d
Sawdust Based Compost	162.20d	42.0d	2628.13d	2.63c	265.70c	6.64c	4151.60c	41.5c	0.73d
Rice bran Based Compost	187.10c	4.67c	2918.80c	2.92b	214.30d	5.36d	3350.0d	3.35d	3.13c
Cocoa husk Based Compost	290.90ab	7.27ab	4545.3b	4.55a	389.20a	9.73a	6081.3a	6.08a	5.32a
NPK 15-15-15	292.10a	7.30a	4562.50a	4.56a	381.00b	9.53b	5953b	5.95b	5.25a

+ Mean seed weight of castor bean contains values of 1999 and 2000 growing seasons.

TABLE 5
Effects of experimental treatments on plant height (cm) of castor bean during the 1999 and 2000 growing season

Treatments	1999	2000	Mean
Control (no fertilizer)	8.00e	7.80e	7.90e
Sawdust based compost	18.00d	21.30d	19.70d
Rice bran-based compost	27.60c	30.20c	28.90c
Cocoa husk based compost	40.40b	46.30b	43.40b
NPK 15 – 15 – 15	45.40a	50.00a	47.80a

Treatment means within each column followed by the same letters are not significantly different from each other using Duncan Multiple Range Test at 5% level.

TABLE 6
Effects on experimental treatments on leaf area index (m^2 of leaf area per m^2 of soil area) of castor bean during 1999 and 2000 growing seasons

Treatments	1999	2000	Mean
Control (no fertilizer)	2.5d	2.80e	2.70d
Sawdust based compost	3.6c	4.5d	4.10c
Rice bran-based compost	3.8c	4.8c	4.30c
Cocoa husk based compost	4.0b	5.00b	4.50b
NPK 15 – 15 – 15	4.5a	5.5a	5.00a

Treatment means within each column followed by the same letters are not significantly different from each other using Duncan Multiple Range test at 5% level.

TABLE 7
Effects on experimental treatments on stem girth of castor bean during 1999 and 2000 growing seasons

Treatments	1999	2000	Mean
Control (no fertilizer)	0.31d	0.30e	0.30d
Sawdust based compost	0.80c	1.10d	0.95c
Rice bran-based compost	0.90c	1.30c	1.10c
Cocoa husk based compost	1.30b	1.60b	1.45b
NPK 15 – 15 – 15	1.50a	2.30a	1.90a

Treatment means within each column followed by the same letters are not significantly different from each other using Duncan Multiple Range test at 5% level.

CONCLUSION AND RECOMMENDATION

Cocoa husk, rice bran and sawdust can be effective sources of nutrients because their additions to the soil enhanced the leaf and soil N, P, K, Ca, Mg content, soil pH, O.M, seed weight, plant height, leaf area index and stem weight, plant height, leaf area index and stem girth of castor bean.

Based on experimental findings, it is recommended that cocoa husk applied at 6t/ha can be used as a fertilizer material for improving the nutrient availability and increasing production of castor bean on low fertility soils in the humid tropics. This recommendation stems from the fact that inorganic fertilizers are scarce and expensive for the growers of castor bean in most tropical countries.

TABLE 8
Effort of experimental treatments on soil chemical composition after harvesting castrol oil bean during 1999 and 2000 growing season

Treatments	Crop 1							Crop 2						
	O.M	N	P	K	Ca	Mg	pH	O.M	N	P	K	Ca	Mg	pH
	%		mg/kg soil			mmol/kg		%		mg/kg soil			mmol/kg	
Control (No fertilizer)	0.41c	0.05d	4.20d	0.09c	0.11d	0.13d	5.20cd	0.28d	0.03d	3.80e	0.05d	0.09d	0.10d	5.00cd
Sawdust-based Compost	1.52b	0.12c	10.20c	2.18b	0.35b	0.25c	6.60b	1.60b	1.16c	11.30d	2.23c	0.40c	0.32c	6.80b
Rice bran-based Composed	1.62b	0.13c	9.35c	2.24b	0.30c	0.28b	6.70b	1.68bc	0.18c	12.40c	2.52b	0.45b	0.48b	6.85b
Cocoahusk-based Compost	2.24a	0.23b	15.26b	3.42a	0.80a	0.78a	7.10a	3.10a	0.52b	23.82b	3.63a	1.20a	1.40a	7.20a
NPK 15 - 15 -15	0.32c	0.45a	21.45a	3.46a	0.08de	0.07de	5.60c	0.26d	0.58a	27.52a	3.92a	0.03e	0.04e	5.30c

Treatments means within each column followed by the same letters are not significantly different from each other using Duncan Multiple Range Test at 5% level.

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Distribution of Food Items of Six Commercially Important Demersal Fishes in the South China Sea

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ABSTRAK

Taburan jenis makanan bagi enam spesies komersial yang penting di perairan pantai timur Semenanjung Malaysia telah dikaji. Tinjauan pukat tunda telah dijalankan di 24 stesen di kawasan antara 12 hingga 200 batu nautika daripada pantai yang meliputi keluasan anggaran 27,785.54 batu nautika persegi. Perut spesies ikan yang dipungut dikeluarkan segera, diawet dan dibawa ke makmal untuk dianalisis kandungannya. Kaedah 'occurrence' digunakan untuk menentukan kuantiti makanan. Penaeus sp. *telah didapati sebagai jenis makanan utama ikan* Carangoides malabaricus, Nemipterus marginatus, Priacanthus tayenus, *dan* Upeneus bensasi *sementara* Loligo sp. *bagi* Saurida undosquamis *dan* Sphyraena forsteri. *Kawasan sub 0, I, II dan III merupakan kawasan paling produktif bagi ikan kajian dan jenis makanan. Keputusan menunjukkan bahawa terdapat perhubungan ketara di antara jenis makanan dan taburan ikan. Maklumat mengenai kedapatan dan taburan jenis makanan adalah penting bagi pengurusan sumber perikanan dan pengeksploitasian spesies ikan dengan efisien.*

ABSTRACT

Distribution of food items of six commercially important fish species in waters off the east coast of Peninsular Malaysia were studied. Trawl surveys were conducted in 124 stations in areas between 12 to 200 nautical miles from shore covering an estimated area of 27,785.54 square nautical miles. Stomachs of fish species collected were removed onboard, preserved and taken to the laboratory for analysis of the contents. The occurrence method was used to quantify the diet. Penaeus sp. *was found to be the main food item of* Carangoides malabaricus, Nemipterus marginatus, Priacanthus tayenus, *and* Upeneus bensasi *while* Loligo sp. *for* Saurida undosquamis *and* Sphyraena forsteri. *Sub-areas 0, I, II and III appeared to be the most productive areas for the studied species and the food items. The results also show that there exist significant relationships between the food items and fish distribution. Information on the availability and distribution of food items is important for the management of fishery resources as well as for the efficient exploitation of the species.*

INTRODUCTION

The decline in the abundance of demersal fish resources is always an issue in fishing industry (Hadzley 1997). This decline is thought to prevail due to either over-exploitation of the demersal resources using highly efficient harvesting gears or factors relating to availability of food in the area. A few reports have discussed this issue but the distribution of fish has not been studied extensively, so until now not much information

on the location of potential fishing grounds is available.

According to Hadzley (1997), the distribution of fish in the sea is related to certain physical and chemical parameters of the water. Since these parameters in Malaysian waters have not changed much over the years, it may be assumed that the distribution of species has also not changed in the whole area. But the availability and distribution of food resources as well as seabed conditions are the main factors that affect the distribution of fish.

The objective of this study is to determine the distribution of food items of the commonly found species of *Carangoides malabaricus, Nemipterus marginatus, Priacanthus tayenus, Saurida undosquamis, Sphyraena forsteri* and *Upeneus bensasi.* In addition, the relationship of the food items and fish distribution is also studied.

MATERIALS AND METHODS

Survey Area

The survey was carried out in the Exclusive Economic Zone (EEZ) off the east coast of Peninsular Malaysia in the months of September to November 1999 using K.K. Manchong, a research vessel of the Southeast Asian Fisheries Development Centre (SEAFDEC) of Malaysia. The survey areas extended from 12 nautical miles to 200 nautical miles offshore, bounded by latitudes 7.73 °N and 1.53 °N, and longitudes of 103.00 °E and 104.61 °E with an estimated total area of 27,785.54 sq. nm. This area was further divided into 5 sub-areas and 124 stations were selected to cover the whole study area (*Fig. 1*).

Selection of Species

Six important demersal fish species were selected based on factors such as the high demand for the species for downstream industries and the increase in annual landings in the last decade (DOF 1990-1998).

Sampling Methods

Fish samples were collected using a high-opening trawl net. The net was made of polyethylene materials with a cod-end mesh size of 38 mm. The net was towed at approximately 4 knots for a one-hour duration at specific stations.

During the survey, the total catch of each haul was sorted out into commercial fish and trash fish categories. Subsequently, the commercial fish species were sorted according to their family group. The selected species were identified and sorted out from each family group. The Total Length (TL) of individual fish was measured to the nearest mm.

The stomachs of the fish were then removed and preserved in 10% formalin to prevent any further digestion and decomposition of the contents. The fish species from each sampling station were kept frozen and brought back to the laboratory for further examination. In the laboratory, the stomachs were dissected to remove the contents. The stomach contents were then identified to the lowest practical taxon.

The occurrence method was used to quantify the stomach content where the number of stomachs in which each food type occurs is expressed as a percentage of the total number of stomachs containing food (Gunn and Milward 1985; Kennedy and Fitzmaurice 1972). This method requires minimum time and apparatus and is simple to apply when food items are readily identifiable. The presence of food items that could not be enumerated (e.g. digested matter) is regarded as one occurance of that item.

RESULTS AND DISCUSSIONS

Percentage of Food Items

The result of stomach content analysis using occurrence method is given in Table 1. The result from this study indicates that *C. malabaricus* feed primarily on crustacean, particularly *Penaeus sp.*, being present in 16.8% of stomach containing food and this is in agreement with the results of the study conducted in the same waters by Mansor *et al.* (1998) and Mohsin and Ambak (1996). The fact that *N. marginatus* was found to feed mainly on crustacean in particular *Penaeus sp.* (59.1%), supplements the work done by Mansor *et al.* (1998), and Mohsin and Ambak (1996) which generally stated that this species feeds on small animals. Crustaceans (*Penaeus sp.*) are also the most commonly occurring identifiable food item in *P. tayenus* (78.5% of stomach containing food) demonstrating the importance of this food item for the species. Work done by other researchers in the same waters also found that *P. tayenus* feed mainly on *Penaeus sp.* (Mansor *et al.* 1998; Daud and Taha 1986; Mohsin *et al.* 1987; Mohsin *et al.* 1988).

Eighteen types of food items were identified from the stomach contents of *S. undosquamis.* Cephalopod in particular *Loligo sp.* (25.0%) is

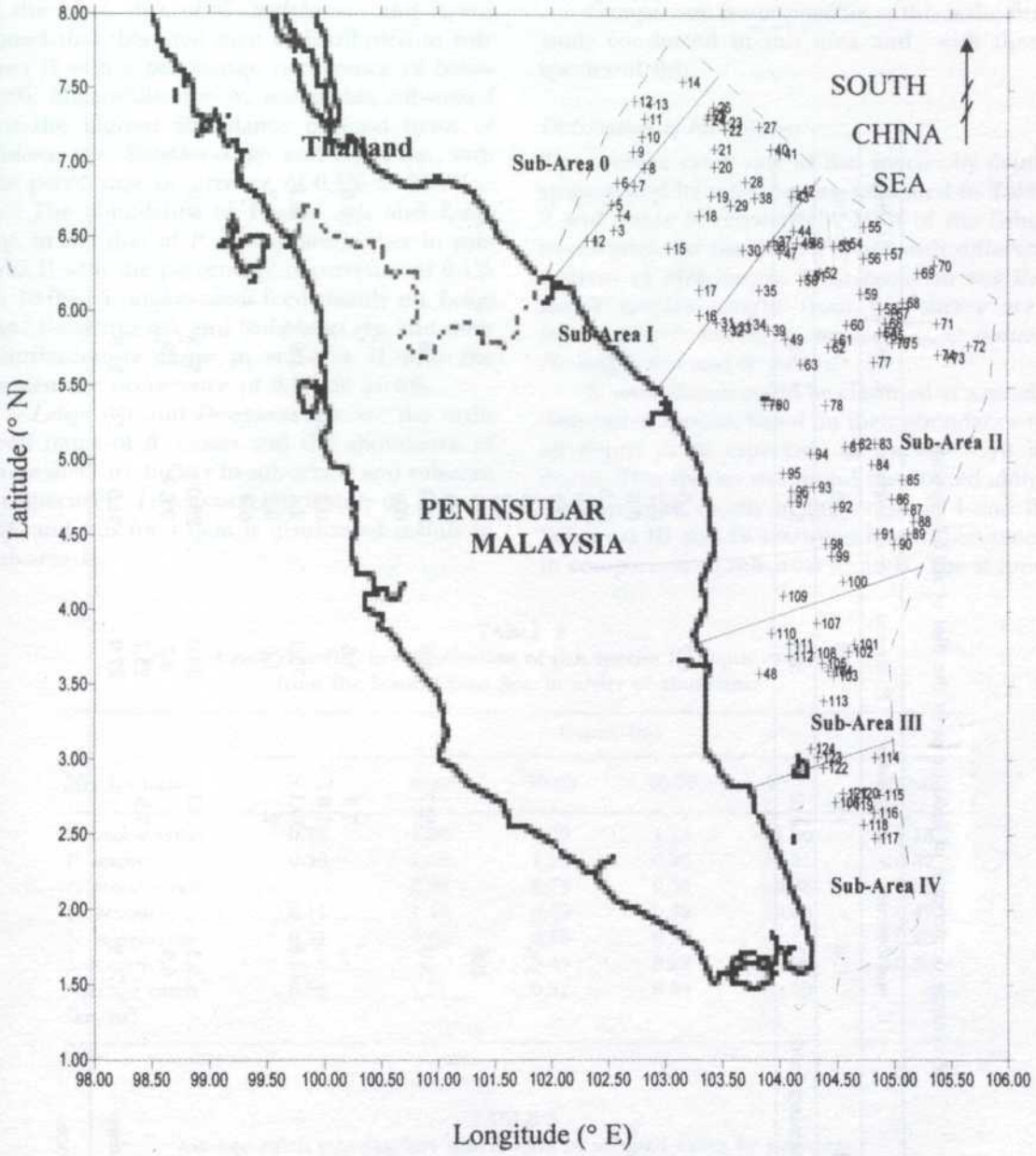

Fig. 1: Map showing the sampling stations in the study area

the most important diet of this species, followed by *Stolephorus spp.* (16.4%). The high diversity of prey items found in the stomachs of *S. undosquamis* suggests that this species is a generalist predator. *S. forsteri* is found to be dependent on *Loligo sp.* (30.8%) and small fishes for their food. Mohsin and Ambak (1996) reported that this species could be found around coral reef areas hunting for small fishes and squids.

Penaeus sp. is the most frequently occurring food item in the stomach contents of *U. bensasi* (98.8%). During one of the research expeditions conducted in similar waters, Daud and Taha (1986) found that 74.6% of the food items of *Upeneus sp.* were crustaceans.

Distribution of Major Food Items in the Sea Areas

The distribution of major food items of the selected species is given in Table 5. *Penaeus spp.*

TABLE 1
Percentage occurrence of food items in the diet of six fish species

Fish species	*C. malabaricus*	*N. marginatus*	*P. tayenus*	*S. undosquamis*	*S. forsteri*	*U. bensasi*
Number of fish examined	156	128	329	558	54	181
Number of stomachs containing food	131	110	304	531	52	160
Food items						
Pisces						
Apogon sp.	-	-	0.7	1.3	-	-
Ariomma indica	-	-	-	0.2	-	-
Brachypleura novazeelandiae	-	-	-	0.6	-	-
Decapterus sp.	-	-	-	11.5	11.5	-
Dipterygonatus batteatus	-	-	-	0.6	-	-
Fistullaria villusa	-	-	-	0.7	-	-
Leiognathus sp.	1.5	-	-	5.5	-	-
Nemipterus sp.	-	-	-	0.2	-	-
Saurida sp.	0.8	-	-	0.9	-	-
Sphyraena sp.	-	-	-	0.4	-	-
Stolephorus sp.	3.0	32.7	2.6	16.4	3.8	-
Upeneus sp.	-	-	-	0.9	-	-
Crustacean						
Crab	-	7.3	1.6	-	-	0.6
Metapenaeus sp.	-	1.8	-	0.2	-	-
Penaeus sp.	16.8	59.1	75.3	2.4	1.9	98.8
Squilla sp.	-	2.7	1.6	0.2	-	1.2
Trachypenaeus sp.	-	-	-	0.4	-	-
Cephalopod						
Loligo sp.	5.3	19.1	20.0	25.0	30.8	0.6
Sepia sp.	6.9	-	0.7	0.6	1.9	-
Polychaete	-	7.3	23.7	-	-	-
Decomposed unidentified tissue	70.2	-	27.0	31.5	51.9	-

- Represents zero occurrence.

is the main diet of *C. malabaricus* and it was found that this food item is distributed in sub-area II with a percentage occurrence of below 10%. Meanwhile, for *N. marginatus*, sub-area I has the highest abundance of food items of *Penaeus spp.*, *Stolephorus spp.* and *Loligo spp.*, with the percentage occurrence of 0.1% to 20.0%.

The abundance of *Penaeus spp.* and *Loligo spp.* in the diet of *P. tayenus* are higher in sub-area II with the percentage occurrence of 0.1% to 10.0%. *S. undosquamis* feed mainly on *Loligo spp.*, *Decapterus spp.* and *Stolephorus spp.* and their distribution is dense in sub-area II with the percentage occurrence of 0.1% to 10.0%.

Loligo spp. and *Decapterus spp.* are the main food items of *S. forsteri* and the abundance of these diets are higher in sub-area 0 and sub-area I respectively. *U. bensasi* feeds mainly on *Penaeus spp.* and this food item is distributed mainly in sub-area II.

Comparison is not possible as this is the first study conducted in this area and with these species of fish.

Distribution of Fish Species

The average catch rate of fish species by depth stratum and by sub-areas are tabulated in Table 2 and Table 3, respectively. Most of the fishes were caught in the deeper water with different degrees of abundance. *S. undosquamis* was the major species caught from the survey area followed by *P. tayenus*, *C. malabaricus*, *U. bensasi*, *N. marginatus* and *S. forsteri*.

S. undosquamis could be classified as a widely distributed species, based on their abundance in all depth strata especially in the 40 – 70 m depth. This species was found distributed along the east coast mostly in the sub-areas I and II. Sub-areas III and IV recorded lower abundance in comparison to sub-areas I and II. The second

TABLE 2
Catch rate (kg/hr) distribution of fish species by depth caught from the South China Sea, in order of abundance

Species name	Depth (m)					Mean
	30-40	40-50	50-60	60-70	70 - >	
S. undosquamis	0.72	1.06	1.70	1.11	1.06	1.13
P. tayenus	0.36	1.28	1.13	0.96	0.35	0.82
C. malabaricus		0.56	0.73	0.55	0.88	0.68
U. bensasi	0.11	1.46	0.40	0.32	0.06	0.47
N. marginatus	0.31	0.69	0.50	0.10		0.40
S. forsteri			0.45	0.22		0.34
Average catch (kg/hr)	0.38	1.01	0.82	0.54	0.59	

TABLE 3
Average catch rate (kg/hr) distribution of selected fishes by sub-areas

Sub-Area	Fish species					
	Cm	Nm	Pt	Su	Sf	Ub
0	1.33	0.59	0.41	0.71	1.39	0.3
I	0.52	0.77	1.4	2.14	0.58	0.36
II	0.62	0.15	0.91	1.39	0.17	0.37
III	1.06	0.05	1.25	0.41	0.14	0.86
IV	0.34	0.03	0.75	0.18	0.17	0.05

Note: Cm – *Carangoides malabaricus*; Nm – *Nemipterus marginatus*; Pt – *Priacanthus tayenus*; Su – *Saurida undosquamis*; Sf – *Sphyraena forsteri*; Ub – *Upeneus bensasi*.

selected species was *P. tayenus* and this species was found mostly in the 40 – 60 m depth and distributed in the sub-areas I, II and III. Sub-area IV recorded the lowest abundance of this species compared to the other sub-areas.

C. malabaricus was found in 40 – 70 m depth especially in sub-areas 0, II and III with the average catch of 1.33 kg/hr, 0.62 kg/hr and 1.06 kg/hr respectively. *U. bensasi* was distributed in all depth strata but is most abundant in 40 – 50 m depth. The highest density in abundance of this species was found in sub-areas I, II and III. Sub-area IV recorded the lowest abundance as compared to the others.

N. marginatus was found distributed in the 40 – 60 m depth especially in sub-areas 0 and I. There was no catch of this species recorded in the water deeper than 70 m depth. Sub-area IV, with the average catch of 0.03 kg/hr was the lowest abundance of this species as compared to other locations. Although *S. forsteri* was found distributed along the east coast (mostly in sub-area 0), this species was not widely distributed, as it was abundant only in 50 - 70 m depth.

The result from this study indicates that *P. tayenus* was found mostly in the 40 – 60 m depth and distributed in sub-areas I and III and this is in agreement with the result of the study conducted by Hadzley (1997). The fact that *N. marginatus* was found distributed in the depth of 40 – 60 m especially in sub-areas 0 and I, supplements the work done by Hadzley (1997).

Previous surveys (Pathansali *et al.* 1974; Jothy *et al.* 1975; Lamp and Shaari 1976; Ahmad 1990) concluded that progressive decline in yield occurred in the deeper zones. The depths from 21 to 40 meters usually were more productive areas. The fish resources off the east coast of Peninsular Malaysia appear to be poor beyond the 40-mile line. This is probably due to a relatively lower content of *chlorophyll a*, zooplankton and fish larvae (Mohsin *et al.* 1987a). The present study indicates that the average catch at different depth strata is lower towards deeper areas.

In this survey, sub-areas 0 to III showed high abundance of the studied fish species and appeared to be the most productive areas as compared to the other sub-areas. This distribution pattern is probably due to the bigger number of sampling stations in these particular sub-areas. The least productive area is Sub-area IV which is located near the busy shipping lane where fishing activities are restricted.

Relationship of Food Items and Fish Distributions

Four out of six species, namely *Penaeus spp.*, *Loligo spp.*, *Stolephorus spp.*, and *Decepterus spp.*, were selected to analyse their relationship with fish distribution. These food items were selected from the percentage occurrence of the identifiable food items with the percentage of 10.0% and above and consumed at least by two of the selected species. The total percentage occurrence of main food items was calculated for each sub-area. The average percentage occurrence was calculated by dividing the total percentage by the number of the sampling station in each sub-area (Table 4).

The result shows that the distribution of all main food items is higher in sub-areas 0, I and II. Distribution on catches of six fish species as presented in Table 3 shows that sub-areas 0, I, II and III recorded the highest catch rates for all species. It can be stated that there is a significant relationship between the food item and fish distribution ($P<0.05$). This result suggested that the distribution of fish species could be affected by the availability and distribution of food resources.

TABLE 4
Average percentage occurrence of the main food items by sub-areas

Sub-Area	Food items			
	Penaeus spp.	*Loligo spp.*	*Stolephorus spp.*	*Decapterus spp.*
0	0.81 ± 0.08	1.41 ± 0.12	2.21 ± 0.31	0.19 ± 0.07
I	1.02 ± 0.05	1.25 ± 0.04	1.75 ± 0.08	2.51 ± 0.11
II	0.88 ± 0.01	0.77 ± 0.01	0.42 ± 0.01	0.42 ± 0.01
III	0.39 ± 0.04	0.19 ± 0.02	0.19 ± 0.03	0
IV	0.08 ± 0.01	0.15 ± 0.02	0.28 ± 0.09	0.19 ± 0.06

TABLE 5
Distribution of dominant food items with sub-areas (in parenthesis)

Fish species	Food items			
	Penaeus sp. (%)	*Loligo sp.* (%)	*Stolephorus sp.* (%)	*Decapterus sp.* (%)
C. malabaricus	0.1 – 10.0 (II)			
N. marginatus	0.1 – 10.0 (I)	0.1 – 10.0 (I)	10.1 – 20.0 (I)	
		0.1 – 10.0 (I)	10.1 – 20.0 (I)	
P. tayenus	0.1 – 10.0 (II)	0.1 – 10.0 (II)		
S. undosquamis		0.1 – 10.0 (II)	0.1 – 10.0 (II)	0.1 – 10.0 (I)
S. forsteri		10.1 – 20.0 (I, II, III)		
		20.1 – 30.0 (0)		10.1 – 20.0 (0, I)
U. bensasi	0.1 – 10.0 (II)			

CONCLUSION

The study on the distribution of six selected fish species and their food items suggests that these fish species were predominantly located in a few specific areas. Based on the distribution of each species as shown in Table 5, it was found that sub-areas 0, I, II and III appeared to be the most productive areas. The depth of 40 to 60 meters recorded the highest abundance of the fish species in the whole survey areas. The occurrence of *Penaeus sp.* in the stomachs of *C. malabaricus, N. marginatus, P. tayenus* and *U. bensasi* shows that these fish species depend mainly on *Penaeus sp.* as food. However, *S. undosquamis* and *S. forsteri* depend on *Loligo sp.* as their food.

It was also found that the distributions of the main food items of the studied fish species are higher in sub-areas 0, I and II. Since these sub-areas are the most productive areas for the fish species and their food items, it can thus be postulated that there is a significant relationship between the food items and fish distributions. This information is very important for those involved in the management and efficient exploitation of fishery resources.

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ABSTRAK

Dua spesies periuk kera iaitu Nepenthers gracilis *Korthals dan* N. mirabilis *(Loureiro) Druce direkod di dalam plot berkeluasan 0.1 hektar.* Nepenthes gracilis *berbeza daripada* N. mirabilis *dengan keratan rentas batangnya yang bersudut, daun yang tidak bertangkai, peristom yang nipis (≤ 1 mm tebal), permukaan bawah tudung periuk ditutupi oleh kelenjar madu yang jarang, dinding dalam periuk ditutupi sebahagiannya oleh kelenjar penghadaman jenis terdedah sebaliknya pada* N. mirabilis *batangnya berbentuk bulat, daun yang bertangkai, peristome yang tebal (≥1 mm tebal), permukaan bawah tudung periuk ditutupi oleh kelenjar madu yang padat, permukaan dalam dinding periuk ditutupi sebahagiannya oleh kelenjar penghadaman jenis populasi berbumbung. Kepadatan populasi* N. gracilis *mengatasi populasi* N. mirabilis *masing-masing mempunyai 147 dan 15 individu. Kepadatan rendah* N. mirabilis *adalah kerana spesies ini sangat cenderung hidup di kawasan tanah yang sangat lembap terutamanya di kawasan berpaya. Corak taburan fasa anak cambah dan dewasa* N. gracilis *dan* N. mirabilis *adalah signifikan berkelompok.*

ABSTRACT

Two species of pitcher plants namely Nepenthes gracilis *Korthals and* N. mirablis *(Loureiro) Druce were recorded in the plot with an area of 0.1 hectare. The former species differed from the later species by its angular stem, sessile leaves, very thin peristome (≤ 1 mm thick), under surface of pitcher lids were sparsely glandular, inner surface of the pitcher cavities were partly covered with exposed type of digestive glands whereas the later species have cylindrical stem, petiolate leaves, thick peristome (≥ 1 mm thick), under surface of pitcher lids were densely glandular, inner surface surface of the pitcher cavities were partly covered with overarched type of digestive glands. The population density of* N. gracilis *is greater than that of* N. mirabalis, *each contained 147 and 15 individuals respectively. The low density of the former species was attributed to its strong tendency to grow in wet soil particularly in temporary and permanent inundated secondary vegetation. The population dispersion pattern of seedlings, saplings and matured plants of* N. gacilis *and* N. gracilis *were significantly aggregated.*

PENGENALAN

Nepenthes genus tunggal dalam famili Nepenthaceae adalah salah satu daripada jenis tumbuhan karnivor yang terdapat hidup di dunia ini. Tumbuhan karnivor lain ialah spesies-spesies daripada famili seperti Dorseraceae (*Drosera* spp., *Dionaea* spp – Venus's Fly Trap), Sarraceniaceae (*Sarracenia* spp & *Heliamphora* spp.) dan Lentibulariaceae (*Utricularia* spp.). Tumbuhan karnivor ini terutamanya *Nepenthes* cenderung untuk hidup di kawasan hutan terganggu atau terbuka, tanah yang kurang subur. Struktur periuk atau kantung yang terbentuk pada hujung salur paut daun tumbuhan ini berupaya memerangkap haiwan kecil terutamanya serangga seperti semut, belalang, lipas, anai-anai dan lalat (Adam 1997). Serangga tersebut akan dihurai melalui proses penghadaman oleh cecair mengandungi enzim yang dirembes oleh kelenjar penghadaman dan kelanjar yang sama menyerap nutrien untuk pertumbuhannya (Adam 1997).

Tumbuhan *Nepenthes* mempunyai pelbagai nama tempatan di Malaysia iaitu periuk kera (Melayu Semenanjung Malaysia), somboi-somboi (Melayu Brunei), tetuyud (Melanau, Sarawak), entuyud (Iban, Sarawak) dan kekuanga (Dusun, Sabah). Tumbuhan periuk kera adalah tumbuhan pemanjat dan kadangkala menjalar, dioesius iaitu jambak bunga jantan dan betina terletak pada pokok yang berasingan (Adam 1998; Phillipps & Lamb 1996; Holttum 1954). Kebanyakan spesies periuk kera adalah terestrial (hidup di lantai hutan) tetapi beberapa spesies boleh ditemui hidup sebagai tumbuhan epifit seperti *Nepenthes veitchii, Nepenthes lowii* dan juga akuatik seperti *N. bicalcarata, N. ampullaria* dan *N. mirabilis.* Genus ini diwakili oleh 81 spesies di dunia (Adam 2002b) dan taburannya dilaporkan terhad di kawasan tropika dan subtropika (Adam 1995; Adam *et al.* 1994 & 1992; Smythies 1965). Pusat taburan utama genus ini ialah di Kepulauan Borneo, Sumatra, Filippina, Semenanjung Malaysia, New Guinea (Adam & Wilcock 1996 & 1992; Adam *et al.* 1994 & 1992). Genus ini juga boleh ditemui di New Caledonia, Isles of Pines, Sri Lanka, Seychelles, Madagascar, India, Thailand, Indo-China, China Selatan dan Australia (York Peninsular).

Objektif kajian ini adalah untuk menentukan komposisi spesies *Nepenthes* dan kepadatannya di kawasan kajian, menyediakan peta populasi mengikut peringkat umur dan spesies, mengenal pasti struktur populasi mengikut peringkat umur dan menentukan corak taburan populasi mengikut fasa hidup dan spesies dengan menggunakan Indeks Taburan Populasi Morisita.

BAHAN DAN KAEDAH

Kawasan Kajian

Kajian ini dilakukan di kawasan Hutan Pendidikan Alam, Universiti Kebangsaan Malaysia, Bangi, Selangor Darul Ehsan. Lokasi tapak kajian adalah terletak di lereng bukit di tepi jalan pada altitud 21 m. Kawasan ini dilitupi oleh belukar dan didominasi oleh paku pakis resam, *Dicranopteris linearis,* dan beberapa jenis pokok dan pokok renek dan rendang seperti *Dillenia suffructicosa, Melastoma malabathricum, Vitex pubescens* dan *Mallotus* sp.

Penyediaan Plot

Satu plot berukuran 20 m x 50 m dengan keluasan 0.1 hektar telah didirikan di lereng bukit di tepi jalan raya. Kedudukan plot tersebut ialah pada 02° 55.40' Utara dan 101° 46.93' Timur. Plot tersebut dipecah kepada 10 subplot yang lebih kecil dan setiap satunya berukuran 10 m x 10 m. Pembinaan plot dan subplot ini dilakukan dengan menggunakan alat 'theodolite'. Pembahagian plot tersebut kepada subplot ialah pertama untuk memudahkan kerja pemetaan populasi dan kedua keperluan wajib untuk mengira nilai Indeks Taburan Morisita (Brower & Zar 1977).

Pemetaan Populasi Periuk Kera

Setiap individu tumbuhan periuk kera yang ditemui di dalam setiap subplot ditentukan nama spesiesnya dan ditanda kedudukannya di atas kertas graf pada skala yang sepadan dengan dengan pita pengukur di plot. Spesimen setiap spesies periuk kera berbeza yang direkod dikutip sebagai spesimen baucer herbarium. Spesimen baucer yang diawet kering dengan menggunakan kaedah piawai herbarium akan digunakan untuk pengecaman taksa ke peringkat spesies dengan kekunci kepada spesies periuk kera di Semenanjung Malaysia dan Singapura oleh Shivas (1984). Dalam kerja pemetaan populasi, pengecaman yang tepat amat diperlukan untuk mencapai objektif kajian. Setiap individu tumbuhan periuk kera dalam plot diukur ketinggiannya dan ditentukan peringkat atau fasa hidupnya iaitu anak cambah, juvena dan dewasa. Dewasa juga ditentukan jantinanya kerana tumbuhan periuk kera adalah doiesius iaitu bunga jantan dan bunga betina terdapat pada pokok yang berasingan.

Kepadatan

Kepadatan ditakrif sebagai bilangan individu yang menempati di dalam satu unit luas kawasan (Brower & Zar 1977; Cintron & Novellii 1984). Kepadatan populasi mengikut fasa anak cambah, juvena dan dewasa diperoleh dengan mengira bilangan individu masing-masing dalam plot yang telah ditetapkan keluasanya. Rumusan kepadatan (D) populasi diringkas seperti berikut:

$$Di = ni / A$$

Dengan, Di = Kepadatan spesies i
ni = Bilangan individu spesies i
A = Luas kawasan sampel

Indeks Taburan Populasi

Corak taburan populasi tumbuhan *Nepenthes* dikira dengan menggunakan Indeks Taburan Populasi Morisita (I_d) dan perbezaan corak taburan cerapan daripada rawak diukur dengan

menggunakan Ujian Khi-Kuasa Dua (c^2) (Brower & Zar 1977).

Indeks Taburan Populasi Morisita (I_d) = n ΣX^2-N/N(N-1)

Dengan n: Bilangan subplot; N: Jumlah hitungan individu dalam semua n subplot dan; ΣX^2 : Jumlah Kuasa Dua Hitungan Individu dalam satu subplot.

Jika I_d = 1, corak taburan ialah rawak, I_d= 0, corak taburan ialah seragam; dan I_d = n, corak taburan ialah berkelompok. Perbezaan signifikan nilai I_d daripada rawak boleh dirumus dengan Ujian Khi Kuasa Dua (χ^2) (Brower & Zar 1977) seperti di bawah:

$$\chi^2 = n\Sigma X^2/N - N$$

dengan n: Bilangan subplot; N: Jumlah hitungan individu dalam semua n subplot dan; ΣX^2: Jumlah Kuasa Dua Hitungan Individu dalam satu subplot.

Jika χ^2 dikira lebih besar daripada $\chi^2_{\alpha = 0.05, Df=n-1}$, tolak hipotesis nol (Ho: Taburan Populasi adalah rawak). Jika I_d < 1 or >1, rumusannya ialah corak taburan populasi adalah seragam dan berkelompok masing-masing.

HASIL DAN PERBINCANGAN

Komposisi dan Kepadatan Populasi Spesies

Daripada kajian ini, dua spesies dijumpai di dalam plot kajian dengan keluasan 0.1 hektar. Spesies-spesies tersebut ialah *Nepenthes gracilis* Korthals dan *Nepenthes mirabilis* (Loureiro) Druce. *Nepenthes gracilis* boleh dibezakan daripada *N. mirabilis* berdasarkan kepada pemerhatian sifat morfologi di lapangan. *Nepenthes gracilis* mempunyai bentuk batang yang bersudut atau segi tiga, daun yang tidak bertangkai, pangkal daun yang bersayap, bentuk periuk tubulos-ventrikos, bibir mulut periuk yang nipis, kelenjar penghadaman pada permukaan dalam periuk yang terdedah, taburan kelenjar madu pada permukaan bawah tudung periuk yang sangat jarang. Manakala itu, *Nepenthes mirabilis* pula mempunyai bentuk batang yang bulat, daun bertangkai, pangkal tangkai daun yang tidak bersayap, bentuk periuk tubulos-infundibulat, bibir mulut yang mendatar dan lebar, kelenjar penghadaman pada permukaan dalam periuk terlindung, taburan kelenjar madu pada permukaan bawah tudung periuk yang banyak dan rapat. Sifat-sifat tersebut di atas boleh dilihat dengan mata kasar dan juga kanta tangan (X10). Dalam kajian ini pengecaman spesies yang tepat adalah penting untuk mencapai objektif-objektif lain kajian ini.

Kedua-dua spesies tersebut tergolong ke dalam kumpulan spesies tanah rendah (Adam 2002a & 2002b; Kurata 1976) dan kerap ditemui dari paras laut hingga ke 100 m altitud. Bilangan spesies periuk yang boleh ditemui pada satu komuniti periuk kera lazimnya terdiri daripada 1 hingga 5 spesies (Adam 2002a; Selle Suep 2002; Nazuha Alias 2002; Nor Atiza Mohamed Akil 2002; Tang Chun Keat 2002; Som 1988).

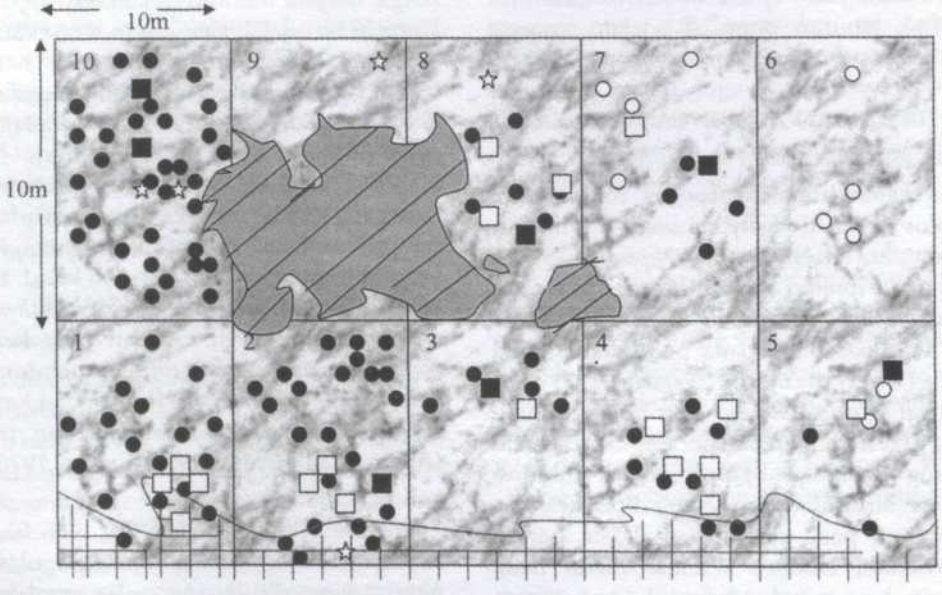
Kepadatan ditakrif sebagai jumlah bilangan individu yang hadir dalam satu unit luas kawasan tertentu (Brower & Zar 1977; Cintron & Novellii 1984; Safiah Salwana Mohd Noor 1992). Daripada keluasan plot 0.1 ha (20 m x 50 m), kepadatan tumbuhan periuk kera di kawasan kajian mempunyai 147 individu *Nepenthes gracilis* dan 15 individu *Nepenthes mirabilis*. Hasil tersebut jelas menunjukkan kepadatan *Nepenthes gracilis* melebehi kepadatan *Nepenthes mirabilis* iaitu 90.74% berbanding 9.26%. *Nepenthes gracilis* sangat berjaya tumbuh di kawasan kajian kerana kawasan ini adalah kawasan hutan sekunder yang sederhana curam dan senantiasa berkeadaan kering iaitu tidak digenangi oleh air pada keadaan biasa. Keadaan habitat seperti ini adalah faktor penghalang untuk pertumbuhan *Nepenthes mirabalis* yang sangat cenderung menjana dengan baik pada habitat terbuka dengan keadaan tanah yang senantiasa lembap dan digenangi oleh air bersifat sementara atau berpaya kekal. Para penyelidik lepas melaporkan *Nepenthes gracilis* ditumbuhi hidup di kawasan yang kering dan *Nepenthes mirabilis* lebih mudah mendominasi di kawasan berpaya yang terbuka atau kawasan air yang bertakung dan berlopak dan bercahaya (Adam *et al.* 1994 & 1992; Kurata 1976).

Pemetaan Populasi Periuk Kera

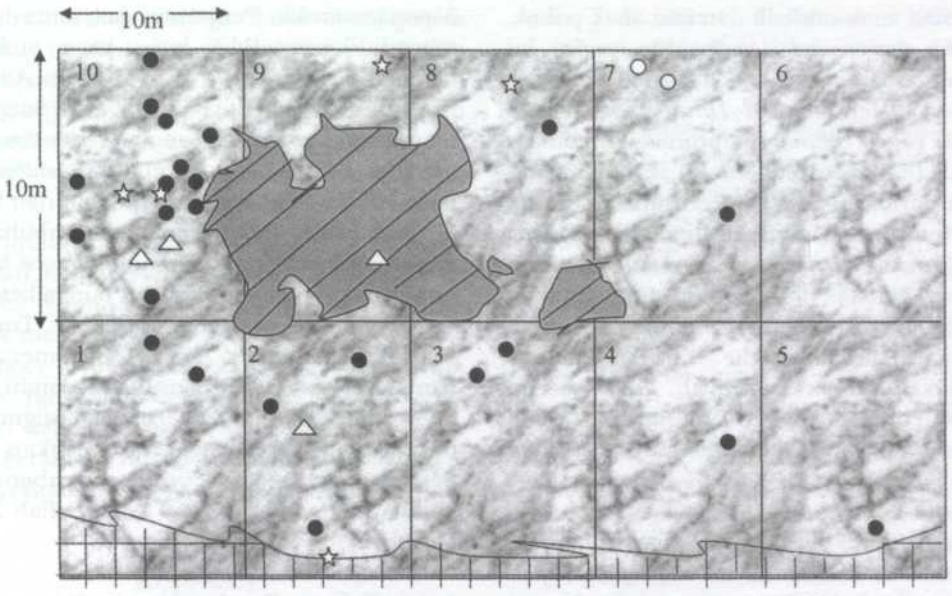
Rajah 1 menunjukkan taburan populasi dewasa *Nepenthes gracilis* dan *Nepenthes mirabilis* dalam plot kajian. Kedua-dua spesies tersebut didapati tidak dapat menjana pada kedudukan subplot 9 kerana kawasan ini berbatu dan mempunyai permukaan tanah yang berkematu berbanding dengan 9 subplot yang lain. Adam (2002c) juga mendapati, *Nepenthes villosa* di Gunung Kinabalu, sukar untuk tumbuh dengan baik di kawasan yang mempunyai permukaan batuan yang

terdedah dan juga pada kawasan kedalaman tanah yang cetek. *Nepenthes gracilis* banyak ditemui pada subplot 10, 2 dan 1 masing-masing dengan 32, 22 dan 17 tumbuhan dewasa. Tumbuhan dewasa *N. gracilis* dalam ketiga-tiga plot ini mewakili fasa lewat sesaran. Pokok dewasa ini mampu bersaing dengan belukar paku-pakis *Dicranopteris linearis* untuk mendapat cahaya dengan memanjat di celah-celah belukar dan pada pokok renek *Dillenia suffructicosa.* Populasi dewasa *Nepenthes gracilis* ini pada satu masa yang akan datang akan tersesar keseluruhannya apabila habitat hutan sekunder ini tersesar oleh pokok-pokok cepat membesar seperti *Macaranga, Mallotus, Dillenia suffructicosa, Vitex pubescens* dan lain-lain. *Nepenthes gracilis* dilaporkan sebagai tumbuhan perintis kawasan cerang baru pada tanah rendah di kawasan tropika (Green, 1967). *Nepenthes mirabilis* didapati tumbuh di kawasan subplot tertentu sahaja iaitu di subplot 5, 6 dan 7. Tiga subplot tersebut mempunyai kawasan permukaan tanah yang mampu menakung air sementara dan keadaan seperti ini merangsang pertumbuhan *Nepenthes mirabilis.*

Populasi *Nepenthes gracilis* juga berpotensi untuk terus hidup kerana 17 dan 7 tumbuhan dewasanya menghasilkan bunga jantan dan bunga betina masing-masing. Biji benih yang dihasil oleh proses persenyawaan mampu untuk bercambah jika habitat asalnya terubah suai menjadi kawasan terbuka kerana gangguan aktiviti manusia dan kematian belukar paku-pakis *Dicranopteris linearis. Nepenthes mirabilis* berupaya membiak kerana populasi dewasanya juga didapati boleh menghasil bunga jantan dan bunga betina. *Rajah 2* jelas menunjukkan anak benih *Nepenthes gracilis* boleh bercambah menghasil populasi anak cambah dan membesar menjadi anak pokok pada subplot 1, 2, 3, 4, 5, 7, 8 dan 10. Cerapan ini menunjukkan anak benih *Nepenthes gracilis* dan *Nepenthes mirabilis* boleh bercambah di kawasan luang, celah dan sisi atau pinggir belukar paku-pakis *Dicranopteris linearis* yang boleh ditembusi oleh cahaya matahari. Spesies periuk kera amat sukar untuk hidup di kawasan yang terlindung daripada cahaya matahari seperti hutan berkanopi rapat pada tanah rendah seperti hutan dipterokap (Adam



Rajah 1: Taburan populasi dewasa Nepenthes gracilis dan Nepenthes mirabilis



Petunjuk :

- Anak cambah *N. gracilis*
- Anak cambah *N. mirabilis*
- Anak pokok *N. gracilis*
- Pokok Rendang *Dillenia suffructicosa*
- Batuan
- Rumput renek
- Belukar Pakis *Dicranopteris linearis*

Rajah 2: Taburan populasi anak cambah dan anak pokok Nephentes gracilis dan Nepenthes mirabilis

2002a & 2002 b; Adam 1995). Kajian oleh Adam (2002c) juga mendapati spesies seperti *N. villosa* tumbuhan pada kawasan hutan tanah tinggi yang mempunyai kanopi yang jarang dan juga kawasan yang separa terbuka di atas batun ultrabes di Gunung Kinabalu.

Struktur Populasi Periuk Kera

Peringkat hidup tumbuhan periuk kera boleh dikelaskan kepada fasa anak cambah, fasa juvena dan fasa dewasa (Adam 2002a; Safiah Salwana Moh Noor 1992; Nor Atiza Mohamed Akil 2002; Nazuha Alias 2002). Fasa anak cambah adalah fasa roset dengan susunan daun yang rapat dan bertindih (<10 cm panjang); fasa juvena diwakili oleh tumbuhan menegak, mempunyai antara ruas yang jelas dan hujung salur paut membentuk periuk atau kantung lantai sahaja. Fasa dewasa adalah fasa tumbuhan yang mampu memanjat di atas pokok renek, belukar sebagai sokongan, membentuk periuk lantai pada bahagian bawah tumbuhan dan periuk udara pada bahagain atas tumbuhan. Tumbuhan dewasa juga berpotensi menghasilkan bunga dan buah pada hujung batangnya. Dalam semua spesies *Nepenthes*, periuk lantai membentuk sepasang sayap pada bahagian depannya dan sayap tersebut bertukar menjadi tetulang yang tebal pada periuk udara.

Jadual 1 menunjukkan kepadatan tumbuhan dewasa mengatasi kepadatan tumbuhan juvena dan anak cambah bagi *Nepenthes gracilis* dan *Nepenthes mirabilis*. Populasi kedua-dua spesies ini mewakili fasa lewat sesaran. Mengikut Adam (2002a) melaporkan populasi spesies tumbuhan periuk kera fasa lewat sesaran terdiri daripada banyak individu dewasa berbanding bilangan

individu fasa anak cambah dan fasa anak pokok. Tumbuban dewasa bagi kedua-dua spesies ini adalah tumbuhan perintis pada sesaran sekunder dan tersesar oleh belukar *Dicranopteris linearis* dan pokok renek *Dillenia suffructicosa*. Tumbuhan paku-pakis *Dicranopteris linearis* ini akan menyesar komuniti periuk kera dan seterusnya menutupi kawasan tersebut. Tumbuhan dewasa kedua-dua spesies periuk kera boleh bersaing dengan tumbuhan belukar lain untuk mendapatkan cahaya dengan cara memanjat dan terdedah di atas permukaan belukar dan seterusnya menghasilkan bunga dan buah. Kejayaan ini ditunjukkan oleh sebanyak dua puluh enam serta tiga tumbuhan dewasa *Nepenthes gracilis* dan *Nepenthes mirabilis* boleh menghasilkan jejambak bunga jantan dan bunga betina atau buah. Biji benih yang dihasil oleh buah kedua-dua spesies periuk kera ini disebar di kawasan plot kajian dan pada mikrohabitat yang sesuai iaitu dengan cahaya dan kelembapan yang cukup boleh merangsang percambahan dan pertumbuhannya. Bukti kejayaan komuniti ini boleh terus hidup ialah dengan kehadiran sebanyak 25 dan 2 fasa anak cambah dan juvena *Nepenthes gracilis* dan *Nepenthes mirabilis* masing-masing. Bagi *Nepenthes mirabilis*, kepadatannya yang sangat rendah kerana kawasan yang tidak sesuai untuk pertumbuhannya berbanding kepadatan *Nepenthes gracilis* yang sangat tinggi. Mikrohabitat dengan kehadiran lopak terhad dalam plot kajian menghalang perkembangan dan penjanaan *Nepenthes mirabilis*. Penjelasan yang sama diperoleh penyelidik-penyelidik lepas yang melibatkan kedua-dua spesies yang sama (Nazuha Alias 2002; Nor Atiza Mohamed Akil 2002). Selle Suep (2002) mendapati kepadatan populasi *Nepenthes mirabilis* mengatasi kepadatan populasi *Nepenthes gracilis* pada habitat yang senantiasa basah dan lembap.

Kajian ini juga mendapati tumbuhan yang menghasilkan bunga jantan mengatasi bilangan tumbuhan yang menghasilkan bunga betina bagi kedua-dua spesies tersebut (Jadual 1). Tumbuhan yang dioesius seperti periuk kera memerlukan banyak bunga jantan untuk menjamin pemindahan debunga kepada permukaan stigma bunga betina dan seterusnya menentukan proses persenyawaan gamet untuk membentuk biji benih yang subur (Adam 1998; Safiah Salwana Mohd Noor 1992).

Indeks Taburan Populasi Morisita (I_d) dan Khi Kuasa Dua (χ^2)

Pengiraan yang dibuat mengikut rumus Indeks Morisita (Brower & Zar 1977) mendapati corak taburan fasa anak cambah dan dewasa bagi *Nepenthes gracilis* dan *Nepenthes mirabilis* adalah signifikan berbeza daripada corak taburan rawak. Nilai I_d bagi dua fasa hidup kedua-dua spesies ini melebehi nilai 1 iaitu berjulat antara 1.667 hingga 10 menunjukkan corak taburan berkelompok (Jadual 2). Corak taburan berkelompok populasi kedua-dua spesies ini bagi fasa anak cambah dan dewasa adalah

JADUAL 1
Struktur populasi *Nepenthes gracilis* dan *Nepenthes mirabilis*

Spesies (Kepadatan/0.01 ha)	Anak cambah	Juvena	Dewasa	♂	♀
Nepenthes gracilis (121)	23	3	95	19	7
Nepenthes mirabilis (12)	2	0	10	2	1

JADUAL 2
Taburan populasi *Nepenthes gracilis* dan *Nepenthes mirabilis* mengikut fasa hidup

Spesies	Fasa Hidup	I_d	χ^2	$\chi^2_{0.05,9}$	Corak Taburan
Nepenthes gracilis	Matang	2.051	189.907	16.919	Kelompok
	anak pokok	1.667	11	16.919	Kelompok
	anak cambah	2.806	48.7391	16.919	Kelompok
Nepenthes mirabilis	Matang	2.889	26	16.919	Kelompok
	anak pokok	tiada	Tiada	Tiada	Tiada
	anak cambah	10	18	16.919	Kelompok

I_d = 1, taburan adalah rawak; I_d = 0, taburan adalah seragam; I_d > 1, taburan adalah berkelompok; Jika nilai χ^2 yang dicerap melebihi nilai $\chi^2_{0.05,9}$ =16.919, maka hipotesis nol ditolak.

dipengaruhi faktor cahaya. Tumbuhan periuk kera sukar untuk hidup di kawasan plot kajian yang menerima keamatan cahaya yang rendah akibat daripada lindungan tumbuhan seperti paku-pakis *Dicranopteris linearis.*

PENGHARGAAN

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Differential Responses in Growth, Physiological Processes and Peroxidase Activity of Young Mango (*Mangifera indica*) and Citrus (*Citrus sinensis* L) Plants to Water Deficit

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Keywords: Water deficits, mango(*Mangifera indica*), citrus *(Citrus sinensis)*, water relations, stomatal conductance, peroxidase activity

ABSTRAK

*Pengaruh tegasan air terhadap pertumbuhan, proses fisiologi, aktiviti prolina dan peroksida telah dikaji pada tanaman mangga (*Mangifera indica*) dan limau (*Citrus sinensis*) didalam rumah tanaman. Dalam keadaan pengurangan air secara berperingkat, pokok mangga dan limau menunjukkan perbezaan dalam potensi air, konduksi stomata, perkembangan daun dan aktiviti peroksida. Perubahan stomata tidak bergantung pada perubahan potensi air daun pada kedua-dua tanaman apabila tegasan air berlangsung. Pengurangan konduksi stomata pada pokok mangga adalah lebih tinggi dibandingkan dengan limau, menunjukkan bahawa limau berupaya mengawal kehilangan air terhadap perubahan kedapataan air. Aktiviti peroksida menunjukkan peningkatan bererti dalam keadaan pengurangan air tanah bagi tanaman limau. Terdapat peningkatan di antara 6 hingga 10 kali ganda kandungan prolina bagi tanaman mangga dan limau apabila pokok didedahkan kepada tegasan air. Peningkatan prolina yang ketara bagi tanaman limau berbanding mangga menunjukkan ketahanan tegasan air yang tinggi tanaman limau berbanding mangga. Bukti ini diperkukuhkan dengan pemulihan semula yang cepat selepas pemberian air kepada pokok limau dibandingkan pokok mangga melibatkan regenerasi pucuk baru yang cepat.*

ABSTRACT

*The effects of water deficit on growth, plant physiological processes and peroxidase activity were studied for young mango (*Mangifera indica*) and citrus (*Citrus sinensis *L) plants in the greenhouse. Under gradually decreasing soil moisture content, mango and citrus differed in their leaf water potential, stomatal conductance, leaf growth and peroxidase activity. Stomata of both plants responded independently to the changes in leaf water potential as soil drying progressed. The reduction in stomatal conductance in mango was greater than citrus suggesting that citrus was able to control water loss better than mango to the changing condition of water availability in the root zone. Peroxidase activity increased significantly in water stressed citrus plants. There was a 6-10 fold increase in proline content when both species were exposed to water stress. Citrus plants accumulated higher proline levels suggesting that they can tolerate water stress compared to mango. This was also evident by a faster recovery after rewatering in citrus compared to mango plants that involved regeneration of new shoots.*

INTRODUCTION

Water deficit is one of the environmentally limiting factors for fruit tree establishment in the field from the nursery. Roots of young fruit trees are often not fully developed at transplanting so a small degree of water deficit can cause plant water stress and mortality. Although Malaysia is tropical and characterized by adequate sources of water, the incidence of water shortage occurs from time to time in some of the agricultural production areas. The problem of plant mortality is often serious in conditions where cultivation is to be done in undulating areas where there are difficulties in installing a proper irrigation system.

Plants usually developed physiological responses as well as ecological strategies to cope with water stress. These responses allow them to

survive and to sustain some growth under adverse conditions. Plants' responses depend on the species differences, nature of water shortage inducing physiological responses to short term changes, acclimation to a certain level of water availability and adaptation to drought (Jones 1983; Ruiz-Sanchev *et al.* 2000). Plant adaptability to localized water deficit has been attributed mainly to maintenance of water status by utilizing available soil and by chemical signaling. Various metabolites and chemicals have been observed to accumulate in plant tissues during water and salt stress and contribute to osmotic adjustment. Some species that adapt to mild and/or moderate drought stress exhibit increases in activities of antioxidant enzymes such as super oxide dismutase, catalase and peroxidase (Fu and Huang 2001). Although the concentration of some amino acids increase during water stress, there is general agreement that proline concentration is profound (Kohl *et al.* 1991; Albernethy *et al.* 1998). The information gathered on drought resistance mechanism makes it easier to plan for crop zoning based on the water availability and to designed irrigation strategies for optimizing water used.

Mango and citrus are two of the fifteen fruit species that had been identified for future development as stated in the Third National Agricultural Policy 1998-2010 (Ministry of Agriculture Malaysia 1999). The area of cultivation is to be expanded and these crops will be cultivated on a large commercial scale throughout Malaysia. The aim of this study was to determine the growth and physiological responses of young mango and citrus plants exposed to water deficit, as well as to improve understanding on the drought tolerance mechanism involved in the responses of these fruit trees to water deficit. The role of proline and peroxidase in drought tolerance mechanism in both species was determined. The response of plant on recovery to water stress was assessed by the ability of plants to rejuvenate their vegetative stage.

MATERIAL AND METHODS

Eighteen months mango (*Mangifera indica*) cultivar Chukanan and fourteen months citrus (*Citrus sinensis L*) cultivar Limau Madu were grown in plastic containers containing 40 kg of soil mixtures. The plants were grown in well-watered conditions for 6 weeks prior to water stress treatments allowing roots to grow and become established in the bottom section of the containers. During this 42 –day period, plants were watered daily until water drained freely from the drainage holes at the bottom and fertilized weekly with full strength of Cooper Solution (Cooper 1973). The experiment was conducted at the Department of Crop Science, Universiti Putra Malaysia, Serdang, Selangor.

The experiment consisted of two soil moisture treatments; control (well watered) and water stress. In the well-watered control, plants were irrigated daily until water drained freely. In water stress treatments, the whole soil in the container was allowed to dry down by withholding irrigation. Each group of watering treatments consisted of twenty plants arranged in a completely randomized design with four replicates. At each harvest, 4 plants were sampled for destructive samplings of leaf water potential and peroxidase determination. Soil moisture content was measured with a time domain reflectometry technique (Topp *et al* 1980). After the drying cycle was completed, plants grown under water stress condition were irrigated to observe the potential of recovery on both plants species.

Leaf length increment was determined on the leaves that were tagged before treatments began. The leaf length increments were calculated at each sampling date by measuring the differences between the length on the sampling date and the initial measurements expressed in rate of leaf increment per day. The differential recovery of mango and citrus were assessed by regeneration of new shoots from the plants.

The onset of responses of water deficit was observed by measuring leaf water potential at mid-day from one young fully expanded leaf per plant and four plant plants per treatment, using a pressure chamber, following the recommendation of Turner (1986). Leaf stomatal conductance and net photosynthetic rate were measured at mid-day for a similar number and type of leaves as for leaf water potential. Stomatal conductance was measured on the abaxial surface of leaf using a diffusive porometer (AP-4, Delta-T Devices Ltd, Cambridge, UK). The photosynthesis rate was determined using a LCA-3 portable infrared gas analyzer (LCA3, Analytical Development Co. Hoddesdon, UK).

Peroxidase activity in the leaf tissue was determined on the youngest fully-developed leaf

sampled from both plant species. 0.5 g of leaf tissue was homogonised with a mortar and pestle, using 1 ml cold 0.05M sodium acetate buffer (pH5.0). Five milligrams of polyvinylpyrrolidone (PVPP) was added to each sample in order to decrease interaction of phenol-proteins during extraction. The homogenate was centrifuged at 14000 Xg for 20 minutes at 4-6 C. The supernatant was used to determine the enzyme activities. Extraction of ionically bound peroxidase was performed by re-homogenizing the pellets from the above extraction with the same buffer containing 1M sodium chloride (NaCl). Samples were incubated at 4-5 °C for 24 h and centrifuged as described above. The peroxidase activities of both soluble and ionically bound supernatant were determined. A 20 ml sample of the supernatant was added to 3 ml of the assay mixture, which consisted of a solution of 0.1M sodium phosphate buffer (pH6.0), 1mM hydrogen peroxide (H_2O_2) and 0.1mM)-methoxyphenol (guaiacol). The increase in absorbance density at 470nm was recorded with a spectrophotometer (Spectronoc 20 Genessys) and the enzyme was expressed as the change in absorbance per minute per gram fresh weight. Determination of free proline levels was based on the method described by Bates *et al.* (1973). Proline was extracted from liquid nitrogen – frozen tissue by homogenizing 5 g of the sampled leaves with 10 ml of 3% sulfosalicylic acid at 25 C. The homogenate was filtered through Whatman No 2 filter paper. Two ml of the filtrate was reacted with two ml of glacial acetic acid and two-ml acid ninhydrin in a test tube for one hour in a water bath at 95 °C. The reaction mixture was then cooled in an ice bath. Following that, 4 ml of toluene was added to the reaction mixture and mixed rigorously with a test tube stirrer for 20 seconds. The toluene layer on the top, which has a pink-red color, was collected with a pipette. The absorbency of the toluene layer was read at 520 nm with a spectrophotometer using toluene as a blank. Standard curve was produced ranging 0 to 30 ug/ml of L-proline (Sigma chemical Co. St Louis, Mo.) dissolved in 3% sulfosalicylic acid. Proline standard curve was used to calculate proline concentration in sample on fresh weight basis.

RESULTS

Fig. 1 shows changes in soil moisture content and rate of leaf length increment as influenced by withholding water for 15 days. Soil moisture declined from 0.26 to 0.12 g cm^{-3} by withholding water for 15 days in the containers. This reduction in soil moisture content had significantly reduced the rate of leaf length increment and plant physiological processes. Under well-watered conditions, leaf length increment was higher for mango compared to citrus plants (*Fig. 2*). The well-watered mango plants grew steadily for the first six days of plants in the treatments. Thereafter, leaf expansion declined or plateaud as the leaf reached its maximum growth. The rate of leaf increment of water-stressed plants declined progressively after day 3 of withholding water. The reduction in the rate of leaf length increment in water-stressed mango plants had resulted in a reduction of 8.84 cm in the final leaf length compared to well watered plants. In citrus, final leaf length was 2.1 cm shorter in water-stressed compared in the well watered plants. After rewatering, regeneration of shoots was faster in citrus than in mango plants (*Fig.* 3).

Fig. 4 shows changes in leaf water potential, stomatal conductance and photosynthetic rate of mango and citrus plants as influenced by water stress. Leaf water potential shows a significant difference between these two plant species. On the onset of water stress, leaf water potential of citrus declined rapidly compared to mango plants. Lower leaf water potential values in citrus were also observed for plants grown in well-watered conditions. The change in stomatal conductance during water stressed period followed quite a different pattern in comparison to the leaf water potential. This reduction in leaf water potential had caused similar reduction in stomatal conductance and leaf photosynthesis rate of plants exposed to water stress. The reduction in stomatal conductance was greater in mango than citrus plants. By day 6, stomatal conductance was reduced by 84% and 54 % in water stressed mango and citrus, respectively. Further exposure to water stress, both mango and citrus reached the same stomatal conductance values. The photosynthetic rate declined progressively with increased duration of water stress in both mango and citrus plants. There were significant reductions in the photosynthetic rates in both water-stressed mango and citrus plants when measurements were made on day 3 after withholding water. The results showed similar trends as observed in stomatal conductance where photosynthetic rate started to reach its

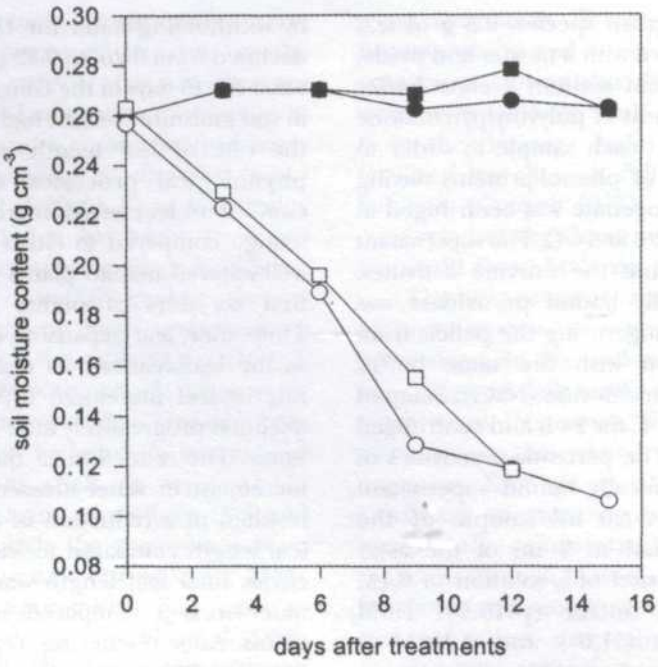

Fig. 1: Bulk soil water content in soil supporting plants under well-watered (closed symbol) and water stressed (open symbol) for mango (0) and citrus (□). Means of SE ± 3 replicates

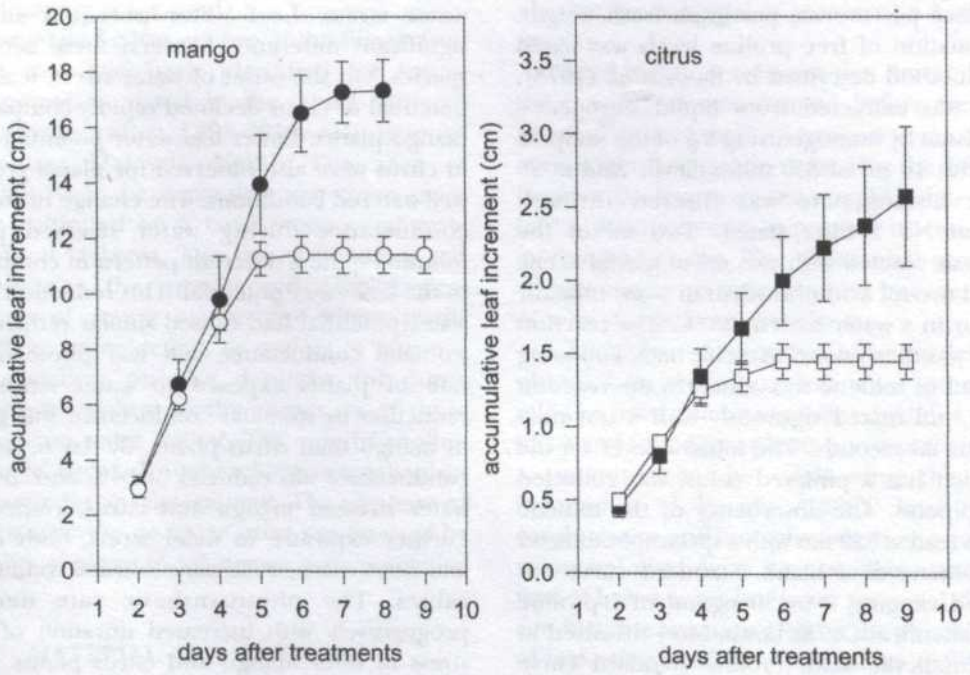

Fig. 2: Accumulative leaf length increment of mango (o) and citrus leaves (□) which were either well-watered (closed symbol) or water-stressed (open symbol). Bars represent ±SE. of five replicates

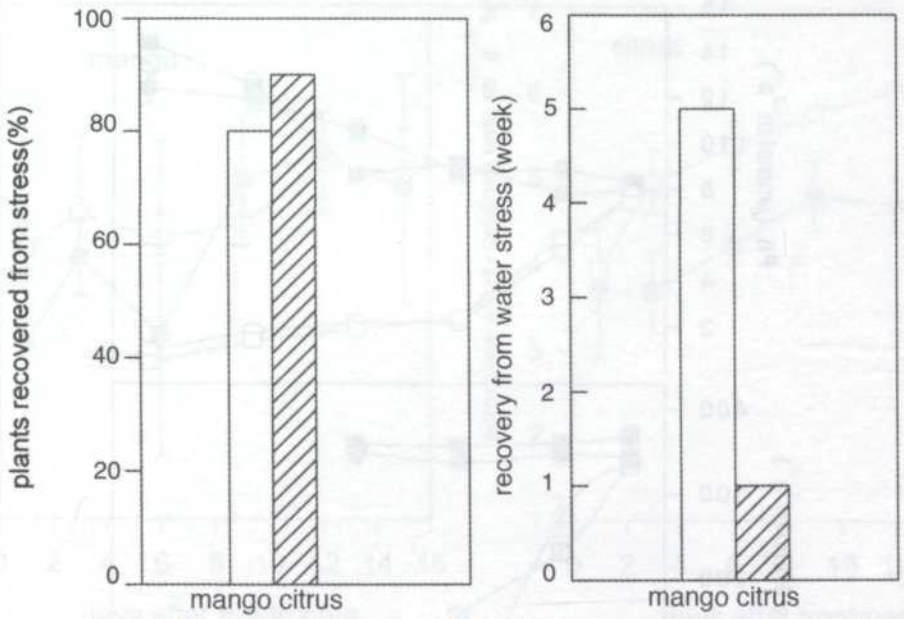

Fig. 3: Effects of re-watering of plants on percentage and duration for recovery in mango and citrus plants. Data was based on observation of plant numbers recovered from pre-stress treatment and no SE of mean presented

minimum values after 6 days of withholding water. Thereafter, the photosynthetic rate remained lower and reached the lowest values after prolonged exposure to water stress in both mango and citrus plants.

Measurements on peroxidase activity show significant higher values observed in water-stressed citrus compared to mango plants (*Fig. 5*). In both species, proline content increased with exposure to water stress. There were marked differences in proline content between citrus and mango in both well-watered and water-stressed conditions. Higher concentration of proline was observed in citrus compared to mango plants. There was a 6-10 fold increase in proline content when both species were subjected to water stressed conditions.

DISCUSSION

Leaf growth was very sensitive to the reduction in soil water availability. Rapid reduction in leaf increment suggests that available water is one of the major factors that determines growth of mango plants at establishment in the field. Nonami and Boyer (1989) suggest that leaf growth could be inhibited at low water potential despite complete maintenance of turgor in the growing region. However, the explanation for this situation of growth inhibition might be metabolically regulated through osmotic adjustment or cell wall associated enzymes possibly serving an adequate role by restricting the development of transpiring surface areas. Nevertheless, leaf growth of these plants species is sensitive to soil drying. In retrospect, leaf growth can be influenced through the mechanism of non-hydraulic factor synthesis from the roots (Bacon *et al.* 1998) or inhibition of leaf growth as a result of decrease in cell wall extensibility (Lu and Newman 1998). The threshold value of around -1.20MPa and –0.95MPa can be observed below which leaf increment was almost zero in citrus and mango, respectively.

In this study, leaf water potential was used as an indicator for water stress in both plant species. The decline in water availability by withholding water had significantly reduced leaf water potential and stomatal conductance on both plant species. In the water-stressed plants, minimum leaf water potential at midday was around –2.5 MPa and -2.0 MPa for citrus plants, respectively. The values of leaf water potential measured here for stressed plants were comparable with those usually reported for other citrus species under severe stress in different climates (Kaufman and Levy 1976; Levy 1983). As shown in *Fig. 4*, stomatal conductance was higher in mango than citrus plants in well-

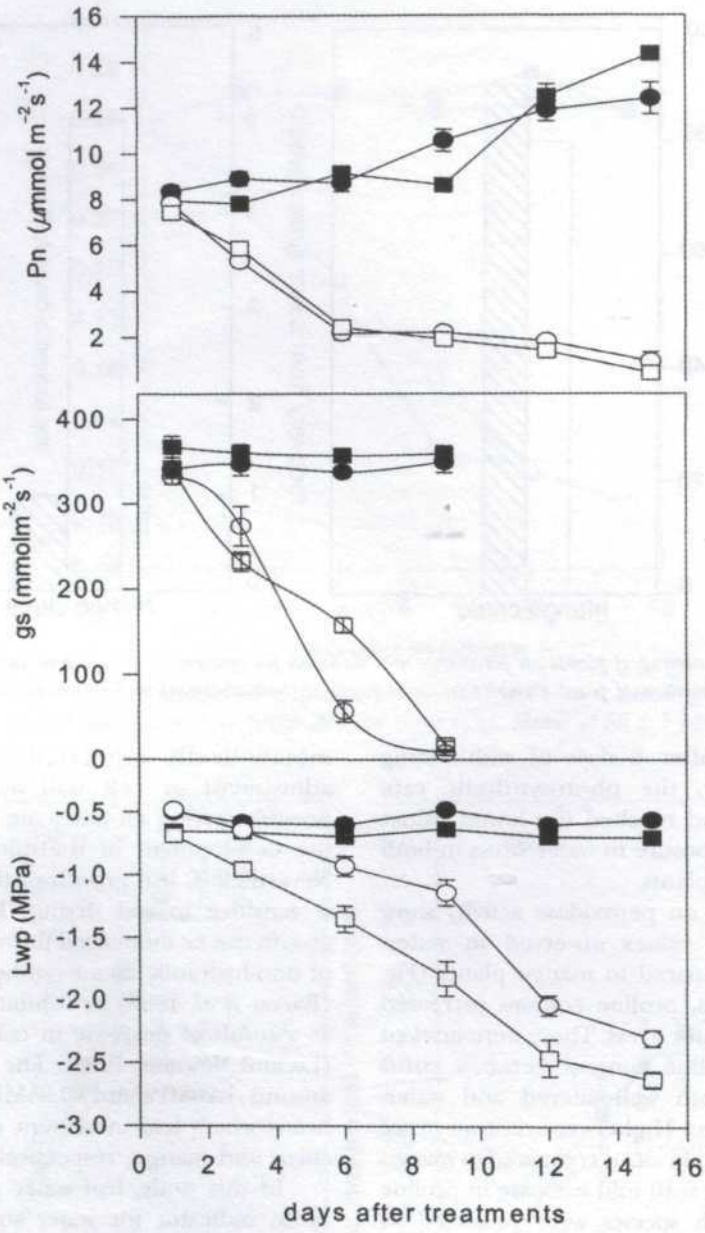

Fig. 4: Changes in leaf water potential (Lwp). stomatak conductance)gs) and photosynthesis rate (pn) of mango (o) and citrus (□) which were either well watered (closed symbol) and water stress (open symbol). Bars represent ± SE of 4-5 replicates

watered plants. Both plant species showed no significant differences in stomatal conductance between stressed and control plants at 1 d after withholding water. Imposition of water stress had resulted in a further reduction in stomatal conductance. The differences between both plant species were obvious on days 6 and 9 of imposing stress with a higher stomatal conductance recorded on citrus compared to mango plants. This could explain findings from the present study that show a greater drought tolerance and recovery at a faster rate after rewatering in citrus compared to mango plants. The results agreed with the findings on drought tolerance of *Eucalyptus* clones that show that plants that have strong stomatal control of water loss enables rapid responses to changing conditions of water availability and possessed characteristics of

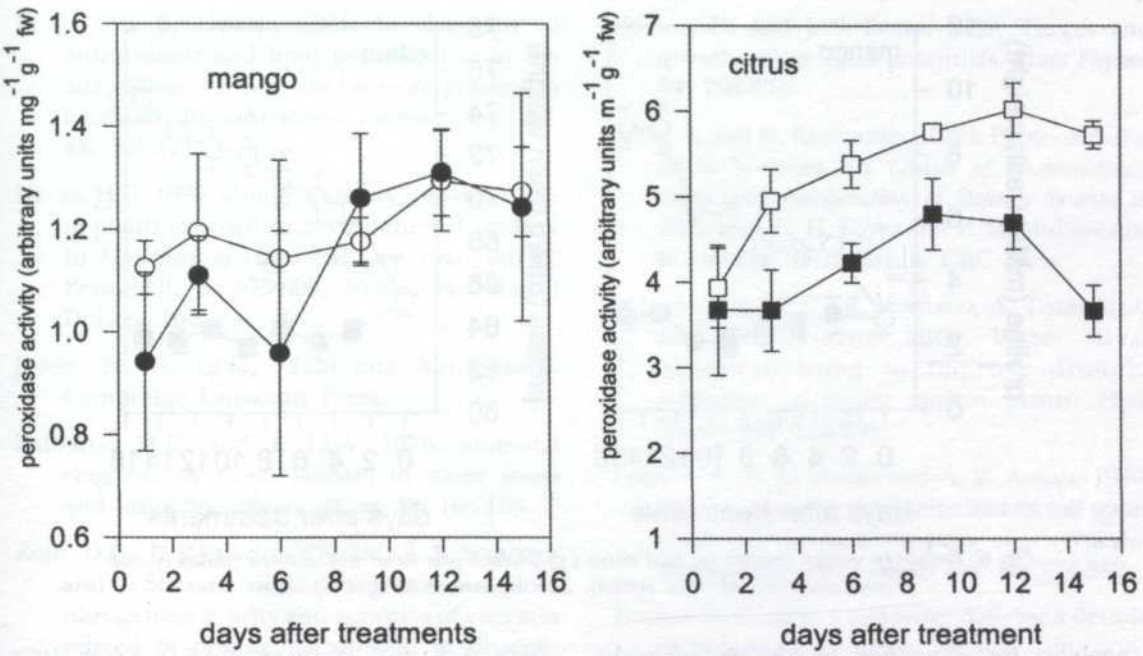

Fig. 5: Peroxidase activity in mango (o) and (□) citrus which were either well watered (closed symbol) and water stressed (open symbol). Bars represent ± SE of four replicates

tolerance to environmental stresses(Farrell *et al.* 1996). The more drought tolerant species control stomatal function to allow some carbon fixation at stress, thus improving water use efficiency or open stomata rapidly when water deficit is relieved (Yordanov *et al.* 2000). There was a similar trend of reduction in photosynthetic rate as in stomatal conductance with exposure of plants to water deficits. The results clearly indicate that stomatal limitation had contributed to a decrease in the photosynthesis rate. This is in agreement with Farquhar *et al.* (1989) who indicated that stomatal factors are more important than non-stomatal factors in affecting photosynthesis rate under water deficit, mainly because of leaf stomatal heterogeneity.

Water deficit can result in an increased production of reactive oxygen species and therefore requires elevated levels of antioxidants for stress compensation. The ability of plants to overcome the effects of different stresses and to sustain their productivity may be related to increased enzymes such as super oxide dismutase, peroxidases and reductases (Polle and Rennenberg 1994; Awad *et al.* 2000). The response of increase peroxidase activity with water stress was clearly evident in citrus plants. In contrast, the present study shows that water deficit did not cause a significant increase in peroxidase activity in mango plants. Our results on the insignificant effects of water stress to caused elevation of peroxidase activities agreed with other findings (Zhang and Kirkham 1996; Brown *et al.* 1995; Fu and Huang 2001). Both citrus and mango plants were found to accumulate proline when exposed to increasing water stress (*Fig. 6*). Proline is considered to be involved in the adaptation mechanism in drought stress. The accumulation of proline in plants under identical stress condition is species-specific (Heuer 1999). In Citrus macrophylla seedlings, proline accumu-lation was proportional to the severity of water stress and not to leaf water potential (Levy 1983). As proline plays an osmoregulatory role in plants, species that can accumulate higher proline levels during water stress can be considered more tolerant to water stress. Our data suggest that citrus accumulated higher proline and tended to survive water stress more readily and regenerate shoots rapidly following stress relief compared to mango plants.

The present study shows the differential plant responses to water deficit for both mango and citrus plants. This is important in optimizing water resources for cultivation of these plants in the field conditions. A proper water management needs to be adopted in establishing mango plants to avoid plant desiccation and mortality. The

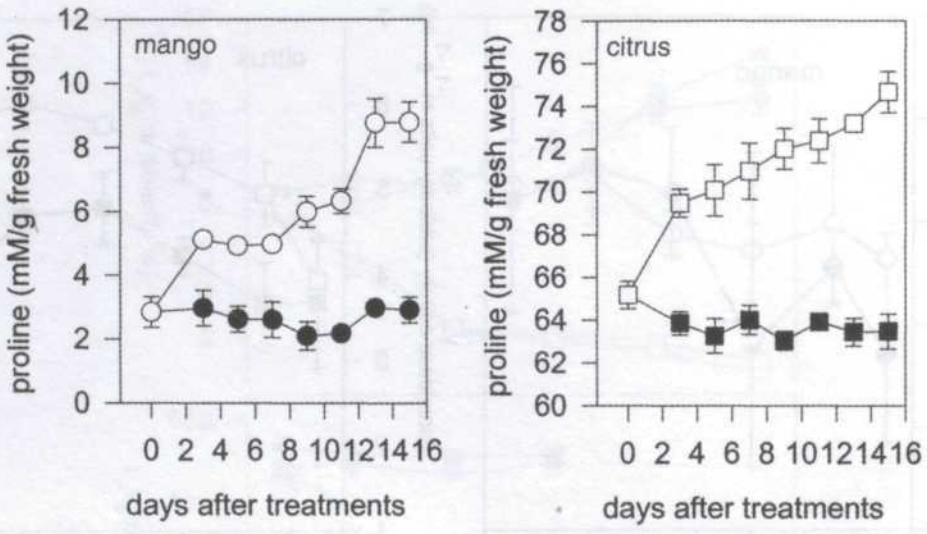

Fig. 6: Proline in mango (mango (o) and citrus (□) which was either well watered (closed symbol) and water-stressed (open symbol). Bars represent ± SE of 4 replicates

capability for adaptation to localized drought stress can be related to a sustained stomatal conductance, higher proline and peroxidase activity in citrus than mango plants. Further studies need to be conducted to identify the possible candidate that controls leaf expansion and stomatal conductance for improvement of water use efficiency in mango and citrus plants during water deficit condition.

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Predominant Weeds of Some Cereal Crops in the Scrub Savannah Region of Nigeria

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Keywords: Weed, cereal, Bauchi, rice, maize, sorghum

ABSTRACK

Satu kajian telah dikendalikan untuk menentukan populasi spesies rumpai yang tumbuh di ladang-ladang bijirin (jagung, padi dan betari) di sesetengah tempat kawasan savanah semak-samun Nigeria semasa musim penanaman 1996-1998 dengan tujuan penyediaan maklumat untuk pengurusan rumpai yang berkesan. Enam puluh kawasan di Gubi, Miri, Inkil, Lukshi dan Birshin Fulani dipilih untuk kajian tersebut. Sampel-sampel rumpai dikutip dalam kuadran 50 cm x 50 cm dan dikenal pasti menggunakan teks standard dan koleksi herbarium Abubakar Tafawa Balewa University, Bauchi, Nigeria. Di kawasan kajian,66 spesies rumpai yang tergolong pada 58 genera dalam 18 keluarga dikenal pasti. Daripada jumlah tersebut, 41 (62.12%) spesies adalah daun lebar, 17 (25.76) rumpai rumput dan 8 (12.12%) sendayan. Rumpai dominan adalah spesies Cyperus, Commelina, Kyllinga, Digitaria, Echinochloa, Imperata, Cynodon, Leucas *dan* Chloris. *Taburan spesies rumpai adalah mengikut jenis tanaman dan kawasan pengumpulan.*

ABSTRACT

A survey was conducted to determine the weed species populations inhabiting cereal farms (maize, rice and sorghum) in some parts of the scrub savannah region of Nigeria during the growing seasons of 1996-1998 with the aim of providing information for effective weed management. Sixty sites in Gubi, Miri, Inkil, Lukshi and Birshin Fulani were selected for the study. Weed samples were collected within 50cm x 50 cm quadrants and were identified using standards texts and collections of the herbarium of the Abubakar Tafawa Belawa University, Bauchi, Nigeria. In the survey sites, 66 weed species belonging to 58 genera within 18 families were identified. Of these, 41 (62.12%) species were broad-leaves, 17 (25.76%) were grass weeds and 8 (12.12%) were sedges. The dominant weeds were Cyperus, Commelina, Kyllinga, Digitaria, Echinochloa, Imperata, Cynodon, Leucas *and* Chloris *species. The distribution of weed species varied with crop type and site of collection.*

INTRODUCTION

Maize (*Zea mays* L.), sorghum (*Sorghum bicolor* (L.) Moench) and rice (*Oryza sativa* L.) are the major cereals grown in the scrub savannah region of Nigeria. The yields of these cereals are generally low despite their importance as staple foods and the interest of farmers in production. The factors associated with low yields include erratic rainfall, diseases, low soil fertility, weed infestation and use of un-improved local varieties.

Of these factors, weed infestation is considered a major limitation to cereal production in this area. Although no accurate data on losses caused by weeds in this area are available, crop losses of between 40-66% (Weber *et al.*, 1995; Udensi *et al.*, 1999) and as high as 100% (Akobundu 1987) have been reported for the Northern Guinea savannah.

Despite the recent development of highly intensive cereal based production systems, weeds and the labour required for weed control are still the most important production constraints in this area. The objective of weed control in cereals is to reduce weed population to levels that do not affect yield, quality and harvesting.

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To achieve this effectively and economically will require a strategy based on the knowledge of the type and distribution of weeds in different localities. However, very little is known about the weed populations affecting cereals in this area. A study of weeds of the northern Guinea Savannah by Weber *et al.* (1995) was limited to Kaduna and Katsina States. Okafor and Adegbite (1991) only carried out a survey of weeds of cowpea fields in Bauchi area.

The objective of this study is to identify the various weeds affecting cereals in some parts of scrub savannah region of Nigeria. The results will provide baseline data for developing weed management strategies.

MATERIALS AND METHODS

A survey of common weed flora of cereal farms was carried out from 1996-1998. The study areas were Inkil, Gubi, Miri, Lukshi and Birshin Fulani. These areas were selected because they are intensively cultivated. The study areas are located in the scrub savannah of Nigeria at an elevation of 609.45m and latitude and longitude of 10° 22'N and 9° 47'E respectively. The area has an average annual rainfall of 905 mm distributed over a growing period of between 150-180 days, which is followed by a pronounced dry season from October to April.

Four farms each devoted to rice, maize or sorghum were randomly selected in each location and were tagged for sample collection. There was no interference in the management practices of the farmers. Weed samples were collected within 50cm x 50 cm quadrants from 10 locations in each farm. Collected weed samples were counted and identified using standard texts (Rains 1968; Akobundu and Agyakwa 1987; Terry and Michieka 1988). Identification was later confirmed at the herbarium of the Abubakar Tafawa Balewa University, Bauchi.

Frequency of occurrence was calculated as the percentage of farms in which a certain weed species was present. The relative occurrence of weed species in relation to crop and site of collection was also estimated.

Data obtained were subjected to two way analysis of variance to determine whether there were significant differences in weeds occurrences between sites of collection and crop type.

RESULTS AND DISCUSSION

Sixty six weeds species belonging to 58 genera within 18 families were identified (Tables 1 and 2). This flora represents about 73% of the total number of weed species identified in the Northern Guinea savannah of Nigeria (Weber *et al.*, 1995). This value is low compared to the 275 plant species recorded around Kano town by Hussain and Karatela (1989). Forty of the genera identified were mong the 60 genera recorded by Weber *et al.* (1995). An earlier study by Okafor and Adegbite (1991) in cowpea fields in the Bauchi area recorded 21 weed genera, which were all recorded in this study. Weed species identified consisted of 41 (62.12%) broad-leaves (Table 1), 17 (25.76%) grasses and 8 (12.12%) sedges (Table 2). A similar study by Kandasamy *et al.* (2000) in India recorded a proportion of 46.7% broad-leaves, 43.5% grasses and 9.8% sedges. Jung *et al.* (1999) recorded 9 species of grasses, 44 species of broad-leaves and 3 species of sedges in apple orchards in Korea. About 101 weed species belonging to 32 families were reported from vegetable farms in Kangwon alphic region of Korea (Kim *et al.* 1999). These trends tend to suggest that the distribution of weed species varies with location and crop under cultivation. About 47.69% of all species recorded belonged to the families of Poaceae, Cyperaceae and Asteraceae with the family Poaceae having the highest number of representative species (17). A striking feature of the flora in the cereal farms was the dominance of *Commelina, Kyllinga, Imperata, Digitaria, Echinochloa, Cyperus, Cynodon, Leucas* and *Chloris* species which constituted about 13.64% of the total weed species and were found in more than 70% of the fields. *Commelina* and *Digitaria* species regarded as the most common weeds of Bauchi area by Okafor and Adegbite (1991) were also found to be dominant in this study. However, there was a significant increase in the number of dominant weeds in the study area. This increase could be attributed to the rapid changes towards intensive land use and cropping patterns. Earlier studies have shown that the distribution and abundance of weed species in cereal crops depend on the system of cultivation, infestation in previous crops and recurrent bush fires (Tottman and Wilson 1990; Garrity *et al.* 1997).

TABLE 1
Broad-leaf weed species and the frequency of occurrence (% fields infested) of cereal farms in the scrub savannah region of Nigeria

Family	Genus	Species	Occurrence (% of farms)
Amaranthaceae	*Amaranthus*	*hybridus*	8.76
	Amaranthus	*spinosus*	6.63
	Alternathera	*sessiles*	4.81
	Celosia	*leptostachya*	3.57
Asteraceae	*Ageratum*	*conyzoides*	42.78
	Acanthospermum	*hispidum*	51.09
	Aspilia	*africana*	27.71
	Chrysanthemum	*americanum*	17.54
	Bidens	*pilosa*	24.92
	Tridax	*procumbens*	34.78
	Synedrella	*nodiflora*	15.76
Caesalpiniaceae	*Cassia*	*obtusifolia*	15.29
	Daniella	*oliveri*	7.68
Commelinaceae	*Commelina*	*benghalensis*	82.54
	Aneilema sp		9.46
Cleomaceae	*Cleome*	*afrospinosa*	6.73
Convolvulaceae	*Evolvus sp*		2.97
	Ipomoea	*septaria*	19.32
	Ipomoea	*dichroa*	13.17
Euphorbiaceae	*Acalypha*	*hispida*	16.70
	Euphorbia	*heterophylla*	13.08
	Euphorbia	*hirta*	5.76
Labiatae	*Hyptis sp*		21.50
	Leucas	*martinicensis*	78.18
Malvaceae	*Abuliton sp*		17.91
Nyctaginaceae	*Boerhavia*	*erecta*	32.11
	Boerhavia	*diffusa*	4.43
Papilionaceae	*Aeschynomene*	*virginica*	16.24
	Crotalaria	*cuspida*	4.90
	Desmodium sp		9.10
	Indigofera sp		18.10
Portulacaceae	*Talinum*	*triangulare*	2.31
	Portula	*olerocea*	8.55
Rubiaceae	*Oldenlandia*	*corymbosa*	43.36
	Borreria sp		16.37
	Mitracarpus	*villosus*	22.37
Solanaceae	*chwenkia*	*americanum*	41.68
Scrophulariaceae	*Buchnera*	*hispida*	9.67
	Striga	*hermonthica*	62.33
	Scoparia	*dulcis*	10.56
Tiliaceae	*Corchorus*	*olitorius*	31.43

TABLE 2
Grasses/sedges weed species and the frequency of occurrence (% field infested) of cereal farms in the scrub savannah region of Nigeria

Family	Genus	Species	Occurrence (% of farms)
Cyperaceae	*Cyperus*	*esculentus*	60.14
	Cyperus	*rotundus*	52.50
	Cyperus	*tuberosus*	35.22
	Cyperus	*sphacelatus*	20.29
	Kyllinga	*squamulata*	76.63
	Mariscus	*alternifolium*	44.20
	Mariscus	*umbellatus*	25.40
	Setaria	*verticillata*	4.96
Poaceae	*Brachiaria*	*deflexa*	21.19
	Andropogon	*gayanus*	10.60
	Chloris	*pilosa*	71.20
	Eluesine	*indica*	29.10
	Pennisetum	*polystachion*	35.60
	Eragrotis	*cilianensis*	19.60
	Setaria	*verticillata*	27.28
	Imperata	*cylindrical*	73.17
	Paspalum	*orbiculare*	7.98
	Digitaria	*longiflora*	75.65
	Panicum	*maximum*	58.30
	Scleragrotis sp		16.48
	Rottboellia	*cochinchinensis*	44.73
	Cynodon	*dactylon*	66.01
	Echinochloa	*colona*	86.43
	Ageratum	*conyzoides*	51.06
	Euclasta sp		25.84
	Dactyloctenium	*aegyptium*	57.23

The distribution of weed species according to crop type and area of collection is presented in Tables 3, 4 and 5. Weeds species distribution varied significantly ($P \leq 0.05$) with cereal crop type and site of collection. Rice farms with 38 species which is equivalent to 58.46% of overall species identified had the highest number of species per crop type (Table 3), while sorghum with a record of 32 species (49.23%) had the lowest number of weed species (Table 5). Of the 38 species from rice farms, 8 were sedges, 10 grasses and 20 broad-leaves, with *Cyperus* species and *Echinochloa colona* being the most dominant species. On maize farms, 14 grass species, 2 sedges and 25 broad-leaves were identified. Similarly, sorghum farms recorded 11 grass, 20 broad-leaves and 1 sedge species. Cynodon dactylon and Striga hermonthica were the most dominant species in maize and sorghum farms respectively. Nineteen of the species identified were common to rice and maize farms, while 12 species were common to all three crops.

Results of this study provide evidence that there is an increase in the number of dominant weeds and that the distribution of weeds species varies with the area of collection and crop types. Further studies need to be carried out on the determinants of weed communities and the implication in the management of theses weeds.

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TABLE 3
Weed flora of rice farms in the scrub savannah region of Nigeria

Family	Genus	Species	Occurrence by Location (%)				
			Miri	Gubi	Inkil	B/Fulani	Lukshi
Amaranthaceae	*Amaranthus*	*hybridus*	6.21	41.87	3.37	-	-
	Amaranthus	*spinosus*	58.13	11.80	8.59	15.88	9.27
	Alternathera	sessiles	35.10	30.08	28.22	17.70	17.05
	Celosia	*leptostachya*	3.21	4.43	3.56	43.35	4.26
Asteraceae	*Ageratum*	*conyzoides*	10.67	10.70	53.09	10.01	13.17
	Acanthospermum	*hispidum*	26.59	21.26	33.10	25.10	30.19
	Aspilia	*africana*	7.56	17.70	28.22	30.08	35.10
	Chrysanthemum	*americanum*	-	1.46	-	-	8.81
	Bidens	*pilosa*	15.08	13.25	12.73	12.89	3.13
	Synedrella	*nodiflora*	20.26	27.93	11.05	39.84	-
Commelinaceae	*Commelina*	*benghalensis*	31.09	41.15	17.13	44.53	31.24
	Aneilema sp		3.13	2.89	-	5.08	3.25
Convolvulaceae	*Evolvulus sp*	*septaria*	17.54	24.92	51.09	-	42.26
	Ipomoea		-	4.14	58.06	11.38	23.02
Cyperaceae	*Cyperus*	*esculentus*	32.57	55.46	32.12	39.86	58.61
	Cyperus	*rotundus*	58.06	62.15	32.39	40.61	51.92
	Cyperus	*tuberosus*	20.20	14.63	18.41	36.99	31.67
	Cyperus	*sphacelatus*	10.39	5.71	19.65	-	-
	Kyllinga	*squamulata*	57.62	65.03	70.67	51.92	68.03
	Mariscus	*alternifolium*	41.87	24.26	33.10	30.19	25.10
	Mariscus	*umbellatus*	6.21	3.37	14.46	-	3.64
	Setaria	*verticillata*	10.01	10.67	35.01	13.17	-
Euphorbiaceae	*Acalypha*	*hispida*	26.15	17.93	-	5.88	32.39
	Euphorbia	*heterophylla*	1.74	5.91	14.51	40.67	22.08
Poaceae	*Brachiaria*	*deflexa*	21.19	9.18	38.70	-	0.69
	Chloris	*pilosa*	71.20	33.60	48.14	62.11	59.33
	Pennisetum	*polystachion*	38.60	13.86	27.31	7.89	41.23
	Paspalum	*orbiculare*	7.98	26.43	-	18.93	13.54
	Imperata	*cylindrical*	44.20	73.17	16.48	66.01	57.23
	Digitaria	*longiflora*	65.60	47.14	75.65	38.13	53.01
	Panicum	*maximum*	29.20	55.23	30.91	36.55	64.38
	Roettboellia	*cochinchinensis*	41.06	18.97	58.32	60.23	39.87
	Cynodon	*dactylon*	53.13	79.67	74.89	55.23	63.22
	Echinochloa	*colona*	76.51	93.80	69.73	54.21	83.28
Portulaceae	*Talinum*	*triangulare*	0.00	1.76	-	-	-
	Portula	*oleraceae*	1.84	1.16	8.87	5.02	-
Rubiaceae	*Mitracarpus*	*villosus*	7.68	19.32	21.50	-	7.68
Tiliaceae	*Corchorus*	*olitorius*	19.05	34.12	28.07	37.22	16.76

TABLE 4
Weed flora of maize farms in the scrub savannah region of Nigeria

Family	Genus	Species	Occurrence By Location (%)				
			Miri	Gubi	Inkil	B/Fulani	Lukshi
Amaranthaceae	*Celosia*	*leptostachya*	3.28	4.21	7.51	5.39	8.83
	Amaranthus	*spinosus*	15.74	1.92	9.15	7.68	24.62
Asteraceae	*Bidens*	*pilosa*	8.28	13.04	15.53	9.34	2.76
	Tridax	*procumbens*	41.07	10.08	3.25	16.57	35.11
	Acanthospermum	*hispidum*	33.56	11.73	39.04	28.67	25.94
Caesalpiniaceae	*Cassia*	*obtusifolia*	-	-	-	6.59	-
	Daniella	*oliveri*	-	-	2.60	1.73	-
	Cleome	*afrospinosa*	-	0.85	-	-	6.59
Commelinaceae	*Commelina*	*benghalensis*	30.31	9.30	15.68	37.12	22.60
	Aneilema sp		1.61	-	-	1.09	3.88
Convolvulaceae	*Evolvulus sp*		2.98	3.64	-	2.09	1.85
	Ipomoea	*dichroa*	6.93	13.86	10.22	12.61	26.27
Cyperaceae	*Cyperus*	*esculentus*	34.98	20.16	37.62	26.17	32.23
Euphorbiaceae	*Acalypha*	*segetalis*	-	1.06	1.70	1.58	5.24
	Euphorbia	*hirta*	9.79	15.76	28.25	37.52	34.17
	Leucas	*martinicensis*	25.84	4.60	-	12.20	9.63
Labiatae	*Hyptis sp*		-	4.45	1.64	3.21	3.01
Nyctaginaceae	*Boerhavia*	*erecta*	33.41	19.92	34.66	35.07	18.34
	Boerhavia	*diffusa*	3.03	1.56	0.93	-	-
Papilionaceae	*Crotalaria*	*cuspida*	2.32	-	-	3.03	1.61
	Indigofera sp		-	-	-	0.97	1.13
Poaceae	*Euclasta sp*		13.14	8.22	8.91	2.23	14.67
	Pennisetum	*polystachion*	8.55	2.98	1.93	3.64	-
	Setaria	*verticillata*	2.09	1.85	1.01	-	4.03
	Paspalum	*orbiculare*	6.13	2.23	2.41	1.70	6.11
	Imperata	*cylindrical*	21.47	16.11	18.22	4.98	-
	Digitaria	*longiflora*	13.43	18.07	25.28	43.34	14.31
	Panicum	*maximum*	39.38	34.09	46.53	37.33	23.18
	Rottboellia	*cochinchinen*	62.12	23.12	61.67	25.28	62.76
	Cynodon	*sis*	58.12	76.62	67.21	53.10	39.33
	Eragrotis	*dactylon*	45.35	25.11	15.16	24.08	41.18
	Scleragrotis sp	*cilianensis*	4.41	7.23	29.57	17.50	23.53
	Chloris sp		18.63	30.09	42.13	28.41	37.16
	Brachiaria	*pilosa*	5.20	9.61	1.25	12.57	2.59
	Eleusine	*deflexa*	28.48	31.23	25.02	21.14	13.86
		indica					
Rubiaceae	*Oldenlandia*	*corymbosa*	-	-	-	-	3.88
Scrophylariaceae	*Striga*	*hermonthica*	55.16	19.93	60.33	47.21	34.76
	Buchnera	*hispida*	2.04	1.11	-	1.31	3.46
	Alectra sp		14.13	2.82	-	3.67	6.41

TABLE 5
Weed flora of sorghum farms in the scrub savannah region of Nigeria

Family	Genus	Species	Occurrence By Location (%)				
			Miri	Gubi	Inkil	B/Fulani	Lukshi
Amaranthaceae	*Amaranthus*	*spinosus*	43.81	21.67	25.43	21.87	33.52
Asteraceae	*Acanthospermum*	*hispidum*	29.42	21.90	10.61	19.80	20.53
	Synedrella	*nodiflora*	9.78	22.44	27.90	11.12	5.61
Caesalpiniaceae	*Cassia*	*obtusifolia*	2.58	-	3.23	-	1.82
	Daniella	*oliver*	1.38	-	-	-	1.13
	Cleome	*afrospinosa*	-	2.13	-	-	-
Commelinaceae	*Commelina*	*benghalensis*	3.29	3.07	5.39	3.53	6.98
Cyperaceae	*Cyperus*	*esculentus*	34.98	20.16	37.62	26.17	32.23
Labiatae	*Hyptis sp*		13.36	3.76	15.23	-	11.21
	Leucas	*martinicensis*	29.19	13.74	26.38	7.89	32.01
Malvaceae	*Abuliton sp*		3.75	8.19	2.04	16.93	-
Papilionaceae	*Aeschynomene*	*virginica*	-	-	5.05	-	0.82
	Crotalaria	*cuspida*	33.49	-	-	-	-
	Indigofera sp		4.51	5.01	23.01	1.92	4.09
Poaceae	*Brachiaria*	*deflexa*	35.04	25.41	60.12	38.20	51.32
	Eragrostis	*cilianensis*	37.47	8.19	20.44	12.12	16.93
	Andropogon	*gayanus*	25.48	28.41	43.12	50.93	25.48
	Scleragrotis sp		2.33	11.84	7.24	2.82	-
	Eleusine	*indica*	54.20	7.33	36.73	16.10	39.20
	Dactyloctenium	*aegyptium*	5.94	6.26	9.45	-	4.36
	Digitaria	*longiflora*	49.95	40.16	36.03	77.81	26.85
	Rottboellia	*cochinchinen*	4.91	31.22	72.63	51.16	21.35
	Cynodon	*dactylon*	61.09	23.53	27.83	7.92	50.20
	Panicum	*maximum*	1.39	3.32	-	2.97	2.78
	Chloris	*pilosa*	8.03	37.42	13.01	12.70	34.59
	Ageratum	*conyzoides*	33.76	45.11	57.03	28.67	46.43
Rubiaceae	*Oldenlandia*	*corymbosa*	6.42	2.51	-	9.47	4.13
Solanaceae	*Schwenkia*	*americanum*	4.43	3.16	-	6.27	3.67
Scrophulariaceae	*Striga*	*hermonthica*	87.42	51.09	58.82	63.83	75.03
	Buchnera	*hispida*	2.71	-	7.81	3.31	1.56
	Scoparia	*dulcis*	9.27	4.39	3.55	7.89	-
Tiliaceae	*Corchorus*	*olitricus*	18.03	51.18	40.71	29.16	13.34

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Effect of Varying Levels and Sources of Dietary Fat on Growth Performance and Nutrient Digestibility in Rabbits

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ABSTRAK

Kesan sumber lemak diet dan tahap ke atas prestasi pembesaran dan kebolehcernaan nutrient dikaji ke atas arnab-arnab baka kacukan Lopx New Zealand. Lima puluh arnab yang bercerai susu secara rawak diberikan lima rawatan diet yang mengandungi satu kawalan (tiada lemak) dan empat yang lainnya dengan lemak, sama ada daripada sumber tumbuhan (minyak kacang tanah) atau haiwan (mentega), setiap satunya pada dua tahap pemasukan (3% dan 6%). Terdapat 10 arnab untuk setiap diet. Arnab-arnab yang 6% berasaskan diet lemak haiwan direkodkan lebih tinggi (P<0.01) daripada arnab berasaskan 6% diet haiwan. Pengambilan bahan organic (OMI) diperhatikan sama antara kawalan pemakanan arnab-arnab dan 3% diet lemak haiwan. Walau bagaimanapun, 6% diet lemak mentega direkodkan terendah. Kebolehcernaan protein kasar (CPD) adalah sama dalam 3% pemakanan arnab dan 6% tahap lemak haiwan yang tertinggi manakala 6% tahap diet lemak tumbuhan mempunyai CPD yang terendah. Dapatan ini menunjukkan bahawa pemasukan lemak haiwan pada tahap 6% meningkatkan tambahan berat dan keberkesanan penggunaan makanan kepada arnab-arnab.

ABSTRACT

The effects of dietary fat sources and levels on growth performance and nutrient digestibility were investigated in cross-breed Lopx New Zealand rabbits. Fifty weaned rabbits were randomly allotted to five dietary treatments consisting of a control (no fat) and four others with fat, either from plant (groundnut oil) or animal (butter) sources, each at two levels (3% and 6%) of inclusion. There were ten rabbits per diet. Rabbits on 6% animal fat-based diet recorded the highest (P<0.01) better in rabbits on 6% animal diet. Organic matter intake (OMI) was observed to be similar between rabbits fed control and 3% animal fat diets. However, the 6% butter fat diet recorded the lowest. Crude protein digestibility (CPD) was similar in rabbit fed 3% and 6% level of animal fat which were the highest while 6% level of plant fat diet had the lowest CPD. These findings show that the inclusion of animal fat at 6% level improved the weight gain and efficiency of feed utilization in rabbits.

INTRODUCTION

Fats are frequently used in commercial feed formulae. Fats are added to rations for several reasons. Nutritionally, fat are exclusively energy sources, because they contain very little, if any, protein, minerals or vitamins. As a source of energy, fats are highly digestible (especially by monogastric animals). Digestible fat supplies have about 2.25 times as much energy as digestible starch or sugar, thus fat can be used to increase energy density of a ration. Fats generally increase absorption of fat soluble nutrients, such as the fat soluble vitamins (Mc Donald *et al.* 1987). In addition linolenic acids in fat are required by monogastric animals. According to NRC (1977), Barge *et al.* (1984) and Lebas (1980), the requirement for dietary fat by rabbits has been set at 2% of the diet. Since plant feedstuffs are the basis of rabbit diet, the essential fatty acid requirement will be satisfied at this level. Also, this level probably guarantees a sufficient absorption of fat-soluble vitamins (Barge *et al.* 1984). Pote *et al.* (1980) used 20% added fat in

a high oil alfalfa diet and found a significant improvement in feed conversion efficiency. Beynen (1988) stated that there was a need to raise the fat content of diets for rabbit fryers to enhance body weight gain and improve feed conversion and that carbohydrate can be replaced by fat on calorie for caloric basis. A high fat level is not recommended in hot environment (Ekpenyong 1988), probably because of the high temperature and high cost of supplementary fats. The dry ration for rabbits should contain between 1% and 8% crude fat (Ekpenyong 1988). Maize is a major source of energy for monogastric animals. However, the quantity produced annually in the Northern states of Nigeria is not enough for human consumption, therefore, there is a shortage in animal feeding. The search for an alternative source of energy now becomes inevitable. The present experiment was therefore designed to study the effects of source and levels of replacement of maize with fat on nutrient intake, digestibility and growth rate of rabbits.

MATERIALS AND METHODS

Animals and their Management

The experiment was conducted with weanling Lopx New Zealand cross-bred rabbits, purchased from the National Veterinary Research Institute (NVRI) in Vom. The rabbits were weighed and randomly allocated to the metabolic cages. Fifty weaned rabbits (533g average body weight) were randomly allotted to five dietary treatments. Each treatment had ten rabbits with 2 rabbits per replicate (5 replicates). One group of rabbits was fed a control diet containing 3% and 6% of either cow milk butter or groundnut oil. The rabbits were provided six days adaptation to the diets and each animal was fed 100g/day of feed at 8.00 hour daily and had free access to drinking water. The design of this experiment was completely randomized. The composition of the dietary treatment is shown in Table 1. The diets were formulated to contain about 18% CP and 11.30KJ/g digestible energy (Table 2).

Data Collection

Daily feed allowance (100g/rabbit) was offered to the rabbits and the residue was weighed the following day to give the daily feed intake. The rabbits were weighed at the beginning of the experiment and thereafter weekly. Faecal collection, which lasted for seven (7) days was conducted during the third week of the experiment. Faeces voided daily by each rabbit were collected and oven dried at 80° for 12 hours. All samples from each rabbit were bulked and kept in sample bottles until required for chemical analysis. The feeding trial lasted for 5 weeks.

Chemical Analysis

Proximate analysis of the experimental diets and the faecal samples were carried out using AOAC (1985) standard methods of analysis. Acid Detergent Fibre (ADF) and Neutral Detergent Fibre

TABLE 1
Percentage composition of the diets fed to rabbits

Ingredients	Dietary Sources of Energy				
	Control No. Added Fat $Diet_1$	Animal Fat		Plant Fat	
		3% $Diet_2$	6% $Diet_3$	3% $Diet_5$	6% $Diet_5$
Maize	46.41	33.82	25.1	33.75	25.00
Groundnut cake	24.00	26.00	25.00	26.00	25.00
Butter	-	3.00	6.00	-	-
Groundnut oil	-	-	-	3.00	6.00
Maize offal	12.00	15.00	10.00	15.00	10.00
Bone meal	2.00	2.00	2.00	2.00	2.00
Premix	0.50	0.50	0.50	0.50	0.50
Salt	0.50	0.50	0.50	0.50	0.50
TOTAL	100.00	100.00	100.00	100.00	100.00

(NDF) of the samples were determined by the method of Goering and Van Soest (1970).

Statistical Analysis

All the data collected were subjected to analysis of variance technique according to Steel and Torrie (1980) based completely on randomized design. Where significant differences between means existed, the least significant difference (LSD) method was used to test the differences between the means. The data were analyzed using Minitab for window statistical package.

RESULTS

The feed intake and growth performance of rabbits fed diets with different levels and sources of fat are shown in Table 3.

There were differences (P<0.01) in feed (fresh) intake (DM) of the diets fed to the rabbits. The rabbits fed 6% animal fat diet had the lowest DM intake of 74.82g/day and this value is even lower for that obtained for the control-diet (75.36g/day). Those on 6% plant fat diet had the highest feed intake of 79.58g/day. The rabbits fed control diet and 3% animal fat diet had similar daily weight gain (DWG).

There were significant differences (P<0.01) in daily weight gain (DWG) of the animals fed the different diets. Rabbits fed 6% animal fat diet had the highest weight gain of 31.10g/day, while those fed 6% plant fat had the lowest (17.85g/day). There were significant differences (P<0.01) in DWG of the animals fed control diet (27.53g/day) and the plant fat diet at both levels (23.28g/day and 17.85g/day) at 3% and 6% level respectively. The results showed that the feed conversion ratios (FCR) of the diets fed to the rabbits were significantly (P<0.01)

TABLE 2
Chemical composition (%) of the diets fed to rabbits

	Diets				
Nutrient	1	2	3	4	5
Energy (KJ/g)	11.30	-	-	-	-
Crude protein (g)	18.38	18.63	18.56	18.50	18.33
ADF	16.74	18.04	18.96	18.64	18.33
NDF	25.12	25.68	26.82	26.22	26.93

TABLE 3
Nutrient intake and growth performance of rabbits fed diets with varying levels and sources of fat

	Control	Butter		Level and Source of Dietary Fat Groundnut Oil		
Parameters	No added fat $Diet_1$	3% $Diet_2$	6% $Diet_3$	3% $Diet_4$	6% $Diet_5$	SE
FI	73.36^{ab}	76.30^{b}	74.82^{a}	77.24^{b}	79.58^{c}	0.329
DWG	27.53^{c}	27.08^{c}	31.10^{d}	23.28^{b}	17.85^{a}	0.45
FCR	2.74^{b}	2.83^{b}	2.40^{a}	3.23^{c}	4.46^{d}	0.06
DMI	64.50^{b}	65.77^{c}	64.19^{a}	67.37^{d}	68.91^{e}	0.28
OMI	60.06^{b}	60.75^{b}	58.99^{a}	62.37^{c}	63.28^{c}	0.26

FI = Feed Intake
DWG = Daily Weight Gain
FCR = Feed Conversion Ratio
DMI = Dry Matter Intake
OMI = Organic Matter Intake

$^{a, b, c, d, e}$ Means in the same row with different superscripts are different (P>0.01)

different. The rabbits on 6% animal recorded the lowest FCR of 2.40, while rabbits fed 6% plant fed diet had the highest FCR of 4.46. The rabbits fed 3% and 6% plant fat diets had significantly (P<0.01) higher DMI than those fed the control and the 3% and 6% animal fat diets. However, those rabbits fed control and 6% animal fat diets had similar DMI, which was followed by the animals fed 3% animal fat diet.

The results showed that the rabbits fed 3% and 6% plant fat had significantly (P<0.01) higher OMI than those fed control, 3% and 6% animal fed diets. Those rabbits fed the control and 3% animal fat diets have similar OMI (P<0.05). The rabbits fed the control and 6% animal fat diet recorded the lowest OMI (58.99g/day) while those on 6% plant fat recorded the highest (63.28g/day). Digestibility of diets containing various levels and sources of fat are presented in Table 4. The rabbits fed the control and 3% plant fat diets had similar dry matter digestibility (DMD). Those rabbits fed 3% plant fat diets had also similar DMD, which were significantly (P<0.01) higher than for those on the control, 3% and 6% plant fat diets. The rabbits fed 6% plant fat diet had the lowest DMD (55.79%) and those on 6% animal fat diet had the highest (66.33%). The rabbits fed 3% and 6% animal fat diets had similar CPD, which were significantly (P<0.01) higher than those fed the control and the 6% plant fat diets. The rabbits fed 5% animal had the highest CPD (91.45%) while those fed 6% had the lowest (59.27%).

The results showed that the rabbits fed 3%, 6% animal fat diets and 3% plant fat diet had similar ADFD, which were significantly (P<0.01) higher than the control and the 6% plant fat diets. The rabbits fed 3% plant fed diet had the highest ADFD (31.88%) while those fed 6% plant fat diet had the lowest (14.15%). The rabbits fed the control, 3%, 6% animal fat and 3% plant fat diets had similar NDFD, which were significantly (P<0.01) higher than those of rabbits fed 6% plant fat diet. The rabbits fed control diet had the highest NDFD (42.13%), while those fed 6% plant fat diet had the lowest NDFD (27.71%).

Effects of control vs. fat diets on nutrient intake, growth performance and nutrient digestibility in rabbits are shown in Table 5.

The rabbits fed fat diets had significantly (P<0.05) higher feed intakes than those on control diet. There was no significant difference in overall weight gain of the rabbits fed control and fat diets. The rabbits fed fat diets had significantly higher dry matter (P<0.01) and organic matter (P<0.05) intake than those fed control diet. Inclusion of fat in the diet had significant effect on Organic Matter Intake (OMI) of the rabbits. There was no significant difference in Dry Matter Digestibility (DMD) and Organic Matter Digestibility and Organic Matter Digestibility (OMD) of the rabbits fed

TABLE 4
Nutrient digestibility of diets containing varying levels and sources of fat by rabbits

Parameters	Control	Animal fat		Level and Source of Dietary Fat		
		3%	6%	3%	6%	SE
	fat $Diet_1$	$Diet_2$	$Diet_3$	$Diet_4$	$Diet_5$	
DMD	63.91^{b}	65.15^{b}	66.33^{d}	64.78^{c}	55.79^{a}	0.35
CPD	90.67^{b}	91.20^{cd}	91.45^{cd}	90.93^{bc}	87.00^{a}	0.12
OMD	65.73^{b}	67.85^{c}	70.07^{d}	67.86^{c}	59.27^{a}	0.32
ADFD	26.37^{b}	31.08^{c}	30.23^{c}	31.88^{c}	14.15^{a}	0.93
NDFD	42.13^{b}	40.66^{b}	41.26^{b}	41.11^{b}	27.71^{a}	0.62

PMD = Dry Matter Digestibility
CPD = Crude Protein Digestibility
OMD = Organic Matter Digestibility
ADFD = Acid Detergent Fibre Digestibility
NDFD = Neutral Detergent Fibre Digestibility

$^{a, b, c, d}$ Means in the same row with different superscripts are different (p<0.01).

TABLE 5
Effect of control versus fat diets on nutrient intake digestibility and growth performance in rabbits

Parameters	Control Diets	Fat	Diets Level of Significance
FI	75.36	76.99	*
DWG	27.53	24.83	NS
FCR	2.47	3.25	NS
DMI	64.50	66.56	**
OMI	60.05	61.35	*
DMD	63.91	63.01	NS
OMD	65.73	66.25	NS
CPD	90.61	90.17	NS
ADFD	26.37	26.85	NS
NDFD	42.13	37.68	NS

* - Significant at 5%
** - Significant at 1%
NS - Not significant

TABLE 6
Multiple regression equation relating performance parameters to the nutrient content of diets in rabbits fed varying sources and levels of fat

Dependent Variables	Independent Variables				
	Constant	CP	ADP	NDF	R^2
DMI	51.80***	-2.10***	0.77***	0.78***	71.60***
DWG	-119.90***	5.79***	-1.72***	1.80***	77.30***
OMI	51.00***	-2.04***	0.73***	-0.79**	67.40***
DMD	-59.30***	3.65***	-0.50*	3.54	62.60***
OMD	-64.70***	3.72***	-0.502***	4.51	60.6***
ADFD	-74.30**	4.75***	0.53	4.54	58.80***
ADFD	-63.30	3.94**	-6.12	2.33	31.10

*** Significant at 0.1%
** Significant at 1%
* Significant at 5%

CP = Crude Protein
ADF = Acid Detergent Fibre
NDF = Neutral Detergent Fibre
R^2 = Coefficient of Determination
DMI = Dry Matter Intake
DWG = Daily Weight Gain
DMD = Dry Matter Digestibility
OMD = Organic Matter Digestibility
ADFD = Acid Detergent Fibre Digestibility
NDFD = Neutral Detergent Fibre Digestibility

control and fat diets. No significant difference was observed in CPD, ADFD and NDFD of the rabbits fed control and fat diets.

Multiple regression equations, relating weight gain, nutrient intake and digestibility in rabbits to the chemical composition of the diets are shown in Table 6. For each unit increase in Crude Protein (CP) and Neutral Detergent Fibre (NDF). Dry Matter Intake (DMI) decreased by 2.1g and 0.78g respectively. The DWG decreased by 1.72g for each unit increase in NDF but increased by 5.8g for each unit increase un CP.

The DMD and OMD increased by 3.63% and 3.72%, respectively for each unit increase in CP and decreased by 0.50 for each unit increase in ADF. For each unit increase in CP, the ADFD increased by 4.75%.

DISCUSSION

Fernandez and Fraga (1996) reported significant variation in feed intake of the control plant and animal fat diets fed to rabbits as shown by the results in this study. The DWG attained with the control diet (27.53g/day) was not different from that obtained from 3% animal fat diet (27.08g/day). This showed that maize could be favorably replaced at 3% with animal fat. Increased levels of animal fat by 6% improved the DWG (31.10%/day) and are comparable to the findings of Fernandez and Fraga (1996) who obtained higher DWG values (34.0-36.40g/day).

Increase in plant fat inclusion level decreased the DWG mean values for 3% and 6% level respectively as was reported by Odunsi (1999) who used 2.50 and 5.0% level palm oil and reported DWG of 21.40 and 15.0g/day respectively. The OMI results obtained in this study are comparable to those of Fernandez and Fraga (1996), who observed significant differences in OMI in rabbits fed the animal and plant fat diets. The results showed improvement in DMD with animal fed addition as observed by Cobos *et al.* (1993), who reported that fat improves the diet and generally increases absorption of fat soluble nutrients. The CPD values obtained in this study are comparable to those of Patridge *et al.* (1986) and Santoma *et al.* (1987), who reported that fat addition and increase in fat level did not affect the crude protein digestibility. The rabbits fed 3% animal and plant fat and 6% animal fat diets had similar DFD, which were significantly higher than those on control diet. These findings indicated that fat addition increased ADF digestibility. The ADFD increased by 4.75% with each unit increase of CP, and these results are comparable to those reported by Fernandez *et al.* (1994). The ADFD obtained from the diets are comparable to Patridge *et al.* (1986) and Santoma *et al.* (1987), who reported that fat inclusion in the diet and increase in fat level did not affect NDFD. The NDFD increased by 3.94% with each unit increase in CP. These findings are similar to those reported by Fernandez *et al.* (1994).

CONCLUSION

According to the above findings, fat is effective in rabbit's diets at the levels studied. Animal fat at 6% level produced the highest weight gain whereas plant fat at 3% level can also be used in rabbit diets without affecting their performance. Animal fat at 6% level could be used to improve the weight gain of rabbits and fat generally can be used as an energy booster in diets low in energy.

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SHORT COMMUNICATION

The Impact of Anthropogenic Activities on Heavy Metal (Cd, Cu, Pb and Zn) Pollution: Comparison of the Metal Levels in the Green-Lipped Mussel *Perna viridis* (Linnaeus) and in the Sediment from a High Activity Site at Kg. Pasir Puteh and a Relatively Low Activity Site at Pasir Panjang

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It has been a common practice to select different environmental backgrounds in biomonitoring studies of heavy metal contamination (Yap *et al.* 2002a; 2002b; 2003a; 2003b). It is expected to obtain different pollutant concentrations in a selected biomonitoring agent. An interesting question that may arise here is 'Are all heavy metal levels high in a known high human activity sampling site ?' This question comes to mind since most researchers would assume a positive answer while ecotoxicologists would like to know which metal(s) is(are) high (maybe not all heavy metals) at that sampling site. The environmental background is closely related to the description of the sampling site which can range from an uncontaminated or pristine site to a highly contaminated site that is known to receive a lot of anthropogenic inputs.

In the present study, sediment samples and the green-lipped mussel *Perna viridis* were used to assess human impacts on the aquatic environment. Bryan and Langston (1992) documented several advantages in using sediment and mussels for this purpose. Firstly, sediment plays a major role in the transport and storage of metals; secondly, sediment is frequently used to identify sources of pollutants spatially and temporally; and thirdly, sediment can also be used to locate the main sinks for heavy metals as these elements are persistent in the marine environment (Nriagu 1978). The sediment samples are also geochemically fractionated and thus the percentages of anthropogenic input of metal can be calculated.

We chose the green-lipped mussel *Perna viridis* because marine mussels are often chosen for biomonitoring studies. The advantages of choosing this species are that they are sedentary organisms, long lived, easily identified and sampled, reasonably abundant and available throughout the year, tolerant to natural environmental fluctuations and pollution. In addition, these organisms have good net accumulation capacities and are ecologically important (Phillips 1980; Farrington *et al.* 1987). Yap *et al.* (2003a) reported the background levels of heavy metals in *P. viridis* collected from the west coast of Peninsular Malaysia.

In this study, two sites with contrasting environmental conditions were selected for the study. Kampung (Kg.) Pasir Puteh at the Straits of Tebrau (Johor) was selected since it has been observed to have a lot of activities such as moorings and petro-chemical plants, shipping, land reclamation, urbanization and other industrial activities. This area is close to Pasir Gudang, which is one of the major industrial areas in Malaysia. In addition, Pasir Gudang Port, one of the largest ports in Malaysia, is also located less than a few kilometers away from this area. There are no baseline data on the heavy metals for Kg. Pasir Puteh before the development of the town, port, marina and

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industries. Therefore, it is necessary to use a reference area to allow the effects of human activities to be assessed by comparison. For this purpose, we selected Pasir Panjang in Port Dickson (Negeri Sembilan) since only a few human activities could be seen around the area, namely land reclamation and offshore shipping.

On scientific grounds, although it is a high activity site, Kg. Pasir Puteh is not considered as being contaminated until scientific data have proven it to be so although this is expected. Therefore, samples of an organism, the green-lipped mussel *Perna viridis* and sediment were collected from Kg. Pasir Puteh and Pasir Panjang and analysed for their metal concentrations.

In this study, the levels of heavy metals in the soft tissues, shells and byssus of *P. viridis* and the geochemical fractions of the sediments were determined and the results for the two sites were compared. The objective of this study is to ascertain if the contrasting environmental backgrounds do play important roles in the contamination of Cd, Cu, Pb and Zn at the sites.

The sampling sites at Pasir Panjang (latitude-north: 2°25' and 101°56') and Kg. Pasir Puteh (longitude-east: 1°26' and 103°55') in Peninsular Malaysia are shown in *Fig.* 1. Sampling was conducted between January and May 2000. About 20 similar size mussels (5-7 cm) were selected and analysed individually for heavy metals. The soft tissue of the mussel was dissected by removing the byssus and the shell. Total soft tissues, shells and byssus were dried at 105°C until they reached constant dry weights (dw) (Tanner *et al.* 2000). The analytical procedures for the mussel analysis followed those as described by Yap *et al.* (2003a) while for the analyses of the geochemical fractions of the sediments, the sequential extraction technique (SET) that was described by Badri and Aston (1983) was used. The T-test between any two variables was conducted using the STATISTICA software package.

The levels of Cu, Pb and Zn in their soft tissues, shells and byssus of the mussels collected from Kg. Pasir Puteh were found to be significantly ($P < 0.05$) higher than those collected

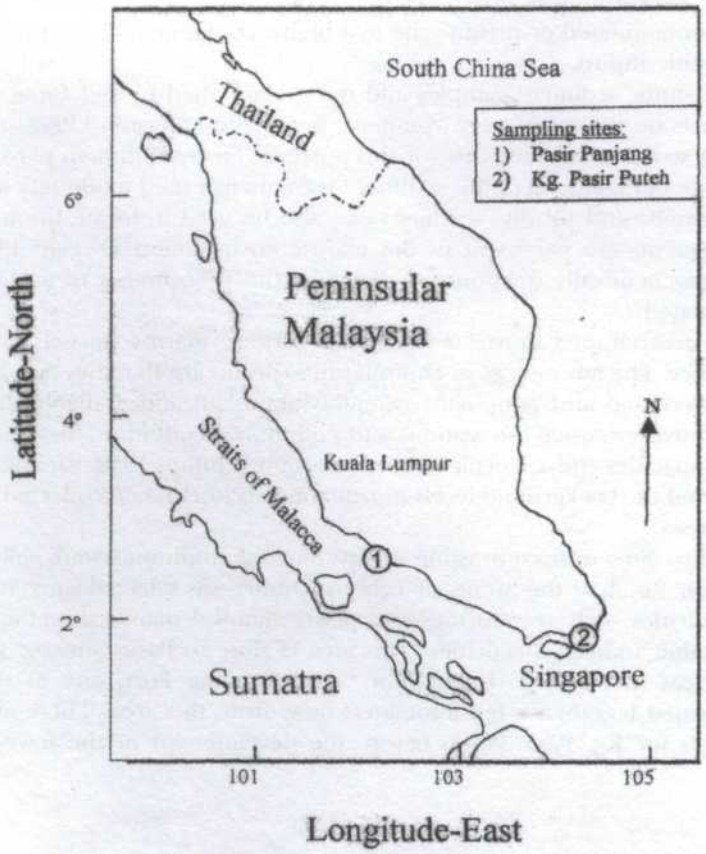

Fig. 1: Map showing the locations of the sampling sites

from Pasir Panjang (Table 1). The similar (not significantly different; P> 0.05) Cd levels in the soft tissues and shells of *P. viridis* collected from both locations were in line with the results for sediment samples in that the easily, freely, leachable or exchangaeble (EFLE) and resistant fractions and total Cd concentration in the sediments were not significantly (P> 0.05) different for the two sampling sites (Table 1).

Since both natural and anthropogenic metals were accumulated together in the sediment, it is difficult to identify which proportion of the total metal concentrations measured in the sediment is natural and which is anthropogenic in origin. The summation of the EFLE, acid-reducible and oxidisable-organic fractions in the SET used were calculated as the non-resistant fraction of the sediment which is believed to be mostly attributable to anthropogenic sources (Badri and

TABLE 1
Concentrations (mg/g dry weight) of Cd, Cu, Pb and Zn in the mussel tissues and sediments with their levels of significance

	Pasir Panjang	Kampung Pasir Puteh	Significance level
Soft tissue mussel			
Cd	0.77 ± 0.20	0.84 ± 0.03	P> 0.05
Cu	7.64 ± 0.20	15.45 ± 0.27	P< 0.01
Pb	4.54 ± 0.09	8.72 ± 0.06	P< 0.001
Zn	74.37 ± 2.36	102.8 ± 1.18	P<0.05
Shell mussel			
Cd	9.44 ± 0.12	9.34 ± 0.09	P> 0.05
Cu	6.92 ± 0.06	7.78 ± 0.07	P< 0.01
Pb	20.70 ± 0.45	32.72 ± 0.14	P< 0.01
Zn	3.99 ± 0.06	7.43 ± 0.04	P< 0.001
Byssus mussel			
Cd	1.06 ± 0.03	1.80 ± 0.06	P< 0.01
Cu	20.56 ± 1.44	51.99 ± 1.06	P< 0.01
Pb	13.52 ± 0.87	17.76 ± 0.72	P< 0.01
Zn	81.22 ± 1.93	192.17 ± 7.72	P< 0.01
Cd			
EFLE	0.23 ± 0.01	0.21 ± 0.01	P> 0.05
Acid-reducible	0.14 ± 0.01	0.25 ± 0.02	P< 0.05
Oxidisable-organic	0.09 ± 0.01	0.33 ± 0.04	P< 0.05
Resistant	0.58 ± 0.08	0.45 ± 0.07	P> 0.05
Total Cd	1.03 ± 0.09	1.24 ± 0.05	P> 0.05
Cu			
EFLE	1.01 ± 0.05	6.19 ± 0.05	P< 0.01
Acid-reducible	0.07 ± 0.00	0.35 ± 0.02	P< 0.05
Oxidisable-organic	2.59 ± 0.08	110.06 ± 3.51	P< 0.01
Resistant	12.50 ± 0.05	23.55 ± 5.36	P< 0.05
Total Cu	16.82 ± 0.13	140.2 ± 1.78	P< 0.01
Pb			
EFLE	5.43 ± 0.33	0.35 ± 0.02	P< 0.05
Acid-reducible	5.64 ± 0.43	15.65 ± 0.08	P< 0.05
Oxidisable-organic	8.02 ± 0.24	42.41 ± 3.05	P< 0.05
Resistant	31.64 ± 0.58	15.57 ± 1.00	P< 0.05
Total Pb	50.78 ± 0.50	77.10 ± 7.91	P< 0.05
Zn			
EFLE	0.15 ± 0.01	0.72 ± 0.08	P< 0.05
Acid-reducible	0.16 ± 0.01	50.00 ± 0.53	P< 0.01
Oxidisable-organic	21.16 ± 1.67	72.68 ± 4.05	P< 0.05
Resistant	92.70 ± 2.60	113.90 ± 19.79	P> 0.05
Total Zn	116.9 ± 3.94	243.9 ± 15.32	P< 0.05

Note: P indicates the significance level of metal levels between the two sites.

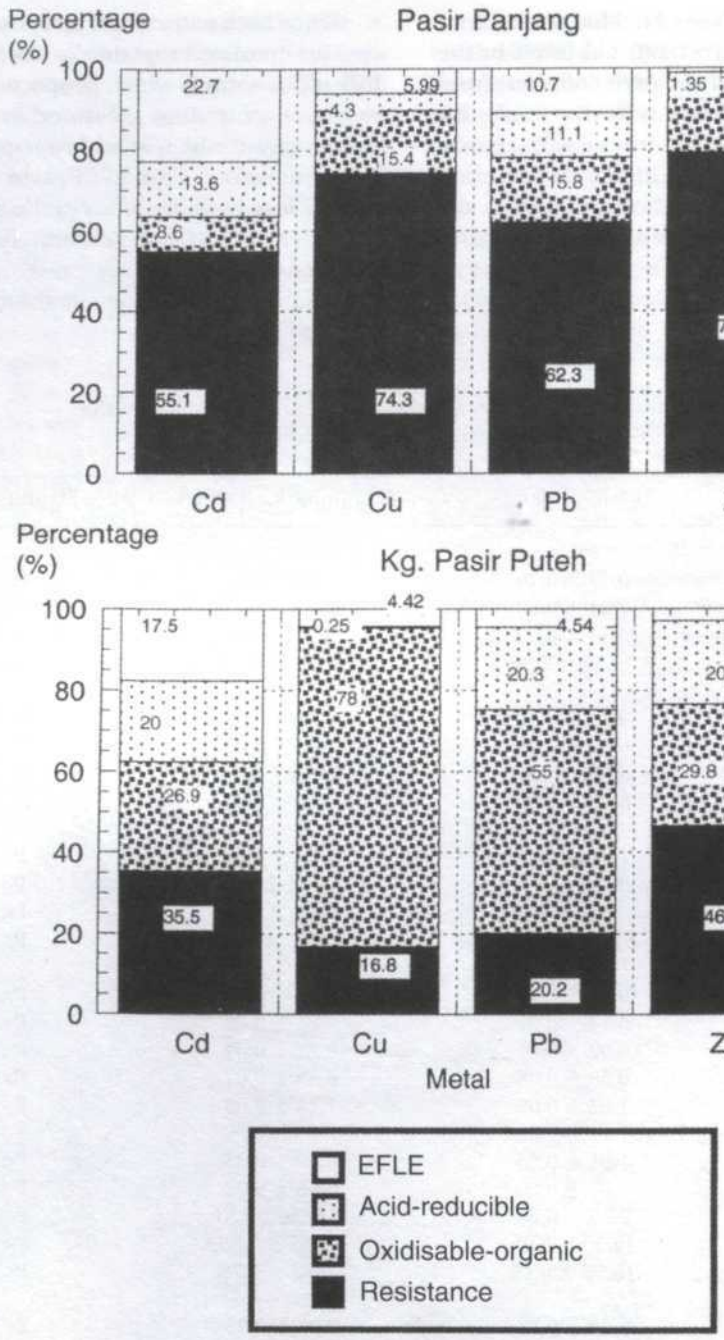

Fig. 2: The distribution of Cd, Cu, Pb and Zn in the four geochemical fractions (easily, freely, leachable or exchangeable [EFLE], acid-reducible, oxidisable-organic and resistant) of sediments from Pasir Panjang and Kg. Pasir Puteh

Aston 1983; Yap *et al.* 2002b). *Fig.* 2 shows that the non-resistant fractions of the geochemical extraction in Pasir Panjang were 44.9, 25.7, 37.7 and 10.7% for Cd, Cu, Pb and Zn, respectively. In comparison, the non-resistant fractions from Kg. Pasir Puteh were higher with 64.5, 83.2, 79.8 and 53.3% for Cd, Cu, Pb and Zn, respectively. This probably indicates that Kg. Pasir Puteh has received more anthropogenic input of the metals.

The conditions of the two sites were probably the reason why Mashinshian *et al.* (2002) chose Kampung (Kg.) Pasir Puteh and Pasir Panjang

for their kinetics studies on polycyclic aromatic hydrocarbons (PAHs), conducted in October 1999. In their results, they found that Kampung (Kg.) Pasir Puteh had significantly ($P< 0.05$) higher levels of PAHs than Pasir Panjang. In the samples collected in December 1997 and September 1998, Mashinshian *et al.* (1999) found that the PAH levels in the soft tissues of *P. viridis* collected from Kg. Pasir Puteh were 8 to 16 times higher than those in mussels collected from Pasir Panjang during the two sampling periods, respectively.

Based on the present data, we can conclude that higher levels of Cu, Pb and Zn can be expected judging from the environmental backgrounds of Pasir Panjang and Kg. Pasir Puteh. The results of this study show the importance of site descriptions with different environmental backgrounds. Observation at the sampling site can provide useful information on the association between the contaminants being studied and the activities in the vicinity of the sampling site. We can assume that the high levels of Cu, Pb, Zn and PAHs could have been released from industrial, urban and port activities since these activities could be observed in the vicinity at Kg. Pasir Puteh. This study showed significant differences between the two sites for Cu, Pb and Zn but not for Cd levels. This indicated that although we might expect relatively high levels of contaminants to be found at the sampling site with observable human activities, actually which contaminant(s) that is(are) high should be verified by chemical analyses done in the laboratory.

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