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Potential for Biological Control of *Sclerotium* Foot Rot of Chilli by *Trichoderma* spp.

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Keywords: *Sclerotium* foot rot, chilli, organic fertilizers, *Trichoderma harzianum*, *T. virens*

ABSTRAK

Penyediaan kering udara Trichoderma harzianum *dan* T. virens *telah dinilai kemandirian danpotensinya sebagai calon kawalan biologi terhadap reput pangkal Sclerotium cili (*Capsicum annuum L*) sendirian atau sebagai campuran dengan baja organik Amina, Avanti Greeen dan Greeen Supergrow sebagai pembawa.* T. harzianum *dan* T. virens *apabila dicampurkan sendirian dengan Amina dan Avanti Green masih kekal kebernasannya sehingga 180 hari tetapi apabila di campurkan dengan Green Supergrow hilang kebernasannya dengan cepat kepada sifar selepas 30 hari dalam simpanan. Kegiatan calon-calon kawalan biologi juga signifikan dengan Amina apabila diuji dalam tanah yang diinoklat secara tiruan. Di ladang, penambahan pembawa organik hanya meningkatkan penetapan awal calon-calon kawalan biologi. Kejadian reput pangkal* Sclerotium *berkurangan dengan bererti dengan pengurangan sclerotia pathogen yang bernas dan pengasingan semula calon-calon kawalan biologi dalam rizosfera cli dan akar adalah tinggi* T. Virens *adalah calon yang lebih baik dengan memberi 100% dan 92.1% pengawalan terhadap reput pankal* Sclerotium *dalam tanah yang diinokulat secara tiruan dan tanah yang dijangkiti semulajadi berurutan.*

ABSTRACT

Air-dried preparations of Trichoderma harzianum *and* T. virens *were evaluated for their survival and potential as biocontrol candidates against* Sclerotium *foot rot of chilli* (Capsicum annuum, *L*) *singly or as mixtures incorporated into organic fertilizers Amina, Avanti Green and Green Supergro as carriers.* T. harzianum *and* T. virens *incorporated singly into Amina and Avanti Green remained viable for 180 days but when incorporated into Green Supergro lost viability rapidly to zero after 30 days' storage. The performance of the biocontrol candidates was also significant with Amina when tested in the artificially-inoculated soils. In the field, the incorporation of an organic carrier only improved initial establishment of the biocontrol candidates. The incidence of* Sclerotium *foot rot was significantly reduced with the reduction in the viable sclerotia of the pathogen and recovery of the biocontrol candidates in the chilli rhizosphere and roots was high.* T. virens *was a better candidate, giving 100 and 92.1% control of* Sclerotium *foot rot in the artificially-inoculated and naturally - infested soils respectively.*

INTRODUCTION

Sclerotium rolfsii Sacc. is one of the most destructive pathogens of chilli (*Capsicum annuum L*). Various strategies for controlling *S. rolfsii* have been introduced over the years including soil disinfestation, cultural practices and fungicide treatments but losses still occur, largely because the effectiveness of these approaches is variable and short lived. In addition, chilli cultivars with strong resistance to *S. rolfsii* are not commercially available. As a result, research efforts are directed toward developing effective and environmentally safe means of combating foot rot of chilli. Indigenous isolates of *Trichoderma harzianum* and *T. virens* (= *Gliocladium virens*) have been identified as potential biocontrol agents for use against *S. rolfsii* (Jinantana and Sariah 1993, 1997). However, applications of these biocontrol agents have been difficult, mainly because their efficacy not only requires an excessively large amount of inoculum but also varies with soil type and environmental conditions.

Recent advances in our understanding of the infection mechanism of *S. rolfsii* on chilli have led to the conclusion that the target sites for infection are the stem collar and underground stem (Jinantana and Sariah 1994). Accordingly, the biocontrol agent should be placed near the collar region of the chilli plant in order to achieve satisfactory control. Application of the biocontrol agent to soil mixture in nursery bags may assist in the early establishment of the biocontrol agent around the collar region before plants are transferred to the field.

The present study attempted to evaluate the shelf-life of air-dried *T. harzianum* and *T. virens* individually and as mixtures incorporated into 3 types of commercially available organic fertilizers widely used by chilli growers. The potential of the biocontrol preparations was also evaluated for the control of *Sclerotium* foot rot of chilli.

MATERIALS AND METHODS

Fungal Cultures

T. harzianum (IMI 378843) and *T. virens* (IMI 378842) isolated from parasitized sclerotia and confirmed to have antagonistic properties against *S. rolfsii* (Jinantana and Sariah 1993, 1997) were maintained on potato dextrose agar (PDA) as the biocontrol candidates tested in this study.

Preparation of Inoculum

The inoculum of the biocontrol candidates consisted of hyphae, conidia and chlamydospores grown on wheat grains. Three hundred grams of commercial wheat grains were soaked in water for 3 h, rinsed and autoclaved at 103 kPa twice for 30 min in 100 ml of distilled water. After cooling, four 5-mm diameter discs from the margin of a 4-day-old colony of the candidate isolate growing on PDA were transferred to the wheat grain preparation and incubated for 14 days at room temperature (28°C). The preparation was then ground and air-dried at 28°C for 48h. Colony forming units (cfu) per g air-dried preparation were determined by dilution plate technique on *Trichoderma* medium E (TME) (Papavizas 1981).

Preparation of Dried Formulation

The air-dried biocontrol preparations were incorporated into each of the three commercial organic fertilizers (Table 1) in the ratio of 1: 5 w/w (biocontrol candidate: organic fertilizer) and kept in sealed polythene bags. For the mixture, *T. harzianum* and *T. virens* were mixed in equal proportions before adding to each of the organic fertilizers. The biocontrol-fertilizer preparations were stored at room temperature (28 ± 2°C) in a completely randomized design with 3 replications. Samples (1 g) were taken from each replication at 0, 30, 60, 90, 120, 150 and 180 days and the rate of survival and proliferation of the biocontrol candidates were determined by dilution plate technique. The data were expressed as cfu per g air-dried biocontrol-fertilizer preparation. Data were statistically analysed by ANOVA and means compared by using Duncan's multiple range test.

Application of Biocontrol Candidates and Rating of Disease in Sclerotium-chilli System

Glasshouse trial: Potting medium was prepared by mixing sterilized soil mixture (3 : 2 : 1 v/v mixture of soil, sand and organic matter) with the respective biocontrol preparations. For each organic carrier evaluated, treatments consisted of organic fertilizer alone; amended with each air-dried biocontrol candidate (2-weeks-old dried formulation) or mixtures of the biocontrol candidates at 0.08% and 0.05% (w/w) respectively. Four hundred grams of each mixture were placed in pots, sampled and assayed for initial population densities of the biocontrol candidates. Soil mixture alone was also used as the control. One-month-old chilli seedlings were transferred to each of the pots and maintained in the glasshouse for 28 days with normal watering.

These plants were then transferred to sterilized soil mixture which had been artificially inoculated two weeks earlier with 4-week-old *S. rolfsii* inoculum raised on wheat grains at a rate of 3% w/w. There were 24 plants in each treatment. The treatments were arranged in completely randomized design.

TABLE 1

Trade name, formulations and compositions of the commercial organic fertilizers used in the study

Organic fertilizer	Trade name	Formulation	Application rate for chilli	Organic matter[1/]	Total N[2/] (%)	Avail. P[3/] (%)	Avail. K[4/] (%)	pH	Moisture content (%)
Tapioca organic fertilizer	Amina	5.5:5.5:5.5:5.1+TE	Not labelled	10.69	4.70	1.64	0.03	6.80	18.52
Oil palm organic fertilizer	Green Supergro	5 : 5 : 5 : 1 +TE	Not labelled	20.61	4.89	1.81	0.04	3.48	25.08
Chicken dung organic fertilizer	Avanti Green	Not labelled	40 kg/100-200 m^2	21.47	1.41	0.71	0.03	5.97	18.74

[1/] analytical analysis by Walkly and Black Method (Nelson and Sommers 1982)
[2/] analytical analysis by Micro Kjeldahl method (Bremmer and Mulvaney 1982)
[3/] analytical analysis by Bray II method (Bray and Kurtz 1945)
[4/] analytical analysis by using NH4AC (pH 7.0) (Anon. 1996)

The number of infected (wilting with white mycelium at the collar) chilli plants was determined 28 days after transplanting to the *Sclerotium* infested soil. Determination of the populations of the biocontrol candidates in the chilli rhizospheres (within 5-cm radius from the stem and 10-cm depth) and on the roots was carried out 20 and 40 days after transplanting. Soil and roots were sampled, homogenized and placed on a shaker for 20 min in sterilized water. The homogenate was serially diluted with sterile water. One-ml aliquots of the dilution (10^{-4}) was spread with a glass rod over the surface of the agar plate containing TME, and incubated for five days in the dark, after which typical greenish colonies were counted. Similarly, populations of surviving *S. rolfsii* propagules were assessed following the method of Rodriquez-Kabana *et al.* (1980).

Field Trial: The organic fertilizer Amina was chosen as the carrier for the biocontrol candidates based on the results of the survival and glasshouse evaluation trials. The preparation of the seedlings was similar to that used in the glasshouse trial. There were eight treatments (Table 5), arranged in randomized complete block design with three replicates; each replicate consisted of two rows and each row comprised nine chilli plants. The initial population of *Sclerotium* propagules in the experimental plots was determined.

Six-week-old chilli plants previously grown in the nursery bags containing different biocontrol-amended and non-amended (fungicide and control) soil mixtures were transplanted to the naturally infested field. For the fungicide treatment, 500 ml of aqueous Brassicol suspension (200 mg a.i./l H_2O) was applied to each plant by drenching soon after transplanting. Watering, fertilization and pest control were as recommended for chilli cultivation in the area. No fungicide was subsequently used. The number of chilli plants infected was assessed two and four months after transplanting. Determination of the populations of biocontrol candidates and *S. rolfsii* in the soil around the chilli rhizospheres (within 5-cm radius from the stem and 10-cm depth) was determined 1, 2 and 4 months after the plants were transferred to the infested fields.

RESULTS

Survival of Biocontrol Candidates

The survival of air-dried biocontrol propagules differed with the organic fertilizer used as carrier, resulting in different shelf-lives of each biocontrol preparation. *T. harzianum* and *T. virens* singly or as mixtures incorporated into Amina and Avanti Green had a significantly longer ($P< 0.01$) shelf-life than those incorporated into Green Supergro (Table 2). The population levels of both fungi dropped to zero within 30 days of incorporation into Green Supergro while those incorporated into Amina and Avanti Green could be detected for 180 days and 120 days for *T. harzianum* and *T. virens* respectively, after storage at room temperature (28 ± 2°C). Amina and Avanti Green support significantly higher numbers of viable *T. harzianum* propagules than *T. virens* during storage. This was further observed when both biocontrol candidates were used as mixtures, the majority of the biocontrol population recovered was that of *T. harzianum* rather than *T. virens.*

Application of Biocontrol Candidates and Rating of Disease in the Sclerotium-chilli System

The biocontrol candidates parasitized the sclerotia of *S. rolfsii*, resulting in the failure of these sclerotia to germinate when plated on PDA (Jinantana and Sariah 1997). The numbers of viable sclerotia per 100 g air-dried soil sampled from the respective treatments were significantly lower ($P < 0.01$) than those taken from the control at 3 and 40 days after transplanting (Table 3). The lowest number of viable sclerotia was achieved by treatments of biocontrol candidates with Amina and highest when incorporated into Green Supergro at day 3, but all treatments were equally as good at day 40.

The incidence of *Sclerotium* foot rot on chilli plants was evaluated 20 days after transplanting. *T. virens*, either singly or incorporated together with the organic fertilizers, gave 100% control of the disease. However *T. harzianum* supplemented with Amina only gave 83.3% reduction in disease incidence (Table 3). The significant reduction in foot rot of chilli was due to the higher initial populations of the biocontrol candidates in the respective treatments and these populations were maintained throughout the experiments as shown by samplings made after 20 and 40 days after

TABLE 2

Populations of air-dried *T. harzianum, T. virens* and *T. harzianum + T. virens* culture incorporated into the organic fertilizers and stored at room temperature for 0-180 days

Biocontrol preparations	cfu per g air-dried preparation						
	0 Day	30 Days	60 Days	90 Days	120 Days	150 Days	180 Days
T. harzianum + Amina	$1.6x10^{14}$ a*	$10.7x10^{5}$ a	$4.2x10^{2}$ bc	$3.0x10^{2}$ a	$2.4x10^{2}$ a	$1.5x10^{2}$ b	$10.8x10^{1}$ a
T. virens + Amina	1.4x1014 a	$3.6x10^{5}$ c	$4.2x10^{2}$ bc	$0.9x10^{2}$ d	$0.1x10^{2}$ c	$0.1x10^{2}$ c	$0.9x10^{1}$ b
T. harzianum + *T. virens* + Amina	$1.0x10^{14}$ b	$3.1x10^{5}$ c	4.9x102 a	$1.5x10^{2}$ c	$1.4x10^{2}$ b	$0.1x10^{2}$ c	0.0 b
	Th $=0.6x10^{14}$	Th$=2.0x10^{5}$	Th$=2.5\ x10^{2}$	Th$=1.2x10^{2}$	Th$=1.3x10^{2}$	Th$=0.1x10^{2}$	
	Tv $=0.4x10^{14}$	Tv $=1.1x10^{5}$	Tv $=2.4x10^{2}$	Tv $=0.3x10^{2}$	Tv $=0.1x10^{2}$	Tv =0.0	
T. harzianum + Green Supergro	$0.2x10^{14}$ c	0.0 d	0.0 d	0.0 e	0.00 c	0.0 c	0.0 b
T. virens + Green Supergro	$0.1x10^{14}$ c	0.0 d	0.0 d	0.0 e	0.0 c	0.0 c	0.0 b
T. harzianum + *T. virens* + Green Supergro	$0.2x10^{14}$ c	0.0 d	0.0 d	0.0 e	0.00 c	0.0 c	0.0 b
	Th$=0.1x10^{14}$						
	Tv $=0.1x10^{14}$						
T. harzianum + Avanti Green	$1.6x10^{14}$ a	$8.4x10^{5}$ b	$4.0x10^{2}$ c	$2.4x10^{2}$ b	$2.3x10^{2}$ a	$2.2x10^{2}$ a	$8.8x10^{1}$ b
T. virens + Avanti Green	$1.5x10^{14}$ a	$2.9x10^{5}$ c	$4.0x10^{2}$ c	$0.6x10^{2}$ d	$0.1x10^{2}$ c	0.0 c	0.00 b
T. harzianum +*T. virens* + Avanti Green	$0.8x10^{14}$ b	$3.7x10^{5}$ c	$4.6x10^{2}$ ab	$1.5x10^{5}$ c	$0.1x10^{2}$ c	$0.1x10^{2}$ c	0.00 b
	Th$=0.5x10^{14}$	Th$=2.5x10^{5}$	Th$=2.3x10^{2}$	Th$=1.2x10^{2}$	Th$=0.1x10^{2}$	Th$=0.1x10^{2}$	
	Tv $=0.3x10^{14}$	Tv $=1.2x10^{5}$	Tv $=2.3x10^{2}$	Tv $=0.3x10^{2}$	Tv $=0.03x10^{2}$	Tv =0.0	

* Means in the same column with different letters are significantly different ($p<.01$) using DMRT

Th = *T. harzianum* Tv = *T. virens*

TABLE 3
Number of viable Sclerotia and percentage of surviving plants from the artificially-inoculated soil

Treatments	Number of viable *S. rolfsii* propagules per 100 g air-dried soil		% Surviving Plants
	3 Days	40 Days	20 Days
T. harzianum + Amina	5.0 c*	2.3 b	83.3 a
T. harzianum + Green Supergro	18.0 b	4.6 b	61.1 b
T. harzianum + Avanti Green	8.3 c	2.6 b	77.7 ab
T. harzianum	5.6 c	3.0 b	77.7 ab
T. virens + Amina	2.6 c	2.3 b	**100**
T. virens + Green Supergro	13.0 b	3.3 b	**100**
T. virens + Avanti Green	11.0 b	3.0 b	**100**
T. virens	11.0 b	3.0 b	**100**
T. harzianum + *T. virens* + Amina	5.6 c	2.3 b	**100**
T. harzianum + *T. virens* + Green Supergro			
T. harzianum + *T. virens*	3.3 c	1.6 b	**100**
T. harzianum + *T. virens*	3.3 c	1.6 b	**100**
Control			
Soil mixture alone	73.3 a	58.3 a	**0**
Amina	72.0 a	60.1 a	**0**
Green Supergro	70.8 a	59.4 a	**0**
Avanti Green	72.5 a	60.6 a	**0**

* Means in the same column of each group of dta with different letters are significantly different (p<0.1) using DMRT

transplanting and also on the root rhizosphere (Table 4) . Cfu of *T. harzianum* recovered from the soil was reduced whereas that of *T. virens* increased. Amina enhanced the survival and proliferating ability of *T. virens* in the chilli rhizosphere. The population of biocontrol isolates recovered from roots was higher than soil rhizospheres with respect to the different treatments analysed.

Field trial: T. harzianum and *T. virens* in all preparations with Amina as the carrier or singly, and Brassicol significantly reduced the population of sclerotia in the infested soil as compared to control (Table 5). The symptoms of *Sclerotium* foot rot were observed two months after transplanting. Symptoms appeared as wilting with the production of white rhizomorphs at the base of the stems. *T. virens*-organic carrier and *T. virens* alone gave highest percentages of surviving plants compared to *T. harzianum* or the control (Table 5).

The initial populations of the biocontrol candidates in the soil were assessed in the nursery bags one week after amendment, prior to transplanting and subsequently 1, 2 and 4 months after transplanting. It was found that there were significant differences (p<0.01) in the populations among the treatments (Table 6). Results showed that within one month after transplanting, populations of *T. virens* when applied singly had increased from the number found prior to transplanting to the infested field, while the population of *T. harzianum* decreased, suggesting that *T. virens* was sustainable in the *Sclerotium*-infested soil.

DISCUSSION

T. harzianum and *T. virens* isolates evaluated have been proven to effectively parasitize mycelium and sclerotia of *S. rolfsii* (Jinantana and Sariah 1993, 1997) under laboratory conditions, and their potential as biocontrol agents against *Sclerotium* foot rot on chilli has been clarified in this study. Connick *et al.* (1990) have stated that a successful biocontrol formulation is one that is economically produced, safe and stable in the environment, easily applied

TABLE 4
Populations of the biocontrol candidates in the artificially-inoculated soil

Treatments	cfu per g aor-dried soil (x10[4])		cfu per g air-drried root (x10[4])		
	In nursery bags	In soil around the chilli plants			on roots
	initial[1/]	on transplanting[2/]	20 Day[3/]	40 Days[3/]	at 40 Days[3/]
T. harzianum + Amina	7.8 a*	5.9 a	0.7 a	0.7 a	2.0 a
T. harzianum + Green Supergro	2.9 b	0.4 c	0.6 a	0.0 c	1.6 b
T. harzianum + Avanti Green	7.1 a	3.2 b	0.7 a	0.3 b	1.5 b
T. harzianum	4.3 b	0.9 c	0.7 a	0.3 b	1.5 b
T. virens + Amina	1.2 a	0.7 a	3.1 a	6.8 a	9.5 a
T. virens + Green supergro	0.3 c	0.5 a	1.6 b	1.4 b	8.9 b
T. virens + Avanti Green	0.8 b	0.6 a	1.9 b	5.8 a	1.3 d
T. virens	1.2 a	0.6 a	1.6 b	1.5 b	6.9 c
T. harzianum + *T. virens* + Amina	3.1 a	1.2 a	2.3 a	3.5 a	7.4 a
	Th=2.2	Th=0.8	Th=0.3	Th=0.1	Th=2.3
	Tv=0.9	Tv=0.4	Tv=2.0	Tv=3.4	Tv=5.1
T. harzianum + *T. virens* + Green Supergro	1.1 b	0.9 b	1.50 b	2.9 ab	6.2 b
	Th=0.6	Th=0.2	Th=0.2	Th=0.1	Th=1.8
	Tv=0.5	Tv=0.4	Tv=1.2	Tv=2.8	Tv=4.4
T. harzianum + *T. virens* + Avanti Green	1.2 b	0.9 b	1.5 b	2.4 b	1.5 d
	Th=0.8	Th=0.5	Th=0.4	Th=0.1	Th=0.9
	Tv=0.4	Tv=0.4	Tv=1.1	Tv=2.3	Tv=0.6
T. harzianum + *T. virens*	3.0 a	0.8 b	1.4 b	2.9 ab	4.0 c
	Th=2.1	Th=0.5	Th=0.2	Th=0.1	Th=1.7
	Tv=0.9	Tv=0.3	Tv=1.2	Tv=2.8	Tv=2.3

* Means in the same column of each group of data with different letters are significantly different (P<01) using DMRT
Th = *T. harzianum* Tv = *T. virens*
[1/] at 1 wk after amendment and prior to transplanting of the chilli into the bags
[2/] on the day the chilli plants were transferred to *S. rolfsii*-infected soil
[3/] days after transplanting the chilli to *S. rolfsii*-infested soil

using conventional agricultural equipment and gives effective and consistent results under a variety of environmental conditions. Three types of commercially available organic fertilizer (Amina, Green Supergro and Avanti Green) were tested as carriers for the biocontrol candidates. Results showed that Amina was superior to the other two organic fertilizers as the delivery system for the biocontrol candidates in the glasshouse. The recovery of biocontrol isolates incorporated into Amina around the plant rhizospheres and roots was high, and viability of *S. rolfsii* propagules in the soil was low, resulting in low incidence of *Sclerotium* foot rot. However, in the field experiment, addition of Amina did not significantly reduce viability of sclerotia compared to biocontrol alone, even though it improved the initial establishment of the biocontrol candidates in the *Sclerotium*-infested soil.

Differences in effectiveness of different organic fertilizers used as carriers may have resulted from the interaction between the biocontrol candidates and chemical and physical characteristics of the carriers (Table 1). The moisture and nutrient content of the carriers possibly affected the effectiveness of the preparations in controlling diseases. Watanabe

TABLE 5
Numbers of viable sclerotia and percentage of surviving chilli plants in the naturally-infested field

Treatments	Number of viable *S. rolfsii* pripagules per 100 g air-dried soil				% Surviving plants
	initial[1/]	1 month[2/]	2 months[2/]	4 months[2/]	4 months[2/]
T. harzianum + Amina	14	5.0 bc*	3.6 bc	2.6 cd	74.7 b
T. virens + Amina	13	3.0 c	2.6 c	1.6 d	92.1 a
T. harzianum + *T. virens* + Amina	15	6.6 b	5.3 b	3.6 bcd	81.4 ab
T. virens	16	7.0 b	5.3 b	4.0 bc	83.3 ab
T. harzianum	14	6.3 b	3.1 b	2.8 bc	73.7 ab
T. harzianum + *T. virens*	12	5.3 bc	5.3 bc	4.3 bc	75.0 b
Brassicol	13	8.0 b	5.6 b	5.3 b	69.9 bc
Control	17	16.0 a	14.3 a	13.7 a	58.3 c

* Means in the same column with different letters are significantly (P<0.1) using DMRT
[1/] 1 day prior to tranplanting of the chilli plants to the field
[2/] after transplanting of the chilli plants to the field

TABLE 6
Populations of the biocontrol candidates sampled from naturally-infested field

Treatment	cfu per g air-dried ($x10^3$)				
	In nursery bags		In soils around the chilli plants		
	initial[1/]	on transplanting[2/] day	1 month[3/]	2 months[3/]	4 months[3/]
T. harzianum + Amina	5.2 a*	3.6 a	1.3 cd	1.1 de	0.6 d
T. virens + Amina	4.9 a	3.7 a	4.6 a	3.1 a	2.3 a
T. harzianum + *T. virens*	3.7 b	2.5 b	2.6 bc	1.8 bc	1.3 bc
+ Amina	Th=2.3	Th=1.3	Th=0.4	Th=0.2	Th0.1
	Tv=1.4	Tv=1.2	Tv=2.2	Tv1.6	Tv=1.2
T. harzianum	4.8 a	2.7 b	0.9 d	0.7 c	0.3 d
T. virens	3.1 c	2.7 b	4.1 ab	2.3 b	1.9 ab
T. harzianum + *T. virens*	3.2 bc	2.2 b	2.0 cd	1.3 cd	0.9 cd
	Th=2.0	Th=1.3	Th=0.5	Th=0.2	Th=0.1
	Tv=1.2	Tv=0.9	Tv=1.5	Tv=1.1	Tv=0.8

Means in the same colimn with different letters are significantly different (P<01) using DMRT
Th = *T. harzianum* Tv = *virens*
[1/] at 1 wk after amendment and prior to transplanting of the chilli into the bags
[2/] on the day the chilli plants were transferred to *S. rolfsii*-infested soil
[3/] months after transplanting the chilli to *S. rolfsii*-infected soil

et al. (1987) found that nitrogen fertilizers stimulated growth and production of conidia of *Trichoderma* in culture and may have a synergistic effect on biocontrol ability. Soil condition is another factor that could affect the availability of nutrients dissolved from organic fertilizers, thus influencing the proliferation and activity of biocontrol candidates. The efficiency of biocontrol agents have been related to rhizosphere competence (Ahmad and Baker 1987a, b). The survival of *Trichoderma* in soils was studied by Papavizas (1981) who concluded that the antagonist did not survive well in the rhizosphere. However, survival of the indigenous *T. harzianum* and *T. virens* isolates in the chilli rhizosphere was significantly improved by incorporating into organic fertilizer in the delivery system for early establishment although effect was diluted with time.

The application of the biocontrol candidates to control soil-borne diseases was mostly through soil amendments in which high quantities of the preparations were needed. Seed treatment and application of the antagonist to rooting or seedling mixtures are alternative methods which could improve establishment and colonization ability in the plant rhizosphere. This will enhance the effectiveness of disease control with the added advantage of using small volumes of biocontrol preparations. Elad *et al.* (1981) applied *T. harzianum* to rooting mixture for carnation and found that disease incidence due to *Rhizoctonia solani* was decreased when the carnations with the whole rooting mixture were transplanted to infested soil. Chilli seedlings are normally prepared in the nursery before being transferred to the field, thus application of the biocontrol formulation to soil mixture in the nursery bags would be the best option. It was found in the present study that this approach provided effective control of *Sclerotium* foot rot of chilli plants in the artificially –inoculated and naturally-infested soils.

Even though *T. harzianum* and *T. virens* possessed no inhibitory effect on each other when used in combinations (Jinantana and Sariah 1993; 1997), the effectiveness of the preparation was mainly due to the activity of *T. virens*. This was confirmed by the results of the recovery (cfu) of *T. virens* from soils with time of application. Although *T. harzianum* survived better in storage, its proliferation in the plant rhizosphere was poor, thereby making *T. virens* a better candidate for the biocontrol of *Sclerotium* foot rot. In addition, biomass of *T. harzianum* and *T. virens* used for incorporation into the carrier in the present study comprised mainly of conidia and mycelia. It has been reported that chlamydospores were more important than conidia in survival and proliferation of the biocontrol candidates (Lewis and Papavizas 1984; Beagle-Ristaino and Papavizas 1985 a, b). Therefore, using biomass comprising abundant chlamydospores of *T. harzianum* and *T. virens* incorporated into Amina may enhance further the effectiveness of the biocontrol candidates in controlling *Sclerotium* foot rot of chilli in Malaysia.

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The Behaviour of Boron Compounds in Treated Rattan when Dehydrated at High Temperatures

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ABSTRAK

Sifat kompaun boron dan kompaun boron di dalam batang rotan manau (Calamus manan Miq.) yang telah dirawat dan diikuti dengan pengeringan pada suhu tinggi diselidiki. Apabila boraks dan asid borik dicampurkan terjadi natrium 1-5 borat. Selepas pengeringan pada suhu 100±3, 50±1, atau < 0°C, peratus berat hilang dari campuran menurut pengukuran dan perkiraan (seperti dalam kurungan) adalah masing-masing 40.34 (40.10), 39.31 (36.39) dan 29.43% (30.11%). Nilai penurunan yang diukur melalui percubaan digunakan untuk menganggarkan bahan larut yang terkandung di dalam sampel rotan yang telah dirawat menggunakan campuran asid borik dan boraks dan dikeringkan. Penurunan berat campuran yang berlebihan di.dalam rotan selepas dikeringkan pada suhu tinggi berkemungkinan diakibatkan oleh 1. peruapan komponen rotan yang berat molekulnya rendah 2. peruapan asid borik di dalam wap air semasa pengeringan dan 3. Kehilangan cecair pengawet secara mekanikal di awal peringkat pengeringan. Untuk menjamin penahanan persamaan asid borik (BAE) yang optimum, rawatan rotan atau bahan berselulosa lain yang melibatkan asid borik dalam larutan berair, pengeringan menggunakan suhu tinggi perlu dihindari

ABSTRACT

The behaviour of boron compounds per se and boron compounds in treated stem of rotan manau (Calamus manan Miq.) dried at elevated temperatures wasstudied. When borax and boric acid are mixed, sodium 1:5 borate is formed. After drying at 100±30, 50±10, or <0°C, the measured and the calculated (in parentheses) percentage weight losses from the mixtures were 40.34 (40.10), 39.31 (36.39) and 29.43% (30.11%), respectively. The experimentally measured values were used to estimate the amount of solute retained in the rattan samples which had been treated with such a boric acid-borax mixture and then dried. The further weight losses of the mixture in treated rattan dried at higher temperatures are presumably attributable to: 1. the volatilization of low molecular weight rattan components; 2. the volatilization of boric acid in water vapour during drying; and 3. the mechanical loss of permeating liquid during the early stage of drying. Treatments of rattan or other cellulosic material which involve boric acid in aqueous solution should avoid high temperature drying in order to ensure optimum retention of boric acid equivalent (BAE).

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INTRODUCTION

Rattans can be treated with boron compounds to enhance their durability against pests. Zaidon (1995) reported that by using vacuum-pressure, thermal and diffusion processes boron compounds can be successfully impregnated into the rattan stems. A standard retention requirement of boric acid equivalent (BAE, % w/w) and a complete penetration of BAE were achieved in the rattans when treated by these processes. According to Zaidon (1995), a significant reduction of BAE in the treated rattan was noted when the materials were dried at elevated temperatures following treatments. This decrement ranged from 10.93% for *rotan jelayang* (*Calamus peregrinus* Furtado) to 23.37% for *rotan manau* (*Calamus manan* Miq.) after they were dried at ca.100°C overnight. It is believed that the reduction of BAE in the treated rattans is probably through the loss of boric acid in the steam during drying.

Kininmonth (1963) also reported that during kiln drying at high temperature, boron compounds can be lost from the treated wood as a consequence of steam volatilization. Parkes (1967) stated that boron compounds, for instance orthoboric acid are appreciably volatile in steam. To understand the behaviour of the boron compounds in treated rattan dried at high temperatures, it was thought advisable to investigate the behaviour of the boron compound itself when subjected to dehydration at similar temperatures.

This paper reports the behaviour of boron compounds in treated rattan stems dried at high temperatures.

MATERIALS AND METHODS

Preparation of Materials

Three different boron solutions were prepared for this study: 5% (w/w) aqueous solutions of orthoboric acid (H_3BO_3, molecular weight (MW) 247.32 g), and of sodium tetraborate decahydrate ($Na_2B_4O_7.10H_2O$, MW 381.36 g), and a solution of boric acid (2.5 g) and borax (3.85 g) in water (100 ml), i.e. 6.35% (w/v) solute. The mixture of boric acid and borax was equivalent to 5% (w/v) boric acid equivalent (BAE) since

$4H_3BO_3$	=	$Na_2B_4O_7.10H_2O$
i.e. (247.32 g)	=	(381.36 g)
or (2.5 g)	=	(3.85 g) molar equivalent.

The concentration of the mixture was converted from w/v to w/w by determining its density. The weight of 10 ml of the solution was 10.22 g, giving a density of 1.022 g/ml. The concentration of solute in the treatment solution on a w/w basis was (6.35/102.2) 6.21% solute, i.e. 2.44 g boric acid and 3.77 g borax in 100 g solution, corresponding to (5.0/102.2) 4.89% BAE.

Approximately 2 - 4 g of each of the solutions was placed in a preweighed flask (25 ml). A small funnel was used to cover the mouth of the flask to prevent any loss of solution by splashing at high temperature. Each of the solutions was evaporated for one day at 100±3°C, or for three days at 50±1°C in a conventional drying oven, or for one day at < 0°C in a freeze-dryer at 0.1 mm Hg. The calculated weights of solute from the aqueous solution before drying were compared with the measured weights of solute after drying. The concentration of the BAE in the solute after the drying processes was measured by spectrophotometric measurement of the rosocyanin complex formed between boric acid and curcumin (after Williams 1968).

Preparation of Samples

The material used in this study was mature stem of Calamus manan Miq. (*rotan manau*). Small samples of approximately 3 cm diam. x 3 cm long were cut from the internode section of the rattan stem. The samples were then oven-dried at 100±3°C to constant weight. The oven-dried samples were then treated with an aqueous solution of boric acid (2.44 g) and borax (3.77 g) in 100 g solution by a vacuum-pressure process. The rattan samples were placed in a glass vessel which was evacuated to 0.1 mm Hg for 10 min. The boron solution was introduced into the vessel and the samples fully immersed. The pressure was allowed to rise to atmospheric and the samples were kept in the liquid until no more solution was absorbed; this took approximately 6 h. The solution was then drained out of the vessel and the vessel with the treated samples was again evacuated for 10 min to remove surface liquid. The samples were then removed, wiped free of solution, and weighed.

The weights of the samples before and after treatment were used to calculate the weights of solute and of BAE contained in them. For ease of comparison, the amounts of solute and the

BAE retention were calculated as percentages of the oven-dried weight of the rattan. The initial BAE retention and the weight of retained solute were calculated using the following equations:

$$\text{BAE }(\%) = [(\text{Wat-Wo}) \times \text{conc. (w/w) of BAE} \times 100] / \text{Wo} \quad [1]$$

$$\text{Solute }(\%) = [(\text{Wat-Wo}) \times \text{conc. (w/w) of solute} \times 100] / \text{Wo} \quad [2]$$

where Wat is the weight of the treated sample after final vacuum treatment and Wo is the od-weight of the sample.

The treated samples were dried for one day at 100±3°C, or for three days at 50±1°C, or for one day at < 0°C in a freeze-dryer. After being dried the samples were again weighed (Wff), and the weight of retained solute was calculated:

$$\text{Retained solute (\%, w/w)} = [(\text{Wff - Wo}) \times 100] / \text{Wo}] \quad [3]$$

All the dried treated samples were analysed for the retention of BAE (% w/w) using UV-spectrophotometer (after Williams 1968).

Analysis of Boric Acid Equivalent (BAE)

The dried material was then ground separately into sawdust (40 mesh) using a Wiley mill. The boron-containing compounds were leached from the sawdust using aqueous sodium hydroxide solution. The weighed sawdust (0.1 g) was transferred into a boron-free soda lime boiling tube, after which 5 M sodium hydroxide solution (10 ml) and water (20 ml) were added. The boiling tube and its contents were heated in a water-bath at 65°C for 30 min with occasional swirling to ensure thorough mixing of the contents. The boiling tube was then removed from the water bath, water (15 ml) added and the tube swirled to mix the contents. It was then cooled to room temperature. The mixture from the boiling tube was transferred to a 50-ml graduated flask and diluted to 50 ml with water. The flask was shaken several times at 5-min intervals before being allowed to settle for 10 min.

A 0.5 ml aliquot of the leachate solution was transferred by pipette to a clean soda lime boiling tube. Three ml of curcumin solution, made by dissolving curcumin (0.12 g) in glacial acetic acid (100 ml), was added using a burette, followed by gentle swirling of the tube to mix the contents. After being allowed to stand for 5 min, a mixture of concentrated sulphuric acid and acetic acid (1:1, v/v; 3 ml) was added from a burette, the mixture shaken and allowed to stand in a water-bath at 30°C for 30 min. With the aid of a filter funnel, the mixture was then slowly poured with swirling into a 100-ml graduated flask containing acetone-water solution (1:1 v/v; 70 ml). Any of the mixture remaining in the boiling tube was washed into the graduated flask with acetone-water solution, the mixture diluted to 100 ml with the solution, and thoroughly shaken.

Optical density (absorbance) of the rosocyanin complex was measured against a reagent blank (prepared in a similar way with the omission of the sawdust) in 1-cm cells at 555 nm on a CECIL 6000 series UV spectropho-tometer (Cambridge, England). The calibration curve was produced by plotting the boric acid mass of standard solutions optical density (*Fig. 1*). The fitted calibration curve is represented by the equation below:

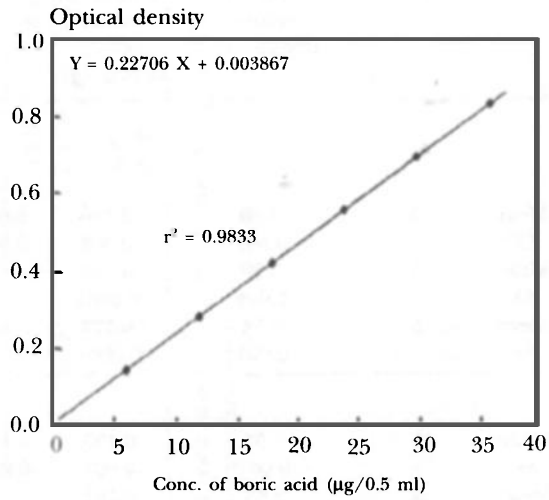

Fig. 1. Calibrationcurve for boric acid obtained form spectrophotometric measurements of the rosocyanin complex at 555 nm

$$Y = 0.003867 + 0.227056\,X \quad (r^2 = 0.983) \quad [4]$$

where Y is optical density measured for X μg of BAE in 0.5 ml of solution.

Having obtained the amount of BAE (μg/0.5 ml solution) from the calibration graph, the concentration of boric acid in the original sawdust from each layer can be calculated using the equation below (Suhaimi 1989):

$$\text{BAE}\ (\%) = \frac{\text{conc. of } H_3BO_3\ (\mu g/0.5\ ml) \times 100}{\text{oven-dry weight of rattan sawdust}} \times 100 \qquad [5]$$

where 100 is the dilution factor.

RESULTS AND DISCUSSION

The Nature of Boron Behaviour

The weights of the boron compounds before and after the different types of drying processes are presented in Table 1, whilst the weights and concentrations of the boric acid-borax solution expressed as BAE before and after the drying processes are shown in Table 2.

From Table 1, the boric acid solution lost 50.0% (SE, 0.002%) of the solute when dried at 100±3°C, 4.74% (SE, 0.0001%) at 50±10C, and almost none when freeze-dried at < 0°C. Orthoboric acid itself when heated at ca. 100°C forms metaboric acid $(HOBO)_3$, usually referred to as "HBO_2" (Parkes 1967).

$$\underset{(61.83\ g)}{H_3BO_3} \xrightarrow{100^{\circ}C} \underset{(43.83\ g)}{HBO_2} + \underset{(18.016}{H_2O} \qquad [6]$$

The loss of one mole of water is equivalent to 29.11% of the original weight of boric acid. The additional loss of 20.89% at 100±3°C may be attributed to the steam volatilization of some of the orthoboric acid. Solid boric acid heated under the same condition lost 31.91% of its

TABLE 1
Weights of boron compounds before and after evaporation of aqueous solutions at different temperatures

	No. of samples	Weight of solution before drying (g)	Calculated weight of solute before drying (g)	Weight of solute after drying (g) and (% loss in weight)					
				At 100°C		At 50°C		At < 0°C	
				Boric acid solution (5% w/w)					
Mean	5	3.60	0.180	0.090	(50.00)	-	-	-	-
SE[1]		0.001	0.002	0.002					
Mean	5	3.80	0.190	-	-	0.181	(4.74)	-	-
SE		0.002	0.0001			0.0001			
Mean	5	3.54	0.177	-	-	-	-	0.176	(0.56)
SE		0.001	0.0001					0.0001	
				Borax solution (5% w/w					
Mean	5	3.56	0.175	0.112	(36.00)	-	-	-	-
SE		0.001	0.001	0.001					
Mean	5	3.62	0.181	-	-	0.116	(35.91)	-	-
SE		0.002	0.0001			0.0001			
Mean	5	3.16	0.158	-	-	-	-	0.151	(7.59)
SE		0.011	0.0001					0.0001	
				Boric acid-borax solution (6.21 % w/w)[2]					
Mean	5	2.38	0.119	0.071	(40.34)	-	-	-	-
SE		0.002	0.001	0.002					
Mean	5	3.10	0.155	-	-	0.093	(39.31)	-	-
SE		0.003	0.0001			0.001			
Mean	5	3.44	0.214	-	-	-	-	0.151	(29.43)
SE		0.012	0.0001					0.0001	

• [1] SE, standard error
• [2] The solution contained boric acid (2.44 g) and borax (3.77 g) in 100 gm solution

TABLE 2
Concentrations (% w/w) and weights (g) of boric acid equivalent (BAE) in an aqueous solution of borax-boric acid before and after evaporation at different temperatures

No. of samples	Initial concentration	Weight of solution before drying	Calculated weight of BAE before drying1	After drying at 100°C, measured by chemical analysis		After drying at 50°C measured by chemical analysis		
				Conc. of BAE	Weight of BAE	Conc. of BAE	Weight of BAE	% loss in weight
	(% w/w)	g	g	(% w/w)	g	(% w/w)	g	
3	4.89	5.07 0.021	0.248 (0.001)	4.04 (0.295)	0.205 (0.001)	-	-	17.30 (0.035)
3	4.85	5.11 0.018	0.250 (0.001)	-	-	4.40 (0.210)	0.225 (0.003)	10.00 (0.041)

- [1]The solution contained boric acid (2.44 g) and borax (3.77 g) in 100 g solution
- Values in parentheses are standard errors

original weight, i.e. only slightly more than the theoretical amount. In this case, of course, a much smaller quantity of water is involved.

After drying at either 100±3°C or 50±1°C the borax ($Na_2B_4O_7.10H_2O$) solutions had lost 36% of the original weight of borax. Borax itself contains 10 molecules of water of crystallization and when heated at 88°C loses 8 of these; this is equivalent to a weight-loss of 37.79% (Menzel *et al.* 1935):

$$\underset{(381.38\ g)}{Na_2B_4O_7.10H_2O} \xrightarrow{88°C} \underset{(237.25\ g)}{Na_2B_4O_7.2H_2O} + \underset{(144.13)}{8H_2O} \quad [7]$$

The loss of a similar amount of water after drying at 50±10C is clearly a consequence of the much longer drying time employed. When the solution was evaporated in a freeze-dryer at < 0°C, the weight loss was only 7.59% which corresponds approximately to the loss of 1.5 molecules of water (theoretically 7.09%).

The Reaction of Borax with Boric Acid

When solid borax and boric acid are mixed a reaction occurs and water is liberated with the formation of sodium decaborate decahydrate ($Na_2O.5B_2O_3.10H_2O$) (Kemp 1956). Due to the ratio of Na_2O:B_2O_3 the latter is conveniently referred to as sodium 1:5 borate.

$$6H_3BO_3 + Na_2B_4O_7.10H_2O \longrightarrow Na_2O.5B_2O_3.10H_2O + 9H_2O \quad [8]$$

Sodium 1:5-borate crystals rapidly lose six molecules of water at ambient temperature and then a further two, more slowly, at 80°C. One molecule of water remains at 200° and is lost only at higher temperatures (Atterberg 1906).

$$Na_2O.5B_2O_3.10H_2O \xrightarrow{20°C} Na_2O.5B_2O_3.4H_2O + 6H_2O \quad [9]$$

$$\xrightarrow{80°C} Na_2O.5B_2O_3.2H_2O + 2H_2O \quad [10]$$

$$\xrightarrow{200°C} Na_2O.5B_2O_3.H_2O + H_2O \quad [11]$$

From Equations 8, 9 and 10, it is apparent that the number of molecules of water lost after heating a suitable mixture of boric acid and borax at 50±1°C will be 15 and at 100±3°C will be 17.

Weight Loss of Boric Acid-Borax Mixture after Drying at 100±3°C

The amount of water lost from a mixture of 3.77 g borax and 2.44 g boric acid can be calculated as follows. From Equation 8,

$$\underset{370.98\ g)}{4H_3BO_3} = \underset{(381.38\ g)}{Na_2B_4O_7.10H_2O}$$

Therefore 2.44 g of H_3BO_3 = (2.44/370.98) 3 381.38 = 2.51 g of borax. This weight was used in the formation of sodium 1:5-borate. The original weight of borax was 3.77 g, and it follows that 1.26 g of borax remained after the formation of the sodium 1:5-borate.

From Equations 8, 9 and 10, six moles of boric acid (370.98 g) react with 1 mole of borax (381.38 g) with the loss of 17 moles of water (306.27 g). Hence 2.44 g of boric acid corresponds to the loss of [(306.27/370.98) x 2.44 g]= 2.01 g of water. When a borax solution is evaporated at 100±30C the loss of weight is 37.79%, corresponding to the loss of 8 molecules of water. As the excess of borax over that used for reaction with boric acid was 1.26 g the loss of water from this was [(144.13/381.38) x 1.26 g] = 0.48 g. Thus the total weight of water lost from the mixture after drying at 100±30C was 2.49 g. As the total weight of solute used was 6.21 g, the calculated loss of weight is 40.10%. This is in excellent agreement with the value (40.34%) found by experiment (Table 1).

Weight Loss of Boric Acid-Borax Mixture after Drying at 50±1°C

If 15 molecules of water are lost from the boric acid-borax mixture (Equations 8 and 9) after drying to constant weight at 50±1°C, the weight loss from 2.44 g of boric acid is 1.78 g. When dried for three days at 50±1°C, borax loses 8 molecules of water (Table 1). Hence the loss of water from the excess of borax over that used in reacting with the boric acid, namely 1.26 g, is 0.48 g. So the calculated total loss of water from the mixture is 2.26 g i.e. 36.39%. This value is comparable with the value (39.31%) found experimentally (Table 1).

Weight Loss of Boric Acid-Borax Mixture after Freeze-drying at < 0°C

Nine molecules of water are liberated during the formation of sodium 1:5-borate and another six molecules are lost during freeze-drying (Equations 8 and 9). Consequently the weight of water corresponding to 2.44 g boric acid is again 1.78 g. Under those conditions borax lost about 1.5 molecules of water (observed, 7.59%,

TABLE 3
Mean retention of solute in rotan manau treated with a mixture of borax and borax and boric acid dried at different temperatures

Drying conditions	No. of samples	Od weight of samples	Weight of solution absorbed	Calculted weight of solute in sample[1]		Weight of retained solute after drying				Weight loss of solute
						Estimated[2]		Measured[3]		
			g	g	%w/w[3]	g	%w/w	g	%w/w	g
At 100°C										
Mean	5	6.87	6.95	0.432	6.29	0.258	3.75	0.180	2.62	0.078
SE		0.514	0.497	0.031	0.139	0.018	0.113	0.012	0.096	0.008
At 50°C										
Mean	5	5.24	5.30	0.329	6.29	0.200	3.81	0.183	3.27	0.017
SE		0.418	0.387	0.024	0.097	0.015	0.062	0.019	0.170	0.006
At < 0°C	5	3.55	3.59	0.223	4.84	0.157	4.53	0.150	4.34	0.007
Mean		0.475	0.312	0.019	0.078	0.014	0.063	0.010	0.253	0.005

- [1]The treatment solution contained boric acid (2.44 g) and borax (3.77 g) in (100 g) solution, (6.21% solute w/w)
- [2]Estimation values based on the loss of solute after drying, 40.34% at 100°C, 39.31% at 50°C and 29.43% at <0°C (Table 1).
- [3]Measured retentions as percentages of the od-weights of rattan
- SE, standard error

calculated, 7.09%) during the drying process. The calculated loss of water from the excess of borax was [(27.02/381.38) x 1.26 g] i.e. 0.089 g. Hence the total loss of weight on freeze-drying the boric acid-borax mixture should be 1.87 g i.e. 30.11%. This is in excellent agreement with the value (29.43%) found experimentally (Table 1).

Loss of BAE Concentration from the Mixture of Boric Acid-Borax after Drying at Higher Temperatures

From Table 2, it can be seen that the concentrations of BAE (w/w) in the solution of boric acid-borax mixture after drying at ca. 100°C and 50°C were reduced respectively by 17.3% to 4.04% and by 10.0% to 4.40% from its original concentration, 4.89%. The reduction of the BAE concentration appears to be mainly due to the loss of some of the boric acid in water vapour during the drying. The volatilization of boric acid in the steam produced during the evaporation of an aqueous solution at high temperature (*ca.* 100°C) has been described earlier; some 20.89% was lost in this way.

The results of weight loss of solute obtained by experiment and by calculation are in good agreement and show that when borax and boric acid are mixed, sodium 1:5 - borate ($Na_2O.5B_2O_3.10H_2O$ i.e. $Na_2B_{10}O_{16}.10H_2O$) is formed. After drying at 100±3, at 50±1 or < 0°C, the measured and the calculated (in parentheses) percentage weight losses from the mixtures were 40.34 (40.10), 39.31 (36.39) and 29.43% (30.11%), respectively. These measured values will be used to estimate the amount of solute retained in the rattan samples treated with such a boric acid-borax mixture and then dried. The percentage loss of H_3BO_3 (% w/w) from the boric acid-borax mixture in the steam volatilization will be used to estimate the amount of BAE in the same samples, i.e. 17.3% after drying at 100°C and 10.0% at 50°C. Preliminary experiment showed that there was no loss of BAE in treated rattan after drying at < 0°C in a freeze-dryer.

The Behaviour of Boron Compounds in Rattan when Dehydrated at Different Temperatures

The mean calculated and the measured values for the retention of the solute after the different drying processes are summarized in Table 3, while the mean retention, expressed as the BAE values, is presented in Table 4. The values under the headings '% w/w' in the Tables are the percentage retentions of the oven-dried weights of the rattan samples. The mean measured weight of the solute (0.180 g) for the treated samples dried at *ca.* 100°C was significantly lower than the mean estimated weight (0.258 g) (Table 3). The difference (30.23%) was too big to be attributed to experimental error. The same was also observed for the BAE values. The mean measured retention BAE value (0.248 g) was 11.74% lower than the mean estimated value (0.281 g) (Table 4). For samples which had been dried at *ca.* 50°C (Table 3), the mean measured solute retention (0.183 g) was 8.50% lower than the mean estimated retention (0.200 g). Under these drying conditions, the measured weight of the BAE (0.210 g) in the treated samples was lower by 7.59% than the estimated value (0.227 g) (Table 4).

Results in Table 3 show that the mean measured weight of the solute (0.150 g) in the samples which had been freeze-dried was slightly lower than the calculated weight (0.157 g). The BAE values (Table 4) in the material before and after freeze-drying were similar.

Several factors could be responsible for the weight loss of the solute and the decrease in the BAE in the treated rattan samples which had been dried at the higher temperatures. These include:

1. the loss of steam-volatile organic compounds,
2. the loss of steam-volatile boric acid, and
3. the loss of some of the permeating liquid during the early stage of drying.

Loss of lower molecular weight components of the rattan may occur by steam volatilization. Many organic compounds which are not themselves appreciably volatile at 100°C are readily volatile in steam (Ault 1968). It seems likely that the drying of the rattan samples which had initially been saturated with water would be accompanied by the loss of some lower molecular weight components. To test this idea six small pieces of rattan which had been dried previously to constant weight at 100°C were saturated with water and then again dried at 100±3°C. All the samples showed a decrease in weight (an average of 0.307% of the original od weight). As the average oven-dried weight of the samples used is 6.87 g (Table 3), the loss of steam volatile components would be 0.021 g. This, however, is

TABLE 4
Mean retention of boric acid equivalent (BAE) in rotan manu treated with a mixture of borax and boric acid and dried at different temperatures

Drying temperatures	No. of samples	Od-weight of sample	Before drying: Weight of solution absorbed	Before drying: Calculated weight of BAE in sample[1]		Weight of retained BAE after drying: Estimated[2]		Weight of retained BAE after drying: Measured by chemical analysis[3]		Weight loss of BAE
	g	g	g	g	% w/w[3]	g	% w/w	g	% w/w	g
At 100°C										
Mean	4	6.87	6.95	0.340	4.94	0.281	4.09	0.248	3.61	0.043
SE		0.514	0.497	0.024	0.152	0.021	0.170	0.012	0.152	0.025
At 50°C										
Mean	4	5.24	5.30	0.253	4.84	0.227	3.41	0.210	4.01	0.017
SE		0.418	0.387	0.019	0.078	0.019	0.117	0.022	0.117	0.010
At < 0°C										
Mean	4	3.55	3.59	0.176	4.99	-	-	0.175	4.95	-
SE		0.312	0.312	0.023	0.077			0.016	0.078	

- [1] The treatment solution is equivalent to 4.89% (w/w) BAE
- [2] Estimation values based on the loss of H_3BO_3, 17.30 at 100°C, 10.0% 50°C (Table 1)
- [3] Measured retentions as percentages of the od-weights of rattan
- SE, standard error

still not enough to account for the average observed weight losses (0.258 - 0.180 g) i.e. 0.078 g (Table 3) in the treated samples.

The volatilization of boric acid in the steam produced during the evaporation of an aqueous solution at 100±30C has been described earlier; some 20.89% was lost in this way. From Table 3 it can be seen that an average 0.078 g of boric acid was lost when rattan samples containing an average 0.432 g of boric acid and borax were dried at ca. 100°C. The weights of boric acid and borax in the original solution (100 g) were 2.44 and 3.77 g, respectively, and it follows that the average weight of boric acid in the rattan samples initially was [(0.432 x 2.44)/6.21] = 0.169 g while the weight of borax was [(0.432 x 3.77)/ 6.21] = 0.262 g.

Since 0.078 g of the boric acid would be lost in the drying process, that remaining is 0.091 g. As shown earlier (Equation 8) this weight would react with 0.094 g of borax with the formation of sodium 1:5 borate. As the original weight of borax present was 0.262 g, it follows that 0.169 g remained after the formation of the sodium 1:5 borate.

From Equations 8, 9, and 10, the loss of water corresponding to 0.091 g boric acid is 0.075 g. The loss of water from partial dehydration of the excess of borax present, namely 0.169 g, is 0.064 g. So the calculated loss of weight resulting from dehydration and volatilization of boric acid is 0.075 + 0.064 + 0.078 = 0.217 g. To this must be added the weight of the steam volatile components, 0.021 g. The total loss of weight of the treated rattan samples is therefore 0.238 g and the average weight of solute remaining will be (0.432 - 0.238), i.e. 0.194 g. This is somewhat higher than the measured value (0.180 g) (Table 3) and an explanation of this discrepancy is needed.

The further loss in weight (0.194 -0.180) i.e. 0.014 g could be a result of loss of the permeating liquid from the sample during the early stage of drying. The imbalance of temperature in the samples with the oven condition might have caused the air in the samples to expand, which would force the liquid out of the samples through the porous end surfaces. The loss of permeating liquid in this way could have been minimized if the samples had been end-coated before drying. This precaution was not taken in this particular experiment.

Similar calculations to those detailed above show that loss of solute also occurred from the samples which were dried at ca. 50°C for a longer period of time (Table 3). The estimated weight of solute after drying was 0.20 g, which was slightly higher than the measured value (0.183 g). The calculation was made assuming that under the milder drying conditions there had been no loss of steam-volatile components from the rattan. The further loss of weight of solute under these conditions would be due mainly to the loss of some boric acid in the water vapour during the drying process together with a little loss of the permeating liquid.

The decrease in the BAE of treated rattan (Table 4) which had been dried at *ca.* 100°C and or 50°C appears to be due mainly to the loss of some boric acid in the water vapour. The percentage loss of BAE from the mixture of boric acid-borax after drying at *ca.* 100°C (17.3%) found in this study (Table 2) seems to be comparable with the loss of BAE calculated for rattans which were treated by the hot and cold-bath process (Zaidon 1995), i.e. between 11-23%, but a higher loss of BAE was found in treated ***rotan manau*** in the present study; i.e. 0.033 g or 11.74% higher (Table 4). The significant difference in the loss of the BAE between these two treated rattans is probably due to the process of drying the samples. The hot and cold-bath treated samples were initially dried at a milder temperature (50°C) for approximately 3 days before being dried at a higher temperature (100°C). In contrast, the vacuum-pressure treated samples in the present study were dried at 100°C following treatment; the loss of permeating liquid from the porous end surfaces on being heated is greater, thus resulting in further loss of boric acid.

CONCLUSION

When borax and boric are mixed, sodium 1:5-borate is formed. After drying at 100±3, 50±1, or at < 0°C, the measured percentage weight losses from the mixtures were 40.34, 39.31 and 29.43%, respectively. The further weight losses of the mixture in treated rattan which had been dried at higher temperatures are presumably attributable to: (1) the volatilization of low molecular weight rattan components, (2) the volatilization of boric acid in water vapour during drying and (3) the mechanical loss of permeating liquid during the early stage of drying.

The decrease in the BAE of the treated rattan which had been dried at ca. 100°C, or at 50°C for a longer time appears to be due mainly to the loss of boric acid in the water vapour. The measured BAE (% w/w) values in treated rattan in the previous experiments would have been *ca.* 17% higher if a lower temperature had been used in the drying process.

Freeze-drying of rattan which has been treated with a mixture of boric acid-borax or with boric acid itself is essential if all the boric acid is to be retained. On a commercial scale, treatments of rattan or wood which involve boric acid in an aqueous solution should avoid high temperature drying to ensure optimum retention of BAE.

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Plantain Squirrel *Callosciurus notatus* in a Plantation Habitat

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ABSTRAK

Corak aktiviti harian atau aktogram Callosciurus notatus *dalam ladang kelapa sawit dan koko di bawah kelapa ditentukan dengan menggunakan radiotelemetri . Hasil menunjukkan* C. notatus *mempamerkan corak aktiviti harian dari jam 0630 hingga 1730 dengan beberapa puncak aktiviti. Dua puncak utama dikenal pasti iaitu pada awal pagi (jam 0730) dan sebelum tengah hari (jam 1130). Ini diikuti dengan fasa rehat yang relatif pada waktu tengah hari (1430 hingga 1630). puncak yang lebih kecil dicerap pada waktu petang (jam 1730). Kepelbagaian mungkin wujud kerana satu individu menunjukkan empat puncak yang diselang seli dengan beberapa fasa rehat yang sengkat. Puncak aktiviti yang kerap mungkin menggambarkan strategi penggelintaran untuk memenuhi keperluan makanan yang tertentu. Kajian isi perut menunjukkan serangga merupakan mekanan utama pada awal pagi tetapi menunjukkan makanan yang pelbagai meliputi serangga dan bahan-bahan tumbuhan selepasnya.*

ABSTRACT

The daily activity pattern or actogram of four Callosciurus notatus *in a mixed plantation crop of oil palm and cacao under coconut in Peninsular Malaysia was determined by using radiotelemetry. Results suggest that* C. notatus *exhibits a daily activity pattern from 0630 to 1730 with several peaks. Two major peaks are identified, one at daybreak (about 0730) and another before midday (about 1130). The two peaks were followed by a relative cessation of activity in the afternoon (1430 - 1630). a third smaller peak was recorded in the evening (1730). Individual variation exists as one individual showed four small peaks interspersed by short periods of rest. The multiple peaks may reflect the foraging strategy in meeting specific dietary needs. A survey of stomach contents suggests that insects constitute the preferred item in the first couple of hours and a mixed diet of insects and plant matter later during the day.*

INTRODUCTION

Rats (*Rattus spp*). and the plantain squirrel (*Callosciurus notatus*) are important vertebrate pests of many plantation crops in Malaysia (for pests of cacao, see Kamarudin and Lee 1981; Hafidzi 1992). To gain a better understanding of the foraging behaviour of plantain squirrels, the present study investigated their activity pattern. Such a study may contribute to more effective control programmes.

Several techniques have been used in studying the activity pattern of small mammals. Among these are unaided sighting (Shorten 1962), time-scheduled trapping (Kamarudin 1982) and radio telemetry (Tonkin 1983). While unaided sighting causes the least disturbance, it may not be suitable for less conspicuous animals like especially in a habitat characterized by dense foliage. Live-trapping techniques are suitable for studying population dynamics but will reveal little of the ranging behaviour as animals are immobilized at points of capture. Radiotelemetry was employed in this study as one clear advantage of this technique is that it allows continuous monitoring of animals with minimal disruption of their activities in their natural environment.

MATERIALS AND METHODS

Radio-tracking Equipment

Basic radio-tracking equipment was used; consisting of radio collars, a portable radio receiver (Model M57, Mariner Radio, UK) and a three-element Yagi antenna. Each radio-collar was made up of a SS-1 transmitter (Biotrack UK Ltd.), a 1.5-V battery and an external antenna mounted on a cable tie collar with self-locking ratchet. The transmitter and battery were both coated in epoxy for protection against gnawing and water-proofing. The whole package weighed approximately 10 g. All animals fitted with radios weighed more than 200 g so that the transmitter-animal weight percentage did not exceed 5%. A greater weight probably affects behaviour and movement of animals (Wolton and Trowbridge 1985; Pouliquen *et al.* 1990)

Study Site and Radio-tracking Techniques

The study area was a mixed plantation of mature oil palm and cacao planted under coconut. The squirrels were trapped using ordinary trap cages set in a row on every fourth cacao tree approximately 10 m apart. Trapping was carried out at the edge of the cacao near the cacao-oil palm boundaries. A previous trapping exercise showed most captures were recorded at intercrop boundaries. Squirrels were identified individually by radio transmitters operated at unique frequencies. The sex, weight, breeding condition and capture locations were recorded for each squirrel (Table 1). Radio collars were attached while the animals were lightly anaesthetized for approximately 60 seconds with either diethyl ether or chloroform. The radio-tagged squirrels were kept in a cage overnight, allowed to recover full locomotor activity, and released at their respective site of capture. This measure was necessary to ensure that the radio collars were fully secured and did not physically harm the animal.

A total of six squirrels (three males and three females) were radio-tracked for up to seven days. The tracking period for each animal is shown in Table 1. Location was determined every hour from 0530 to 1730 h either throughout or for part of the period. Tracking was not done on rainy days when animals seemed less active. Radio signals were detectable from at least 100 m awayand were pin-pointed by walking along the path of the strongest signal. Apparent animal location was determined to be accurate within 1 – 2 metres as confirmed by actual sighting. The presence of coconut and oil palms grown at 15-m intervals across the study area conveniently served as grid markers to mark animal locations on the map. When radio location was uncertain, triangulation (Kenward 1987) was employed; this involves taking bearings from at least three different points. The point at which the bearings intersect was designated as the animal location. The distance between successive radio locations was taken to represent the level of activity during a 1-hour period. If the animal did not move from its previous location over a period of time, it was assumed for practical reasons that it had remained inactive. To further qualify this assumption, animal location was checked every 10 - 15 minutes. The mean distance moved was used to plot the daily activity pattern for each animal. Palomares and Delibes (1991) showed that distance travelled gave similar results to net activity time in estimating daily activity patterns in the Egyptian mongoose (*Herpestes ichneumon*).

Survey of Stomach Contents

A 1-day shooting exercise was organized in the study area to determine the feeding habits of *C. notatus* based on the stomach contents. Shooting started at daybreak and ended before noon to cover the time of the day when animals were observed to be most active. The exact time of

TABLE 1
Summary of breeding condition, weight and tracking dates of four Callosciurus notatus

	Breeding condition	Wt (S)	Tracking dates
Male A	Breeding	246	June 5 -8, 10 - 12, 1990
Male B	Breeding	232	Jul 6 - 7, 9, 12 - 16, 1990
Female A	Non-breeding	201	July 2 - 5, 7 - 8, 1990
Female B	Breeding	226	July 20 - 28, 1990

the shooting of each individual was recorded. The gut was opened and contents broadly identified as oil palm fruit, cacao mucilage, unidentified plant matter and insects. The proportion of each item was subjectively quantified.

RESULTS

Of the six squirrels, sufficient data for analysis was collected from only four. Signals were not picked up from the other two and their transmitters were assumed to be lost. Daily activity profiles were plotted for each squirrel (*Fig. 1*).

In terms of habitat utilization, Male A was tracked in both cacao and oil palm, Female A in cacao only and Male B and Female B in oil palm only. The small sample size precludes comparison of activity patterns in the two habitats. Individual daily activity patterns (*Fig. 1a-d*) are quite consistent. Differences in the mean distance travelled during the tracking period were not significant between Male A and B (Z=1.345, P > 0.1) and between Female A and B (z=0.275, P > 0.1). All squirrels started movement at first light i.e. around 0600 h and maintained a high level of activity until 0930 h. Since tracking started 30 min earlier i.e. at 0530 h, it is assumed that squirrels stayed at their nest site within that period. In 12 out of 14 instances where tracking was carried out on consecutive days, squirrels were located at their last position (1730 h the previous day), suggesting that they remained inactive throughout the night. Two morning peaks were identified, the first at 0730 h and the second from 1030 - 1130 h. The first peak was higher than the second except for Male A, where the level of activity was similar at both peaks. The afternoon period (1230 - 1630 h) was marked by a period of ærestÆ except for Female A, which showed a third peak about 1530 h. All squirrels showed increased activity from 1730 h to just before nightfall. In terms of the daily activity profile, they generally showed a progressively decreasing level of peak activity, except for Female A where the four peaks were similar. Female A, the smallest (201 g) was

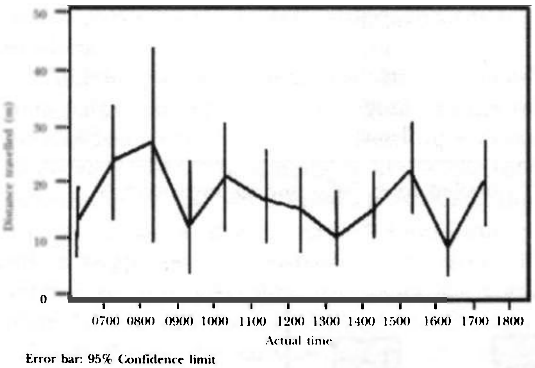

Fig. 1a. Daily activity pattern of female A (radio-tracked in cacao only)

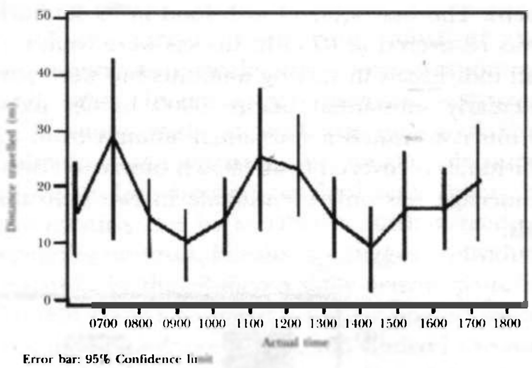

Fig. 1b. Daily activity pattern of female B (radio-tracked in oil palm only)

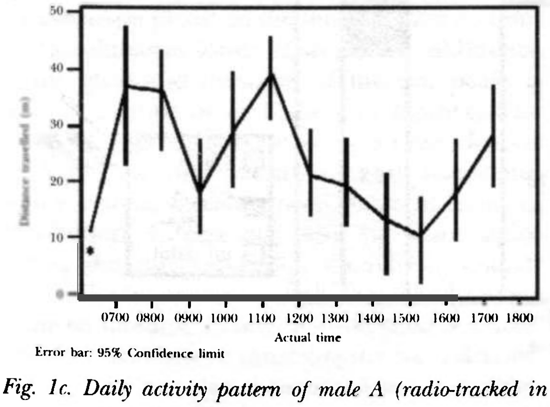

Fig. 1c. Daily activity pattern of male A (radio-tracked in cacao and oil palm)

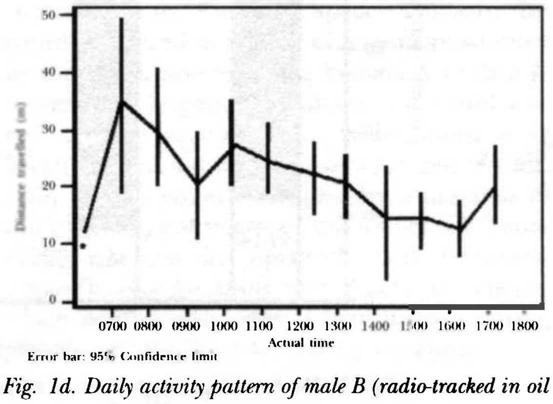

Fig. 1d. Daily activity pattern of male B (radio-tracked in oil palm only)

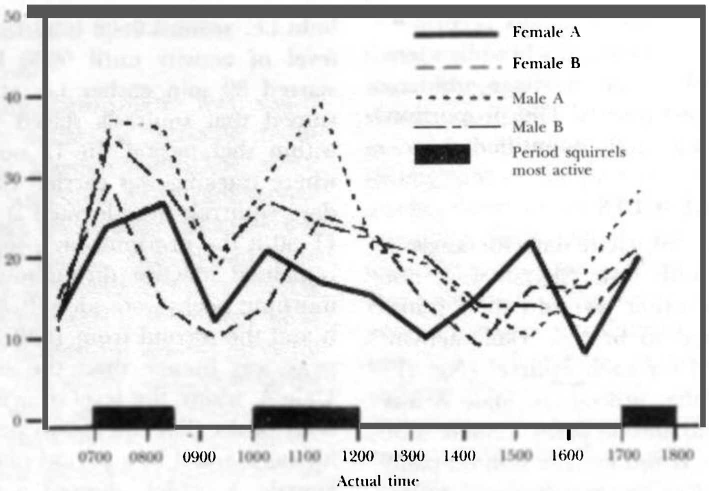

Fig. 2. Comparison of the duration of the active phases of the four radio-tracked Callosciurus notatus

therefore active for a relatively longer period. A comparison of the duration of the active phases is shown in *Fig. 2.*

A total of 18 shot squirrels were recovered for feeding analysis (*Fig. 3*). The first two squirrels, recovered at 0700 h, both had empty stomachs. The first squirrel with food in its stomach was recovered at 0735 h. Insects were found in all individuals in varying amounts but were particularly substantial before 0900 h. Oil palm fruits constituted a substantial amount from individuals recovered from 0955 h onwards. Cacao mucilage was only identifiable in two individuals.

TABLE 2
Number of squirrels shot every hour from 0700 - 1200

Time (1-hour period)	No. of squirrels analysed
0800	8
0900	4
1000	3
1200*	3
Total	18

* - pooled from 1000 due to few squirrels

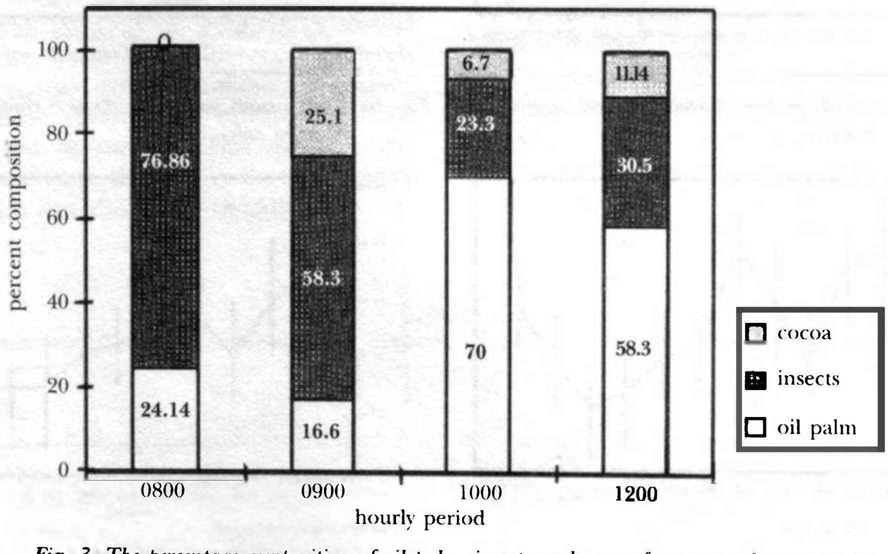

Fig. 3. The percentage composition of oil palm, insects and cacao from stomach contents of Callosciurus notatus shot in the study area

Due to the small sample size, squirrels collected within a one hour period beginning from 0700 were grouped together. The respective food items from each stomach were pooled and the mean proportion estimated for each group. The percentage composition of each food item for every 1-hour period is shown in *Fig. 3*.

DISCUSSION

The present study suggests that *C. notatus* exhibits a daily activity pattern with at least three distinct peaks; two in the morning and one in the late afternoon. However there is individual variation, as exemplified by one individual, which had four peaks. Three or more activity peaks per day constitute a variation of the typical biphasic or bimodal pattern that has been observed in many animals (Aschoff 1966). Tonkin (1983) suggested that this type of behaviour is either a manifestation of the physiological need for rest or that a break following the morning feeding period is needed when animals have eaten their fill. Whereas this might be reflected in the present study, the existence of double morning peaks punctuated by a short break and variability among individuals raised some interesting questions.

The double peaks may also be associated with a particular resource or foraging strategy to optimize food searching effort, hunting success and energy return. The difference in the intensity and duration of the active phases may reflect the nutrient value of the associated food source. This is supported by evidence from stomach contents of squirrels shot in the study area, which showed that insects formed the primary food item during the early morning hours (0730 - 0930 h). Oil palm was only identified in substantial amounts from individuals recovered from about 1000 h onwards. This suggest that the two active phases in the morning may account for two different kinds of food. The difference in the level and duration of the two peaks is again indicative of such food preference. The first peak may account for an insect diet. Insects are highly nutritive but are relatively scarce and highly mobile, thus less predictable in terms of distribution. Insects may also be more active during the early morning. Therefore, animals have to intensify their search to take advantage of the temporarily abundant insect food resource. The short rest before resuming the second active phase may indicate the point whereby foraging for insects is no longer economic in terms of energetics. Energetic cost is an important factor in the foraging decisions in animals (Krebs *et al.* 1983). Oil palm fruits and cacao pods, on the other hand, are more evenly distributed, highly predictable, conspicuous and immobile, and thus require less effort to secure. The second active phase could be associated with a primarily oil palm diet. A longer phase may suggest a greater volume consumed to meet dietary and energetics requirements. In general, foraging time and intensity are influenced by availability, nutrient value, predictability and handling time of food source (Lewis 1980).

There are other possible explanations for the double morning peak. The first of the two peaks may account for the daily re-establishment of exclusive feeding ranges. *C. notatus* has been suggested to exhibit some form of territorial behaviour (Duckett 1982). Such behaviour was also observed in this study. *C. notatus* is most conspicuous at daybreak, when it can be seen in hot pursuit of other individuals apparently driving away intruders from a dray or a favourite feeding site.

From direct observation, most of the afternoon was normally spent dozing, grooming and other less energy expensive activities. Continuous radio surveillance indicated that animals sometimes stayed in the same location for up to four hours. The final active phase in the evening can be ascribed to a final feeding round or nest run. Results also suggest individual variation in the observed daily activity pattern. Tonkin (1983) observed a similar phenomenon among a population of the red squirrel (*Sciurus niger*). He suggested that newly independent young adults tend to be active during periods when fewer adults were active to avoid direct competition for food and space. Similarly, this could be viewed as a form of a strategy adopted by the small, non-breeding Female A (Table 1) to reduce competition. However, it could also be random variation since observations were based on one individual from a sample of four. Daily activity pattern can also be a function of the physiological needs of the individual. Maier (1992) showed in pipistrelle bats *Pipistrellus pipistrellus* that variation existed between females which exhibit unimodal activity pattern during pregnancy and bimodal during lactation.

CONCLUSION

The results of this study show that *C. notatus* is more active during early morning and just before noon. Therefore, shooting exercises to control squirrel infestation in plantations, particularly oil palm, should be carried out during those two periods i.e. when squirrels are more active and thus more conspicuous.

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Effects of Nitrite and pH on a Tropical Fish Fry, *Puntius gonionotus* (Bleeker)

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ABSTRAK

Kesan pendedahan jangka pendek dan panjang anak ikan tropikal, Banbodes gonionotus *(Bleeker) terhadap pH dan nitrite secara berasingan dan gabungan telah ditentukan dengan menggunakan biocerakinan statik dan sistem aliran terus. Nilai* LC_{50} *96-jam pH dan nitrit masing masing adalah pH 4.9 dan 7.91 mg* NO_2*-N/l. Walau bagaimanapun, nilai* LC_{50} *96-jam bagi pH adalah lebih tinggi (pH 5.39) dalam kepekatan nitrit (5 gm* NO_2*-N/l) dibandingkan dengan nilai tanpa nitrit. Pada tahap pH 5.00 100% kematian berlaku dalam 4.00 mg* NO_2*-N/l selepas pendedahan selama 48 jam. Di bawah pendedahan jangka panjang, kadar tumbesaran anak ikan menurun dengan peningkatan kepekatan nitrit. Anak ikan di dalam 2.00 mg* NO_2*-N/l (pH 7.33-7.56) menunjukkan kadar tumbesaran yang lebih rendah (*$p < 0.05$*) dibandingkan dengan kawalan, dan lebih tinggi (*$p < 0.05$*) dibandingkan dengan yang didedahkan pada tahap 4.00 mg* NO_2*-N/l (pH 7.33-7.56). Seratus peratus kematian berlaku dalam masa 30 hari pada tahap pH 5.00-7.00 apabila ikan didedahkan kepada 4.00 mg* NO_2*-N/l pada masa yang sama. Kajian menunjukkan kesan pH dan nitrit secara gabungan ke atas kemandirian dan kadar tumbesaran anak ikan adalah lebih serius dibandingkan dengan kesan setiap faktor secara berasingan.*

ABSTRACT

The effects of short term and long term exposure of a tropical fish fry, Barbodes gonionotus *(Bleeker), to pH and nitrite separately, and in combination, were evaluated using static and flow-through bioassays respectively. The 96-hour* LC_{50} *values of pH and nitrate were 4.9 and 7.91 mg/l* NO_2*-N respectively. However, the 96-hour* LC_{50} *of pH was higher (5.4 pH unit) in the presence of nitrite 5.00 mg/l* NO_2*-N) than that without nitrite. At pH 5.00, 100% mortality was found at 4.00 mg/l* NO_2*-N concentration after 48-hour exposure. Under long-term exposure, the growth rates of the fish fry decreased with increased nitrite concentrations. Fish fry grown at 2.00 mg/l* NO_2*-N had significantly lower growth rate (*$P < 0.05$*) than the control, but had a significantly higher rate (*$P < 0.05$*) than in the 4.00 mg/l* NO_2*-N (pH 7.33-7.56). One hundred per cent mortality occurred within 30 days at pH 5.00 - 7.00 when the fish were exposed to 4.00 mg/l* NO_2*-N concentration at the same time. The study demonstrated that the effects of combined pH and nitrite on the survival and growth rates of the fish fry were more serious than the effects of each factor separately.*

INTRODUCTION

Barbodes gonionotus (Bleeker), locally known as Javanese carp, is one of the most popular cultured fishes in the South-East Asian region such as Indonesia, Malaysia and Thailand. In Malaysia, it contributes about 21% of the total freshwater fish production (Department of Fisheries 1991). Thus, hatchery production of *B. gonionotus* fry is very important to supply sufficient seed to fish farmers. One of the problems preventing efficient fry production is water quality. Nitrite toxicity to fish is common in hatchery tanks and intensive culture ponds. Although nitrite accumulation is rare in natural waters with an average concentration of less than 10 µg/l (Wetzel 1983), concentrations of nitrite nitrogen in culture ponds may attain 5.00 mg/l (Boyd 1982).

The major action of nitrite in fish is oxidation of hemoglobin to methemoglobin, which is incapable of oxygen binding, thus affecting oxygen transport in blood. Fish can be adversely affected if the blood contains more than 50% methemoglobin (Bowser *et al.* 1983). The presence of nitrite in water, even at a low concentration of 15 µg/l, increases the methemoglobin concentrations in fish blood (Smith and Williams 1974; Smith and Russo 1975; Brown and McLeay 1975). Wise and Tomasso (1989) reported that plasma nitrite concentrations increased with increasing exposure time in fish exposed to both 9.1 and 5.1 mg/l nitrite-N.

Warm water fish species seem to be more tolerant to nitrite than the cold water species. Twenty-four-hour LC_{50} for rainbow trout fry was 55 µg/l NO_2-N (Smith and Williams 1974). Russo *et al.* (1974) and Brown and McLeay (1975) reported that 96-hour LC_{50} values for 12-g and 9-g rainbow trout were 190 and 230 µg/l respectively. Westin (1974), who worked with salmonid fishes, suggested that maximum NO_2-N concentration in fresh water should be 36 µg/l. On the other hand, Palachek and Tomasso (1984b) reported that the 96-hour LC_{50} for fathead minnow was 147.4 mg/l NO_2-N, and Tomasso and Carmichael (1986) found that the 96-hour LC_{50}, for guadalupe bass (*Micropterus treculi*) was 187.6 mg/l NO_2-N. Yusoff and Subasinghe (1995) reported that *B. gonionotus* fingerlings of 6-7 cm in total length were able to survive high nitrite concentrations of 20.00 mg/l NO_2-N. However, exposure to high concentration of nitrite caused sufficient stress to make fish more susceptible to a ubiquitous bacterium such as *Aeromonas hydrophila*. Other adverse effects of nitrite in fishes include reduced growth (Colt *et al.* 1981) and decreased disease resistence (Hanson and Grizzle 1985; Yusoff and Subasinghe 1995).

Nitrite toxicity is affected by other water quality parameters. Huey *et al.* (1982) reported that at low pH, bluegills (*Lepomis macrochirus*) exhibited immediate stress at NO_2- of 6.9 and higher. Fish exposed to low pH resulted in changes of gill morphology, such as hyperplasia and hypertrophy, necrosis and edema in filament epithelium (Nelson 1982, Woods 1989, Leino and McCormick 1984). McCormick *et al.* (1989) reported that these symptoms were more serious under low pH conditions and long exposure time.

Although nitrite and pH are two of the main water quality parameters in management of fish culture activities, their acute toxicity values for tropical fishes are lacking. Safe chronic exposure levels are largely unknown. Since nitrite and pH affect not only the survival, but also growth rate and disease resistance, this study was undertaken to evaluate the effects of short- and long-term exposure of Javanese carp fry to these water quality factors.

MATERIALS AND METHODS

Javanese carp fry ranging from 18-20 mm and 70-80 g, obtained from the Hatchery of the Department of Fisheries in Bukit Tinggi Selangor, were acclimatized in 500-l tanks for approximately two weeks before being used in the experiments. Fish fry were treated with 5.0 mg/l potassium permanganate and fed daily at 2-3% body weight. The feeding was stopped 24 hours before the start of the experiment.

Four experiments were carried out in the acute toxicity test for the determination of 96-hour LC_{50} for nitrite, 96-hour LC_{50} for pH, 96-hour LC_{50} of nitrite at pH 5.0, and 96-hour LC_{50} of pH at nitrite concentration of 4.00 mg/l NO_2-N. For each experiment, 25-l glass tanks containing 15 l water were used. At the beginning of the experiment, the water in the tanks was saturated with oxygen. No aeration or feeding was given during the experiment to avoid changes in the quality of the test water. Twenty fish fry were randomly selected from the holding tank and placed in each test container. Nitrite solutions were prepared using analytical grade sodium nitrite. Desired pH levels were achieved by using sulphuric acid (H_2SO_4) or sodium hydroxide (NaOH).

In the first experiment, high nitrite concentrations of 0, 30.00, 60.00, 90.00 and 120.00 mg/l NO_2-N and lower concentrations of 0, 5.00, 10.00, 15.00, 20.00 and 25.00 mg/l NO_2-N were tested. In the second experiment to determine 96-hour LC_{50} of pH, levels of 4.0, 4.5, 5.0, 5.5, 6.0, 7.0, 8.0 and 10.0 were tested. Results from the first two experiments were used to decide pH and nitrite levels employed in the third and fourth experiments.

In the third experiment, pH levels in all treatments were held constant at 5.0 and nitrite concentrations of 4.00, 6.00 and 8.00 mg/l NO_2-N were tested. In the fourth experiment, nitrite levels were held constant at 5.00 mg/l NO_2-N, and pH levels tested were 5.0, 5.5 and 6.0. The controls for these experiments consisted of 0 mg/l NO_2-N and the pH ranged between 7.4-7.6. All experiments was done in triplicate.

Observations were made after 6, 12, 24, 48, 72 and 96 hours. The number of dead fish at each observation was noted. Death was assumed when there was no response to a light touch (Reish and Oshida 1986). During the experiments, nitrite-N and pH were monitored daily using sulphanilamide-naphthylethylene-diamine method (APHA-AWWA-WPCE 1989) and Orion pH meter model 230, respectively, and adjusted to the tested values as necessary. Water temperature and dissolved oxygen were also monitored daily using an oxygen meter equipped with a thermistor (YSI, model 57). Alkalinity (potentiometric method using pH meter) and total ammonia (phenate method according to APHA-AWWA-WPCF (1989)) were determined at the beginning and end of each experiment. Water temperature, dissolved oxygen, alkalinity and total ammonia-N were measured to ensure that no significant differences in their values occurred in all treatments (Table 1).

For the chronic toxicity test, another 3 experiments were carried out. The first and second experiments were to test the chronic effects of nitrite and pH separately. The third experiment was to test the combined chronic effects of nitrite and pH. In these experiments, a flow-through system was used. Test solutions were placed in 10-l containers set above the tanks. Solution from each container flowed into three test tanks. All tanks were aerated using air

TABLE 1
Range of water quality parameters in the acute toxicity experiments

Nitrite-N (mg/l)	Temperature (°C)	Dissolved O_2 (mg/l)	pH	Alkalinity (mg/l $CaCO_3$)	Total Ammonia-N (mg/l)
0.00	25.8 - 26.2	4.1 - 7.8	7.2 - 7.5	20.5 - 23.0	0.005 - 0.273
5.00	26.0 - 27.4	6.3 - 8.2	7.4 - 7.8	26.3 - 29.5	0.013 - 0.267
10.00	26.0 - 27.4	6.4 - 8.2	7.4 - 7.8	26.8 - 30.5	0.004 - 0.186
15.00	26.0 - 27.4	6.3 - 8.2	7.4 - 7.8	26.1 - 28.7	0.005 - 0.108
20.00	26.0 - 27.4	6.6 - 8.2	7.4 - 7.8	25.4 - 29.2	0.003 - 0.115
25.00	26.0 - 27.4	6.4 - 8.2	7.4 - 7.8	26.5 - 28.6	0.012 - 0.085
30.00	25.8 - 26.2	5.9 - 7.8	7.6 - 7.4	21.0 - 26.5	0.005 - 0.125
60.00	25.8 - 26.2	5.3 - 7.8	7.2 - 7.4	22.7 - 25.8	0.010 - 0.132
90.00	25.8 - 26.2	6.0 - 7.9	7.2 - 7.5	21.6 - 25.5	0.008 - 0.086
120.00	25.8 - 26.2	6.8 - 7.7	7.3 - 7.4	23.1 - 26.7	0.011 - 0.093
pH					
4.0	25.8 - 26.2	6.8 - 7.9	3.97 - 4.01	21.7 - 23.4	0.016 - 0.063
4.5	26.0 - 27.4	7.0 - 8.2	4.47 - 4.55	22.3 - 23.7	0.015 - 0.082
5.0	26.0 - 27.4	5.9 - 8.2	4.98 - 5.03	21.7 - 23.1	0.009 - 0.243
5.5	26.0 - 27.4	5.8 - 8.2	5.46 - 5.53	22.5 - 23.3	0.007 - 0.281
6.0	25.8 - 26.2	5.5 - 8.1	5.98 - 5.05	22.5 - 24.1	0.012 - 0.182
7.0	25.8 - 26.2	5.4 - 7.8	6.98 - 6.05	21.3 - 23.8	0.014 - 0.215
8.0	25.8 - 26.2	5.5 - 7.6	7.96 - 8.03	25.6 - 28.9	0.010 - 0.194
10.0	25.8 - 26.2	5.2 - 7.8	8.92 - 9.02	24.8 - 29.2	0.009 - 0.136

which was bubbled through the deionized water to remove carbon dioxide and suspended particles.

Results from the acute toxicity tests were used to determine nitrite and pH concentrations employed in the chronic toxicity trials. In the first experiment, nitrite concentrations of 0, 2.00, 4.00, 6.00 and 8.00 mg/l NO_2-N were used. Levels of pH were maintained at 7.3 - 7.6. In the second experiment, pH levels of 5.0, 6.0, 7.0, 8.0 and 9.0 were used and the test water was free of nitrite. The concentrations used in the third experiment were based on the results of the first and second experiments of the long-term exposure trial. The nitrite concentration was held constant at 4 mg/l NO_2-N and pH levels used were 5.0, 6.0, 7.0, 7.50, 8.0 and 9.0. Similar to the acute toxicity experiments, 20 fish fry were placed in each tank and each treatment was carried out in triplicate.

Fish were fed at 5% body weight twice a day at 0900 and 1700 hours. Excess feed was siphoned out every day. Fish were sampled twice to measure growth rate. Water temperature, dissolved oxygen, total ammonia, nitrite, alkalinity and pH were monitored weekly. Experiments were terminated after 30 days. at the end of the experiments, live fish were used for histology preparation and observation (Humason 1979).

Values of LC_{50} with 95% confidence level were obtained by using probit analysis (Finney 1977). One-way ANOVA was used to determine significant differences amongst various treatments in the chronic toxicity experiments.

RESULTS AND DISCUSSION

In the acute toxicity test of nitrite, all concentrations above 20.00 mg/l NO_2-N (at pH 7.4-7.8) caused 100% mortality within 96 hours (Tables 1 and 2). The fish in this study seemed to be more sensitive to nitrite than those reported by Yusoff and Subasinghe (1995), perhaps due to their smaller size. Wedemeyer and Yasutake (1978) also reported that larger steelhead trout, *Salmo gairdneri*, were more resistant to nitrite than smaller fish. However, other workers have reported that smaller fish were more tolerant to nitrite than larger fish of the same species (Russo *et al.* 1974; Perrone and Meade 1977; Palachek and Tomasso 1984a). Further studies are needed to resolve the conflicting observations of the nitrite toxicity due to fish size and species.

In this study, the value for 96-hour LC_{50} for nitrite was 7.91 mg/l NO_2-N when pH was in the neutral range (7.4-7.6). No fish survived (100% mortality) when nitrite-N concentrations were 20.00 mg/l NO_2-N and above (Table 2). However, 100% mortality occurred at a much lower nitrite concentration (4.00 mg/l NO_2-N) when the water pH level deviated (pH 5) from the natural range (Table 3). Wedemeyer and Yasutake (1978) showed that 96-hour LC_{50} for 10 g steelhead trout was 5.80 mg/l NO_2-N when pH and hardness were 7.3 and 150.0 mg/l respectively. On the other hand, Russo *et al.* (1981) showed that 96-hour LC_{50} value for 6.3 - 387 g rainbow trout, Salmo gairdneri, at pH 6.44 - 9.04 ranged from 0.11 - 1.67 mg/l NO_2-N. Colt and Tchobanoglous (1976) reported that 96-hour LC_{50} for nitrite was 43 mg/l for channel catfish. Results for nitrite acute toxicity are widely variable, depending on fish size, fish species and water chemistry. The suppression of nitrite toxicity by chloride ions has been reported for rainbow trout (Russo and Thurston 1977), coho

TABLE 2
Mean percentage mortality of fish fry exposed to different nitrite and pH levels separately after 96 hours

Nitrite-N concentrations (mg/l NO_2-N) (pH ranged from 7.4 - 7.6)	Mean % Mortality ± SD
0.00	5.5 ± 2.0
5.00	8.0 ± 4.0
10.00	83.0 ± 6.0
15.00	95.0 ± 4.0
20.00	100.0 ± 0.0
25.00	100.0 ± 0.0
30.00	100.0 ± 0.0
60.00	100.0 ± 0.0
90.00	100.0 ± 0.0
120.00	100.0 ± 0.0
pH Levels (Nitrite = 0.00 mg/l NO_2-N)	**Mean % Mortality ± SD**
4.0	100.0 ± 0.0
4.5	100.0 ± 0.0
5.0	8.0 ± 2.0
5.5	3.0 ± 2.0
6.0	3.0 ± 2.0
7.0	3.0 ± 2.0
8.0	7.0 ± 2.0
10.0	12.0 ± 5.0

TABLE 3
Combined effects of pH and nitrite in terms of mean percentage mortality on fish fry after 96-hour exposure

Nitrite-N Concentration (mg/L NO_2-N)	pH Levels	Mean % Mortality ±SD
5.00	5.00	100.0 ± 0.0
5.00	5.50	33.0 ± 4.0
5.00	6.00	15.0 ± 4.0
4.00	5.00	100.0 ± 0.0
6.00	5.00	100.0 ± 0.0
8.00	5.00	100.0 ± 0.0

salmon (Orcorhynchus kisutch) (Perrone and Meade 1977), steelhead trout (Wedemeyer and Yasutake 1978) and channel catfish (*Ictalurus punctatus*) (Tomasso *et al.* 1979). In the pH acute toxicity test of this study, 100% mortality occurred at pH 4.5 and below in the absence of nitrite (Table 2). However, in the presence of nitrite (> 4 mg/l NO_2-N), 100% mortality occurred at pH 5.0 (Table 3). Without nitrite, only 8% mortality was observed at pH 5.0 (Table 2). The lowest mortality (3%) occurred between pH 5.5 and 7.0 in treatments without nitrite. Thus, toxic effects of pH were more serious in the presence of nitrite ions. The 96-hour LC_{50} for pH was 4.9 in the absence of nitrite, but increased to 5.39 in 5.00 mg/l NO_2-N solution.

Similarly, as pH deviates from neutral value, the safe concentration of nitrite becomes lower. Assuming a safety factor of 0.001 of 96-hour LC_{50} (Bresch 1993), this study showed that the safe nitrite concentration was about 8 µg/l NO_2-N at neutral pH range, but lower than 4 µg/l NO_2-N at pH 5.0. Huey *et al.* (1982) reported that bluegill (*Lepomis macrochirus*) (17.3 - 7.4 g) exposed to pH 4.00 showed immediate stress at concentra-tions of 6.9 mg NO_2/l (2.1 mg/l NO_2-N). Wedemeyer and Yasutake (1978) reported that increasing pH from 6 to 8 reduced nitrite toxicity by a factor of 3 for 10 g steelhead. Huey *et al.* (1980) attributed the increased nitrite toxicity to the permeability of the uncharged nitrous acid form of nitrite predominant at low pH. Although both forms of nitrite (HNO_2 and NO_2) are known to be toxic, Huey *et al.* (1980) suggested that HNO_2 uptake is much more rapid and the sudden nitrite load converts most of the fish haemoglobin to methemoglobin, resulting in death. Bath and Eddy (1980), on the other hand, reported that acidity of the water except at extreme values (below pH 5 and above pH 10) had no significant effect on nitrite toxicity.

TABLE 4
Mean percentage mortality and growth rate of fish fry in chronic toxicity test at different nitrite and pH levels (separately) after 30-day exposure (Growth rates were not considered in treatments with high mortality

Nitrite-N Concentrations (mg/l NO_2-N) pH ranged from 7.33 - 7.56	Mean % Mortality ± SD	Mean Growth Rates (mg/day) ± SD
0.00	7 ± 2	8.3[a] ± 0.1
2.00	15 ± 4	7.7b ± 0.1
4.00	43 ± 2	6.1c ± 0.2
6.00	80 ± 7	–
8.00	100.0 ± 0	–
pH Levels (Nitrite = 0.00 mg/l NO_2-N)		
5.00	100.0 ± 0	–
6.00	68 ± 5	–
7.00	18 ± 5	7.6a ± 0.1
8.00	12 ± 2	8.5b ± 0.1
9.00	62 ± 2	–

Means in column with different superscripts are significantly different at $p < 0.05$

TABLE 5
Combined effects of pH and nitrite on the growth rates and mortality of fish fry after 30-day exposure (Growth rates were not considered in treatments with high mortality)

Nitrite-N Concentrations (mg/l NO_2-N)	pH	Mean % Mortality ± SD	Mean Growth Rates (mg/day) ± SD
4.00	5.0	100 ± 0 (within 48 hrs)	-
4.00	6.0	100 ± 0 (within 14 days)	-
4.00	7.0	100 ± 0 (within 30 days)	-
4.00	7.5	8 ± 2	11.3[b] ± 0.1
4.00	8.0	32 ± 6	8.8[a] ± 0.1
4.00	9.0	88 ± 5	-

Means in column with different sueerscripts are significantly different at $p < 0.05$

Under chronic exposure, the growth rates of the fish fry decreased with increased nitrite concentrations (Table 4). Fish fry grown at 2 mg/l NO_2-N had a significantly lower growth rate ($P < 0.05$) than the control, but had a significantly higher rate ($P < 0.05$) than in the 4.00 mg/l NO_2-N. Mortality of 100% occurred in 8 mg/l NO_2-N (in neutral pH range) after 12 days. In the absence of nitrite, 100% mortality occurred after 25 days at pH 5.0 (Table 4). Growth rates of 7.6 mg/day and 8.5 mg/day were found at pH 7.0 and 8.0 respectively. Below pH 6.0 and above pH 9.0, more than 60% mortality occurred.

Although nitrite concentration of 4.00 mg/l NO_2-N did not significantly affect fish growth when pH was 8.0, the mortality was almost three time higher in the treatment compared to the treatment without nitrite (Tables 4 and 5). In the absence of nitrite, 18% mortality occurred at pH 7.0. However, 100% mortality occurred when nitrite concentration was 4.00 mg/l NO_2-N at the same pH level. Mortality was lowest and mean growth rate was highest when pH was 7.5 although the nitrite concentration was 4.0 mg/l NO_2-N. Thus nitrite toxicity was highly influenced by pH level.

Chronic exposure to 6.0 mg/l NO_2-N for 30 days in neutral pH caused gill histological changes such as hyperplasia and oedema. Below this concentration, there was slight hyperplasia and oedema observed in the gills. Serious gill epithelial changes including hyperplasia, oedema and necrosis were observed in fish exposed to pH 6 without nitrite. At pH 7.0 and 9.0, only hyperplasia occurred. Gills appeared normal at pH 7.5 and 8.0. When nitrite concentrations were 4.00 mg/l NO_2-N, serious gill hyperplasia and necrosis were observed at pH 8.0 and 9.0. Wedemeyer and Yasutake (1978) reported that 6-month exposure of steelhead trout to 0.06 mg NO_2-N/l caused minimal gill epithelial changes with no adverse effects on survival and growth.

This study showed that both nitrite and hydrogen ions are toxic to fish, but their toxicity is enhanced by the presence of the other. Control of pH can be used to reduce nitrite toxicity as nitrite is less toxic when pH is in the neutral range.

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Influence of Organic Matter on AM Colonization and Associated Rhizosphere Mycoflora in *Vigna unguiculata* subsp. *unguiculata* (L.) Walp

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ABSTRAK

A greenhouse investigation was undertaken to determine the influence of organic amendments on the colonization of arbuscular mycorrhizal (AM) symbiosis and rhizosphere microfungal population in Vigna unguiculata *subsp.* unguiculata *(L.) Walp. grown in sandy loam.* Pongamia glabra *leaves (PL) and goat pellets (GP) were applied at the rates of 5, 10 and 15 g kg^{-1} soil either alone or along with* Acaulospora scrobiculata, Glomus aggregatum *and* G. etunicatum. *Plant dry weights, nodulation and tissue nutrients differed with AM fungi, types of organic matter and their concentrations. Increased rates of PL amendment enhanced AM colonization, in contrast higher rates of GP amendment suppressed AM fungi colonization. Among plant nutrients, potassium content increased profoundly with increasing rate of organic matter amendments with few exceptions at 90 d but at 45 d it showed a reverse trend at higher concentration. Microfungal populations were higher in PL amended soils than in GP amended soils. Among the various types of microfungal genera isolated,* Aspergillus *had the most diverse species.*

ABSTRACT

A greenhouse investigation was undertaken to determine the influence of organic amendments on the colonization of arbuscular mycorrhizal (AM) symbiosis and rhizosphere microfungal population in Vigna unguiculata *subsp.* unguiculata *(L.) Walp. grown in sandy loam.* Pongamia glabra *leaves (PL) and goat pellets (GP) were applied at the rates of 5, 10 and 15 g kg^{-1} soil either alone or with* Acaulospora scrobiculata, Glomus aggregatum *and* G. etunicatum. *Plant dry weights, nodulation and tissue nutrients differed with AM fungi, types of organic matter and their concentrations. Increased rates of PL amendment enhanced AM colonization; in contrast, higher rates of GP amendment suppressed AM fungi colonization. Among plant nutrients, potassium content increased profoundly with increasing rate of organic matter amendments with few exceptions at 90 d but at 45 d it showed a reverse trend at higher concentration. Microfungal populations were higher in PL-amended soils than in GP-amended soils. Among the various types of microfungal genera isolated,* Aspergillus *had the most diverse species.*

INTRODUCTION

Agriculture in the tropics is very intensive and is characterized by high inputs in terms of machinery, fertilizers and crop protection chemicals. Chemical fertilizers added to the soil are frequently unavailable to plants because many are easily absorbed or readily precipitated from are soil solution or relatively immobile in soil (Bolan 1991). Less than 50% of the applied fertilizer remains available to plants. Organic farming, which relies heavily on the use of natural resouces, biological processes and crop rotations, is an alternative to conventional farming. Amendment of soil with decomposable organic matter is recognized as an effective method of altering the rhizosphere microbial life cycle, thereby enabling plants to resist pathogenic attack through better vigour and/or altered root physiology (Singh and Singh 1984).

The development of arbuscular mycorrhizal (AM) infection in the host roots is influenced by edaphic, biotic and abiotic factors (Avio and

Giovannetti 1988). Organic residues have variable effecfs on the physio-chemical properties of the soil and AM association in non-acidic soils (Hepper and Warner 1985; Aziz and Habte 1988; Harinikumar and Bagyaraj 1989). Addition of organic matter such as cellulose to soil is known to suppress several soil-borne plant pathogens and therefore has been suggested as an effective method for biological control of pathogens (Cook and Baker 1983).

The role of fresh green manure and goat pellets on AM symbiosis and other microbial populations in sandy loam soils is largely unknown (Wander *et al.* 1995). Studies are necessary to establish clear-cut conclusions on the inter-relationship between organic matter-AAM symbiosis-microbial populations. The purpose of the present study was to determine (i) the effect of different types of organic amendments on AM formation and function and (ii) the changes in microfungal population due to organic amendments.

MATERIALS AND METHODS

Substrate

The soil used in the study was a phosphate-deficient sandy loam soil with pH 6.8. The soil contained 2.01% organic matter; 9.48 mg kg^{-1} nitrogen (N); 0.95 mg kg^{-1} phosphorus (P) and 37.79 mg kg^{-1} potassium (K). It was steamed to kill the indigenous mycorrhizal fungi, air dried and 1.5 kg soil used to fill each polybag after the addition of organic matter.

Organic Matter

Two organic manures viz., leaves of *Pongamia glabra* Vent. (25 mg/g N, 3.4 mg/g P and 9.8 mg/g K) and goat pellets (14 mg/g N, 0.75 mg/g P and 11.8 mg/g K) were dried and powdered before application. The manures were applied at three concentrations: 5, 10 and 15 kg^{-1} and thoroughly mixed with soil.

Endophytes

Soil inoculum consisting of extramatrical spores and infected root bits of cowpea infected with AM fungi *Acaulospora scrobiculata* Trappe, *Glomus aggregatum* Schenck & Smith emend. Koske and *Glomus etunicatum* Becker & Gerd., served as inoculum. Mycorrhizal inoculum (15 g) were placed as a thin layer, 5 cm below the soil surface in mycorrhizal treatments. Twenty ml of nodulating bacterial suspension containing 100 cells ml^{-1} obtained from fresh nodules of horse gram was added to each polybag. The normal microbial flora except AM fungi was reintroduced by adding the soil filtrate. For this, freshly collected rhizosphere soil (500 g) of horse gram was suspended in Sl water and passed through 38 µm sieve. Since AM fungal spore/sporocarps normally exceed 38 µ in diameter, the sieved soil suspension contained micro-organisms other than AM fungi. Fifteen ml of the soil extract was added to both mycorrhizal and non-mycorrhizal bags.

Plant Source

Seeds of horse gram (*Vigna unguiculata* subsp. *unguiculata* (L.) Walp.) were procured from the Tamil Nadu Agricultural University, Coimbatore. Seeds were soaked in water overnight and two seeds were sown in each polybag prepared as mentioned earlier. After germination one seedling was removed and the treatments were arranged in randomized block designs with 10 replicates per treatment, under greenhouse conditions (26 ± 2°C and 65% RH). The plants were watered when necessary; no nutrients were added.

Measurements

Five plants were harvested with their entire root system intact at 45 and 90 days after emergence. The roots were washed free of soil and the number of nodules was counted visually. The shoot and root dry weights were determined after drying the plants at 70°C for 48 h.

The roots were cleared in 2.5% KoH at 90°C for 30 minutes; acidified with 5N HCl and stained with trypan blue (0.05% in lactophenol). AM fungal infection was quantified according to magnified intersect method (McGonigle *et al.* 1990).

The soil microfungal populations were enumerated by dilution plate method using rose bengal agar medium (Martin 1950) with three replicates. The Petri dishes were incubated for four days at room temperature (21-24°C) and light. The fungal colonies were counted and identified according to the descriptions by Barron (1969), Ellis (1971), Subramaniam (1971), and Domsch *et al.* (1980).

Dry matter from 45- and 90- day-old shoots and roots was ground and digested in a triple acid mixture and plant tissue P was estimated by the molybdenum blue method (Jackson 1958). Tissue N was estimated following the micro-kjeldahl digestion of the samples (Humphries 1956) and K was estimated by flame photometric method (David 1962). Soil nutrients were analysed according to Jackson (1958).

Statistical Analysis

The data were subjected to analysis of variance and the means were separated using Duncan's new multiple range test (DMRT).

RESULTS

Plant Biomass

The shoot dry weight of mycorrhizal plants at 45 and 90 d had significant variations compared to non-mycorrhizal plants grown in different concentraitons of *Pongamia* leaf (PL) amended soils (Table 1), but no significant variations were observed in goat pellet (GP) amended soils (Table 2). Though root dry weight did not vary much between treatments at 45 d in both the amendments, plants inoculated with *G. etunicatum* in 5 g kg^{-1} PL amended soil had a significantly higher root dry weight at 90 d than the control. Plants inoculated with either *G. aggrregatum* or *G. etunicatum* in 15 g kg^{-1} GP amended soil had a significantly low root dry weight at 45 d.

Root: Shoot Ratio

Root: shoot ratios did not vary significantly between mycorrhizal and non-mycorrhizal plants (Table 1 and 2).

AM Fungal Root Colonization

The Percentage root of colonization by *A. scrobiculata* and *G. etunicatum* was enhanced by increasing rate of PL amendment, except for *G. etunicatum* at 90 d (Table 3). However, root colonization by *G. aggregatum* was significantly less at 15 g kg^{-1} PL amendment at 45 d and at 5 and 10 g kg^{-1} at 90 d. In contrast, increasing rate of GP inhibited AM colonization, except for *G. aggregatum* at 90 d (Table 4).

Increased concentration of PL amendment favoured arbuscular formation in *A. scrobiculata* and *G. etunicatum* inoculated plants initially, but at 90 d arbuscules were absent in 15 g kg^{-1} PL amended soils (Table 3). Increasing concentrations of GP amendment reduced arbuscular formation. At 90 d arbuscules were present only in *G. etunicatum* (5 g kg^{-1}) and *G. aggregatum* (15 g kg^{-1}) inoculated plants (Table 4).

Percentage of root length colonized by vesicles in mycorrhizal plants significantly increased with increasing amount of PL application at 45 d (except in *G. aggregatum* inoculation) (Table 3). But at 90 d vesicle

TABLE 1
Effects of AM fungi and three different amounts of *Pongamia* leaf amendment on the plant dry weights and R/S ratios of horse gram

Treatments	Dry matter production (g plant^{-1})						R/S Ratio		
	Shoot			Root					
Concentration (g kg^{-1})	5	10	15	5	10	15	5	10	15
45 d									
Control	0.08a*	0.18a	0.15a	0.06a	0.07a	0.07a	0.49a	0.32a	0.39a
A. scrobiculata	0.20b	0.18a	0.32a	0.07a	0.07a	0.07a	0.44a	0.43a	0.28a
G. aggregatum	0.20b	0.22ab	0.39ab	0.07a	0.07a	0.07a	0.44a	0.31a	0.19a
G. etunicatum	0.12ab	0.29b	0.44b	0.06a	0.09a	0.09a	0.45a	0.30a	0.19a
90 d									
Control	0.47a	1.15a	1.12a	0.12a	1.20a	0.29a	0.22a	0.24a	0.31a
A. scrobiculata	0.94a	1.71a	1.96b	0.26ab	0.24a	0.36a	0.30a	0.38a	0.31a
G. aggregatum	1.39b	1.63a	1.88b	0.25ab	0.29a	0.27a	0.21a	0.22a	0.31a
G, etunicatum	1.10ab	1.52a	1.87b	0.35b	0.28a	0.23a	0.36a	0.25a	0.24a

* Means in a column followed by same letter(s) are not significantly different (P < 0.05) according to Duncan's multiple range test

TABLE 2
Effects of AM fungi and three different amounts of Goat pellet amendment on the plant dry weights and R/S ratios of horse gram

Treatments Concentration ($g\ kg^{-1}$)	Dry matter production ($g\ plant^{-1}$) Shoot 5	Shoot 10	Shoot 15	Root 5	Root 10	Root 15	R/S Ratio 5	R/S Ratio 10	R/S Ratio 15
45 d									
Control	0.40a*	0.46ab	0.38a	0.09a	0.11a	0.12a	0.25a	0.32a	0.27a
A. scrobiculata	0.40a	0.42b	0.31b	0.12a	0.16a	0.13b	0.29a	0.14a	0.17a
G. aggregatum	0.44a	0.52a	0.36a	0.09a	0.11a	0.07c	0.18a	0.19a	0.14a
G. etunicatum	0.38a	0.46a	0.39a	0.12a	0.12a	0.09b	0.39a	0.18a	0.12a
90 d									
Control	2.01a	2.43a	1.62a	0.19a	0.32a	0.39a	0.11a	0.14a	0.26a
A. scrobiculata	1.86a	2.07a	2.06a	0.26b	0.38b	0.39a	0.14a	0.18a	0.19b
G. aggregatum	1.90a	2.32a	1.90a	0.28b	0.32a	0.27b	0.15a	0.14a	0.16a
G, etunicatum	2.07a	2.68a	1.63a	0.37c	0.37b	0.31c	0.17a	0.14a	0.19b

* Means in a column followed by same letter(s) are not significantly different ($P < 0.05$) according to Duncan's multiple range test

TABLE 3
Effects of *Pongamia* leat amendment on AM root colonization in horse gram after 45 and 90 days of growth

Treatments Concentration ($g\ kg^{-1}$)	Arbuscules (%) 5	Arbuscules 10	Arbuscules 15	Vesicles (%) 5	Vesicles 10	Vesicles 15	Total colonization (%) 5	Total colonization 10	Total colonization 15
Control 45 d	0.00*c	0.00b	0.00d	0.00a	0.00c	0.00c	0.00b	0.00b	0.00d
A. scrobiculata	3.05bc	13.21a	19.66a	2.42a	6.05b	15.56b	41.50a	53.08a	73.17b
G. aggregatum	13.30a	15.74a	5.39c	5.42a	0.81c	1.79c	45.64a	50.55a	22.09c
G. etunicatum	10.92ab	11.55a	13.99b	3.62a	17.03a	22.97a	48.05a	61.87a	85.92a
Control 90 d	0.00a	0.00b	0.00a	0.00c	0.00c	0.00b	0.00c	0.00c	0.00c
A. scrobiculata	3.72a	11.34a	0.00a	33.14ab	23.90b	39.04a	75.04b	75.83a	80.48b
G. aggregatum	1.75a	0.59b	0.00a	25.88b	26.72b	40.61a	59.18b	52.21b	75.21b
G, etunicatum	5.95a	4.32b	0.00a	45.37a	39.82a	43.45a	94.06a	88.28a	89.82a

* Means in a column followed by same letter(s) are not significantly different ($P < 0.05$) according to Duncan's multiple range test

occurrence in *G. etunicatum* infected roots were higher than *A. scrobiculata* and *G. aggregatum* infected roots. Increased rate of GP application either reduced or inhibited vesicle formation at 45 d and 90 d except in *G. aggregatum* at 90 d in 15 $g\ kg^{-1}$ GP amended soils (Table 4).

Nodulation

Plants in soils amended with PL at the rates of 5 and 10 $g\ kg^{-1}$ and inoculated with either *A. scrobiculata* or *G. aggregatum* developed fewer nodules than the non-mycorrhizal plants at 45 d (Table 5). But plants inoculated with the same fungi at 15 $g\ kg^{-1}$ PL amendment had more nodules for the same period. Nodule numbers did not vary between mycorrhizal plants at 90 d except for plants inoculated with *G. etunicatum* and *A. scrobiculata* which had significantly higher nodule numbers at 5 and 15 $g\ kg^{-1}$ PL amendment, respectively. Mycorrhizal plants in GP amended soils had reduced nodule numbers at 45 d, but at 90 d nodule numbers in

TABLE 4
Effects of goat pellet amendment on AM root colonization in horse gram after 45 and 90 days of growth

Treatments Concentration (g kg^{-1})	Arbuscules (%)			Vesicles (%)			Total colonization (%)		
	5	10	15	5	10	15	5	10	15
Control 45 d	0.00*a	0.00a	0.00a	0.00b	0.00b	0.00a	0.00b	0.00b	0.00b
A. scrobiculata	3.34a	4.25a	0.00a	16.71a	6.22ab	0.00a	26.03a	15.33a	8.82a
G. aggregatum	5.01a	0.00a	1.09a	2.71b	0.00b	0.49a	11.67ab	0.62b	8.16a
G. etunicatum	7.85a	2.96a	0.00a	7.71ab	11.61a	0.11a	13.05ab	21.35a	6.51ab
Control 90 d	0.00b	0.00a	0.00a	0.00c	0.00b	0.00c	0.00d	0.00c	0.00d
A. scrobiculata	0.00b	0.00a	0.00a	30.46a	10.47a	3.07c	75.16b	25.92b	10.11c
G. aggregatum	0.00b	0.00a	0.55a	20.95b	4.54a	33.80a	47.43c	82.49a	69.60a
G, etunicatum	6.63a	0.00a	0.00a	35.46a	6.51a	8.59b	90.21a	15.01b	20.38b

* Means in a column followed by same letter(s) are not significantly different (P < 0.05) according to Duncan's multiple range test

TABLE 5
Effects of different organic matter amendments and AM fungi on nodulation of horse gram

Type of Organic Matter	Treatments	Concentration of organic matter (g kg^{-1}) 5		10		15	
days		45 D	90 D	45 D	90 D	45 D	90 D
	Control	22*a	10a	4a	8a	8a	5a
Pongamia leaf	*A. scrobiculata*	10b	12ab	8b	11a	12b	7b
	G. aggregatum	8c	9a	8b	11a	14b	5a
	G. etunicatum	27d	14b	12a	10a	8a	6a
	Control	27a	12a	16a	13a	20a	13a
Goat pellets	*A. scrobiculata*	20b	19c	16a	16a	11b	13a
	G. aggregatum	14c	14ab	18a	16a	7c	11a
	G. etunicatum	16d	17bc	14b	15a	11b	20b

* Means in a column followed by same letter(s) are not significantly different (P < 0.05) according to Duncan's multiple range test

mycorrhizal plants were either high or did not vary significantly compared to non-mycorrhizal plants.

Microfungal Populations

Microfungal populations were generally higher in soils amended with PL (Table 6) than GP (Table 7). However, the dominance of microfungal components varied for different treatments and concentrations of organic matter amendment. In soils amended with PL but devoid of mycorrhizal fungi, *Aspergillus fumigatus* was dominant at both 5 and 10 g kg^{-1} concentrations, whereas *A. flavus* was dominant at 15 g kg^{-1} concentration at 45 d. *Aspergillus fumigatus* was dominant in soils inoculated with *A. scrobiculata* and *G. aggregatum* at all concentrations of PL amendments, whereas in *G. etunicatum* inoculated soils *A. flavipes* in 5 g kg^{-1}, *A. flavus* in 10 g kg^{-1} and *A. fumigatus* in 15 g kg^{-1} were dominant. However at 90 d *A. carneus* in 5 g kg^{-1}, *A. pulverulentus* in 10 g kg^{-1} and *A. flavipes* in 15 g kg^{-1} were dominant in PL amended non-mycorrhizal soils. In PL amended

TABLE 6
Microfungal populations in *Pongamia* leaf amended soils

Concentration (g kg^{-1})	Microfungal population (x10^3)											
	5				10				15			
Fungal species	C	V1	V2	V3	C	V1	V2	V3	C	V1	V2	V3
45d												
Aspergillus carneus	26	66	44	30	36	36	48	30	12	12	20	14
A. fumigatus	152	186	204	54	192	86	86	70	60	140	112	108
A. flavus	-	24	24	38	62	112	86	318	194	82	52	76
A. flavipes	78	48	32	64	42	70	68	8	12	14	34	14
A. pulverulentus	28	22	20	44	38	16	40	54	-	4	10	8
Mucor racemosus	38	52	38	30	22	16	6	18	18	8	4	12
Penicillium rubrun	2	-	-	2	2	-	10	8	-	-	2	-
Trichoderma koningii	32	50	46	32	46	72	48	86	12	-	26	20
Total	356	448	408	294	440	408	392	592	308	260	260	252
90d												
Aspergillus carneus	90	62	50	108	42	50	70	110	20	28	32	36
A. fumigatus	44	20	10	28	34	6	18	26	-	-	28	14
A. flavus	12	20	18	44	30	14	44	26	2	70	50	20
A. flavipes	72	106	74	84	38	36	48	54	76	36	30	32
A. pulverulentus	44	18	10	44	52	60	98	88	14	24	28	32
Mucor racemosus	-	-	2	-	2	-	2	-	2	2	2	-
Penicillium rubrun	18	16	16	14	12	16	2	18	6	26	22	8
Trichoderma koningii	24	36	14	48	30	10	18	54	8	16	10	18
Total	304	278	194	370	240	192	300	376	128	202	202	160

C - control; V1 - *A. scrobiculata;* V2 - *G. aggregatum;* V3 - *G. etunicatum*

soils *A. flavipes* in 10 g kg^{-1} and *A. pulverulentus* in 15 g kg^{-1} were dominant, respectively in *A. scrobiculata* and *G. aggregatum* inoculation; whereas *A. carneus* dominated *G. etunicatum* inoculated PL soils at all concentrations.

In GP amended soils *A. fumigatus* was the dominant species throughout the study. *A. flavus* in 5 and 15 g kg^{-1}, while *A. flavipes* at 15 g kg^{-1} GP application were dominant at 45 d in *G. aggregatum* inoculated soils (Table 7).

Host Nutrient Contents

No appreciable changes were observed in N concentration in shoots at 45 d in both PL and GP amended soils (*Fig. 1a* and *4a*). At 90 d mycorrhizal plants in 5 g kg^{-1} PL amended and 15 g kg^{-1} GP amended soils (except *G. etunicatum*) showed decreased N content in their shoot tissues (*Fig. 1b* and *4b*). The root N content decreased with increasing concentrations of PL and GP amendments at 45 d. But at 90 d N content in root tissues of mycorrhizal plants was equal to control in both 10 g kg^{-1} PL and GP amended soils (*Fig. 1c, d* and *4c, d*).

The shoot tissue P decreased with increasing PL application at 45 d but generally increased in 90 d (*Fig. 2 a, b*). *A. scrobiculata* inoculated plants in 10 g kg^{-1} GP amended soil had the maximum root tissue P at 45 d and 90 d (*Fig. 5c, d*).

At 45 d shoot K content had no notable variations in plants grown in PL amended soils (*Fig. 3a*), but at 90 d shoot tissue K content slightly increased with rate of PLA (*Fig. 3b*). Though root K content declined with increasing concentrations of PL application at 45 d (*Fig. 3c*), such variations were not evident at 90 d (*Fig. 3d*). Further plants grown in 15 g kg^{-1} GPA soil had the lowest shoot K at 45 d and non-mycorrhizal plants in 10 g kg^{-1} GPA had more shoot K than the mycorrhizal counterparts (*Fig. 6a, b*). Potassium concentrations in roots were low at 45 d, with increasing rate of GP application, whereas at 90 d mycorrhizal plants

TABLE 7
Microfungal populations in goat pellet amended soils

	Microfungal population (x 10^3)											
Concentration (g kg^{-1})	5				10				15			
Fungal species	C	V1	V2	V3	C	V1	V2	V3	C	V1	V2	V3
45 d												
Aspergillus carneus	2	2	8	2	-	-	4	4	2	16	16	80
A. fumigatus	22	70	58	140	6	-	-	44	26	96	48	32
A. flavus	14	28	64	24	4	4	4	12	20	30	98	16
A. flavipes	-	4	6	6	-	2	6	10	6	10	20	10
A. pulverulentus	-	-	-	-	-	-	-	-	-	-	-	-
Mucor racemosus	4	-	-	-	-	-	2	4	-	-	-	-
Penicillium rubrun	-	2	-	-	-	-	-	-	-	-	-	-
Trichoderma koningii	6	-	-	-	-	-	-	2	-	2	2	4
Total	48	106	136	172	10	6	16	76	54	154	184	142
90 d												
Aspergillus carneus	16	22	70	26	-	20	06	30	-	-	30	12
A. fumigatus	28	46	2	20	-	52	50	62	22	48	64	74
A. flavus	22	24	18	116	8	20	14	10	8	-	14	10
A. flavipes	8	20	-	4	-	-	-	-	-	10	16	2
A. pulverulentus	4	22	-	62	-	28	-	-	4	-	2	2
Mucor racemosus	6	2	2	4	10	-	4	4	6	2	1	-
Penicillium rubrum	4	4	26	-	-	2	2	6	8	4	8	6
Trichoderma koningii	2	8	-	-	-	-	-	-	-	-	-	-
Total	90	148	118	232	18	122	76	148	48	64	135	106

C - control; V1 - *A. scrobiculata;* V2 - *G. aggregatum;* V3 - *G. etunicatum*

had more K in their root tissue in 5 and 15 g kg^{-1} GP amendments than non-mycorrhizal plants (*Fig. 6c, d*).

DISCUSSION

The reported results indicate a selective influence of organic amendments on AM fungi and plant growth. Increasing rate of PL application along with mycorrhizal inoculation stimulated plant growth. However, high levels of GP (15 g kg^{-1}) application reduced plant growth of mycorrhizal and non-mycorrhizal plants. Brechelt (1989) observed a similar reduction in growth of *Capsicum annuum* at high levels of staple manure application. The intensity of growth response to organic amendments and mycorrhizal inoculation is low compared to other reports in different plant species (Ramos *et al.* 1993; Mappaona *et al.* 1994; Sorensen *et al.* 1994). Mycorrhizal benefit in the form of enhanced nutrient and water uptake could be altered to a certain degree due to organic amendments. Studies by Mohan *et al.* (1991) suggests that soil microfungal populations like *A. fumigatus* and *A. flavus* could influence plant growth, as culture filtrates of these fungi reduced shoot growth in soybean. Further, the increase in soil microbial activities due to the carbon source applied can produce antibiotic substances, enzymes, organic acids or influence other microorganisms which could affect the efficiency of AM fungi in different ways (Azcon *et al.* 1989).

Plants inoculated with either *G. aggregatum* or *G. etunicatim* at 15 g kg^{-1} GP amended soils had lower root dry weight. Similar observations have been made in maize (Kothari *et al.* 1990), Citrus (Graham and Syvertson 1984) and Cotton (Price *et al.* 1989). The reduction in root dry weight has been attributed to decreased root lengths due to increased soil microbial activities and fierce competition among the micro-organisms and the roots for the available nutrients

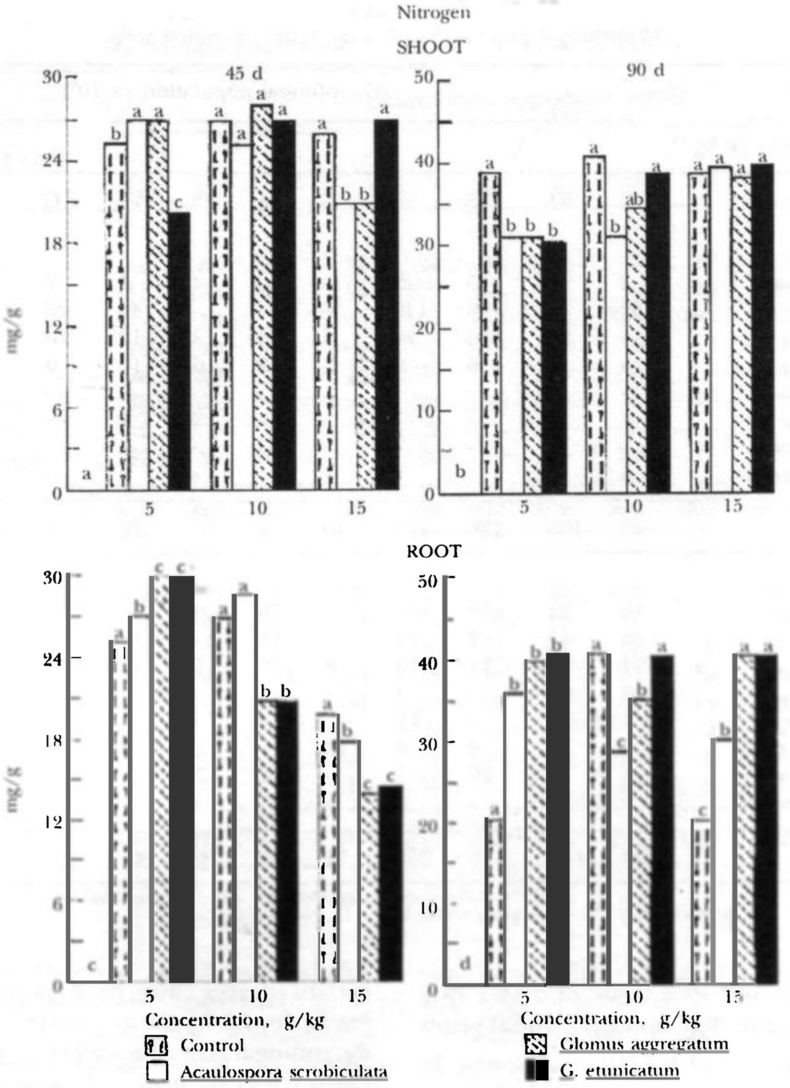

Fig. 1a-d. Effects of AM fungi and three different levels of Pongamia *leaf amendment on nitrogen content of plant shoot and root of horse gram at 45 and 90 days. Bars bearing the same letter(s) in each concentration are not significantly different according to Duncan's new multiple range test ($P \leq 0.05$).*

(Schonwitz and Ziegler 1989; Kothari *et al.* 1990). In addition, the rate of plant growth is determined by interactions between mycorrhizal infection and a number of nutritional and non-nutritional aspects of symbiont physiology (Smith and Gianinazzi-Pearson 1988).

The root: shoot ratios of mycorrhizal plants in organic matter amended soils had no significant variation compared to non-mycorrhizal plants. This contradicts the more common observation that mycorrhizal symbiosis generally lowers root: shoot ratio (Fitter 1982; Bass and Lambers 1988) and also indicates the less dependence of mycorrhizal fungi owning to the presence of organic matter (Azcon and Ocampo 1981).

Even though no significant variations existed for plant tissue N and P, mycorrhizal plants in general had more nutrients in their tissue than non-mycorrhizal plants. The inflow rates of nutrients from soil solution into roots for mycorrhizal plants is faster than non-mycorrhizal plants, which may attribute for the increased rates of plant growth and increased concen-

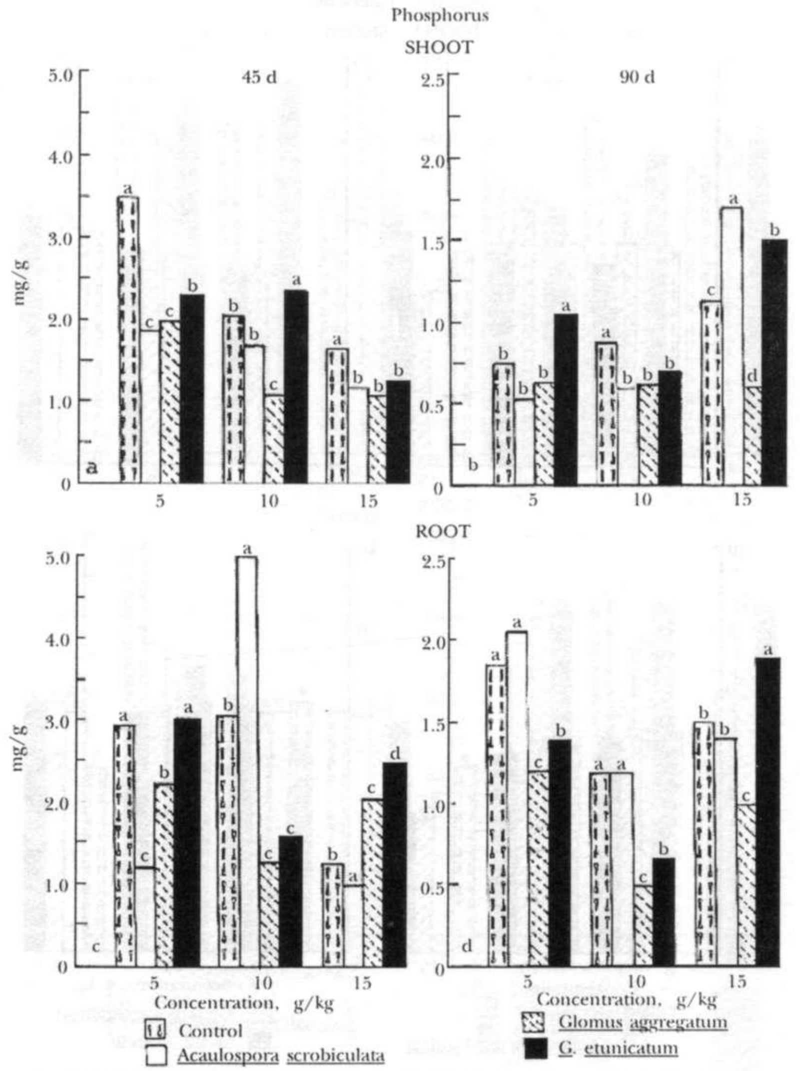

Fig. 2a-d Effects of AM fungi and three different levels of Pongamia *leaf amendment on phosphorus content of plant shoot and root of horse gram at 45 and 90 days (For further explanation see Fig. 1 footnote).*

tration of N and P in the tissues (Smith and Gianinazzi-Pearson 1988). Further, mycorrhizal roots exploit the soil profile, with extramatrical hyphae extending beyond the depletion zone surrounding the absorbing root and its hairs. The test plant horse gram is a nodulating legume; it is not surprising for nodulated mycorrhizal plants to accumulate more N since AM fungi have been reported to enhance N_2 fixation by the bacterial symbiont (Barea and Azcon-Aguilar 1983; Bethlenfalvay and Newton 1991). Though K accumulation was higher in mycorrhizal plants at low levels of organic amendments, higher rates of their application reduced K concentrations, which could be attributed to the effect of organic amendment on mycorrhizal colonization since AM fungi has been reported to aid plants in K uptake.

Root infection by *A. scrobiculata* was enhanced by increased rates of PL application. A similar effect was observed in *G. etunicatum* at 45 d *G. aggregatum* at 90 d, which is in accord with Harinikumar and Bagyaraj (1988) who also observed high mycorrhizal infection in response to PL application. Sheikh *et al.* (1975) indicated that addition of organic manure to soils that are

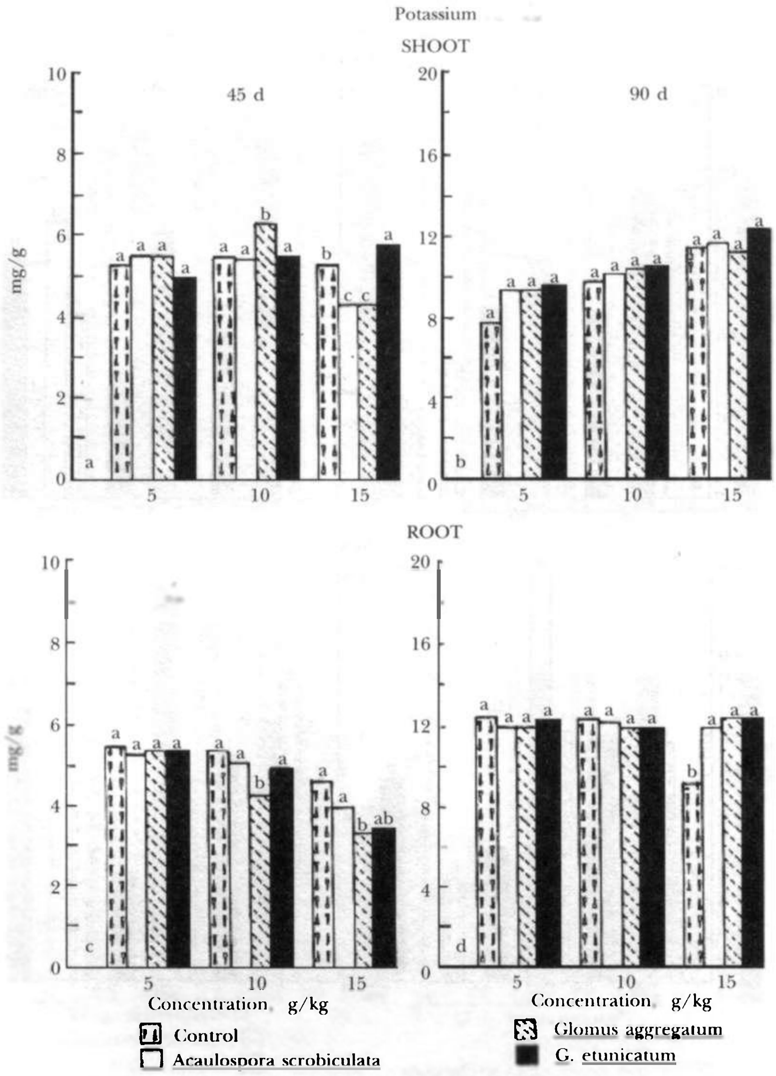

Fig. 3a-d Effects of AM fungi and three different levels of Pongamia *leaf amendment on potassium content of plant shoot and root of horse gram at 45 and 90 days. (For further explanation see Fir. 1 footnote).*

low in organic matter may enhance mycorrhizal development, but higher rates of GP amendment reduced mycorrhizal infection. Similar suppression was reported for pig and cow slurry application in a grassland by Christie and Kilpatrick (1992). These variations may be due to the indirect effect of different organic matter on their varying effect on soils structure, water holding capacity, nutrient mineralization etc.

Arbuscule formation in mycorrhizal plants raised in GP amended soils were low compared to mycorrhizal plants raised in PL amended soils at similar application rates. Arbuscules have a short life span and are presumably formed at times of P demand by the host (Dodd and Jeffries 1986; Dunne and Fitter 1989).

Although nodulation in mycorrhizal plants was either enhanced or unaffected by organic amendments, mycorrhizal plants raised in low (5 g kg^{-1}) levels of PL and high levels of GP (15 g kg^{-1}) amendments had fewer nodules compared to non-mycorrhizal plants. Nodulation and N_2 fixation are characterized by a high phosphorus demand (O' Hara *et al.* 1988). The influence of phosphorus on symbiotic N_2 fixation may be indirect, i.e. by stimulation of host plant growth (Robson *et al.* 1981) or direct, by more specific effects on nodule initiation, growth and

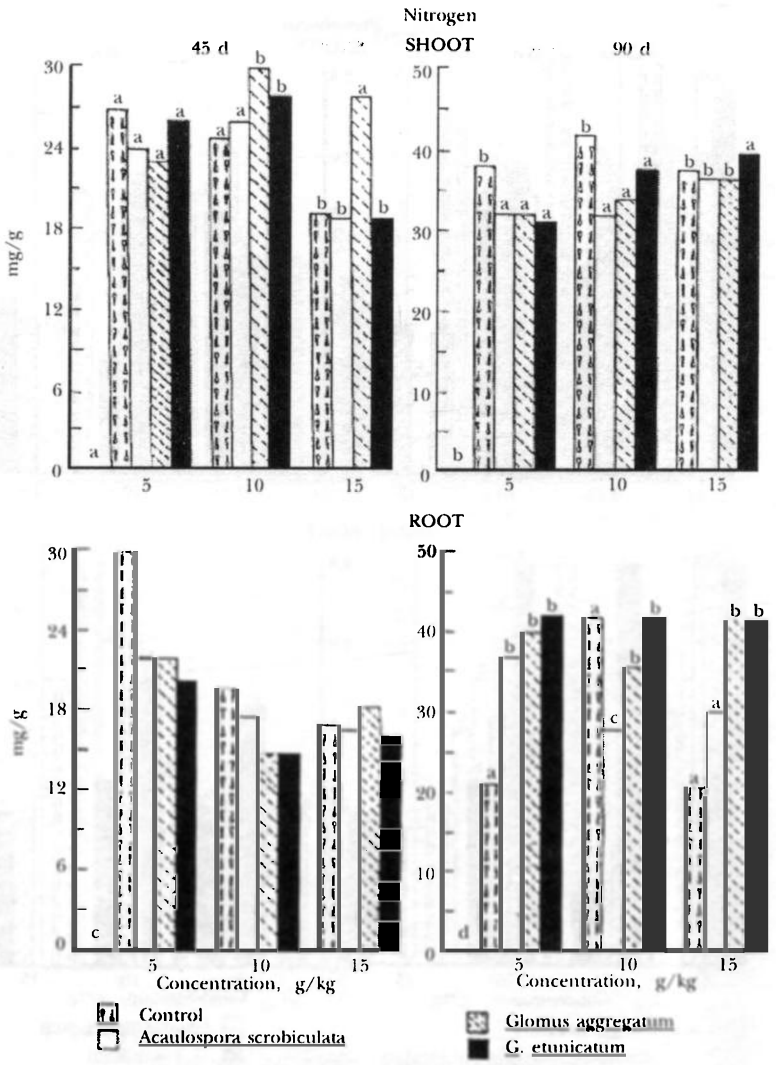

Fig. 4a-d Effects of AM fungi and three different levels of goat pellet amendment on nitrogen content of plant shoot and root of horse gram at 45 and 90 days. (For further explanation see Fig. 1 footnote).

functions. The reduction observed in the present study in some treatments might be due to the activities of soil fungi, since several species of aspergilli, *Penicillium* and *Trichoderma* are known to produce antibiotics which reduce nodulation (Lebed *et al.* 1978; Mohan *et al.* 1991). The antagonistic effect of soil fungi on nodule formation has also been recorded in *Trifolium* (Chhonkar and Subba Rao 1966) and soybean (Mohan *et al.* 1991).

Addition of organic matter altered the microfungal populations. This is in accord with Popova (1993), who demonstrated a direct relationship between microfungal populations and soil fertility. Microfungal populations in GP amended soils were low compared to PL amended soils. Further, a decrease in microfungal population at 90 d can be attributed to the depletion of organic resources due to hastened decomposition. Sivapalan *et al.* (1993) reported that organic matter amended soils supported twice the number and a wider range of fungal species than in unamended soil. But our study does not support this. Among various fungal genera isolated, *Aspergillus* had the most diverse species. *Aspergillus* species are known to

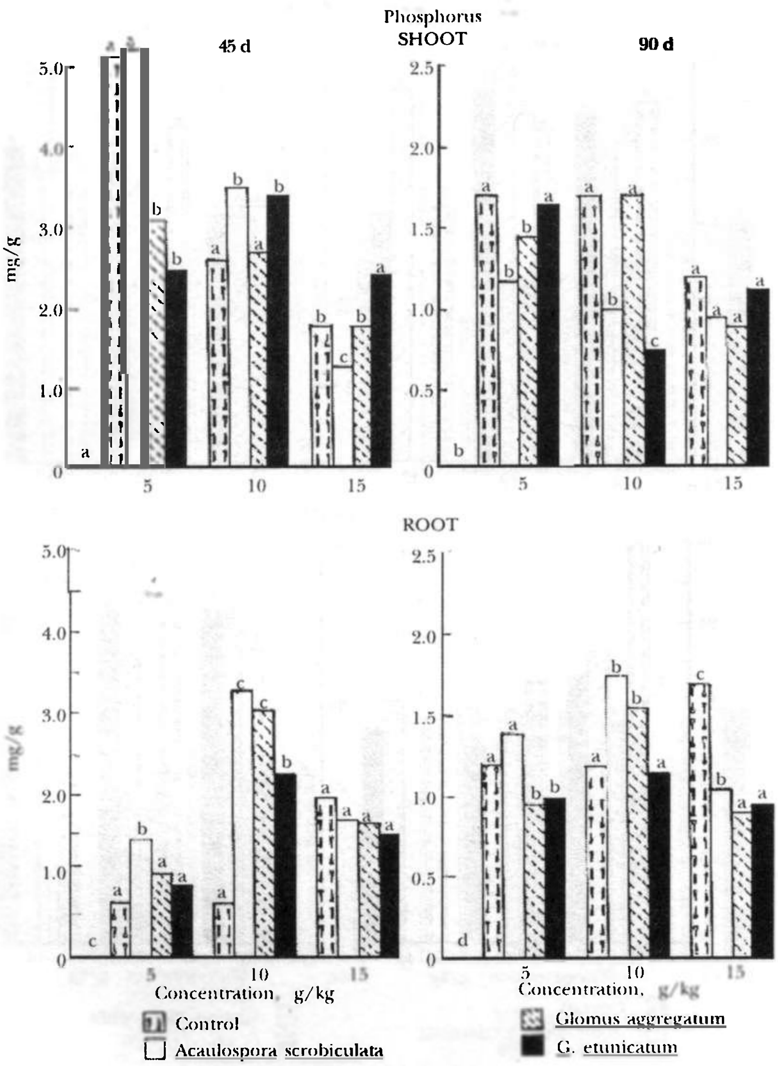

Fig. 5a-d Effects of AM fungi and three different levels of goat pellet amendment on phosphorus content of plant shoot and root of horse gram at 45 and 90 days. (For further explanation see Fig. 1 footnote).

tolerate different environmental conditions; this has already been proven through laboratory experiments (Dubost 1969; Rai *et al.* 1970; Venkataraman and Rajyalakshmi 1971). The genus *Penicillium* was represented by *P. rubrum* and populations of *Mucor racemosus* was fewer than to other microfungal populations. These observations are in accord with those of Popova (1993) who reported a decline in *Penicillium* species diversity and biomass of *Mucor* with increasing soil fertility. The low abundance of these species in the present study implies an increase in soil fertility owing to the absence of antibiosis.

Population and diversity of microfungi in the present study were unaffected by AM fungal inoculation, which is in agreement with Ames *et al.* (1987) and Secilia and Bagyaraj (1988). These authors have also reported the absence of alterations in the rhizosphere microfungal population due to AM fungal infections.

This study clearly indicates the varied influence of organic matter on plant growth, AM fungi, soil microfungi and nodulation with organic manure types and concentrations of their application.

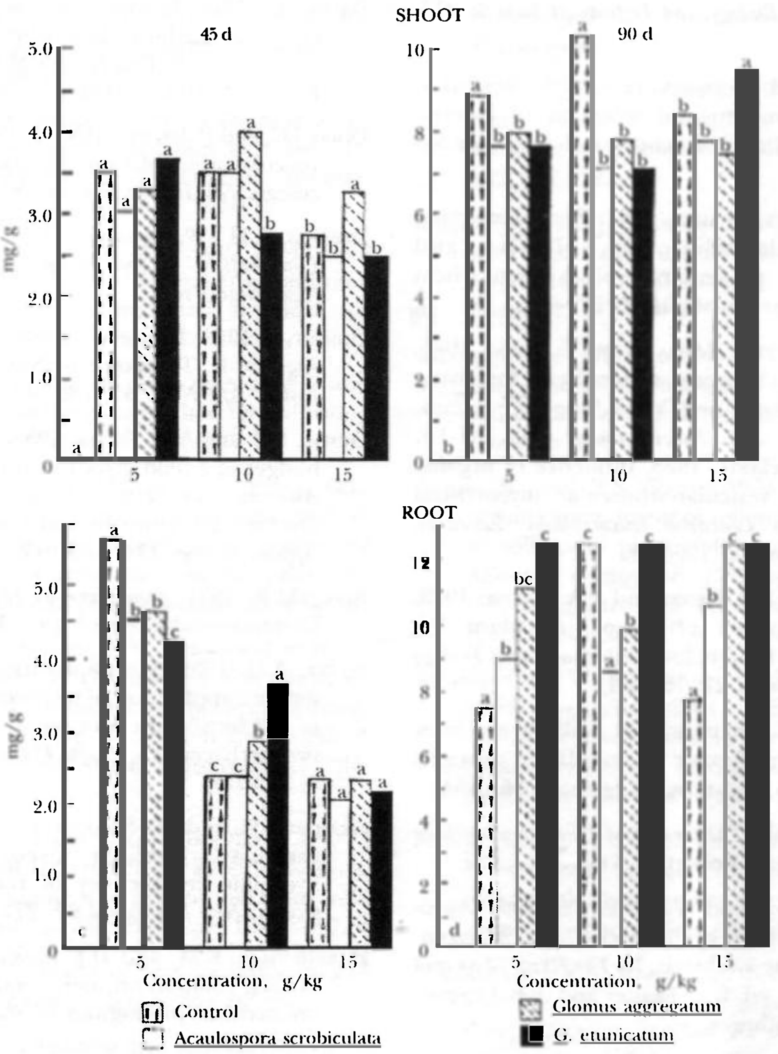

Fig. 6a-d Effect of AM fungi and three different levels of goat pellet amendment on potassium content of plant shoot and root of horse gram at 45 and 90 days. (For further explanation see Fig. 1 footnote).

CONCLUSION

The present study reveals that application of organic matter improved plant growth, rhizobial nodulation and plant nutrient content, which varied with endophytes, organic matter types and their concentrations. Higher rate of PL amendment and low rate of GP amendment favour AM colonization. The plant K concentration increased with increasing rate of organic matter at 90 d. Higher rate of PL application favours microfungal establishment than GP application. The genus *Aspergillus* is dominant and most diverse species isolated. Work is in progress to identify the most effective/ favourable AM fungal and organic matter for crop improvement under field conditions.

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Analytical Attributes of Humic Acids Derived from Tropical-based Resources

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ABSTRAK

Asid Humik (HA), sejenis kompaun yang mempunyai sifat fizik dan kimia yang unggul, telah banyak dikaji dan dieksploitasi. Namun, maklumat tentang HA hasil dari sumber tropika masih kurang. Percubaan telah dilakukan untuk mencirikan HA yang diekstak dari gambut tropika dan efluen kilang kelapa sawit (POME), menggunakan kaedah-kaedah fizik dan kimia. Analisis elemen terhadap 3 jenis HA yang dikaji mendapati kandungan C berjulat dari 48.94 hingga 57.87%, H dari 4.90 hingga 8.20%, N dari 1.93 hingga 8.05%, dan O dari 30.96 hingga 40.85%. Analisis kumpulan berfungsi menunjukkan bahawa HA yang diekstrak dari gambut adalah lebih reaktif berbanding dengan HA dari POME. Ketumpatan optik dan ujian spektra memaparkan HA sebagai mempunyai tahap kearomatikan yang berbeda. Secara am, data HA daripada kajian ini (khasnya HA yang diekstrak dari gambut) menyokong data daripada kajian lain.

ABSTRACT

Humic acids (HA) are widely researched and exploited compounds dae to their exceptional chemical and physical properties. However, information on tropical-based HA is still lacking. This attempted to characterize HA derived from tropical peat and POME using chemical and physical methods. Elemental analysis of the HA showed that C ranged between 48.94 and 57.87%, H between 4.90 and 8.26%, N between 1.93 and 8.05% and O between 30.96 and 40.85%. The functional group analysis indicated that peat-derived HA were more reactive than those derived from POME. Optical density and spectral examinations revealed that the HA had varying degrees of aromaticity. Generally, data obtained from the HAs studied (particularly from peat) agreed closely with those reported elsewhere.

Keywords: humic acids, peat, POME

INTRODUCTION

Organic matter content strongly affects soil fertility by increasing the availability of plant nutrients, improving the soil structure and water-holding capacity, and acting as an accumulation phase for toxic heavy metals in the soil environment (Stevenson and Fitch 1981; Stevenson 1985). To this end, the recycling of organic materials via their application to the soil can be an important and promising practice for agricultural activities. Humic substances are bulk constituents of organic matter, and have been shown to possess exploitable properties that fit agricultural, environmental, industrial and medical applications (Schnitzer 1986). By definition, humic acid is the fraction that is soluble in a dilute base, but is precipitated upon acidification.

Analytical data on HA isolated from diverse sources, particularly soils, peat, marine and lake sediments, and natural waters, have been steadily accumulating. However, information on HA derived from tropical resources is still limited and thus merits further exploration. It is well established that HA originating from different sources are different, and vary as a function of multiple factors such as climate, vegetation and nature of the substrate.

The present investigation was aimed at characterizing HA derived from tropical peat and palm oil mill effluent (POME), both of which are common sources of organic matter added to Malaysian soils. A better understanding of the chemical and physical properties of HA is imperative to comprehend the transformations the acids will undergo over time within the soil environment.

MATERIALS AND METHODS

Organic Materials

Peat was sampled between 0.5 and 2.0 m depth from the surface of a peat deposit in Dengkil, Selangor, Malaysia. Meanwhile, POME samples were sourced from a Guthrie palm oil mill in Rantau, Negri Sembilan. Two categories of POME, namely a decomposing POME and a clarified POME, were used in the study. The decomposing POME was generated from a 3-month (approx.) ponding process while the clarified POME emanated from a post-pressing process within the settling tank. Peat and both POME were then oven-dried at 60°C for 3 d, finely ground to pass through a 1.0-mm sieve, and extracted for HA.

Extraction of HA

Fifteen grams of peat/POME were weighed into a 250-ml polypropylene flask, 150 ml of 0.2 M NaOH solution was added, and the system was shaken intermittently for 24 h at room temperature. The resulting dark coloured supernatant was separated from the residual peat/POME by centrifugation at 10,000 rpm for 15 min, then acidified to pH 1 with 3 M H_2SO_4 and allowed to stand at room temperature for 24 h to facilitate coagulation of HA (Schnitzer 1982).

The coagulated HA was purified by reprecipitation and redissolution using H_2SO_4 and NaOH, respectively. After the final precipitation, the HA was placed in cellulose tubes and dialysed against distilled water for 7 d with diurnal water change. The mixture was then centrifuged. Residues of HA were subsequently shaken for 48 h with excess HCl-HF mixture (0.5%, v/v) to desorb the HA completely of silicate impurities. The acid mixture was removed by centrifuging (10,000 rpm for 30 min) and decanting the supernatant. The residual HA was thoroughly washed with distilled water untill free of chloride (as shown by tests with silver nitrate) and finally dried at ambient temperature (< 40°C). Yield of HA were expressed as percentage derived on a weight per weight basis.

Characterisation Techniques

Purified HA were subjected to elemental and funtional group analyses, optical density determination and spectroscopic examination. The ash contents of HA were obtained by igniting the acids at 700oC for 4 h. Carbon, H and N were estimated by dry combustion using a CHN analyser (VARIO-EL), and O was calculated by difference. Carboxyl groups were measured by the Ca(OAc)2 method (Schnitzer and Gupta 1965) and phenolic hydroxyl groups by the colorimetric method using Folin-Cicocaltyeu's phenol reagent (Tsutsuki and Kuwatsuka 1978). Total acidity was ascribed to the sum of carboxyl and phenolic hydroxyl groups. Quinonoid C=O groups were determined by ferrous reduction in alkaline TEA solution using bulk electrolysis (Glebko *et al.* 1970). The E_4:E_6 ratio was determined by dissolving 2 mg of HA in 10 ml of 0.005 N NaHCO3 solution and measuring its optical density at 465 and 665 nm. Fourier transform infra-red analysis of HA was carried out by mixing 0.5 mg of HA with 400 mg infra-red grade KBr, and pressing into a pellet (Tan 1982). Spectra were recorded on a Perkin Elmer 1650 FTIR spectrophotometer for wavenumbers ranging from 4000 to 400 cm^{-1}.

RESULTS AND DISCUSSION

The chemical and physical attributes of HAs studied are given in Table 1. Peat yielded 16.73% (w/w) HA while the decomposing and clarified POME registered 1.82 and 3.47 HA, respectively. Higher yield of peat-derived HA, as comparaed to both POME, could be attributed to its higher organic matter content. Yield of HA derived from peat obtained is comparable to those reported by Husni *et al.* (1996) and Norhayati (1989).

Loss on Ignition

Percentage loss on ignition relates, indirectly, to the purity of HA. Higher loss on ignition means lower ash content, thus higher purity of the HA. Results indicated that ash contents of the acids were exceptionally low, which was possibly due to the exhaustive purification steps employed.

TABLE 1
Chemical attributes of humic substances derived from POME and peat

	Humic acid		
	POME[a]	POME[b]	Peat
% yield	3.47	1.82	16.73
% LOI[c]	97.50	99.50	96.00
Elemental make-up:			
%			
C	57.87	48.94	52.32
H	**8.26**	5.76	4.90
N	**2.91**	8.05	1.93
O	30.96	37.25	40.85
O:H	3.75	6.47	8.34
C:N	19.89	6.08	27.11
C:H	7.01	8.50	10.68
Functional groups:			
meq. g^{-1}			
Quinonoid			
C = O	**2.52**	2.85	3.34
[x]Carboxylic			
COOH	**2.22**	2.08	4.06
[y]Phenolic			
OH	**3.34**	3.27	2.63
zTotal acidity	**5.56**	5.35	6.69
Optical density:			
$E_4:E_6$ ratio	4.22	6.09	7.67

[a]clarified, [b]decomposed
[c]loss on ignition
z = x + y

Elemental Analysis

Elemental composition of peat-derived HA conform to those reported elsewhere (Visser 1987, Garcia *et al.* 1991, Husni *et al.* 1996). Acids derived from POME showed higher values of H and N but lower value of O compared to peat-derived HA. Nevertheless the C level of peat-derived HA was intermediate between the HA derived from POME.

Data on atomic ratios showed higher values in peat-derived HA than those of POME-derived HA. Higher O:H value indicates poor aromatization and/or poor condensation, while narrower C:H ratio reflects the occurence of smaller number of saturated groups (Kononova 1966). The C:N ratio of HA submitted to the order of peat>clarified POME>decomposing POME. Lower C:N value infers that mineralization was more intense (Norhayati 1989).

Functional Group Analysis

The reactivity of HA is greatly influenced by the nature and amount of oxygen-containing functional groups present. According to Schnitzer (1982), the acidity of HA is primarily due to the occurrence of ionizable hydrogens in aromatic and aliphatic carboxylic and phenolic OH groups, and is largely responsible for the exchange capacities of HA, which may have chelating effects that influence plant nutrition.

Comparatively, total acidity, carboxylic and quinonoid contents were higher in peat-derived HA, while POME-derived HA recorded higher phenolic OH content. However, disparity in functional group values between both POME-derived HA was narrow. Carboxylic and phenolic OH values obtained for peat-derived HA are higher than those reported by Husni *et al.* (1996) for tropical peat and Norhayati and Verloo (1984) for tropical soil. Meanwhile, HA from POME appear to be similar in carboxylic and phenolic OH contents to HA derived from a commercial humate (Enersol Micronutrient), as reported by Lobartini *et al.* (1992). Peat-derived HA contained approximately 1.5-fold higher amounts of carboxylic than phenolic OH, thus suggesting that the pH-dependent charge in peat is regulated by carboxyls. In POME-derived HA, phenolic OH dominated over carboxylic OH.

Optical Density

The $E_4:E_6$ values were highest in peat-derived HA, followed by decomposing POME-derived HA and clarified POME-derived HA. Wider ratios reflect a low degree of aromatic condensation and infer the presence of relatively large proportions of aliphatic structures (Kononova 1966). HA derived from clarified POME indicated greater aromatic condensation (narrower E4:E6) than HA derived from decomposing POME. Incidentally, the C:N ratio of clarified POME-derived HA was also higher than that from decomposing POME-derived HA. Thus, reduced aromaticity of HA from the decomposing POME could be attributed to intense mineralization, as indicated by the lower C:N ratio.

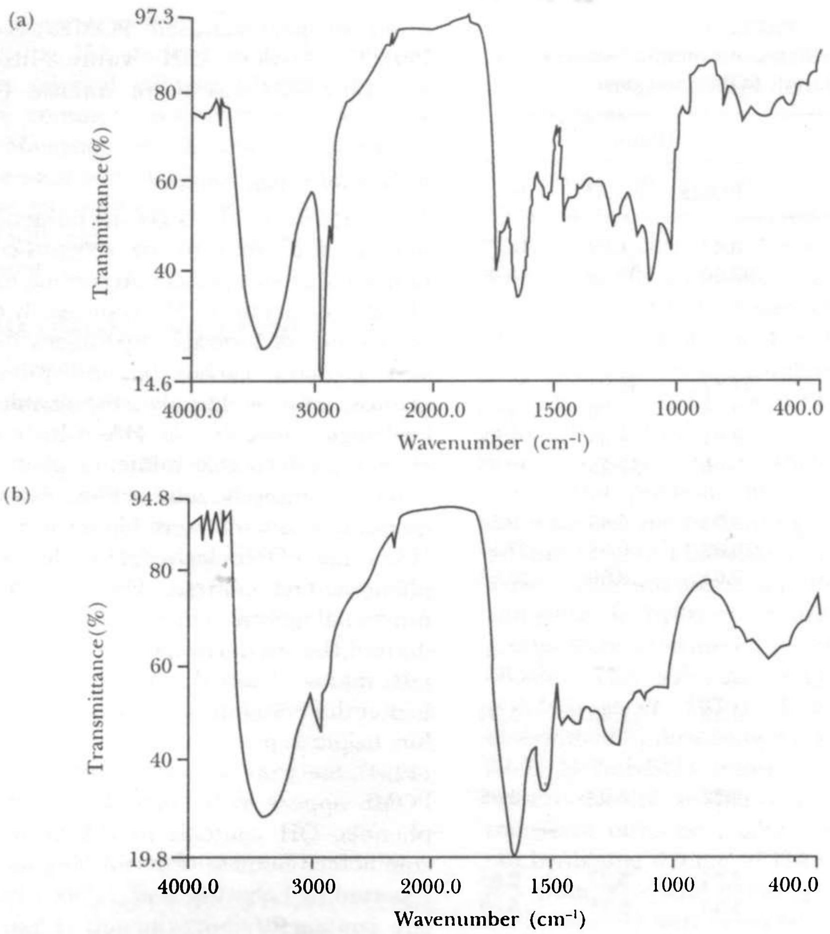

Fig. 1. FTIR spectra of HA derived from a) clarified POME and b) decomposed POME

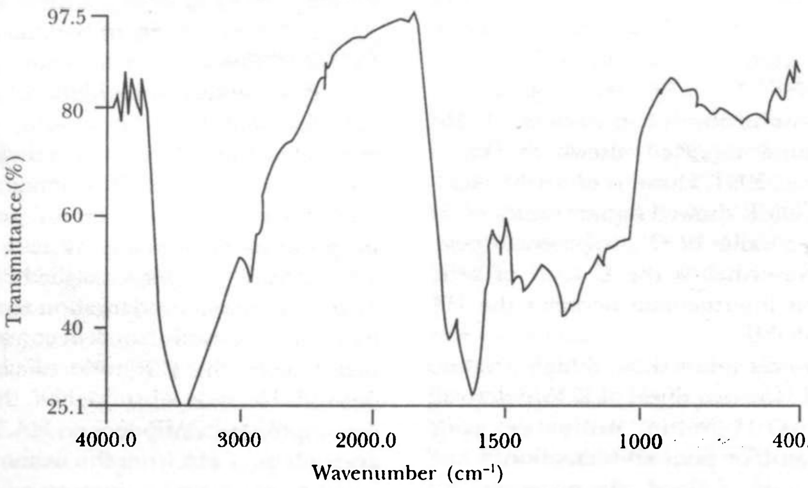

Fig. 2. FTIR spectra of HA derived from peat

Spectral Characteristics

Fourier transform infra-red (FTIR) spectra of the HA are shown in *Figure 1, 2.* Interpretation of the FTIR spectra are based on Schnitzer and Khan (1972). The HA displayed main absorption bands in the regions of 3400 cm^{-1} (hydrogen-bonded OH), 2900-2850 cm^{-1} (aliphatic C-H stretch), 1730-1715 cm^{-1} (C=O of C_2H, C=O of ketonic carbonyl), 1650-1630 cm^{-1} (C=O stretch of quinones, COO^-, hydrogen-bonded C=O), 1550-1520 cm^{-1} (C=C of aromatic rings), 1460-1450cm^{-1} (CH_2) and 1420-1400 cm^{-1} (CH_2, COO^-). Absorption bands in the 1290-1200 and 1160-1130 cm-1 regions were assigned to symmetrical bonding of aliphatic CH_2, OH or C-O stretch of various groups. Meanwhile, absorbances in the 930-660 cm^{-1} range included symmetrical and unsymmetrical C-H bonding, structural and out of plane vibrations of C-H in aromatic rings.

The 3400 cm^{-1} assignment is supportive of Stevenson's suggestion (1982) that HA engage in pronounced hydrogen bonding. The 2900-2850 cm-1 assignment, usually superimposed in the shoulder of the broad O-H stretching band, confirmed the presence of large concentrations of aliphatics. The stronger band at 1650 cm^{-1} infers higher concentration of COOH groups. Weak bands at 1550-1520 and 1460-1450 cm^{-1} observed in HA under study are in agreement with those reported by Husni *et al.* (1996) and Garcia *et al.* (1991). They deduced that HA extracted using alkali extractant exhibited more pronounced aromaticity compared to that using pyrophosphate. The weak band obtained at 1290-1200 cm^{-1} can be attributed to OH, from COOH deformation.

CONCLUSION

Comparatively, peat yielded higher HA than POME. Elemental composition of peat-derived HA compared closely with data reported by Husni *et al.* (1996). The HA derived from POME, however, varied in elemental composition. Clarified POME-derived HA registered higher C and H but lower N and O contents than decomposing POME-derived HA. Contents of C, H and O of peat-derived HA and that of decomposing POME-derived HA were similar. Quinonoid C=O and carboxylic groups, and total acidity were recorded marginally higher in peat-derived HA, compared to POME-derived HA, whilst the reverse was found for phenolic OH. This suggests that the reactivities of the HA are comparable. Optical measurements revealed aromatic characteristics for the HA, which were confirmed by the FTIR spectra. The Degree of aromaticity varied only marginally among the HA.

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Survival and Early Growth of *Acacia mangium, Ceiba pentandra* and *Casuarina equisetifolia* on Sandy Tin Tailings

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ABSTRAK

Satu kajian di ladang bekas lombong telah dijalankan untuk menilai tumbesaran tiga spesis pokok (Acacia mangium, Ceiba pentandra *dan* Casuarina equisetifolia) *dengan menabur dan tidak menabur baja dan penanaman tiga spesis penutup bumi* (Centrosema pubescens, Calopogonium muconoides *dan* Puereria phaseoloides). *Kajian ini telah dijalankan di Kampung Pasir, Semenyih, Ulu Langat, Selangor. Baja (NPK) sebanyak 300 g telah ditaburkan pada anak pokok tiga bulan sekali dalam masa setahun. Ketinggian dan perepang pokok telah dikira selepas 23 bulan dari hari pokok ditanam. Sampal tanah juga telah diambil untuk analisa makmal. Keputusan menunjukkan bahawa tiga spesis pokok boleh tumbuh dengan baik walaupun tanpa baja dan tumbesaran terdapat perbezaan yang ketara diantara pokok-pokok itu. Acacia mangium menunjukkan kadar pertumbuhan yang tertinggi diikuti oleh* Ceiba pentandra *dan* Casuarina equisetifolia. *Tanaman penutup bumi telah meningkatkan nutrien-nutrien dalam tanah. Kesan daripada kajian ini adalah spesis pokok Acacia mangium boleh digunakan untuk memulihkan tanah bekas lombong manakala* Ceiba pentandra *dan* Casuarina equisetifolia *juga boleh digunakan tetapi tumbesaran tidak setanding dengan* Acacia mangium.

ABSTRACT

A field study was carried out on tin tailings to evaluate the growth performance of three timber species (Acacia mangium, Ceiba pentandra *and* Casuarina equisetifolia) *with and without fertilization and with three species of cover crops* (Centrosema pubescens, Calopogonium muconoides *and* Puereria phaseoloides). *The experiment was carried out at Kampung Pasir, Semenyih, Ulu Langat, Selangor. NPK compound fertilizer was applied at the rate of 300 g per seedling every three months during the first year of the study. Height and diameter were measured 23 months after planting. Soil samples were also collected for laboratory analysis. The results showed that the three timber species can grow well even without fertilizer and the growth rates of the three species differ significantly. The fastest growth rate was recorded by* Acacia mangium *followed by* Ceiba pentandra *and* Casuarina equisetifolia. *The planting of cover crops slightly increased the nutrient status of the soil. Thus this experiment shows that timber species, particularly* Acacia mangium, *could be successfully used to rehabilitate abandoned ex-mining land, while* Ceiba pentandra *and* Casuarina equisetifolia *could also be used, but have slower growth rates than* Acacia mangium.

INTRODUCTION

Active tin mining in Malaysia began in the late nineteenth century and has been a major contributor to the nation's economy (Lim *et al.* 1981). Most tin production is obtained from dredging, gravel pumps and open mines (Anon 1991). The mining operations have resulted in environmental destruction such as siltation of river beds and drainage systems and the destruction of agricultural land. Tin tailing areas in Peninsular Malaysia are estimated to be cover about 113,500 ha (Chan 1990).

The tin mining activities have left three types of tailings: sand tailings, slime tailings and sandy slime tailings. Slime tailings with a proper drainage system have been successfully used for producing fruits and vegetables. However, there are problems with sand tailings. Many studies have been conducted to rehabilitate the ex-tin mining land and to better utilize these tin tailings for agriculture, including the use of natural rubber skim latex, palm oil mill effluent, sewage sludge, bitumen and emulsion to improve the physical and chemical properties of the tin tailings (Lim *et al.* 1981). Afforestation and agroforestry practices have been recognized as suitable for rehabilitation of ex-tin mining areas (Mitchell 1957; Ang 1986, 1994; Nik Muhamad *et al.* 1994).

The main objective of this study was to evaluate the growth performance of three timber species with two levels of fertilization and three species of cover crops on the ex-tin mining land.

MATERIALS AND METHODS

Site Description

The study was conducted at Kampung Pasir Semenyih, Selangor, about 20 km from Universiti Putra Malaysia campus, on soil belonging to the order Ultisol (Nik Muhamad *et al.* 1994) which is sandy in texture. The study area is relatively flat and has an average rainfall of about 2506.9 mm per year. The monthly rainfall figues for April 1994 - February 1996 are presented in Table 1. Average annual temperature ranges from 20 - 33°C. The water table is 2 m from the soil surface, determined by the digging of a 2-m deep soil pit. The soil was moist above this level due to capillary rise of water, but the water was available only at a depth of 2 m. The physical and chemical properties of the soil before planting are given in Table 2.

TABLE 1
Monthly rainfall (mm) at Semenyih during the study period

Month	1994	1995	1996
	Rainfall (mm)		
January		75	80
February		120	128
March		165	
April	205	218	
May	245	240	
June	182	170	
July	195	200	
August	230	245	
September	348	370	
October	300	280	
November	290	270	
December	185	160	

TABLE 2
Soil properties (before planting)

A. Physical	
Coarse sand (%)	49.10
Fine sand (%)	37.65
Silt (%)	4.29
Clay (%)	8.06
Moisture content (%) (0-40 cm depth)	0.55
B. Chemical	
pH (H_2O)	4.47
N (%)	0.02
P (ppm)	6.75
K (meq/100 g soil)	0.09
Ca (meq/100 g soil)	1.35
Mg (meq/100 g soil)	0.50
CEC (meq/100 g soil)	1.54

Experimental Layout

Seedlings of *A. mangium, C. equisetifolia* and *C. pentandra* were planted in early April 1994 at a spacing of 3 x 3 m. The experimental area was divided into 4 blocks (replicates) of 45 x 45 m, each block consisting of nine subplots (Table 3), each with 25 seedlings. Three cover crops (*Centrosema pubescens, Calopogonium muconoides* and *Puereria phaseoloides*) were planted in rows between the tree species. The cover crops were planted only once, at the beginning of the experiment, and gave 100% coverage for each of the tree species. A 9-m buffer zone was established between the blocks.

Fertilizer was applied to two blocks at 3-monthly intervals during the first year of the

TABLE 3
Plot layout

	R1			R2	
T2A3	T1A1	T2A1	T3A1	T2A3	T1A3
T3A1	T2A2	T3A2	T1A2	T1A1	T2A1
T1A2	T3A3	T1A3	T3A3	T3A2	T2A2
	R3			**R4**	
T3A2	T2A2	T1A2	T1A1	T1A2	T1A3
T1A3	T3A3	T2A3	T2A1	T2A2	T2A3
T2A1	T1A1	T3A1	T3A1	T3A2	T3A3

Note: T1- *Acacia mangium*
A1- *Centrosema pubescens*
T2- *Ceiba pentandra*
A2- *Calopogonium muconoides*
T3- *Casuarina equisetifolia*
A3- *Puereria phaseoloides*

study period at the rate of 300 g NPK blue (15:15:15) per seedling. The remaining two blocks were not fertilized. The fertilizer was applied 24 hours after rainfall 0.3 m away from the base of the seedlings in a 10-cm deep circular trench and lightly covered with soil.

Data Collection

Growth in terms of total height and diameter was monitored for 23 months during the study period (April 1994-February 1996). The initial average height and diameter of the seedlings were as follows: Height - *A. mangium* (68.3 cm), *C. pentandra* (73.6 cm) and *C. equisetifolia* (44.7 cm); Diameter - *A. mangium* (8.2 mm), *C. pentandra* (10.3 mm) and *C. equisetifolia* (8.5 mm). Survival rate one year after planting was 93% for *A. mangium*, 87% for *C. equisetifolia* and 89% for *C. pentandra*.

Soil samples were collected randomly from each subplot prior to and 23 months after planting. Soil sampling was done at depths of 0-20 and 20-40 cm, randomly from five sampling points within each of the subplots and composited to form a sample. A soil auger was used to collect the samples, which were kept in plastic bags before being oven dried. The results are presented as average values of two soil depths.

Data Analysis

The soil samples collected were air dried and sieved through a 2-mm sieve to ensure that soils with very coarse sand (1-2 mm particle size) could also be incorporated for analysis. The samples were analysed to determine the physical and chemical properties. The physical properties determined were soil texture (determined by the pipette method) and moisture content (determined by the gravemetric method).

The soil chemical properties determined were total N, available P, exchangeable Ca, Mg, K, pH and cation exchange capacity (CEC). Total N was determined by the Kjeldahl digestion procedure (Bremner 1962). Available P was determined using a spectronic-20 spectrophotometer. Exchangeable Ca, Mg, K were determined by the leaching method (1N NH_4OAc at pH 7.0) and analysed by using an atomic absorption spectrophotometer. Soil pH was determined at 1:2.5 soil/water solution by a glass electrode pH-meter. Total organic carbon was determined by the Walkley and Black method (1934).

The data were subjected to analysis of variance (ANOVA) to test the effects of the fertilizers and cover crops on the growth parameters of three tree species and soil properties.

RESULTS AND DISCUSSION

Soil Physical Properties

The results of the analysis of soil physical properties are shown in Table 4. These show that the plots planted with *A. mangium* had significantly higher moisture content (0.57%) than the *C. pentandra* (0.43%) and *C. equisetifolia* (0.41%) plots. This is probably due to the higher organic matter accumulated through litterfall under the *A. mangium* plot compared to the plots of the other two species. The moisture content of the *P. phaseoloides* (0.54%) and *C. muconoides* (0.49%) plots was significantly ($P<0.05$) higher than that of *C. pubescens* (0.39%) plot. There was a significant ($P<0.05$) difference in moisture content between the fertilized and unfertilized plots.

Generally, soil moisture content was very low compared to other types of soil. For instance, the moisture content of a normal agricultural soil is about 25%. According to Letey (1985), low soil moisture content will affect plant growth because of the direct relationship between water potential and soil water content.

The sand content was significantly ($P<0.05$) higher in the *C. pentandra* (86.79%) plot than in the plots of the other two tree species. The silt and clay contents were, however, significantly ($P<0.05$) higher in the *A. mangium* plot than in the other two plots. For plots on the cover crops, the sand content was higher in the *C. muconoides* plot whereas silt and clay contents were higher in the *C. pubescens* plot as than in the other two cover crop plots. Similarly, silt and clay contents were significantly ($P<0.05$) higher in the unfertilized plots than the fertilized ones whereas there was no significant ($P<0.05$) difference for sand content between these two plots. However, in quantitative terms, the differences in soil physical properties apparently caused by planting tree species and cover crops are too small to have any real impact on site quality.

The high percentage of sand (85.07-86.72%) causes high soil temperature during the day time. This is a limiting factor for tree growth (Ang 1994). High sand content in the soil also

TABLE 4
Soil physical properties

(a) between three timber species

Soil physical properties	Acacia mangium	Ceiba pentandra	Casuarina equisetifolia
Sand (%)	85.48b	86.79a	85.75b
Silt (%)	5.16a	4.35b	4.10b
Clay (%)	10.16a	8.02b	7.64b
M. C. (%)	0.57a	0.43b	0.41b

(b) between the cover crop species

Soil physical properties	Centrosema pubescens	Calopogonium muconoides	Puereria phaseoloides
Sand (%)	86.02ab	86.51a	85.48b
Silt (%)	4.75a	4.50a	4.36a
Clay (%)	9.04a	8.14b	8.64ab
M.C. (%)	0.39a	0.49b	0.54b

(c) between two levels of fertilizer

Soil physical properties	with fertilizer	without fertilizer
Sand (%)	86.14b	85.26b
Silt (%)	4.29b	4.78a
Clay (%)	8.06b	9.15a
M.C. (%)	0.55a	0.39b

Note: Means with the same letter are not significantly different ($P<0.05$) as determined by Duncan's new multiple range test
M.C.- moisture content

increases the porosity and reduces the water retention capacity (Ang 1994) and will cause excessive drainage and leaching of nutrients. According to Shamsuddin *et al.* (1986), a high sand levelwill slow down the process of soil structure development.

Soil Chemical Properties

The results of the analysis of soil chemical properties are shown in Table 5. The pH value, exchangeable Ca, Mg and CEC showed significant ($P<0.05$) difference among the three tree species. A. mangium recorded the highest values, followed by *C. equisetifolia* and *C. pentandra* plots, probably due to the higher accumulation of organic matter through litterfall under *A. mangium* plots. There was no significant ($P<0.05$) difference between plots of the three tree species for total N, available P and exchangeable K. However, available P was highest in *C. equisetifolia,* followed by *A. mangium* and *C. pentandra* plots. Organic carbon was highest in the *A. mangium* plot, and this was significantly ($P<0.05$) different from the other two tree species plots.

In the plots under cover crops, exchangeable Ca, organic carbon and CEC values showed significant ($P<0.05$) difference between the plots, *C. pubescens* plot giving the highest value for exchangeable Ca whereas the *C. muconoides* plot recorded the highest value for CEC. Similarly, only exchangeable Ca and CEC values were significantly ($P<0.05$) higher in fertilized plots than the unfertilized ones.

The results show that total soil N after planting with tree crops, cover crops with and without fertilizer application was still low (0.04%) compared to the other agricultural

TABLE 5
Soil chemical properties

(a) between three timber species

Soil chemical properties	*Acacia mangium*	*Ceiba pentandra*	*Casuarina equisetifolia*
Org C	1.12a	0.92b	0.88b
pH	4.87a	4.73c	4.76b
N (%)	0.04b	0.03b	0.03b
P (ppm)	9.59a	8.60a	10.40a
K (meq/100 g soil)	0.11a	0.12a	0.11a
Ca (meq/100 g soil)	1.12a	0.84c	0.95b
Mg (meq/100 g soil)	0.57a	0.48b	0.52ab
CEC (meq/100 g soil)	1.95a	1.87c	1.88b

(b) between the cover crop species

Soil chemical properties	*Centrosema pubescens*	*Calopogonium muconoides*	*Puereria phaseoloides*
Org C	1.08a	0.82b	0.88b
pH	4.80a	4.80a	4.78b
N (%)	0.03a	0.03a	0.03a
P (ppm)	10.12a	9.32a	9.15a
K (meq/100 g soil)	0.12a	0.11a	0.12a
Ca(meq/100 g soil)	1.04a	0.97b	0.88c
Mg (meq/100 g soil)	0.54a	0.52a	0.51a
CEC (meq/100 g soil)	1.90b	1.96a	1.84c

(c) between two levels of fertilizer

soil chemical properties	with fertilizer	without fertilizer
Org C	0.96a	0.92a
pH	4.95a	4.95a
N (%)	0.03a	0.03a
P (ppm)	11.02a	10.82a
K (meq/100 g soil)	0.13a	0.12a
Ca (meq/100 g soil)	0.81a	0.74b
Mg (meq/100 g soil)	0.53a	0.54a
CEC (meq/100 g soil)	2.09a	2.06b

Note: Means with the same letter are not significantly different ($P<0.05$) as determined by Duncan's new multiple range test

soils under Malaysian conditions, which is about 0.12% (Law and Tan 1973). The results of the present study confirmed the findings of Mitchell (1957) because of the high leaching process in the soil and low organic matter content. This is also related to the high sand content and the high soil temperature (Black 1968). Similarly, CEC in the soil is very low (2.09 meq/100g soil) compared to the normal soils under Malaysian conditions (>100 meq/100g soil) (Law and Tan 1973), probably due to the low clay content (<10%) in tin tailing areas. In general, it can be concluded that soil chemical properties were little influenced, quantitatively, by the planting of tree or cover crops.

Height Growth

A. mangium showed the fastest height growth of the three tree species (Table 6). There was, however, no significant ($P<0.05$) difference in

TABLE 6
Growth performance

Growth parameters	Cover crops	*A. mangium* F	*A. mangium* WF	*C. pentandra* F	*C. pentandra* WF	*C. equisetifolia* F	*C. equisetifolia* WF
Height	*C. pubescens*	615c	620c	270bc	280b	170d	290b
(cm)	*C. muconoides*	580d	760b	290b	380a	280b	300a
	P. phaseoloides	780a	758b	270bc	220d	180d	240c
Diam	*C. pubescens*	78c	81bc	58b	68a	26c	28c
(mm)	*C. muconoides*	75c	85b	67a	72a	34b	38a
	P. phaseoloides	120a	83b	57b	55b	33b	34b

Note: F- fertilized WF- without fertilizer
Means with the same letter(s) are not significantly (P<0.05) different as determined by Duncan's new multiple range test

height growth between *C. pentandra* and *C. equisetifolia. A. mangium* interplanted with *P. phaseoloides* (fertilized) recorded the highest height growth followed by *A. mangium* with *C. muconoides* (unfertilized) and *A. mangium* with *P. phaseoloides* (unfertilized). The other two tree species (*C. pentandra* and *C. equisetifolia*) recorded the maximum height growth in combination with *C. muconoides* (unfertilized). Interestingly, the results show that generally the trees in the unfertilized plots have better height growth than trees in the fertilized plots. This is possibly due to the nutrients taken by the cover crops in fertilized plots. In simultaneous agroforestry where the tree and crop components grow at the same time and sufficiently close to each other, there is competition for light, water or nutrients (Sanchez and Palm 1996). Thus it might be possible that the competition for nutrients between trees and cover crops led to reduced height growth in fertilized plots.

Diameter Growth

A. mangium recorded the greatest diameter growth, followed by *C. pentandra* and *C. equisetifolia* (Table 6), and the growth was significantly (P<0.05) different between the three tree species. A. mangium interplanted with *P. phaseoloides* (unfertilized) showed the highest diameter growth followed by *A. mangium* with *C. muconoides* and *C. pubescens* (both unfertilized), respectively. *C. pentandra* and *C. equisetifolia* showed maximum diameter growth with *C. muconoides* (unfertilized). Similar to height growth, unfertilized plots generally had higher diameter growth than the fertilized ones, possibly for the reason explained earlier.

The results clearly demonstrated better growth performance of *A. mangium* than the other two tree species on sandy tin tailings because *A. mangium* is a pioneer species that can grow very well in rocky, disturbed and even on sandy soils. Ramli (1995) reported that *A. mangium* recorded the highest growth on ex-tin mining land. Similarly, Zakari (1990) also reported the successful planting of *A. mangium* Willd. on sandy ex-tin mining land in Semenyih.

C. pentandra has also established well on this ex-tin mining land. Earlier, Paudyal and Nik Muhamad (1992) reported that *C. pentandra* can be used to rehabilitate the ex-tin mining land. Similarly, *C. equisetifolia* has shown promising results for such rehabilitation.

Fertilizer application at the rate of 300 g NPK per seedling may not be sufficient as there was significantly poorer tree growth. Similarly, there was little quantitative effect on soil properties before and after planting tree species with cover crops. This is probably because of high leaching of nutrients and also changes in soil properties in poor soils, such as, tin tailings, take a longer time period to occur.

CONCLUSION

All the three tree species can grow well on sandy tailings. *A. mangium* showed the best growth performance followed by *C. pentandra* and *C. equisetifolia.* The planting of the cover crops and tree species improved, in smaller quantities, some soil chemical properties. This combination might be a viable option for reducing the input of chemical fertilizers as growth was enhanced even without the application of fertilizers.

RECOMMENDATIONS

As there was no significant effect on height and diameter growth of the three tree species by the application of fertilizer, more research needs to be conducted to determine the cause of this effect. Another area for further research is to determine the optimum dose of fertilizers for boosting growth of trees. Other types of slow release fertilizers should be used for longer retention in the soil. Similarly, further studies on other indigenous species need to be conducted in the rehabilitation of ex-tin mining land as information in this area is lacking.

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Fibre Saturation Point of Lesser-Known Timbers from Sabah

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ABSTRAK

Nilai takat tepu gentian beberapa spesis 'OT' dari negeri Sabah telah dikaji. 'OT' spesis merujuk kepada nama beberapa spesis kayu yang belum mempunyai nama dagangan atau spesies kayu yang belum terkenal. Semua 30 spesies kayu OT yang dikaji mempunyai julat nilai takat tepu gentian dari 17 - 33%. Nilai takat tepu gentian bagi kayu-kayu ini amat berguna dalam aspek pengeringan, pemprosesan dan penggunaan kayu secara umum.

ABSTRACT

The fibre saturation point (FSP) values of some 'OT' timbers from Sabah, Malaysia were evaluated. 'OT' is a term used to refer to a number of unidentified or unknown timbers and also some identified lesser known timbers of Sabah. The fibre saturation point values of these 30 species of OT timbers range from 17 - 33%. Determination of fibre saturation point in relation to OT wood properties is very useful in timber drying, conversion and timber utilization.

INTRODUCTION

Early in the 20th century, Tiemann (1906) introduced the term fibre saturation point in connection with his early work on strength-moisture relations. Since then, fibre saturation point has become the subject of numerous investigations. The concept of fibre saturation point is defined in terms of theoretical condition of wood when its cell cavities are completely devoid of water whilst the cell walls are saturated with water.

The natural transition point between bound water (water held with a force greater than that of water to water) and free water is of considerable interest in wood science as it represents the transition point of many important wood properties (Stamm 1964).

Nine methods for determining fibre saturation point were described by Stamm (1964). Four of these requires some extrapolation.

1. Moisture content adsorption isotherms to unit relative vapour pressure.
2. Differential heat of wetting: Moisture content plot to zero heat evolved.
3. Volumetric shrinkage-moisture content plot to zero shrinkage.
4. Another shrinkage involved determining the ratio of the total volumetric shrinkage to the green volume specific gravity and a correction for the average density of the absorbed water.

Two methods involved in determining the transition point due to the changing relationship:

5. Logarithm of electrical conductivity versus moisture content.
6. The logarithm of strength properties versus moisture content.

All of these require measurements over practically the full range of relative vapour pressure or moisture content. The remaining three, newer methods require a negligible reduction in vapour pressure.

7. The porous plate method, or no change in moisture content.
8. The non-solvent water technique.
9. The non-freezing water technique.

Nine methods for determining fibre saturation points of wood are given together with the limitations of each. The six older methods all depend upon making measurements over a broad range over a relative water vapour pressure or moisture content. They involve either an extrapolation of moisture content-relative vapour pressure, of differential heat of wetting-moisture data to zero evolved, or of external volumetric shrinkage moisture content data to zero shrinkage, or determining the shrinkage per unit specific gravity, the break in the logarithm of electrical conductivity-moisture content relation, or the break in the logarithm of a strength property-moisture content relationship.

The newer porous plate method, the non-solvent water method and the non-freezing water method involve only a slight drying or none at all. Fibre saturation points of never-dried wood are appreciably higher than those of pre-dried woods and vary only slightly with variation in the chemical composition of the wood substance. It is, however, substantially affected by extractives and ash content, especially when these are deposited within the cell walls, causing bulking and thus lowering the amount of water that can be held within the cell wall.

Excluded from the above methods is a method of measuring FSP using Hailwood-Horrobin sorption equation (1946) which involves the use of desiccators kept at 20C, where the relative humidity is controlled by various saturated salt solutions. Recently, this equation was applied by Yasuda and Minato (1992).

MATERIALS AND METHODS

Thirty samples, each 2 mm (T) x 25 mm (W) x 25 mm (L), were prepared from each of 30 identified OT timbers to ensure that the examined samples were at the exact EMC of the relative humidity in which they were kept.

Five desiccators filled with 5 different saturated salt solutions were prepared to provide five different relative humidity levels. These desiccators were kept at the controlled 20°C temperature. The relative humidity was monitored and recorded using a hygrometer inside each of the desiccators. Six of the 30 prepared samples from each of the OT timbers were conditioned into the prepared desiccators for 2 weeks. The desiccator set-up is shown in *Fig. 1*.

After two weeks, a few samples were selected and weighed repeatedly within two days at 6-h

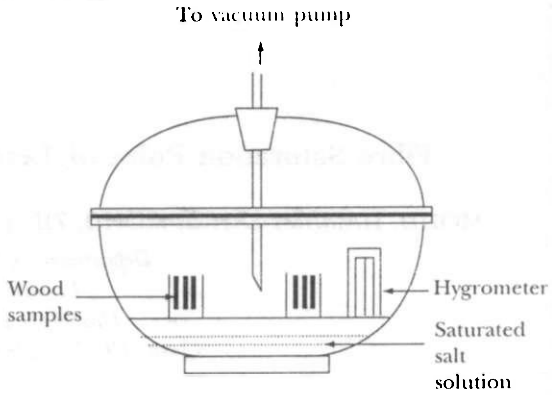

Fig. 1. Set-up of desiccator for fibre saturation point determination

intervals to ensure the EMC was constant. The purpose of the pump was to avoid air condensation and to create a slightly vacuumed condition to enable the moisture sorption to reach the EMC in which they were kept. The salt solutions used to create various relative humidity levels,at 20°C are listed in Table 1.

TABLE 1
Types of salt used to create five levels of relative humidity

Salts Solutions	Molecular Formula	Relative Humidity (%)
Magnesium chloride	$MgCl_2 6H_20$	33
Sodium nitrite	$NaNO_3$	66
Sodium chloride	NaCl	76
Ammonium sulphate	$(NH_4)_2SO_4$	81
Potassium nitrite	KNO_3	93

The results obtained from the desiccation were simplified and calculated using Minitab (curvilinear regression) after Thomas (1982). The FSP values were calculated using the following formulas:

where H = relative humidity, M = moisture content, K_1 = equilibrium constant, K_2 molecular weight of dry wood, Mh = hydrated water and Ms = dissolved water

$$H/M = A + BH + CH^2 \qquad (1)$$

where

$$A = \frac{W}{18K_2(K_1+1)} \qquad (2)$$

$$B = \frac{(K_1-1)W}{1800\,(K_1+1)} \qquad (3)$$

$$C = \frac{K_1K_2W}{180000\,(K_1+1)} \qquad (4)$$

$$K_1 = 1 + \frac{B^2 + B\,(B^2+4AC)^{1/2}}{2AC} \qquad (5)$$

$$K_2 = \frac{50[-B+(B^2+4AC)^{1/2}]}{A} \qquad (6)$$

$$W = 1800\,(B^2+4AC)^{1/2} \qquad (7)$$

$$Mh = \frac{1800\,K_1K_2H}{W(100+K_1K_2H)} \qquad (8)$$

$$Ms = \frac{1800K_2H}{W(100-K_2H)} \qquad (9)$$

$$FSP(\%) = Mh + Ms \qquad (10)$$

RESULTS AND DISCUSSION

The FSP values of the OT timbers calculated to 100% relative humidity are tabulated in Table 2. The mean FSP (21%) of the OT timbers falls in the range of 17 - 33%. The assumption of FSP values made by many well-known authors which ranges between 25 - 30% (Frederick 1967) is smaller in range than the determined FSP value of OT timbers. This is due to the species variety and greater differences in magnitude of wood properties (Kollmann 1968).

Below FSP value, shrinkage and swelling take place; these are associated with changes in dimension, strength and other properties (Tsoumis 1968). Drying of any timbers below FSP needs extra energy to evaporate the moisture from the cell walls (Walker 1993). Therefore, at FSP a harsher drying condition is necessary to facilitate better drying.

Drying of timbers under harsher conditions above FSP could result in serious seasoning defects (Desch 1974). Two species with different FSP values will shrink at different rates. For example, species of Bawing will start to shrink at an FSP of 33%, whereas Magas starts to shrink at 17%. If harsher conditions are applied at the FSP of 33%, Magas will be dried rapidly, which could result in serious seasoning defects. Thus kiln dry operators should not mix timbers with great differences in FSP values in a single chamber during kilning of timbers.

TABLE 2
Fibre saturation point values of OT timbers of Sabah

Vernacular Name	Scientific Name	FSP (%)
Magas	*Duabanga* sp.	17.24
Rengas	*Melanorrhoea* sp.	17.35
Terap	*Parartocarpus* sp.	17.60
Ramin	*Gonystylus* sp.	17.74
Sendok-sendok	*Endospermum* sp.	17.77
Geronggang	*Cratoxylon* sp.	17.80
Laran	*Neolamarckia* sp.	17.99
Kedondong	*Canarium* sp.	18.20
Talisai	*Terminalia* sp.	18.22
Ipil	*Afzelia* sp.	18.23
Obah	*Syzygium* sp.	18.25
Bintangor	*Calophyllum* sp.	18.97
Kasai	*Pometia* sp.	19.34
Sepetir	*Sindora* sp.	19.45
Binuang	*Octameles* sp.	19.69
Pengiran	*Anisoptera* sp	19.72
Malulok	*Gordonia* sp.	19.80
Bangkulat	*Linoceira* sp.	19.84
Limpaga	*Azadirachta* sp.	20.04
Darah-darah	*Myristica* sp.	20.24
Sedaman	*Macaranga* sp.	20.28
Sengkuang	*Dracontomelon* sp.	20.92
Minyak Berok	*Xanthophyllum* sp.	21.05
Mempening	*Lithocarpus* sp.	22.26
Durian	*Durio* sp.	22.50
Ranggu	*Koordersiodendron* sp.	24.09
Bayor	*Pterospermum* sp.	27.54
Tembusu	*Fragraea* sp.	29.06
Karpus	*Hydnocarpus* sp.	31.25
Bawing	*Adinandra* sp.	33.33
N		29
Mean		20.73
Std. deviation		4.05
Min		17.24
Max		33.33

The FSP values of OT timbers can be tabulated into percentage ranges as shown in Table 3, which group the species with similar FSP values. In agreement with the theory that timbers shrink and swell at FSP (Walker 1993), the feasibility of this grouping or classification needs to be studied further to foresee the practical use of drying timbers of different species which have similar FSP values. This could help in the proper

TABLE 3
Classification of FSP values of the identified 'OT' timbers (range of 5%).

Fibre Saturation Point (%).			
15.00-19.95	20.00-24.95	25.00-29.95	30.00-34.95
Magas	Limpaga	Bayor	Karpus
Rengas	Darah-darah	Tembusu	Bawing
Terap	Sedaman		
Ramin	Sengkuang		
Sendok-sendok	Minyak Berok		
Geronggang	Mempening		
Laran	Durian		
Kedondong	Ranggu		
Talisai			
Ipil			
Obah			
Bintangor			
Kasai			
Sepetir			
Binuang			
Pengiran			
Malulok			
Bangkulat			

the feasibility of this grouping or classification needs to be studied further to foresee the practical use of drying timbers of different species which have similar FSP values. This could help in the proper utilization of timbers of varying species and occurrence. This could also probably be an economic way of drying timbers.

The FSP value is also an important indicator in the prevention of decay and insect attack (Kollmann 1968). Therefore, knowing the FSP values of these OT timbers the millers can be guided into controlling the timbers to reduce handling and storage costs.

CONCLUSION AND RECOMMENDATIONS

Thirty OT timbers were identified. Their basic densities range from 285 - 732 kg/m^3, and their FSP values range from 17 - 33%.

OT timbers consist of a broad range of species with differences in basic densities and FSP values. Attempts to utilize and manage these timbers need clear understanding of each and every species. Identification is necessary for proper utilization and management of these species. The density of OT timbers in relation to FSP should be extended into mill practices under kiln drying and other manufacturing processes.

OT timbers or lesser known timbers, which are assumed to be underutilized, are highly utilized by mills throughout Sabah. A study on their actual composition and processing needs to be carried out to enhance and improve the management of forests in Sabah, especially in an attempt to solve the problems of species heterogeneity, scattered occurrences of low availability species, size, and their market penetration. New training programmes for log and/or timber graders should be formulated by the Malaysian Timber Industry Board (MTIB) or the Forestry Department. This could help timber users to identify logs or timbers correctly to ensure accelerated promotion of these OT timbers in commercial activities.

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