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Etiology of Bacterial Soft Rot of Orchids

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ABSTRAK

Gejala penyakit reput lembut bakteria telah diperhatikan pada pokok-pokok orkid jenis Phalaenopsis *dan* Dendrobium. *Penyakit ini menyebabkan kematian banyak pokok-pokok orkid terutama sekali jenis* Phalaenopsis *pada peringkat benih dan pokok muda. Bakteria telah berulangkali diasingkan daripada pokok-pokok yang berpenyakit. Ujian menunjukkan asingan-asingan bakteria adalah patogenik pada orkid. Langkah-langkah mengikut dalil-dalil Koch telah dijalankan. Berdasarkan kepada ujian-ujian kultur, morfologi, fisiologi dan biokimia, asingan-asingan bakteria telah dikenalpasti sebagai* Erwinia chrysanthemi *Burk., Mc Fadden and Dimock, 1953.*

ABSTRACT

Symptoms of bacterial soft rot were observed on the Phalaenopsis *sp. and* Dendrobium *sp. orchids. The disease caused death in many plants, especially those of the* Phalaenopsis *sp. at the seedling stage and of young plants. Bacteria were consistently isolated on diseased plants. Tests proved the pathogenicity of the isolates on orchids. Steps were carried out to complete Koch's postulate. Based on the cultural, physiological and biochemical properties the pathogen was identified as* Erwinia chrysanthemi *Burk., McFadden and Dimock 1953.*

Keywords: Bacterial soft rot, *Phalaenopsis, Dendrobium, Erwinia chrysanthami*

INTRODUCTION

Orchids have been known to be infected by bacteria from the genus *Erwinia.* Strider (1985) described soft rot caused by *Erwinia carotovora* (Jones) Holland, which affected a wide range of vegetable and ornamental plants, as being not too common on orchids, but can be the most destructive disease. In Malaysia, Singh (1973) listed soft rot of *Phalaenopsis* sp. caused by *E. carotovora* (Jones) Holland and indicated that the disease was not serious and of rare occurrence. However, since early 1989, rotting of *Dendrobium* sp. and *Phalaenopsis* sp. was commonly observed in the campus of Universiti Pertanian Malaysia on all stages of plant growth. The disease was observed to be more severe during the wet periods and on *Phalaenopsis* hybrids. The objective of this study was to determine the etiology of the disease on these orchids.

MATERIALS AND METHODS

Isolation of bacterial strains

Leaves of plants showing soft rot symptoms were brought to the laboratory and washed under running tap water. The epidermis of the leaves between the rotted and healthy tissue were aseptically removed. A small portion of the tissue was then removed and squashed in a drop of sterile distilled water and allowed to stand for 15 min. A loopful of this was streaked on Difco nutrient agar (NA) plates and incubated at 30 ± 1°C for 24hr. Isolated colonies were purified by serial dilutions and spread on NA plates and incubated in the same manner. Isolated colonies were selected and streaked on NA and modified yeast extract-dextrose-calcium carbonate (YDC) agar (Dye, 1968) slants for stock preparation. Stocks were kept at 4 and 15°C for further studies. *Bacterial cultures:* In addition to the five bacterial isolates from orchids, an isolate

of *Erwinia carotovora* pv. *carotovora*, that caused soft rot of cabbage was also included in the morphological, cultural, physiological and biochemical tests. All cultures were maintained at the Department of Plant Protection, Universiti Pertanian Malaysia.

Morphological and cultural properties

All bacterial strains were tested for Gram's stain and examined for shape. Gram's stain reaction was further confirmed with the KOH solubility test. Colour of growth on modified YDC and on glucose yeast extract calcium carbonate (GYCA) agar (Dye, 1968) was observed daily up to 1 week.

Physiological and biochemical properties

All tests were made using a 24-48hr culture from NA and incubated at 30 ± 1°C unless indicated otherwise. Cultures were tested for their ability to cause rotting of potato slices, phosphatase production and sensitivity to erythromycin (15 ul). These were carried out as described by Kelman and Dickey (1980). The methods described by Dye (1968) were used to test for : acetoin production, oxidation fermentation, gas from glucose, catalase, oxidase, growth in 5% NaCl, reducing substance from sucrose, glatin hydrolysis (Cowen's method), growth at 40°C, production of nitrite from nitrate and production of acid from glucose, sucrose, lactose, maltose, trehalose, cellobiose, rhamnose, arabinose, sorbitol, dulcitol, mannitol, melibiose and alpha-methyl-d-glucoside using medium C. In addition, acid production from glucose, sucrose, lactose, maltose, trehalose, cellobiose, sorbitol, dulcitol and mannitol were also tested using Bacto OF medium (Difco). A 10% (w/v) aqueous solution of the above carbon sources was filter sterilized were aseptically added to the basal medium to give a final concentration of 1.0% (w/v). A change in the color of the medium from green to yellow was scored as a positive reaction. Readings were done at 3, 7, 14 and 21 days. To test for the production of indole, bacterial strains were cultured in 3 media for indole production as given in i) Lelliott (1957), ii) Bradshaw (1963) and iii) Dye (1968). Cultures were tested after 2 and 5 days by addition of 0.5 ml xylene which was mixed with the culture before addition of Kovacs' reagent. Hydrogen sulphide production was tested from cystein hydrocloride by the method described in Dye (1968) and from sodium thiosulphate by using Kligler Iron agar (Oxoid). Bacto Malonate broth (Difco) and Bacto-Koser Citrate Medium (Difco) were used to test for the utilization of malonate and citrate respectively. Production of lecithinase was determined as described in Fahy and Hayward (1983).

Pathogenicity test

Bacterial suspensions were made from a 24 - 48hr culture in sterile distilled water. These were adjusted to approximately 6 x 10^9 cfu/ml using a spectrophotometer. Fifteen ul of the bacterial suspension was then placed on the surface of the leaves of *Phalaenopsis* hybrids and the leaves were lightly pricked twice through the bacterial suspension. Control plants were similarly inoculated but with sterile distilled water.

All bacterial strains from the pathogenicity test that produced soft rot after 24 - 48hr were reisolated. Morphological, cultural, physiological and biochemical test as indicated above were repeated with these isolates.

RESULTS AND DISCUSSION

Morphological and cultural properties

Five bacterial isolates examined were all rod shaped with peritrichous flagella. All grew readily on modified YDC and GYCA. Orchid isolates consistently produced non-diffussible blue pigment on GYCA media. On modified YDC medium, pigment production was variable and was observed only on the third or fourth day while on GYCA pigment production was observed on the first day. *E. carotovora* pv. *carotovora* did not produced any pigment on both YDC and GYCA. On NA, all isolates produce small translucent colonies, that could not be differentiated.

Physiological and biochemical properties

Distinct differences could be seen in the physiological and biochemical properties of bacterial isolates from orchids and cabbage (Table 1). The distinctive properties of *E. chrysanthemi* according to Dickey (1979); Dye (1969); Cother and Sivasithamparam (1983), such as: gas production from glucose, production of phosphatase and lecithinase, sensitive to erythromycin (15 ug), produced blue non-diffusible pigment on modified YDC and GYCA media; utilization of sodium malonate was apparent for the orchid isolates (Table 1). Based on their cultural, physi-

TABLE 1
Physiological and biochemical properties of isolates of *Erwinia* spp. from orchid and cabbage

Property	Origin of *Erwinia* species	
	orchids (5 isolates)	cabbage (1 isolate)
Acid production from:		
Glucose (aerobic & anaerobic)	+	+**
Sucrose	+	+
Maltose	–	–
Cellobiose	+	+
Lactose*) (in 1 week)	–	+
Trehalose*)	–	+
Rhamnose	+	+
Arabinose	+	+
Sorbitol	–	–
Dulcitol	–	–
Mannitol	+	+
Alpha–methyl–d–glucoside	–	–
Melibiose	+	+
Gas from glucose*	+	–
Potato soft rot	+	+
Gelatin liquefaction	+	+
Sensitivity to erythromycin (15 g)*	+	–
Phosphatase*	+	–
Lecithinase*	+	–
Blue non–diffusible pigment on GYCA media*	+	–
Catalase	+	+
Oxidase	–	–
Indole*	+	–
Methyl Red	–	+
H_2S production	–	–
Nitrite from nitrate	+	+
Reducing sub. from sucrose (48 hr.)	–	–
Beta–galactosidase	+	+
Arginine dihydrolase	+	+
Utilization of:		
Sodium citrate	+	+
Sodium malonate*	+	–
Gram stain	–	–
KOH test	+	+

* Determinative properties according to Dye (1969)
** + = Positive reaction; – = Negative reaction
GYCA = Glucose yeast extract calcium carbonate agar (Dye, 1969).

ological and biochemical properties isolates from *Dendrobium* sp. and *Phalaenopsis* sp. were thus identified as *E. chrysanthemi* Burk., Mc Fadden & Dimock, 1953. This findings corroborate the work of Lim and Khaw (1984) who indicated that the causal organism of bacterial soft rot of orchids, previously attributed to *Erwinia carotovora* (Jones) Holl. in Singapore and Peninsular Malaysia, to be *Erwinia chrysanthemi* Burk., Mc Fadden & Dimock, 1953.

In Malaysia, *E. chrysanthemi* had so far been isolated from two other hosts, namely, *Ananas comosus* (L.) Merr. (Lim, 1974) and *Zea mays* (Abdullah, 1982).

Symptoms and pathogenicity

Soft rot symptoms on orchids were observed on *Phalaenopsis* sp. and *Dendrobium* sp. at all stages of plant growth (Plates 1a & 1b). However, the disease was most severe on seedlings and young plants of the *Phalaenopsis* sp. during wet periods. On seedlings, soft rot commonly occurs at the base of the leaves, thus resulting in the death of the plants soon after infection (Plate 2). On inoculated plants, initial symptom was a watersoaked rot which enlarged rapidly with no apparent yellowing of the margin after 1-2 days (Plate 3). On mature plants, the margin of the

A

B

Plate 1. Natural infection of soft rot on A) Phalaenopsis *sp. and B)* Dendrobium *sp.*

Plate 2. Infection of Phalaenopsis *seedlings at the base of the leaves resulted in the death of the plants.*

Plate 3. Symptoms of soft rot on Phalaenopsis *seedlings 2 days after inoculation.*

Plate 4. Symptoms of soft rot on mature Phalaenopsis *plants 5 days after inoculation.*

rotted area usually produce yellowing, 4-6 days after infection (Plate 4). *E. chrysanthemi* isolates were found to be highly pathogenic to *Phaelaenopsis* hybrids while *E. carotovora* pv. *carotovora* from cabbage was not.

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Growth Inhibition and Stimulation by Groundnut Plant Residue

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ABSTRAK

Beberapa kedudukan yang berbeza sisa pokok kacang tanah telah dikaji kesan fitotoksiknya ke atas tumbesaran dan perkembangan pokok kacang tanah dan jagung. Keputusan menunjukkan sisa mengeluarkan bahan yang merencatkan tumbesaran dan perkembangan pokok kacang tanah semasa penghuraiannya. Sisa yang digaul dengan tanah atau yang terletak dibawah biji benih paling merencatkan tumbesaran kacang tanah. Sebaliknya, sisa di atas permukaan tanah atau di dalam tanah merangsangkan tumbesaran awal jagung.

ABSTRACT

Groundnut plant residue at different placements in the soil was tested for phytotoxic and other effects on the growth and development of groundnut and maize plants. Results indicated that the residue released substances during decomposition that inhibited growth and development of groundnut. Residues mixed with the soil or banded in a layer below the seed was the most inhibitory to the growth of groundnut. However, early maize growth was stimulated by the presence of residue on the soil surface or in the soil.

Keywords: allelochemicals, groundnut plant residue, phytotoxic

INTRODUCTION

Groundnut (*Arachis hypogaea*) yields from second and subsequent croppings were reported to decrease by more than 50% of the first crop (Chan, 1968; Cheah, C.H. - personal communication). In most cases the yield reduction was attributed to poor pest and disease management or depletion of soil nutrients. This decrease in yield, however, may also be partly explained by the type of residue remaining from the previous crop. Substantial evidence from the literature shows the presence of phytotoxic substances, called allelochemicals, that are produced by most crops (Guenzi *et al.* 1967; Cochran *et al.* 1977; Robinson and Burdick, 1978; Elliot and Roy, 1982; Yakle and Cruse, 1983, 1984). These allelochemicals may be responsible for the reduced growth and yield observed. However, genotypes of various crop species may differ in their ability to produce or tolerate allelochemicals. Kimber (1967) reported difference in the level of inhibition of wheat *(Triticum aestivum)* growth caused by residues of several wheat genotypes. Maize (*Zea mays*) hybrids also showed some differences in their responses to maize residue (Zakaria and Kaspar, 1990). Growing the same maize hybrids continuously yielded lower than continuous maize when hybrids were rotated (Hicks and Peterson, 1981). The lower yields may have resulted because the hybrids either differed in their tolerance to allelochemicals or in their residue toxicity.

Likewise, groundnut plant residue and groundnut hulls were also reported to inhibit the germination and shoot growth of groundnut, okra *(Hibiscus esculentus)* and cucumber *(Cucumis sativa)* as well as caused decrease in yield and grade of tobacco (*Nicotiana tabacum)* leaves (Robinson and Burdick, 1978; Elliot and Roy, 1982; Zakaria and Razak, 1990). The extract from fresh groundnut plants was more toxic than extracts from partially decomposed or heat-traeted residues (Zakaria and Razak, 1990). However, the inhibitory effect of the residues decreased as time of residue decomposition increased.

The objective of this study was to examine the inhibitory and stimulatory effects of ground-

nut plant residue at different placements in the soil on the growth and development of groundnut and maize plants.

MATERIALS AND METHODS

Groundnut plant residue of Matjan was collected after harvest, air-dried and then cut to pieces ranging from 1 to 2 cm in length. The potting medium was a 3:2:1 mixture of soil, sand and organic matter. Five treatments were compared: residue on the soil surface, residue banded 2.5 cm below soil surface, residue banded 5.0 cm below soil surface, residue mixed with the soil, and no residue as the control. Eighty grams of residue (equivalent to approximately 10000 kg residue ha^{-1}) were either banded or mixed with approximately 0.0212 m^3 of soil mixture in a 36-cm diameter clay pot. Seeds of either groundnut (Matjan) or maize (Thai Supersweet) were planted in each pot at a depth of 3 to 4 cm. Each pot was given an equal volume of water every two days. Each pot also received 0.7g urea, 1.3g Triple Superphosphate (TSP) and 1.0g Muriate of Potash (MOP) for groundnut and 4.3g urea, 2.2g TSP and 1.6g MOP for maize at planting. Two sets of experiments were conducted using a randomized complete block design with four replications. One set of experiment was harvested at maturity. Plants in each pot were thinned to four seedings and one seedling after emergence for set one and two, respectively. Parameters measured for set one were: extended leaf height of each plant, shoot and root dry weight of the four plants, and shoot to root dry weight ratio. For set two, the following were determined: a) for groundnut - days to flowering, pegging and podding and pot and kernel dry weight per plant at maturity; b) for maize - days to tasseling, silking and maturity and ear and kernel dry weight at maturity. Plants in set one were harvested by washing off soil mixture form the roots. The roots were further cleaned by hand. The cleaned roots were separated from the shoots and dried in an oven at 60°C for 48 h. The shoot and root dry weights and ratios were determined thereafter. In set two, the plants were harvested at physiological maturity. For groundnut, maturity was determined by the method of Boote (1982).

RESULTS AND DISCUSSION

Toxicity of residue on growth of groundnut

Groundnut plant residue used as green manure, compost or mulch may inhibit early crop growth. Bioassay of fresh and partially decomposed residue extracts were shown to inhibit germination and radicle elongation of several crop species (Zakaria and Razak, 1990). Table 1 shows the mean height of groundnut plants treated with residue at different placements in the soil. At 6 DAP, all residue-treated plants were shorter than the control. However, from 10 DAP onwards plants grown in the residue mixed with the soil were the shortest compared to plants in the other treatments. Residues mixed with the soil probably decomposed much faster than surface residues and because allelochemicals sometimes result from decomposition, a greater concentration of allelochemicals may have been produced when residues were mixed with the soil (Yakle and Cruse, 1983; Zakaria and Kaspar, 1990). Additionally, incorporating residues with the soil results in direct contact between residues and roots growing in the soil and thus, may

TABLE 1
Effect of groundnut residue on mean height (cm) of groundnut plants.

Residue placement	Day after planting				
	6	10	14	18	22
Soil surface	0.4b	5.9a	10.1b	12.9b	16.2b
2.5 cm below soil	0.6b	6.3a	10.2a	13.4b	16.0b
5.0 cm below soil	0.7b	6.1a	10.2a	13.4b	16.3b
Mixed with soil	0.8b	4.9b	9.1b	12.2c	15.6c
No residue	2.1a	6.5a	10.4a	14.1a	17.8a

All means in a column not followed by the same letter were significantly different from one another at 5% probability as determined by Duncan Multiple Range Test (DMRT).

result in a greater effect. The residue on the soil surface or banded in the soil also resulted in plants that were shorter than the control (Table 1). The results indicated that the effect of the surface or banded residue was not only caused by just physical restriction but possibly also by chemical interaction. The groundnut plants might be sensitive to substances released by the residue during decomposition, thus, causing autointoxication. This effect was observed with rice, maize and wheat treated with their respective residues (Guenzi *et al.* 1967: Chou and Lin. 1976).

Plants grown with the residue were lighter in shoot, root and total dry weight compared to the control (Table 2). The shoot and the root dry weight were reduced by between 25-41% and 45-60%, respectively. In addition, the total dry weight was reduced by 32-46%. Zakaria and Razak (1990) reported that the groundnut plant residue extract caused browning and distorted elongation of the radicle of several crop species. This indicated that root growth was the most sensitive to substances produced by the residue, irrespective of its placement in the soil. However, the residue mixed with the soil or banded below the seeds had a higher chance of inhibiting early root growth. The reduction in root growth was also manifested by the larger shoot to root dry weight ratio when the residue was placed on the soil surface, below the seeds or mixed with the soil (Table 2).

Table 3 shows the effect of residue placements on days to flowering, pegging and podding, and pod and kernel dry weight of groundnut at harvest. Early reproductive development of the plants in terms of days to flowering, pegging and podding was not delayed by the residue. The results implied that although early vegetative growth was inhibited by the residue, groundnut plants were able to overcome the effect at the later growth stages. Thus, increasing the time of residue decomposition or weathering of the residue eventually decrease residue toxicity under most conditions (Yakle and Cruse, 1984; Zakaria and Kaspar, 1990). In addition, the pod and the kernel dry weight of plants grown in soil with the residue were lighter than those of the control by 34-56% and 42-66%, respectively (Table 3). The residue not only inhibited early vegetative growth but also the pod and kernel development during the reproductive stage. The placement of the residue relative to the seed also affected the pod and the kernel development. The residue placed below the seed or mixed with the soil resulted in plants having the lightest pod and kernel dry weight. The developing pod might have been in direct contact with the residue, and substances produced during residue decomposition might have influenced both the pod and the kernel development. These findings might account for the reduction in groundnut yield as a result of continuous croppings and which were not totally attributed to insect pests and diseases (Chan, 1968). Another possibility is that the residue might have immobilized nutrients to the developing parts, especially nitrogen and phosphorus (Parker, 1962; Bhowmik and Doll, 1984).

Stimulation of maize growth by the residue

Groundnut plant residue might be stimulatory to early maize growth even though maize germination was inhibited by fresh extract from groundnut plant residue (Zakaria and Razak, 1990). They also noted a stimulatory effect on

Table 2
Effect of groundnut residue on mean dry weight (g) and ratio of groundnut vegetative parts at 22 DAP

Residue placement	Shoot Weight	Root Weight	Total Weight	Shoot:root Ratio
Soil surface	1.83b	0.36b	2.19b	5.08a
2.5 cm below soil	1.66b	0.42b	2.08b	3.95b
5.0 cm below soil	1.45b	0.31b	1.76b	4.68a
Mixed with soil	1.44b	0.31b	1.75b	4.65a
No residue	2.45a	0.77a	3.22a	3.18b

All means in a column not followed by the same letter were significantly different from one another at 5% probability as determined by DMRT.

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Influence of Position of Panicle on Seed and Seedling Characteristics of Rice *(Oryza sativa L)*

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ABSTRAK

Ciri-ciri biji benih diperoleh daripada cabang-cabang bulir primer, sekunder dan tertier dalam empat jenis padi Indica (Oryza sativa) *telah dinilai. Pola-pola percambahan dan viabiliti biji benih yang diperoleh serta kekuatan dan ciri-ciri anak benih yang membesar daripada biji-biji benih ini juga telah dikaji. Ciri-ciri biji dan anak benih daripada tiga jenis dahan yang sama bagi empat jenis padi ini adalah serupa. Sebaliknya perbezaan yang ketara kelihatan antara biji benih yang diperoleh daripada tiga jenis dahan. Biji benih daripada dahan primer bulir mempunyai berat lebih 100 biji. Berat terendah diperhatikan pada biji-biji benih daripada cabang-cabang tertier. Perbezaan ini pada asasnya disebabkan endosperma yang lebih bagi biji-biji benih di dahan primer, yang seterusnya meningkatkan nisbah endosperma: embrio. Daya tahan dan daya percambahan ketiga-tiga biji benih adalah sama dengan ciri-ciri biji benih tersebut. Biji benih daripada cabang primer bulir mempunyai daya tahan yang lebih tinggi. Anak benih daripada biji-biji benih ini juga lebih kuat. Perbezaan pertumbuhan anak benih daripada ketiga-tiga dahan bulir padi tersebut adalah sama daripada segi ciri-ciri dan dayatahan bijinya. Kajian tersebut menunjukkan bahawa anak benih daripada biji-biji benih cabang primer memperlihat pertumbuhan yang lebih unggul disebabkan ciri-ciri biji benih yang lebih baik. Beberapa implikasi praktikal kajian tersebut akan dibincangkan.*

ABSTRACT

The characteristics of seeds obtained from primary, secondary and tertiary branches of panicles in four varieties of Indica rice (Oryza sativa) *were evaluated. The germination patterns and viability of procured seed and the vigour and characteristics of seedlings developed from these seeds were also studied. The characteristics of seeds of the three branch types of the four varieties and their seedlings were similar. In contrast, marked differences were observed between the seeds obtained from the three types of branches. Seeds of primary branches of the panicle had a greater 100-seed weight. The lowest weight was observed in seeds from tertiary branches. This difference was principally due to the heavier endosperm of the seeds of primary branches, which in turn increased the endosperm:embryo ratios. The viability and germinability of the three categories of seed were similar to those of seed characteristics. Seed from primary branches of the panicle had a higher viability. The vigour of seedlings from these seeds was also greater. Differences in growth of seedlings from the these seeds was also greater. Differences in growth of seedlings from the three branches of the rice panicle were similar in terms of characteristics and viability of seeds. The study revealed that seedlings from seeds of primary branches manifested superior growth because of better seed characteristics. Some practical implications of the study are discussed.*

Keywords: Branches, panicles germination patterns, seed and seedling characteristics, *Oryza sativa L*

INTRODUCTION

Seeds play an important role in determining the success of rice culture. Thus, selection of good quality seeds is considered a primary factor in ensuring high yields.

Seedling emergence and subsequent early growth affect final yields in cereals (Evans and Bhatt, 1977). Variations in field emergence, particularly under poor management are attributed to differences in seed vigour (Matthews. 1980). Seed with low vigour either emerge late or produce abnormal seedlings, resulting in poor yields.

Seed vigour is affected by the storage material in the seed (Heydecker, 1969). This has been reported to be the cause in regard to cereals (eg. Evans and Bhatt, 1977) and legumes (e.g. Grotechusmann and Robbeler, 1985). Rice (Tashiro *et al.*, 1988) also showed differences in storage material within and between varieties. These differences have been attributed to sev-

eral factors, ranging from panicle position and development to variations in growth and environmental conditions.

Rice panicles have three types of branches, namely primary, secondary and tertiary. The position of the seed on the panicle also affects grain filling during seed development (Vijayalaksmi *et al.* 1988). This study was carried out to determine the physical characteristics of seeds on different branches of a rice panicle and their germination patterns. The study also evaluated differences in seedlings grown from seeds obtained from different branches of the rice panicle.

METHDOLOGY

The study was carried out in a plant house at the University of Peradeniya, Sri Lanka (7°N, 81°E, 385m above sea level). The mean environmental conditions during the experimental period were: Temperature - 27.4° ± 1.8°C; Relative humidity 76.8% ± 3.45% and a daylength of 10 - 11 hours. Uniform seeds of four rice varieties (3 1/2 - 4 month maturity group, germination 94.5 ± 1.5%), namely BG 34/8; 94/1, 278/5 and 379/2 were selected for the study. The 100-seed weights of these varieties were 2.811g, 2.801g, 2.849g and 2.816g respectively. Thus the mean 100 seed weight of the four varieties was 2.811 ± 0.427g.

The selected seeds were immersed in distilled water for six hours and planted in plastic pots containing approximately 6 kg of a saturated low humic gley soil. The soil selected had a clay loam texture with 65.1% clay. Planting was carried out to ensure 4 plants per pot.

The lay out of the plants was a completely randomized design with four replicates per variety. The crop was managed on the basis of recommendations for rice in Sri Lanka (Gunasena, 1974). Soil moisture was maintained over field capacity until seed ripening. and then left to dry under normal farm conditions until harvesting.

At full ripening, seeds of primary, secondary and tertiary branches of the selected varieties were removed carefully. A sample of each catergory was dried to determine the moisture content, and the seed weights calculated at 14% moisture and the 100 seed weights determined. The following studies were carried out to determine the seed and seedling characteristics from seeds obtained from the three types of branches of a panicle -

A. Samples of 50 seeds from each category were soaked for 24 hours in distilled water, and dissected to determine the weights of husk, endosperm and embryo. The dissected samples were dried, and the weights calculated at a moisture content of 14%.

B. Germination characteristics of the three types of seed were determined by planting 100 seeds per category from each variety on trays containing 2.5 cm of fine river sand. The seeds were placed in rows and lightly covered with sand. These were watered daily. Seed germination was measured daily until 70% emergence was obtained, the tray were maintained for 21 days to obtain the final germination. The percentage abnormal seedling and percentage dead seedling were also determined as described by ISTA (1985) from these samples at the 21st day after planting. In addition seed viability was tested by the tetrazolium method on 50 seeds per sample suggested by ISTA (1985).

C. Seedling vigour was determined by planting 50 seeds of each catergory from the four varieties at different depths in fine sand. The depths of planting were at 1, 3, 5 and 7cm. Final emergence of seedling was determined at 20 days after planting.

D. Growth of seedling emerging from seeds obtained from different branches of the panicle was determined by planting 100 seed of each category per replicate in trays containing 2.5 cm of fine sand. Seedlings were harvested at 21 days, and the shoot and root dry weight were determined by drying 20 healthy seedling per replicate from each category of the four varieties at 80°C to a constant weight. In addition, the total root length of the seedling was determined by the Grid technique as described by Tennent (1975).

The data were analised accordig to methods described by Gomez and Gomez (1981). As the results of all four varieties were similar, they were pooled for the final analysis to determine treatment differences.

RESULTS AND DISCUSSION

Seeds of the three types of branches on rice panicles had significant differences in terms of seed weight (Table 1). The weight of 100 seeds from the primary branches was 3% and 13% greater than those from secondary and tertiary branches respectively. The seed weight components appear to be the causal factors for these

TABLE 1
Characteristics of rice grain on different branches of the panicle

CHARACTERISTIC	BRANCH TYPE			
	Primary	Secondary	tertiary	LSD (P=0.05)
100 Seed weight (g)	2.946	2.862	2.571	0.031
Wt of embryo and endosperm	2.340	2.272	2.008	0.050
Wt of husk (g)	0.606	0.590	0.563	0.041
Wt of endosperm	2.291	2.223	1.995	0.022
Wt of embryo (g)	0.049	0.049	0.053	0.009
Endosperm:Embryo ratio	46.75	45.37	36.88	

differences. The weight of husks of seeds from the different branches were low in all cases, suggesting that the husk does not contribute to of the variaton of seed weights. The weight of embryo and endosperm of seeds obtained from tertiary branches are significantly less than those from the primary and secondary branches. The weights of the embryo and endosperm of seeds of the secondary branches are less than those of seeds from primary branches. The differences in the measurements of these two components are primarily caused by the variations in weights of the endosperm, as seeds of all branches have similar embryo weights. Thus differences in weights of the rice seeds obtained from the different branches of a panicle could be attributed to the variations in the endosperm weights.

In their study of panicle development Tashiro *et al.* (1988) highlight the early pollination and seed set in primary branches and suggest that seeds on the primary branches have a greater sink effect, thereby obtaining carbohydrates from the sources at a faster rate. This could be considered the causal factor for the differences in weights of the endosperms, and the greater weights of seeds on primary branches in our study. The endosperm:embryo ratios also illustrate differences between the three seed types (Table 1). The greater endosperm:embryo ratios of the seeds from primary branches indicate the ability of the food reserves to support the growing embryo to a greater degree than seeds of secondary and tertiary panicle branches.

Germination characteristics (Table 2) of seeds from the different branches of the rice panicle are significantly different. Seeds from primary branches germinate earlier. The 70% germination of seeds from tertiary branches occurs at least a day later than seeds of other branches. This in turn can affect their subsequent growth and also determine competitive effects and final yields (Harper, 1977).

The higher final germination rate also illustrates the superior performances of seeds from primary branches. The germinability of seeds from tertiary branches is 16% and 9% less than that of seeds from primary and secondary branches respectively. This suggests that the use of seeds from tertiary branches alone could reduce plant populations. Examination of viability (Table 2) also illustrates differences in characteristics among seeds of different branches. Seeds from the tertiary branches of the panicle show a lower percentage of viability, suggesting poor capacity for germination.

Table 3 presents the vigour of emerging seedlings from the selected seed classes. Seeds of all categories placed on the surface show the greatest percentage of germination. This suggests that burial of seeds which deprives the

TABLE 2
Germination of rice grain on different branches of the panicle

CHARACTERISTIC	BRANCH TYPE			
	Primary	Secondray	Tertiary	LSD (P=0.05)
Time to 70% germination	7.05 days	7.48 days	8.47 days	0.32
Final germination (%)	92.6	85.9	76.4	2.65
Viability (%)	98.5	94.6	86.5	1.48

TABLE 3
Influence of planting depth on emergence of rice seedling from seeds obtained from different branches of the panicle

Planting depth	Primary	Secondary	Tertiary	LSD (p=0.05)
		Percentage Emergence		
Surface	88.5	86.7	79.2	0.13
1 cm	85.9	81.5	74.6	0.67
3 cm	76.5	70.5	61.7	0.98
5 cm	66.4	58.3	42.6	1.89
7 cm	58.9	49.6	35.3	3.98
LSD (P=0.05)	4.65	5.96	2.99	

seeds of light could retard fermination. Germination of seeds decreases with increasing depth. The differences between the emergence of seedling from seeds of the three categories are clearly significant. Seedling emergence from seeds of the primary branches is greater at all depths. This shows high seedling vigour. In contrast, reductions in emergence of seedling from seeds of tertiary branches indicate low seedling vigour. This again highlights the superior perfomance of seeds of primary branches of the rice panicle.

Characteristics of seedling also follow the trends of seed characteristics of the different branches. This clearly indicates the influence of seed characteristic on seedling growth. Seedlings emerging from seeds of primary branches have a greater shoot and root dry weight at 21 days. Dry weights of shoot of the secondary and tertiary branches are approximately 10% and 35% lower by comparison, whereas the differences are greater in terms of root weight (i.e. 13% and 37% lower than those of seedlings from seeds of primary branches respectively). Thus shoot growth seems to be affected more by seed size than root growth; this phenomenon warrants further study.

The differences in total root length are less marked than those of weight. The reductions in total root length of seedling from seeds of secondary and tertiary branches of the panicle are 14% and 29% respectively when compared to those of seedlings from seeds of primary branches. This suggests that root dry matter accumulation, especially in seedlings from seeds of tertiary branches , exceeds that of root length. Evaluation of the types of seedlings also clearly illustrates differences between the seed types. Seeds from tertiary branches produce a greater quantity of abnormal seedlings. The number of seedlings that do not survive is also greater among this category of seeds. This occurrence is significantly reduced in seedlings emerging from seeds of secondary branches. In contrast, seeds from the primary branches produce the highest number of normal healthy seedlings.

In the process of panicle development of rice, primary branches set seed earlier. The secondary and tertiary branches set seed later (Vijayalaksmi *et al.* 1988). This phenomenon can have a significant impact on seed development (Rao, 1989). The current study indicates that seeds from tertiary branches tend to have a greater number of partially filled grain. The weights of the fully filled grain are also less than those from secondary and primary branches of the same panicle.

Seed weight has a significant impact on seedling growth (Perry, 1972), which can influence establishment and subsequent competitive relationships. This study illustrates that seeds of primary branches of the rice panicle are heavier, mainly due to the well developed endosperm. Thus, the developing embryos of these seeds, which are similar in weight to those of seeds from other branches have a greater source of carbohydrates for seedling growth, as shown by the endsperm:embryo ratios.

The differences in seed characteristic affect all aspects of germination and seedling development. The greater viability of seeds on primary branches, the higher germination percentages and seedling vigour together with better growth of the emerging seedling, could be considered the resultant effects of superior seed characterisric.

Farmers establishing rice strive to obtain the optimum populations with seed available. Thus seed characteristics are very important. This study

TABLE 4
Characteristics of seedlings from seeds of primary, secondary and tertiary branches of the rice panicle

Characteristic	Primary	Secondary	Tertiary	LSD (P=0.05)
Shoot dry wt at 20 days (g)	0.65	0.58	0.42	0.04
Root dry wt at 20 days (g)	0.59	0.51	0.37	0.11
Total root lenght at 20 days (cm)	247.5	210.0	174.5	18.96
% Normal seedlings	84.5	78.5	60.7	6.31
% Abnormal seedlings	7.4	10.8	21.4	1.96
% Dead seedlings	8.1	10.7	17.9	0.92

clearly illustrates that heavy seed, preferably from primary or secondary branches of the panicle, could provide better germination and healthy seedlings. These factors are important considerations in breeding and selection programmes where the seed supply is limited. However, in practice, procuring seeds only from selected branches of a panicle is a difficult task especially when the whole plant is harvested, unless there are breeding and selection programmes. Rao (1989) has suggested that the development of rice plants with panicles having a greater number of primary and less of tertiary branches should be considered a useful source for obtaining healthy seedlings of rice which could produce sturdy plant with the promise of higher yields; such a programme with also ensure good planting material for a crop that is primarily propagated by seed.

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Characterization of Diazotrophs Associated with Roots of *Leptochloa fusca* (L) Kunth

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ABSTRAK

Sekumpulan sembilan diazotroph diasingkan daripada rumput kallar rizosfora dan dikultur di atas nitrogen bebas sederhana. K_5, K_8, K_9, K_{10}, K_{11}, K_{12}, K_{13}, menunjukkan keaktifan nitrogenase yang tinggi (718 n mol C_2H_4 h^{-1} $riat^{1}$). Sebaliknya, dibandingkan dengan K_7 dan K_{14} ia adalah lebih rendah (< 5 n mol C_2H_4 h^{-1} $riat^{1}$). Keaktifan maksimum nitrogenase didapati dalam kultur muda (24 jam selepas pengeraman) kecuali untuk K_9 dan K_{13} di mana maksimum keaktifan masing-masing ialah selepas 36 dan 48 jam. Semua terikan Gram-negatif, mengeluarkan koloni licin dan pelikel di atas media semi-pepejal. Sel K_5, K_7, K_{10}, K_{11}, K_{12} dan K_{14} adalah pleimortik; K_8 berbentuk rod-rod yang panjang; K_9 kecil dan berbentuk bulat ataupun bujur; manakala K_{14} berbentuk rod-rod yang berlubang. Hanya K_8, K_{13} dan K_{14} didapati dalam bentuk motil. Kesemua pencilan ini berupaya mengecilkan nitrat dan positif untuk oksidase dan katalase. Tiada satupun pencilan-pencilan boleh dinitrifi atau mempunyai keaktifan urase kecuali K_{14} yang positif urease. K_8, K_{13} dan K_{14} difermentasi dan mengeluarkan pigmen merah. Pencilan-pencilan K_5, K_{10} dan K_{12} telah dikaitkan dengan genus Azotobacter *sementara yang lainnya masih tidak dikenalpasti.*

ABSTRACT

A group of nine diazotrophs were isolated from the rhizosphere of kallar grass and cultured on nitrogent-free medium. K_5, K_8, K_9, K_{10}, K_{12}, K_{13} showed high nitrogenase activity (> 18 n mol C_2H_4 h^{-1} $vial^{1}$) whereas in K_7 and K_{14} it was comparatively low (< 5n mol C_2H_4 h^{-1} $vial^{1}$). Maximum nitrogenase activity was found in young cultures (after 24 hours of incubation) except for K_9 and K_{13} where it was maximum after 36 and 48 h, respectively. All strains were Gram-negat, produced smooth colonies and pellicles on semi-solid media. Cells of K_5, K_7, K_{10}, K_{11}, K_{12} and K_{14} were pleiomorphic: K_8 formed long fine rods; K_9 was small, round or oval shaped; while K_{14} formed beaded rods. Only K_8, K_{13} and K_{14} were found to be motile. All isolates were able to reduce nitrate, and were positive for oxidase an catalese. None of them could denitrify or had urease activity except for K_{14} which was urease positive. K_8, K_{13} and K_{14} were fermentative and produced red pigments. The isolates K_5, K_{10} and K_{12} are assigned to the genus Azotobacter *while others remained unidentified.*

Keywords: Diazotrophs, nitrogenase activity, physiological and biochemical characteristics, kallar grass, *Leptochloa fusca*, *Azotobacter*

INTRODUCTION

A number of nitrogen fixing bacteria have been isolated from the roots of different plants (Ahmad, 1979: Cappone and Budin, 1982; Malik and Zafar, 1985; Bilal and Malik, 1987; Reinhold *et al.* 1987; Cavalcante and Dobereiner, 1988; Zafar *et al.* 1988). In Pakistan, attempts have been made to reassess the possible contribution of nitrogen-fixing bacteria on the fertility of saline soil. Attention is being focussed on a salt-tolerant grass, *Leptochloa fusca* (L), which can grow well on low-fertility saline and sodic soils without the addition of nitrogenous fertilizers. Dinitrogen fixation associated with the roots of kallar grass has been reported Malik *et al.* (1980, 1982). Aerobic nitrogen-fixing bacteria have been reported in the rhizophere, rhizoplane and histoplane of *Leptochloa fusco.* Bilal and Malik (1987b) have isolated a nitrogen-fixing zoogloea - forming bacterium from the histoplane of kallar

grass while Niemann *et al.* (1985) and Reinhold *et al.* (1986, 1987) have identified diazotrophs associated with the roots of the same grass as *Azospilirillum*. Facultative anaerobic diazotrophs *Klebsiella* (Malik and Zafar, 1985) and *Enterobacter* (Zafar *et al.* 1988) from the roots of kallar grass have also been reported. The isolation of nine diazotrophs from the rhizophere of *Leptochloa fusca* (L) (Kunth) is reported here.

MATERIALS AND METHODS

After washing with saline (0.85% NaCl) and sterile, distilled water, soil-free roots of kallar grass were excised into 1 cm long pieces and incubated in 5 ml of semi-solid nitrogen free medium (NFM) which contained (g/l in distilled water) $CaCl_2.2H_2O$, 0.02; $MgSO_4.7H_2O$, 2.0; Malic acid, 5.0; KOH, 4.5; NaCl, 0.1; $NaMoO_4.2H_2O$, 0.002; Bromothymol blue, 0.5% in 3 ml of ethyl alcohol; Biotin, 0.1; Yeast extract, 0.002; Agar - for semi-solid, 2.0, for solid, 20.0; K_2HPO_4, 0.5; Na_2Fe (EDTA), 1.64% in 4 ml of water; pH,6.8. Cotton-plugged bottles containing root pieces in NFM medium were incubated at 30°C for 24 - 48 h. Thereafter, fresh vials were inoculated and incubated at 30°C. The cultures were assayed for nitrogenase activity by the acetylene reduction assay (ARA) as per Zafar *et al.* (1988). Cultures from ARA positive vials were streaked on NFM plates and incubated for 48 h. Cultures were then purified by routine streaking methods on nutient agar plate. Pure cultures were transferred to semi-solid NFM medium and incubated at 30°C When growth was observed (between 24- 48 h) the acetylene reduction assay was performed and positive cultures were maintained for further study.

Morphological, cultural and biochemical characters of the isolates were studied using standard bacteriological methods (Gerhardt *et al.* 1981) and QTS-20 and cyto chrome oxidase strips obtained from DESTO (Defence Science and Technology Organisation) Laboratories, Karachi.

RESULTS

ARA performed on freshly-grown cultures (24 h) showed that all the vials gave positive results for nitrogenase activity (> 18 n mol C_2H_4 h^{-1} $vial^{-1}$). These cultures were further streaked to single colonies on plates containing NFM medium. After purifying on nutrient agar plates, twenty single colonies were picked at random and tested for nitrogenase activity. Of these, eleven isolates gave week positive results while nine others (K_5, K_7, K_8, K_9, K_{10}, K_{11}, K_{12}, K_{13} and K_{14}) produced rather strong ARA activities and were selected for subsequent studies. Nitrogenase activity of the selected isolate was determined after 24, 36, 48 and 72 h of incubation. ARA was found to be different for each of these isolates (*Fig.1*) Nitrogenase activity was comparatively low for K_7 and K_{14} and rather strong, with maximum activity after 24 hours of incubation, for K_5, K_8, K_{10}, K_{11} and K_{12}. In K_9 and K_{13}; maximum nitrogenase activity was observed after 36 and 48 hours of incubation, respectively.

All isolates produced round colonies with entire margins (with some variation in size) on NFM and nutrient agar media. The sizes were 1.5 mm for K_5, K_7, K_9, K_{10} and K_{12}; 2.5 mm for K_{11}, K_{13} and K_{14}; and 5.0 mm for K_8 after 24 hours of incubation. All strains formed pellicles in semi solid media. Colony colour variation was observed on different media by different isolates. Colour of all isolates grown on NFM was cream except for K_{14} which was white; on nutrient agar it was yellowwish for K_5, offwhite for K_8 and K_{10}, and cream for K_7, K_9, K_{11}, K_{12}, K_{13} and K_{14}. Colour of colonies cultured on potato extract medium was cream for K_8, K_{11} and K_{14}, and brown to dark brown for K_5, K_7, K_9, K_{12}, and K_{13}. On MacConkey's agar, weak positive results were observed only for K_8, K_{13} and K_{14}. Variation in the cell shape of the different isolates was also recorded. K_5, K_7, K_{10}, K_{11}, K_{12} and K_{13} were irregular or pleiomorphic. K_8 formed long fine rods; K_9 was small, rounded or oval; K_{14} formed beaded rod (*Fig 2*). Only K_8, K_{13} and K_{14} were motile. All strains were Gram-negative.

All isolates, except K_8, showed positive NO_3^- reduction test (Table 1). Denitrification test was negative for all strains except for K_9 which was positive. Urease activity was negative (except for K_{14}) while oxidase and catalase tests were positive for all the isolates. only K_8, K_{13} and K_{14} were able to ferment glucose and manitol (with acid and gas production), and they also produced red pigment. Results or identification strip using QTS-20 reflect great variation in the biochemical characters of the isolates (Table 2).

DISCUSSION

NFM media, which contained nitrogen-free malate compound, were used for the isolation of diazotrophs. Use of N-free semi-solid isolation

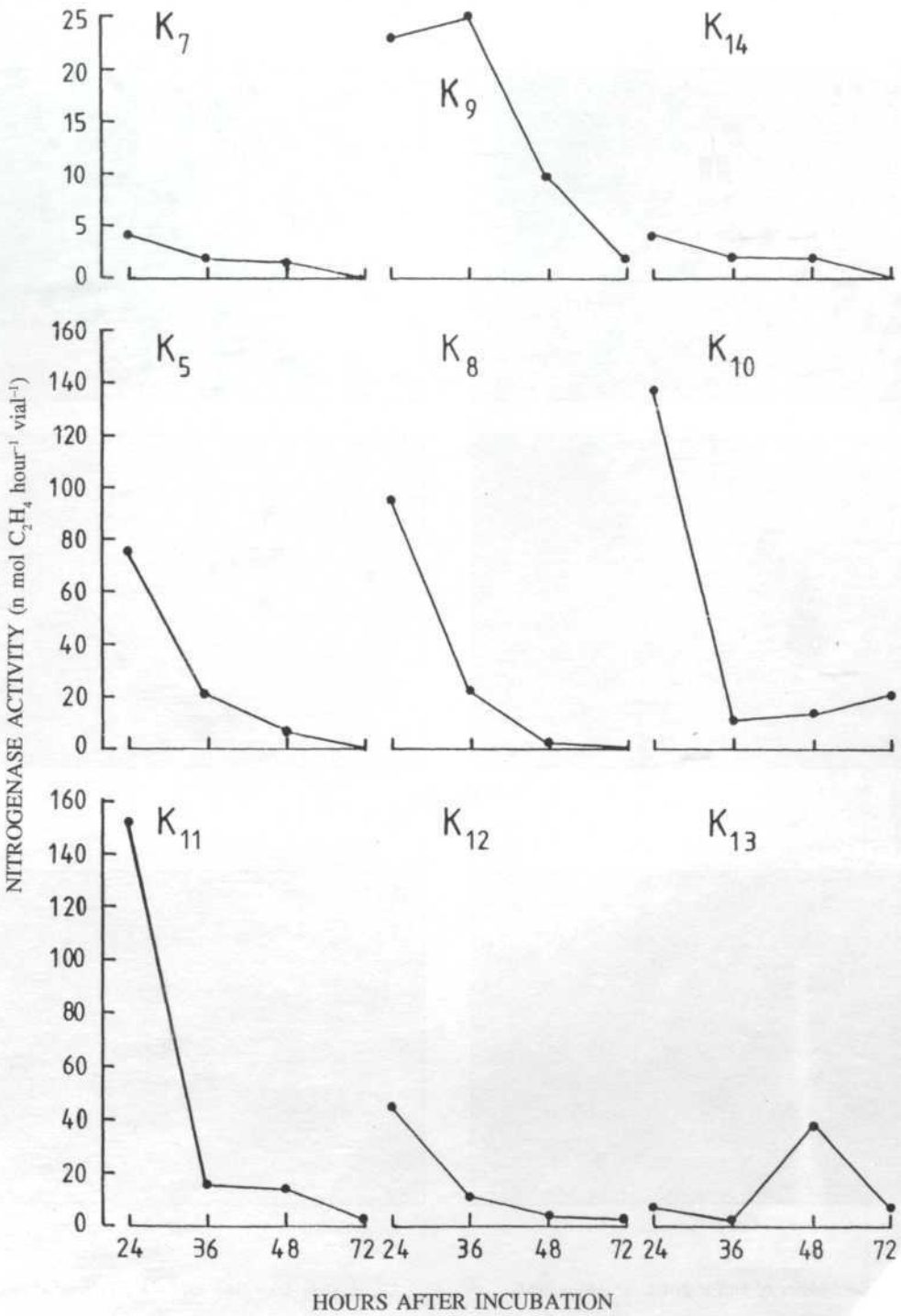

Fig. 1: Nitrogenase activity of the isolates after 24, 36, 48 and 72 hours of incubation

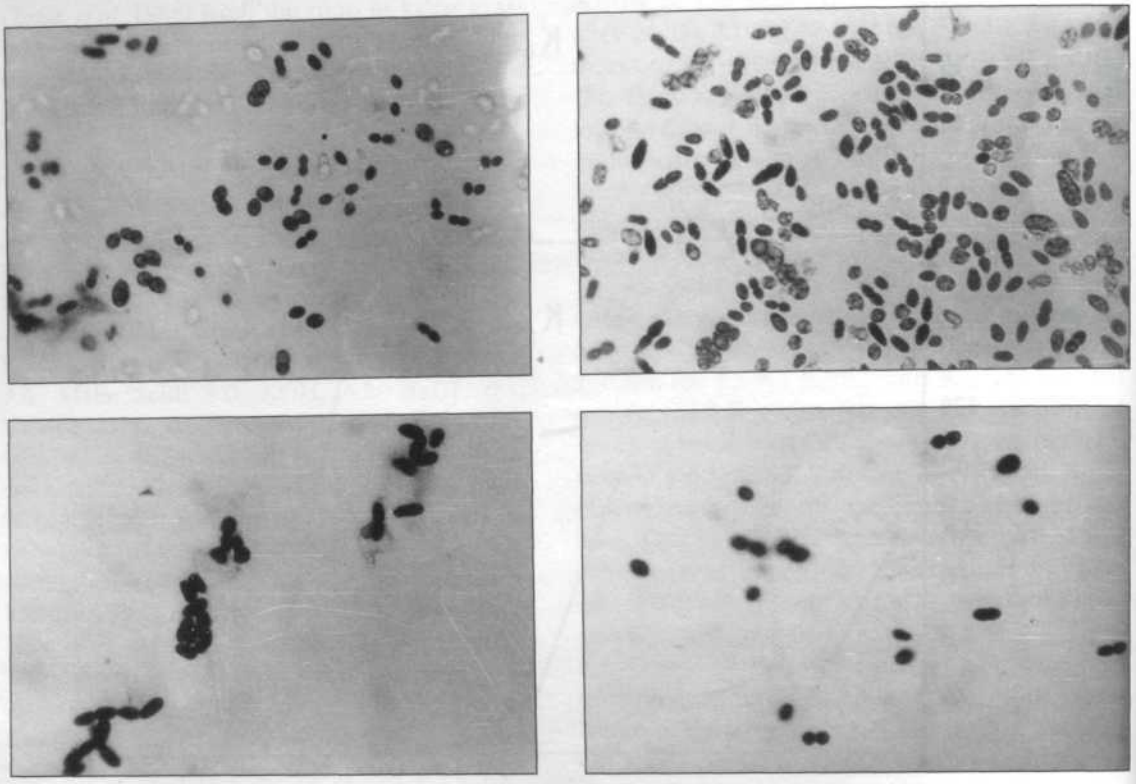

Fig. 2a

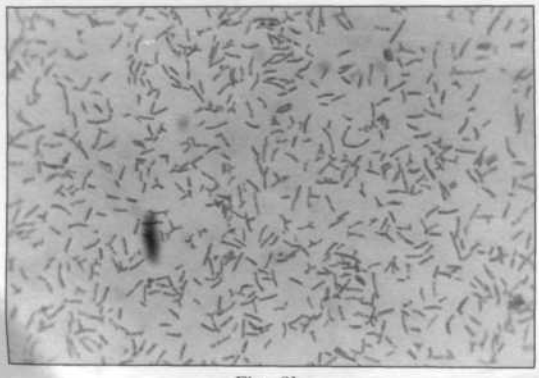

Fig. 2b

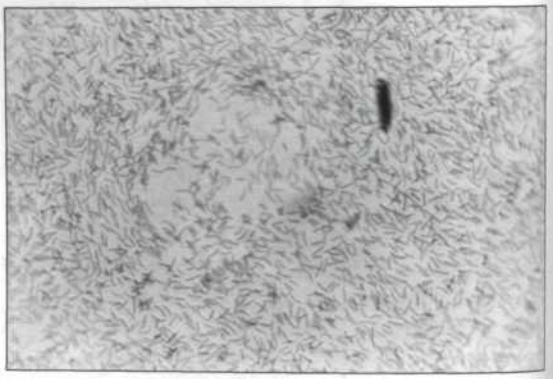

Fig. 2c

Fig. 2: Some isolates of kallar grass, a) pleiomorphic - K_5, K_{10}, K_{12}, K_{13}; b) long fine rods - K_8; c) beaded rods - K14

TABLE 1
Some physiological and biochemical characteristics of the isolates

Isolates	Catalase	Urease	Oxidase	Pigment	NO^-_3 red	Denitrifi cation	Sucrose Utilization	OF	Gram staining
K5	+	–	+w	–	+	–	+	–	–
K7	+	–	+	–	+	–	ND	–	–
K8	+	–	++	red	–	–	–	AG	–
K9	+	–	+W	–	+	+	+	–	–
K10	+	–	+W	–	+	–	+	–	–
K11	+	–	+W	–	+	–	ND	–	–
K12	+	–	+W	–	+	–	+	–	–
K13	+	–	++	red	+	–	–	AG	–
K14	+	+	++	red	+	–	–	AG	–

+, positive; +W, weak positive; –, negative; ND, not determined; AG, acid and gas

TABLE 2
QTS –20 Biochemical characterization of the diazotrophic isolates from the rhizosphere of kallar grass

Isolates Code	a	b	c	d	e	f	g	h	i	j	k	l	m	n	o	p	q	r	s	t	u	v
K_5	-	+	+	+	+	+	-	-	-	-	-	-	-	+	-	+	-	-	+	-	-	+
K_7	-	+	+	-	+	+	-	-	-	-	+	-	+	+	+	+	-	-	+	-	+	+
K_8	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	+	-	-	+
K_9	-	+	+	-	-	-	-	-	-	-	-	-	+	+	+	+	-	-	+	-	-	+
K_{10}	-	-	-	-	-	-	-	-	-	-	-	-	+	+	+	+	+	+	+	+	-	+
K_{11}	-	-	+	+	-	-	-	-	-	-	+	-	+	+	-	+	-	-	+	+	-	+
K_{12}	-	+	+	-	-	-	-	-	-	-	+	-	+	+	+	+	-	-	-	-	-	+
K_{13}	-	-	+	+	+	-	-	-	-	-	-	-	+	+	-	-	+	+	-	+	-	-
K_{14}	+	-	+	+	+	+	-	+	+	-	+	-	+	+	+	+	+	-	+	+	-	-

a, ONPG; b, cilrate; c, malonote; d, lysine; e, arginine; f, ornithine; g, H_2S; h, Urea; i, TDA; j, indole; k, vp; l, gelatine; m, glucose; n, nitrate; o, maltose; p, sucrose; q, mannitol; r, arabinose; s, rhamnose; t, sorbitol; u, inositol; v, oxidase; W+, weak positive.

media provided considerable progress for the isolation of nitrogen fixers (Boddey and Dobereiner, 1984). Species of *Azospirillium* and *Pseudomonas* have been mainly isolated on N-free medium (Barraquio *et al.* 1983; Falk *et al.* 1985; Reinhold *et al.* 1987). K_5, K_8, K_{10} and K_{11} showed reasonable nitrogenase activity (> 70 n mol C_2H_4 h^{-1} $vial^{-1}$) whereas in K_7 and K_{14} nitrogenase activity was rather low (< 5 n mol C_2H_4 h^{-1} $vial^{-1}$). The range of activity was between 4 and 168 n mol C_2H_4 h $vial^{-1}$ and it decreased with the age of the culture, and after 48 hours it was very low except for K_{13} where it was maximum. Tropical grasses show high rates of nitrogenase activity (Ahmad, 1979) and while xeric grasses do show activity, they do so at low rates (Wullstein *et al.* 1979). High nitrogenase activity has been found to be associated with the roots of kallar grass (Malik *et al.* 1982).

Bilal and Malik (1987b) have isolated nitrogen-fixing bacteria from the histoplane of kallar grass, which show very high (500 - 600 n mol C_2H_4 h^{-1} culture-$bottle^{-1}$) nitrogenase activity. Low nitrogenase activity of the isolates described here may be attributed to the low temperature of soil (Thompson *et al.* 1984), since samples for this study were collected in December.

Diazotrophs isolated in this study are Gram-negative, and with the exception of some bacilli (Seldin *et al.* 1984) the majority of N2 fixers are Gram-negative (Oken, 1982; Reinhold *et al.* 1987; Bilal and Malik, 1987b; Zafar *et al.* 1988). On the basics of cytochrome oxidace and fermenting abilities, K_8, K_{13} and K_{14} have been included in Gram-negative, facultative anaerobic rods, while the rest of the isolates were Gram-negative aerobic rods (Krieg and Holt, 1984). K_8, K_{13} and K_{14} were compared with all genera of Enterobacteriaceae but they remained unidentified. Previously, nitrogen-fixing *Klebsiella pneumoniae* have been reported from the rhizosphere of kallar grass (Malik and Zafar, 1985) while aerobic diazotrophic *Zoogloea* (Bilal and Malik, 1987b) and *Azospirilium* (Niemann *et al.* 1985; Reinhold *et al.* 1987) have been isolated from the roots of kallar grass. Zoogloea-forming bacteria were isolated on CCM (Carbon Combined Medium) and *Azospirillum* on NFM. *Zooglae* was pleiomorphic while *Azospirillum* formed vibroid to S-shaped cells. Aerobic rods of K_5, K_7, K_{10}, K_{11} and K_{12} showed pleiomorphic morphology while while K_9 formed small oval rods. Those which had 2 m m dia 3 3 m m length were taken as presumptive *Azotobacter* and *Azomonas*. Considering other biochemical and morphological characters K_5, K_{10} and K_{12} can initially be placed in the genus Azotobacter. These colonies were smooth, glistening, opaque and low convexed. All of them were non-motile, catalase-positive and had ovoid cells which occur singly, in pairs, in irregular clumps or small chains (*Fig. 2a*). Slime formation was also observed. Cysts were also formed in old cultures. All of them could reduce nitrate but none denitrified. All of them can utilize sucrose as the sole carbon source. K_5 and K_{10} utilize rhamnose while K_{12} did not. Table 3 shows the comparison of some biochemical characters of K_5, K_{10} and K_{12}. with six species of *Azotobacter* from Bergy's Manual of Systematic Bacteriology (Krieg and Holt, 1984). Ni % G + C or DNA/RNA or DNA/DNA homology studies were performed with these strains which can escertain the taxonomics position of these isolates. Both *Azotobacter* and *Azimonas* are included in RNA super family II (De Smedt *et al.* 1980) and both of them are able to fix N_2 under atm pO_2.

From the above account it can be surmised that three *Azotobacter* strains (K_5, K_{10}, K_{12}) with comparatively high nitrogenase activity were obtained from the rhizosphere of *Leptochloa fusca*. Two other strains (K_8, K_{11}) also showed high nitrogenase activity but their affinities remain to be established. The extent of nitrogenase activity depends upon the incubation period. Generally, it is highest after 24 hours of incubation but was reduced with the prolonged incubation period.

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TABLE 3
Some biochemical characteristics of K_5, K_{10} and K_{12} along with selected strains of *Azotobacter* from Bergy's Mannual of Systematic Bacteriology (Krieg and Holt, 1984)

	A.chroococcum	*A.Vinelandii*	*A. beijerinchii*	*A.nitrican*	*A. arme niacus*	*A.paspali*	K_5	K_{10}	K_{12}
Catalase	+	+	+	+	+	+	+	+	+
Oxidase	+	+	+	+	d	+	+	+	+
ND_3 reduction	+	+	+	+	–	–	+	+	+
Denitrification	–	–	–	–	–	–	–	–	–
Rhamnose	–	+	–	–	–	–	+	+	–
Sorbitol	+	+	d	d	+	–	–	+	–
Inositol	–	+	d	–	d	–	–	–	–
Mannitol	+	+	d	d	+	–	–	+	–
Malonate	d	+	+	d	–	–	+	–	+
Sucrose	+	+	+	+	+	+	+	+	+
H_2S production	d	+	d	d	–	+	–	–	–
Pigment	+	–	+	+	–	–	–	–	–
Motility	+	+	–	–	+	+	–	–	–

+. all strains positive; –, all strains negative; d, 11–8 stains are positive

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Leaching Losses and Nutrient Build-Up in the Soil through Application of Raw and Digested Palm Oil Mill Effluent (POME)

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ABSTRAK

Kehilangan nutrien melalui larut lesap dari penggunaan effluen kilang kelapa sawit (POME) yang mentah dan juga yang dihadamkan secara anaerobik, telah diuji dalam lysimeter dengan Tanah Siri Segamat sedalam 75sm. POME mentah telah ditabur pada kadar 1.78 mm persamaan hujan (rey)/taburan selama 1175 hari sementara POME yang dihadam telah ditabur pada kadar 3.54 rey/taburan untuk selama 475 hari. Purata penaburan untuk setahun adalah masing-masing sebanyak 463 mm dan 936 mm setara hujan untuk POME mentah dan POME hadam. Kadar kehilangan nutrien Ca, K dan Mg melalui larut lesap dari penaburan POME mentah adalah masing-masing sebanyak 49.8%, 37.7% dan 7.7% manakala dari penaburan POME yang dihadam secara anaerobik pula adalah masing-masing sebanyak 30.7%, 16.3% dan 11.5%. Larutan lesap N dan P dari kedua-dua jenis effluen tersebut adalah kecil.

ABSTRACT

The leaching losses as a result of application of nutrient rich raw and anaerobically digested palm oil mill effluent (POME) onto Segamat Soil Series were studied using lysimeters of 75 cm soil depth. Raw POME was applied at a rate of 1.78mm rain equivalent per year (rey)/application over 1175 days while anaerobically digested POME was applied at a rate of 3.54 rey/application over 475 days. The average yearly application amounted to 463 mm and 936mm rain equivalent for raw and digested POME respectively. The percentage of nutrients leached as a result of applying raw POME was 49.8% for Ca, 37.7% for K and 7.7% for Mg, while for anaerobically digested POME it was 30.7% for Ca, 16.3% for K and 11.5% for Mg. For both effluent types there was very little leaching of total N and P.

Keywords: Palm oil mill effluent (POME), leaching, lysimeter

INTRODUCTION

Palm oil mill effluent (POME) is a by-product in the processing of oil plam fresh fruit bunches (ffb) to produce crude palm oil. About 0.5 - 1.0 tonne of waste-water is produced for every tonne of ffb processed. The raw POME, a golden brownish liquor, is nutrient rich apart from containing carbohydrates, proteins and oil. Untreated fresh POME has a Biochemical Oxygen Demand of about 20,000 ppm making the liquid a strong pollutant if discharged into the water ways in its raw form. With the introduction of the Environmental Quality Act in 1974, the discharge of raw POME into rivers was disallowed. The Environment Quality Regulation (Prescribed Premises) (Crude Plam Oil) 1977 prescribed permissible standards of the water quality which can be discharged.

As an option to dischaging POME into waterways, land application of the effluent both raw (untreated) and anaerobically digested POME have been investigated by many workers (Wood 1981, Tan *et al.* 1982, Lim *et al.*1983, Tan 1983, Lim *et al.* 1983, Tajuddin dan Zakaria 1984, Dolmat 1985).

Little imformation exists on the impact of land application of POME, either raw or anaerobically digested, on the soil and its influence on underground water (Lim 1987, Dolmat *et al.* 1987). To add some understanding in this area, a study on leaching of certain nutrients was conducted at Pusat Perkhidmatan Pertanian Tun Razak, Sg Tekam using lysimeter. Information on nutrient build-up in the soil was also obtained.

MATERIALS AND METHODS

Simple lysimeters were constructed using 200 liter drums of diameters 58.4 cm. The lysimeters were sheltered from the rain. The base of each lysimeter was curved outward and an outlet using a hollow metal rod welded to the base was constructed. This was designed to help in easy collection of the leachate. The base of the lysimeters were filled with stones and pebbles to a depth of about 9cm. Segamat Series Soil (Haplic Acrorthox) a deep friable, heavy textured well drained soil, was selected for this study and it was back filled layer by layer with the lowest profile dug going first into the lysimeter. The depth of each layer was 15 cm and after each layering it was irrigated to facilitate soil settling. The entire soil depth of the lysimeter was 75 cm.

Water was irrigated for a month at a rate of 5370 ml three times a week. This was to help stabilise the soil before the start of the study. Two lysimeters were used for the raw POME application study of which one was used as a control. The study was initiated in August of Year 1. To the treatment lysimeter, 1.78 mm rain equivalent per year (rey) or 456 ml of raw POME was applied each time. To the control, the same volume of water was applied. The amount of raw POME applied per year was 46.3 cm rain equivalent. For land disposal of raw POME, studies by Pillai *et al.* (1978) have shown that application of 480 rain equivalent mm/yr could be applied on land. This high rate was selected for this study. To simulate rainfall, 5370 ml water was applied twice weekly or 2159 rem per year, to both the lysimeters. The leachate was collected the following day and volume recorded. The leachate was stored in a glass container kept in a dark room. Formic acid was added to prevent microbial growth. Fortnightly, samples of water from, both treatment and control leachate were sent for chemical analysis of total-N, P, K Mg and Ca concentrations. The study was terminated in December Year 4 after 1175 days. For every 15 cm depth, a bulk soil sample was taken from each of the four lysimeters at the end of the study. Two sub-samples from each bulk sample were analysed for pH, total-N, available P, and exchangeable K, Mg and Ca. However only the top (0-15 cm) and bottom (61-75 cm) depths are reported. Samples at other depths were used for computing the nutrient bulid-up.

Two more lysimeters were constructed and soil filled in as described earlier. To one, anaerobically digested POME was irrigated at a rate of 3.56 mm rey or 912 ml per application and the same quantity of water was applied to the other which served as a control. As an average the amount of digested POME applied per year was 936 mm rain equivalent. The amount is twice that applied for raw POME. One of the problems attributed to high rates of raw POME application was the rapid build-up of solids. In digested POME the suspended solids are only about 30% of that of raw POME. In view of this the double rate of raw POME application was selected for this study. As before 5370 ml of water was irrigated twice weekly to simulate rainfall. The study was terminated after 475 days. The same procedure was followed for collection of leachate and soil and their analysis.

RESULTS AND DISCUSSION

The data have been analysed and presented on a calendar year basis. For the amount of water applied, only about 40-60% was collected as leachate after each irrigation over the 4 years.

The raw and digested POME vary greatly in the nutrient contents. The amount of nutrients present depends on a number of factors e.g. dilution as a result of water usage during processing, nutrient content in oil palm fresh fruit bunches, etc. As such only an average figure was computed for use in calculations. Table 1 gives the nutrient content of raw and digested POME.

The volume of raw POME added over 4 years (1175 days) was 382 litres while for digested POME 312 l were applied over 2 years (475 days). Table 2 give the total quantity of

TABLE 1
Certain characteristics of raw and anerobically* digested (Supernatant) POME

	Raw POME (ppm)	Digested POME (ppm)
N	380	227
P	70	31
K	990	1542
Mg	242	247
Ca	330	256
Total solid	34260	12408
Suspend solid	19990	5456
BOD	20790	2240
pH	4.0	7.0

* 20 days retention time in a tank digester.

TABLE 2
Quantity of nutrients added in lysimeter through raw and digested POME application

POME	Volume Applied (l)	Nutrient Content (g) N	P	K	Mg	Ca
Raw	382	152.8	24.3	378.1	92.4	126.0
Digested	312	70.8	9.7	480.7	77.0	79.8

Note: Raw POME applied over 1175 days and digested POME over 475 days.

nutrients added over the peroid of study through application of raw and digested POME. The amount of nutrients applied in order of descending quantity for raw POME was K > N > Ca > Mg > P and for digested POME it was K > Ca > Mg > N > P. It is most probable that Ca and Mg had leached out faster from the organic matter into the liquid fraction of the anaerobically digested POME than for N where mineralisation could have been slow.

Table 3 gives the total amount of nutrients leached out from the lysimeters as a result of raw and digested POME application. The amount of POME nutrients leached out is computed by subtracting the control leacheate from the treatment leacheate on a yearly basis. For the raw and digested POME treatments the nutrient content leached in descending order was the same i.e K > Ca > Mg > N > P. This order was similar to the amount of nutrients applied via digested POME but differed from raw POME application where N content was substantially higher.

TABLE 3
Total quantity of nutrients leached through application of raw* and digested** POME over the period of study

POME	Nutrient Content Leached (mg) N	P	K	Mg	Ca
Raw	550	60	142410	7080	62750
Digested	650	50	78380	8850	24460

* Period of study 1175 day, Volume applied 382 l
** Period of study 475 days, Volume applied 312 l

TABLE 4
Percentage of nutrients leached as compared to amount added via raw POME application

(Year)	% Leached Period N	P	K	Mg	Ca
1	Neg	1.4	1.2	1.7	22.4
2	Neg	0.1	13.1	8.4	34.9
3	0.6	0.4	74.8	13.3	43.6
4	0.7	0.3	39.5	3.3	48.5
Over 4 years*	0.4	0.2	37.7	7.7	49.8

*1175 days

TABLE 5
Percentage of nutrients leached as compared to amount added via digested POME application

Period (Year)	% Leached N	P	K	Mg	Ca
1	1.1	0.2	2.9	2.9	28.5
2	0.9	0.7	22.2	15.3	31.6
Over 2 years*	0.9	0.5	16.3	11.5	30.7

* 475 days

Tables 4 and 5 give the percentage nutrient leached as compared to the amount of nutrients applied through raw and digested POME applications, respectively. For raw POME the first two years showed no traces of N leached although the remaining two years showed only neglible leaching. In the case of P, the amount leached was generally less than N over 4 years. In digested POME application, the N and P leached were also low although the percentages were higher than in raw POME application. The lower leaching of P is expected as its mobility through the soil is slow. Phosphorus is generally fixed in acidic soils. The bulk of N, especially in raw

POME, could have been immobilised as it is probably organically bound. The mineralisation rate of N as a result of POME application has been reported to be slow (V. M. Palaniappan and S. Palaniappan 1980).

The nutrient that leached most over the 1175 days of raw POME application was calcium (49.8%), follwed by potassium (37.7%) and to a lesser extent magnesium (7.7%). For digested POME aplication over the 475 days the same order of leaching prevailed with Ca (30.7%), K (16.3%) and Mg (11.5%).

Table 6 gives the soil nutrient status of the top 15 cm of soil and the lower most 15 cm depth (61-75 cm) for control and POME treated lysimeters. For the 0-15 cm depth, marked increases as compared to control were noted in pH (70%), total-N (76%), available P (340%) and exchangeable K (140%), exchangeable Ca (270%) and exchangeable Mg (270%) for raw POME application. For digested POME the changes were pH (55%), N (18%), available P (230%), exchangeable K (110%), exchangeable Ca (160%) and cxchangeable Mg (150%) for the top 15 cm. For soil treated with raw POME over 1175 days, the 61-75 cm soil depth also showed significant increases in all parameters measured. However, to digested POME applied over 475 days this was not apparent.

Table 7 gives an extrapolation of the nutrient bulid-up in the 75 cm soil profile as a result of raw and digested POME appplication using the same rates over 1 ha. The extrapolation was based on converting the extra nutrient content (treated soil content - control soil content) for each 15 cm soil depth over a hectare basis. The value for each nutrient for the five 15 cm depths was added to give the total nutrient build-up over the 75 cm soil profile. It can be observed that at these rates of application the nutrient accumulation in the soil can be significant over time. The abundance of 'excess' nutrients in the soil through raw and digested POME application was the same following the order K > N > Mg > Ca > P.

The enrichment of the soil through POME application (raw or digested) can be observed

TABLE 7
Amount of nutrients (kg/ha) accumulated in the 75 cm soil depth as a result of raw and digested POME application*

Nutrient	Nutrients Accumulated (kg/ha)	
	Raw POME Applucation	Digested POME Application
N	5400	2620
P	1000	360
K	8800	15030
Mg	3190	2550
Ca	2360	2070

*Equivalent to - 14,258,914 l/ha of raw POME applied over 1175 days
- 11,646,024 l/ha of digested POME applied over 475 days.

TABLE 6
Some chemical analysis of Segamat series soil at 0-15 cm and 61-75 cm depth treated with raw and digested POME application

Soil Depth (cm)	pH	Total N (%)	Available P (ppm)	Exchangeable (meq/100g soil)		
				K	Mg	Ca
Control						
0-15	4.7	0.17	23	2.25	1.68	2.29
61-75	5.0	0.15	4	0.19	0.20	5.62
Raw POME Application						
0-15	8.0	0.30	102	5.48	6.24	8.54
61-75	6.0	0.20	25	2.44	2.13	7.42
Digested POME Application						
0-15	7.3	0.20	75	4.75	4.24	5.96
61-75	4.3	0.14	8	0.20	0.21	1.42

by studies on responses to growth and yield of plants (S. Palaniappan and V.M. Palaniappan 1981, Yeow and Zin 1981, Palaniappan and Rusdi 1986). Nevertheless differential build-up and leaching of nutrients in the soil over long periods of application, could lead to an imbalance of nutrients for plant uptake. The high K build-up could lead to a suppression of Mg uptake. High Ca could in turn affect K and Mg uptake. The increase in Ca would reduce phosphate availability through the formation of calcium phosphates. Nevertheless with close monitoring of POME (raw or digested) application on land, through regulated quantities, an imbalance situation could be minimized. Futhermore if some imbalances persist they can be rectified through the use of inorganic fertilisers to amend the situation. Application of POME onto land not only helps overcome the disposal of the polluting waste water but its ulitisation in the field would help improve yield and cut down production cost.

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Nature of Genetic Control of the Length and Number of Elongated Internodes in Deepwater Rice under Non-flooded Condition

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ABSTRAK

Turunan jumlah panjang dan bilangan ruas yang dipanjangkan dalam beberapa jenis padi apungan telah di kaji dalam 7 × 7 dialel (tidak termasuk salingan) melalui prosedur yang tidak memerlukan air yang banyak. Anggaran keupayaan menggabung dan analisis komponen genetik menunjukkan kesan gen berdaya tambah dan tidak berdaya tambah begitu ketara dengan keadaan yang lebih kepada jenis berdaya tambah. Anggaran yang tinggi terhadap kemampuan mewaris selanjutnya menyokong kepentingan kesan gen berdaya tambah. Jalmagna dan IR40905 - 11 - 3 - 1 - 5 - 2 - 21 diputuskan sebagai penggabung terbaik. Walau bagaimanapun benih yang baik tidak selalunya penggabung terbaik. Keketaraan taburan gen menunjukkan wujudnya isimetri gen. Hitung panjang darjah dominan adalah 0.83 bagi kedua-dua jumlah panjang dan bilangan ruas yang dipanjangkan menunjukkan dominan separa.

ABSTRACT

Inheritance of total length and number of elongated internodes in some varities of deepwater rice were studied in a 7 × 7 diallel (excluding reciprocals) by a procedure that does not require flooding. Estimates of combining ability and genetic component analysis showed highly significant additive and non-additive gene effects with preponderance of additive type. The high estimate of narrow sense heritability further supported the importance of additive gene effects. Jalmagna and IR40905-11-3-1-5-2-21 were not always the best combiners. The significance of the gene distribution indicated the presence of gene asymmetry. The average degree of dominance was 8.83 for both total length and number of elongated internodes indicating partial dominance.

Keywords: *Oryza sativa* L., deepwater rice, plant elongation

INTRODUCTION

Deepwater rice possess the ability to elongate rapidly to keep pace with rising water to as much as 5 - 6 meters. The total elongation during flooding is usually a cumulative effect of three elongating plant components: leaf sheath, leaf blade and internodes. Internode elongation is the most important. Leaf elongation is limited. The length of an elongated internode may vary from 9 - 30cm. However, the number of elongated internodes does not indicate actual elongation capacity (Datta 1982, Hasanuzzaman *et al.* 1975). Lack of suitable screening methods for elongation ability have been considered a major bottleneck in the study of the trait. Available methods are destructive in nature. Experiments conducted at the International Rice Research Institute (IRRI) with elongating and non-elongating rice varieties grown under normal irrigated condition showed developmental-morphological differences between the two types, which can be detected even in the absence of flooding. This suggested that the length and the number of elongated internodes of rice grown under non-flooded condition could be used as traits suitable for genetic studies (Thakur and Hillerislambers 1989; Dwivedi *et al.* 1992). Therefore, the present study was undertaken to determine the nature of genetic control of the length and number of elongated internodes without flooding.

MATERIALS AND METHODS

The materials consisted of seven eco-culturally different rice varieties viz fast elongating tall traditional (Jalmagna), slow elongating tall (IR40905-11-3-1-5-2-21), tall traditional deepwater (NDGR 207), slow elongating modern type (IR11141-6-1-4 and NDGR 150), submergence tolerant, non-elongating, modern type

(BKNFR76106-16-0-1) and submergence sensitive, non-elongating, modern type (IR42). Performance of these parents with respect to the number of elongated internodes and length of internodes under non-flooded condition is shown in Table 1. These were crossed in all possible combinations excluding the reciprocals. The seven parents and their F_1's were grown in a randomized block design with four replications under normal irrigated condition during the wet season of 1991 at IRRI. Each entry consisted of three rows, each with 15 plants. Plant spacing between and within rows was 30 cm. Standard crop management practices were followed. At the time of complete panicle emergence, 10 plants were selected randomly from each replication and main tillers were dissected to record the length and number of elongated internodes. Internodes with more than 6 cm length were considered elongated.

TABLE 1
Average number of elongated internodes and length of internodes of parents used

Parent	Type*	Average	
		Number of elongated internodes	Length of internodes (cm)
NDGR207	1	4	68.1
NDGR150	1	4	67.9
IR11141-6-1-4	1	5	63.5
IR40905-11-3-1-5-2-2-1	1	5	101.5
BKNFR76106-16-0-1	2	3	47.8
IR42	2	3	48.2
	LSD (.05)	0.5	5.0

*1 = elongating, 2 = non-elongating

Analysis of combining ability was carried out according to Griffing (1956) using Model 1, Method 2. The genetic components of variances were computed following Hayman (1954).

RESULTS AND DISCUSSION

General Combining Ability (gca) and Specific Combining Ability (sca) Effects

An analysis of variance demonstrated the presence of highly significant genetic variability within diallel population for total length and number of elongated internodes. The mean square due to gca and sca effects were highly significant indicating the importance of both additive and non-additive types of gene effects in controlling these two traits (Table 2). However, gca variances were higher than sca variances indicating the predominance of additive gene action in the expression of these characters.

TABLE 2
Analysis of variance for combining ability

Character	Mean square[a]		
	gca	sca	error
Length of internodes	1379.467**	123.678	4.954
Number of elongated internodes	3.779**	0.393**	0.085

** Significant 1% level
a Mean squares for gca, sca, and error are based on 6, 21 and 81 degrees of freedom, respectively.

Jalmagna was the best general combiner followed by IR40905-11-3-1-5-2-21 for internode length and number of elongated internodes as these genotypes showed highly significant and positive gca effects for these traits (Table 3). NDGR 207 had positive and highly significant gca effects for length of internodes while having negative and significant effects for number of elongated internodes. The rest of the genotypes were adjudged to be poor general combiners due to significant but negative gca effects. The parents which were good combiners for length of

TABLE 3
Estimates of gca effects in 7 parent F1 diallel for number and length of internodes based on Griffing (1956)

Parent	Length of internodes	Number of Elongated internodes
Jalmagna	22.32**	1.286**
NDGR207	2.54**	-0.187*
NDGR150	-3.52**	-0.492**
IR11141-6-1-4	6.80**	-0.048
IR40905-11-3-1-5-2-21	8.43**	0.341**
BKNFR76106-16-0-1	-8.271**	-0.270**
IR42-14.704**	-0.631**	
S.E.(gi)	.679	.09

* Significant at 5%
** Significant at 1%

internodes were also good combiners for number of elongated internodes except for BDGR 207. Thus, the parents particularly Jalmagna and IR40905-11-3-1-5-2-21 are considered good donors for the improvement of length and number of elongated internodes.

Jalmagna/BKNFR76106-16-0-1, NDGR207/NDGR150, NDGR207/IR11141-6-1-4, NDGR207/BKNFR76106-16-0-1, NDGR 150/IR40905-11-3-1-5-2-21, NDGR150/BKNFR76106-16-0-1, IR11141-6-1-4/IR40905-11-3-1-5-2-21, IR40905-11-3-1-5-2-21/BKNFR76106-16-0-1, IR40905-11-3-1-5-2-21/IR42 possessed high and significant sca effects for internode length (Table 4). These best cross combinations involved at least one good general combiner. Of the 21 crosses, four showed negative and significant sca effects. The remaining crosses had either positive or negative but non-significant sca effects. Therefore, it could be concluded that sca effects varied greatly from cross to cross; and that moderate to poor general combiners also produced good combinations. However, low internode length parents showed very high negative general and specific combining ability effects.

The crosses, Jalmagna/IR40905-11-3-1-5-2-21, IR40905-11-3-1-5-2-21/BKNFR76106-16-0-1, NGDR150/BKNFR76106-16-0-1 showed positive and significant sca effects for number of elongated internodes. The first two crosses involved high x high and high x low general combiners. The high and highly significant sca effect of NDGR 150/BKNFR 76106-16-0-1 involving both poor general combiners indicated that poor general combiners may not always produce poor F_1 combinations for number of elongated internodes. The remaining cross combinations showed low magnitude of sca effect, either positive or negative except for Jalmagna/NDGR 207 and Jalmagna/NDGR150 which possessed significant negative sca effects. The presence of non-additive genetic variance or sca effects offer scope for exploiting heterosis in these traits through the development of hybrid rices.

TABLE 4
Estimates of sca effects in 7 parent F_1-diallel for number and length of internodes based on Griffing (1956) (normal irrigated condition)

Cross	Length of internodes	Number of elongated internodes
Jalmagna/NDGR207	-18.186**	-1.653**
Jalmagna/NDGR150	-10.314**	-0.597
Jalmagna/IR1141-6-1-4	1.319	-0.042
Jalmafna/IR40905-11-3-1-5-2-21	1.425	0.569*
Jalmagna/BKNFR76106-16-0-1	11.381**	0.431
Jalmagna/IR42	-3.586	-0.458
NDGR207/NDGR150	4.769*	0.431
NDGR207/IR4040905-11-3-1-5-2-21	-2.142	-0.458
NDGR207/ bknfr76106-6-0-1	8.364**	0.403
NDGR207/IR42	-1.103	0.014
NDGR150/IR11141-6-1-4	-2.525	-0.014
NDGR150/IR40905-11-3-1-5—2-21	10.381**	0.097
NDGR150/BKNFR76106-16-0-1	11.336**	0.708**
NDGR150/IR42	2.769	-0.181
IR11141-6-1-4/IR40905-11-3-1-5-2-21	16.264**	0.403
IR11141-6-1-4/BKNFR76106-16-0-1	-8.031**	-0.236
IR11141-6-1-4/IR42	-2.547	-0.125
IR40905-11-3-1-5-2-21/BKNFR76106-16-0-1	12.175**	0.625*
IR40905-11-3-1-5-2-21/IR42	4.358*	0.236
BKNFR76106-16-0-1/IR42	-9.786**	-0.403
S.E.(S_{ij})	1.977	0.262
S.E. (S_{ij}-S_{ik})	2.937	0.389

*, ** Significant at 5 and 1% respectively.

Genetic component analysis

Tests for the validity of the additive-dominance model for length and number of elongated internodes satisfied their assumptions. The regression coefficients for length of internodes and the number of elongated internodes, b= 8.11 and 3.24 respectively, were signicantly different from zero, but their deviation from unity was not significant indicating the presence of non-allelic interaction (epistasis) at very low intensity. Estimated genetic components of variation and proportional values for these characters are presented in Table 5. The significance of additive effects and three components of dominance (H_1, H_2 and h^2) for length of internodes and two components of dominance (H_1 and H_2) for number of elongated internodes indicated the importance of both additive and dominance genetic effects. This was supported by the importance of gca and sca effects in the combining ability analysis. However, the larger magnitude of D as compared to H_1 revealed the greater importance of additive gene action. This was further substantiated by high narrow sense heritability observed for these traits. The significance of F value suggested the presence of asymmentry in the distribution of genes among parents. This was also corroborated by the ratio estimates of $H_2/4H_1$ for length and the number of elongated internodes (0.171 and 0.159 respectively) whose maximum possible value of .25 is expected under equal frequencies of posi-

TABLE 5

Estimates of genetic components of variation and proportional values for number and length of internodes (normal irrigated condition)

Component	Length of internodes	No. of Elongated internodes
D(Additive effects)		808.113*
2.488*		
	+11.311	+0.255
H(Dominance effects)	555.719*	1.706*
H_1	+77.023	+0.615
H_2	381.078*	1.089*
	+67.868	+0.542
H_2	169.342*	-0.042^{ns}
	+45.583	+0.364
F (Gene distribution)		388.523*
1.556*		
	+76.751	+0.613
E (Environment effects)	4.739^{ns}	0.089^{ns}
	+11.311	+0.090
Proportional values		
$H1/D)^{1/2}$(Average dominance)	0.829	0.828
$H_2/4H_1$ (Gene asymmetry)	0.171	0.159
K_D/K_R + (Proportion of dominance and recessive genes in the parents)	1.816	2.213
r between Wr+Vr and Y_r	-0.161	0.579
Heritability narrow sense (%)	74.817	68.182

\+ KD.KR= $[(4DH1)^{1/2}+1/2\ F]/[(4DH1)^{1/2}-1/2\ F]$

* Significant at 5% level

^{ns} Nor significant

tive and negative alleles. This further implies that positive and negative alleles were not present in equal proportion in each parent used. The average degree of dominance $(H_1/D)^{1/2}$ for both length and number of elongated internodes was 0.83 showing partial dominance.

CONCLUSION

Combining ability and genetic components analyes revealed the importance of both additive and non-additive types of gene effects with preponderance of additive and non-additive type for the number and length of internodes. Jalmagna and IR40905-11-3-1-5-2-21 proved to be the best general combiners among the varieties studied for the two traits. They could be used in breeding to develop improved deepwater rices.

The length and number of elongated internodes measured in the absence of flooding could be used to determine the genetic architecture of donors and breeding materials but may not be useful in practical breeding since the procedure is laborious and consuming.

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Supplementing Artificial Diets for Catfish (*Clarias macrocephalus*) Fry with *Tubifex*

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ABSTRAK

Fri ikan keli (Clarias macrocephalus) *yang berumur tiga minggu diberi makanan uji pada paras protein 30% dan 35% serbuk ikan dominan serta 35% dan 40% kacang soya dominan selama 8 minggu. Sembilan rawatan telah diuji di mana empat rawatan diberi makanan uji sahaja, satu diberi* Tubifex *sahaja (kawalan) dan selebih empat lagi diberi Tubifex diikuti dengan makanan uji. Fri yang diberi makanan tambahan* Tubifex *makan lebih aktif dan menujukkan kadar pertumbuhan dan kecekapan pemakanan yang baik dibandingkan dengan makanan uji sahaja. Di antara rawatan yang menerima makanan* Tubifex, *makanan 30% dan 35% serbuk ikan dominan menunjukkan pertumbuhan dan penggunaan makanan yang paling bererti.*

ABSTRACT

Three-week-old catfish (Clarias macrocephalus) *fry were fed exprimental diets at protein levels of 30% and 35% fish meal dominant and 35% and 40% soybean dominant types for 8 weeks. Nine treatments were tested with four treatment consisting wholly of experimental diets, one of* Tubifex (*control) and the remaining four diets supplemented with live* Tubifex. *Fish reared on experimental diets supplemented with live* Tubifex *fed more actively and showed better growth and feed efficiency than those reared without supplemented diets. Those on the 30% and 35% fish meal dominant feed showed significantly better growth and feed conversion than those fed 35% 40% soybean dominant diet.*

Keywords: Artificial diets, Catfish fry, Tubifex

INTRODUCTION

Intensive culture of catfish (*Clarias macrocephalus*) in Malaysia in recent years is the result of higher demand and good market prices offered for this fish (FAMA, 1990). However, limited fry production due to poor survival has made it necessary to improve hatchery techniques and upgrade nutritional qualities of diets for fry and fingerlings.

The nutritional requirements of several species of catfish such as *Clarias gariepinus* (Henken *et al.* 1986) and *Clarias batracus* (Chuapoehuk, 1987) have been determined in previos studies. This information has been used to formulate low cost feeds utilizing local ingredients. However, among the problems encountered when these ingredients (especially those of plant origin) were used, were inconsistensies in quality and digestibility of the diet; and the unattractive feeds, although the nutritional requirements were satisfied. Thus various ingredients such as lumbricid worms (Tacon *et al.* 1983) krill, silkworm pupae powder, etc. (Akiyama *et al.* 1984) have been incorporated into fish feeds to act as feed attractants or feeding stimulants. This work investigates the feasibility of using *Tubifex* worms as a feed attractant and appetite stimulant for catfish (*Clarias macrocephalus*) fry.

MATERIALS AND METHODS

Four experimental diets containing 30%, 35% and 40% protein levels of varying proportions of animal and plant protein were prepared by mixing the feed ingredients and pelletised with an extruder. The percentage composition and nutrient levels of the diets are presented in Table 1. The diet preparations were on based earlier feeding studies (Hashim and Ali, 1989) Diets 1 and 2 contained 30% and 35% protein respectively, at a ratio of 2:1 animal to plant protein i.e. fish meal to soybean meal. Diets 3 and 4 contained 35% and 40% protein respectively, at a ratio of 2:1 plant to animal protein (soybean to fish meal). Diet 5, (control), consisted wholly

TABLE 1
Formulation and proximate composition of the experimental diets (% by dry weight) for *Clarias macrocephalus* fry

Component	Diet				
	1	2	3	4	*Tubifex*
Ingredient					
Fish Meal	40.49	47.23	23.62	26.98	
Soybean Meal	17.39	20.28	48.20	55.07	
Broken Rice	8.93	10.40	5.22	5.94	
Wheat Flour	28.49	18.09	14.16	3.91	
Crude Palm Oil	4.5	3.8	8.6	7.9	
Vitamin Mix[1]	0.10	0.10	0.10	0.10	
Mineral Mix[1]	0.10	0.10	0.10	0.10	
Proximate analysis					
Moisture	7.0	9.1	8.3	8.3	-
Crude Protein	29.4	36.2	35.4	39.8	69.8
Crude lipid	11.5	12.1	11.9	11.0	3.8
Ash	13.1	14.0	11.8	12.8	8.3
N. F. E.[2]	39.0	28.6	32.6	28.1	18.1
Gross energy[3] (kcal/g)	432.8	435.7	445.2	443.8	506.4

[1]Recommended by the National Research Council (1983).
[2]N.F.E. = Nitrogen Free Extract (100 - [moisture + crude protein + crude lipid + ash])
[3]Based on 5.7 kcal/g protein; 9.5 kcal/g lipid and 4.0 kcal/g carbohydrate

of live *Tubifex*. The *Tubifex* was treated with 20 ppm formalin for 10 to 15 minutes and washed thoroughly with tap water prior to feeding to avoid the introduction of pathogens and parasites normally associated with live natural food (Uys and Hecht, 1985).

Catfish fry were obtained by induced spawning using human chorionic gonadotropin (Mollah and Tan, 1983) at the laboratory in the School of Biological Sciences. Forty-five 40-liter aquariums were then randomly stocked with 20 fry (average weight 0.1g) at 5 replicates per treatment. The water exchange was maintained at a rate of 0.5 1/min and all tanks were aerated. Throughout the feeding trial the temperature, pH and dissolved oxygen were maintained at between 27.9 - 31.7°C, 7.16 - 7.74 and 5.1 - 6.8 mg/l, respectively. The fry in treatmentas A - D were fed only the experimental diets 1 - 4 respectively, whereas those in Treatment E with *Tubifex* only. In treatments F - I, the fry were fed *Tubifex* in the morning at a feeding level of 2% body weight before experimental diets. In all the nine treatments, the fry were fed to satiation three times daily according to the prescribed diets.

At the end of 8-week feeding trial, the fish were weighed and the relative growth rate, feed conversion ratio, protein efficiency ratio and survival rate were determined. The differences in the parameters for each treatment means were tested by Analysis of Variance (Anova) followed by the Duncan's Multiple Range Test (Steel and Torrie, 1960).

RESULTS AND DICUSSION

Fry fed with *Tubifex* alone (Treatment E) and those supplemented with *Tubifex* in the morning followed by the experimental diets (Treatments F - 1) fed more actively from the beginning of the feeding trial compared with fry maintained entirely on the respective experimental diets (Treatments A to D). The optimal growth of *Clarias macrocephalus* fry obtained at 30% - 40% protein is consistent with previous studies (Hashim and Ali 1989, Chuapoehuk, 1987). A significantly higher growth rate and improved feed conversion were observed among fry fed on diets supplemented with *Tubifex*, indicating that the poorer growth performance of the fry fed solely with the experimental diets (Diets 1 - 4

could be improved by supplemental feeding with *Tubifex*. The poorer growth rate and feed conversion of these fry could be attributed to the poor digestibility and assimilation of the diet (Wilson *et al.* 1981) or its unattractiveness. Several studies have been conducted to improve the acceptability and feed consumption of experimental diets. Recently, Lovshin and Rushing (1989) used feed attractants, also known as gustatory additives, in pelleted diets which showed improved diet acceptance for largemouth bass fingerlings. Oligochaetes such as earthworms have also been know to contain food attractants for catfish, *Ameiurus nebulosus* (Olmsted, 1981). Sugars such as glucose and sucrose in cat (Rabin *et al.* 1976) and chum salmon fry (Akiyama *et al.* 1982) feeds have been reported to act as appetite stimulants. However, the improvement in feeding activity for the experimental diets after a morning feed of *Tubifex* observed in this experiment, suggests that the *Tubifex* not only contains food attractants but it also stimulates the fry appetite for the test diets.

The data obtained from this feeding trial is summarised in Table 2. Mortalities throughout the feeding trial for all treatments were negligible except for the fry maintained entirely on *Tubifex* (Treatment E). It is likely that the nutrients present in the live *Tubifex* were insufficient to sustain the fry over the feeding trial period. No significant difference in growth and feed conversion ratio was obtained when the fry were fed the experimental diet alone. This indicates that either fish meal or soybean meal can be used as the dominant protein source when experimental diets are used alone. Our study shows that, supplementing the experimental diets with a small amount of *Tubifex* (at 2% body weight) resulted in fry having a significantly higher relative growth compared to those maintained on *Tubifex* alone or on experimental diets alone. Fry maintained on the supplement 35% fish meal dominant diet (Treatment G) showed the highest improved relative growth rate (780.8%) but this was not significantly different from that of Treatment F (683.6%) where the diet was supplemented by 30% fish meal. This observation suggests that when *Tubifex* is used to supplement fish meal dominant diets, a 30% instead of 35% protien feed is sufficient for optimal growth. Tacon *et al.* (1983) reported that partial replacement of a commercial rainbow trout diet with frozen lumbricid worms resulted in growth and feed efficiency that were similar to those associated with the commercial feed. No difference was observed in this study probably because freezing reduces the nutritive quality of some natural feeds and hence are unsuitable for the fish (Grabner *et al.* 1981). However, the significant improvement in growth and feed efficiency observed in the supplementary feeding regime could be attributed to the fact that live

TABLE 2
Effects of the dietary treatments on relative growth rate (RGR), feed conversion ratio (FCR), protien efficiency ratio (PER) and survival rates on catfish (*Clarias macrocephalus*) fry.

Treatment	Diet (% protein)	RGR (%)	FCR	PER	SR (%)
A	1 (30%)	141.6a	2.43a	1.50a	98.8a
B	2 (35%)	120.4a	2.37a	1.35a	99.0a
C	3 (35%)	110.1a	2.99a	1.07b	90.0a
D	4 (40%)	189.6a	3.06a	0.88b	97.9a
E	Tubifex	284.4b	2.24a,b	0.90b	60.0b
*F	1(30%) + Tubifex	683.6c	1.31c	1.51a	97.6a
*G	2(35%) + Tubifex	780.8c	1.24c	1.63a	97.2a
*H	3(35%) + Tubifex	314.8b	1.75b	1.71a	97.4a
I*	4(40%) + Tubifex	460.0d	1.66b	1.31a,b	97.0a

Means in columns with the same superscripts are not significantly different ($P < 0.05$)
* Supplementation with Tubifex at 2% body weight.
RGR = Final wt. - Initial wt./Initial wt. X 100
FCR = Total dry feed fed (g) + Total Tubifex (dry matter)/Total wet weight gain (g)
PER = Wet weight gain (g)/Amount of protein fed (g)

unprocessed *Tubifex* retained its nutritive qualities, its feed stimulants and was an attractant. However, Akiyama *et al.* (1984) observed that the incorporation of various processed ingredients such as krill meal, earthworm powder, glucose, etc, to replace 5% of fish meal in experimental diets was able to improve the growth and feed efficiency of chum salmon fry.

CONCLUSION

This study indicates that supplementation of artificial diets with live *Tubifex* at 2% body weight can effectively promote growth and feed conversion efficiency of the *Clarias macrocephalus* fry. This type of feeding regime is a practical and economic propositon since it requires only treatment of the *Tubifex* with formalin prior to feeding at no extra cost; and the abundance of this natural feed in this region ensures a suitable source for supplemental diet for *Clarias macrocephalus* fry production.

ACKNOWLEDGEMENTS

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Effect of Adding Mineral Oil to the Effectiveness of Permethrin Emulsion Spray Droplets on *Plutella xylostella* Larvae

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ABSTRAK

Keberkesanan titis semburan emulsi permetrin dicampur dengan (10% v/v) dan tanpa minyak mineral telah dinilai dalam makmal dengan bioassai menggunakan larva Plutella xylostella. *Nozel mikro telah digunakan untuk mendapatkan saiz titis semburan berukuran 52-274 µm median diameter isipadu (VMD). Instar larva kedua telah diletakkan di atas cakera daun yang telah dirawat dan catatan rebahan dan kematian larva dibuat selepas 1 jam dan 24 jam berturutan. Bila titis semburan bertambah kecil, dos juga menurun tanpa kehilangan keberkesanan permetrin ke atas larva. Ini menunjukan titis semburan yang kecil adalah lebih cekap jika dibandingkan dengan titis semburan kasar. Semburan menggunakan saiz titis semburan di antara 70 µm hingga 100 µm tidak menghasilkan peningkatan keberkesanan permetrin dari campuran titis semburan emulsi dengan minyak mineral apabila dibandingkan dengan titis semburan emulsi sahaja. Walau bagaimanapun pada saiz titis semburan yang melebihi 125 µm, keberkesanan campuran titis semburan emulsi permetrin dengan minyak mineral didapati menurun.*

ABSTRACT

The effectiveness of permethrin emulsion spray droplets with (10% v/v) and without mineral oil was evaluated in the laboratory by bioassay using larvae of Plutella xylostella. *A microtip nozzle was used to produce spray droplets ranging from 52 - 274 µm in volume median diameter. The 2nd instar larvae were placed on treated leaf discs, and knockdown and mortality were recorded after 1 h and 24 h respectively. In both treatments, as droplet size decreased the dose also decreased without losing the effectiveness of permethrin indicating that small droplets were more efficient than large droplets. When sprayed using droplet size between 70 µm to 100 µm, there was no increase in the effectiveness of permethrin from emulsion spray droplets plus mineral oil compared to emulsion spray droplets alone. However, for droplet sizes greater than 125 µm, the effectiveness was reduced.*

Keywords: permethrin, mineral oil, spray droplets, *Plutella xylostella*

INTRODUCTION

In pesticide spraying, the evaporation of water-based spray droplets is one of the major routes of pesticide loss. This could be severe especially in the tropics where the weather is hot. Thus, oil is sometimes added to reduce evaporation and to increase the life span of water-based formulation spray droplets before the target is reached (Wodagenah and Matthews, 1981). However, little is known about the biological effect on the leaf surfaces when oil is added to the spray droplets. The combined use of mineral oil and synthetic pyrethroid insecticide have been shown to reduce the incidence of virus transmitted by aphid on potatoes (Gibson and Rice, 1986; Gibson and Cayley, 1987). In those studies, the movement of the insect on the treated area was very limited. The present study evaluates the extent to which the addition of mineral oil affects the efficacy of permethrin spray droplets against the mobile larvae of the diamondback moth, *Plutella xylostella* L.

MATERIALS AND METHODS

The diamondback moth was cultured on brussels sprout leaves in the laboratory at 20°±2°C and 70±10% RH. The emulsifiable concentrate (EC) formulation of permethrin (Ambush 25

EC - ICI Plant Protection Division, Jealott's Hill, Berks) was diluted with water. One dilution contained 10g of active ingredient (a.i.)/litre, while the other dilution contained 10g a.i./litre and 10% (w/v) mineral oil (Ulvapron - British Petroleum Oil Ltd, Victoria Street, London). To both dilutions, 0.75% (w/v) of flourescent tracer, Uvitex Stardust (Ciba-Geigy) was added. The spray mixture was freshly prepared and used within 24 hours. A microtip nozzle (Coggins and Baker, 1983) was used to produce spray droplets between 52 - 274 µm in volume median diameter.

Bioassay

The bioassay procedure adopted was according to Omar and Matthews (1987). Leaf discs (5 cm^2) of brussels sprout plants were sprayed at various droplets density under the UV light. They were then grouped into their respective class of droplet density (no. of droplets/cm^2±15% S.E.). Samples of droplets were collected on magnesium oxide-treated slides before and immediately after treatment for measurement of droplet size. The undersurface of leaf discs were coated with petroleum jelly to discourage larvae from crawling underneath them. The leaf discs were then placed in petri dishes lined with moist filter papers. Control discs were sprayed with water containing 0.75% (w/v) flourescent tracer for EC treatment and water containing 10% (v/v) of mineral oil and 0.75% (w/v) flourescent tracer for EC plus mineral oil treatment. Three 2nd instar larvae were placed on each disc. A minimum of seven discs were used for each class of droplet density and at least four droplet densities (representing doses) were used to obtain a dose response curve. All tests were carried out at 20±2° C and 70±10% RH.

Assessment of treatment effect

Knockdown (lack of co-ordination, twitching and convulsion) and mortality (no movement when probed with a fine camel hair brush) of the larvae were recorded after 1 hour and 24 hours respectively. Data were analysed by probit analysis (Finney, 1971) and the median quantity for 50% knockdown (KQ_{50}) and mortality (LQ_{50}) were obtained. If mortality occurred in control, treatment mortality was corrected using Abbott's formula. Droplet size was measured using the Optomax computer based image analyser.

RESULTS AND DISCUSSION

As the spray droplet size of the EC formulation and EC formulation plus oil decreased, the KQ_{50} and LQ_{50} also decreased indicating the utilization of permethrin deposit for knockdown and mortality from small droplets was more efficient than large droplets (Figure 1 and 2). A similar result was obtained using different formulations and insecticides (Munthali and Wyatt, 1986; Omar and Matthews, 1987). Small droplets distribute toxicant more efficiently than large droplets, hence increasing the probability of the mobile larvae coming into contact with the toxicant. Thus, dosage could be reduced without decreasing the larval responses by using small droplets.

When sprayed at a droplet size between 70 µm and 100 µm in diameter, adding mineral oil to the EC formulation did not improve the knockdown and mortality effectiveness of permethrin (Figure 1 and 2). Earlier work also showed no increase in toxicity when spraying was done under low volume conditions, using a mixture of petroleum oil and fenpropathrin for control of adult *Bemisia tabaci* (Ishaaya *et al.*, 1986). With larger droplet sizes (>125 µm), the LQ_{50} and KQ_{50} values of EC formulation plus mineral oil with droplet sizes tested were greater (Table 1) when compared to the LQ_{50} values of the EC alone, indicating the reduction of permethrin efficacy. The reduction of the effectiveness of permethrin with the addition of mineral oil could be due to the permethrin penetrating the leaf interior. Oil has been documented to enhance the penetration of the pesticide into plants (Worthing and Walker, 1986). The permethrin, having a major contact mode of action, depends on contact with the larva for the transfer of toxicant to produce the response. Since oil enhanced the penetration of permethrin into the plant, the toxicant on the surface was less than when EC alone was used.

The present study shows that when a mixture of mineral oil and permethrin is sprayed with droplets greater than 125 µm in diameter, the effectiveness of permethrin is reduced. For field control, it appears that a higher dosage of permethrin would be needed if the EC formulation is mixed with oil and sprayed with larger

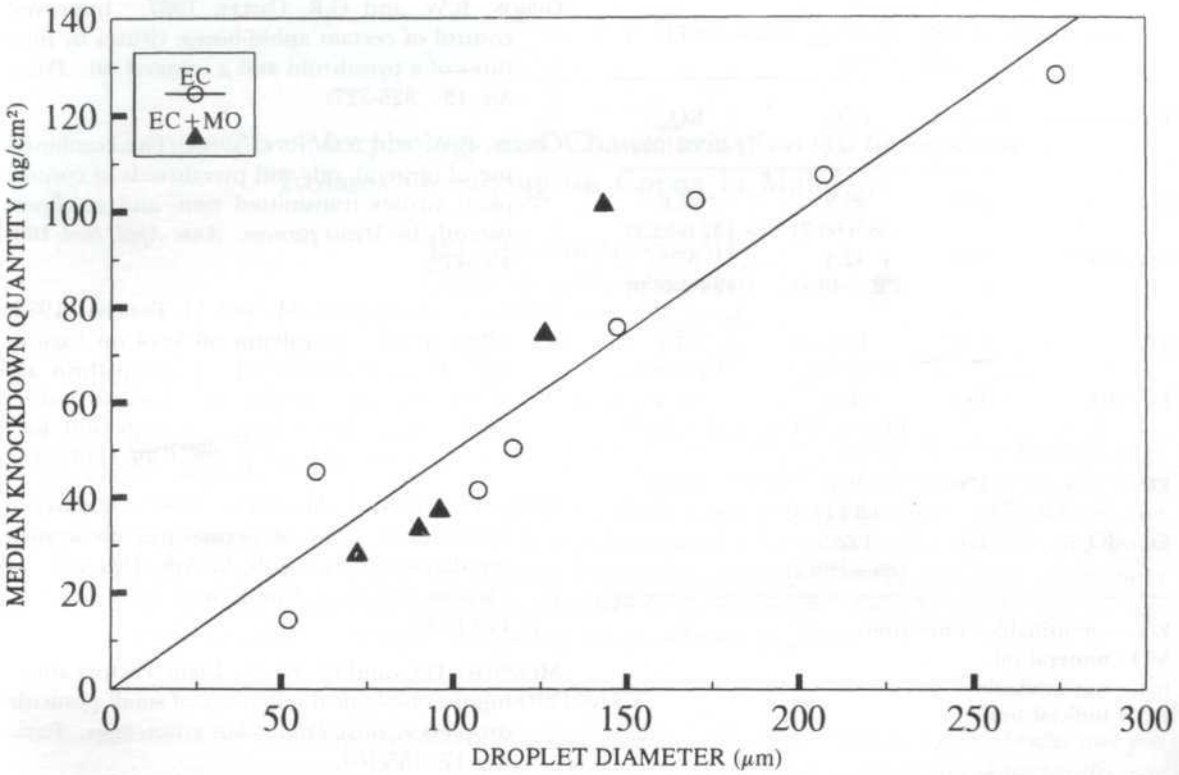

Fig. 1: Effect of adding mineral oil to EC formulation on KQ_{50} of 2nd instar P. xylostella *larvae*

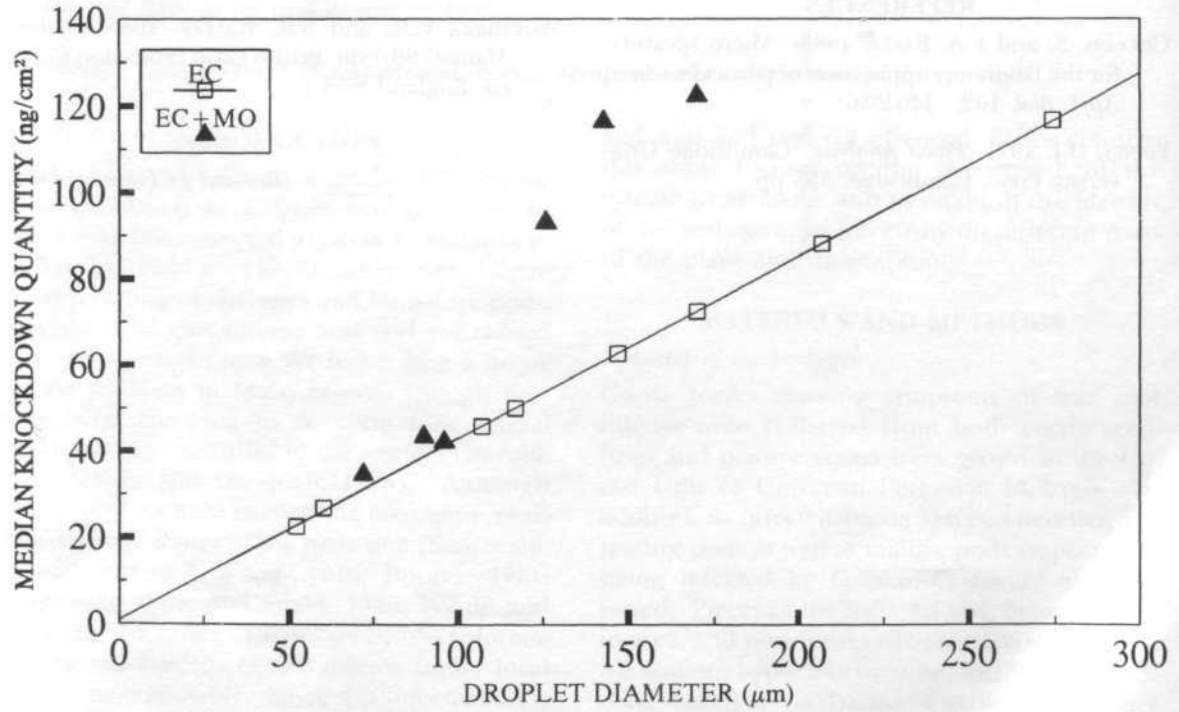

Fig. 2: Effect of adding mineral oil to EC formulation on LQ_{50} of 2nd instar P. xylostella *larvae*

TABLE 1
Comparison of LQ_{50} and KQ_{50} values for EC and EC+MO

Formulation	Droplet Size (μm)	LQ_{50} (95% f.l.)	KQ_{50} (95% f.l.)
EC	107	45.9 (38.9-60.7)	41.6 (32.6-52.2)
EC+MO	96	42.4 (35.9-48.8)	37.3 (24.8-46.9)
EC	147	62.9 (49.6-89.2)	37.3 (54.5-95.8)
EC+MO	143	116.2 (94.3-136.9)	101.2 (79.1-121.7)
EC	170	72.6 (79.2-117.5)	102.1 (84.3-125.2)
EC+MO	170	122.5 (89.4-196.7)	n.a.

EC = emulsifiable concentrate
MO= mineral oil
n.a. = not available
f.l. = fudicial limit

droplets. Further research is needed to confirm the present finding.

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Comparative Morphology and Characterization of *Colletotrichum* Isolates Occurring on Cocoa in Malaysia

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ABSTRAK

Pemencilan daripada daun-daun koko yang menunjukkan gejala "shot-hole", hawar dan bintik daun, juga daripada putik dan buah yang reput menghasilkan Colletotrichum gloeosporioides. Tiada perbezaan jelas dari segi ciri-ciri kultur dan morfologi dicatatkan daripada pemencilan-pemencilan yang berbeza. Kulat didapati hidup dan membentuk spora dengan baik pada 30°C. Agar Ekstrak Daun Koko (CLEA) merupakan medium yang sesuai untuk pertumbuhan miselium manakala Agar Kentang Dekstros (PDA) menggalakkan pensporulaan. Daun-daun koko dan juga buah yang dicederakan mudah dijangkiti oleh C. gloeosporioides. Anak benih koko berumur tiga minggu dan putik menunjukkan peringkat yang paling rentan terhadap jangkitan.

ABSTRACT

Isolation from cocoa leaves showing symptoms of shot-hole, blight or irregular leaf spot and from cherelles and pod rot yielded Colletotrichum gloeosporioides. No distinct differences in cultural and morphological characteristics were noted between the various isolates. The fungus was found to grow and sporulate well at 30°C. Cocoa Leaf Extract Agar (CLEA) was the best medium for mycelial growth while Potato Dextrose Agar appeared to favour sporulation. Both cocoa leaves and injured pods were liable to infection by C. gloeosporioides. Three week old cocoa seedlings and cherelles were noted as the most susceptible stages.

Keywords: ***Colletotrichum gloeosporioides*, cocoa, temperature, culture media, infectivity**

INTRODUCTION

Leaf spot and pod rot of cocoa (*Theobromae cocoa,* L) incited by *Colletotrichum gloeosporioides* which were first reported to occur in Malaysia in 1975 by Lin and Liew (1975), cause severe blighting and rotting of cherelles and immature pods. However, leaf spot disease and pod rot caused by *C. gloeosporioides* have yet to become a major disease problem in Malaysia even though they have been recorded to be serious in several cocoa growing countries in the world (Thorold, 1975; Dakwa and Danquah, 1978). Although several workers have studied the taxonomy, morphology and biology of the pathogen (Stoneman, 1898, Shear and Wood, 1913; Burger, 1921; Simmonds, 1965; McDonald, 1926; Wastie and Shanker, 1970; Arx, 1970), very little information on the etiology of this disease under local conditions is available. Since this information is required for the formulation of a comprehensive disease control programme for *Colletotrichum* leaf spot and pod rot of cocoa in this country, this study focuses on the occurrence of the disease in Malaysia; and to establish the identity of the pathogen, its infectivity on different parts of the plant and its symptoms.

MATERIALS AND METHODS

Isolation of the pathogen

Cocoa leaves showing symptoms of leaf spot disease were collected from both cocoa seedlings and mature cocoa trees grown in the Cocoa Unit of Universiti Pertanian Malaysia. In addition to infected cocoa leaves, cherelles, immature pods as well as mature pods suspected of being infected by *Colletotrichum* were also collected. Pieces of the infected leaf, 3mm × 3mm in area, and pod tissues were surface sterilised in 5% sodium hypochlorite solution (NaOCl) for 5 min., washed in two changes of sterile distilled water and plated on potato dextrose agar (PDA). The resulting cultures were sub-cultured onto fresh

PDA plates until pure cultures were obtained. All cultures of the isolates were subcultured onto and maintained on PDA throughout this study unless otherwise stated.

Effect of Environmental Factors on Growth, Sporulation and Cultural Characteristics of Colletotrichum gloeosporioides *isolates*

For this study and subsequent studies, the four *Colletotrichum* isolates as shown in Table 1 were used as the test fungi. To study the effect of temperature, each PDA plate was centrally inoculated with 5mm diameter fungus plug taken from the advancing margin of a 5-day old culture and incubated in the dark at 20, 25, 30 and 35°C for five days.

TABLE 1
Isolates of *Colletotrichum gloeosporioides* from infected cocoa leaf and pod associated with leaf spot and pod rot disease

Isolate	Source
Sh	cocoa leaf with shot hole symptom
Lb	cocoa leaf with blight symptom
Is	cocoa leaf with irregular spot symptom
Pr	cocoa pod with pod rot symptom

The effect of culture media on the growth, sporulation and cultural characteristics were determined by centrally inoculating PDA, CDA (Czapek Dox Agar), MEA (Malt Extract Agar), OMA (Oat Meal Agar), Cooks (Cooks medium) and CLEA (Cocoa Leaf Extract Agar) plates with a 5mm diameter fungus plug taken from the advancing margin of a five-day old culture and incubated at 30°C in the dark for five days.

Each treatment was replicated four times. Growth measurements, degree of sporulation and cultural characteristics were assessed at the end of the experiment. Mycelial growth was assessed by taking the average of the two perpendicular distances across the centre of the colony. Spore concentration was determined with the aid of a Neubauer haemocytometer.

Infectivity Studies

Cocoa seedlings of mixed hybrid of 2, 3 and 4-week old were inoculated with spore suspension of 10^6 conidia/ml until run-off. The spore suspension was prepared by flooding a five-day old culture; three drops of Tween 80 were then added to the resultant spore suspension before spraying. Sterile distilled water was used as the control. Seedlings were maintained in a moist environment for infection to occur. The experiment was replicated ten times, arranged in randomized complete block design (RCBD) with each replication consisting of a single seedling. Assessment of percentage leaf area infected was conducted on the tenth day.

Infectivity studies were also carried out on detached cocoa pod; Cherelle (25-30 mm long), young pod (40-50 mm long) and green mature pod (85-100 mm long) of mixed hybrid which were surface sterilised with 5% NaOCl and rinsed with sterilised water. Pods were inoculated directly or injured with a 0.5mm diameter sterilised inoculating needle prior to inoculation. Pods were spot inoculated with 10µl of spore suspension of 10^6 conidia/ml. The inoculated pods were incubated in a moist chamber at 30°C with 98% relative humidity. Ten pods of each size were used for each treatment. The control treatments were inoculated with sterile distilled water. Lesions were assessed on the tenth day by taking the average of the two perpendicular distance across the centre of the lesion.

Data were statistically analysed and the difference between individual means was tested using Duncan Multiple Range Test (DMRT).

RESULTS

The Pathogen

Isolation made from the three types of foliar symptoms viz: shot-hole, leaf blight and irregular spot and a pod rot symptom consistently yielded the fungus *Colletotrichum*. Cultural studies on the pure cultures obtained showed that the cultures isolated from infected tissues showing the same type of symptom, were similar to one other; however, they were different from those isolated from other types of symptom. Results as shown in Table 2 suggest the identity of the four cultures was close to that of *C. gloeosporioides* (Penz.) Sacc. The identification was confirmed by IMI, Kew, England. No other distinct characteristics were noted which could facilitate strain differentiation within the species.

TABLE 2
Cultural and morphological characteristics of 5-Day old *C. gloeosporioides* isolates from cocoa grown on PDA

Isolate	Cultural characteristics	Morphological characteristics
Sh	Colony appeared white and gradually turned greyish salmon in colour as the culture grew older. Aerial mycelium slightly flocculose with orange conidial pustules evident at the centre of the colony. Reverse of colony appeared smoky grey in colour.	Conidia cylindrical with obtuse ends, hyaline, aseptate, uninucleate, 5-22 µm x 2-6 µm, formed in setose or globose acervuli or on solitary phialides on mycelium. Acervulus round to elongated to irregular 60-240 µm in diameter. Setae sparse to profuse, dark brown to black, straight to slightly curved, 1-4 septate, swollen at the base and tapering towards the apex, 50-170 µm long.
Lb	Colony appeared white and gradually turned olivaceous grey in colour as the culture grew older. Aerial mycelium flocculose with orange conidial pustules apparent at the centre of the colony. Reverse of colony appeared smoky grey in colour.	Conidia cylindrical with obtuse ends, hyaline, aeptate, uninucleate, 4-24 µm x 2.5 µm, formed in setose or globose acervuli or on solitary phialides on mycelium. Acervulus round to elongated to irregular, 70-250 µm in diameter. Setae sparse to profuse, dark brown to black, straight to slightly curved, 1-4 septate, swollen at the base and tapering towards the apex, 50-80 µm long.
Is	Colony appeared white and gradually turned greyish white as the culture grew older. Aerial mycelium slightly flocculose with orange conidial pustules evident at the centre of the colony. Reverse of colony appeared smoky grey in colour.	Conidia cylindrical with obtuse ends, hyaline, aseptate, uninucleate, 4-23 µm x 2-6 µm, formed in setose or globose acervuli or on solitary phialides on mycelium. Avervulus round to elongated to irregular 65-190 µm in diameter. Setae were sparse to profuse, dark brown to black, straight to slightly curved, 1-4 septate, swollen at base and tapering towards the apex, 70-165 µm long.
Pr	Colony appeared smoky grey in colour with thick floccose aerial mycelium and orange conidial pustules at the centre. Reverse of colony appeared in the form of distinct olivaceous grey zonation alternated with rosy buff zonation.	Conidia cylindrical with obtuse ends, hyaline, aseptate, uninucleate, 4-22 µm x 2-5 µm, formed in setose or globose acervuli or on solitary phialides on mycelium. Acervulus round to elongated to irregular, 80-230 µm in diameter. Setae sparse to profuse, dark brown to black, straight to slightly curved, 1-4 septate, swollen at the base and tapering towards the apex, 80-170 µm long.

Effect of Environmental Factors on Growth and Sporulation of Colletotrichum gloeosporioides Isolates

Studies on the effect of temperature on the mycelial growth and sporulation (Fig. 1A & 1B) of the four *Colletotrichum* isolates proved to be significant at P = 0.05. All the four isolates were found to grow better at 30°C over an incubation period of 5 days Sh and Lb isolate attained a mycelial growth of 51 mm. in diameter Is isolate 37 mm. and Pr isolate, 30mm. The fungi were also found to sporulate better at 30°C with the highest number of spores being harvested from Is isolate ($9x10^6$ spores/ml), followed by Pr isolate ($8x10^6$ spores/ml), Sh isolate ($2x10^6$ spores/ml) and Lb isolate ($1x10^6$ spores/ml).

Growth media influence the growth rate and sporulation of *Colletotrichum* isolates. However, the variation in growth and sporulation was insignificant at P = 0.05. CLEA was noted as the best medium for mycelial growth followed by OMA, Cook's, PDA, MEA and CDA. On the other hand, CLEA, failed to maintain favourable

sporulation. PDA was the best sporulation medium followed by CDA, OMA, MEA, Cook's and CLEA.

Infectivity Studies

Each isolate could produce more than one type of symptom depending on the leaf age and the

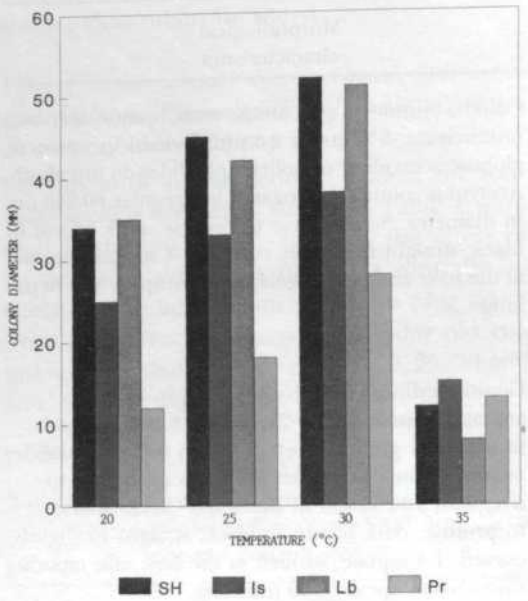

Fig. 1A: Effect of Temperature on the Linear Growth of C. gloeosporioides *Isolates from Cocoa on PDA* ($L.SD_{0.05}$ = 8.47)

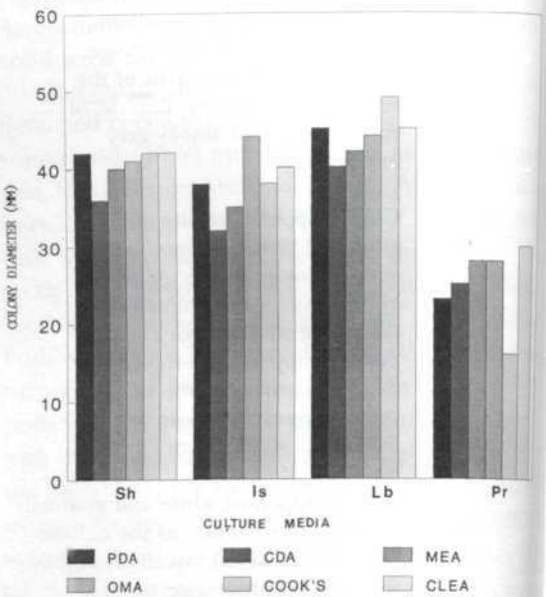

Fig. 2A: Effect of Culture Media on the Linear Growth of C. gloeosporioides *Isolates from Cocoa on PDA*

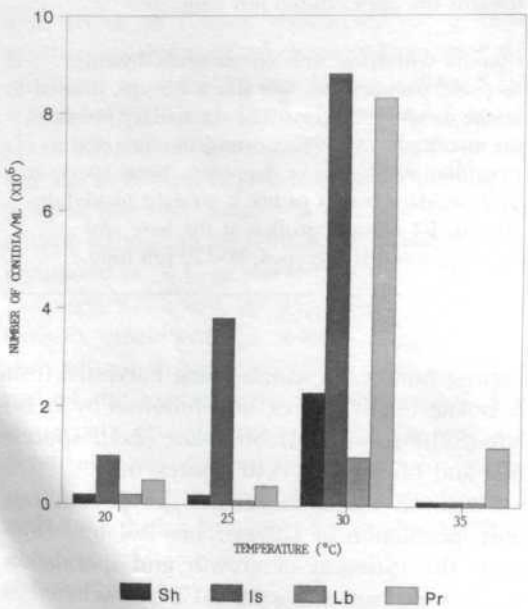

Fig. 1B: Effect of Temperature on Sporulation of C. gloeosporioides *Isolates from Cocoa on PDA* ($L.SD_{0.05}$ = 8.47)

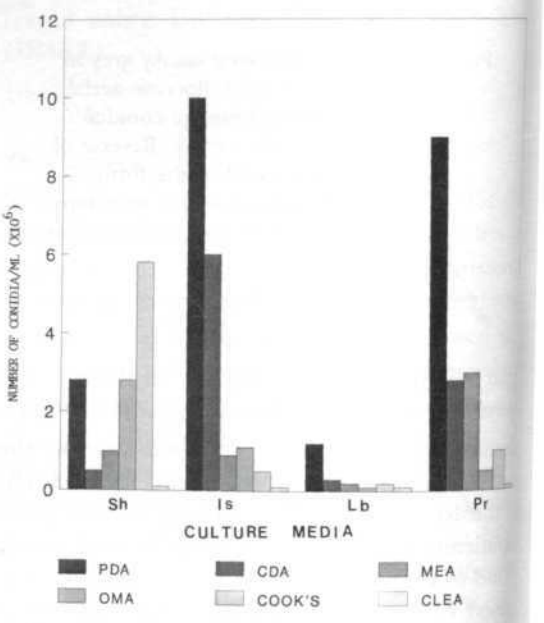

Fig. 2B: Effect of Culture Media on the Sporulation of C. gloeosporioides *Isolates from Cocoa on PDA*

extent of the infection. Blight and shot-hole symptoms were more frequently observed on younger leaves although they were also seen on older leaves. Irregular spot symptom, however, dominated on the older leaves with blight symptoms being occasionally observed. The first evidence of infection was noted after four days of incubation. The symptoms caused by the various isolates of *Colletotrichum* were undistinguishable at the initial stage of infection. The lesion first appeared as minute yellowish specks, and later discernible as circular reddish brown lesions with a chlorotic halo. As the infection progressed, three clearly distinguishable foliage symptoms viz, shot-hole, irregular spot and blight were apparent on the affected seedlings. The results presented in Table 3 indicate that Pr isolate was the most virulent one tested on the cocoa seedlings, recording a mean percentage leaf area infection of 2.2%, 12.2% and 11% on 2-week, 3-week and 4-week old cocoa seedlings respectively. Isolate Is was the least pathogenic, exhibiting a mean percentage leaf area infected of 1.1%, 6.5% and 6.0% on 2-week, 3-week and 4-week old seedlings respectively.

Lesions incited by the various cocoa isolates of *Colletotrichum* on detached injured pods are shown in Table 4. The severity of infection on cherelle was significantly different from that of green mature pod. Isolate Pr was noted to be the most pathogenic strain followed by Sh, Is and Lb. Infection was first observed on the inoculation spot 5 days after incubation in the form of a small brownish round spot with a yellow halo. The affected area later became darker and formed a depressed lesion, followed by the production of greyish white mycelium with pink coloured masses of conidia. Lesions caused by the various isolates of *Colletotrichum* appeared identical and reisolation from pod lesion yielded only the isolate with which the particular pod was inoculated. No infection was noted in any of the non-wounded inoculated pods.

TABLE 3
Severity of infection incited by *Colletotrichum gloeosporioides* isolates on cocoa seedlings of different ages

Treatment	Mean percentage leaf area infected*				
Seedling Age	Is	Lb	Sh	Pr	Control
2 weeks	1.1[a]	1.7[a]	1.8[a]	2.2[a]	0[a]
3 weeks	6.5[b]	8.8[b]	7.4[b]	12.2[b]	0[a]
4 weeks	6.0[b]	8.0[b]	6.8[b]	11.0[b]	0[a]

* Any two means within the column followed by the same letter are not significantly different at 5% level based on Duncan Multiple Range Test.

TABLE 4
Lesion development caused by *Colletotrichum gloeosporioides* isolates on injured detached cocoa pods of various sizes

Treatment	Lesion size (mm)*				
Pod size	Is	Lb	Sh	Pr	Control
Cherelled	4.8[a]	3.5[a]	10.4[a]	13.0[a]	0[a]
Young pod	4.7[a]	0[b]	8.3[b]	12.5[a]	0[a]
Green mature pod	0[b]	0[b]	0[c]	7.5[b]	0[c]

* Any two means within the column followed by the same letter are not significantly different at 5% level based on Duncan Multiple Range Test.

DISCUSSION

Isolation of the disease pathogen from the respective types of foliar symptoms viz., shot-hole, leaf blight and irregular spot and pod rot yielded *Colletotrichum* isolates. These isolates were identified as *Colletotrichum gloeosporioides* (Penz.) Sacc. and subsequently confirmed by the International Mycological Institute. Cultural and morphological studies showed no distinct differences in characteristics among the *C. gloeosporioides* isolates which could facilitate strain differentiation within the species, except for slight variation in culture colour and consistency of the mycelium. All the *C. gloeosporioides* isolates produced cylindrical conidia with obtuse ends, hyaline, aseptate, uninucleate and measured 4-24µm x 2-6µm which were formed in setose or globose acervuli. The shape of the acervuli ranged from round to elongated to irregular and measured 60-250µm in diameter. Setae were sparse to profuse, dark brown to black, straight to slightly curved, 1-4 septate, swollen at the base and tapering towards the apex.

Studies on the species of *Colletotrichum* elsewhere have shown them to be very variable in their morphological (Arx, 1970) and cultural

characteristics (Stoneman, 1898; Shear and Wood, 1913; Burger, 1921). Mohanan (1983) was able to classify the isolates of *C. gloeosporioides* from cocoa associated with irregular spot, blight and shot-hole symptom into white, dark and light types. Dakwa and Danquah (1978) also observed distinct variation in morphological and cultural characteristics among the isolates of *C. gloeosporioides* which cause leaf blight of cocoa in Ghana.

Studies on the effect of temperature on mycelial growth and sporulation of *C. gloeosporioides* isolates proved to be significant at P = 0.05. The isolates were found to grow and sporulate better at 30°C on PDA. Similar observations were made by Quimio (1974) and Scattar and Malik (1938) on *C. gloeosporioides* from mango, and Lii (1972) on *C. gloeosporioides* from guava. Earlier work by Wastie and Shanker (1970) and Muthappa (1971) were confirmed by our studies that growth media could influence the growth rate and sporulation of *C. gloeosporioides*. However, the effects were insignificant (P = 0.05). CLEA was observed to be the best medium for mycelial growth followed by OMA, COOK'S, PDA, MEA and CDA. In our study, however, CLEA failed to maintain its status in favouring sporulation; we noted that PDA was the best sporulation medium followed by CDA, OMA, MEA, COOK'S and CLEA. Radziah (1985) claimed that PDA and CDA favour sporulation while working on *C. gloeosporioides* from rubber.

Results obtained from our infection studies reveal that *C. gloeosporioides* could infect both cocoa leaves and pods. Wilting of cherelles and young pods were generally considered to be due to physiological factors, but recent observations by Mohanan and Kaveriappa (1983) showed that a considerable percentage of pod rot of cherelles and young pods was due to *Colletotrichum* infection. Similar observations were reported by Bailey (1966) from Nigeria and Reddy and Mohanan (1976) from India. Mohanan and Kaveriappa (1983) in their studies on the symptomatology of *Colletotrichum* disease of cocoa reported that the occurrence of three different types of symptoms on the foliage of cocoa plant caused by *C. gloeosporioides* could be attributed to the existence of different varieties of the same species or pathological strain. However, in our studies, we found that each isolate could produce more than one type of symptom depending on the leaf age and the extent of infection. Wastie and Shankar (1970) claimed that apart from climatic factors, leaf age has an influence on the severity of *Colletotrichum* infection. Blight and shot-hole symptoms were more prominent on younger leaves although they were also spotted on older leaves. On the other hand, irregular spot symptoms dominated on the older leaves with blight symptom being occasionally observed. Dakwa and Danquah (1978) reported similar observations. Spotting of older leaves caused little damage; however, infection on newly formed young leaves could result in impairment of the functional photosynthesis (Sarma and Nambiar, 1976) and leaf fall, producing bare tips which could subsequently be invaded by *Botryodiplodia theobromae* Pat. (Shell Chemicals Technical Bulletin).

Laboratory studies with detached cocoa pods showed that only injured cocoa pods were liable to infection by *C. gloeosporioides* conidia and that cherelles and young pods were more susceptible than green mature pods. Although pathogenicity tests were only carried out in the laboratory, this information suggests a possible mode of penetration for field infection. Various factors have been linked to the initiation of spore germination and disease spread. Mohanan (1983) suggested that free water available on leaf surface could facilitate conidial germination and infection. Sarma and Nambiar (1976) reported that although shot-hole incidence was found throughout the year in Kasaragod district of Kerala, the intensity was higher when a temperature range of 19 - 33°C and relative humidity of 77 - 98% prevailed. On the other hand, Dakwa and Danquah (1978) were of the opinion that the high incidence of leaf blight in Ghana could be attributed to the availability of tender leaves coupled with the high relative humidity and moisture on leaf surface. Although *Colletotrichum* disease of cocoa is not very serious at present in this country, its wide distribution and occurrence makes it necessary for effective control measures to be taken to prevent the fungus from attaining epiphytotic proportions as has happened in Ghana.

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Allantoin and Amino Acid Composition in Xylem Exudates of Nodulated and Nitrate-dependent Cowpea Plants

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ABSTRAK

Kesan suntikan Rhizobium dan kepekatan N eksogen [0, 0.5, 1.0, 2.0, 4.0 dan 8.0 mM N sebagai $Ca(NO_3)_2$] ke atas tumbesaran pokok, pembintilan, pengikatan N_2, komposisi asid amino dan kelimpahan ureida dalam eksudat xilem dikaji bagi pokok kacang panjang (Vigna unguiculata L. Walp cv. Kausband) yang ditanam dalam kultur pasir dalam rumah kaca. Tanpa pemberian N, suntikan meningkatkan dengan ketara pengikatan N_2, tumbesaran bahagian atasan pokok dan kepekatan alantoin dalam eksudat berbanding dengan kawalan tanpa suntikan. Nitrogen eksogen, dibekalkan cuma kepada pokok berbintil sahaja, tidak menimbulkan apa-apa faedah ke atas tumbesaran pucuk, akar dan keseluruhan pokok berbanding dengan suntikan tanpa N, tetapi berat dan saiz bintil dan pengikatan N_2 merosot secara ketara dengan pertambahan kepekatan N melebihi 1.0 mM. Walau bagaimanapun, bilangan bintil tidak dipengaruhi oleh kepekatan N. Kepekatan alantoin dan indeks relatif ureida [(ureida-N/ jumlah N dalam eksudat) x 100] merosot secara drastik dengan kepekatan N, tetapi kelimpahan NO_3-N dalam eksudat menunjukkan trend yang bersetentangan. Dalam pokok yang bergantung kepada simbiosis Rhizobium, alantoin adalah hasil eksport yang predominan (94%) dalam eksudat, dengan baki bahan larutan terdiri daripada NO_3-N (4.5%) dan amino-N (1.5%). Di sebaliknya NO_3-N adalah bentuk N utama (87%) dieksport dalam eksudat xilem bagi pokok bergantung penuh kepada NO_3. Walaupun 20 asid amino berlainan dikenalpasti dalam eksudat, glutamina adalah komponen yang terbanyak (65%) dalam tanaman bergantung kepada bintil, dan asparagina adalah asid amino predominan (43%) dalam pokok bergantung kepada NO_3. Berat bintil dan indeks relatif ureida berkorelasi secara tinggi dan positif ($r = 0.959^{**}$). Kepekatan alantoin dan pengikatan N_2 mempunyai korelasi yang rendah ($r = 0.655^{**}$). Kepentingan data ini dalam kajian pengikatan N_2 simbiosis dibincangkan.

ABSTRACT

The effects of Rhizobium inoculation and exogenous N concentrations [0, 0.5, 1.0, 2.0, 4.0 and 8.0 mM N as $Ca(NO_3)_2$] on plant growth, nodulation, N_2 fixation, amino acid composition and ureide abundance in xylem exudates of nodulated cowpea (Vigna unguiculata L. Walp cv. Kausband) were investigated in glasshouse-grown plants in sand culture. Without applied N, inoculation markedly increased N_2 fixation, top growth and allantoin concentration in exudates compared with the uninoculated control. Exogenous N, applied only to nodulated plants, had no beneficial effects on top, root or whole plant growth compared with inoculated control, but nodule mass and size and N_2 fixation decreased substantially with increasing N concentrations exceeding 1.0 mM. However, nodule number was unaffected by any N concentration. Allantoin concentration and relative ureide index [(ureide N/ total N in exudates) x 100] declined drastically with N concentration, but the abundance of NO_3-N in the exudates followed the reverse trend. In plants fully dependent on Rhizobium symbiosis, allantoin was the predominant (94%) export product recovered in xylem exudates, with the remaining N solutes comprising of NO_3-N (4.5%) and amino-N (1.5%). Conversely, NO_3-N was the principal (87%) form of N exported in xylem of NO_3-dependent plants. Although 20 different amino acids were identified in the exudates, glutamine was the most abundant component (65%) in the nodule-dependent crop whereas asparagine was the predominant (43%) amino acid in the NO_3-dependent plants. Nodule mass and relative ureide index were highly and positively correlated ($r = 0.959^{**}$).

Allantoin concentration and N_2 fixation had low correlation coefficient ($r = 0.655^{**}$). The significance of these data in biological nitrogen fixation studies is discussed.

Keywords: Asparagine, correlation, cowpea, glutamine, NO_3-N, nodule mass, N-free nutrient solution, relative ureide index, sand culture

INTRODUCTION

Investigations on the composition of nitrogenous solutes in xylem exudates of plants of cowpea (*Vigna unguiculata* L. Walp) and soybean (*Glycine max* L. Merrill) and detailed tracer studies using ^{14}C and ^{15}N reveal that ureides (allantoin and allantoic acid) are products of N_2 fixation (Herridge *et al.* 1978; Matsumoto *et al.* 1976; Matsumoto *et al.* 1977a, 1977b). In many tropical legumes ureides form the major soluble nitrogen pool exported from the nodules to the shoots *via* the xylem stream (Schubert 1986; Peoples *et al.* 1989a; Peoples and Herridge 1990).

The relative abundance of ureides in the xylem sap of legumes is regarded as an indicator of plants' dependency on *Rhizobium* symbiosis in a number of different legumes and can provide a quantitative assay of N_2 fixation. There is a progressive and predictable change in xylem sap composition from non-nodulated plants, which largely export nitrate and amino acids to the shoots when supplied with mineral N, to a xylem stream dominated by ureides in nodulated plants supplied with N-free nutrient solution (McClure *et al.* 1980; Pate *et al.* 1980; Rerkasem *et al.* 1988; Peoples *et al.* 1989b; Herridge and Peoples 1990; Herridge *et al.* 1990).

Although the effects of mineral N application on nodulation, ureide transport and composition of N solutes in xylem exudates of soybean have been widely studied (e.g McClure and Israel 1979; McClure *et al.* 1980; Herridge and Peoples 1990), less is known about the correlation between nodule mass, allantoin concentration, N_2 fixation and the proportional changes in the export products with the plants' decreasing relative dependency on N_2 fixation in other species. This paper examines the spectra of N solutes and amino acid composition exported in xylem exudates of nodulated cowpea plants subjected to a wide range of mineral N concentration supplied in the rooting medium.

MATERIALS AND METHODS

Plant Culture and Maintenance

Four seeds of cowpea (*Vigna unguiculata* (L.) cv. Kausband) were either uninoculated or inoculated with *Rhizobium* strain CB 756 and sown (on 27 December 1989) to a depth of 2 - 3 cm in a sand culture contained in 10 L pots (26 cm diameter x 26 cm height). Each pot contained 16 kg of washed river sand of the following chemical analysis: total N(1.0 g kg^{-1}), total P(58 mg kg^{-1}), K(8.3 g kg^{-1}), Mg(5.4 mg kg^{-1}) and organic matter (3 g kg^{-1}).

After 2 weeks the seedlings were thinned to 2 per pot. The pots were placed in a glasshouse (from 27 December 1989 to 15 February 1990) located at the Department of Field Crops and Grassland Science, Wageningen Agricultural University, The Netherlands. The environmental conditions in the glasshouse were similar to those of previous experiment (Othman *et al.*, 1991). The plants were supplied weekly with 300 ml per pot of basal N-free nutrient solution (pH 6.10) of the following composition (μM): P (200), K (2000), Mg (400), S (1000), Ca (160), Mn (36), B (20), Zn (12), Fe (10), Mo (3.5), Cu (2.5), Cl (2.1), Na (0.8), Co (0.2). The plants were watered daily to field capacity with deionised water. The calibration for the exact volume of water to achieve field capacity (with no excess drainage) was accurately determined prior to the experiment. All nutrients applied (including the applied N) were assumed to be taken up by the plants.

Treatments

The inoculated plants were subjected to 6 concentrations of mineral N solution containing 0, 0.5, 1.0, 2.0, 4.0 and 8.0 mM N [as $Ca(NO_3)_2$]. The uninoculated plants were supplied with 0 mM N. This treatment is referred to as the uninoculated control. The N accumulated by this "reference crop" was used in the calculation of N_2 fixation by total N assays. Each pot received 300 ml of its respective N treatment solution per week.

The 7 treatments were arranged in a randomised complete block design with 4 replications. The data were subjected to an analysis of variance and Duncan multiple range test and least significant difference (LSD).

Plant Harvesting and Xylem Exudate Collection

All plants were harvested 50 days after sowing. At harvest, plant tops were cut below the cotyledonary nodes and a tightly-fitted 3 cm

long silicon tube was placed over each stump. Xylem exudates were collected over a period of 1 h. The techniques of exudate collection were the same as the ones described in an earlier experiment (Othman *et al.* 1991). The exudates dispensed into 1.0 ml vials were stored in a freezer (-15° C) until analyses were carried out.

After exudate collection, the root systems were harvested. The sand was washed off the roots, the nodules separated and then counted. The roots, nodules and plant tops were dried in an oven at 75° C for 48 h and weighed. These plant parts were later ground in a Ritz plant grinder, passing through a 1.0 mm mesh screen. The ground plant samples were digested by the micro Kjeldahl techniques and the N concentrations determined on a Technicon autoanalyser.

Determination of Nitrogenous Solutes and Amino Acid Composition in Exudates

The concentrations of allantoin (ureide-N), amino-N and nitrate-N in the exudates were determined colorimetrically on a Vitatron spectrophotometer using the procedures described by Young and Conway (1942), Yemm and Cocking (1955) and Cataldo *et al.* (1975) respectively. These analytical techniques have been modified by Peoples *et al.* (1989a).

The composition of amino acids in the exudates was assayed using an amino acid analyser (Biotronik LC 6000 E) fitted with Durrum DC 6A column (25 cm long and 6 mm diameter). A 250 µL mixture, consisting proportionally of 100 µL of exudate sample, 320 µL diluting buffer (for physiological fluid separations) and 80 µL Norleucine solution (external standards), was injected into the column for analysis.

RESULTS

Top and Root Growth

Without inoculation and devoid of mineral N, cowpea plants showed extremely poor growth of tops and whole plants, yielding about 6.8 and 9.5 g dry matter per pot respectively (Fig. 1a and 1c). However, top and overall plant growth were significantly increased ($P < 0.05$) by inoculation alone without the need for application of mineral N. This increase in dry matter production was 81 and 70% for top growth and whole plant respectively. Inoculated plants showed no positive response to increasing concentration of mineral N. In fact top and whole plant growth showed at first a tendency to increase with N application from 0 to 2.0 mM and then a decline thereafter. However, these increases and decline were non-significant compared with the inoculated control (+ *Rhizobium* + 0 mM N). Unlike top growth, root dry weight was apparently unaffected by either inoculation or applied N (Fig. 1b).

Nodulation

Nodule number, size and mass showed differential responses to inoculation and N application. Unfortunately, the uninoculated plants produced appreciable amounts of small and white-coloured nodules at harvest, indicating a rhizobial contamination in the glasshouse. Nodule mass and size of inoculated plants declined sharply ($P < 0.01$) with increasing concentrations of mineral N applied, but nodule number appeared to be unaffected by N application (Fig. 2), despite a declining trend, due to the large variability in nodule numbers between plants and the large standard errors. Nitrogen concentration of more than 1.0 mM was deleterious to nodulation, with both nodule mass and size greatly suppressed. The reduction in nodule mass and size caused by increasing concentration of applied N was highly significant ($P < 0.01$).

Concentration and Composition of N Solutes in Xylem Exudates

Without applied N, inoculation significantly ($P < 0.05$) increased allantoin concentration compared with the uninoculated control. However, allantoin concentrations declined drastically ($P < 0.01$) from 425 to 50 nmol ml^{-1} when the concentration of mineral N was increased from 0 to 8.0 mM (Fig. 3a). In contrast, the concentration of NO_3-N was the lowest (40 nmol ml^{-1}) at 0 mM N, but increased sharply to 650 nmol ml^{-1} at 4.0 mM N and then levelled off at 8.0 mM N.

The concentrations of amino-N showed erratic trends with applied N between 0 to 2.0 mM, but further increases in N supply had little effect on this soluble nitrogen component. Nonetheless, the concentration of amino-N remained fairly constant at 7 nmol ml^{-1} between 2.0 to 8.0 mM mineral N application.

The composition of soluble N, pool in xylem exudates, expressed as a percentage of total N followed similar patterns as their concentrations described earlier. In plants fully depend-

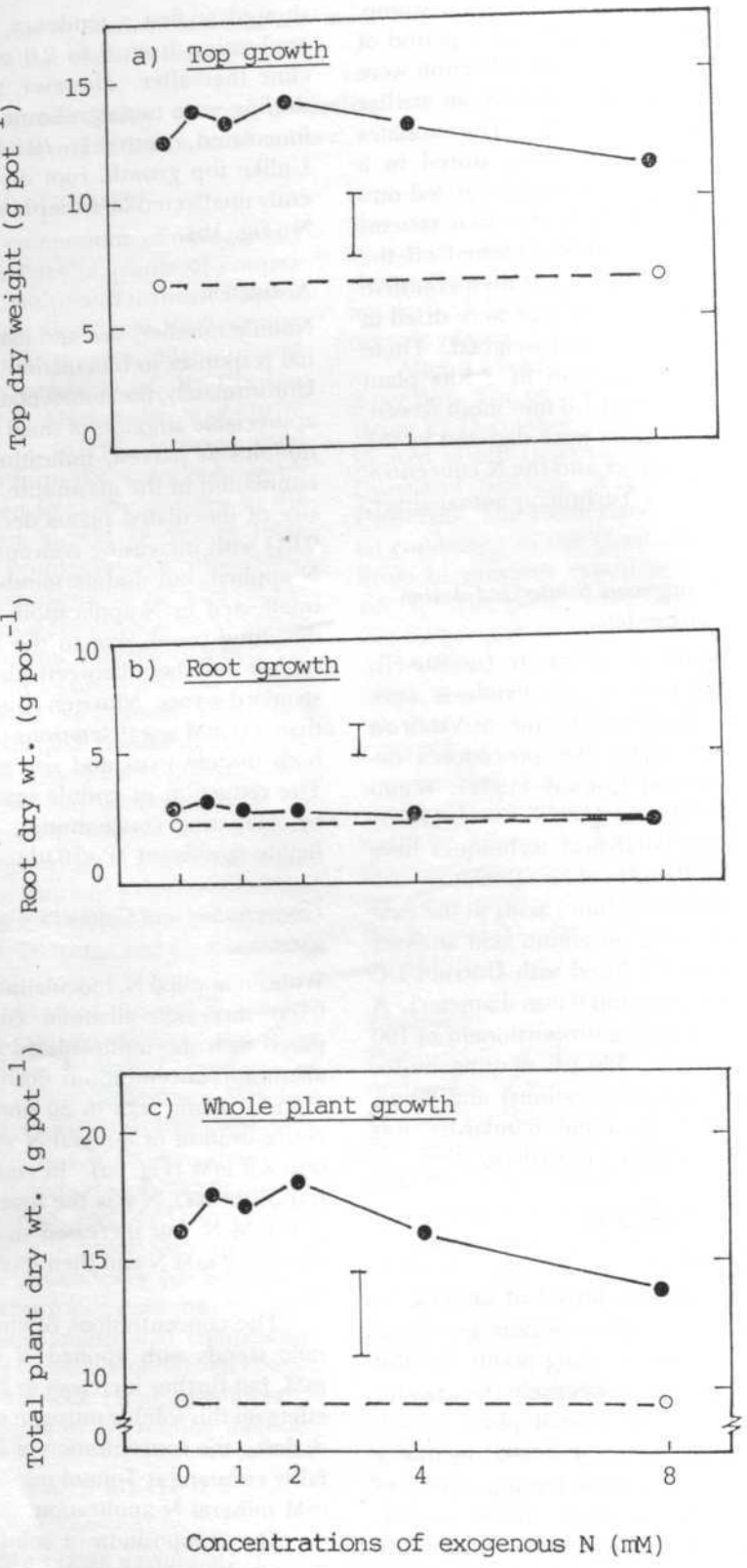

Fig. 1: Effects of Rhizobium *inoculation and exogenous N on top, root and growth of whole cowpea plants. Vertical bars denote LSDs at $P < 0.05$. (• = inoculated plants, o = uninoculated + 0 mM N)*

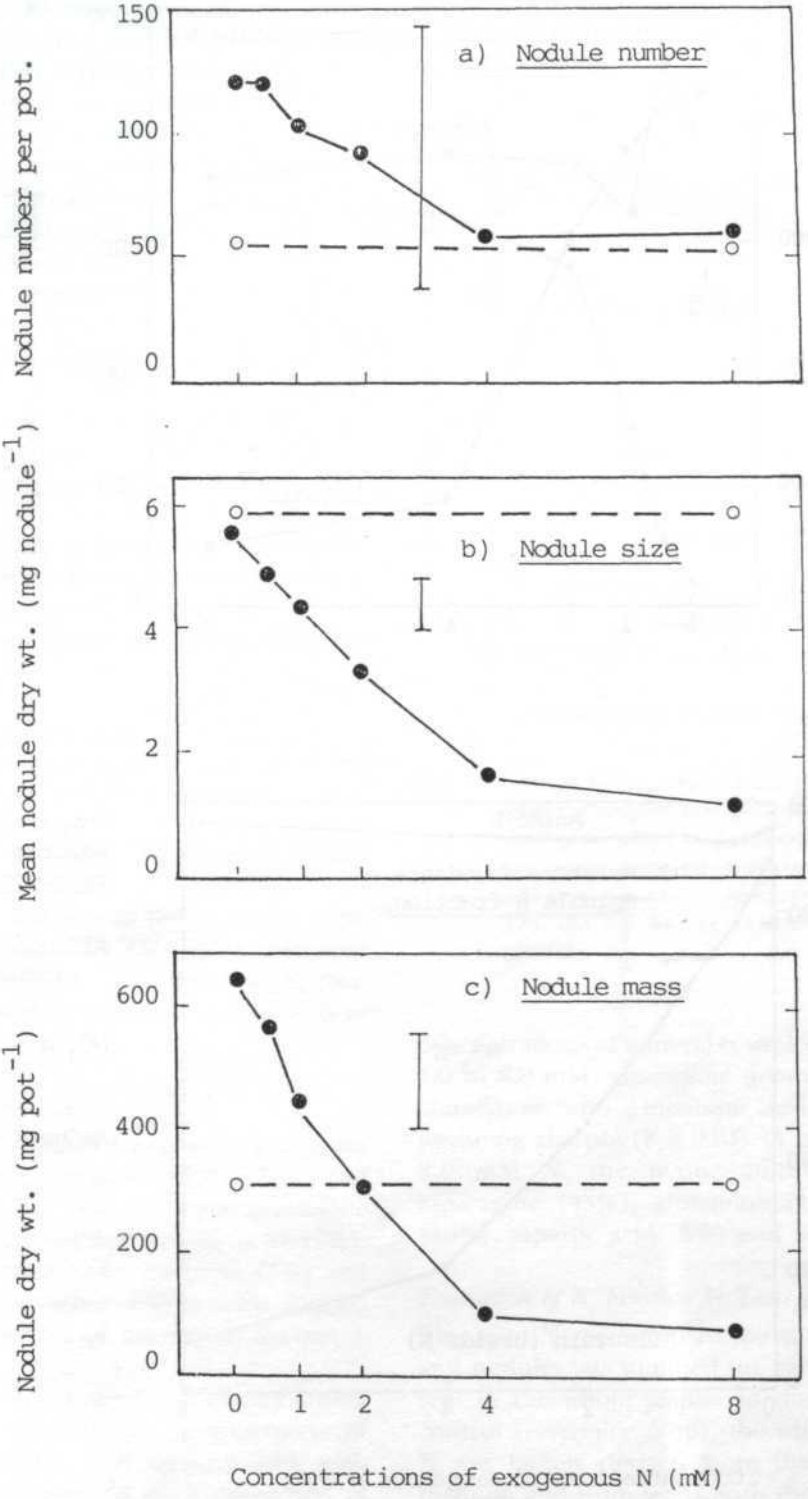

Fig. 2: Effects of Rhizobium *inoculation and exogenous N on nodule number, size and mass. Vertical bars denote LSDs at P < 0.05. (• = inoculated plants, o = uninoculated + 0 mM N)*

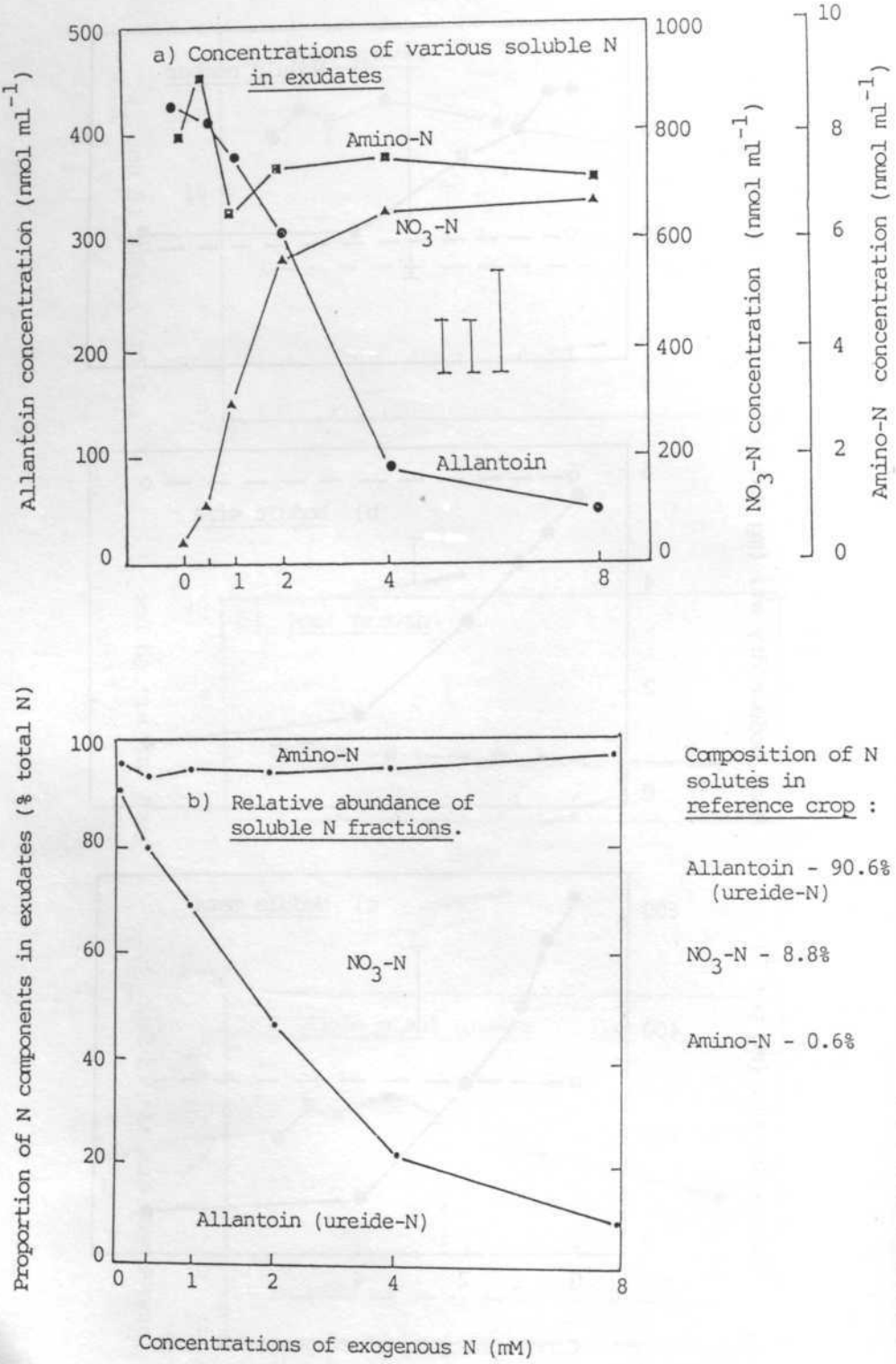

Fig. 3: (a) Concentrations of allantoin, NO_3 -N and amino-N in xylem exudates of cowpea plants and (b) the relati abundance of various soluble N fractions as affected by mineral N application. Vertical bars, from left to right, deno LSDs at P < 0.05 for allantoin, amino-N and NO_3 -N respectively. (The soluble N concentration (nmol ml^{-1}) reference crop: Allantoin (241), amino-N (2.6) and NO_3 -N (31)

ent on symbiosis (*Rhizobium* inoculation + 0 mM N), allantoin (a form of ureide-N) formed the predominant (94%) export product in the exudates with the remainder comprising of NO_3-N (4.5%) and amino-N (1.5%) (Fig. 3b). However, as the supply of exogenous N in the nutrient solution was increased from 0 to 8.0 mM N, the proportion of allantoin declined drastically, tapering to only about 10% at 8.0 mM N when the plants were presumed to be almost fully dependent on mineral N (Fig. 3b). On the other hand, the proportion of NO_3-N increased ($P < 0.01$) rapidly from about 4.5% in the nodule-dependent plants to about 87% in plants dependent on mineral N. The proportion of amino-N in the soluble N pool was relatively small, (ranging between 1.0 and 2.5%) and showed erratic trends with exogenous N concentrations. These inconsistent effects were fairly similar to that of amino-N concentration.

The relative ureide index calculated using the formula :

$$\frac{4 \text{ allantoin conc.}}{(4 \text{ allantoin conc} + NO_3\text{-N} + \text{amino-N})} \times 100$$

(After Peoples *et al.* 1989a)

followed similar patterns as the allantoin composition (%) in Fig. 3b. This index indicated the declining dependency of plants on N_2 fixation as the mineral N was increased from 0 to 8.0 mM.

Composition of Amino Acids

On analysis, the amino-N component was found to contain 20 different amino acids. The most abundant ones, on average, were glutamine (48%), asparagine (13%), aspartic acid (7%), arginine (7%), lysine (4%), histidine (3%) and valine (2%). The other amino acids formed only a minor fraction of the amino-N component.

The application of mineral N caused drastic changes in the composition (percentages) of glutamine, asparagine and aspartic acid, with only marginal variations in the composition of other amino acids (Fig. 4). In fully symbiotic plants and those subjected to 0.5 and 1.0 mM N, glutamine was the predominant (65%) amino acid, with asparagine and aspartic acid comprising about 8 - 10 and 7 - 10% respectively. As the

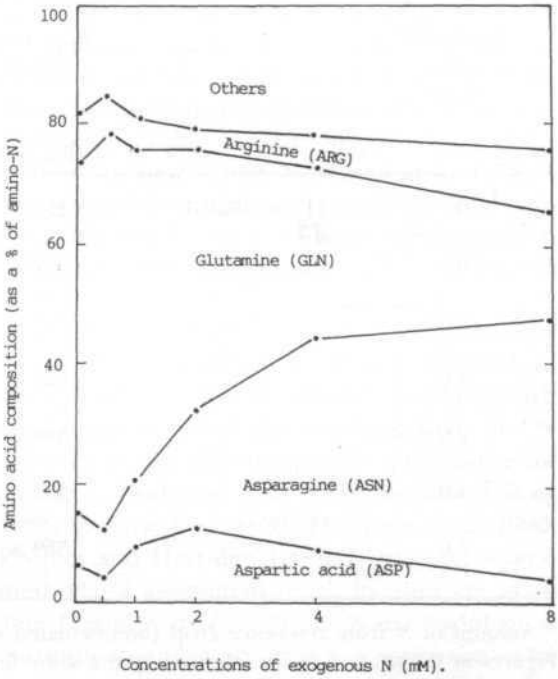

Fig. 4: Changes in the composition of predominant amino acids (asparagine and glutamine) in xylem exudates of nodulated cowpea plants subjected to various concentrations of exogenous N. (The amino acid composition in the reference crop: GLN (60%), ARG (6.6%), ASN (4%), ASP (3%) and others (26.4%).

concentrations of mineral N were increased from 1.0 to 8.0 mM, asparagine greatly increased in abundance with glutamine and aspartic acid declining sharply ($P < 0.01$) in proportion. At 8.0 mM N, the major amino acids were asparagine (43%), glutamine (17%), arginine (10%), aspartic acid (5%) and others (25%).

Estimation of N_2 Fixation by Total N Assays

The total N recovered in the plant tops, roots and nodules was summed up, giving the N content in the whole plant. In the uninoculated control (reference crop), the whole plant total N was largely derived from the sand culture medium and from seeds, with the basic assumption that no symbiotic fixation occurred. The second basic assumption was that all plants, irrespective of N treatments or inoculation, took up the same amount of N from the planting medium.

TABLE 1
Effects of applied N on N content in whole plant and N_2 fixation

Applied N conc. (mM)		N in whole plant (mg pot^{-1})	N from fertiliser (mg pot^{-1})	N from sand/ ref. crop* (mg pot^{-1})	N from symbiotic fixation (mg pot^{-1})
0	Uninoculated	156 b	0	156*	-
0	Inoculated	532 a	0	156	376 a
0.5	"	563 a	29.4	156	378 a
1.0	"	521 a	58.8	156	306 ab
2.0	"	550 a	117.6	156	276 b
4.0	"	632 a	235.2	156	240 b
8.0	"	569 a	470.4	156	0 c (-57)

* Amount of N from reference crop (uninoculated + O N) = 156 mg pot^{-1}.
Figures in the same column followed by the same letters are not significantly different at $P < 0.05$.

TABLE 2
Correlation coefficients between nodule dry weight, allantoin concentration, relative ureide index (RUI) and N_2 fixation (total N assay)

Parameters	Correlation coefficients (r)
Nodule d. wt and RUI+ (%) (mg pot^{-1})	0.959 **
Nodule d. wt and allantoin conc. (mg pot^{-1}) (nmol ml^{-1})	0.900 **
Nodule d. wt and N_2 fixation (mg pot^{-1}) (mg N pot^{-1})	0.731 **
Allantoin conc. and RUI+ (%) (nmol ml^{-1})	0.914 **
Allantoin conc. and N_2 fixation (nmol ml^{-1}) (mg N pot^{-1})	0.655 **
RUI+ (%) and N_2 fixation (mg N pot^{-1})	0.736 **

** Significant at $P < 0.01$
+ RUI = relative ureide index

With inoculation and N application, the whole plant N was derived from 3 sources, namely from symbiotic fixation, from the applied mineral N and from the sand culture medium. Since the amount of N derived from fertiliser and reference crop were known, N from symbiotic fixation was calculated from the difference between N in whole plant and N from the other 2 sources (Table 1).

Nitrogen from fixation was significantly reduced by N application only when the concentration exceeded 1.0 mM, compared with the inoculated control. A further increase in mineral N concentration caused a further reduction in N_2 fixation, although this detrimental effect was not as prominent as that of relative ureide index outlined in the previous section. Without applied N, inoculation significantly increased N_2 fixation compared with no inoculation. However, applied N between 0.5 and 1.0 mM had little effect on N_2 fixation compared with the inoculated control. As the applied N concentration was increased further from 2.0 to 8.0 mM, the plants' symbiotic activity decreased progressively and was completely inhibited at 8.0 mM N, when the plants were fully dependent on mineral N for nutrition.

A relationship between nodule dry weight, allantoin concentration relative ureide index and N_2 fixation was computed to establish the correlation of these nodule activity parameters (Table 2).

Nodule dry weight and relative ureide index were highly and positively correlated ($r = +0.959^{**}$). Although nodule dry weight, allantoin concentration and N_2 fixation were also highly correlated, the coefficients of correlation were much lower than those of nodule mass and relative ureide index. Similarly, allantoin concentration and N_2 fixation, and relative ureide index and N_2 fixation were positively and significantly ($P < 0.01$) correlated, but the former two nodule activities had the lowest coefficient of correlation ($r = +0.655^{**}$).

DISCUSSION

Legume plants often respond to *Rhizobium* inoculation where effective native rhizobia are lacking in numbers and weak in colonisation of root zones. Farmers and investigators often apply low rates of nitrogenous fertilisers to inoculated legume crops mainly to promote initial growth prior to nodule initiation. Our present data indicated that mineral N supply had no beneficial effects on early growth when cowpea plants were well nodulated (Figs. 1 and 2). In fact mineral N exceeding 1.0 mM was detrimental to nodulation, with nodule mass and size adversely affected (Fig. 2) followed by depressed N_2 fixation (Table 1). The negative value of N_2 fixation in the 8.0 mM N treatment in Table 1 was associated with the accidental rhizobial contamination of the uninoculated reference crop which consequently overestimated the N derived from the growth medium.

Over the years much research emphasis has been focussed on direct and indirect methods of estimating symbiotic N_2 fixation of field-grown crop legumes, using the ureide analysis or ^{15}N depleted dilution techniques as quantitative assays (e.g. Norhayati *et al.* 1988; Rerkasem *et al.* 1988; Pereira *et al.* 1989; Herridge *et al.* 1990; Peoples and Herridge 1990). Although several methods of assessment of N_2 fixation are available (Peoples *et al.* 1989a), ^{15}N methodology is currently the most accurate but expensive. The ureide analysis technique employed in the present study is an indirect method but simple, sensitive and inexpensive, requiring no excavation of nodulated root systems as opposed to acetylene reduction assays. The ureide analysis offers an alternative assessment procedure with practical applications and which allows researchers to compare accurately differences between treatments in respect of plant dependency on N_2 fixation where other N sources (soil-N and fertilizer-N) exist and affect nodule functioning. Since tropical legumes relying on N_2 fixation export ureide-N from the nodulated root systems to the shoots and mineral N absorbed by the roots is exported in the form of NO_3-N, this biochemical discriminating factor makes quantitative assays much simpler. The extremely abundant ureide-N (allantoin) and the low level NO_3-N recovered in xylem exudates of plants fully dependent on *Rhizobium* symbiosis obtained in the present study (Fig. 3) distinguished that from NO_3-dependent crops in which NO_3-N was the principal export product. In the latter case the proportion of allantoin in exudates was small and nodule activity was markedly impaired or completely inhibited (Fig. 3b, Table 1). This is consistent with other observations on cowpea (Herridge *et al.* 1978; Pate *et al.* 1980; Peoples *et al.* 1985; Elowad *et al.* 1987; Peoples and Herridge 1990).

The declining trends in allantoin abundance, nodule mass and N_2 fixation in response to exogenous N application correlated strongly with one another and were interdependent (Figs. 2 and 3, Tables 1 and 2). These results suggest that allantoin concentration (and relative ureide index) are reliable indicators of nodule activity and the plants' dependency on N_2 fixation. However, difficulties in collecting root-bleeding xylem sap from root stumps of field-grown plants, as experienced by Streeter (1972), may limit the widespread adaptation of this technique. However, alternative techniques of recovering xylem sap have more recently been developed (Herridge *et al.* 1988; Peoples *et al.* 1989a).

The exceptionally large proportion of glutamine in exudates of nodule-dependent plants and the abundant asparagine in NO_3-fed crop is another biochemical determinant of nodule activity (Fig. 4) in response to applied N (or other factors, see Pate *et al.* 1980). The amino acid analysis may be particularly useful in studies with peanut or other legumes which are known to be non-ureide exporters, but transport amides from their nodules (Peoples *et al.* 1986, 1987; Norhayati *et al.* 1988).

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COMMUNICATION I

Financial Analysis of Managing Bamboo Stands in a Natural Forest

ABSTRAK

Kertas ini membincangkan analisis kewangan bagi pengurusan projek buluh dalam kawasan hutan asli. Bagi tujuan penganalisaan projek, kos-kos sebenar bagi penyediaan petak penyelidikan buluh Institut Penyelidikan Perhutanan Malaysia (FRIM), bertempat di negeri Kedah telah digunakan. Keputusan analisis menunjukkan projek ini berdaya maju sekiranya pelaburan dibiayai dengan pinjaman pada kadar faedah rendah walaupun pada harga minima RM 0.80/batang. Projek ini amat menguntungkan jika harga buluh dapat mencapai paras yang lebih tinggi atau hasil dapat ditingkatkan.

ABSTRACT

This paper discusses the financial analysis of managing bamboo stands in natural forest. Throughout the analysis, actual costs incurred by the Forest Research Institute Malaysia (FRIM) in setting up trial plots in Kedah were studied. Results from the analysis show that the project is financially viable at a price of RM 0.80/culm provided that the project is financed at a lower interest rate. The project would be very attractive if bamboo culm could fetch a higher price or if yield could be improved.

INTRODUCTION

Bamboo, formerly categorized as a minor forest produce and now replaced by the more appropriate term 'non- wood forest products' has recently become increasingly important. Bamboo is used in making chopsticks, toothpicks, skewers and blinds. The use of bamboo as an industrial raw material for these products continues to be significant.

Available resources of Bamboo in the natural forest of Peninsular Malaysia are significant. McGrath (1970) estimated that the total area of bamboo in Peninsular Malaysia is about 20,250ha. Razak and Abdul Latif (1988), estimated the area under bamboo was about 329,000 ha. The standing stock was estimated at 7.0 million tons (average 20 tons per ha). Out of the 7.0 million tons only 6,000 tons comprise commonly used species with an estimated value of RM 3 million.

The vastly different figures clearly indicate that there has been no thorough assessment of bamboo resources in Peninsular Malaysia. The National Inventory II (1981-82) carried out by the Forestry Department has only given a general estimate of available resources of rattan and bamboo. Therefore, an updated information on these resources is vital for long term planning of the bamboo-based industries.

The Government has increasingly emphasised the development of small-scale industries based on wood, rattan and bamboo since 1985. Bamboo in the past has been used as supplementry material in housing construction (scaffolding), in the making of numerous home utility items, handycrafts, bridges, rafts, waterpipes, vegetable-growing supports, traps, blowpipes, and musical instruments. Todate, there are about 1032 bamboo-based industrial units varying in scale in Peninsular Malaysia (Wong, 1988). Most of these industrial units are located close to areas of bamboo extraction for ease of transportation.

One of the important requirements for the viability of these bamboo-based industrial units is the sustainability of bamboo supplies. Salleh and Wong (1985), Hsiung (1987). Sustainability of bamboo supply can be ensured by establishing bamboo plantations and improving the existing stands through silvicultural efforts.

The Forest Research Institute Malaysia (FRIM) has initiated setting up trial plots in Kedah with the objective of managing natural bamboo stands for sustained supply of high and uniform quality culms. This paper evaluates the financial feasibility of managing bamboo stands in a natural forest through systematic silvicultural operations. Cash-flow estimates and the results

from this analysis can provide information for potential investors of bamboo plantations of the future.

METHODS

Management data on the bamboo stands relate to actual labour costs incurred in setting up trial plots in the state of Kedah by the Forest Research Institute Malaysia (FRIM). Costs of building, vehicles and a fire tower relate to costs incurred on an existing teak plantation in Jitra, Kedah.

All costs incurred and revenue received throughout the duration of the project were discounted to present values. Two discount rates were used in calculating the Net Present Value and the Benefit Cost Ratio. The discount rates used were 15.0 percent (assuming current costs of capital or cost of borrowing) and 10.0 percent (if the project is financed at a lower interest rate or subsidised by the government (ISIS, 1986)). Three of the most commonly used measures of a project's worth, namely (i) Net Present Value (NPV) (ii) Benefit Cost Ratio (B/C Ratio), and (iii) Internal Rate of Return (IRR) were used in this analysis (Gittinger, 1984). Sensitivity analysis was also carried out to examine the impact of changes in price, yield and cost.

MANAGEMENT COSTS

The management of bamboo stands in a natural forest can be broadly classified into three stages viz.: (i) site preparation which includes survey , road construction and application of fertilizer; (ii) silvicultural treatments; and (iii) harvesting. It is assumed that the project will be established in a logged-over forest.

Detailed data on the expected cost per annum for managing 100 ha of bamboo stands in a natural forest leased out for five years are presented in Appendix 1. The project area is assumed to be established partly in year one and two at the rate of 50 ha /year. The following paragraphs summarize principal characteristics of the data.

(i) Stage 1 : Site preparation.

Stage 1 of the project involves four main operations:

(a) Site-survey

Site-survey of the project area is assumed to cost about RM 30/ha which is based on a wage-rate per man day of RM 15.00. Two casual labourers are needed to complete the survey of one hectare within three days.

(b) Site-preparation

Costs of site-preparation, include land clearing (RM 264/ha), cleaning (RM176/ha), recruitment counts of number of shoots (RM 66/ha) and recruitment counts of existing culms in clumps (RM 176/ha). The total cost of site-preparation for the project is therefore RM 616/ha.

(c) Fertilizer.

Applications of fertilizer (N : P : K) at a cost RM 200/ha in the proportion of 15 : 15 : 15 are carried out in year one and year two.

(d) Road maintenance

The average cost of repairing the existing ex-logging roads was about RM 25/ ha with an annual maintenance estimated at RM 6/ ha. The repairs to the existing ex-logging roads was contracted out to a private company.

(ii) Stage 11 : Silvicultural treatments.

Number of weedings required is dependent on the type of forest area. For this project 50 percent of the area will be subjected to weeding in the first year while the other 50 percent will be in the second year. The cost of each weeding is estimated to cost approximately RM 176/ha. Silvicultural thinnings are carried out both at year one and year two at the cost of RM 264/ha.

(iii) Stage III : Harvesting

Harvesting is carried out thoughout the leased period. Based on field observations the average number of bamboo clumps/ha is estimated at about 200/ha. This will produce an equavalent of about 1,600 culms/ha. The minimum number of clumps was observed at about 46/ha. Field observations indicated that by carrying out silviculture operations, viz as application of fertilizer, weeding and thinning improved the yield by at least 30 percent.

Harvesting of these 200 clumps requires five man-days with four full time casual laborers. The cost of harvesting is about RM 220/h

(iv) Other expenses incurred are as follows

a) Fire Protection

For the purpose of this project, a fire-lookout tower at the cost of RM 5,000 will be set up.

b) Salary and Wages

The bulk of the total cost for managing the bamboo stands constitute salaries and wages. Salaries and wages for this project are based on one supervisor (RM 600.00/month) and four labourers (RM 300.00/labourer/month). It is assumed that salaries and wages would increase at a normal six percent per annum (Personal Communication[1])

c) Field Vehicles, Equipment, and Building

Since the area under this project is on a five year lease, a second-hand lorry is sufficient for transportation within the project area. The lorry will be purchased at RM 30,000 and it is assumed that it will last for the whole project duration. Therefore the residual value is zero. For the harvesting operations, two chainsaws at RM 300/each will also be purchased. The economic life of these chainsaws is about five years with zero residual values.

No permanent building is required for this project. A temporary wooden building costing about RM 20,000 will be built to cater for office space and the storing of field equipment. It is assumed that the building has a zero residual value.

d) Administration and other expenses

Administration and other expenses include the cost of maintenance of building (10% of building cost), vehicles (5% of vehicle cost), equipment (5% of equipment cost), fees paid to the Forestry Department (e.g. royalty, license, etc), stationery, telephone, and other sundry items.

(v) Price Dtermination

The price of bamboo culm varies among States. The present price quoted by the Forestry Department ranges from as low as RM 0.80 (farm price) to as high as RM 1.20 per culm (ex-mill). For the purpose of this analysis, the price of bamboo is taken at M$0.80/culm.

RESULTS AND DISCUSSION

Financial Analysis

Based on the various costs mentioned earlier, the total cost of operatig 100 ha of bamboo stands for five years amounts to RM 518,172. Management and Operational costs in year one and year two make up about 62 percent of the total cost. The high cost recorded for these two years is mainly due to the high initial operational costs of managing the bamboo stands.

Using a price of RM 0.80/culm and an average production of 1,600 culm/ha , the total revenue for the whole project is RM 576,000. This will give a net cash flow of RM 57,828 or an average of RM 578/ha. The IRR for the project is 13.88 percent. The NPV values at 10.0 percent and 15.0 percent are RM 11,926 and RM -3,014 respectively (Table 1). The B/C ratio at 10.0 percent discount rate is 1.03 and at 15.0 percent is less than one. Thus, the project is financially feasible only if funding is at low discount rate or the project is subsidized by the government.

TABLE 1
Results of financial analysis of bamboo plantation in natural stand

Investment criteria	
Discounted at 10.0 %	
NPV (M$)	11,926
B/C Ratio	1.03
Discounted at 15.0 %	
NPV (M$)	-3,014
B/C Ratio	0.992
IRR (%)	13.88

Sensitivity Analysis

A sensitivity analysis with respect to price, total cost and yield was carried out to further examine the financial viability of the project. The results of the sensitivity analysis are summarised in Table 2.

The IRR values increase considerably when an increase in culm price is assumed, making the project more financially feasible. The sensitivity analysis also suggests that, if a culm of bamboo could fetch a price of RM 1.20, managing the existing stands would be a very lucrative business.

TABLE 2
Sensitivity analysis of managing the bamboo stand with respect to price, total cost and yield

Variable Simulations	NPV at 15.0% (M$)	NPV at 10.0 % (M$)	IRR
Based ($0.80)	-3,014	11,926	13.88
Price increased by			
10% ($0.88)	34,328	54,629	28.05
15% ($0.92)	52,999	75,981	35.41
25% ($1.00)	90,341	118,685	50.90
50% ($1.20)	183,697	225,455	96.80
Total Cost increased by			
10%	-40,657	-29,586	1.31
15%	-59,479	-50,341	4.16
25%	-97,123	-91,853	13.88
50%	-191,234	-195,632	34.80
Yield increased by			
10%	34,328	54,630	28.05
15%	52,999	75,981	35.41
25%	90,342	118,685	50.90
30%	153,210	192,651	72.26

By varying the total cost of the project, it seems that the project is not financially viable at a discount rate of 10 percent. An increased in yield of about 10 percent on the other hand, would increase the IRR value.

CONCLUSION

The results of the financial analysis of managing bamboo stands in a natural forest show that the investment is financially viable and feasible if the capital is subsidised or financed at a low interest rate. If bamboo culms could fetch a higher price or if the yield could be improved financially with silviculture operations then the project would be very attractive. Based on this analysis, this project offers more returns in comparison with the planting of rattan in a rubber plantation which yields an IRR of 12.76 percent (Salleh and Aminuddin 1988).

At present, bamboo has been utilized extensively and the demand for the raw material has increased yearly. With the goverment policy of promoting small-scale industries such as basket making, chopsticks, skewers and tooth picks production, it is anticipated that the bamboo-based industries using this source as raw material would ultimately face the problem of sustained bamboo supply . It is timely for investors to take the opportunity to manage the existing bamboo stands or to establish bamboo plantations, thus ensuring sustainable supplies of bamboo.

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COMMUNICATION II

Potassium Supplying Capacity of Representative Soils of South Western Nigeria as Measured by Intensity, Quantity and Capacity Factors.

ABSTRAK

Kajian-kajian telah dilakukan dimakmal dan rumah tanaman untuk mempastikan kegunaan faktor-faktor kuantiti, keamatan dan keupayaan K bagi menilai keupayaan-keupayaan bekalan K, dan dalam meramalkan pengambilan K oleh jagung dalam 14 tanah terpilih di Selatan Barat Daya Nigeria. Keputusannya menunjukkan bahawa nisbah aktiviti adalah satu indeks yang lemah bagi K yang boleh digunakan, dan tidak berkemampuan dalam peramalan yang tepat bagi pengambilan K semasa penanaman yang panjang. Potensi keupayaan penampanan adalah lebih tinggi dalam tanah-tanah pada kompleks paling bawah daripada mendakan azal, menunjukkan bahawa pengosongan K terhadap tanaman akan lebih cepat dalam tanah yang diambil daripada bantuan mendak daripada tanah-tanah pada kompleks paling bawah. Keputusan tersebut juga menunjukkan bahawa ketetapan K, faktor kuantiti dan potensi keupayaan penampanan akan memberikan maklumat yang berguna untuk menyatakan perbezaan tabiat penampanan tanah.

ABSTRACT

Laboratory and greenhouse studies were undertaken to determine the usefulness of K Quantity, Intensity and Capacity factors in evaluating K supply capacities and in predicting K uptake by maize in 14 representative soils of South Western Nigeria. The results showed that the activity ratio was a poor index of the available K and was incapable of correct prediction of K uptake during prolonged cropping. The potential Buffer Capacity was higher in the soils on basement complex than in soils derived from sedimentary origin, indicating that depletion of K on cropping will be faster in soils derived from sedimentary rocks than those on the basement complex. The results also showed that the 'fixed K', Quantity factor and the Potential Buffer Capacity could provide useful information in characterising the differential Buffer behaviour of soils.

INTRODUCTION

Several researchers (Unamba-Oparah, 1985; Udo and Ogunwale, 1978; Fagbami *et al.* 1985) have shown that potassium deficiencies occur in many Nigerian soils, especially in acid sands. The exact levels of soil K at which deficiencies will occur in many Nigerian soils under continuous cropping still cannot be predicted accurately. Quantification of K needs under continuous corpping requires knowledge of the soil K buffer capacity. The nature of the k buffer capacity is determined by the K quantity-Intensity relationships as well as the character of the non exchangeable K in the soil. In order to improve the reliability of predicting soil K so that sound K management decisions can be made, indices of K availability should be carefully considered.

The present study was conducted to examine the K supplying potential of South Western Nigerian soil by evaluating the major soil K supplying indices.

MATERIALS AND METHODS

Soil

Bulk samples from surface 15 cm soil were collected from 14 locations of different agroecological zones of South Western Nigeria. The soil samples were air dried and sieved to pass through 2mm sieve. A portion was retained for laboratory analysis and the remainder was used for the greenhouse trials.

Soil Analysis

Exchangeable Na, K, Ca, and Mg in the soil samples were extracted with neutral 1M NH_4OAC. K and Na in the extract were determined by flame photometry while Ca and Mg were determined by atomic absorption sepctrophotometry. The K released from the non-exchangeable form ('fixed' - K) plus the exchangeable K in the samples were extracted by boiling in HNO_3 and determined by flame photometry. The K released from the non-ex

changeable forms ('fixed' K) was estimated by difference (Wood and De Turk, 1941;). Particle size analysis of the soils was determined by the hydrometer method. Soil pH was measured by the glass electrodes in a 1:1 Soil - Water ratio. Organic matter was determined by the wet digestion suggested by Walkley and Black (1935). The total K in the soil samples was digested with a mixture of HF and $HClO_4$. The K in the digest was further dissolved in 6N HCL and determined with a flame photometer. The intensity factor (ARO^K), the quantity factor (Δ K) and the Potential Buffer Capacity (PBC), were estimated according to the procedure of Beckett (1964).

Greenhouse Studies

Two kg soil was weighed into each of several pots; K was applied at 2 levels , 0 and 100 mg kg-1 soil. The pots were arranged in the greenhouse in a randomised complete block design with 3 replicates. All treatments received initially 100mg Nkg^{-1} N as NH_4NO_3 and 50 mg ρkg^{-1} as Na_2HPO_4 $12H_2O$. Each pot contained 3 seed of maize (*Zea Mays L*) which were later thinned to 2 seedlings per pot. Five cycles of the crop of 4 weeks each were studied. Whole plant tops were harvested at the end of each cycle, oven dried at 70°C for 72 hours and weighed. A portion of the plant tissue was milled and digested with H_2SO_4 - H_2O_2 mixture; the K content of the digest was determined by flame photometry. The K response was calculated from the uptake data as:

$$\frac{\text{Uptake of Plus K plots - uptake of minus K plots}}{\text{uptake of minus K plot.}}$$

RESULTS AND DISCUSSION

Table 1 shows properties of the soils used in this experiment. Table 2 shows the K status of the 14 soils studied .

The intensity factors (ARo^K) (Table 2) varied widely among soils and showed no definite trend. No relationship was found between ARo^K values and any of the soil properties. However, the Quantity factor (ΔK) and the Potential Buffer Capacity (PBC) appeared to be related to the parent material; PBC values were found to be lower in soils derived from sedimentary rocks than in soils on the basement complex. The PBC and ΔK values correlated significantly with the soil clay content ($r = 0.969^{***}$ and 0.979^{***} respectively). The dynamic equilibrium between exchangeable and non exchangeable K determines the ability of a soil to buffer K supply to

TABLE 1
Some properties of the experimental soils

		Exchangeable cations meg/ 100g.					Mechanical Analysis (%)				
		K	Ca	Na	Mg	Ex Acidity	Sand	Silt	Clay	Organic Matter %	pH in Water
Soils derived from Basement complex	Ile-Ife	0.354	2.8	0.007	0.83	0.3	66.8	14.7	18.5	2.48	5.9
	Modakeke	0.573	2.0	0.004	0.87	1.0	72.2	14.8	13.0	2.08	6.0
	Ise-Ijesha	.564	5.6	0.026	0.73	1.0	58.4	16.8	24.8	1.88	6.1
	Ibodi	.449	3.2	0.013	0.33	1.0	48.5	16.9	34.6	2.15	5.7
	Eduabon	.462	3.6	0.022	0.77	0.8	81.0	12.6	6.4	1.07	5.8
	Ede	.449	4.0	0.022	1.52	0.7	79.2	8.0	12.8	2.88	6.2
	Ikare	.585	0.9	-	0.33	0.8	68.4	20.5	10.1	0.54	6.3
	Efon	.237	1.4	-	0.12	0.9	80.2	8.8	11.0	4.19	5.3
	Ado Ekiti	.500	2.8	0.007	0.73	1.0	65.0	12.6	22.4	1.27	6.1
	IyinEkiti	.179	3.0	0.035	-	1.4	71.0	12.2	16.8	1.81	6.2
Soils derived from Sedimentary origin	Owode	.147	2.0	0.035	0.83	0.8	74.6	10.2	15.2	1.34	6.4
	Odogbolu	.173	1.7	0.017	0.50	0.9	72.9	16.0	11.1	2.01	5.7
	Ilaro	.128	0.6	-	0.64	0.8	71.6	6.0	22.4	1.68	5.1
	Iperu	.115	2.1	0.004	0.81	0.8	72.5	17.4	10.1	1.74	6.1

crops; and this is dependent on the amount and type of clay (Beckett, 1964). Illite and vermiculite are know to be the major K fixers in the soil.

However, these Nigerian soils do not contain appreciable amounts of clay minerals. Some workers (Adepetu *et al.* 1990) have observed a phenomenon similar to the K fixation tendency found in South Western Nigerian soils where illite and vermiculite probably do not influence the soil content. These workers observed that the zone of K depletion about the plant root is a portion of the non exchangeable K ('fixed' K) that contributes to soil available K. The rate of release of this 'fixed' K depends on the buffer capacity of the soil which is characterised by the Quantity factor and the Potential Buffer Capacity. Thus the depletion of K on cropping should be faster in soils derived from sedimentary rocks than in soils on the basement complex.

Table 3 shows the results of the correlations of some of the indices studied with crop uptake. They show that the exchangeable K is the dominant contributor to the K available for uptake by the crop. However, this contribution decreased with cropping. while 97% of variation in uptake (R^2 = 0.97) is due to exchangeable K alone in the first crop; its cumulative contribution accounts for 72% after the 4th crop. This relationship could be used to predict cumulative uptake after 4 croppings according to the following regression equation:

$$\text{Uptake} + 0.79 + 35.96 \text{ exch-K}$$

The contribution of 'fixed' K was insignificant in the first two crops; however, its contribution increased with cropping. After the 4th crop its cumulative contribution increased by 33%. This may indicate that the 'fixed' K is a potentially important buffer of available K in these soils, although it does not appear to represent a definite fraction of the labile K in the soils studied. The contribution of the activity ratio ARo^K was found to be insignificant and erratic. This shows that ARo^K is a poor index of the available K and is an unreliable predictor of K uptake at initial or after prolonged cropping. The Quantity factor (ΔK), on the other hand,

TABLE 2
Potassium station and quantity-intensity relationships of potassium in the experimental soils

	Soil	K Status in C mole (+) Kg^{-1} Exch.K.	Fixco K.	Exch + fixed K	Mineral K	Total K	ARo^k (ML^{-1}) $X10^3$	K C mole (+) Kg^{-1}	IBC CM(+) (ML^{-1}) $X10^3$
Soils on Basement Complex	Ile-Ife	.358	0.236	0.588	40.437	41.026	5.7	0.14	24.4
	Modakeke	.573	1.462	2.035	51.176	53.205	3.8	0.15	40.0
	Ise-Ijesha	.564	0.744	1.308	46.769	48.077	3.2	0.20	63.2
	Ibodi	.449	0.314	0.762	37.699	38.461	2.0	0.14	70.6
	Edunabon	.462	0.868	1.329	49.952	51.282	10.0	0.10	9.8
	Ede	.449	0.314	0.762	30.006	30.769	5.6	0.13	23.1
	Ikare	.385	0.868	1.765	30.286	32.051	4.8	0.10	21.0
	Efon	.237	0.026	0.263	4.224	4.467	6.1	0.10	16.4
	Ado Ekiti	.500	0.917	1.417	65.891	67.308	2.8	0.18	64.5
	Iyin-Ekiti	.179	0.823	1.003	50.279	51.282	3.6	0.11	30.6
	Mean	0.415	0.708	1.124	38.107	39.231	4.7	.14	36.4
Soils from sedimentary origin	Owode	.147	0.071	0.218	40.167	40.385	2.6	0.08	30.4
	Odogbolu	.173	0.110	0.283	34.973	35.256	2.0	0.05	25.0
	Ilaro	.128	0.090	0.218	36.321	36.538	1.6	0.04	25.0
	Iperu	.115	0.103	0.244	37.577	37.821	6.2	0.10	
	Mean	0.141	0.093	0.241	37.259	37.50	3.1	0.09	24.1
	Grand Mean	0.337	0.533	0.871	37.865	38.736	4.3	0.12	32.9

TABLE 3
Relationships between K uptake and K status indices (cummulative).

Factor	1st Crop	1st & 2nd Crop	1st to 3rd Crop	1st to 4th Crop
Exch.K	0.985***	0.864***	0.846***	0.850***
Fixed K	0.311	0.400	0.585*	0.580*
Exch. + fixed K	0.620*	0.686**	0.744**	0.700**
ARo^k	0.110	-0.320	-0.186	0.040
K	0.620*	0.588*	0.720**	0.878***
PBC	0.462	0.642*	0.585*	0.542*

* Significant at 5% level of probability
** Significant at 1% level of probability
*** Signigicant at 0.1% level of probability

related significantly to the K uptake and its contribution increased rapidly with cropping; after the 4th crop its cumulative contribution to crop uptake was 78% which can be demonstrated by the regression equation: Uptake = 14.986 - 12.43K. The capacity factor (PBC) showed no significant relationship with crop uptake at the first crop but was significant and essentially constant thereafter, indicating that the capacity of the soils to replenish depleted K in solution and on the exchange, varies widely according to cropping dependent on the status of the cropping. The rate of repletion tends to increase with prolonged cropping.

These results indicate that 'fixed' K, exchangeable K, K Quantity and K capacity can provide useful information in characterising the differential buffer behaviour of individual soils of South Western Nigeria. However, further work is needed to verify the practical usefulness of this information under field conditions.

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