ISSN 0300 - 9254

Appropriate Technology Service
121, POINT PEDRO ROAD
NALLUR, JAFFNA
No. 1866

JOURNAL OF THE NATIONAL SCIENCE COUNCIL OF SRI LANKA

Volume 15 No. 2

December 1987

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Journal
of the
National
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PUBLICATION: One volume of two issues (June and December) is published annually by the Natural Resources, Energy and Science Authority of Sri Lanka.

Subscription

Annual subscription—Foreign $ 27.00; Local Rs. 45.00. Accepted on a calendar year basis. Rates include postage.

Single issues—Foreign $ 13.50; Local Rs. 27.50 each Rates include postage.

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J. Natn. Sci. Coun. Sri Lanka 1987 **15** (2) : 117–132

FERTILIZER FROM EPPAWELA APATITE : CONVERSION USING ALKALI HYDROXIDE AND QUARTZ

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(Date of receipt : 07 November 1986)
(Date of acceptance : 08 April 1987)

Abstract : The reaction of Eppawela apatite with alkali hydroxide and quartz has been investigated in an attempt to convert this mineral into a more soluble phosphate fertilizer. For complete breakdown four moles of sodium hydroxide are required for each mole of apatite. A molar ratio of apatite : sodium hydroxide : quartz, 1 : 4 : 2 at 950°C after four hours heating yielded a product possessing a maximum percentage of available phosphorus. Quartz may be replaced by potashfeldspar to obtain a potash containing phosphorus fertilizer but this requires an extended heating time. Replacement of sodium hydroxide by potassium hydroxide in the process gave a product containing high citric acid soluble phosphorus and potassium. The potassium and phosphorus containing phase in the product was identified as potassium calcium phosphate, $KCaPO_4$, which is apparently isostructural with rhenanite, $NaCaPO_4$. Extraction of phosphates from apatite using hot aqueous alkaline solutions followed by crystallisation of the extracts yielded crystalline soluble phosphates suitable for use as a fertilizer.

1. Introduction

A large deposit of rock phosphate,[8] apatite occurs in the North Central Province of Sri Lanka at Eppawela. It has been shown that the composition of this apatite from the "leached zone" at Eppawela is close to chlorfluorapatite[2,6] of formula $Ca_5(PO_4)_3(Cl,F)$. Available phosphorus content of this mineral is low and as such it is not suitable for direct application as a phosphate fertilizer especially for short terms crops like paddy. Therefore, it is desirable that this mineral be processed to obtain more soluble phosphate fertilizers.

For this conversion it is necessary as far as possible to use locally available and cheap raw materials. Furthermore, capital investment should be low in order to make the process commercially viable.

With this in view, the acidulation[7] of Eppawela apatite with Paranthan hydrochloric acid and the reaction[3,4,5] of apatite with soda ash and quartz

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have been investigated. The product developed in the latter case was similar to rhenania phosphate[11] and it was made by sintering apatite with soda ash and quartz in the molar ratio of 1 : 2 : 1 at 900°C for 2 hours. The product contains about 26–28% available P_2O_5.

This method may be employed to produce phosphate fertilizer when the rock is not suitable for acidulation or for phosphoric acid production by the Wet Process. The method can also be used in developing countries where mineral acids such as sulphuric and phosphoric acids are not readily available.

In the present study, the solid state reaction of Eppawela apatite with alkali hydroxide (NaOH or KOH) and quartz has been investigated in an attempt to replace soda ash by locally produced sodium hydroxide in the process. Furthermore, investigations have also been made on the extraction of phosphate content in apatite using aqueous alkaline solutions.

2. Experimental

2.1 Heating experiments

The compositions were prepared by mixing the apatite samples (100 mesh) with dried sodium hydroxide/potassium hydroxide or 60% aqueous solution of sodium hydroxide and quartz/potashfeldspar (120 mesh) and heating the mixtures in a muffle furnace using platinum crucibles. After heat treatment the samples were allowed to cool in air for subsequent examination.

2.2 Solubility studies

In general, phosphate content available for plant nutrition is determined by estimating either water soluble or citric acid soluble P_2O_5. Two per cent citric acid method[10] has been employed in the present study. Approximately 1.0g samples (100 mesh) were extracted with 100 ml of 2% citric acid using a mechanical shaker operating at about 260 oscillations per minute for ½ hr. The total phosphorus contents of the products were determined by extracting with a mixture of conc. HNO_3 and conc. HCl followed by 65% $HClO_4$. The extracts were analysed for phosphorus by the vanado-molybdate method[9] using a Unicam SP 600 colorimeter at a wavelength of 460 nm.

2.3 Alkali extraction

5 g samples of Eppawela apatite (100 mesh B.S.) were treated with 50 ml of aqueous alkaline solutions of different concentrations and stirred for 24

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hours using a magnetic stirrer. The extractions were carried out using 1M – 5M solutions of (i) sodium hydroxide, (ii) potassium hydroxide and (iii) sodium carbonate at ambient to 85°C. The higher temperatures were maintained during the extraction with the aid of a thermostat. The solutions were then filtered and the pH of the extracts was adjusted in the range 5 – 6 with dilute HNO_3 for the determination of phosphorus content by the vanadomolybdate method.[9]

2.4 Identification and analysis

Sintered products, crystallisation products of some alkali extracts and the residues obtained after extraction were examined by powder X-ray diffraction using Cu K_α radiation. Potassium contents of some citric acid extracts were estimated using a Perkin-Elmer 305 Atomic Absorption Spectrophotometer with an emission mode at a wavelength of 766 nm.

One g portions of the products were extracted with 50 ml of de-ionized water for 30 minutes using a mechanical shaker operating at about 260 oscillations per minute for pH measurement using a CORNING model 5 pH meter.

3. Results and Discussion

3.1 Reaction with sodium hydroxide and quartz

The results of heating experiments with solid sodium hydroxide and quartz along with the pH of water extracts of the products are given in Table 4.

3.1.1 Reaction conditions and products

In the compositions containing only apatite and sodium hydroxide in the molar ratios 1 : 1, 2 : 3, 1 : 2, 1 : 3 and 1 : 4, after heat treatment at 950°C for 4 hours, the available P_2O_5 percentage increases with increase in the alkali/apatite ratio. For instance only about 21% conversion was observed in 1 : 1 composition while almost 99% conversion was observed in the 1 : 4 composition. This variation is due to the increase in extent of the reaction between apatite and alkali. Analysis of these products by powder X-ray diffraction showed the presence of large amounts of free lime in addition to α or β –rhenanite, $CaNaPO_4$ and some unreacted apatite. Presence of lime was further confirmed by the pH values (Table 1) of the water extracts of these products.

In the apatite : sodium hydroxide mixtures mentioned above, the pH values increase with increase in the proportion of sodium hydroxide. This is

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Table 1. Results of heating experiments with solid NaOH and quartz
Temperature = 950°C; Heating time = 4 hours

Composition Molar ratio of Apatite: $NaOH: SiO_2$	2% citric acid solubility wt. % P_2O_5	2% citric acid solubility % out of total P_2O_5	phases present	pH of water extract
1 : 1 : 0	7.8	21.1	Ap. + CaO + βR	9.7
2 : 3 : 0	10.8	29.5	βR + CaO + Ap.	9.8
1 : 2 : 0	14.0	38.4	βR + CaO + Ap.	10.0
1 : 2 : 1	8.4	26.0	βR + Ap. + Quartz	9.6
2 : 3 : 3	5.7	17.8	βR + Ap. + Quartz	8.3
4 : 8 : 3	5.4	16.3	βR + Ap. + Quartz	8.3
1 : 3 : 0	30.0	88.5	βR + CaO + Ap.	11.1
1 : 3 : 1	24.0	77.8	βR + Ap.	10.0
1 : 3 : 2	16.0	56.6	α & βR + Ap.	9.4
1 : 4 : 0	32.0	99.2	βR + CaO	11.3
1 : 4 : 1	26.8	91.1	α & βR + Ap.	10.6
1 : 4 : 2	26.8	98.9	α & βR	10.3

Note: R = Rhenanite, $CaNaPO_4$; α and β are the high and low temperature forms respectively.
Ap. = Apatite.

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due to the increase in the extent of the reaction between alkali and apatite with the formation of $CaNaPO_4$ and the liberation of increasing amounts of free lime.

Therefore, it is apparent that the mixtures of apatite and sodium hydroxide, in the absence of silica, react to produce free lime. Free lime is considered as an undesirable constituent in a fertilizer. Presence of free lime would create additional problems in storage and transport of fertilizer. Therefore, silica should be added to combine with free lime released in the reaction.

The low values of the percentage conversion observed in the compositions 1 : 2 : 1, 2 : 3 : 3 and 4 : 8 : 3 (Table 1) could be explained to be due to the incomplete reaction and the dilution of the mixtures with silica. Of the mixtures studied, higher percentage conversion values (> 90%) were obtained with molar ratios of apatite, sodium hydroxide and quartz, 1 : 4 : 1 and 1 : 4 : 2. The 1 : 4 : 2 composition records the highest value ($\sim$ 99%). The pH values of the water extracts of these two compositions are relatively low indicating the absence of free lime. Furthermore, X-ray powder pattern of the 1 : 4 : 2 composition confirmed that this composition does not contain any trace of free lime.

In all these products the major constituent was identified as rhenanite (α or β $CaNaPO_4$). Dicalcium silicate, Ca_2SiO_4, formed by the reaction of quartz with lime goes into solid solution with rhenanite as shown in previous studies.[6] As such, only rhenanite solid solution has been detected in the products by powder X-ray diffraction.

3.1.2 Rate of conversion

The reaction of Eppawela apatite with sodium hydroxide resembles its reaction with soda ash.[6] Furthermore, the phases present in the products are in complete agreement with previous phase equilibrium studies.[6] Complete conversion to rhenanite has been achieved using soda ash at 900°C after 2 hours heating. On the other hand, kinetic studies on the reaction of apatite with solid sodium hydroxide and quartz revealed that the most suitable temperature is 950°C. For complete conversion heating duration should be increased to 4 hours unlike in the case of soda ash process.

In order compare the rates of the two reactions, the compositions apatite : soda ash : quartz 1 : 2 : 1 and apatite : sodium hydroxide : quartz 1 : 4 : 2 were heated in a muffle furnace from ambient to 1000°C at a heating rate 5°C per minute. Over the temperature range 500° to 1000°C small samples were removed at 50° intervals and cooled rapidly in air and the available P_2O_5 contents determined. Under these conditions the effect of temperature on percentage conversion of the two compositions is compared in Figure 1.

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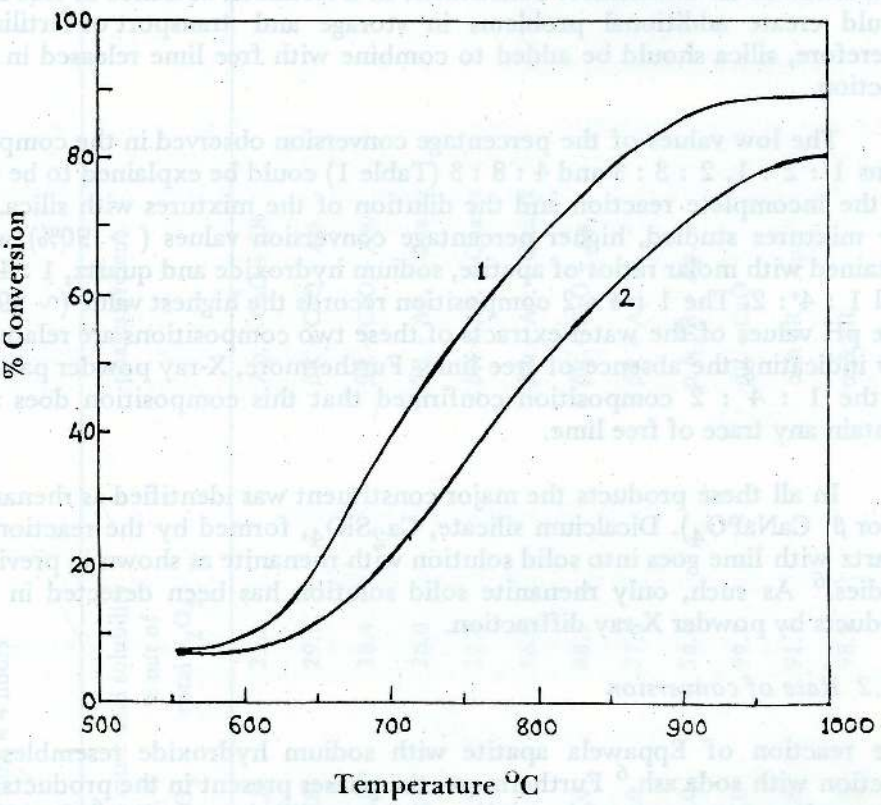

Figure 1. Relationship between temperature and percentage conversion (% wt P_2O_5 out of total P_2O_5) of the compositions,
(1) apatite : Na_2CO_3 : quartz, 1 : 2 : 1
(2) apatite : NAOH : quartz, 1 : 4 : 2, at a heating rate of 5°C per minute.

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In both cases the percentage conversion increases with rise in temperature from 600°C reaching the highest value (87%) in the composition containing soda ash around 900°C. In the composition apatite : sodium hydroxide : quartz, 1 : 4 : 2, maximum conversion (81%) was observed at about 950°C. Heat treatment of the latter sample at 950°C for further two hours resulted in almost 100% conversion. This confirms that the most suitable temperature for the conversion using sodium hydroxide and quartz is 950°C and a further reduction of reaction temperature cannot be achieved without appreciably affecting the available P_2O_5 content. This study also confirms that soda ash is more effective than sodium hydroxide in the conversion.

3.1.3 Use of aqueous NaOH

Since aqueous sodium hydroxide is manufactured locally it is of interest to investigate the possible use of aqueous solution of sodium hydroxide in the process. Experiments carried out using 60% aqueous solution of sodium hydroxide and the molar ratio of apatite : NaOH : quartz, 1 : 4 : 2, revealed that an additional drying stage is in fact necessary prior to firing at 950°C. Initial mixing was found to be easy with the aqueous solution and drying sould be done at 120 – 150°C for 1 hour. The final product obtained after firing at 950°C contained rhenanite as the major phase with about 26% available P_2O_5. Thus it is possible to replace solid sodium hydroxide by an aqueous solution in the process.

3.2 Reaction with sodium hydroxide and potashfeldspar

It is theoretically possible to replace quartz by potashfeldspar to produce a fertilizer containing potassium in addition to phosphorus. This is advantageous because both phosphorus and potassium are important plant nutrients.

Assuming the ideal formula of potashfeldspar as $KAlSi_3O_8$, the molar ratio of apatite, sodium hydroxide and feldspar, 3 : 11 : 1 respectively, has been calculated to be equivalent to the optimum ratio observed for compositions containing apatite, sodium hydroxide and quartz. Therefore, molar ratios close to this calculated value (3 : 11 : 1) has been investigated and the results are shown in Table 2.

About 80% conversion has been observed in all the compositions (Table 2) after firing at 950°C for 4 hours. When heating time was increased to 8 hours the percentage conversion was raised to $\sim$ 85%. Almost 100% conversion was effected by raising the temperature to 1100°C for 8 hours. Therefore, to obtain 100% conversion of available phosphorus with feldspar, increase of reaction temperature or heating time or both may be necessary.

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Table 2. Results of heating experiments with solid NaOH and potash feldspar

Apatite	Composition (Molar ratio) : NaOH	: Feldspar	: Quartz	Temp. °C	Heating time hrs.	2% citric acid solubility Wt. % P_2O_5	% out of total P_2O_5	Phases Present
3	: 10	: 1	: 0	950	4	21.5	82	α & β R + Ap
				950	8	23.0	88	α & β R + Ap
				1100	4	24.9	95	α & β R + tr. Ap
				1100	8	25.7	98	α & β R + tr. Ap
3	: 11	: 1	: 0	950	4	20.5	80	α & β R + Ap
				950	8	22.0	86	α & β R + tr. Ap
				1100	4	24.1	94	α & β R
				1100	8	24.8	97	α & β R
6	: 20	: 1	: 1	950	4	21.1	77	α & β R + Ap
				950	8	23.6	86	α & β R + tr. Ap
				1100	4	26.1	95	α & β R + tr. Ap
				1100	8	27.5	100	α & β R
6	: 22	: 1	: 1	950	4	22.5	84	α & β R + Ap
				950	8	23.6	88	α & β R + tr.Ap
				1100	4	25.7	96	α & β R
				1100	8	26.8	100	α & β R

Note : Ap. = Apatite; R = rhenanite, $CaNaPO_4$; tr. = trace; α & β are high and low temperatures forms respectively.

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No specific potassium containing phase has been identified in the products. The major phase in the product was identified as α or β rhenanite by powder X-ray diffraction. It is likely that potassium goes into solid solution with rhenanite. This was evident from potassium analysis of the citric acid extracts of the products [Table 3 (i)].

Table 3. Potash contents of citric acid extracts of some products (950°C/4h).

Composition (Molar ratio)	Wt. % K_2O	
	% K_2O	% of total K_2O
(i) Apatite : NaOH : Feldspar : Quartz		
3 : 10 : 1 : 0	1.0	97%
3 : 11 : 1 : 0	1.0	95%
6 : 20 : 1 : 1	0.5	96%
6 : 22 : 1 : 1	0.5	99%
(ii) Apatite : KOH : Quartz		
1 : 3 : 1	16.2	99%
1 : 3 : 2	15.1	98%
1 : 4 : 1	19.6	99%
1 : 4 : 2	18.6	100%

The feldspar sample used in the present study, when chemically analysed was found to contain 9.2% wt K_2O (approximately half the theoretical value of 16.9% wt K_2O) and 2.9% wt Na_2O. Hence this sample contained 30% less alkali than theoretical : the deficiency presumably being made up by calcium.

The potassium contents of the citric acid extracts of the products [Table 3(i)] show low values (0.5 – 1.0 % wt K_2O) owing to the unexpected low content of potassium in the feldspar sample. However, about 95–99% of the total potassium in the product is extractable in 2% citric acid.

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Insoulble residues after citric acid extraction, typically 1–2 wt %, were collected, dried and examined. X-ray powder diffraction studies indicated that the residues contain carnegieite, $(Na,K)AlSiO_4$, residual apatite and calcium monoferrite, $CaFe_2O_4$.

3.3 Reaction with solid potassium hydroxide and quartz

The results of heating experiments of apatite with solid KOH and quartz are presented in Table 4.

Table 4. Results of heating experiments with solid KOH and quartz
Temperature = 950°C; Heating time = 4 hours

Composition Molar ratio of Apatite:KOH:SiO_2	2% citric acid solubility wt. % P_2O_5	% out of total P_2O_5	Phases present
1 : 1 : 0	5.9	16.8	Ap. + CaO + $CaKPO_4$
2 : 3 : 0	8.0	24.5	$CaKPO_4$ + Ap. + CaO
1 : 2 : 0	10.3	33.9	$CaKPO_4$ + Ap. + CaO
1 : 2 : 1	6.0	21.4	$CaKPO_4$ + Ap.
2 : 3 : 3	5.9	20.6	Ap. + $CaKPO_4$ + tr. Quartz
4 : 8 : 3	5.2	18.2	$CaKPO_4$ + Ap. + tr. Quartz
1 : 3 : 0	21.9	81.6	$CaKPO_4$ + CaO + Ap.
1 : 3 : 1	18.4	74.1	$CaKPO_4$ + Ap. + tr. Cao
1 : 3 : 2	14.0	60.1	$CaKPO_4$ + Ap.
1 : 4 : 0	22.6	94.1	$CaKPO_4$ + CaO
1 : 4 : 1	20.7	92.2	$CaKPO_4$ + tr. Ap. + tr. CaO
1 : 4 : 2	20.4	96.6	$CaKPO_4$

3.3.1 Reaction conditions

The same trends observed in the compositions containing apatite, NaOH and quartz have been observed in the compositions containing apatite, KOH and quatz as well. The extent of the reaction increases with increase in potassium hydroxide content from the molar ratio of apatite : KOH, 1 : 1 up to 1 : 4. As in the case of sodium hydroxide reaction, it is evident that one mole of apatite requires four moles of potassium hydroxide for complete reaction. Apatite and potassium hydroxide react together to give a large amount of free lime and a phase containing both phosphorus and potassium.

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Among the compositions studied 1 : 4 : 0, 1 : 4 : 1 and 1 : 4 : 2 gave high yields (> 92% wt P_2O_5) of available phosphorus. Since 1 : 4 : 0 contains excess lime it is not suitable as a fertilizer. A composition of 1 : 4 : 2 (Table 4) fulfills the requirements of a P,K—fertilizer possessing ~ 20% wt available P_2O_5 and ~ 18% wt K_2O. The 2% citric acid soluble potash contents of sintered apatite, KOH and quartz compositions are shown in Table 3 (ii). These results show that almost 100% of the potassium in the product is soluble in 2% citric acid and thus available for plant nutrition.

Therefore, the use of potassium hydroxide instead of sodium hydroxide has an added advantage of producing a fertilizer possessing high phosphorus as well as high potassium contents.

3.3.2 Nature of products

The powder X-ray diffraction pattern of K,P—containing phase in the product was found to be very similar to β rhenite. Synthetic and X-ray powder diffraction investigations revealed that this phase is in fact $CaKPO_4$,[1] which is isostructural with rhenanite. Similar to α and β rhenanite $CaKPO_4$ is almost 100% soluble in 2% citric acid, making its total phosphorus and potassium contents available for plant nutrition.

DTA was performed on $CaKPO_4$ using Stranton-Redcroft Model DTA 673 up to 1000°C at a heating rate of 10°C per minute. A reversible transition has been observed in the temperature range 660 – 680°C. The hysteresis effect of this transition is not very prominent indicating that the structural change is not very drastic. It has also been observed using TG that there is no weight loss or gain associated with the transition. This shows that $CaKPO_4$ exists in two crystalline forms which possibly have slightly different crystal structures. It was, however not possible to differentiate the two forms by using powder X-ray diffraction.

3.4 Alkali extraction of phosphates

Results of extraction studies with aqueous sodium hydroxide and potassium hydroxide solutions are given in Table 5. With both these solutions it has been observed that the extractable P_2O_5 increases with increase in concentration from 1M to 5M. The extractable phosphorus content increases with increase in temperature from ambient up to 60°C and then decreases with rise in temperature in all the concentrations studied using aqueous sodium hydroxide. However, in the case of potassium hydroxide gradual increase in extractable P_2O_5 was observed with increase in temperature up to 75°C.

It appears therefore that the optimum temperature for extraction with NaOH is around 60°C. Five M NaOH solution at 60°C gave the maximum

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Table 5. Results of extraction studies with aqueous alkali hydroxide solutions

Strength of alkali hydroxide in moles l^{-1}	Temperature °C	NaOH Extraction		KOH Extraction	
		Extractable P_2O_5 in % wt.	Extractable P_2O_5 out of total in %	Extractable P_2O_5 in % wt.	Extractable P_2O_5 out of total in %
1.0	room temp. (27°C)	5.6	16.4	–	–
2.0	room temp. (27°C)	6.1	17.8	4.8	14.0
	40°C	6.4	18.7	–	–
	60°C	6.9	20.2	5.1	14.9
	75°C	5.5	16 1	5.5	16.1
	85°C	5.6	16.4	–	–
3.0	room temp. (27°C)	6.5	19.0	5.7	16.7
	40°C	6.8	19.9	–	–
	60°C	7.2	21.0	6.2	18.1
	75°C	5.6	16.4	6.2	18.1
	85°C	5.4	15.8	–	–
4.0	room temp. (27°C)	7.1	20.8	5.9	17.3
	40°C	7.4	21.6	–	–
	60°C	7.8	22.8	7.3	21.3
	75°C	6.2	18.1	7.8	22.8
	85°C	5.6	16.4	–	–
5.0	room temp. (27°C)	7.2	21.0	6.4	18.7
	40°C	7.6	22.2	–	–
	60°C	8.2	24.0	9.2	26.9
	75°C	6.5	19.0	9.4	27.5
	85°C	5.9	17.3	–	–

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value (8.2%) of extractable P_2O_5 which is equivalent to 24% of the total P_2O_5 in the rock sample. A slightly higher maximum value of 9.4% extractable P_2O_5 was observed using 5M KOH solution at 75°C. This value amounts to 27.5% of the total P_2O_5 in the sample, which is only slightly higher than the value observed for aqueous NaOH.

Results of the extraction studies using aqueous sodium carbonate solutions are presented in the Table 6. With aqueous sodium carbonate it has been observed that the extractable P_2O_5 content increases with increase in concentration from 1M to 5M and at concentrations higher than 5M no appreciable increase has been observed. As in the case of NaOH, extractable P_2O_5 content increases with rise in temperature from ambient to 60°C and then decreases with increase in temperature. Therefore, available data indicate that 5M aqueous sodium carbonate solution and a temperature of about 60°C are suitable for extraction. Under these conditions a maximum extractable P_2O_5 value of 17.3% was obtained, which is equivalent to 50% of the total P_2O_5 in the original sample. The total P_2O_5 content of the Eppawela apatite sample used in the present study was experimentally estimated to be 34.2% wt.

The solubility of Eppawela apatite in water is very small, only about 0.5 – 1.0% wt P_2O_5 being soluble. However, 2% citric acid soluble P_2O_5 is about 6%. In this context the maximum extractable P_2O_5 contents observed using aqueous NaOH and KOH ($\sim$ 8 – 9% wt P_2O_5) are not very significant. On the other hand, aqueous sodium carbonate can be considered as a fairly efficient extracting agent.

Crystallization of the alkaline extracts has been attempted by concentrating the extracts by evaporation followed by cooling. Crystallisation of sodium carbonate extracts after concentration yielded large needle shaped crystals of high birefringence. X-ray powder diffraction studies on the crystalline products indicate that they are not pure sodium phosphates. It is most likely that the crystalline products contain mixed crystals of carbonate-phosphates of sodium. These crystalline products are water soluble and as such it may be possible to use this material as a phosphate fertilizer in certain acidic soils.

4. Conclusions

For complete conversion of apatite to citric acid soluble product four moles of sodium hydroxide (or 2 moles of Na_2O) are required for each mole of apatite. The optimum molar ratio of apatite: NaOH : quartz was found to be 1 : 4 : 2. Unlike in the case of soda ash process, use of sodium hydroxide necessitates higher temperature (950°C) and increased heating time

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Table 6. Results of extraction studies with aqueous sodium carbonate solutions

Strength of Na_2CO_3 in moles l^{-1}	Temperature °C	Extractable P_2O_5 in % wt.	Extractable P_2O_5 out of total in % wt.	2% Citric acid soluble P_2O_5 in the residue in % wt.
2.0	room temp. (27°C)	5.7	16.7	2.7
	40	9.7	28.4	2.0
	60	14.9	43.6	
	75	5.1	14.9	
	85	5.4	15.8	
3.0	room temp. (27°C)	5.8	16.9	2.8
	40	10.7	31.3	
	60	16.8	49.1	
	75	5.5	16.1	
	85	5.6	16.4	
4.0	room temp. (27°C)	6.2	18.1	2.5
	40	11.8	34.5	
	60	17.1	50.0	
	75	5.5	16.1	
	85	5.8	16.9	
5.0	room temp. (27°C)	6.5	19.0	2.9
	40	15.9	46.5	
	60	17.3	50.6	
	75	5.7	16.7	
	85	6.0	17.5	

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(4 hours). It is possible to replace solid NaOH by 60% aqueous solution of NaOH in the process but in this case an additional drying stage is necessary before sintering.

Quartz may be replaced by potashfeldspar in the composition to obtain a potassium containing phosphate fertilizer. However, the reaction appears to be sluggish and as such either a higher temperature (1100°C) or increased heating time (8 hrs.) may be necessary to obtain a fertilizer with a high yield of available phosphorus.

The replacement of NaOH by KOH results in the formation of a fertilizer containing both phosphorus and potassium. The major phase in the product was identified as $CaKPO_4$. $CaKPO_4$ is completely soluble in 2% citric acid, thus its total phosphorus and potassium contents are available for plant nutrition.

It is evident that an aqueous solution of sodium carbonate is more effective in extracting phosphate from Eppawela apatite than the alkali hydroxide solutions. About 50% of the total P_2O_5 in the rock can be extracted with 5M aqueous sodium carbonate solution at 60°C. These extracts may be crystallised to obtain crystalline water soluble phosphates.

Acknowledgements

We are grateful to the International Seminar in Chemistry of the Uppsala University, Sweden for financial assistance.

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J. Natn. Sci. Coun. Sri Lanka 1987 **15**(2) : 133–144

LACTATIONAL AMENORRHOEA AND CONTRACEPTIVE PRACTICE

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(Date of receipt : 25 August 1986)
(Date of acceptance : 20 April 1987)

Abstract : Lactational amenorrhoea represents an important child spacing mechanism in many developing countries. The contribution made by lactation as a method of contraception needs to be assessed for a given situation depending on the infant feeding practices and the pattern of postpartum amenorrhoea. A community based study on a sample of mothers from 'urban', 'town' and 'rural' sectors provides the above information and also highlights the differences between these sectors. Such information need to be taken into consideration in formulating policies related to family planning programmes in order to identify appropriate timing for the introduction of such programmes.

1. Introduction

The contraceptive effects of lactation has been recognised in various cultures for centuries. Following childbirth, each woman experiences a period of temporary infecundability, during which there is no ovulation, hence a period of amenorrhoea follows on childbirth. Since amenorrhoea is more easily observed than ovulation, the period of amenorrhoea is taken as indicative of post-partum non-susceptible period.

Breastfeeding practices appear to be a principal determinant of lactational amenorrhoea. Many studies have shown that in the absence of breastfeeding, post-partum amenorrhoea commonly lasts about two months and where breastfeeding is prolonged, this would last between one to two years.[3,9] An analysis of breastfeeding patterns in 25 sub-populations in nine countries showed that variations in the proportion of women breastfeeding explained about 85% of the variance between populations in the proportion of menstruating women.[1] The contribution that prolonged breastfeeding could make towards increasing the birth interval thus influencing the fertility pattern has also been studied.[8]

Even though a positive relationship between the duration of breastfeeding and postpartum amenorrhoea has been identified, considerable

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differences in the patterns have been observed between countries as well as within countries.[10] Differences in the frequency of suckling[4] and the nutritional status of the women have been identified as likely contributory factors.[7]

For an individual woman, the reliability of using breastfeeding per se as a method of family planning, remains doubtful. As the average duration of breastfeeding in many communities decline, family planning programmes need to pay emphasis on providing contraception before an unwanted pregnancy occurs.

2. Materials and Methods

A cross-sectional study to identify infant feeding practices was carried out in twelve of the twenty four districts of Sri Lanka. Approximately 70% of the population of Sri Lanka reside in the districts included in the study.[11]

Thirty study areas from each of the three sectors ie. urban, town and rural, were identified using the technique of "probability proportional to size" sampling, using the data available from the Census. From each of these areas, one study cluster was identified randomly. From each of these clusters, thirty children whose ages were one year and below, on the day of the interview were identified, thus including approximately 900 mother-child pairs from each of the three sectors.

The information was collected using an interviewer administered pre-coded questionnaire, which was pre-tested and modified prior to use in the survey. Part I of the questionnaire contained information related to the household while Parts II and III contained information related to the infant feeding practices and those on the mother (which included data on menstruation and contraceptive practices).

A group of family health workers were trained to collect the information. Field supervisors were responsible for cross-checking the data at the field level and in supervising the work of the field investigators. All questionnaires were checked manually as well as edited using computer facilities.

3. Results

Majority of women initiated breastfeeding as shown in Table 1. The data on infant feeding was obtained by questioning the mother as to the feeding of the child on the day preceeding the interview, thus minimising recall error. Even though the majority of women initiated breastfeeding, most mothers

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Table 1. Initiation of Breast Feeding

	Number of Mothers		
	Urban	Town	Rural
Initiated breastfeeding	892 (98.0%)	896 (98.0%)	901 (99.0%)
Not initiated –			
due to "no milk"	7 (0.9%)	4 (0.4%)	3 (0.3%)
mother's illness	3 (0.3%)	5 (0.5%)	–
child's illness	1 (0.1%)	2 (0.2%)	–
other reasons	4 (0.4%)	2 (0.2%)	1 (0.1%)
No information	3 (0.3%)	3 (0.3%)	2 (0.2%)
Total	910 (100%)	912 (99.6%)	907 (99.6%)

included "other milks" specially powdered milk, in the feeding of their children, early in infancy. Table 2 shows that by the age of three (3) months, 55% of urban, 53% of town and 40% of rural mothers had introduced powdered milks. Data related to menstruation obtained from the mothers do include a certain amount of recall even though the maximum period of recall was one year. In 92% of the interviews, the mothers responded to this question thus improving the quality of this information.

The proportion of women in whom menstruation had returned, was studied in relation to the breastfeeding pattern (Table 3). In each sector, the proportion of women in whom menstruation had returned was significantly higher among those who had discontinued breastfeeding.

The age of the child is an important factor that influences the pattern of feeding as well as the return of menstruation, hence was taken into consideration in further analysis (Table 4). It is observed that for all age groups, the proportion of women in whom menstruation has returned was comparatively higher among the group that had stopped breastfeeding. The exception to this observation is that made for the younger age groups in the 'Town' and 'Rural' sectors, the likely reason being the very small number of women who had stopped breastfeeding, in these age groups. It is seen that as the age of the child increases, the magnitude of the difference between the two groups decline. This is specially marked from 7 – 8 months onwards.

The family planning acceptance pattern was studied in relation to the 'return' of menstruation (Table 5). A total of 22 mothers out of 2665 in

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Table 2. Prevalence of Elusive Breast Feeding, 'Mixed Feeding' and Formula Feeding (Based on Previous Day's Feeding)

	Age of Child in Months												
	0	1	2	3	4	5	6	7	8	9	10	11	12	Total
% exclusively breast fed														
Urban	86	66	61	45	42	32	24	29	21	16	20	20	0	44
Town	92	66	65	47	43	26	26	29	25	24	26	17	0	43
Rural	94	87	72	60	60	46	49	47	48	44	38	32	20	58
% on 'mixed' feeding														
Urban	10	34	33	44	39	41	42	52	52	33	43	30	50	30
Town	8	30	31	37	42	50	46	33	47	40	40	46	50	38
Rural	4	12	28	38	34	38	46	47	43	47	42	50	60	34
% on formula only														
Urban	4	0	6	11	18	26	33	19	46	49	37	50	50	26
Town	0	4	5	16	15	24	28	38	28	36	34	37	50	19
Rural	2	1	0	2	6	17	5	6	9	9	20	18	20	8

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Table 3. Pattern of amenorrhoea in relation to breastfeeding practices.

A. URBAN

		Menstruation Returned		
		Yes	No.	Total
Breastfeeding	Yes	399	278	677
	No	183	17	200
	Total	582	295	877

No information – 33

$x^2 - 37.1$, df – 1, $p < 0.001$

B. TOWN

		Menstruation Returned		
		Yes	No.	Total
Breastfeeding	Yes	394	331	725
	No	148	23	171
	Total	542	354	896

No information – 16

$x^2 - 59.8$, df – 1, $p < 0.001$

C. RURAL

		Menstruation Returned		
		Yes	No.	Total
Breastfeeding	Yes	341	481	822
	No	52	15	67
	Total	393	496	889

No information – 18

$x^2 - 32.6$, df – 1, $p < 0.001$

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Table 4. Percentage of women in whom menstruation has returned

% of women in whom menstruation has returned	Age of child in completed months										
	Less than 2 months completed	3	4	5	6	7	8	9	10	11	Total
URBAN											
Group I*	89	91	93	100	89	94	92	89	81	100	92
Group II*	37	52	62	60	51	60	78	89	89	88	59
TOWN											
Group I*	22	70	91	100	100	95	76	84	88	96	87
Group II*	35	42	50	61	69	57	69	83	76	74	54
RURAL											
Group I*	0	0	60	63	100	80	86	100	92	100	78
Group II*	19	20	42	47	51	63	49	51	60	73	41

* – Group of mothers in whom breastfeeding has been discontinued.

** – Group of mothers who continued 'exclusive' breastfeeding or 'mixed' feeding.

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Table 5. Family Planning Acceptance Pattern, by 'return' of Menstruation*

Sector	URBAN		TOWN		RURAL		TOTAL	
	Menstruation		Menstruation		Menstruation		Menstruation	
Practicing a FP method	Returned	Not Returned	Returned	Not Returned	Returned	Not Returned	Returned	Not Returned
Yes	262(45)	73(25)	207(38)	70(20)	118(30)	98(20)	587(39)	241(21)
No	308(54)	218(74)	329(61)	281(80)	269(69)	395(80)	906(60)	894(78)
Pregnant	7(1)	4(1)	4(1)	1(0)	4(1)	2(0)	15(1)	7(0)
Total	577(100)	295(100)	540(100)	352(100)	391(100)	495(100)	1508(100)	1142(100)

(Percentage given in parentheses)

* Mothers from whom the relevant information was not available were excluded from the analysis.

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whom the information was available i.e. 0.8% were pregnant at the time of the interview. One third of this group had got pregnant before menstruation resumed.

In the total sample, 21% of the group of women in whom menstruation had not returned, had adopted a method of family planning compared to 39% among the group in whom menstruation had returned. Comparatively higher proportions of women in the urban sector had accepted a method of contraception.

In this study, the practice of the following methods of contraception were inquired into i.e. surgical methods (male and female) loop, pills, IUCD, Injectables, Condom, safe period and other natural methods. Such information was available from 76% of the group of women who were practicing a method of family planning. Table 6 shows that the commonest methods adopted were the surgical methods and the 'natural' methods. Of the women in whom menstruation had not returned, 58% had adopted a surgical method compared to 24% among the group in whom menstruation had returned. Inappropriateness of the use of hormonal methods and natural methods was indicated by the comparatively lower proportion of women in whom menstruation had not returned, who practiced these methods.

Table 6. Practice of contraceptive methods in relation to the status of menstruation.

Method of Contraception	Menstruation		Total
	Returned	Not returned	
Oral contraceptives	79 (18)	13(6)	92 (14)
IUCD	41 (9)	12(6)	53 (8)
Sterilization (male and female)	108 (24)	114(58)	222 (35)
Injections	47 (11)	8(4)	55 (9)
Condom	59 (13)	26 (13)	85 (13)
Safe period and other 'natural' methods.	108 (24)	24 (12)	132 (21)
Total	442 (100)	197 (100)	639 (100)

(Percentage given in parentheses)

4. Discussion

This study provides some information on the pattern of lactational amenorrhoea among a group of Sri Lankan women. However, the study

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cannot be considered a national level study, but is based on the information available from a representative sample of women with children under one year of age, living in twelve of the 24 districts of Sri Lanka, where 70% of the population reside.

The WHO collaborative study[13] carried out in eight countries showed that among the non-breastfeeding (NBF) women, 80 – 100% were menstruating by 3 – 4 months postpartum. In this study, comparable proportions were observed among mothers from the 'Urban' and 'Town' sectors. Valid conclusions cannot be drawn as the number of women belonging to the 'NBF' group in the 'Rural' sector is very small.

Among the 'breastfeeding' (BF) group, wider variations were observed in the proportion of women in whom menstruation has returned by 3 – 4 months. They ranged from 1% in rural India to 57% among the 'urban' group in Ethiopia.[13] The observations made in this study compare well with those made in the 'Urbanised' areas of the countries included in the WHO collaborative study.

The frequency and intensity of suckling is thought to influence the duration of infecundity.[12] From the point of view of ensuring adequate child growth, it is necessary to stress the need to introduce complementary foods using locally available foods, as the child grows older. Introduction of such feeding practices is likely to reduce the frequency of suckling, thus influencing prolactin levels, milk production and eventually ovulation. The differences in the proportion of women who had menstruated, between BF & NBF groups became smaller as the age of the child increases. This observation is likely to be due to the reduction of frequency of suckling among the older infants.

Even though a very high proportion of mothers did commence breastfeeding, most mothers did introduce formula milks and other complementary foods in early infancy. This information has important policy implications regarding family planning programmes, as it is necessary that such a program should be developed so as to provide contraception before an unwanted pregnancy occurs. Such decisions involve the identification of a specific time when infant suckling patterns change considerably, which may be an appropriate time to start contraception.

Absence of menstruation does not mean the absence of ovulation and it has been observed in the WHO study that up to 10% of the mothers who had got pregnant at the time of the interview had conceived before menstruation resumed. Only 0.8% of women in the present study had got pregnant at the time of the interview, among whom a third had conceived before menstruation returned, thus indicating the need for the early introduction of family planning programmes.

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Different views have been expressed as to the timing of a programme to promote contraceptive practices. Jeliffe[6] suggested that technical family planning methods need to be introduced on a community basis not later than about two months prior to the usual time of return of menses which need to be known for a particular situation. The concensus at the meeting of the World Health Organization and US National Research Council[14] was that contraception should be initiated at a time when a quarter to one half of the lactating women resume menses. In view of the relevance of food supplementation, Howie[5] observes that if the time at which food supplementation is to be introduced could be clearly defined, then it may be possible to link such a change with the start of contraception or change from a less effective method to a more effective method.

Taking the above views into consideration, from the data available in the present study it could be suggested that emphasis on family planning programmes should be made from about 4 months post-partum.

Another main area of concern are the methods of contraception that needs to be encouraged during lactation. Non-hormonal methods are generally preferred during this period.[9,12]

It is necessary for all health workers to be aware of the link between infant feeding practices and maternal fecundity. This is of special importance as studies have shown that some categories of health workers are often misinformed about breastfeeding practices.[2] They should be able to use relevant information to identify mothers who need protection and should be able to recommend appropriate methods of contraception.

Acknowledgement

The financial support provided by the World Health Organization for this study is gratefully acknowledged.

The authors wish to thank the Director General of Health Services, Professor of Paediatrics, University of Peradeniya, Professor of Paediatrics, University of Ruhuna, Director, National Institute of Health Sciences, Chief Medical Officer of Health, Colombo Municipality and many members of their staff for their assistance during the study.

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J. Natn. Sci. Coun. Sri Lanka 1987 **15** *(2) 145–160*

TRICHLOROACETIC AND SOLUBLE ANTIGENS OF *SETARIA DIGITATA*: CHARACTERIZATION AND CROSS REACTION WITH HUMAN SERUM COMPONENTS

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(Date of receipt : 18 November 1986)
(Date of acceptance : 06 May 1987)

Abstract : Antigenic cross reactivity between 10% trichloroacetic soluble antigens of adult *Setaria digitata* (SDTCA) and components present in human serum and urine were investigated by ELISA and polyethylene glycol precipitation immunoradiometry (PEGIRMA). The usefulness of SDTCA in the serologic diagnosis of *Wuchereria bancrofti* infections was also investigated. The antibody response to SDTCA in *W. bancrofti* infections was predominantly IgM and SDTCA was not a good antigen for immunodiagnosis by antibody determination. Evidence supporting antigenic cross reactivity between trichloroacetic acid soluble antigens of *S. digitata* and human immunoglobulin carbohydrates is presented. Rabbit antisera to human immunoglobulins, human immunoglobulin carbohydrates, 10% trichloroacetic acid soluble components in normal human serum and adult *S. digitata* showed the same degree of reactivity with radiolabelled human immunoglobulins. Immunoreactive components were detected in the urines of filarial patients by dot ELISA with rabbit antisera to SDTCA. False positive reactions were observed due to cross reactions with catabolic products excreted in urine.

1. Introduction

Antigenic similarities between polysaccharides of nematode parasites and mammalian blood group substances were known for many decades.[19,20,21,22,29] Smith *et al.*[28] have shown that such molecules are expressed on the surface and excretions/ secretions of *in vitro* maintained second stage larve of *Toxocara canis.*

Sharing of host antigens and/or antigenic cross reactivities between molecules of filarial parasites and host tissue components have been described previously in *Onchocerca volvulus*[19], *Wuchereria bancrofti*[27] and *Litomosoides carinii*[23, 24, 30] and the discussion has been on the possible mechanisms of evasion of the host immune response.[2,16]

The availability of a specific immunodiagnostic test could be of much value in the control of filarial infections and the current emphasis is on the development of antigen detection assays.[6,7,8,25,26,32] Sharing of antigens between the host and the parasite could affect the specificity and sensitivity of both antigen and antibody detection systems,[7,14] particularly in situations such as bancroftian filariasis where heterologous antigens are

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relatively indispensable as at present.

In this communication we present evidence to demonstrate antigenic cross reactivity between human immunoglobulin carbohydrates and trichloroacetic acid soluble carbohydrate antigens of the heterologous filarial parasite *Setaria digitata.*

2. Materials and Methods

2.1 Blood serum and urine from filarial patients and controls

Blood serum and urine were collected from *W. bancrofti* infected patients (both microfilaraemic and amicrofilaraemic) attending an Anti-Filariasis clinic. Microfilaraemia was determined by the membrane filtration technique. The diagnosis of clinical cases was based on the indirect immunofluorescence test using *W. bancrofti* microfilariae [12] and characteristic symptoms such as elephantiasis, lymphoedoma, lymphangitis and response to DEC treatment. Sera from non-filarial subjects (absence of clinical signs/symptoms and of serum antibodies by ELISA and IFA) from the filariasis endemic and non-endemic areas were also collected.

2.2 Carbohydrate antigens of *S. digitata*

Solubility in 10% Trichloroacetic acid (10% TCA) was the criterion used for isolation of carbohydrate like antigens. Adult *S. digitata* worms were collected from the peritoneal cavity of cattle slaughtered at the municipal abbatoir, Kandy, Sri Lanka. The parasites were transported in normal saline, washed and TCA–soluble antigens were extracted by homogenisation in 10% TCA, dialysed against phosphate buffered saline (PBS pH 7.4), concentrated and stored at -20^{o}C in aliquots. This antigen preparation was denoted as SDTCA.

2.3 Antigen SD2–4 of *S. digitata*

Antigen SD2–4 from adult *S. digitata* was prepared as described previously.[3]

2.4 TCA–Soluble components in normal human serum (NHSTCA)

Trichloroacetic acid (TCA) soluble components in normal human serum (NHSTCA) were prepared by extraction with 10% TCA. To approximately 50ml of pooled human sera, TCA was added to produce a final concentration of 10%, the mixture homogenised and the soluble components isolated by centrifugation. The supernate was dialysed against PBS (pH 7.4), concentrated 5:1 and stored at -20^{o}C.

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2.5 Normal human Immunoglobulin (NHIg)

Normal human immunoglobulin (NHIg) was prepared by DEAE–Cellulose chromatography [10] from Cohn Fraction II (Sigma Chemicals).

2.6 Carbohydrates from human immunoglobulins (HIgCHO)

Human immunoglobulins isolated by DEAE–Cellulose chromatography were digested with protease (insoluble enzyme attached to CM–Cellulose, from *Streptomyces griseus,* Sigma Chemical Co. Ltd) at 37°C for 12 hrs. The carbohydrates were isolated by dialysis and concentrated.

2.7 Concentrated and dialysed urine from non-filarial subjects (CDNU)

Twenty four-hour urine samples were collected from normal subjects living in areas non-endemic for filariasis, pooled, concentrated (10:1) by dialysis against solid sucrose and PBS and stored at −20°C.

2.8 Concentrated and dialysed urine from filarial patients (CDFilU)

Concentrated and dialysed urine samples from filarial patients were prepared similarly. The filarial patients were *W. bancrofti* infected and all the patients were symptomatic and had serum antibodies to *W. bancrofti* microfilarial surface antigens by immunofluorescence.

2.9 Labelling of antigen preparations with 125 Iodine

Antigen preparations were labelled with 125 Iodine (IMS 30, Amersham Radiochemicals, UK) by the method of Hunter & Greenwood.[11]

2.10 Preparation of rabbit antisera to SDTCA, SD2–4, NHIg, NHSTCA, HIgCHO, CDNU and CDFilU

Rabbits were immunised with SDTCA, SD2–4, NHIg, NHSTCA, HIgCHO, CDNU and CDFilU in Complete Freund's Adjuvant, intramuscularly at the hind leg region. Three or four animals were used for each antigen. Booster immunizations were given in Incomplete Freund's Adjuvant at 10 day intervals through the same route. Test bleeding commenced 10 days after the third booster immunization. The sera were tested by ELISA against the immunising antigen and, when the antibody titre was above 1:500, the animals were bled.

2.11 Determination of total protein and carbohydrate levels

The levels of total protein and carbohydrate in antigens and urines were determined by the Lowry method[15] and the Phenol-Sulphuric acid method

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of Norris & Ribbons[18] respectively.

2.12 SDS polyacrylamide gel electrophoresis (SDSPAGE) & Western Blotting

SDSPAGE was performed in vertical gel slabs of 7.5–10.0% gel strength in phosphate buffer (pH 7.1, 0.1M) and Western blotting according to the method of Towbin *et al.*[31] and Batteiger *et al.*[1] SDSPAGE gells were stained with Coomassie Blue or Periodate–Schiff reagent (Sigma Chemicals) and the Western blots were reacted with hyperimmune sera from animals immunised with filarial antigens and sera from *W. bancrofti* infected patients. The development of Western blots was by ELISA using Peroxidase conjugated antisera (Cappel Laboratories, Cochranville, USA) and 0.3% 4–chloro–1–napthol as substrate (Sigma Chemicals).

2.13 ELISA in microtitre plates

Microtitre plate ELISA was carried out using Dynatech PVC round bottomed plates (SDTCA showed better binding to PVC plates compared to polystyrene). The antigen was coated in carbonate buffer pH 9.6, 0.06 M at a predetermined concentration of 10 μg/ml and the reaction volume was maintained at 100 μl per well. Sera were tested at both fixed and serial dilutions. Peroxidase-linked anti-human IgM (Fc specific) and anti-human Ig (IgA+IgM+IgG) were obtained from Cappel Laboratories, USA and urease-conjugated anti-human IgG (affinity purified) was from Commonwealth Serum Laboratories, Melbourne, Australia. ELISA reactivity in peroxidase ELISA was determined by reading absorbance at 492 nm (read on Titretek plate reader). With IgG-urease conjugate, colour change to purple in 20 minutes was considered positive.

2.14 DOT ELISA on Nitrocellulose

A dot ELISA procedure was developed for the detection of antigens in urine. Five μl of urine or predetermined dilutions of antigen preparations were spotted on to nitrocellulose paper and air dried. The unbound sites on the paper were then blocked with bovine serum albumin (BSA) and Tween 20 in PBS (0.5% BSA,0.05%T20) at 37°C for 3 hours. The paper was then reacted with hyperimmune sera followed by the enzyme-antibody conjugate and colour developed with 3% 4–Chloro–1–Napthol (Sigma) in citrate buffer, pH 5.0.

2.15 Polyethylene glycol–Immunoradiometry (PEGIRMA)

All radio-immunoprecipitations were carried out in polystyrene tubes (LP3, Lukham Ltd, England) coated with a 1% solution of gelatin. All reactions were carried out in PBS containing BSA and Tween 20 (PBS, pH 7.4, 0.1 M;

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0.5% BSA; 0.05% Tween 20, PBS–BSA–T). ^{125}Iodine labelled antigens (approximately 20,000 cpm/tube, final reaction volume, 0.5 ml) were incubated in duplicate with different, serially diluted antisera at 37°C for 3 hrs and overnight at 4°C. An equal volume of 20% polyethylene glycol (PEG, MW 6000, Sigma Chemicals) in PBS–BSA–T20 was then added, mixed well and incubated at 4°C overnight. The tubes were then centrifuged at 1000g for 10 minutes at 4°C and the precipitate washed (5 x times) with cold 10% PEG in PBS. The radioactivity in the precipitate was determined.

3. Results

3.1 Composition of SDTCA and concentrated urines

The approximate concentrations of protein and carbohydrate in the 10% trichloroacetic acid extract of adult *S. digitata* (SDTCA) were 300 μg/ml of protein and 20 mg/ml of carbohydrate (as glucose units) respectively (protein : carbohydrate ratio 1:66). SDTCA on SDSPAGE showed seven Coomassie Blue staining bands (molecular weight range 10K–100K). Contrary to expectation, Periodate–Schiff staining was weak and showed two broad bands of approximate molecular weight 50K–100K. More than 95% of total carbohydrate in SDTCA was in the excluded fraction by sephadex G200 gel filtration. CDFilU contained 880 μg/ml of protein and 400 μg/ml of carbohydrate as glucose units. On SDSPAGE, CDFilU showed 4 Coomassie Blue staining bands of molecular weight range 15K–75K and 2 bands of molecular weight 60K–70K with Periodate–Schiff reagent. CDNU contained 790 μg/ml protein, 180 μg/ml carbohydrate and the same SDSPAGE pattern as CDFilU. Sephadex G200 gel filtration of CDNU and CDFilU showed carbohydrates in the excluded fraction and in the molecular weight range 40–60K.

3.2 Serum Antibody to SDTCA in *W. bancrofti* infections

The levels of antibodies reacting with antigen SDTCA in the sera of a representative group of *W. bancrofti* patients and non-filarial subjects are shown in Table 1. Antibodies reacting with SDTCA were not filarial specific. Levels of antibodies in filarial patients were not significantly different from those of non-filarial subjects. A relative absence of IgG antibody reacting with SDTCA determinants was observed (Table 1). The levels of total antibody (IgG+IgM+IgE) reacting with SDTCA in ELISA correlated well with the level of IgM antibody (Pearson's R=0.63, $p < 0.05$) but the total antibody did not correlate with the level of antibodies other than IgM (IgG+IgA+IgE, Pearson's R = 0.05, correlation not significant).

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Table 1. Levels of antibodies reacting with SDTCA in ELISA in a representative group of *Wuchereria bancrofti* infected and control subjects.

Subject Group	Antibody Levels, ELISA OD 492 Mean ± S.D. (% seropositive*)		IgG antibody, Urease** No. positive
	Total Ab.	IgM Ab.	
Elephantiasis. (N=16)	0.31 ± 0.07 (12)	0.19 ± 0.12 (6)	5
Lymphoedema. (N=40)	0.29 ± 0.07 (7)	0.28 ± 0.35 (10)	17
Microfilaremic. (N=5)	0.24 ± 0.09 (0)	0.19 ± 0.06 (0)	1
Nonendemic Controls. (N=16)	0.24 ± 0.08 (0)	0.15 ± 0.09 (6)	3

Pearsons's Correlation between total antibody (Total, A_{492}) and IgM antibody (IgM, A_{492}) for the total subjects R=0.63, $p < 0.05$.

Pearson's Correlation between total antibody (Total A_{492}) and other antibody (Total, A_{492} − IgM A_{492}), R = 0.05, not significant.

* A_{492} greater than mean + 2.SD of the non endemic control group was considered seropositive.

** Urease Conjugated affinity purified anti-human IgG.

Serum antibodies in patients reacting with SDTCA were also investigated by SDSPAGE–Western blotting. Different patients' sera showed different staining patterns and did not have any correlation with the disease type. The Western blot bands were broad and diffuse and estimation of molecular weight was difficult. Some filarial sera showed up to 6 bands of molecular weight range 15k to 70k, while non-filarial sera detected up to 3 bands, generally of molecular weight range higher than 60k. The bands of molecular weight less than 60k appeared to be reacting only with filarial sera. Table 2 gives a representative summary of the results of SDSPAGE Western blotting. The bands detected by Western blotting did not overlap with Coomassie blue staining bands.

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Table 2. Representative summary of SDSPAGE–Western blotting analysis of SDTCA with filarial & non-filarial sera.

Test Serum	Clinical history	Molecular weight range X 10^{-3} (approximate)						
		High M.Wt*	95–90	90–85	65–60	45–40	35–30	20–25
01 F0	microfilaraemia	+	–	–	+	+	+	+
02 F0	lymphodema	+	–	–	–	+	+	+
05 F0	microfilaraemia	+	–	–	–	–	–	+
11 F0	lymphodema.	+	–	–	–	–	–	–
12 F0	lymphodema.	+	–	–	–	–	–	–
14 F0	elephantiasis.	+	–	+	–	–	–	–
15 F0	lymphodema.	+	–	–	+	+	+	+
16 F0	lymphodema.	+	–	–	–	–	–	–
C 1	control.	+	–	–	–	–	–	–
C 2	control.	+	–	–	–	–	–	–
C 3	control.	+	–	+	–	–	–	–
C 4	control.	+	+	+	–	–	–	–

* Origin of Gel.

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3.3 Reactivities of rabbit antisera to antigens SD2–4, SDTCA, NHSTCA, CDFilU, human Ig and HIgCHO with SDTCA in ELISA

In Figure 1 are shown the relative ELISA reactivities of rabbit antisera to SD2–4, TCA–soluble antigens of *S. digitata* (SDTCA), TCA soluble components of normal human serum (NHSTCA), concentrated filarial urine (CDFilU), human Ig and human Ig carbohydrate (HIgCHO) with SDTCA. All antisera showed reactivity with SDTCA as compared to normal rabbit serum.

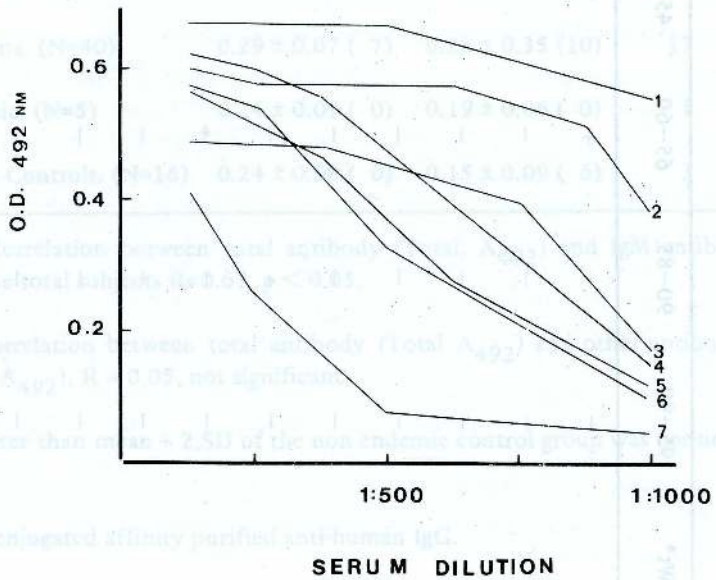

Figure 1. Relative ELISA reactivities of rabbit antisera made against filarial antigens and human serum and urine components with SDTCA antigen. All assays were performed in 5% normal goat serum, 0.5% bovine serum albumin and 0.05% tween 20. Each point represents the mean of 3 assays. Antigen SDTCA was coated at 5 μg/ml. 1 = rabbit anti Sd2–4, 2 = rabbit anti SDTCA, 3 = rabbit anti NHSTCA, 4 = rabbit anti CDFilU, 5 = rabbit anti human Ig, 6 = rabbit anti HIgCHO and 7 = normal rabbit serum pool. The conjugate used was goat anti rabbit Ig–Peroxidase absorbed against CNBr–Sepharose 4B insolubilised human Ig.

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3.4 Reactivity of rabbit antisera to filarial antigens, human serum components and human urine components with radiolabelled antigens as determined by PEGIRMA

Radiolabelled human Ig (NHIg) and TCA–soluble components in normal human serum (NHSTCA) when used as antigen in PEGIRMA reacted to the same extent with rabbit antisera to human Ig, human Ig carbohydrates, TCA soluble components in normal human serum (NHSTCA) and TCA soluble antigens of *S. digitata* (SDTCA). However, radiolabelled SDTCA did not show the converse reaction, possibly because the cross reacting epitope bearing molecules were not radiolabelled. Similarly rabbit antisera to concentrated filarial and non-filarial urines reacted with radiolabelled human Ig and NHSTCA. Antigens SDTCA and concentrated urines could not be labelled by the Chloramine T method to the same specific activities as human Ig and NHSTCA. This may account for the poor reactivity of SDTCA and concentrated urines as antigens in PEGIRMA. These results are summarised in Table 3.

3.5 Dot ELISA with concentrated urines

The goat anti-rabbit Ig–peroxidase conjugate reacted with 100% of the urines as detected by dot ELISA, and this reactivity was abolished when the conjugate was absorbed with human Ig (Table 4). Rabbit antiserum to SDTCA detected reactive components in 26% of the filarial urines and 12% of non-filarial urines. Greater part of this reactivity of the rabbit anti–SDTCA antiserum with urine was abolished when the anti–SDTCA antiserum was absorbed with CNBr–Sepharose 4B–insolubilised human Ig. The residual reactivity with normal urines could not be removed by exhaustive and repeated absorption of the antiserum (see discussion). The antiserum to human Ig carbohydrate showed similar reactivity. These results are summarised in Table 4. The dot ELISA reacting components in concentrated urines were restricted to the excluded fractions in Sephadex G200 gel filtration (data not shown).

4. Discussion

Studies on carbohydrate antigens of filarial parasites are relatively scarce. However the few reports available [4,5,6,8,25,26,32] indicate that carbohydrates form an important group of antigens in filarial infections.

The 10% trichloroacetic acid soluble molecules of adult *S. digitata* were predominantly carbohydrate (protein: carbohydrate = 1:66). Although serum antibodies reacting with these carbohydrates could be detected in *W. bancrofti* infections, the differences between the antibody levels of filarial patients and non-filarial subjects was not sufficient to be of diagnostic

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Tanle 3. Reactivity of rabbit antisera to SDTCA, SD2–4, Human Ig, Human IgCHO, NHSTCA and NRS with radiolabelled antigens, human Ig, SDTCA, NHSTCA, IN PEGIRMA.

Radiolabelled Antigen	Radioactivity in the immunoprecipitate (cpm).			
	High (>4X Mean NRS)	Moderate (<4, >3X Mean NRS)	Weak (<3, >2X Mean NRS)	Negative <2X Mean NRS
1. Human Ig	None	R.anti–human Ig (59%) R.anti–human IgCHO (42 %) R.anti–NHSTCA (57%) R.anti–SDTCA (50%)	R. anti–CDNU (42%) R.anti–CDFILU (46%).	R.anti SD2–4. (27%)
2. NHSTCA	R.anti–humanIg (10%) R.anti–humanIgCHO (38%) R.anti–NHSTCA (51%) R.anti–SDTCA (24%) R.anti–CDNU (36%) R.anti–CDFILU (37%)	None	None	R.anti SD2–4 (2%)
3. SDTCA	R.anti SD24 (15%) R.anti SDTCA (9%)	None	None	R.anti human IgCHO (1–2%) R.anti human Ig (1–2%) R.anti NHSTCA (1–2%) R.anti CDNU (1–2%) R.anti CDFILU (1–2%)
4. CDNU	None	None	None	All antisera.
5. CDFILU	None	None	None	All antisera.

Figures within parenthesis indicate the percentage of radioactivity precipitated.

NRS:Normal Rabbit Serum. SDTCA: 10% Trichloroacetic acid soluble antigens of *S. digitata*. SD2–4: Purified antigen from *S. digitata*. Human Ig: Human Immunoglobulin. Human IgCHO: Human Immunoglobulin Carbohydrate. NHSTCA: 10% TCA extract of normal human serum. NRS: Normal Rabbit Serum. CDNU: Concentrated and dialysed Urine from non–filarial subjects. CDFILU: Concentrated and dialysed Urine from Filarial Patients.

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Table 4. Detection of antigens in urine; Effect of immunoabsorption against human and rabbit immunoglobulins

Detecting Antibody in DOT ELISA	Enzyme Conjugate	Number positive in DOT ELISA. Filariasis Urine	Control Urine.
1. 1a. PBS–BSA–T20 diluent.	Unabsorbed Goat anti Rabbit–Ig–Peroxidase	10/10 (100%)	10/10 (100%)
1b. PBS–BSA–T20 diluent.	Goat anti–Rabbit Ig–Peroxidase absrobed with insolubilised human Ig.	0/10 (0%)	0/10 (0%)
2. Normal rabbit serum.	– do –	0/132 (0%)	0/10 (0%)
3. 3a. Unabsorbed rabbit anti SDTCA	– do –	35/132 (26%)	3/24 (12%)
3b. Rabbit anti–SDTCA absorbed with insoluble human Ig.	– do –	11/132 (8%)	2/24 (8%)
4. 4a. Unabsorbed rabbit anti human Ig–CHO.	– do –	48/132 (36%)	5/24 (21%)
4b. Rabbit anti human Ig–CHO absorbed with insoluble human Ig.	– do –	7/132 (5%)	2/24 (8%)

Composition of filariasis urine : microfilaraemic, n = 17 amicrofilaremic symptomatic. n = 115
Control urines : from endemic areas, n = 21, from non endemic areas n = 3.

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value. However, as observed in Western blot analysis, certain filarial specific epitopes are likely to be present in this complex mixture. Attempts to isolate such epitopes have so far been unsuccessful.

The antibodies in *W. bancrofti* infections reacting with determinants in SDTCA as determined by ELISA were predominantly IgM. The levels of total antibody showed a significant correlation with the level of IgM antibodies. Such dominance of IgM antibody responses has been reported in brugian filariasis.[17]

Immunoprecipitation results summarised in Table 3 demonstrate that rabbit antisera to human Ig, human Ig carbohydrates and 10% TCA extracts of normal human serum and the filarial parasite *S. digitata* show antigenic cross reactivity. Similarly, radiolabelled human Ig and 10% TCA extract of normal human serum showed comparable reaction with antisera to human Ig and carbohydrates of the filarial parasite *S. digitata.* The comparable reactions observed with the rabbit antiserum to human immunoglobulin carbohydrates show that the cross reacting epitopes were present in the carbohydrate fractions.

The observed cross reactivities between immunoglobulins and parasite derived carbohydrates were not due to contaminating cattle immunoglobulins which could have been present on the parasite surface. Adult *S. digitata* dwells in the peritoneal cavity and as such are not exposed to serum immunoglobulins. We were unable to detect cattle Ig in concentrated washings of the parasites. Also, cryostat sections of the parasite did not show detectable staining of immunoglobulins by immunofluorescence.

Table 4 shows that urines of some filarial patients and non-filarial subjects contained substances that reacted with antisera to immunoglobulins, Ig carbohydrates and parasite carbohydrates. While it is very likely that certain filarial antigens are excreted in urine,[6,26] we are unable to explain the residual reactivity seen with non-filarial urines. The possibility of subclinical infection is unlikely in these subjects. Although most of these reactivities could be abolished by absorption with insoluble immunoglobulins (evidence of antigen sharing), 5–8% of the urines remained positive even after several absorptions. We have consistently observed that certain urines contain molecules reacting with anti-immunoglobulin sera (apparently not species specific) which could not be removed by absorption against insoluble Ig. A possible explanation is that these epitopes are hidden in the intact molecule, but are expressed in the catabolic products. It is also possible that these are derived from other tissue components.[9,13]

The findings in this report extend the previous studies on host antigen sharing[2,16,19–24,27–30] to heterologous immunoglobulin carbohydrates. This has not been reported previously. Such cross reacting molecules were

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present in both blood serum and urine. These molecules are likely to affect the specificity and sensitivity of immunodiagnostic tests, particularly when antigen determination is attempted.[7,14]

Acknowledgements

This study received financial support from the Directorate General for Science, Research and Development Joint Research Centre of the Commission of the European Communities (Grant No. TSD–M–409 to S. Dissanayake). Excellent technical and computer assistance of Miss. S.C. Galahitiyawa is gratefully acknowledged.

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J. Natn. Sci. Coun. Sri Lanka 1987 **15** (2) : *161–174*

SALINITY TOLERANCES OF THREE SPECIES OF FRESHWATER ATYID SHRIMPS *(DECAPODA : CARIDEA)* OF SRI LANKA

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(Date of receipt : 02 December 1986)
(Date of acceptance : 12 May 1987)

Abstract : Salinity tolerances of *Caridina fernandoi, Caridina pristis* and *Caridina simoni* were studied in the laboratory by subjecting individuals from three populations of each species to a graded series of concentrations of sea water. *C. pristis* showed the lowest salinity tolerance and *C. simoni* showed the highest salinity tolerance. The ultimate incipient lethal salinities (which just fail to kill 50% of the sample on indefinite exposure) of *C. pristis, C. fernandoi* and *C. simoni* appear to be about 10.5, 13.5 and 16.5 ppt NaCl respectively. The possible influence of the salinity tolerance on the geographic distribution of the three species is discussed.

1. Introduction

Atyids are an important component in the freshwater littoral fauna of tropics and subtropics and they appear to occupy a position similar to that occupied by isopods and amphipods in the temperate littoral fauna. Sri Lanka, although a small island of 65,600 km^2, is rich in atyid fauna and contains ten species, which include *Atya spinipes* and nine species of *Caridina.* Of the nine species of *Caridina*, four, *Caridina gracilirostris, Caridina propinqua, Caridina typus* and *Caridina zeylanica* have a coastal distribution, two, Caridina *fernandoi* and *Caridina simoni*, are widely distributed at altitudes upto 700 m, one, *Caridina pristis*, is found between 480 and 850 m, one, the rare *Caridina singhalensis*, is isolated in a single stream at 2,100 m, and one, *Caridina costai,* is reported only from one location at 500 m.[4] *C. costai, C. fernandoi, C. pristis, C. singhalensis* and *C. zeylanica* are not recorded outside Sri Lanka, except for a doubtful case of *C. fernandoi* in South India.[14] *C. pristis* and *C. singhalensis* have a restricted distribution in the hill country and appear to be truly endemic to the island. Three species, namely, *C. gracilirostris, C. propinqua* and *C. typus* are widely distributed in the Indo–West Pacific region.[1] Therefore it is interesting to know whether *C. zeylanica,* which is more widely distributed in the coastal region of Sri Lanka than the other three species, is truly confined to Sri Lanka. *C. simoni*, which occurs together with *C. fernandoi*, in many low country water bodies, is also widely spread in the Indo–west Pacific.[1] Thus, it is also interesting to know whether *C. fernandoi* is also truly endemic. Since *C. costai* is recorded only from one location so far, more work is obviously necessary before the status of this species is decided.

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Despite the importance of Atyidae in the tropical freshwater ecosystem, not much attention has been paid to the group, except for the systematic studies, and little is known of the ecology of the group. Some preliminary work on the salinity tolerance of *C. pristis* and *C. simoni* has been carried out.[2,10] Apart from these, no work has been reported on the tolerance to ecological factors of Sri Lankan atyids. As part of a broader study of the distribution and ecology of Sri Lankan atyids, the present paper examines the role of salinity tolerance may play in determining the geographic distribution of three species, namely, *C. fernandoi, C. pristis* and *C. simoni.*

2. Materials and Methods

Samples of each species were collected from three populations. *C. fernandoi* was collected from Kandy lake, Kurunegala lake and a small reservoir at Ginigathena; *C. pristis* was collected from three slow-flowing streams at Peradeniya university campus, Galaha and Talatuoya; *C. simoni* was collected from Sarasavi oya at Peradeniya campus, Kandy lake and Kospothu Oya at Galagedera (Figure 1). Samples were kept separately in pond water in the laboratory at 24°C for at least two weeks prior to experimentation in order to acclimatise the shrimps to laboratory conditions. Shrimps were fed with decaying leaves of *Ficus religiosa* (Family Moraceae), which were easily obtained and found to be readily acceptable to all three species. Under these culture conditions the mortalities were negligible.

Individuals of *C. fernandoi* and *C. simoni* in the size range 15—17 mm, and individuals of *C. pristis* in the size range 12—14 mm were selected for experimentation. These size ranges include medium-sized adults. Sexing of live *Caridina* spp. is difficult and therefore, in order to determine whether the response to salinity is different between males and females, a preliminary experiment was carried out in which the response of the two sexes were tested for 10 days at 25%, 50%, 75% and 100% sea water (7.5, 15.0, 22.5 and 30.0 ppt NaCl). Ten males and ten females of each species (*C. fernandoi* and *C. simoni* from the Kandy lake populations and *C. pristis* from the Peradeniya population) were selected from the suitable size ranges and were kept at test salinities. There were no deaths in 10 days at 25% and 50% sea water in any species but mortalities occurred at higher concentrations. (Figure 2). Wilcoxon's two sample test showed that there was no statistically significant difference ($P > 0.01$) between the responses of males and females of any of the three species to salinities equivalent to 75% and 100% sea water. Therefore, no attempt was made to separate sexes in the subsequent experiments, but, ovigerous females were excluded.

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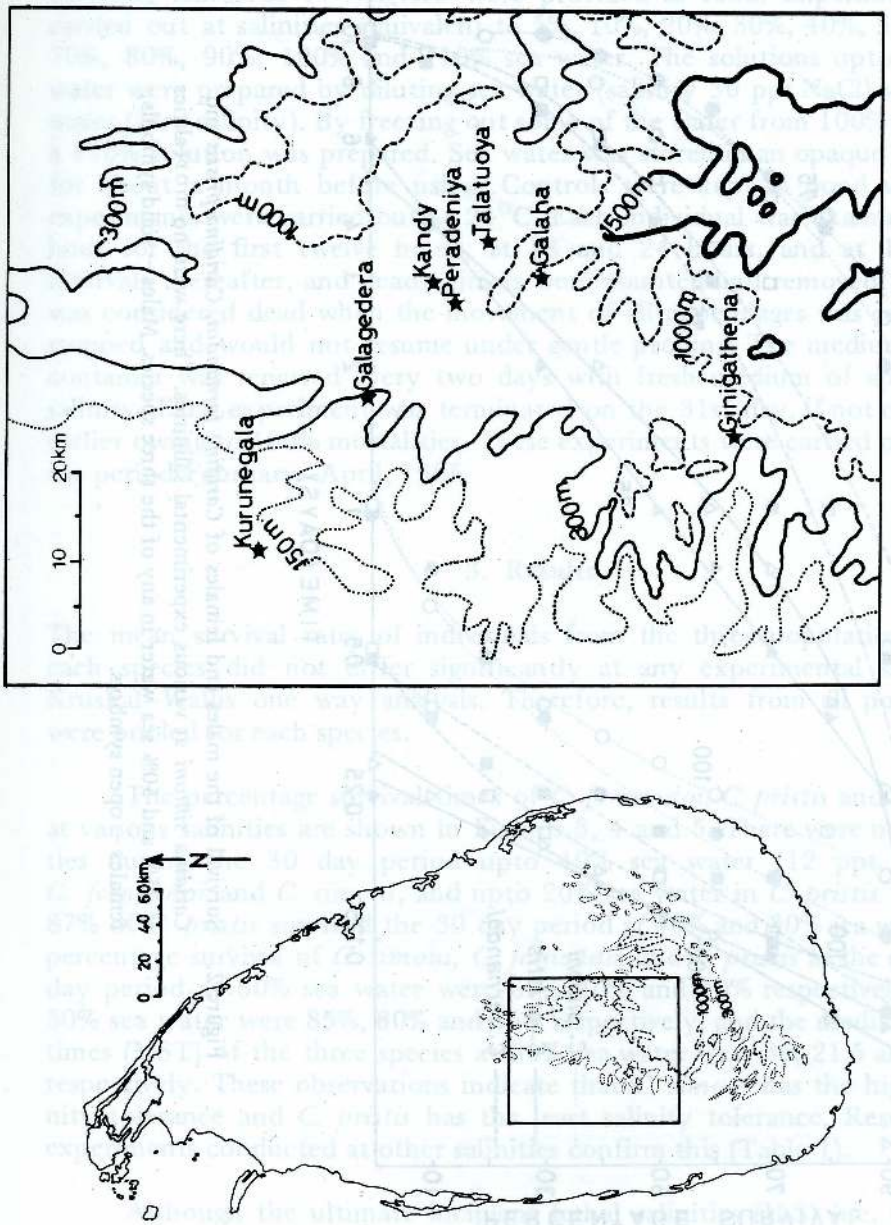

Figure 1. (a) Sri Lanka showing 300 m and 1,000 m contours. Samples were obtained from localities in the sector demarcated in the central region of the map.
(b) Enlarged map of the sector demarcated in the central region of the map in (a). 150 m, 300 m, 1,000 m and 1,500 m contours are shown. Sampling locations are indicated by (★).

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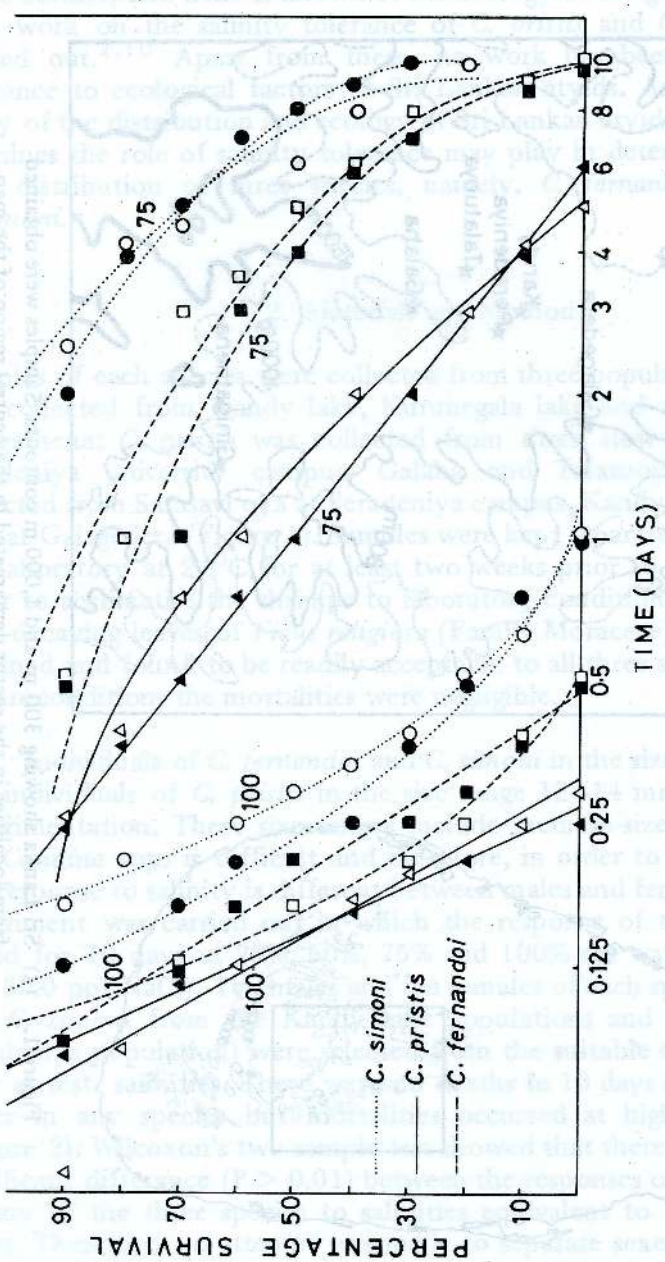

Figure 2. Survival of the males and females of *Caridina fernandoi*, *Caridina pristis* and *Caridina simoni* at various experimental salinities. There were no mortalities at 25% and 50% sea water in any of the three species. Males — solid symbols; females — open symbols.

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Thirty specimens (10 from each location) of each species were used in each experiment. Each specimen was kept in 100 ml of the test medium in a wide-mouthed 250 ml glass bottle (depth of water about 25 mm). Pieces of decaying leaves of *F. religiosa* were provided as food. Experiments were carried out at salinities equivalent to 5%, 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, 100% and 110% sea water. The solutions upto 90% sea water were prepared by diluting sea water (salinity 30 ppt NaCl) with pond water (zero salinity). By freezing out some of the water from 100% sea water a 110% solution was prepared. Sea water was stored in an opaque container for about a month before using. Controls were kept in pond water. All experiments were carried out at 24°C. Each individual was examined every hour for the first twelve hours, at 18 and 24 hours, and at 12 hourly intervals thereafter, and dead animals were counted and removed. A shrimp was considered dead when the movement of all appendages was completely stopped and would not resume under gentle probing. The medium in each container was renewed every two days with fresh medium of appropriate salinity. Each experiment was terminated on the 31st day, if not concluded earlier owing to 100% mortalities. These experiments were carried out during the period February–April, 1985.

3. Results

The mean survival rates of individuals from the three populations within each species did not differ significantly at any experimental salinity in Kruskal–Wallis one way analysis. Therefore, results from all populations were pooled for each species.

The percentage survival times of *C. fernandoi, C. pristis* and *C. simoni* at various salinities are shown in Figures 3, 4 and 5. There were no mortalities during the 30 day period upto 40% sea water (12 ppt NaCl) in *C. fernandoi* and *C. simoni*, and upto 20% sea water in *C. pristis*. 70% and 87% of *C. pristis* survived the 30 day period at 40% and 30% sea water. The percentage survival of *C. simoni, C. fernandoi* and *C. pristis* at the end of 30 day period at 60% sea water were 67%,40% and 10% respectively, and at 50% sea water were 85%, 80% and 55% respectively, and the median survival times (MST) of the three species at 60% sea water were 30, 21.5 and 8 days respectively. These observations indicate that *C. simoni* has the highest salinity tolerance and *C. pristis* has the least salinity tolerance. Results from experiments conducted at other salinities confirm this (Table 1).

Although the ultimate incipient lethal salinities (ILS) (i.e. the lethal salinity levels which on indefinite exposure just fail to kill 50% of the sample) of the three species cannot be determined precisely from the present data, a rough estimate can be made from the probit lines in Figures 3, 4 and 5, and from the MST graphs in Figure 6. The ILSs of *C. pristis, C. fernandoi*

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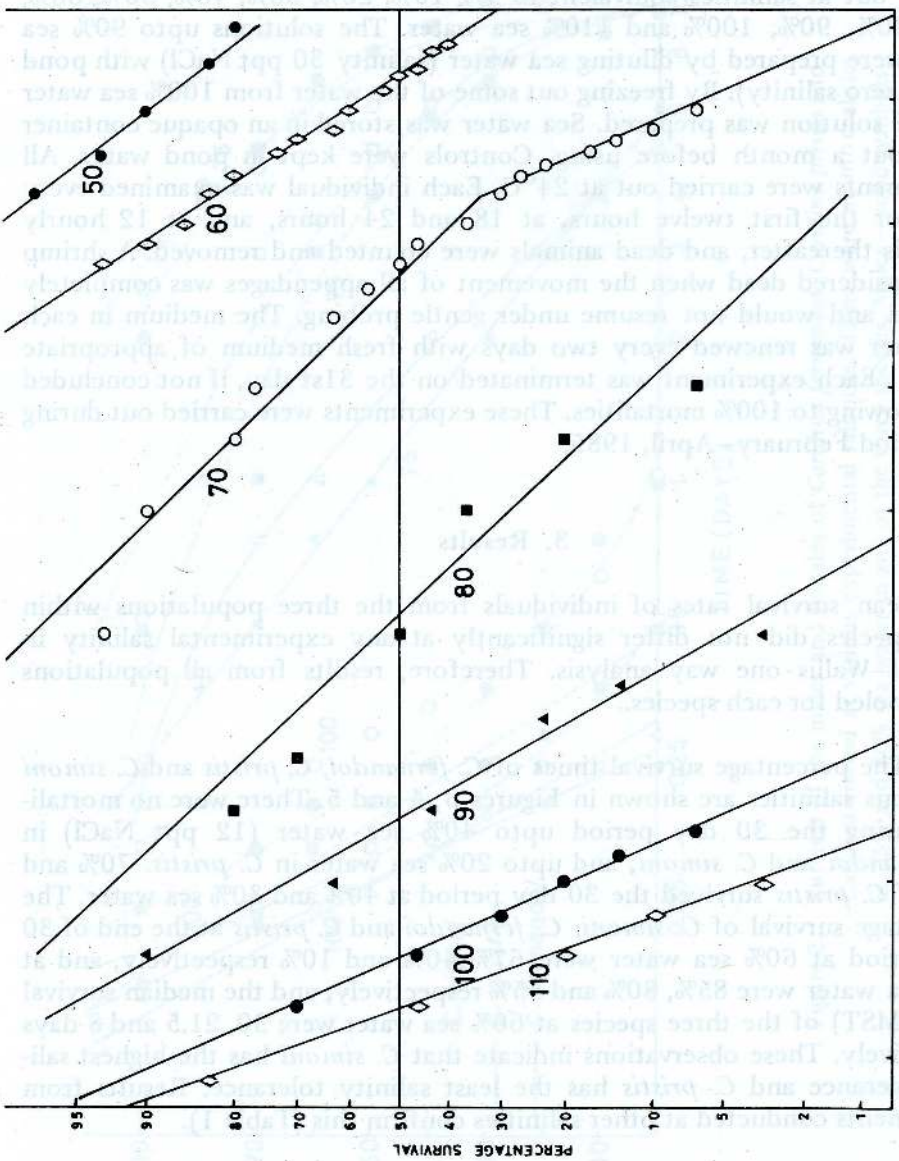

Figure 3. Survival of *Caridina fernandoi* at each experimental salinity. Percentage survival is plotted on a probability scale and survival time on a log scale.

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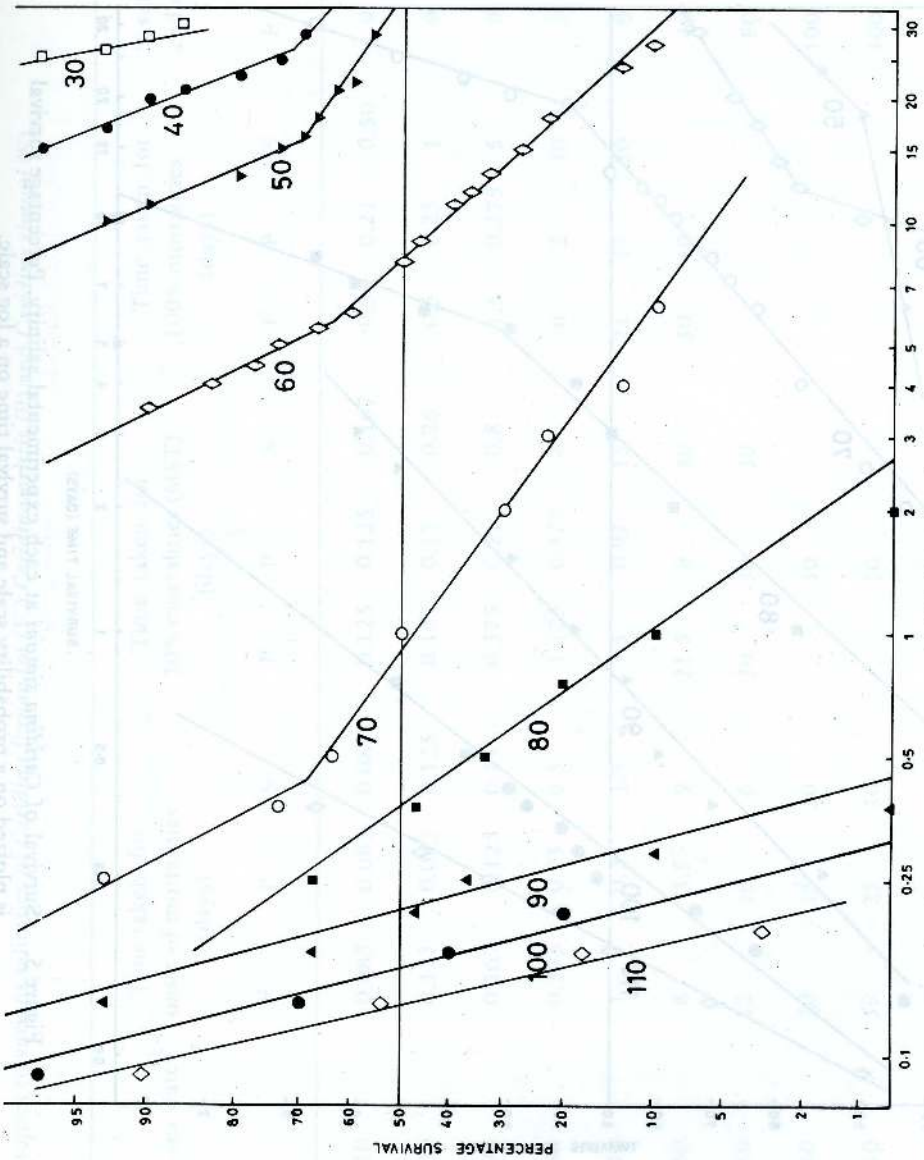

Figure 4. Survival of *Caridina pristis* at each experimental salinity. Percentage survival is plotted on a probability scale and survival time on a log scale.

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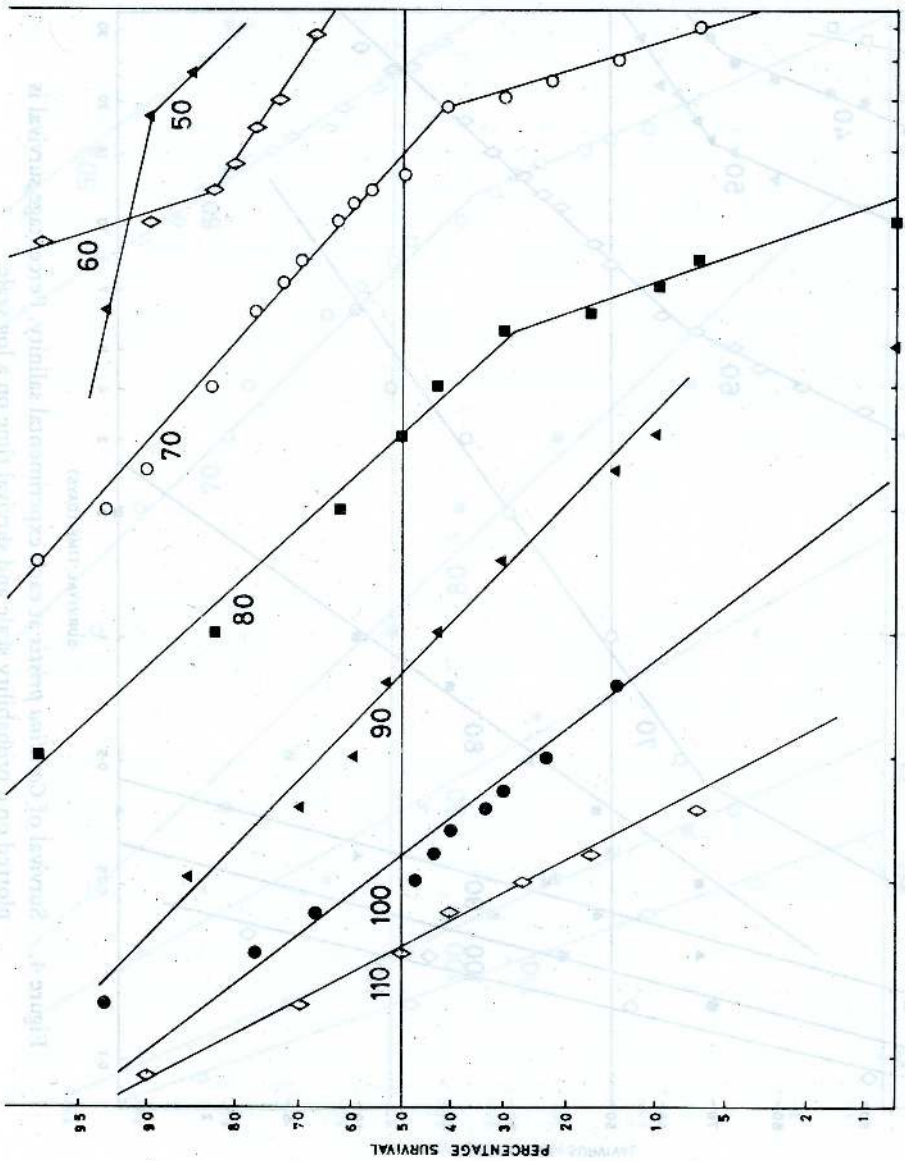

Figure 5. Survival of *Caridina simoni* at each experimental salinity. Percentage survival is plotted on a probability scale and survival time on a log scale.

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Table 1. Responses to various salinities of *Caridina fernandoi* (F.) *Caridina pristis* (P) and *Caridina simoni* (S).

Salinity ppt NaCl	% sea water	Time taken for onset of mortalities (days)			Time taken for 50% mortalities (MST) (days)			Time taken for 100% mortalities (days)			% survival at the end of 30 days		
		F	P	S	F	P	S	F	P	S	F	P	S
33	110	0.083	0.083	0.083	0.125	0.125	0.167	0.33	0.21	0.50	0	0	0
30	100	0.125	0.083	0.125	0.167	0.17	0.26	0.5	0.25	1	0	0	0
27	90	0.167	0.125	0.25	0.375	0.21	0.8	1.5	0.375	5	0	0	0
24	80	0.375	0.25	0.5	1.125	0.375	3	6	2	10	0	0	0
21	70	1	0.25	1.5	7.5	0.92	15	21	13	30	0	0	7
18	60	8	3.5	9	21.5	8	30	30	30		40	10	67
15	50	12	10	6	30	30	30				80	55	85
12	40	30	15	30		30					100	70	100
9	30	30	25	30		30					100	85	100
6	20	30	30	30							100	100	100

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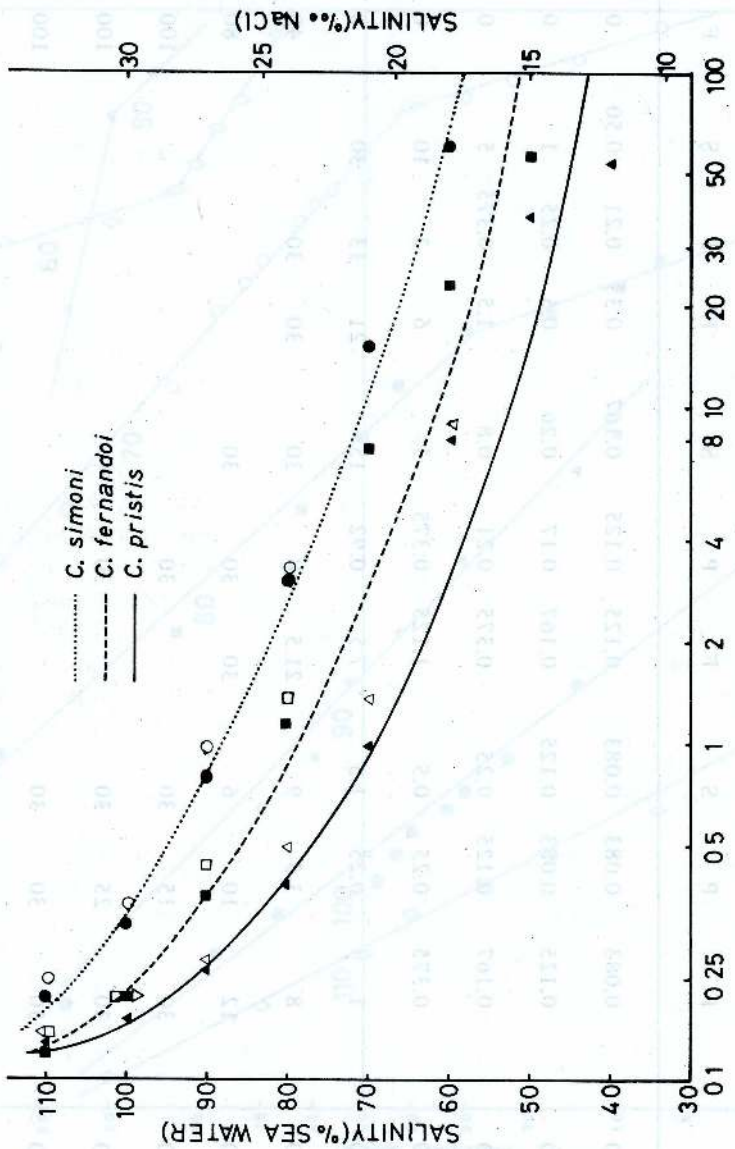

Figure 6. Relationship between median survival time (MST) and salinity in *Caridina fernandoi*, *Caridina pristis* and *Caridina simoni*. MST is plotted on a log scale. Solid symbols indicate the MSTs estimated from the probit lines in figures 3, 4 and 5, and open symbols indicate the geometric mean time to death.

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and *C. simoni* thus estimated appear to be 35%, 45% and 55% sea water (10.5, 13.5 and 16.5 ppt NaCl) respectively.

4. Discussion

The results indicate that all three species can live in low salinity brackish water. However, *C. pristis* has not been recorded below an altitude of 480 m. Therefore, other ecological factors must be responsible for limiting the species to upper elevations. The data on its response to various temperatures indicate that temperature is of major importance in this respect.[5] *C. pristis* also has the lowest salinity tolerance of the three species, with MSTs of > 30 days, 8 days and less than 1 day at 15 ppt, 18 ppt and 21 ppt NaCl respectively. Because of its confinement to upper elevations and its low salinity and high temperature tolerances, it does not appear to be amenable to a marine distribution and hence it is unlikely to find *C. pristis* outside Sri Lanka, if it had evolved within the island.

C. fernandoi has MSTs of > 30 days, 21.5 days and 7.5 days at salinities of 15 ppt, 18 ppt and 21 ppt NaCl respectively. It is found almost down to the sea level. For instance, it has been recorded from Giant's reservoir at Murunkan[6] at less than 10 m altitude. However, it has not been recorded from brackish water. Responses of *C. fernandoi* to various temperatures and pHs[5] indicate that these factors are of no hindrance for the species to colonize at least some of the low salinity brackish water bodies or inland water bodies that become somewhat saline during some part of the year. Thus, the possibility exists that its range of geographic distribution is wider than presently known and extends outside Sri Lanka. In this connection, it is of importance to note that *C. simoni,* with which *C. fernandoi* occur in many water bodies, is widely distributed in the Indo-West Pacific.

C. simoni shows the highest salinity tolerance among the three species, with MSTs of > 30 days at 15 and 18 ppt, and 15 days at 21 ppt NaCl. It has been recorded from slightly brackish water bodies in Sri Lanka, for instance, at 0.15 ppt NaCl at Beira Lake[3] and at the same salinity at Bolgoda lake.[5] Its wide distribution in the Indo-West Pacific includes many islands in the Pacific.[8] (The taxonomic status of *C. simoni* is still not settled and some authors[7] consider it as conspecific with *C. nilotica*, a species found in East Africa from Egypt to Natal.[7]) It has been described as occasionally occurring in slightly brackish waters[7] and entering low salinity tidal rivers.[8] Its pattern of distribution and the ability to tolerate considerable salinity suggest that it is capable of some marine dispersal.

In some Caridea, even though the adult may be purely freshwater, the larva may have a salt water requirement for development (e.g. *Atya* spp. and *Macrobrachium* spp.). Thus, presumably, larva of these species has a higher

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salinity tolerance than the adult and the larva may be capable of marine dispersal.[9] No data are available on this aspect of the three species considered in the present study, except for the observation that there is no brackish water requirement for the development of the larva of all three species[5] and that *C. simoni* could apparently develop in slightly brackish waters.

In an earlier study,[2] *C. simoni*, when offered a choice between diluted sea water and tap water, reacted indifferently to the two media upto 16.5 ppt NaCl at 25°–26°C (the shrimp's response to higher salinities were not tested). This is in agreement with the present results since the MSTs at 15 at 18 ppt NaCl were more than 30 days. However, in another study of salinity tolerance of this species,[10] 30% and 40% mortalities occurred in > 30 days at 3 ppt and 6 ppt NaCl respectively at 24°–26°, and the MST at 11 pt was 22 days, in contrast to the absence of mortalities upto 12 ppt during the same period and a MST of more than 30 days even at 18 ppt observed in the present study (Table 1). The observations on the salinity response of *C. pristis* in the present study differ markedly from those of the two earlier studies.[2,10] It was shown that there was a negative reaction even at 6 ppt NaCl,[2] and that the MSTs at 3, 6 and 9 ppt NaCl were 24 days, 23 days and 21 days respectively,[10] in contrast to the absence of mortalities upto 6 ppt and 13% and 30% mortalities in > 30 days at 9 and 12 ppt respectively observed in the present study. It is difficult to account for the differences observed, since experiments were carried out at similar temperatures and using specimens presumably from the same locality at Peradeniya. However, it is not clear from the description of the earlier experiments[10] whether, (i) shrimps were acclimatised to laboratory conditions prior to experimentation, (ii) food was provided to the shrimps during the experimental period, and (iii) all specimens used in experiments belonged to the same size class, and if so, which size class was used. In this earlier study,[10] 20 shrimps were kept in a single container (size of the container and the amount of medium used not specified) and therefore, the possibility of dead and decaying shrimps contaminating the medium and affecting the condition of the live ones cannot be ruled out, since the observations were made only once a day and the medium was changed only 2–4 days. It was noted that even a few dead and decaying shrimps could contaminate and cause considerable mortalities in cultures within a few hours.

Salinity response of a species may change with the ambient temperature. Although data are lacking for atyids, it is known that in estuarine decapods such as fiddler crabs (*Uca* spp.), tolerance to high salinities decreases with increase of temperature.[11] Thus, it is possible that the ranges of salinity tolerance of the three species are different at the temperatures prevalent at the sea level than those estimated in the present study at 24°C. The monthly mean temperature of Beira lake varied between 26° and 33°C during the period May 1969 to March 1971[3] and that of Bolgoda lake varied

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between 28° and 32°C during the period October 1980 to September 1981.[13] It has also been shown that, in species such as *Uca* spp., exposure to sublethal intensities of two environmental factors such as temperature and salinity could become lethal.[12]. The organism interacts not with individual ecological factors but with the sum total of many separate factors, and these factors may act synergetically to produce an effect which is more extreme than that which results when each factor acts separately. Thus, study of synergetic action of at least temperature and salinity is more appropriate for understanding geographic distribution of atyid species.

Acknowledgements

Thanks are due to Mr. T.S.B. Alagoda of this department for drawing the figures in this paper. I also wish to thank my wife, Kumari, for her unfailing help both in the field work and laboratory work. This work was carried out in the laboratories of Department of Zoology, University of Peradeniya.

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J. Natn. Sci. Coun. Sri Lanka 1987 **15** (2) : *175–182*

THRESHOLD WEED COMPETITION DURATION OF MUNGBEAN *(PHASEOLUS AUREUS. ROXB)*

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(Date of receipt : 05 September 1985)
(Date of acceptance : 02 June 1987)

Abstract : The threshold weed competition duration of mungbean with respect to the natural weed flora was investigated by allowing the weeds to compete with the crop for different durations ranging from 5 to 42 days after seeding (DAS) at the Agricultural Research Station, Angunukolapalessa, during Maha (wet) season 1984/85 and Yala (dry) season 1985. The data showed that the threshold weed competition duration of mungbean to be 35 DAS without any seasonal variation on threshold level. The gain on grain yield at threshold level was 101% and 102% during Maha 1984/85 and Yala 1985, respectively. The pod number was most affected by the weed competition.

1. Introduction

Weeds are nourished by the same nutrients and environmental factors needed by the crop. Because of the limited supply of these elements, weeds compete with the crop to procure them. Weed control attempts to shift competition in favour of the crop to make it more competitive than the weeds.

The duration of weed competition can be defined as the time where competition between weed and crop persist from the time of crop seed germination. Mercardo[7] indicated that weed competition duration critical to crops varies with the type of the crop, and observed it by either allowing the crop to compete with weeds or keep the crop weed-free for different durations. The author further indicated that all crops demand weed control during the early period of growth, particularly the first 1/3 to 1/2 of its life cycle. Klingman *et al.*[4] concluded that the weed competition early in the season is more critical in reducing crop yields than late season competition.

Legumes are very sensitive to weed competition and early season weed control is necessary to get optimum yields. Asian Vegetable Research and Development Centre (AVRDC)[1] reported 27% and Madrid and Manimtim[5] 10% yield loss of mungbean due to uncontrolled weed growth. Further, yield depression of 9% compared to weed free control was observed in 5 mungbean varieties when weeded 2 weeks after emergence.[8] Weed competition reduces branching[2] and pods per plant.[2,9] The present study was undertaken to find out the threshold weed competition duration of mungbean.

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2. Materials and Methods

The experiments were conducted at the Regional Research Station, Angunukolapellessa during Maha 1984/85 (wet) season and Yala 1985 (dry) season using a randomized complete block design, replicated three times. The soils at the experimental site belonged to Ranna series of reddish brown earths in the well drained land class. During the experimental period, the crop received 212.7 mm and 129.6 mm rainfall and daily temperature ranged from 24.6°C – 25.9°C and 27.3°C – 28.2°C during Maha and Yala seasons, respectively.

Mung bean variety M.I. 4 with a growth duration of 65 days was used. Mungbean seeds were dibbled at 30 cm x 10 cm spacing in 4 m x 3 m plots and thinned out to one plant per hill. The crop was allowed to compete with weeds upto 6, 12, 16, 20, 25, 29, 35 and 42 days after seeding (DAS) during Maha and 5, 10, 15, 20, 25, 30, 35 and 40 DAS during Yala and were compared with a weeded and an unweeded control. N.P.K. at the rate of 15:60:47 kg/ha was applied basally with a top dressing of urea at the rate of 30 kg/ha at flowering stage. Pod borer damage was observed at flowering stage in both seasons, which was controlled by spraying Azodrin at the rate of 62 ml/ha.

Plant height and pod number per plant were recorded from 10 randomly selected plants at harvest. Number of seeds per pod from 25 randomly selected pods, 1000 seed weight and yield/ha were recorded. The individual plots are weeded at the end of weed competition duration and weighed. A representative sample of weeds was then weighed and dried in an oven to constant weight. The ratio of fresh weight to dry weight was thus obtained and the weed dry weight of plots were calculated by multiplying the weed fresh weight by this factor. Dry matter production of mungbean in a weeded and an unweeded plot was monitored at weekly intervals in two separate plots treated same way as for experiment. Plants were sampled and dried in an oven to constant weight to obtain the dry matter yield per plant.

3. Results and Discussion

The weed population during the Maha season mainly consisted of grasses and sedges. The presence of sedges was due to the cultivation of wetland rice in the area for several seasons, even though the soil were well drained. Among the grasses, *Echinocloa crusgalli* was dominant. During the Yala season, the experimental site was changed and the weed population was dominated by broad-leaved *Euphorbia geneculata L.* and grasses. Sedges were less and consisted mainly of *Cyperus rotundus*. Of the two seasons, the pod borer outbreak was severe during Yala and was observed at the late flowering stage.

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Table 1. Dry matter yield of mungbean g/plant

Age of plant in weeks	Dry matter yield g/plant			
	1984/85 Maha		1985 Yala	
	Weeded	Unweeded	Weeded	Unweeded
1	0.17	0.16	1.20	1.10
2	0.87	0.76	2.40	2.10
3	4.23	3.39	5.30	4.00
4	5.80	6.28	10.80	7.40
5	16.70	10.70	25.80	18.50
6	30.40	23.50	36.30	35.80
7	41.1*	30.30*	87.70	55.70
8	48.4*	33.30*	95.60	64.30

* – without pods

Mungbean dry matter production increased with the increase in age of the crop, irrespective of the weed competition (Table 1). However, the rate of increase was lower in the unweeded treatment. The maximum growth period extended upto 42 DAS in both seasons. The plant height was drastically reduced when the weed competition duration exceeded more than 35 DAS in Maha (Table 2) where as in Yala there was no such reduction (Table 3). The plant height in Maha season was low (49 – 60 cms) compared to the Yala season (59 – 69 cms). This could be attributed to the higher rainfall received during the Maha season (212.7 mm), compared to Yala season (127.6 mm). Madrid and Vega[6] also observed that mungbean can compete favourably with weeds during the dry season than during the wet season.

The yield components showed no significant difference except in pod number per plant during Maha season (Tables 2 and 3). However pods per plant and 1000 seed weight decreased, with the increase in weed competition duration in both seasons. However, the decrease in these parameters were not consistent and the difference between the weeded and unweeded control was very high. The number of pods per plant was significantly low, when the weed competition duration exceeds 29 DAS. Enyi, and Rethinum *et al.*[2,9] reported that the pod number per plant decrease with the increase in duration of weed competition. The other yield components were not affected by the weed competition except where the competition duration extended upto the harvest. The seed number per pod and pod number per plant were high in Maha season even though the plants were taller in Yala. This could probably be attributed to the severe pod borer damage during the Yala season destroying the early pods which are normally long and bear higher number of seeds per pod.

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Table 2. Plant height and yield components of mung bean at harvest Maha 1984/85 season

Treatment	Plant height cm	Pod No/Plant	Seed No/Pod	100 Seed weight gm
Weed free 2 DAS	54.6	16.8a	12.2	47.1
" 12 DAS	56.8	16.5a	11.1	47.8
" 16DAS	54.9	12.7a	10.8	46.9
" 20 DAS	56.0	13.8a	10.7	48.6
" 27 DAS	52.4	9.8a	11.1	46.2
" 29 DAS	57.8	10.9a	11.4	48.9
" 35 DAS	56.2	9.4b	11.6	47.8
" 42 DAS	44.6	9.3b	10.5	47.6
Unweeded control	50.1	8.5b	9.5	44.9
Weeded control	60.7 N.S.	13.7a	10.9 N.S.	50.5 N.S.

Any two means followed by the same letter are not significantly different at 5% level.

Table 3. Plant height and yield components of Mungbean at harvest – Yala 1985

Treatment	Plant height cm	Pod No/Plant	Seed No/Pod	1000 Seed weight gm
1. Weed free 5 DAS	68.2	10.0	7.5	48.0
2. " 10 DAS	69.1	10.6	8.7	49.3
3. " 15 DAS	66.4	10.6	7.9	47.8
4. " 20 DAS	63.7	10.3	7.7	48.4
5. " 25 DAS	62.7	12.3	7.4	48.1
6. " 30 DAS	64.9	10.1	8.6	48.6
7. " 35 DAS	63.2	10.7	7.5	47.0
8. " 40 DAS	62.2	8.5	8.4	45.7
9. Unweeded control	59.5	9.4	8.2	43.2
10. Weeded control	66.5 N.S.	12.7 N.S.	7.3 N.S.	47.5 N.S.

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Mungbean grain yields obtained during the Maha was higher than the Yala season. The lower yields in the Yala season could be due to the severe pod borer attack observed at late flowering stage.

Table 4. Grain yield and percentage increase in yield

TREATMENT	1984/85 Maha		1985 Yala	
	Yield/ha	% increase over unweeded control	Yield/ha	% increase over unweeded control
Weed free 5 DAS	–	–	566.3 a	109.9
" 6 DAS	976.6 a	208.5	–	–
" 10 DAS	–	–	597.4 a	126.5
" 12 DAS	961.1 a	203.6	–	–
" 15 DAS	–	–	502.3 a	86.2
" 16 DAS	894.0 a	182.4	–	–
" 20 DAS	911.3 a	187.8	592.3 a	119.6
" 25 DAS	–	–	434.3 b	61.0
" 27 DAS	597.2 a	88.6	–	–
" 29 DAS	647.3 a	104.5	–	–
" 30 DAS	–	–	490.4 a	81.8
" 35 DAS	635.3 a	100.6	545.9 a	102.4
" 40 DAS	–	–	367.2 c	36.1
" 42 DAS	440.0 b	39.0	–	–
Unweeded control	316.6 b	–	269.7 d	–
Weeded control	917.9 a	189.9	518.5 a	92.2
C. V. = 22.7%			20.2%	

Any two means followed by the same letter are not significantly different at 5% level.

Significant decrease in grain yield in both seasons was observed when the weed competition duration exceeded 35 DAS. Similar results were observed by Madrid and Vega[6] in mungbean yields if crop-weed competition was more than 4 weeks during the wet and dry seasons under irrigation in Philippines. They also reported that weeds had little effect if they were allowed to compete with mungbean for 2 weeks during the wet season and 4 weeks during the dry season. Present study showed no seasonal variability in threshold weed competition duration. Nevertheless, grain yield decreased significantly in treatment 25 DAS (Table 4) during Yala which could be attributed to exceptionally high weed density observed in these plots (Table 5).

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Table 5. Weed dry weight under different treatments

Treatment	Weed dry weight g/plot	
	Maha 1984/85	Yala 1985
Weed free 5 DAS	–	17.5
" 6 DAS	68.3 a	–
" 10 DAS	–	50.7
" 12DAS	240.8 a	–
" 15 DAS	–	70.8
" 16 DAS	417.2 a	–
" 20 DAS	822.3 a	359.0
" 25 DAS	–	1518.0
" 27 DAS	1114.8 b	–
" 29 DAS	1126.2 b	–
" 30 DAS	–	792.0
" 35 DAS	1460.3 c	748.0
" 40 DAS	–	1413.0
" 42 DAS	2803.4 d	–
Unweeded control	–	–
Weeded control	–	–

Any two means followed by the same letter are not significantly different at 5% level.

The increase in grain yield over unweeded control was subjected to seasonal variability with a higher increase in the Maha season. However, early weeding resulted in a greater increase in grain yield. One late weeding 35 DAS resulted in 100.6% and 102.4% yield increase over unweeded control during Maha and Yala seasons respectively. The seasonal difference in grain yield could be attributed to the differences in competing weed species and their densities. Higher weed density dominated by monocots during the Maha was more competitive and their removal at different competition durations resulted in higher grain yields, unlike the Yala season where the weed population dominated by broad leaved weeds. However, if the weed competition continued upto harvest time broad leaved weeds are equally competitive as monocots at high density (comparable yields of two unweeded controls, Table 4). Moody[8] also reported that one late weeding 30 DAS, an average yield increase of 34% over the unweeded control could be obtained.

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Grain yield data also indicate that yield performance of mungbean during the Maha season was better that the Yala season under irrigation. This however, is doubtful due to pod borer damage in the Yala season.

Conclusion

The results indicate that the threshold weed competition duration of Mungbean was 35 DAS and the yield component most affected by the weed competition was pod number per plant. Further, a late weeding 35 DAS leads to 100.6% and 102.4% increase in yield over the unweeded control during Maha and Yala seasons respectively. The seasonal difference in grain yield could be attributed to the differences in competing weed species and their densities during the two seasons. However, there is no seasonal variation in threshold weed competition duration.

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J. Natn. Sci. Coun. Sri Lanka 1987 15 (2) : *183–200*

STUDIES ON COMPLETE AND PARTIAL ACIDULATION OF EPPAWELA APATITE

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(Date of receipt : 27 April 1987)
(Date of acceptance : 27 July 1987)

Abstract : Acidulation of Eppawela apatite with hydrochloric acid and sulphuric acid has been investigated in an attempt to convert this rock phosphate to an acceptable P–fertilizer using a simple and low cost method. Although direct acidulation with hydrochloric acid leads to a highly hygroscopic product, physical condition of this material can be improved by subsequent treatment with ammonia. Hydrochloric acid acidulation with 15–20% HCl followed by pH adjustment using an alkali, leads to the precipitation of dicalcium phosphate, $CaHPO_4$, which can readily be used as a P–fertilizer. Use of ammonia and ammonium salts has an added advantage in that the product contains nitrogen in addition to phosphorus. Physical condition of H_2SO_4 – acidulated material is superior and the fertilizer value of this product is dependent on the extent of acidulation. Since the cost of this product depends mainly on the cost of H_2SO_4, partial acidulation with sulphuric acid seems to be a potentially useful and economical method. 50% acidulation with sulphuric acid to produce partially acidulated phosphate rock (PAPR–50) containing 17% wt available P_2O_5 and 25% wt total P_2O_5 appears to be suitable.

1. Introduction

Rock phosphate reserves[6] at Eppawela in Sri Lanka can be represented as chlorfluorapatite [3] $Ca_5(PO_4)_3(Cl,F)$. It has been reported[8] that the use of Eppawela apatite as a phosphatic fertilizer is limited due to its very low solubility. Its water solubility is about 0.5% wt P_2O_5, while 2% citric acid solubility[3] is in the range 5–6% wt P_2O_5. Thus, it is not generally recommended for direct application especially for short term crops such as paddy. As such, attempts have been made to convert this mineral to more soluble phosphate fertilizers by low temperature sintering methods.[3,4,5]

Acidulation is one of the common methods[10] to convert rock phosphate to more soluble phosphate fertilizers. Most commonly rock phosphate is treated with either H_2SO_4 or H_3PO_4 to produce singlesuperphosphate (SSP) or triplesuperphosphate (TSP) respectively. SSP contains about 20% wt available P_2O_5 while TSP possesses much higher available phosphorus content ($\sim$ 45% wt P_2O_5).

Eppawela rock phosphate is believed to be of igneous origin[6] and contains more chlorine[3] than fluorine. Its total P_2O_5 content is rather high, the average being about 36%. It has a relatively high and a variable content

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(4–10%) of total Al_2O_3 and Fe_2O_3. Although these special chemical features of Eppawela apatite could make it difficult to use it for conventional acidulation processes, it is of interest to investigate the basic aspects of its acidulation reaction with locally produced mineral acids such as hydrochloric and sulphuric acids.

In the present study complete and partial acidulation of Eppawela apatite with hydrochloric acid and sulphuric acid has been investigated in an attempt to convert Eppawela rock phosphate to a more soluble fertilizer grade phosphate material.

2. Experimental

Two rock phosphate samples labelled I and II have been used in the present study. Sample I was collected from one of the hillocks in the northern part of the "leached zone" of the apatite deposit at Eppawela, Sri Lanka. This rock sample was crushed, powdered and sieved (100 mesh) for subsequent investigation. Sample II is a sample of commercially available powdered (90% passing through 100 mesh) Eppawela rock phosphate.

2.1 Chemical analysis

Analysis of the metallic elements and silicon has been performed using a X-ray fluorescence spectrometer. Total P_2O_5 has been determined by the X-ray fluorescence as well as by the vanadomolybdate method.[9] Analyses for fluoride have been made using Orion model 94–09 fluoride ion electrode[1] against a Beckman type R.L.B. calomel reference electrode. The samples were dissolved in 5M hydrochloric acid for this analysis. Chlorine analyses were made by dissolving the samples in 1:1 nitric acid and titrating it with standard 0.05M silver nitrate to a potentiometric end point. Table 1 shows the results of chemical analysis of the two samples for their major constituents and trace elements. Phases present in some products were identified by using powder X-ray diffraction with Cu Kα radiation.

2.2 HCl acidulation

Rock phosphate samples were treated with hydrochloric acid of known strength and stirred vigorously for 30 minutes in beakers. The resulting slurry was allowed to stand for 6 hours and then transferred to porcelain dishes for curing. After curing period, the product was dried at 100°C.

For dicalcium phosphate precipitation, the rock samples have been mechanically stirred with hydrochloric acid for 6 – 8 hours. The pH of the resulting solution was adjusted in the range 5 – 7 using aqueous sodium

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Table 1. Analytical data of Eppawela apatite

(i) Partial Chemical Analysis: (in wt%)

	Apatite (I)	Commercial apatite (II)
CaO	46.60	43.50
P_2O_5	35.30	32.80
Fe_2O_3	3.80	5.00
Al_2O_3	0.74	2.60
Cl	2.20	2.03
F	1.70	1.60
SiO_2	0.43	0.79
SrO	0.38	0.34

(ii) Trace Elements Analysis: (in ppm)

Element	I	II	Element	I	II
K	300	500	Cu	307	157
Ba	316	1178	La	470	438
Co	8	10	Mn	500	2300
Cr	20	21	Ti	10	200
Mg	300	400	Na	600	1100
Zn	309	239	Zr	9	12
Y	94	108	Ni	23	26
Sr	3217	2936	W	8	5
As	23	27	Pb	111	140
V	46	81			

NOTE: I – A representative sample from the 'leached zone' at Eppawela.

II – A sample of commercially available powdered Eppawela apatite.

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hydroxide, lime or ammonia. After the precipitation is complete, the product was filtered under suction and dried at 100°C prior to analysis.

2.3 H_2SO_4 acidulation

Sulphuric acid acidulation was performed similar to hydrochloric acid acidulation but in this case drying was not necessary. During the curing period the acidulated product gradually transforms into a dry powder.

2.4 Estimation of phosphorus

Water and 2% citric acid soluble P_2O_5 contents of the samples have been determined[11] by using about 1g samples. These were extracted in 250 ml reagent bottles with 100 ml of distilled water or 2% citric acid solution using a mechanical shaker operating at about 250 oscillations per minute for 30 minutes. The total P_2O_5 contents have been determined by extracting the samples with conc. HCl. The extracts were analysed for phosphorus by the vanadomolybdate[9] method using Corning colorimeter model 253 at a wavelength of 460 nm.

3. Results and Discussion

3.1 Direct acidulation with HCl

Acidulation reaction for the complete (100%) acidulation of apatite may be represented as follows.

$$2Ca_5(PO_4)_3\,(X) + 14HCl \longrightarrow 3Ca(H_2PO_4)_2 + 7CaCl_2 + 2HX$$

Several side reactions may also occur depending on the other components and the impurities present in the rock. As such, the acid requirement for acidulation in general is calculated by considering the nature and amounts of impurities present and the total P_2O_5 content of the rock.

Variation of available P_2O_5 with the acid concentration for 100% and 60% acidulations is shown in Figure 1. In both cases the available P_2O_5 increases with increase in concentration up to about 18% and further increase in concentration does not have any effect on the available phosphorus content. Thus, the optimum concentration for HCl acidulation is in the range 15 – 20%. Therefore, it appears that dilute hydrochloric acid solution could be conveniently used for the acidulation process. Completely acidulated product of rock sample I contains $\sim$ 19% available P_2O_5 while

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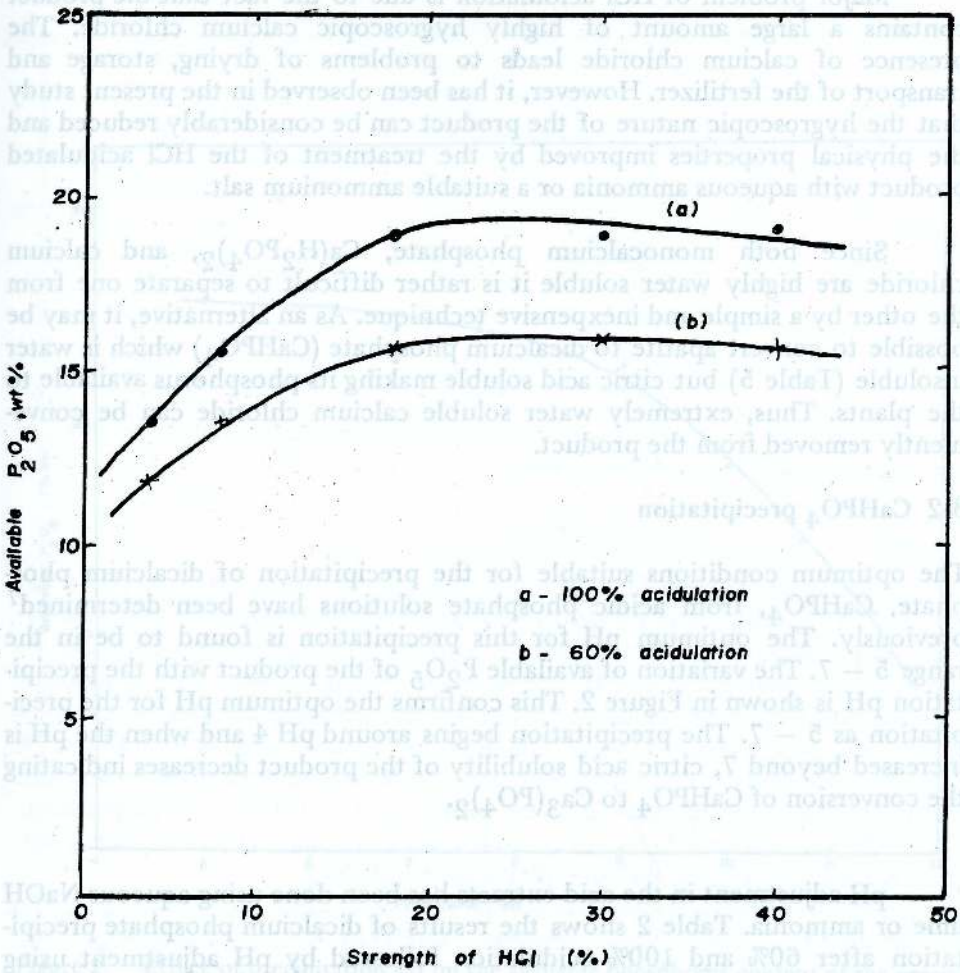

Figure 1. Variation of available phosphorus content of HCl-acidulated product with the acid concentration.

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commercial apatite (sample II) gave a product containing ~ 17% wt P_2O_5. However, a product containing a maximum of ~ 20% available P_2O_5 can be obtained by increasing the amount of acid added. On the other hand partially acidulated (60%) product contains 14 – 16% wt available P_2O_5.

Major problem of HCl acidulation is due to the fact that the product contains a large amount of highly hygroscopic calcium chloride. The presence of calcium chloride leads to problems of drying, storage and transport of the fertilizer. However, it has been observed in the present study that the hygroscopic nature of the product can be considerably reduced and the physical properties improved by the treatment of the HCl acidulated product with aqueous ammonia or a suitable ammonium salt.

Since both monocalcium phosphate, $Ca(H_2PO_4)_2$, and calcium chloride are highly water soluble it is rather difficult to separate one from the other by a simple and inexpensive technique. As an alternative, it may be possible to convert apatite to dicalcium phosphate ($CaHPO_4$) which is water insoluble (Table 5) but citric acid soluble making its phosphorus available to the plants. Thus, extremely water soluble calcium chloride can be conveniently removed from the product.

3.2 $CaHPO_4$ precipitation

The optimum conditions suitable for the precipitation of dicalcium phosphate, $CaHPO_4$, from acidic phosphate solutions have been determined[7] previously. The optimum pH for this precipitation is found to be in the range 5 – 7. The variation of available P_2O_5 of the product with the precipitation pH is shown in Figure 2. This confirms the optimum pH for the precipitation as 5 – 7. The precipitation begins around pH 4 and when the pH is increased beyond 7, citric acid solubility of the product decreases indicating the conversion of $CaHPO_4$ to $Ca_3(PO_4)_2$.

pH adjustment in the acid extracts has been done using aqueous NaOH lime or ammonia. Table 2 shows the results of dicalcium phosphate precipitation after 60% and 100% acidulation followed by pH adjustment using aqueous NaOH. Both sets of results show that there is an increase of 2% citric acid solubility of the product with increase in acid concentration up to 18%. 60% acidulation with 18% HCl followed by neutralization with aqueous NaOH yielded a product containing 23–25% available P_2O_5. The rock sample II, however, yielded a product containing slightly lower (~21%) available P_2O_5 content. The major constituent in the dried product was identified as dicalcium phosphate ($CaHPO_4$) by powder X-ray diffraction. Tricalcium phosphate, $Ca_3(PO_4)_2$, and apatite also have been identified in the product.

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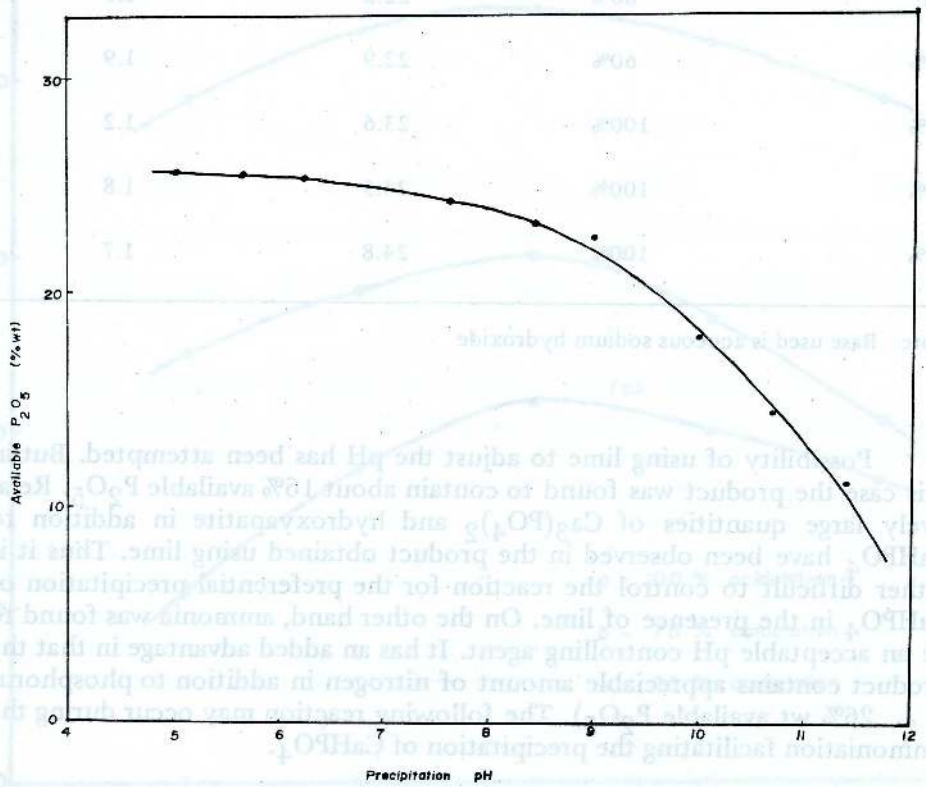

Figure 2. Effect of precipitation pH on the available phosphorus content of the precipitated product.

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Table 2. Results of HCl acidulation followed by precipitation.

Strength of acid	Extent of acidulation	2% citric acid soluble P_2O_5 wt. %	P_2O_5 wt. % in the filtrate
4%	60%	21.3	0.5
8%	60%	22.2	1.6
18%	60%	22.9	1.9
4%	100%	23.6	1.2
8%	100%	24.5	1.8
18%	100%	24.8	1.7

Note: Base used is aqueous sodium hydroxide

Possibility of using lime to adjust the pH has been attempted. But in this case the product was found to contain about 16% available P_2O_5. Relatively large quantities of $Ca_3(PO_4)_2$ and hydroxyapatite in addition to $CaHPO_4$ have been observed in the product obtained using lime. Thus it is rather difficult to control the reaction for the preferential precipitation of $CaHPO_4$ in the presence of lime. On the other hand, ammonia was found to be an acceptable pH controlling agent. It has an added advantage in that the product contains appreciable amount of nitrogen in addition to phosphorus ($\sim$ 26% wt available P_2O_5). The following reaction may occur during the ammoniation facilitating the precipitation of $CaHPO_4$.

$$Ca(H_2PO_4)_2 + NH_3 \longrightarrow CaHPO_4 + NH_4H_2PO_4.$$

3.3 Acidulation with sulphuric acid

Figure 3 shows the variation of the extent of conversion with the concentration of H_2SO_4 used in the acidulation. All three curves representing 100%, 75% and 50% acidulation reactions show a maximum around 70% H_2SO_4. Thus the optimum concentration for complete and partial acidulation of apatite with H_2SO_4 is in the range 65 – 70%. As such, all the H_2SO_4 acidulation experiments have been performed using 70% sulphuric acid in the present study.

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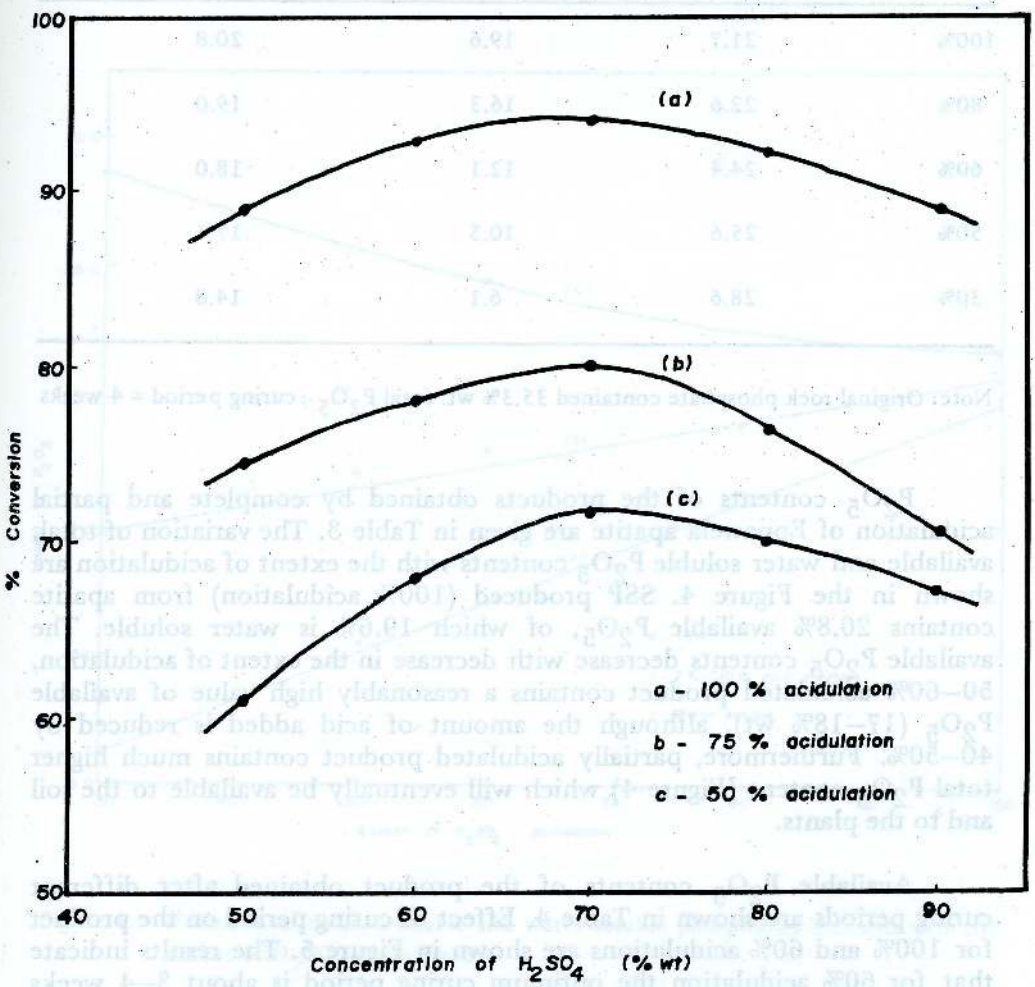

Figure 3. Effect of concentration of H_2SO_4 on the extent of conversion.

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Table 3. Variation of available P_2O_5 content with the extent of H_2SO_4 acidulation.

Extent of acidulation	Total P_2O_5 % wt.	Water soluble P_2O_5 % wt.	2% citric acid soluble P_2O_5 % wt.
100%	21.7	19.6	20.8
80%	22.6	16.3	19.0
60%	24.4	12.1	18.0
50%	25.6	10.5	17.1
30%	28.6	6.1	14.8

Note: Original rock phosphate contained 35.3% wt. total P_2O_5 ; curing period = 4 weeks

P_2O_5 contents of the products obtained by complete and partial acidulation of Eppawela apatite are given in Table 3. The variation of total, available and water soluble P_2O_5 contents with the extent of acidulation are shown in the Figure 4. SSP produced (100% acidulation) from apatite contains 20.8% available P_2O_5, of which 19.6% is water soluble. The available P_2O_5 contents decrease with decrease in the extent of acidulation. 50–60% acidulated product contains a reasonably high value of available P_2O_5 (17–18% wt), although the amount of acid added is reduced by 40–50%. Furthermore, partially acidulated product contains much higher total P_2O_5 content (Figure 4) which will eventually be available to the soil and to the plants.

Available P_2O_5 contents of the product obtained after different curing periods are shown in Table 4. Effect of curing period on the product for 100% and 60% acidulations are shown in Figure 5. The results indicate that for 60% acidulation the optimum curing period is about 3–4 weeks while for SSP production 5–6 weeks curing is required. Thus, partial acidulation has an added advantage of having a less curing period.

50% acidulated product was found to contain unreacted apatite, monocalcium phosphate and trace amounts of dicalcium phosphate by powder X-ray diffraction. The product was not found to be sticky. However, a suitable treatment may be necessary if the product obtained in a commercial scale preparation is found to be sticky.

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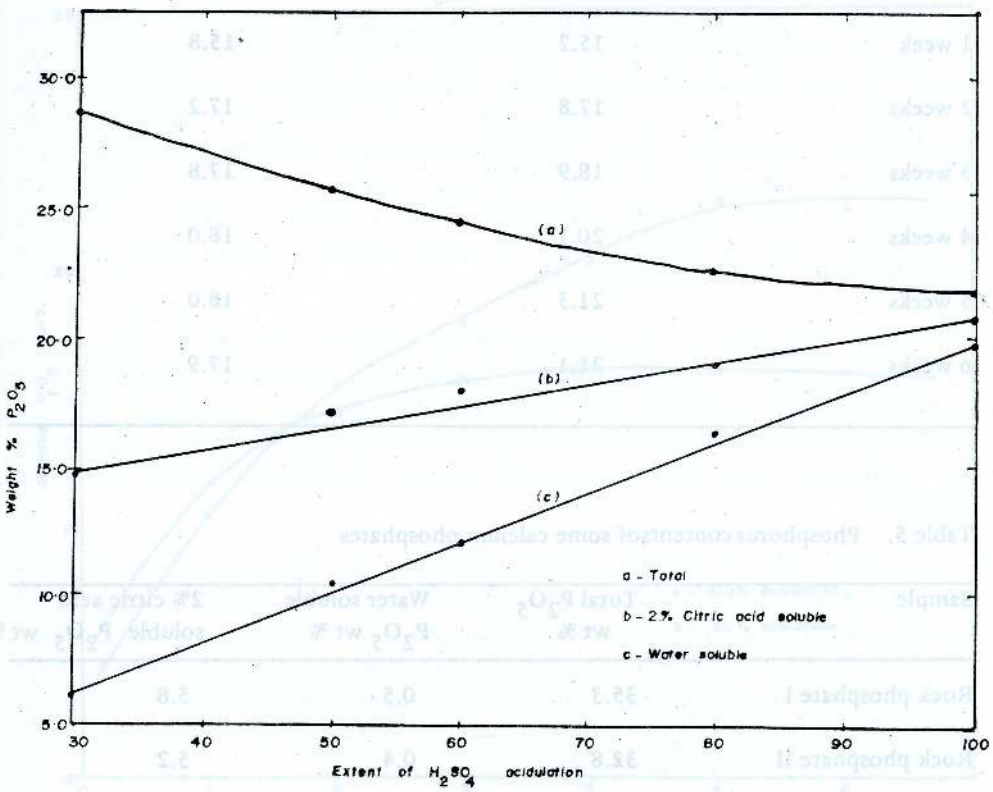

Figure 4. Variation of total, available and water soluble phosphorus contents with the extent of H_2SO_4-acidulation.

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Table 4. H_2SO_4 acidulation : Effect of curing time on the availble P_2O_5 content.

Curing period	2% citric acid soluble P_2O_5 wt %	
	100% acidulation	60% acidulation
34 hrs	12.3	13.0
1 week	15.2	15.8
2 weeks	17.8	17.2
3 weeks	18.9	17.8
4 weeks	20.1	18.0
5 weeks	21.3	18.0
6 weeks	21.1	17.9

Table 5. Phosphorus contents of some calcium phosphates

Sample	Total P_2O_5 wt %	Water soluble P_2O_5 wt %	2% citric acid soluble P_2O_5 wt %
Rock phosphate I	35.3	0.5	5.8
Rock phosphate II	32.8	0.4	5.2
Hydroxyapatite $Ca_5(PO_4)_3$ OH	42.3	0.7	16.2
Monocalcium phosphate $Ca(H_2PO_4)_2$	60.6	58.3	60.4
Dicalcium phosphate $CaHPO_4$	52.1	1.3	44.4
Tricalcium phosphate $Ca_3(PO_4)_2$	45.8	0.9	34.0

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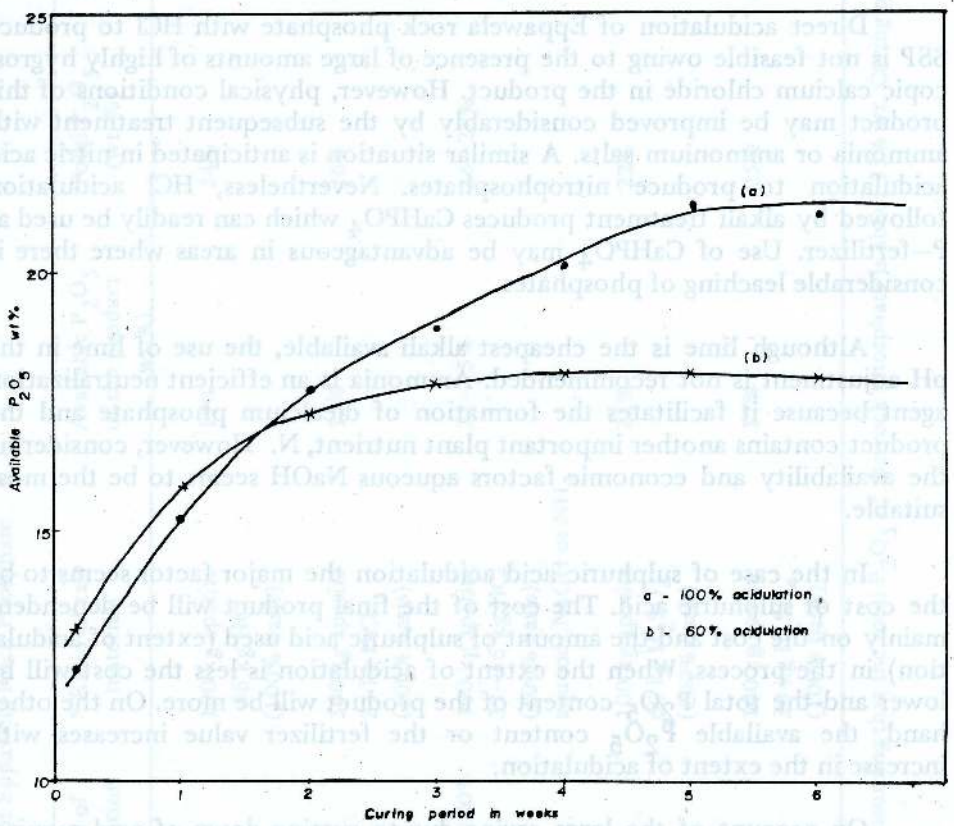

Figure 5. Effect of curing period on the available phosphorus content of the H_2SO_4–acidulated product.

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4. Discussion

Phosphorus contents of the starting materials are compared with those of the major constituents in the fertilizer products in Table 5. Conditions of acidulation of Eppawela apatite are summarised in Table 6. For the production of 50% acidulated product, 30 kg H_2SO_4 (100% basis) diluted to 70% is required for 100 kg powdered apatite.

Direct acidulation of Eppawela rock phosphate with HCl to produce SSP is not feasible owing to the presence of large amounts of highly hygroscopic calcium chloride in the product. However, physical conditions of this product may be improved considerably by the subsequent treatment with ammonia or ammonium salts. A similar situation is anticipated in nitric acid acidulation to produce nitrophosphates. Nevertheless, HCl acidulation followed by alkali treatment produces $CaHPO_4$ which can readily be used as P–fertilizer. Use of $CaHPO_4$ may be advantageous in areas where there is considerable leaching of phosphates.

Although lime is the cheapest alkali available, the use of lime in the pH adjustment is not recommended. Ammonia is an efficient neutralization agent because it facilitates the formation of dicalcium phosphate and the product contains another important plant nutrient, N. However, considering the availability and economic factors aqueous NaOH seems to be the most suitable.

In the case of sulphuric acid acidulation the major factor seems to be the cost of sulphuric acid. The cost of the final product will be dependent mainly on the cost and the amount of sulphuric acid used (extent of acidulation) in the process. When the extent of acidulation is less the cost will be lower and the total P_2O_5 content of the product will be more. On the other hand, the available P_2O_5 content or the fertilizer value increases with increase in the extent of acidulation.

On account of the large saving due to cutting down of acid requirement, and due to the presence of reasonably high phosphorus content of the partially acidulated product, this material can be considered as a potentially useful and economical phosphate fertilizer for Sri Lanka. Thus, 50% acidulation of Eppawela apatite with H_2SO_4 to produce partially acidulated phosphate rock (PAPR) containing about 17% wt available P_2O_5 and 25% total P_2O_5 appears to be suitable.

Free phosphoric acid content in the acidulated product plays an important role in fixing phosphates as aluminium and iron phosphates. Since the free phosphoric acid content is minimal in the partially acidulated product, the effect of the presence of Al_2O_3 and Fe_2O_3 is negligible in

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Table 6. Methods of acidulation of Eppawela Rock Phosphate

Acid	Strength of acid	Extent of acidulation	* Ratio of raw materials	Available P_2O_5 of the product (wt %)	Total P_2O_5 (wt. %)	Machinery
HCl	20%	100%	100 kg apatite 46 kg HCl (100% basis)	19%	24%	Acidulation plant
HCl	20%	50%	100 kg apatite 23 kg HCl (100% basis)	15%	26%	Acidulation plant
HCl	20%	50%–100%	100 kg apatite 23–46 kg HCl (100% basis) aqueous NaOH or NH_3	22–25%	26–28%	Acidulation plant & crystallization tank
H_2SO_4	70%	100%	100 kg apatite 60 kg H_2SO_4 (100% basis)	21%	22%	Acidulation plant
H_2SO_4	70%	50%	100 kg apatite 30 kg H_2SO_4 (100% basis)	17%	25%	Acidulation plant

N.B. * These ratios are calculated assuming that the total P_2O_5 of the rock phosphate is about 35% wt. Curing period is 4 weeks.

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comparison with 100% acidulation to produce SSP.

Presence of high chlorine content in Eppawela apatite leads to corrosion problems in an industrial acidulation process. As such, mechanical mixers and the acidulation tanks should be lined with corrosion resistant material. Furthermore, pollution factors also should be taken into consideration in deciding the location of acidulation plants.

Although chemical tests and laboratory evaluations indicate that these products can be used as P–fertilizers, it is essential to examine the crop response of the products and the financial viability of these processes. Therefore, pilot plant trials together with comprehensive financial evaluation of the processes and long-term field trials have to be carried out prior to commencement of any commercial production.

Acknowledgement

Author wishes to thank the International Seminar in Chemistry of the Uppsala University in Sweden for financial assistance to perform X-ray fluorescence analysis and powder X-ray diffraction.

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J. Natn. Sci. Coun. Sri Lanka 1987 15 (2) : 201–208

ELECTRONIC CONDUCTION IN H_2O DOPED SOLID METHYLENE BLUE

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(Date of receipt : 15 October 1984)
(Date of acceptance : 11 August 1987)

Abstract : Traces of water as an impurity is found to increase the electronic conductivity of the charge transfer solid methylene blue. It is found that hydrogen bonded and free molecules of water decrease the thermal activation energy of charge carriers in different ways. Theoretical arguments based on the small polaron theory are given to explain the mechanism of conduction.

1. Introduction

Experimental and theoretical investigations on electrical transport in molecular solids, especially charge transfer (CT) complexes have attracted much attention.[4,5,7,8] In general the neutral CT solids are insulators or poor semiconductors while ionic CT complexes show much enhanced semiconduction.[2] The mechanism of conduction in these materials is quite involved and often arise from a band structure or hopping of the charge carriers between localized donor-acceptor sites.[1,3,9] When the mobilities are small the latter mode charge transport is explained satisfactorily by the small polaron theory.[1,3,9]

In this note we report our observations on enhancement of electrical conductivity of methylene blue (organic CT complex) by water molecules present as an impurity. A simple qualitative argument based on the small polaron theory is presented to explain how bonded and free water molecules could reduce the energy needed for the thermal activation of charge carriers.

2. Experimental

Methylene blue (BDH and Aldrich brands) are purified by recrystallization from the alcoholic solution. The purified compound dried in vacuo melts sharply at 463.4 K. Anhydrous samples when exposed to the humid atmosphere readily absorb moisture. Data on thermal gravimetric analysis is indicated in Figure 1. It is seen that in addition to water that is lost continously, the material can also hold $\sim$ 14.5% bonded water corresponding to a hydration number of 3.

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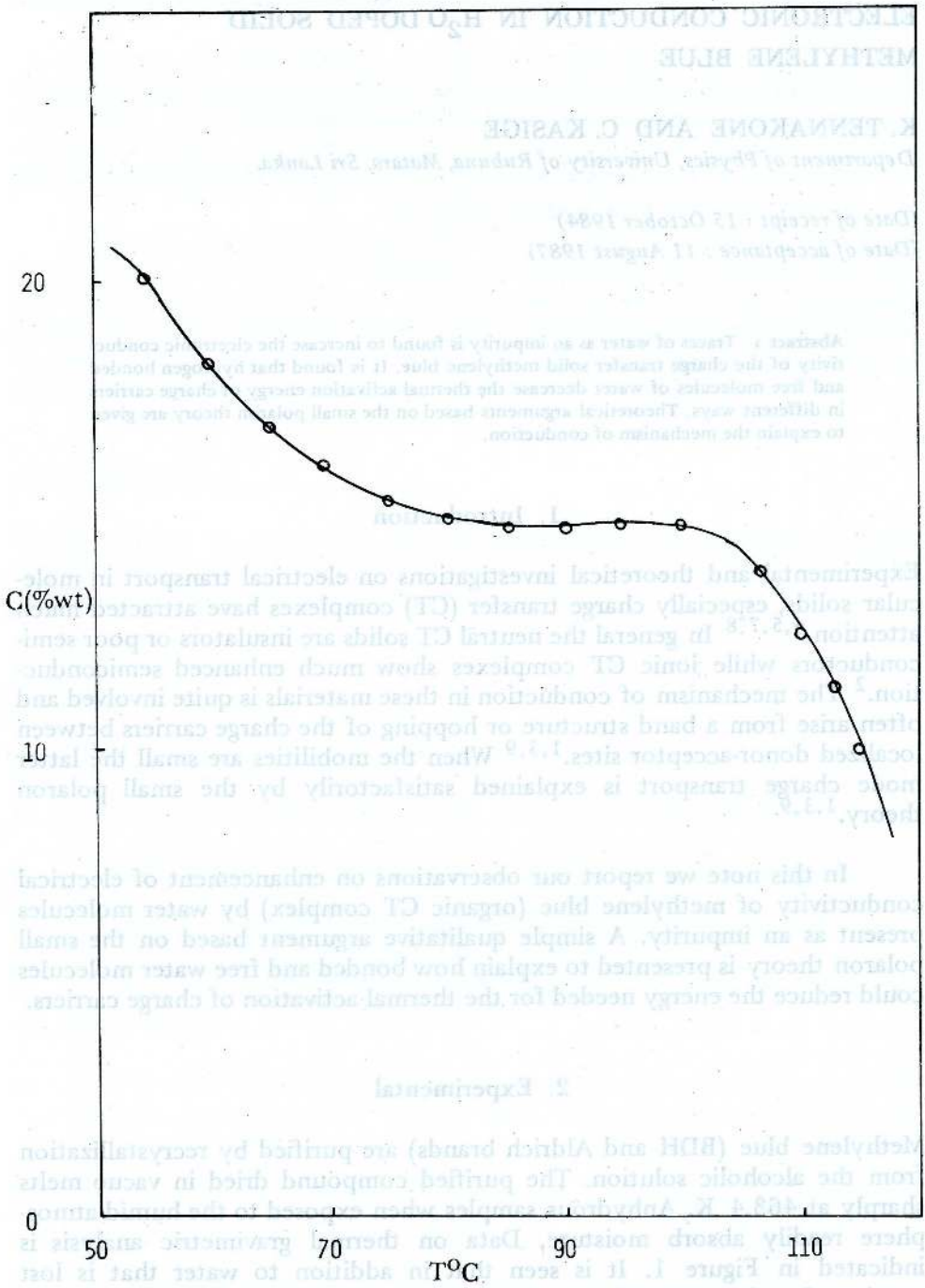

Figure 1. Thermal gravimetric analysis data of a sample of moisture absorbed methylene blue giving the percentage of water (C%) when heated at a constant rate 2 K min^{-1}.

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Varying amounts of moisture are introduced by exposing weighed quantities of the material into the humid atmosphere in desiccators containing water. The samples for conductivity measurements are made by pressing the powder between stainless electrodes in pyrex tubes (diameter $\sim$ 0.4 cm, pellet length $\sim$ 0.3 cm). Ends of the tube are sealed with epoxy resin, they are immersed in a thermostatic oil bath and d.c. conductivities at different temperatures are determined using a resistance meter. D.C. current voltage characteristics are tested and found to be linear at all temperatures < m.p, provided the concentration c of absorbed water does not exceed $\sim$ 10% by weight. Compound containing more than the above quantity of water shows nonlinear and time varying I–V characteristics, at temperatures below $\sim$ 383K. This can be attributed to ionic conduction as electrolytic effects are also seen under these conditions. However when the water content is << 10%, conduction is almost entirely electronic, this can be proved by passing large currents through thin discs of the pressed material, when V.I characteristics remain linear and time invariant. Also polarization effects are not seen when blocking electrodes were used.

Although it is difficult to prepare sizable single crystals, few careful measurements show that there are no significant differences in the conductivity properties of compressed pellets and single crystals. Four terminal and two terminal measurements with compressed pellets give the same results indicating that the surface effects are negligible.

Table 1. Conductivity and activation energies $E_1(T < T_c)$, $E_2(T > T_c)$ for methylene blue at different levels of doping with H_2O.

C (% wt)	$\sigma_{30}/M^{-1}m^{-1}$	E_1/eV	E_2/eV
0	6.9×10^{-11}	–	1.50
0.2	2.8×10^{-7}	0.69	1.45
1	1.4×10^{-6}	0.66	1.20
3	4.6×10^{-6}	0.61	0.92
5	4.5×10^{-4}	0.55	0.54
10	4.5×10^{-3}	0.42	–

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3. Results

The room temperature 303 K conductivity of anhydrous methylene blue is $\sim 10^{-8}\ \Omega^{-1}\ m^{-1}$. Traces of moisture increase the conductivity by several orders of magnitude (Table 1). The plot of $\ln \sigma$ vs T^{-1} at different concentrations C of water is shown in Figure 2. It is seen that at lower values of C the data fit into two straight line portions with a sudden change of the slope at $T_c \simeq 383$ K. As C increases further (C > 10%) the abrupt change tends to become continous. The region of continous change of slope is associated with ionic conductivity and at still higher values of C (>20%) ionic conduction is detectable even at room temperature.

It is also found that the values of thermal activation energy E corresponding to the straight line portions $T < T_c$ and $T > T_c$ in the plots $\ln \sigma$ vs T^{-1} decrease linearly with C as long as C does not exceed $\sim$ 10% (Figure 3).

4. Theory and Discussion

As mentioned in the introduction the electrical transport in most low mobility molecular solids is due to hopping of the charge carriers between localized sites. In methylene blue (structural formula given in Figure 4) Cl^- would act as a electron donor and S^+ with an unpaired electron an acceptor. If I = ionization energy of the donor, A = electron affinity of the acceptor. Thermal activation energy of hopping conduction can be written in the form,[6]

$$E = -\left(\frac{Ne^2}{8\pi\epsilon_o r}\right)(1-\epsilon^{-1}) \qquad (1)$$

where N = Avogarodo constant, e = electronic charge
ϵ_o = permittivity in vacums, r = radius of the polarization cavitiy,
ϵ = static dielectric constant.

The last term in (1) gives the polarization energy (r = radius of the polarization cavity, ϵ = static dielectric constant). As the dipole moment of the water molecules is very high and they diffuse easily through the solid, the polarization cloud around the hopping electron would consist mainly of water molecules. It is seen from (1) that the high dielectric constant of water increases the polarization energy reducing I.

Again I and A could also be changed by H_2O molecules. The high dielectric constant of H_2O will reduce I and it is reasonable to suppose that bonded H_2O is more effective in decreasing I. The thermal energy kT_c ($\sim$ 0.03 eV) is of the same order of magnitude as that of a hydrogen bond and the sudden increase in E at $T > T_c$ may be interpreted as resulting from breaking of these bonds.

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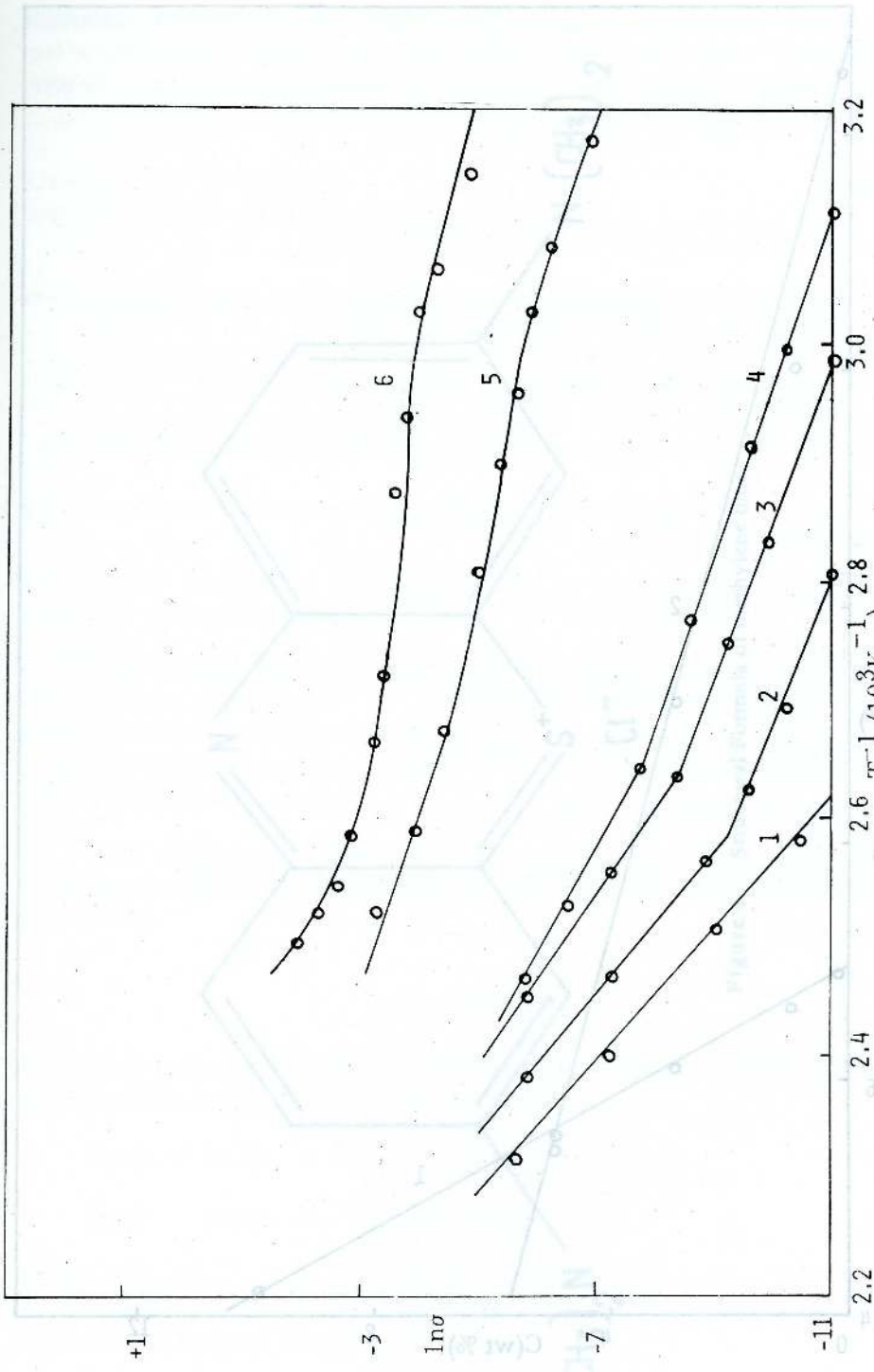

Figure 2. Plot of $\ln \sigma$ vs T^{-1} (σ in Ω^{-1} m^{-1}). The Percentage of H_2O by weight (i) 0% (2) 0.2% (3) 1% (4) 3% (5) 5% (6) 10%

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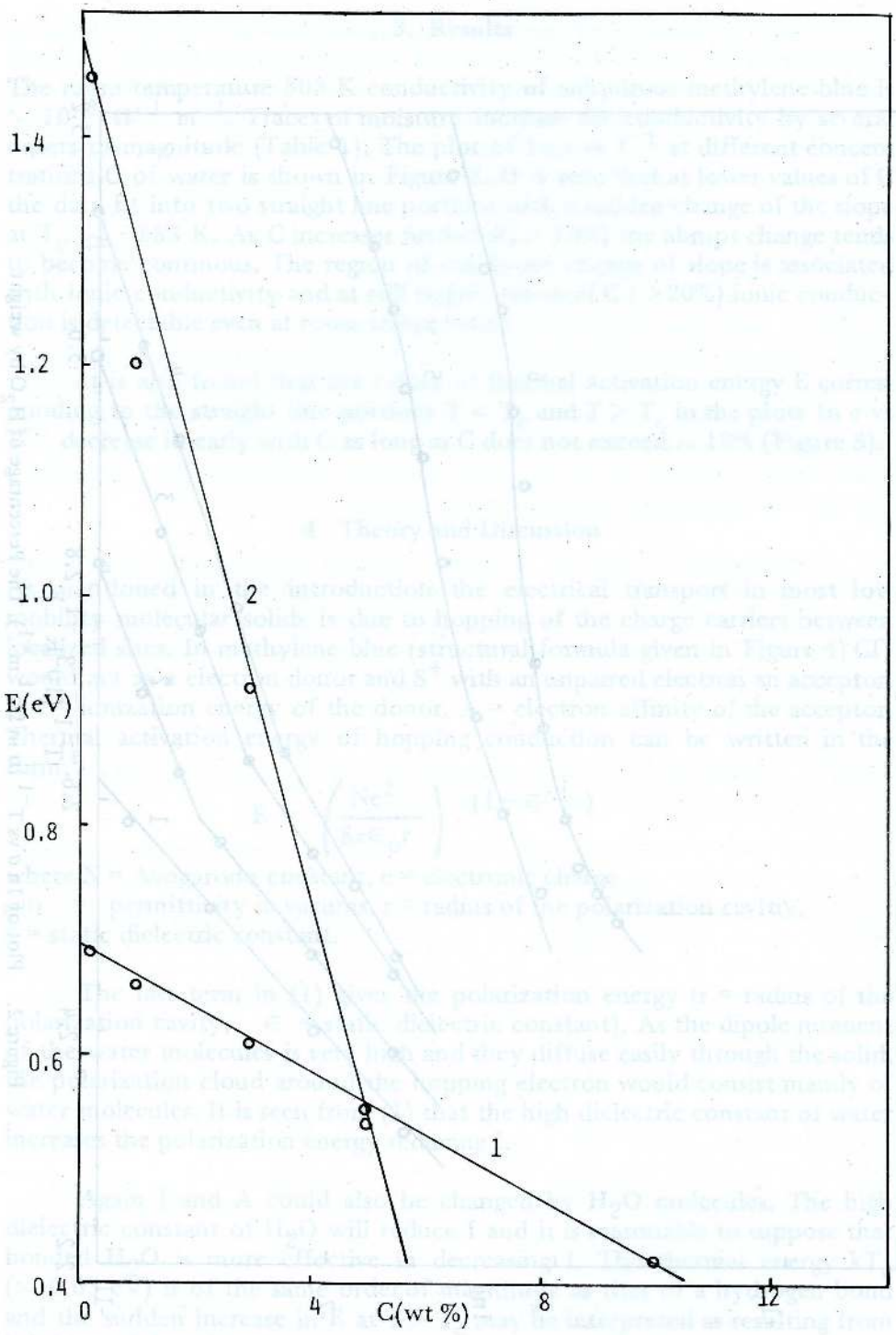

Figure 3. Plot of activation energy E vs concentration of H_2O (% by wt (1) $T < T_c$ (2) $T > T_c$

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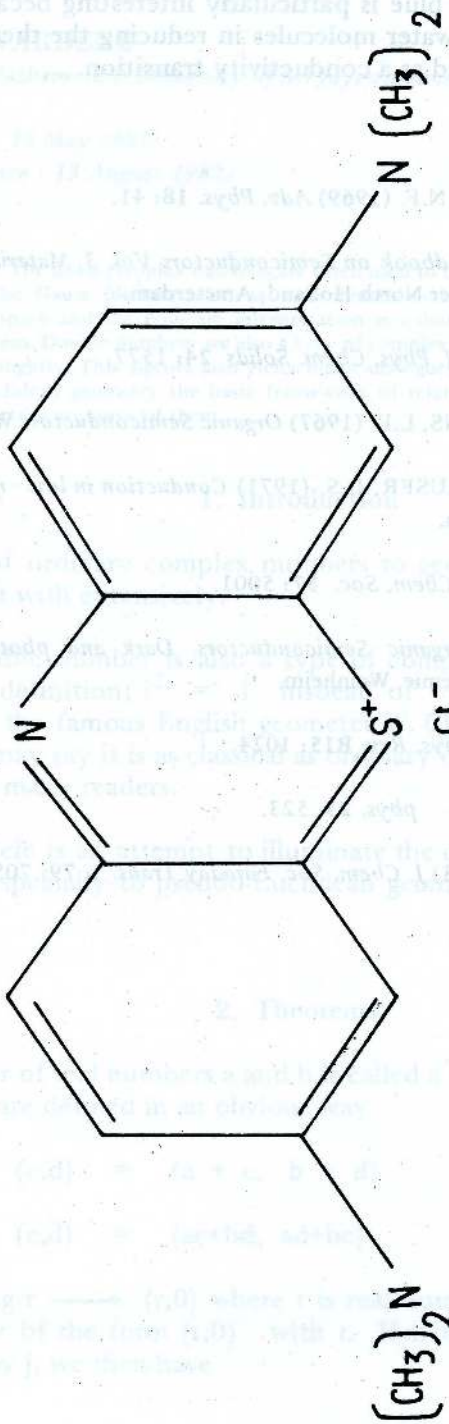

Figure 4. Structural Formula of methylene blue.

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Several other molecular solids exhibit water activated electronic conduction.[10] Methylene blue is particularly interesting because, the effect of the hydrogen bonded water molecules in reducing the thermal activation energy is clearly manifested as a conductivity transition.

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J. Natn. Sci. Coun. Sri Lanka 1987 **15** (2) : *209–216*

DOUBLE NUMBERS IN GEOMETRY

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(Date of receipt : 15 May 1987)
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Abstract : The usual complex numbers are often used to describe many familiar geometries. The Gauss interpretation as a 2–dimensional sphere in 3–dimensional Euclidean space and the Poincare interpretation as a double Lobachevski plane are some of them. Double numbers are also a type of complex numbers which differs the definition slightly. This algebra also yields many analogues applications to geometry. Pseudo-Euclidean geometry the basic framework of relativity, spherical and hyperbolic geometries are some of them.

1. Introduction

The relation of ordinary complex numbers to geometry is well known and have been dealt with extensively.

The double number is also a type of complex number which differs slightly in its definition; $i^2 = 1$ insdead of -1. This algebra was first introduced by the famous English geometer W. Clifford. The theory is quite classical. One may say it is as classical as ordinary complex numbers. Yet it is not familiar to many readers.

This article is an attempt to illuminate the connections of this algebra to geometry, especially to pseudo-Euclidean geometry, the basic framework of realtivity.

2. Theorems

An ordered pair of real numbers a and b is called a double number. Addition, multiplication are defined in an obvious way:

$$(a,b) + (c,d) = (a + c,\ b + d) \tag{2.1}$$

$$(a,b)\ .\ (c,d) = (ac+bd,\ ad+bc) \tag{2.2}$$

By the mapping $r \longrightarrow (r,0)$ where r is real number, we can identify each double number of the form $(r,0)$ with r. Hence if we denote the double number $(0,1)$ by j, we then have

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$$(a,b) = (a,0) + (b,0)(0,1) = a + bj \qquad (2.3)$$

and (2.2) gives

$$j^2 = 1.$$

With these definitions the system of double numbers form a commutative algebra of dimension 2 over reals. We shall denote this algebra by $\mathbb{A}$.

If we write

$$j_1 = 1/2\ (1+j) \quad \text{and} \quad j_2 = 1/2\ (1-j) \qquad (2.4)$$

We find

$$j_1^2 = j_1,\ j_2^2 = j_2 \quad \text{and} \quad j_1 j_2 = 0$$

Hence by considering the basis j_1 and j_2 we see $\mathbb{A} \cong \mathbb{R} \times \mathbb{R}$

Where $\mathbb{R}$ denotes the set of real numbers. Each double number $Z = a + bj$ has a conjugate denoted by $\bar{z}$ which takes the form $a - bj$. It follows immediately

$$Z\bar{Z} = a^2 - b^2 \qquad (2.5)$$

For $Z_1, Z_2 \in \mathbb{A}$ we see

$$\overline{Z_1 + Z_2} = \bar{Z}_1 + \bar{Z}_2 \qquad \overline{Z_1 Z_2} = \bar{Z}_1 \bar{Z}_2 \qquad (2.6)$$

Real numbers

$$|Z| = \pm |a^2 - b^2|^{1/2} \qquad (2.7)$$

are called modulii of Z $(= a + bj)$. Note that if

$|Z| = 0$ then $a = \pm b$ and vice versa. This implies

$$Z = t(1 + j) \quad \text{or} \quad Z = t(1 - j)$$

for some $t \in \mathbb{R}$

The double number with non-zero modulus can be expressed in polar form. For $r = |Z| = \pm |a^2 - b^2|^{1/2}$ we get

$$Z = a + bj = r\ (a/r + b/r\ j)$$

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Since $(a/r)^2 - (b/r)^2 = \pm 1$, it follows

$$a/r = \pm \text{Cosh } \xi \qquad b/r = \pm \text{Sinh } \xi \tag{2.8}$$

or

$$a/r = \pm \text{Sinh } \xi \qquad b/r = +\text{Cosh } \xi$$

Where $\xi \in \mathbb{R}$ and $\cosh \xi$ and $\sinh \xi$ are the hyperbolic cosine and hyperbolic sine of the argument ξ

This gives

$$Z = r(\cosh \xi + j \sinh \xi) \quad \text{or } Z = r(\text{Sinh}\xi + j \cosh \xi),\ r \gtrless 0 \tag{2.9}$$

The argument of the double number Z is denoted by arg Z.

For $Z_1, Z_2 \in \mathbb{A}$, it follows immediately

$$|Z_1 Z_2| = |Z_1||Z_2| \tag{2.10}$$

$$\arg Z_1 Z_2 = \arg Z_1 \arg Z_2$$

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As with complex numbers, the double numbers can be represented as points of the plane. The following figure (a) describes the whole representation.

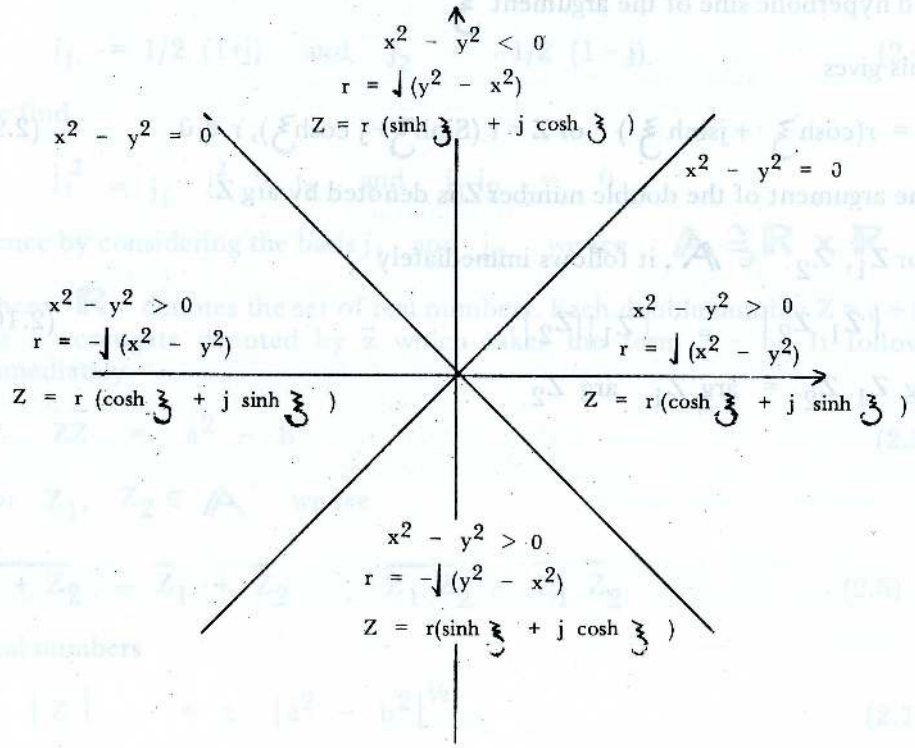

fig. (a)

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It is worth noting how $\mathbb{A}$ behaves as a ring. Other than $\{0\}$ and $\mathbb{A}$ the only ideals of $\mathbb{A}$ are

$$I_+ = \mathbb{R}(1+j) = \{r(1+j) \mid r \in \mathbb{R}\} \tag{4.1}$$
$$I_- = \mathbb{R}(1-j) = \{r(1-j) \mid r \in \mathbb{R}\}$$

It follows easily that

$$I_+ \cap I_- = \{0\}$$

and $\mathbb{A} - (I_+ \cup I_-)$ is the unit group $\cup$ of $\mathbb{A}$.

Now, what are the corresponding elements of $\cup$ in the plane?
To see this let $\Phi = \{\text{Cosh } \xi + j \text{ Sinh } \xi\}$

We see $P = \{r \mid r \in \mathbb{R}, r > 0\}$ and $P\Phi = \{re^{j\xi} \mid r \in \mathbb{R}, r > 0\}$ are subgroups of U. The subgroup $P\Phi$ fills the region I (see fig (a)). $-P\Phi$ represents region III. Since

$$je^{j\xi} = \text{Sinh } \xi + j \text{ Cosh } \xi,$$

we see $jP\Phi$ and $-jP\Phi$ represents the regions II and IV respectively. Thus U represents I $\cup$ II $\cup$ III $\cup$ IV. In general a point in the region is given by $u = re^{j\xi}$. In region II it takes the form $u = rje^{j\xi}$.

The map $Z \longrightarrow \bar{Z}$ is an automorphism of $\mathbb{A}$. As in complex numbers there are only two automorphisms namely the identity automorphism and the involutory automorphism.

The reader may verify whether the other properties in complex numbers do hold in this algebra. We shall now look at the geometries of this algebra.

If we consider as distance between Z_1 and Z_2 of the modulus of the difference $Z_1 - Z_2$, we obtain the planar metric given by

$$d^2 = |Z_1 - Z_2|^2 = |Z_1 - Z_2||Z_1 - Z_2| \tag{5.1}$$

For $Z = (x_1, y_1)$ and $Z = (x_2, y_2)$ we find

$$d^2 = (x_1 - x_2)^2 - (y_1 - y_2)^2 \tag{5.2}$$

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It is evident from (5.2), the algebra with this metric is isomorphic to the 2 – dimensional pseudo – Euclidean (Minowskian) plane $^1\mathbb{R}_2$ of index 1.

The Euclidean straight line

$$bx + cy + d = 0 \tag{5.3}$$

with $b^2 - c^2 \neq 0$ is known as pseudo – Euclidean straight line, while (5.3) with $b^2 - c^2 = 0$ is known as isotropic line in the pseudo – Euclidean plane. Recall in one–dimensional special relativity the line $bt + cy + d = 0$ (t is the time coordinate, y is the space coordinate) with $b^2 - c^2 > 0$ is known as time – like line, and if $b^2 - c^2 < 0$ it is known as space – like; the isotropic lines are known as light – like lines.

To each point (x,y), if we associate the double number $Z = x + yj$, the above (5.3) takes the form

$$(b + cj) Z + (b - cj) \bar{Z} + 2d = 0 \tag{5.4}$$

With $(b + cj)(b - cj) \neq 0$. Thus every pseudo – Euclidean straight line can be represented by an equation of the form:

$$\alpha Z + \bar{\alpha}\bar{Z} + \gamma = 0 \tag{5.5}$$

With $\alpha\bar{\alpha} \neq 0$, The isotropic line also take the form (5.5) with $\alpha\bar{\alpha} = 0$.

The equation of the pseudo – Euclidean circle could be worked out similarly. Observe that, this pseudo–Euclidean circle is a hyperbola. The geometry of pseudo Euclidean plane may be worked out in an analogus manner.

The ordered pair (Z_1, Z_2) is said to be an element of the projective line $P(\mathbb{A})$ over $\mathbb{A}$ whenever not both elements Z_1, Z_2 lie in the same ideal $I (\neq \mathbb{A})$ of $\mathbb{A}$.

The two ordered pairs (Z_1, Z_2) and (Z_1', Z_2') represent the same element whenever $Z_i' = r Z_i$ $(i = 1,2)$ for some $r \in \cup$ (see 4.1). This enables to identify the pseudo – Euclidean points with $P(\mathbb{A})$. The point (x,y) can be identified with $(x+yj, 1)$ $P(\mathbb{A})$. The pseudo – Euclidean point ∞ identified with $(1,0) \in P(\mathbb{A})$. The isotropic lines are identified with $(Z_1, 1 + j)$ and $(Z_1, 1 - j)$ of $P(\mathbb{A})$, where $Z_1 \notin I_+$ and $Z_1' \notin I_-$.

This identification enables one to describe the geometry of $P(\mathbb{A})$. The interested reader may refer (1).

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The elements of $\mathbb{A}$ which do not have inverses are the elements of the ideals I_+ and I_- of $\mathbb{A}$

If we supplement the algebra by these ideal elements then it is possible to introduce into this algebra spherical and hyperbolic metrics:

$$\text{Cos}^2 \frac{d}{2r} = \frac{|1 + Z_1 \bar{Z}_2|^2}{(1 + |Z_1|^2)2 \ (1 + |Z_2|^2)} \tag{6.1}$$

$$\text{Cosh}^2 \frac{d}{2r} = \frac{|1 - Z_1 \bar{Z}_2|^2}{(1 - |Z_1|^2) \ (1 - |Z_2|^2)} \tag{6.2}$$

Where d is the distance between the points Z_1 and Z_2 and r is the radius S_2 the 2 – dimensional sphere in pseudo – Euclidean space ${}^1\mathbb{R}_3$ of index 1.

As in ordinary complex numbers, constructing a stereographic projection, it is possible to represent the supplemented algebra as the unit sphere.

$$S = \{(x_1, x_2, x_3) / x_1^2 + x_2^2 - x_3^2 = 1\}$$

in ${}^1\mathbb{R}_3$. It is easier to verify that this supplemented algebra with spherical metric is isometric to the 2 – dimensional sphere of radius r in ${}^1\mathbb{R}_3$. In the case of hyperbolic metric it is isometric to 2 – dimensional sphere of imaginary radius in the space ${}^2\mathbb{R}_3$ of index 2. The algebra $\mathbb{A}$ with the speherical & hyperbolic metrics was first studied in (4). and (5).

It has been shown in (2) that the pseudo – Euclidean plane supplemented by the ideal elements with the group of motions.

$$Z' = \frac{\alpha Z + \beta}{\gamma Z + \delta} \tag{6.3}$$

Where the elements of the matrix $\begin{pmatrix} \alpha & \beta \\ \gamma & \delta \end{pmatrix}$ satisfy $\alpha\bar{\alpha} + \beta\bar{\beta} = 1$

$\gamma\gamma + \delta\bar{\delta} = 1, \quad \alpha\bar{\gamma} + \beta\bar{\delta} = 0$

in spherical metric and $\alpha\bar{\alpha} - \beta\bar{\beta} = 1$, $\gamma\bar{\gamma} - \delta\bar{\delta} = 1$, $\alpha\bar{\gamma} - \beta\bar{\delta} = 0$ in hyperbolic metric, represent models of geometries of constant curvature. These models are to a large extent similar to the known models of Poincare for non–Euclidean geometries. They are known as tangential models of Poincare.

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7. It is natural to generalize the above geometries to n–dimensions.

The affine space A_n ($\mathbb{A}$) over $\mathbb{A}$ is introduced as follows. The points of A_n ($\mathbb{A}$) are characterised as system of n – arguments.

$$Z_1, Z_2, \ldots\ldots\ldots\ldots, Z_n$$

of $\mathbb{A}$. The affine space over $\mathbb{A}$, with metric

$$d^2 = \sum |Z_i - Z_i'|^2 = \sum (Z_i - Z_i')(\bar{Z}_i - \bar{Z}_i') \qquad (7.1)$$

is known as unitary – Euclidean space $\cup_n$ ($\mathbb{A}$).

It is easy to verify, the space $\cup_n$ ($\mathbb{A}$) is isometric to pseudo – Euclidean space ${}^n\mathbb{R}_{2n}$ of index n.

The projective space P_n ($\mathbb{A}$) over $\mathbb{A}$ is introduced as follows. The points of P_n ($\mathbb{A}$) are characterised as system of n–arguments.

$$\alpha Z_1, \alpha Z_2, \ldots\ldots\ldots\ldots\ldots, \alpha Z_{n+1}$$

where α is an arbitrary element of the algebra.

The projective space P_n ($\mathbb{A}$) with metric

$$\text{Cos}^2 \; d/r = \frac{\sum_i Z_i \bar{Z}_i' \sum_i Z_i' \bar{Z}_i}{\sum_i Z_i \bar{Z}_i \sum_i Z_i' \bar{Z}_i'} \qquad (i = 1,2, \ldots\ldots\ldots n+1) \qquad (7.2)$$

is known as unitary – Elliptic space K_n($\mathbb{A}$). It is shown in (4), The space K_n ($\mathbb{A}$) is isometric to pseudo – Riemann space ${}^nV_{2n}$ of index n. For further study of these spaces, I refer the reader to (2).

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J. Natn. Sci. Coun. Sri Lanka 1987 15 (2) : 217–226

IMMOBILIZATION OF ALPHA–AMYLASE AND GLUCOAMYLASE TO SEPHAROSE – 4B

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(Date of receipt : 17 January 1986)
(Date of acceptance : 20 August 1987)

Abstract : Alpha amylase was coupled to Sepharose – 4B (activated by both electrophilic and nucleophilic methods using cyanogen bromide). Cyanogen bromide concentration was found to be directly proportional to the concentration of the alpha amylase protein coupled. When 120 mg CNBr/g wet gel was used for the activation by electrophilic and nucleophilic methods, the alpha amylase coupled was 70% and 19% respectively. The coupling of alpha amylase and glucoamylase to the cyanogen bromide activated gel was linear up to 10 min and reached a maximum by 0.5 h. When the concentrations of alpha amylase and glucoamylase used for coupling were increased from 1 – 1000 μg/g wet gel, the percentage activity of the immobilized enzymes decreased and was inversely proportional to the log of enzyme concentration added for coupling. The total activity of these enzymes increased with an increase in enzyme concentration.

1. Introduction

The water soluble enzyme proteins are immobilized to water insoluble matrices for their repeated utilization and for continuous systems. Immobilization of proteins has been carried out since the late 1960's by various methods using different water insoluble matrices.[2,10]

To immobilize an enzyme protein to a water insoluble matrix, the matrix should first be activated. Axen *et al* who were the pioneers in the activation of Sepharose – 4B used cyanogen bromide[1,9] as the reagent. This method of activation was carried out in an alkaline medium and the active species produced results in a negative charge hence it is called a nucleophilic method of activation.

Kohn and Wilcheck developed a method to activate the same matrix (Sepharose – 4B) at neutral pH with the reactive species having a positive charge. This method, which is called the electrophilic method of activation, is more effective than the nucleophilic method.[6]

Although general conditions for activation of matrices and coupling of proteins are known, the optimal conditions for coupling of alpha amylase and glucoamylase, (enzymes of industrial importance) are seldom published.

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Further, the electrophilic and nucleophilic methods of activation of matrices have not been compared previously. In our work we have used both the electrophilic and nucleophilic methods of activation and studied the effect of cyanogen bromide concentration on the activation of the Sepharose – 4B by the two methods. In addition, the optimal conditions for the coupling of alpha amylase (1,4– α–D glucan glucanohydrolase; EC 3.2.1.1.) and gluco-amylase (1,4– α–D glucan glucohydrolase; EC 3.2.1.3) to the cyanogen bromide activated Sepharose – 4B were investigated.

2. Experimental

2.1 Materials

Alpha amylase from hog pancreas (Sigma Chemical Company, USA), gluco-amylase from *Aspergillus niger* (CISIR, Sri Lanka) and Sepharose – 4B (Pharmacia Fine Chemicals, Sweden) were used in this study.

2.2 Preparation of cyanogen bromide

Cyanogen bromide was prepared according to the method described previously[5] with minor modifications (see results and discussion).

2.3 Electrophilic activation of Sepharose – 4B by cyanogen bromide

Sepharose – 4B (25 g wet weight) was washed thoroughly with cold distilled water followed by 250 ml portions of 30% cold acetone and 60% cold acetone in a Buchner funnel. The washed gel was divided into 5 different, 5 g portions and each was suspended in 5 ml of 60% acetone. To these 5 g portions of gel different amounts of cyanogen bromide in 60% acetone (10, 20, 40, 80 & 120 mg/g wet gel) were added while the gel portions were stirred continually. Varying amounts of triethylamine (TEA) in 60% acetone (16.4, 32.8, 65.6, 131.2 & 196.8 mg/g wet gel) were added to the gel portions dropwise for 3 min. The pH of the medium was found to be neutral. The CNBr : TEA concentration was 1 M : 1.5 M respectively.[6]

2.4 Nucleophilic activation of Sepharose – 4B by cyanogen bromide

Varying amounts of NaOH in 60% acetone (5.66, 11.32, 22.64, 45.28 and 67.92 mg/g wet gel) were used in place of triethylamine. The rest of the procedure was as in section 2.3. The concentration of CNBr : NaOH added was 1 M : 1.5 M. The pH of the medium was 12.

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2.5 Coupling of alpha amylase to the activated gel

The cyanogen bromide activated Sepharose – 4B (5 g portions) was transferred into screw cap tubes and suspended in 5 ml of coupling buffer (0.1 M bicarbonate – 0.5 M NaCl buffer pH 8.3). To these were added alpha amylase in solution (1 mg/g wet gel). The mixture was agitated for 2 h at room temperature in a shaker water bath. The gel portions were washed thrice with coupling buffer.

In order to block the excess activated groups on the mixture, 20 ml of 1 M ethanolamine in water (pH 8.3) were added to the gel and the mixture agitated for 2 h at room temperature. The gel was washed thrice alternatively with coupling buffer and with 0.1 M acetate – 0.5 M NaCl buffer (pH 4).

The gel was washed finally with 0.02 M phosphate buffer pH 6.9 (in which alpha amylase is more stable). The immobilized enzyme protein was estimated by the Kjeldahl method.[8]

2.6 Effect of time on coupling of alpha amylase and glucoamylase

The time for coupling of enzyme molecules to the activated Sepharose–4B was varied between 0 and 120 min. The coupled alpha amylase and glucoamylase were estimated, indirectly, by measuring the activity of coupled enzymes using the dinitrosalicylic acid method.[3,4] The gel coupled to glucoamylase was finally washed with 0.01 M acetate buffer pH 4.8 (in which the enzyme is more stable).

2.7 Effect of concentration of alpha amylase and glucoamylase on coupling

Varying amounts of alpha amylase and glucoamylase (1 – 1000 μg/g wet gel) were added to the activated gel for coupling. The coupled alpha amylase and glucoamylase were estimated, indirectly, by testing for their activity.

In Figure 4, the activity (%) is the amount of immobilized alpha amylase activity/the soluble alpha amylase activity added x 100; and total activity refers to the amount of activity in the immobilized alpha amylase.

3. Result and Discussion

3.1 Synthesis of cyanogen bromide

A yield of 25.45 g of CNBr was obtained from 50 g of bromine which was 76.83% the theoretical yield. The melting point of the product was 49°C (observed 48° – 50°C) which showed the purity of the product. The

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following experimental details were noted: (a) A short wide air condensor was used since CNBr clogs the narrow long condensor at room temperature. (b) To reduce the volatility of the product the receiving flask was kept in an ice bath. Cyanogen bromide being soluble only in water at 50°C, it is advisable to dissolve it in solvents such as acetonitrile, dimethyl sulphoxide and acetone.[7]

3.2 Activation and coupling

3.2.1 Method of Activation

A linear relationship between the amount of CNBr used and the amount of alpha amylase protein coupled to the Sepharose–4B was obtained for both the electrophilic and the nucleophilic methods (Figure 1). This showed that the degree of activation was directly proportional to the amount of CNBr used for activation.

When 120 mg CNBr/g wet Sepharose–4B was used in the electrophilic and nucleophilic methods of activation and 1 mg alpha amylase/g wet gel was added for coupling, the alpha amylase protein coupled was 70% and 19% respectively. From these observations it was concluded that the electrophilic method was better than the nucleophilic method and needed lesser amounts of CNBr for greater activation.

Kohn and Wilcheck postulated that in the nucleophilic method of activation the cyanate esters were formed and the alkaline medium hydrolysed the active cyanate esters. To compensate for this hydrolysis a high concentration of CNBr is essential. Hence the percentage of activation is very low even at a high CNBr concentration. In the electrophilic method of activation, triethylamine reacts with CNBr to form a highly reactive triethylammonium nitrile complex (Figure 2) and this complex has a higher electrophilicity than CNBr. The complex reacts with the hydroxyl group of the gel and leads to a higher degree of activation. Since the pH of the medium is 7 there is little or no hydrolysis of the cyanate esters.

3.2.2 Coupling

The buffer used for coupling had a high salt concentration (0.5 M NaCl) to minimise the protein – protein adsorption caused by the polyelectrolyte nature of the protein and to reduce the mutual repulsion between the enzyme and the support where the proteins are negatively charged at higher pH values (pH 8.3).

The adsorbed enzymes present in the immobilized enzyme preparations were removed by cyclic washings using high and low pH buffer

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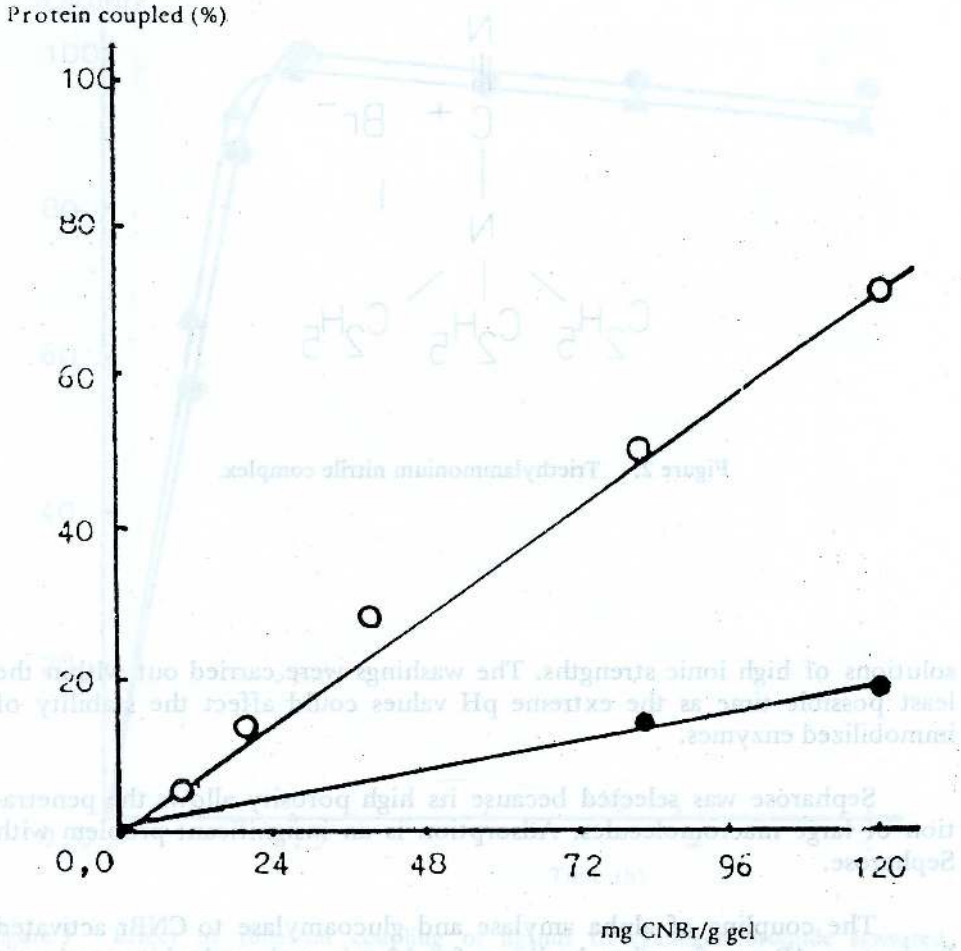

Figure 1. Effect of cyanogen bromide concentration on activation of Sepharose – 4B when NaOH ●—● and TEA ○—○ were used.

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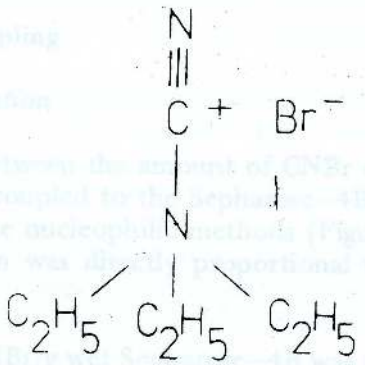

Figure 2. Triethylammonium nitrile complex.

solutions of high ionic strengths. The washings were carried out within the least possible time as the extreme pH values could affect the stability of immobilized enzymes.

Sepharose was selected because its high porosity allows the penetration of large macromolecules. Adsorption is an insignificant problem with Sepharose.

The coupling of alpha amylase and glucoamylase to CNBr activated Sepharose–4B was found to be linear for 10 min and reached its maximum by 0.5 h. After 0.5 h there was no significant increase in coupling (Figure 3).

3.3 Activity of enzymes

When the amount of enzymes added for coupling was increased from 1 – 1000 μg/g wet gel, the percentage activity of the immobilized enzymes decreased and the total activity of enzyme coupled increased (Figures 4 and 5). The decrease in percentage activity may be due to the steric effect of the enzyme molecules coupled to the solid support.[7]

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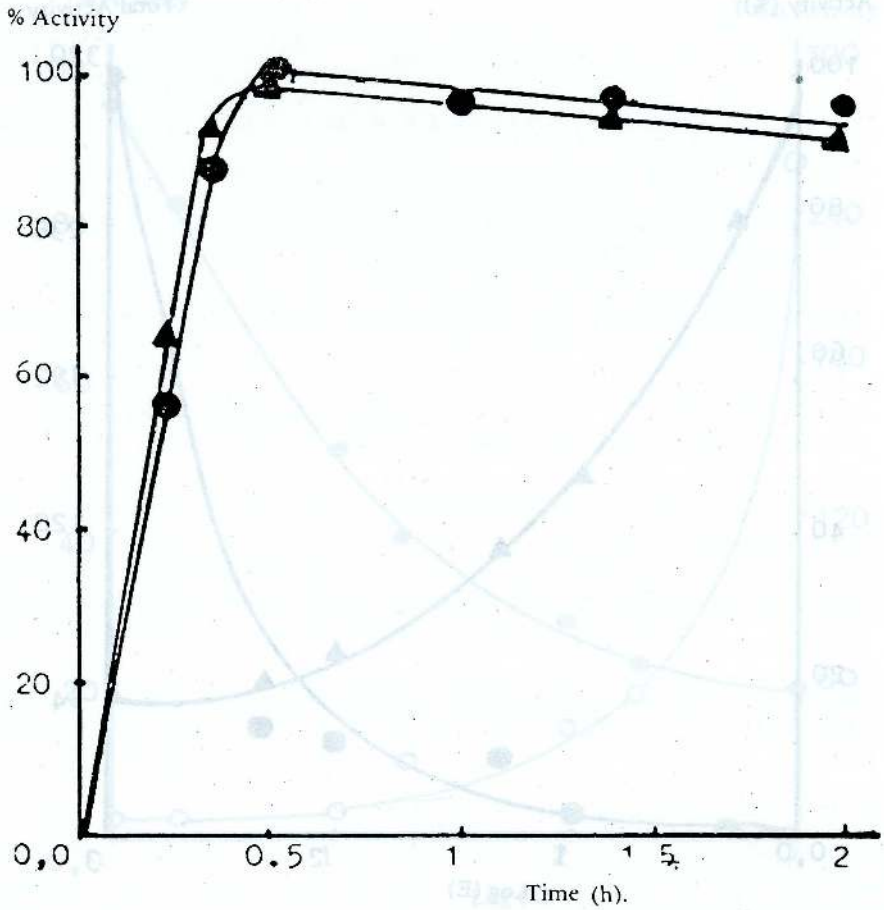

Figure 3. Effect of time on coupling of ligands to cyanogen bromide activated Sepharose–4B

●——● α– amylase ; ▲——▲ glucoamylase
(Each point is the average of duplicate determinations).

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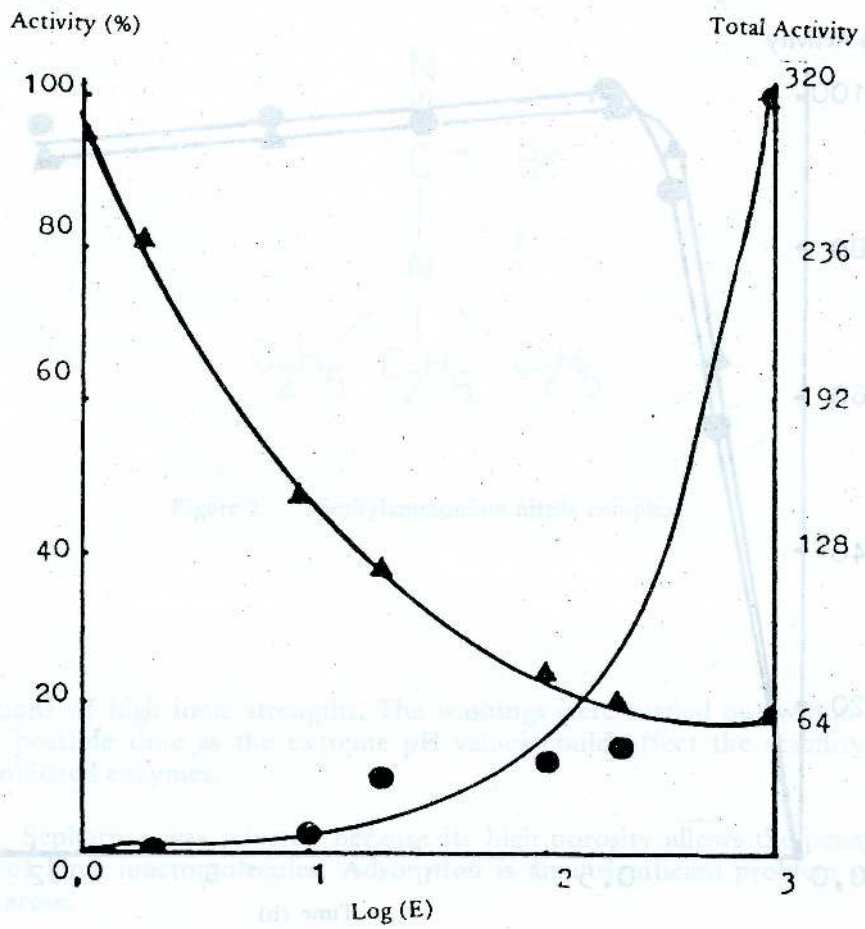

Figure 4. Effect of α–amylase concentration (μg/g wet gel) added on the degree of immobilization. ▲——▲ % Activity ; ●——● Total activity. (Each point is the average of duplicate determination.).

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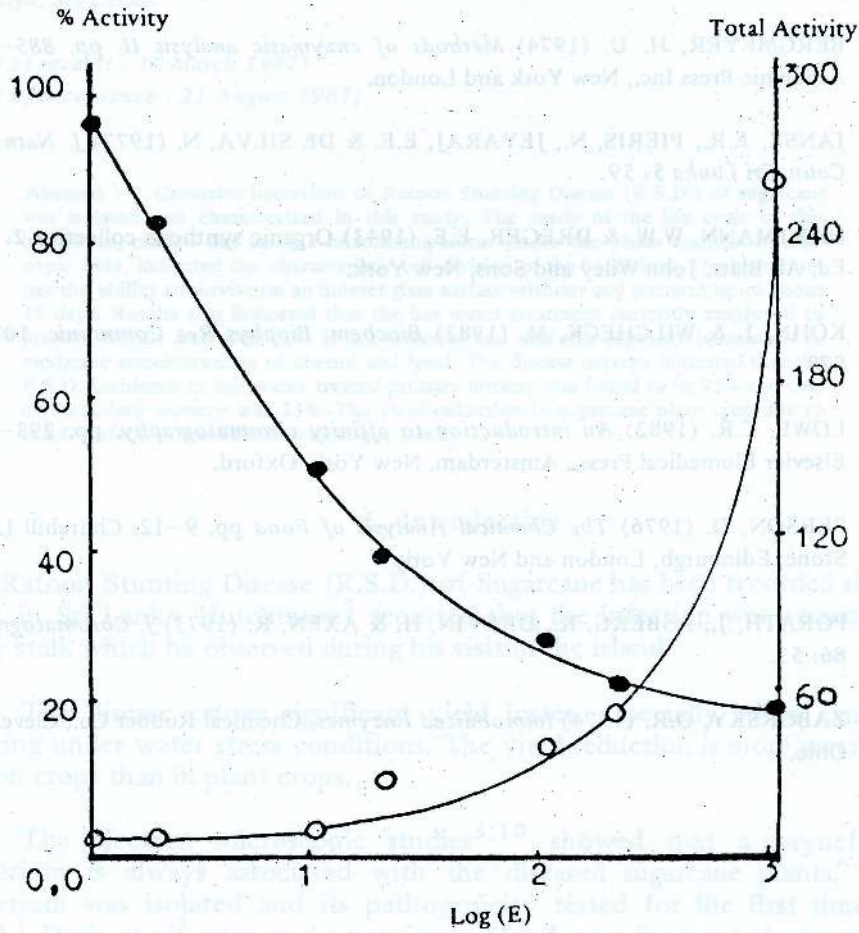

Figure 5. Effect of glucoamylase concentration (μg/g wet gel) added on the degree of immobilization. (Each point is the average of duplicate determinations).
●—● % Activity ; ○—○ Total activity

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J. Natn. Sci. Coun. Sri Lanka 1987 15(2): 227–236

RATOON STUNTING DISEASE OF SUGARCANE IN SRI LANKA

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(Date of receipt : 10 March 1987)
(Date of acceptance : 21 August 1987)

Abstract : Causative bacterium of Ratoon Stunting Disease (R.S.D.) of sugarcane was isolated and characterized in this study. The study of the life cycle of this bacterium, carried out using a micromanipulator under the phase contrast microscopic field, indicated the characteristic cell division of the bacterium. The bacterium has the ability to survive on an inneret glass surface without any nutrient up to about 19 days. Results also indicated that the hot water treatment currently employed to prepare R.S.D. free seed cane is not effective and that this organism is resistant to moderate concentrations of phenol and lysol. The disease surveys indicated that the R.S.D. incidence in hot water treated primary nursery was found to be 25% and that of secondary nursery was 33%. The yield reduction in sugarcane plant crop due to R.S.D. infection was found to be about 10%.

1. Introduction

The Ratoon Stunting Disease (R.S.D.) of Sugarcane has been recorded since 1961 in Sri Lanka. Hutchinson[5] reported that the infection was present in every stalk which he observed during his visit in the island.

The disease causes significant yield losses, especially when cane is growing under water stress conditions. The yield reduction is more severe in ratoon crops than in plant crops.

The electron microscopic studies[3,10] showed that a coryneform bacterium is always associated with the diseased sugarcane plants. The bacterium was isolated and its pathogenicity tested for the first time in 1980.[1] Davis *et al*[2] proposed a new genus *Clavibacter* for some phytopathogenic coryneform bacteria. Accordingly causative bacterium of R.S.D. was classified as *Clavibacter xyli* sub sp. *xyli* sp nov. sub sp. nov.[2]

The disease diagnosis of R.S.D. is often confused as the external and internal symptoms are not specific. Therefore electron microscopic method[3] or phase contrast microscopic method[9] are used for reliable diagnosis.

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The Hot Water Treatment at 50°C for 2 or 3 hours is used to eliminate the infection in seedcane setts. Sterilization of cane knives prior to harvesting and chopping seed cane, prevents further spread of the disease.

The present investigation was carried out on the following lines to obtain more information on R.S.D. in Sri Lanka.

(a) R.S.D. diagnosis and survey.
(b) The effect of the disease on cane yield and juice quality.
(c) Isolation, identification and life cycle studies of the causative bacterium.
(d) Pathogenicity of the bacterium.
(e) Efficiency of the Heat Treatment.
(f) Viability of the bacterium on implements used in sugarcane cultivation.
(g) Sensitivity to the disinfectants.

2. Experimental

2.1 Ratoon Stunting Disease Survey

The disease survey was carried out in hot water treated primary and secondary nurseries at Sugarcane Research Station, Kantale. The bioassay technique developed by Davis *et al.*,[1] was used to diagnose this disease. In this method a "three budded sett" selected from a single stalk was split open longitudinally using a clean sharp knife and examined for vascular bundle discolourations at the nodes just below the leaf scar, with continuous slicing towards the same direction.[1]

The survey area for each site was about 10% of total extent (i.e. sample field). The percentage infestation was determined from randomly selected stools which represented 1% of the sample field.

2.2 The effect of the R.S.D. bacterium on cane yield, juice quality and fibre content of sugarcane

A replicated yield trial was carried out in Reddish Brown Earth at Sugarcane Research Station, Kantale using the commercial Sugarcane variety Co 775. The disease free seed material were selected from the hot water treated primary nursery. The seed materials were tested and selected by both bioassay technique[1] and phase contrast microscopy technique.[9]

Half of the seed materials were treated with sugarcane juice which was extracted from R.S.D. plants. The treated and control (healthy) seed

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materials were planted in 30 feet long furrows x 4 rows in each block. Thirty three budded setts were planted in each row. The statistical design was randomized block design with fourteen replicates.

Pol %, Brix, Fibre content were determined according to the methods used in sugarcane Research Institute.[12]

2.3 Microscopic observations of Diseased plant tissues and vascular sap.

Microtome sections of the diseased plant stems and leaves were mounted on Formaline Acetic Acid Alcohol (FAA) solution and observed under the light microscope to assess the physical damage of vascular elements caused by R.S.D. bacterium.[7]

Vascular sap was extracted by suction pressure method and examined under phase contrast microscope (Olympus – BH2) for R.S.D. causative bacterium.[9]

2.4 Isolation of R.S.D. bacterium

A small piece of stem tissue excised from the nodal region of disease sugarcane plant was surface sterilized with 0.1% Mercuric chloride solution and rinsed in sterilized distilled water. The sap was expressed from the tissue with the aid of sterilized forceps into (a) sterile distilled water (b) sterile 0.01 M phosphate buffer.

Suction pressure method[9] was also used for vascular sap extraction. The plates containing nutrient agar medium, Liao & Chen medium,[7] and sugarcane juice agar medium were inoculated with the prepared extracts using streak plate and spread plate methods.

Micro manipulator[8] was also used for isolation of pure culture derived from a single R.S.D. bacterial cell.

2.5 Pathogenicity test

A suspension of a pure culture of R.S.D. bacterium was prepared in sterilized 0.01 M phosphate buffer solution. Healthy sugarcane plants, (4-5 months old) were inoculated with this suspension.

2.6 Morphological and biochemical characters of the bacterium

The bacterium was observed under the phase contrast microscope. Gram reaction, Acidfastness, Spore formation, (stained with malachite green) and motility of the bacterium were also examined with light microscope.[4]

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The method developed by Davis et al[2] was used to test hydrolysis of starch, esculin, and Tween.[80]

Catalase production was tested by adding a drop of 3% Hydrogen Peroxide solution on to the colonies of the bacterium growing on an agar medium.[4]

Oxidase production was determined by the Kovacs method.[4]

Cell division and generation time of the bacterium were also studied using micromanipulator.[8]

2.7 Survival of the bacterium on an inneret glass surface.

Glass needles prepared from soda glass pasteur pipette tubings (5mm in diameter) were inserted separately in boiling tubes with the aid of the cotton wool plugs. These sets were sterilized by autoclaving at 120°C for 20 min. Sterilized glass needles were contaminated with the bacterium and inserted again in the tubes. Viability of the bacterium was then checked daily by streaking each needle on fresh agar medium followed by incubation at 30°C.

2.8 Effect of temperature on viability of the bacterium

The viability of the bacterium were checked using the actively growing bacterial colonies and bacterial cell suspension in 0.01 M phosphate buffer solution.

The agar plates consisting of five days old growing bacterial colonies were incubated at following temperature, time combinations in an incubator.

At 50°C for 0.5, 1.5, 2.0, 2.5, 3.0 hr respectively and at 56°C for the same time durations.

At the end of the incubation time the bacteria were subcultured in to fresh agar plates and then incubated at 30°C for 5–7 days.

A similar study was carried out by using a bacterial suspension in sterile 0.01 M phosphate buffer. The McCartney bottles filled with the bacterial suspension were kept in the water bath at different temperature, time combination as above. The experiment was replicated four times.

2.9 Sensitivity to the disinfectants

The isolated bacterium was tested for its sensitivity to various concentrations of two phenolic disinfectants "Lysol" and "Phenol". The concentrations

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were 4%, 8%, 12%, 16%, 20%, 24%, 28%, 32% and 36%.

A bacterial colony was picked up from agar plates using a sterilized standard inoculating loop (size 0.36 mm diameter) and was dipped in disinfectant solution for five seconds and streaked on sterilized agar plates after draining off the excess disinfectant.

This procedure was repeated for both disinfectants at the above concentrations. The experiment was replicated twice. The inoculated plates were incubated at 30°C for 5–7 days.

The inhibitory concentration of each disinfectant were determined by observing the colony formation on agar plates (i.e. no colony formation of bacteria takes place at inhibitory concentrations).

3. Results and Discussion

The Ratoon Stunting Disease survey shows that the disease incidence in hot water treated primary nursery is 25% while that in hot water treated secondary nursery is 33%. Untreated commercial sugarcane plantation shows a record of about 80% disease incidence.[5] The percentage of disease incidence shows large variations from block to block in the sample fields (Table 1).

Table 1. Percentage incidence of Ratoon Stunting Disease in hot water treated primary and secondary nurseries.

Block No.	No. of samples tested		R.S.D. positive		Percentage incidence	
	I^{ry} Nursery	II^{ry} Nursery	I^{ry} Nursery	II^{ry} Nursery	I^{ry} Nursery	II^{ry} Nursery
I	64	360	15	65	23	18
II	12	180	04	90	33	50
III	40	330	08	124	20	37
IV	76	150	21	60	27	40
	192	1020	48	339	25	33

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This difference may be due to shortcomings in the treatment such as a drop in temperature, or shortening of the duration of heat treatment or it may also be due to the low heat penetration through thicker seed setts. Poor crop sanitation during the harvesting of the primary nursery may be a further contributing factor.

The samples obtained from Kantale, Moneragala and Pelwatte commercial sugarcane plantations were observed for the bacterium by using phase contrast microscope.

The results indicated that all the samples were contaminated with R.S.D. bacterium. Furthermore this investigation showed that the positive samples of bioassay were always associated with the coryneform bacterium. However some negative samples of bioassay also consisted of coryneform bacterium (Table 2).

Table 2. The comparison of Ratoon Stunting Disease and first ground nursery samples, their relationship in bio assay and phase contrast microsocope.

Site	No. of Samples	Bio assay technique		Phase contrast microscope	
		Positive	Negative	Positive	Negative
Commercial samples					
Kantale	16	14	2	16	0
Monaragala	8	6	2	8	0
Pelwatte	8	0	8	8	0
1st ground nursery samples					
Kantale	8	0	8	0	8

Therefore, it is probable that the actual R.S.D. incidence in the surveyed area could be much higher than the values obtained from bioassay technique.

The experiment carried out to estimate the effect of the R.S.D. on cane yield, showed that there was a significant difference (at P = 0.01) in cane yield between inoculated and R.S.D. free control plants. The calculated cane yield for R.S.D. free crop was 135.65 MT/ha. and for R.S.D. infected crop was 121.53 MT/ha. A significant yield reduction was observed in ten plots out of fourteen replicates. The results of the experiment showed that there was no significant difference (at P = 0.01 and 0.05) in height and girth of stalks between control and treated plants.

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However, it was observed that the R.S.D. infection affected the stalk population by reducing the germination and tillering.

Table 3. The effect of Ratoon Stunting Disease on yield by reduction of both seed-cane germination and tillering.

Repli.	Germination counts		Percentage germination		Tillers per plot	
	Treat.	Control	Treat.	Control	Treat.	Control
1	183	207	50.83	57.50	364	402
2	169	159	46.94	44.17	333	378
3	155	147	43.08	40.83	339	338
4	123	156	34.17	43.33	276	359
5	122	288	33.89	80.00	297	490
6	187	325	51.94	90.28	247	405
7	101	241	28.00	66.94	155	263
8	165	185	45.83	51.39	366	415
9	147	210	40.85	58.33	344	420
10	154	174	42.78	48.33	342	387
11	109	158	30.28	43.89	246	338
12	123	253	34.17	70.28	269	332
13	159	209	44.17	58.06	199	281
14	103	195	28.61	54.17	137	252
Mean	142.85	207.64	39.68	57.67	279.57	361.42
S.D	29.08	52.90	8.08	14.69	75.43	66.45

The investigation also showed that the causal bacterium did not effect on the juice quality and vascular anatomy of the plants.

When compared with healthy plants there was no marked difference in the overall fibre content or the fibre content in the top, middle and bottom regions. This indirectly shows that the R.S.D. does not damage vascular anatomy during the growth period. The direct microscopic observations on the diseased plant stem tissues also confirmed that the R.S.D. bacterium does not physically damage the xylem elements where the bacterium is colonized. The average number of vascular bundles per cross section of a sugarcane stem are about 1200[5]. But it was observed that less than 1% of the vascular bundles were blocked by R.S.D. infection.

Therefore it is unreasonable to believe that the disease can decrease the water translocation in the plant to any extent which can create wilting in the plant. The isolation of R.S.D. causal bacterium was a critical stage of this experiment. Of the three methods employed for isolation the micromanipulator technique was the most reliable and quick..

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Table 4. The effect of Ratoon Stunting Disease (R.S.D.) on fibre content and juice quality in both R.S.D. inoculated (treated) samples and disease free (control) samples.

		Treated (inoculated)	Control (Disease free)
Fibre	Top	11.59	11.75
	Middle	12.73	13.08
	Bottom	14.13	14.15
Brix		16.26	16.50
Pol %		13.78	13.90
Purity		84.21	83.98
C.C.S*		9.32	9.34

* C.C.S. (Commercial cane Sugar) – That percentage by weight of a quantity of cane which would be recovered as pure sucrose if milling and refining operations were conducted at a prescribed standard of efficiency.

The isolated bacterium was able to grow only in a complex medium consisting of L–Asparagine, Glutamic acid, Beef infusion, Casamino acid, Haemoglobin and essential minerals. The bacterial colonies which appeared on the agar medium were circular with entire margins, convex, glistering butyrus, and were white in colour with a maximum diameter of 0.5 mm.

The isolated bacterium was gram positive, non-acid fast, non-endosporeforming, non-motile, pleomorpic rods. The bacterium did not hydrolyse starch, esculin, or Tween 80 and also were catalase positive and oxidase negative. These morphological and biochemical characters were similar to that recorded in previous investigations[2,7] for *Clavibacter xyli.*

The cell division of the isolated bacterium was observed under the phase contrast microscope. The studies indicated that the cells divided by binary fission and was usually followed by a snapping movement. This is a characteristic feature in coryneform bacteria. As a result of a snapping movement the two cells were arranged in "V" shape. The coccoid cells of the bacterium elongate first to form rods and then divide by binary fission. The time taken for a single division was four to five hours.

The contaminant bacterium survived for a long period on implements. The results of this investigation indicated that the organism can survive on glass surfaces without a supply of nutrients up to about 19 days. As the bacterium is a non-spore former the lethal temperature cannot be very high. However the bacterium survived at 50°C for three hours. Actively

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growing cells survived for one hour at 56°C in agar medium. The lethal temperature, time combination for the bacterium appeared to be 56°C for 1½ hr.

These results clearly indicated that the hot water treatment of seed cane setts at 50°C for three hours is not effective against R.S.D. control.

This bacterium was resistant to phenolic disinfectants which are usually employed to sterilize cane knives. The bacterium tolerated lysol concentration up to 24% and phenol concentration up to 28%. During the seed-cane harvesting, the cane knives are just dipped in disinfectant solution for sterilization. The normal disinfectant used for cane knives sterilization is lysol at 20% concentration. Such sterilization practices are not effective against R.S.D. control. The cane knives can be sterilized by dipping in 30% 'Lysol' or 'Phenol'. It has also been suggested that the bacterium can be transmitted from one plant to another via the cane knives used for seed cane harvesting.

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J. Natn. Sci. Coun. Sri Lanka 1987 15 (2) : 237 248

A RECAPITULATION OF THE GENETICS AND EVOLUTION OF INSECT BIOTYPES ON CEREALS: THE CASE STUDY OF THE HESSIAN FLY

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(Date of receipt : 16 June 1987)
(Date of acceptance : 25 August 1987)

Abstract : The host-specific nature of the Hessian fly, *Mayetiola destructor* (Say) and its genetic interaction with the wheat (*Triticum aestivum* L. em. Thell) plant results in the evolution of virulent biotypes. However, carefully planned deployment of resistant genes can delay the evolution of biotypes and maximize the stability of resistant genes. The usefulness of the Hessian fly-wheat interaction model in breeding for insect resistance in rice is discussed.

1. Introduction

1.1 Biotype Concept

The importance of resistant cultivars for use in integrated control strategies of agricultural insect pests has served to emphasize the need for a basic understanding of the variation in the response of resistant crop cultivars. Some insect pests have increased their virulence and cause significant damage to cultivars which previously showed resistance. Such apparently new forms of pests have been termed host races or biotypes. In the glossary to their compendium on breeding plants resistant to insects, Maxwell and Jennings[25] define the term biotype in entomology as 'an individual or a population that is distinguished from the rest of its species by criteria other than morphology; for example, a difference in parasitic ability.'

The terminology employed to describe the genetic and environmentally induced phenotypic variation in phytophagous insects is extensive and include such terms as race (geographic and host race), biotype, ecotype, pathotype, form, morph, strain, variety, subspecies, semispecies, etc.[9] The term biotype is more specifically used for individuals and populations of species which share certain biological characteristics usually concerning virulence on specific host cultivars. The term is more appropriate when genes or genotypes for virulence in a pest are known to correspond with particular genes for resistance in a host plant.[5] This type of interaction has not been demonstrated in all insects where biotypes have been described. The best

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understood case of genetic-interrelationship between virulent genes in the insect and resistant genes in the plant is that of the Hessian fly-wheat interaction. Therefore, this system stands as a model for cereal insect-plant studies. Resistant rice cultivars play a major role in the management of rice insects but their longterm stability is threatened because of the evolution of biotypes. The knowledge available on the Hessian fly-wheat interaction could be used in rice insect host-plant resistance studies.

1.2 Objectives

This paper reviews the mechanisms involved in the development of biotypes of Hessian fly on wheat and the strategies used by plant breeders to manage the plant resistance to control Hessian fly biotype development. Also, the usefulness of some of these strategies in breeding for insect resistance in rice will be discussed.

2. Genetics of resistance in wheat to Hessian fly

The Hessian fly, *Mayetiola destructor* (Say), is a significant pest of wheat in the midwest and other wheat producing regions of the United States.[12] Emphasis has been placed on using resistant wheat cultivars to limit the damage caused by this pest. Sixteen wheat genes for resistance to Hessian fly have been identified to the present time (Table 1). Many of these have been incorporated into high yielding wheat cultivars and are widely grown in the field. The wheat chromosomes carrying some of these genes for resistance have been determined using monosomic analysis. For example, Gallun and Patterson[16] found the H_6 gene, derived from PI 94587 durum wheat *(Triticum turgidum* var. *durum)*, located on chromosome 5A. Subsequent linkage studies by Patterson and Gallun[32] and Stebbins *et al.*[38] located the genes H_3, H_6, H_9 and H_{10} all on chromosome 5A. The H_5 gene has been shown to be independent of the H_3 gene and linked to the H_{11} gene[29]. Also using monosomic analysis, Roberts and Gallun[34] located the H_5 gene on chromosome 1A. The H_{13} gene was derived from *Triticum tauschii* (Coss.); hence the resistance it provides is different from other types of resistance. This gene is presumably associated with the D genome of the wheat plant and is on chromosome 6D.[17,20]

The location of resistant genes on specific chromosomes is important in genetic engineering research and also in studies of evolution of the virulence of Hessian fly biotypes.

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Table 1. Various wheat genes identified as having resistance to the Hessian fly, *Mayetiola destructor* (Say)

Resistant Genes	Nature of resistance	Sources of resistance (cultivars)	Reference No.
H_1H_2	Complementary dominant	'Dawson'	28
H_3	Dominant	'W38'	4
h_4	Recessive	'Java'	39
H_5	Dominant	'Ribeiro'	36
H_6	Dominant	P.I.94586 (diploid durum)	1
H_7H_8	Partially dominant	'Seneca'	31
H_9H_{10}	Independent dominant	'Elva' (tetraploid) *(Triticum turgidum)*	38
H_{11}	Dominant (tetraploid)	P.I. 94587	38
H_{12}	Dominant	'Luso'	29
H_{13}	Dominant	*Triticum tauschii* (D− genome)	20
$H_{14}H_{15}$	Independent dominant	'ELS 6404−160' (diploid durum)	23
H_{16}	Dominant	PI 94 587 (diploid durum)	30

3. Hessian fly biotypes

3.1 Occurrence of Hessian fly biotypes

Since there are sixteen genes for resistance identified in wheat, there are potentially 2^{16} or 65,536 possible Hessian fly biotypes, assuming each biotype differs in at least one gene specifically matching one of the host's 16 resistant genes. However, only a small fraction of this potential diversity has been directly assayed. In most experiments only four resistant wheat varieties have been utilized, each having a different resistant gene or gene pair. Using these four differential resistant varieties to assess the fly's genotype, there is a total of 16 possible biotypes.[12] To date, 12 biotypes have been identified.[12, 21, 37]

3.2 Identification of biotypes

When Hessian fly larvae feed on a susceptible wheat plant, the leaves become stunted and dark green, and the new leaves fail to form. Resistant seedlings

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show initially some leaf stunting, but they recover and remain light-green as do non-infested plants. Larvae that feed on resistant plants usually die. Thus, the virulent larvae can be distinguished from avirulent larvae by the reaction of the plant to the infestation. Biotype designation is based on the virulence or avirulence of larvae to specific wheat cultivars having known genes for resistance. One biotype may be virulent on a specific cultivar, while another may be avirulent. Virulence and avirulence are thus terms describing the insect's reaction to the host plant, whereas resistance and susceptibility are terms describing the plant's reaction to the insect. The phenotype of any biotype can be determined by scoring the reaction of four wheat differentials having five genes (H_3, H_7H_8, H_6 and H_5) to larvae of the same progenitor and the ability or inability of the larvae to survive. Wheat seedlings of known genotypes are grown and infested by a gravid female which lays eggs at random on the plants, without showing any host preference. The reaction of the seedlings is observed 15 days after infestation. The seedling reaction indicates the phenotype of the progeny.

The Great Plains (GP) biotype is the least virulent of the biotypes and it cannot attack wheat cultivars with the H_3, H_7H_8, H_6 and H_5 genes. This biotype can only live on wheats having no known resistant genes and it was probably the first biotype that entered the United States 200 years ago.[12] The most virulent biotype is designated as L, which is capable of attacking all four differentials used. However, newer genes such as H_9 and H_{13}, are resistant to this biotype.

3.3 The gene-for-gene concept

Hybridization experiments with Hessian fly biotypes have provided evidence for a gene-for-gene interaction between the fly and its wheat hosts, which is similar to the genetic interaction that has been demonstrated for rust, *Melampsora lini* on varieties of flax, *Linum usitatissimum*.[11]

According to this concept, for every major gene for resistance in the host species there is a corresponding matching gene for virulence in the parasite. The host plant shows a resistant reaction when it has a resistance gene and the insect has an avirulent allele at the corresponding gene locus. On the other hand, the plant is susceptible when the insect has a virulent gene at the corresponding locus. The gene-for-gene relationship has been called the 'matching gene theory'.[25] According to this concept, a Hessian fly biotype can be virulent to a specific wheat cultivar, only when the biotype is homozygous for a recessive virulent gene at all loci corresponding to the loci at which the wheat plant has dominant alleles for resistance[12] (Table 2). For example, the wheat cultivar 'Turkey' is susceptible to all Hessian fly biotypes because it has no genes for resistance. The wheat cultivars 'Seneca', 'Monon', 'Knox 62' and 'Abe' are resistant to the GP biotype because GP has no genes

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Table 2. Genotypes of eight selected Hessian fly biotypes (after Gallun)

	Wheat Varieties[a]				
Biotype	Turkey	Seneca	Monon	Knox–62	Abe
		H_7H_8	H_3	H_6	H_5
GP	tt	S_	M_	K_	A_
A	tt	ss	M_	K_	A_
B	tt	ss	mm	K_	A_
C	tt	ss	M_	kk	A_
D	tt	ss	mm	kk	A_
E	tt	S_	mm	K_	A_
F	tt	S_	mm	kk	A_
G	tt	S_	mm	kk	A_

a: Symbols designate recessive and dominant alleles that represent virulence in the insect and susceptibility in the plant; and avirulence in the insect and resistance in the plant, respectively (see the section on the gene-for-gene theory in the text for a detailed explanation).

for virulence to these resistant wheats. The wheat cultivars Seneca, Monon, Knox–62 and Abe are susceptible to biotype L because this biotype has recessive alleles for virulence at the loci corresponding to the resistance alleles in the wheat. Therefore, when a biotype has homozygous recessive alleles at a locus, then it is virulent on a wheat that has at least one dominant allele at the corresponding locus for resistance. For example, biotypes A through D, with homozygous recessive alleles at the H_7H_8 locus of Seneca, are virulent.

3.4 Genetics of virulence

Hybridization experiments on different biotypes have shown that virulence in the fly is controlled by recessive genes.[15,19] Also, the virulent genes at different loci are not alleles of the same gene.[13] Gallun and Hatchett[15] and Hatchett and Gallun[19] showed that the segregation ratio of reciprocal crosses of Hessian fly biotypes depended on heterozygosity and the direction of the mating. The heterozygous male bred as if they were homozygous. The differences in virulence observed in these reciprocal crosses were explained on the basis of the loss of the paternally derived chromosomes containing the virulent genes in the male. Metcalfe[26] studied the germ cell cycle of the

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Hessian fly and observed that during spermatogenesis the reduction of 2n = 16 chromosome to n = 8 chromosome is accomplished by two unequal divisions. In each division, four chromosomes are expelled in bud-like processes from the main cell, so that only one functional spermatid remains and the haploid set has then eight chromosomes.

4. Evolution of Biotypes

The formation of new biotypes in the Hessian fly accelerates in response to the wide scale use of wheat cultivars with specific genes for resistance. This is related to changes in the gene frequencies that determine virulence in the insect. According to Mackenzie[24] a stable equilibrium of the gene frequency results from several reasons. The first reason is known as the Hardy-Weinberg law. This law states that unless acted upon by some outside force, gene frequencies will remain unchanged from generation to generation. Outside forces which might upset this equilibrium are nonrandom mating, differential mutation rates between alleles, migration or selection. A second case for genetic equilibrium could result from stabilizing selection.[10] This stabilizing process operates against the extreme classes and maintain the mean, reducing the variance. A third cause of genetic equilibrium is balanced polymorphism. Heterozygote advantage in diploids is one of the ways in which balanced polymorphism is maintained. The heterozygotes (Aa) of one generation produce less fit homozygotes (AA and aa) in the next generation.

Selection intensity expressed by widespread use of a resistant cultivar results in biotypes that can live on these cultivars. Selection proceeds, when possible, in the direction of increased fitness in the pest population. This effect is called the directional selection. The directional selection for parasitic fitness could operate only in those populations with some genetically different types. Genetic variance is necessary for the selection to proceed. The variance could come from several sources, mutation and sexual recombination being two very important and obvious sources.

Since avirulence in the Hessian fly is determined by dominant genes, mutation into recessive alleles could bring about changes in virulence. The most important source of genetic variance in Hessian fly populations is provided, however, by genetic recombination. For instance, a mating between two flies of biotype A with the genotypes ssMmKK (loci for Seneca, Monon and Knox 62) and ssMmKk would result in two other genotypes, ssMMKK and ssmMkk, taking into consideration the fact that only maternally derived chromosomes in the male are transmitted into the progeny.[14] If a female of genotype ssmMKK mates with a male fly having the same genotype, then two kinds of genotypes are produced: ssmmKK (biotype B) and ssMmKK (biotype A). If no selection pressure is exerted against this population, biotype B genotypes would remain in the population

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and be diluted with avirulent biotype A flies. However, if wheat having genes for resistance to biotype A is cultivated, then selection operates against this biotype, with a corresponding increase of biotype B.

This is apparently what has happened in Indiana and in adjoining states where similar wheats were grown. Biotype A occurred when flies having double recessive alleles at the Seneca or S locus appeared in the population due to the selection pressure of Seneca wheat or wheats with similar resistance. This left biotype A in the field because there was no selection pressure by any other kind of wheat resistance. When Monon wheat and other cultivars of the same genetic resistance were used widely, biotype B became prevalent.[19] To combat this new biotype, cultivars having the H_6 gene resistant to biotype A and B were developed.[1] This resulted in the development of biotypes like C and D, which can survive on wheats with the H_6 gene of resistance. The H_5 gene,[36] derived from the Portuguese wheat 'Ribeiro' has been utilized extensively in the Purdue University–U.S. Department of Agriculture wheat breeding program, and is present in wheat cultivars Abe and Arthur 71.[33] Following the cultivation of these varieties, biotypes J and L appeared in the field.[37]

5. Management of Resistance

The present method of deploying Hessian fly resistant genes in most wheat breeding programs is to incorporate a single gene into cultivars and use it until the resistance it provides is no longer effective. When a virulent fly biotype has rendered the currently deployed gene(s) ineffective, new genes are crossed to elite breeding lines and subsequently utilized in the breeding program. This method, however, can eventually use all the available genes for resistance. Therefore, a rational approach of utilizing valuable genes that will afford protection to wheat over the maximum number of years is necessary.

The management of resistant genes to maximize the durability has been discussed in plant pathology literature. Browning *et al.*[3] advocated incorporating genes of major effect into multiline cultivars. These multilines consist of several components, each having a single resistant gene. In contrast, Nelson[27] suggested the accumulation of as many resistant genes as possible into individual cultivars (Pyramiding), in order to provide a cumulative effect.

Recently, Cox and Hatchett[7] have described a rational approach for the deployment of resistant genes against Hessian fly. According to their genetic model, deployment of eight resistant genes in a single cultivar would result in a rapid loss of protection since each allele applies a heavy selection pressure, and the insect loci evolve independently. Dispersed, simultaneous

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deployment in eight different cultivars or isolines would afford protection over 45 years because of the reduced selection pressure exerted by each resistant allele. In this strategy, resistant alleles could be used sequentially, by phasing in each in 25% yearly increments while phasing out the previous allele, then utilizing the new allele exclusively for four years before beginning to phase out. This strategy would supposedly provide more durability than does the dispersed simultaneous deployment.

Gould[18] proposes a different strategy of deploying resistant genes for the control of the Hessian fly. He proposes that mixing 20% susceptible seed with 80% single factor resistant wheat could extend the durability of the sequentially released germplasm. Also, when the antibiotic effects of the resistant factors are high, pyramiding two genes into a single cultivar increases productive life of the variety than does the single gene resistance. The productive life can be further increased by using a cultivar with two genes for resistance interplanted with a susceptible cultivar.

Some problems in implementing resistant germplasm deployment strategies are obvious. For example, it is more difficult to breed and register a pyramided cultivar than does a pure cultivar. The interplanting of a susceptible cultivar with a resistant cultivar can be difficult because farmers may prefer to use a single cultivar. This strategy will demand a more efficient seed production process than does the use of a pure cultivar.

6. Application to rice breeding programs in Sri Lanka

In Sri Lanka several pest resistant high yielding rice varieties have been developed and widely used by farmers.[3,5] Among these varieties 'Bg 400–1', 'Bg 276–5' are resistant to the rice gall-midge, *Orseolia oryzae* (Wood Mason) and 'Bg 379–2' is resistant to the rice brown planthopper, *Nilaparvata lugens* (Stal).

Adaptation of rice pests, particularly the brown planthopper to resistant varieties of rice has been shown to occur both in the laboratory and in the field.[6,22]

Genetic interaction between rice pests like the brown planthopper and resistant varieties is not well understood and far from being conclusive.[8] However, studies by Barrion and Saxena[2] suggested a gene-for-gene relationship between host varietal resistance and virulence of three biotypes of the brown planthopper. This type of interaction is similar to the one discussed for host resistance in wheat and virulence of different biotypes of the Hessian fly.

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The ability of the rice pests to adapt to resistant varieties is most probable; therefore host plant resistance will continue to provide an inexpensive and reliable form of pest control for the Sri Lanka farmer.

Identification of diverse and different sources of resistance for rice pests as accomplished in the Hessian fly-wheat resistant breeding program will likely be the cornerstone of a successful breeding program. Concurrent to the identification of diverse sources of resistance to a particular biotype of an insect pest, the genetic diversity in that pest should be surveyed. The principles used in the identification of biotypes of the Hessian fly should have direct application in the identification of biotypes of the rice gall-midge, which also belongs to the same family Cecidomyiidae.

In a program to develop insect-resistant rice cultivars, consideration should be given to strategies of deploying the resistance in a way that will delay the rate of evolution of biotypes. The sequential release of cultivars with major genes for resistance can play a major role in the pest resistant breeding program in Sri Lanka. The incorporation of major genes for resistance in a high yielding variety is relatively simple. Farmers in Sri Lanka use different age class cultivars according to the season, availability of water and personal preference. When different genes for resistance are used in rice cultivars of different age classes, the adaptation of the pests on these cultivars should be slower than if all these varieties had the same genes for resistance.

Pyramiding several major genes for resistance in the same improved cultivar is another strategy to manage biotype development as discussed earlier for the Hessian fly. Plant varieties with several major genes for resistance are subject to less selection pressure and therefore have more durable resistance than varieties with single genes.

7. Conclusions

The knowledge obtained from the development of biotypes of the Hessian fly and the selection and breeding of wheats that are resistant to the insect can be applied to the selection of rice cultivars resistant to certain insect pests. The success of a pest resistance breeding program on rice will depend on the continous search for new sources of resistance in the crop, the incorporation of resistance genes into high yielding varieties and the planned use of these varieties in a way that will limit biotype development. Close co-operation between entomologists and plant breeders is a pre-requisite for success in breeding for insect resistance.

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J. Natn. Sci. Coun. Sri Lanka 1987 **15** *(2) : 249–265*

RICE -- FIELD WEEDS IN THE COLOMBO AND GAMPAHA DISTRICTS OF SRI LANKA

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(Date of receipt : 18 May 1987)
(Date of acceptance : 31 August 1987)

Abstract : Two rice-growing districts in the low-country Wet Zone were surveyed for the weed-flora in the rice-fields. A comprehensive list of the species, including grass, sedge and broad-leaved weeds found in the rice-fields surveyed, is presented. An estimation of the frequency of occurrence of the major weeds was made in order to evaluate the abundance of the species in the rice agroecosystem. A method of scoring based on visual estimation of cover was used to evaluate the level of infestation of the weeds. *Fimbristylis miliacea, Isachne globosa* and *Cyperus iria* were the most abundant monocotyledonous weed species found in the rice-fields of the two districts. Among the broad-leaved weeds, *Ludwigia hyssopifolia* was by far the most abundant species with high levels of infestation in most of the fields surveyed. A second species of *Ludwigia*, yet unidentified and probably a relatively new weed record for Sri Lanka, was also found to occur in high frequency in the Colombo district. The survey revealed that a rich weed-flora occurs, both within the rice-field habitat and on the raised earthen bunds in the areas studied.

1. Introduction

Rice-cultivation in Sri Lanka covers an area of about 0.53 million hectares and provides employment for a very large section of the peasantry in the country. Recent times have seen an encouraging and steady increase in rice production in the country. However, weed problems are one of the major factors depressing rice yields in many parts of the island.[3,9] Total yield losses in rice due to weeds have been estimated at about 20%,[9] but this figure is dependent on factors such as type of rice cultivar, time of cultivation, degree of available moisture in soil and the composition of the weed flora.

It has been reported that there are about 70–80 weed species which occur commonly as rice-field weeds,[9] but that most of them cause little concern to the farmer. The number of species that comprise the major portion of the weed flora in any rice-field in the country may not exceed 10–15, out of which only 4–5 may be categorized as important to warrant control measures.[9]

Many of the rice-field weeds of Sri Lanka and their distribution[6,8] and weeds found in arable soils which include many of the rice-field weeds

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listed under wetland conditions,[2] have been already described. A more comprehensive survey of rice-field weeds in the island based on visits to 518 villages both in the Wet and Dry zones in 1975/1976 revealed that *Fimbristylis miliacea* (L.) Vahl, *Isachne globosa* (Thunb.) O.Ktze. and *Cyperus haspan* L. were the most serious rice-field weeds in the country.[10]

The present survey was carried out to update and improve the status of knowledge regarding rice-field weeds of Sri Lanka, in the Low Country Wet Zone. In addition to compiling a list of the species in accordance with the newly revised flora of the island,[5] emphasis was placed on determining their frequency of occurrence and levels of infestation.

2. Materials and Methods

Two major rice-producing districts in the low-country Wet Zone, namely Colombo and Gampaha (Figure 1), were chosen for the present survey, which was carried out during the 'Yala' (April–August) season of 1984. The sampled rice fields were the same ones selected by the Department of Census and Statistics for their annual rice-yield estimations,[7] done every season at District level. The Department of Census and Statistics uses a multi-stage stratified sampling frame for the purpose of selecting rice-fields for sampling.[7] Villages are selected at random with probability proportional to the area under rice cultivation. In each selected village, two rice-fields are chosen at random and in each one, a 'Liyadde' (an area surrounded by elevated earthen banks) is also chosen at random. Rice-yield estimations are done by harvesting experimental plots of a standard size located within the selected 'liyaddes'.[7] The present rice-weed survey was also done in the same randomly chosen fields and in many instances incorporating the 'liyaddes' selected for yield estimations by the Department of Census and Statistics. In each of the chosen rice-fields a minimum of 4 liyaddes was surveyed.

Each sample field was surveyed by walking along the bunds, collecting and identifying the weeds present and recording their occurrence. Quadrats of 0.5 m^2 were placed at random, a minimum of 4 times within a liyadde, to study the weeds that were occurring below the crop canopy. The weeds growing above the crop canopy were evaluated by scanning the whole field/liyadde. The bund–weed flora was also studied using the quadrats. The relative abundance of each species was scored using the following key: 0 = weed species not observed; 1 = a few scattered plants; 2 = occasional patches of the weed; 3 = weed widespread throughout field (or bund); 4 = a dense infestation, and 5 = weed completely dominating and masking the crop (or other on bunds). Categories 2, 3, 4 and 5 corresponded approximately with 1–25% weed cover, 26–50% weed cover, 51–75% weed cover and 76–100% weed cover, respectively, based on visual estimates. Whilst the

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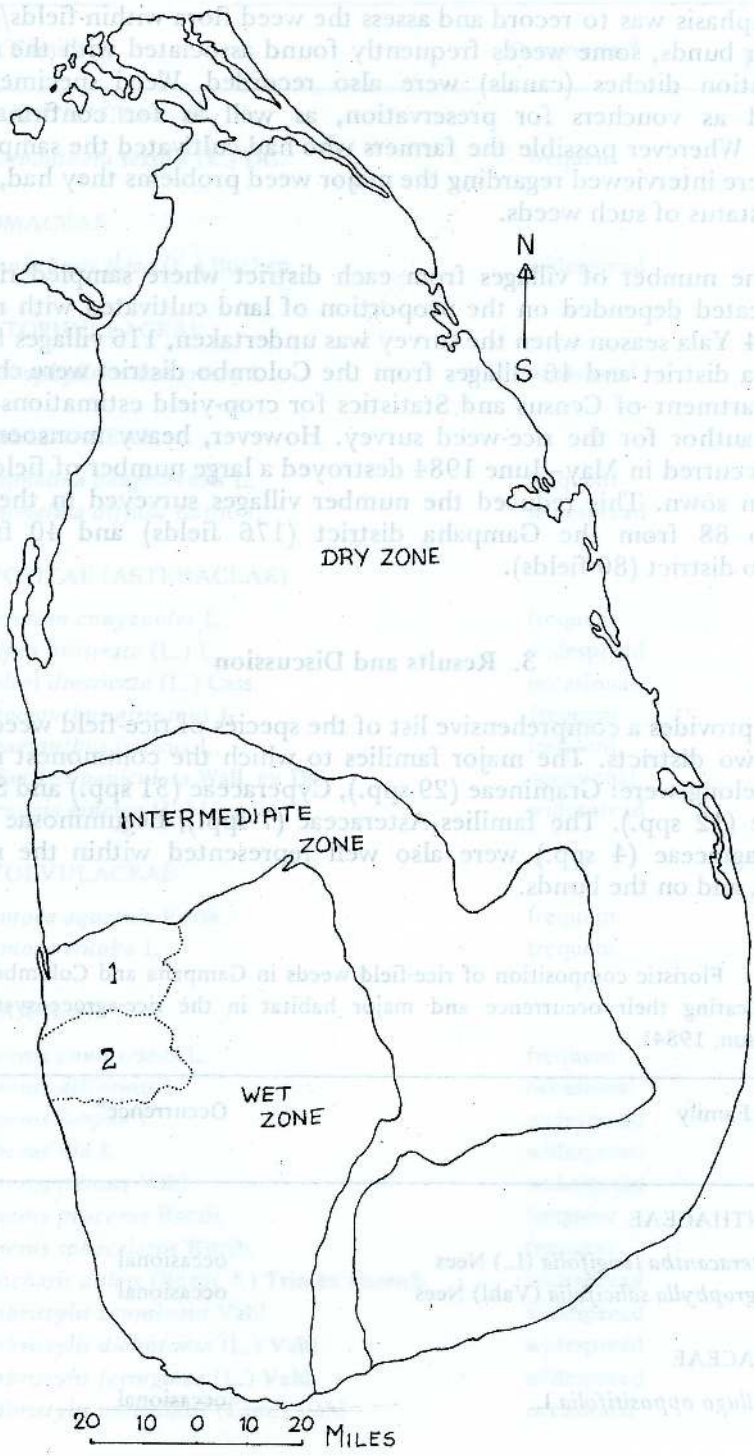

Figure 1. A Map of Sri Lanka indicating the Wet, Intermediate and Dry Zones and the Gampaha (1) and Colombo (2) districts.

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main emphasis was to record and assess the weed flora within fields/liyaddes and their bunds, some weeds frequently found associated with the network of irrigation ditches (canals) were also recorded. Weed specimens were collected as vouchers for preservation, as well as for confirmation of identity. Wherever possible the farmers who had cultivated the sampled rice-fields were interviewed regarding the major weed problems they had, and the present status of such weeds.

The number of villages from each district where sampled rice-fields were located depended on the proportion of land cultivated with rice. For the 1984 Yala season when the survey was undertaken, 116 villages from the Gampaha district and 46 villages from the Colombo district were chosen by the Department of Census and Statistics for crop-yield estimations, as well by the author for the rice-weed survey. However, heavy monsoonal rains which occurred in May–June 1984 destroyed a large number of fields which had been sown. This reduced the number villages surveyed in the present work to 88 from the Gampaha district (176 fields) and 40 from the Colombo district (80 fields).

3. Results and Discussion

Table 1 provides a comprehensive list of the species of rice-field weeds found in the two districts. The major families to which the commonest rice-field weeds belong were: Gramineae (29 spp.), Cyperaceae (31 spp.) and Scrophulariaceae (12 spp.). The families Asteraceae (7 spp.), Leguminosae (9 spp.) and Onagraceae (4 spp.) were also well represented within the rice-field habitats, and on the bunds.

Table 1. Floristic composition of rice-field weeds in Gampaha and Colombo districts, indicating their occurrence and major habitat in the rice-agroecosystem (Yala season, 1984).

Taxon & Family	Occurrence[a]	Major Habitat[b]
1. ACANTHACEAE		
Asteracantha longifolia (L.) Nees	occasional	B
Hygrophylla salicifolia (Vahl) Nees	occasional	B
2. AIZOACEAE		
Mollugo oppositifolia L.	occasional	B

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Taxon & Family	Occurrence[a]	Major Habitat[b]
3. AMARANTHACEAE		
Alternanthera sessilis (L.) DC.	frequent	B,F
4. BUTOMACEAE		
Limnocharis flava (L.) Buchen.	widespread	F,B,D
5. CERATOPHYLLACEAE		
Ceratophyllum demersum L.	occasional	F,B
6. COMMELINACEAE		
Commelina benghalensis L.	frequent	B
Commelina diffusa Burm. f.	widespread	B,F,D
7. COMPOSITAE (ASTERACEAE)		
Ageratum conyzoides L.	frequent	B
Eclipta prostrata (L.) L.	widespread	B,F
Epaltes divaricata (L.) Cass.	occasional	B
Sphaeranthus africanus L.	frequent	B,F
Sphaeranthus indicus L.	frequent	B,F
Spilanthes paniculata Wall. ex DC.	occasional	B
Vernonia cinerea (L.) Less	widespread	B,D
8. CONVOLVULACEAE		
Ipomoea aquatica Forsk.	frequent	B,D
Ipomoea triloba L.	frequent	B,F
9. CYPERACEAE		
Cyperus compressus L.	frequent	B
Cyperus difformis L.	occasional	F,B
Cyperus haspan L.	widespread	F,B,D
Cyperus iria L.	widespread	F,B,D
Cyperus pilosus Vahl	widespread	F,B,D
Cyperus procerus Rottb.	frequent	B
Cyperus sphacelatus Rottb.	frequent	B
Eleocharis dulcis (Burm. f.) Trin ex Hensch	widespread	F
Fimbristylis acuminata Vahl	widespread	B,F
Fimbristylis dichotoma (L.) Vahl	widespread	B,F
Fimbristylis ferruginea (L.) Vahl	widespread	B
Fimbristylis umbellaris (Lam.) Vahl	occasional	B,F

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Taxon & Family	Occurrence[a]	Major Habitat[b]
Fimbristylis miliacea (L.) Vahl	widespread	F,B,D
Fimbristylis schoenoides (Retz.) Vahl	widespread	B,F
Fimbristylis tetragona R. Br.	widespread	B,F,D
Fuirena ciliaris (L.) Roxb.	occasional	B,F
Fuirena umbellata Rottb.	widespread	F,B
Fuirena capitata (Burm.f.) T. Koyama	widespread	B,F
Kyllinga brevifolia Rottb.	widespread	B,F,D
Kyllinga melanosperma Nees	occasional	B,D
Kyllinga nemoralis (J.R. & G. Forst.) Dandy ex Hutchinson & Dalziel	occasional	B,D
Kyllinga bulbosa Rottb.	widespread	B
Mariscus paniceus (Rottb.) Vahl	occasional	B
Pycreus polystachyos (Rottb) Beauv.	frequent	B
Pycreus pumilus (L.) Nees	occasional	B
Rhynchospora corymbosa (L.) Britton	occasional	B,D,F
Schoenoplectus articulatus (L.) Palla	occasional	F
Schoenoplectus juncoides (Roxb.) Palla	widespread	F,B,D
Schoenoplectus grossus (L.f.) Palla	frequent	F,B,D
Scleria poaeformis Retz.	occasional	B,F
Scleria sumatrensis Retz.	occasional	B
10. ERIOCAULACEAE		
Eriocaulon quinquangulare L.	widespread	F
Eriocaulon sexangulare L.	widespread	F
Eriocaulon thwaitsii Koern.	frequent	F
11. EUPHORBIACEAE		
Euphorbia hirta L.	frequent	B
Phyllanthus debilis Klein ex Willd.	frequent	F,B
Phyllanthus urinaria L.	frequent	B
12. FABACEAE (LEGUMINOSAE)		
Aeschynomene aspera L.	frequent	F,B
Aeschynomene indica L.	frequnet	F,B
Alyssicarpus vaginalis DC.	frequent	B
Cassia tora L.	occasional	B
Desmodium heterocarpum (L.) DC.	frequent	B,D
Desmodium heterophyllum (Willd.) DC.	frequent	B,D
Desmodium triflorum (L.) DC.	frequent	B,D
Mimosa pudica L.	widespread	B
Tephrosia purpurea (L.) Pers.	occasional	B

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Taxon & Family	Occurrence[a]	Major Habitat[b]
13. GRAMINEAE (POACEAE)		
Alloteropsis cimicina (L.) Stapf	frequent	B
Axonopus affinis Chase	widespread	B,D
Axonopus compressus (Sw.) Beauv.	frequent	B,D
Coix gigantea Koen. ex Roxb.	occasional	F,B
Echinochloa colonum (L.) Link.	widespread	F,B,D
Echinochloa crus-galli (L.) Beauv.	widespread	F,B
Echinochloa frumentacea Link.	frequent	F,B
Echinochloa stagnina (Retz.) Beauv.	frequent	F,B
Eleusine indica (L.) Gaertn.	widespread	B,D
Eragrostis gangetica (Roxb.) Steud.	frequent	B,D
Eragrostis tenella (L.) Beauv. ex Roem & Schult	occasional	B
Eragrostis unioloides (Retz.) Nees ex Steud.	widespread	B,F
Isachne globosa (Thunb.) Kuntze	widespread	F,B,D
Isachne miliacea Roth ex Roem. & Schult	occasional	B
Ischaemum indicum (Houtt.) Merr.	frequent	B
Ischaemum muticum L.	occasional	B,D
Ischaemum rugosum Salisb.	widespread	F,B
Leersia hexandra Sw.	frequent	B,D,F
Leptochloa chinensis (L.) Nees	frequent	B,D
Oryza perennis Moench	occasional	F
Oryza rufipogon Griff.	frequent	F,B
Panicum brevifolium L.	occasional	B,F
Panicum repens (L.) beauv.	widespread	B,F,D
Paspalum commersonii Lam.	widespread	B,F
Paspalum conjugatum Berg.	widespread	B
Sacciolepis indica (L.) Chase	widespread	B,F
Sacciolepis interrupta (Willd.) Stapf	widespread	F,B
Setaria geneculata (Lam.) Beauv.	frequent	B,F
Sporobolus diandrus (Retz.) Beauv.	frequent	B
14. HYDROCHARITACEAE		
Blyxa octandra (Roxb.) Planch. ex Thw.	occasional	D,F
Ottelia alismoides (L.) Pers.	occasional	D,F
15. HYDROPHYLLACEAE		
Hydrolea zeylanica (L.) Vahl	occasional	F

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Taxon & Family	Occurrence[a]	Major Habitat[b]
16. LAMIACEAE (LABIATAE)		
Hyptis capitata Jacq.	occasional	B
Hyptis suaveolens (L.) Poit.	frequent	B
Leucas zeylanica (L.) R. Br.	widespread	B
17. LENTIBULARIACEAE		
Utricularia bifida L.	frequent	F
Utricularia reticulata Sm.	frequent	F
18. LOBELIACEAE		
Lobelia alsinoides Lam.	occasional	F
19. LYTHRACEAE		
Rotala densiflora (Roth ex Roem. & Schult.) Koehne	frequent	F
20. MARSILIACEAE		
Marsilea quadrifolia L.	frequent	F,D
21. ONAGRACEAE		
Ludwigia adscendens (L.) Hara	occasional	F,D
Ludwigia spp. (decurrens Walt.?)	widespread	F,B
Ludwigia hyssopifolia (G.Don) Exell	widespread	F,B
Ludwigia perennis L.	occasional	F,B
22. POLYGONACEAE		
Polygonum barbatum L.	frequent	B,D
Polygonum glabrum Willd.	occasional	B,D
23. PONTEDERIACEAE		
Monochoria vaginalis (Burm. f.) Kunth	widespread	F,D,B
24. RUBIACEAE		
Hedyotis auricularia L.	widespread	B
Knoxia zeylanica L.	frequent	B
Mitracarpus hirtus (L.) DC.	frequent	B
Oldenlandia corymbosa L.	frequent	B
Spermacoce hispida L.	widespread	B
Spermacoce latifolia Aubl.	widespread	B,F

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Taxon & Family	Occurrence[a]	Major Habitat[b]
25. SCROPHULARIACEAE		
Bacopa monnieri (L.) Pennell	frequent	F,B
Dopatrium junceum (Roxb.) Buch-Ham. ex Benth.	widespread	F
Dopatrium lobelioides (Retz.) Benth.	widespread	F
Limnophila aquatica (Roxb.) Alston	frequent	F,D
Limnophila aromatica (Lam.) Merr.	occasional	F,D
Limnophila repens (Benth.) Benth.	widespread	F,B,D
Lindernia anagallis (Burm.f.) Pennell	widespread	F,B
Lindernia antipoda (L.) Alston	frequent	F,B
Lindernia hyssopioides (L.) Haines	widespread	F,B,D
Lindernia pusilla (Willd.) Boldingh.	frequent	B
Lindernia rotundifolia (L.) Alston	widespread	F,B,D
Scoparia dulcis L.	widespread	B
26. SALVINIACEAE		
Azolla pinnata Lam.	occasional	F,D
Salvinia molesta D.S. Mitchell	widespread	F,D
27. STERCULIACEAE		
Melochia corchorifolia L.	widespread	B,F
28. TILIACEAE		
Corchorus olitorius L.	frequent	B,F
Triumfetta rhomboidea Jacq.	frequent	B
29. XYRIDACEAE		
Xyris indica L.	frequent	F,D

[a]widespread = most common and widely distributed; frequent = less common, yet frequently found also with wide distribution; occasional = least common, patchy in appearence.

[b]B = Bund; D = Ditch (irrigation canal); F = Field

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3.1 Monocotyledonous Weeds

Table 2 gives the occurrence of the commonest monocotyledonous species in the two districts and their levels of infestation. Species have been listed in the order of abundance. In general, the relative abundance of the major weed species found in the two districts was not dissimilar.

In both districts *Fimbristylis miliacea* was the most frequently recorded monocotyledonous weed, found growing in 96.5% of fields sampled in Gampaha and in 97.5% of fields in Colombo. Where it occurred, 38.7% of its infestations were at the level of 3 or above, indicating its seriousness as a weed. However, this major weed completed its life-cycle within the first 3–4 months and often before the rice crop matured. Hence, by the time of harvesting the rice, this weed may not be very conspicuous, as it would be earlier on in the growing season.

Isachne globosa was the second most frequent weed. In the Colombo district its occurrence was as high as that of *F. miliacea,* but in Gampaha it occurred at a lower frequency. However, this grass-weed notably had a greater proportion of the heaviest infestations with scores of 3 or above being given to 48.6% of fields where it occurred.

Two species of the sedge genus *Cyperus,* namely *Cyperus iria* L. and *Cyperus pilosus* Vahl, were the third and fourth ranking monocotyledonous weeds in the two districts, *Cyperus iria* occurred in 82% of fields sampled in Gampaha and 88% of fields in Colombo, while the figures for *C. pilosus* were 70% and 98% respectively for the two districts. Like *F. miliacea, C. iria* was also found most conspicuously in the early part of the growing season. This weed too completed its life-cycle usually before the rice crop matured, and was not prominent at later stages. *Cyperus pilosus*, on the other hand, was found to persist longer and also to display noticeable variation with respect to dimensions of leaves, size and colour of inflorescence, etc., as noted by Koyama.[5]

Echinochloa colonum (L.) Link. and *Echinochloa crus-galli* (L.) Beauv. were both found to be major weeds in the rice-fields surveyed. The former occurred in 68% of fields sampled in Gampaha and 56% of fields in Colombo. *Echinochloa crus-galli* was recorded from 63% of fields in Gampaha and in 49% of fields in the Colombo district. Infestations of both weeds were moderate to heavy in the two districts (Table 2).

Monochoria vaginalis (Burm. f.) Kunth occurred in 64% and 59% of fields in Gampaha and Colombo districts, respectively. Although these infestations were less severe than those of the above, (scores of 1 and 2 in 90% of fields where it occurred), *M. vaginalis* too appeared to be a major weed in

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Table 2. The Relative abundance at five levels of the most frequent monocotyledonous weeds in 256 rice-fields of the Gampaha and Colombo districts (1984)

Taxon	Relative abundance						Total fields infested	Frequency of occurrence	Frequency of occurrence	
	0	1	2	3	4	5			in Gampaha	in Colombo
1. *Fimbristylis miliacea*	8	13	139	82	14	–	248	96.9	96.5	97.5
2. *Isachne globosa*	38	21	91	67	35	4	218	85.2	79.5	97.5
3. *Cyperus iria*	40	58	109	38	11	–	216	84.4	82.3	88.7
4. *Cyperus pilosus*	56	57	107	33	3	–	200	78.1	69.9	97.5
5. *Echinochloa colonum*	90	43	95	21	7	–	166	64.8	68.2	56.3
6. *Monochoria vaginalis*	95	52	94	15	–	–	161	62.9	64.7	58.8
7. *Echinochloa crus-galli*	106	25	74	38	13	–	150	58.6	63.1	48.8
8. *Cyperus haspan*	117	47	76	16	–	–	139	54.3	54.0	55.0
9. *Ischaemum rugosum*	119	24	54	37	20	2	137	53.5	47.7	66.3
10. *Commelina diffusa*	127	71	53	5	–	–	129	50.4	47.7	56.3
11. *Eriocaulon sexangulare*	134	30	76	16	–	–	122	47.7	50.0	42.5
12. *Scirpus juncoides*	177	7	59	13	–	–	79	30.9	28.4	36.3
13. *Limnocharis flava*	190	15	40	11	–	–	66	25.8	19.3	40.0
14. *Fimbristylis tetragona*	216	4	28	8	–	–	40	15.6	9.1	30.0

0 = weed species not observed; 1 = a few plants; 2 = occasional patches; 3 = weed widespread throughout field; 4 = dense and severe infestation; 5 = weed completely dominant and masking crop.

Gampaha district – out of 176 fields sampled.
Colombo district – out of 80 fields sampled.

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both districts, particularly in the fields fed by irrigation waters.

Cyperus haspan, a species recorded earlier[10] to be one of the first three major weeds in an all-island survey, occurred only in 54% of fields sampled in the two districts. Its occurrence in the two districts was not much dissimilar, and their infestations were also relatively less severe (Table 2).

The occurrence of *Ischaemum rugosum* Salisb. in the rice-fields is worthy of special mention. Although this grass weed was the 9th most frequent when both districts were considered, its position in the Colombo district was found to be of a higher ranking. It occurred in 66% of sampled fields in Colombo and was ranked fifth in order of abundance. In Gampaha it occurred in 47% of fields. In both districts the infestations were fairly heavy. Infestation levels of 3, 4 or 5 were recorded from 64% of fields where it occurred in Colombo, while in Gampaha such heavy infestations were found in 30% of fields infested with the weed (data not presented). The farmer interviews revealed that *I. rugosum* is a species that appears to be rapidly becoming a major threat to rice cultivation. According to most farmers this weed has already achieved a greater prominence than *Cyperus, Isachne, Fimbristylis* or *Echinochloa* species in terms of a need for weed control. Many expressed the view that *I. rugosum* was not considered so important 10–20 years ago, suggesting that its present abundance may have been achieved over a relatively short period of time.

Among other monocotyledonous species found in the rice-fields of the two districts, the following also occurred at fairly high frequency: *Commelina diffusa* Burm. f., *Eriocaulon sexangulare* L., *Scirpus juncoides* Roxb., *Limnocharis flava* (L.) Buchen., *Fimbristylis tetragona* R.Br., *Fimbristylis dichotoma* (L.) Vahl, *Eleocharis dulcis* (Burm.f.) Trin. ex Hensch., *Paspalum commersonii* Link., *Sacciolepis indica* (L.) Chase and *Kyllinga brevifolia* Rottb.

3.2 Dicotyledonous Weeds

The occurrence of the most frequent dicotyledonous weeds in the rice-fields sampled and their levels of infestation are given in Table 3. Fifty-two dicotyledonous weed species were recorded from the rice-fields in the present survey. However, only a few species were recorded as of high infestation levels and abundance.

Ludwigia hyssopifolia (G.Don) Exell was by far the most frequently recorded dicotyledonous weed. Its occurrence in the two districts was very similar. All fields sampled in the Colombo district (100%) and 92% of fields in the Gampaha district had this weed. Infestations of *L. hyssopifolia* were also found to be heavy with scores of 3 or higher recorded for 37% of fields

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Table 3. The Relative abundance at five levels of the most frequent dicotyledonous weeds in 256 rice fields of the Gampaha and Colombo districts (1984)

Taxon	Relative abundance						Total fields infested	Frequency of occurrence	Frequency of occurrence	
	0	1	2	3	4	5			in Gampaha	in Colombo
1. *Ludwigia hyssopifolia*	13	35	119	77	12	–	243	94.9	92.6	100.00
2. *Lindernia anagallis*	58	41	119	38	–	–	198	77.3	78.4	75.0
3. *Lindernia hyssopioides*	64	40	110	42	–	–	192	75.0	81.3	61.3
4. *Lindernia rotundifolia*	71	42	105	38	–	–	185	72.3	79.1	59.4
5. *Dopatrium lobelioides*	141	38	70	7	–	–	115	44.9	42.5	39.1
6. *Eclipta prostrata*	147	53	55	1	–	–	109	42.6	45.5	36.3
7. *Ludwigia decurrens*	172	14	39	22	9		84	32.8	10.2	82.5

0 = weed species not observed; 1 = a few plants; 2 = occasional patches; 3 = weed widespread throughout field; 4 = a dense and severe infestation; 5 = weed completely dominant and masking crop.

Gampaha District – out of 176 fields sampled.
Colombo District – out of 80 fields sampled.

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in which it was recorded from both districts. A second species of *Ludwigia,* tentatively identified as *Ludwigia decurrens* Walt.*, a species which had not been previously recorded from Sri Lanka,[3] was also found to occur very commonly, particularly in the Colombo district. It was recorded from the Gampaha district in only about 10% of fields sampled, thus indicating a major difference in the geographical distribution of the weed. Where it occurred in the Colombo district, infestation levels were high with scores of 3 or higher being recorded for 45% of the sampled fields (data not presented).

Three species of *Lindernia,* namely *Lindernia anagallis* (Burm. f.) Pennell, *Lindernia hyssopioides* (l.) Haines and *Lindernia rotundifolia* Alston, were found at high frequency in both districts. However, these infestations were in general less severe than those of the *Ludwigia hyssopifolia* (Table 3).

Other common dicotyledonous weeds found growing in the rice-fields of the two districts included the following: *Dopatrium lobelioides* (Retz.) Benth., *Dopatrium junceum* (Roxb.) Ham., *Eclipta prostrata* (L.) L., *Limnophila repens* (Benth.) *Benth., Limnophila aquatica* (Roxb.) Alston, *Lindernia pusilla* (Willd.) Boldingh., and *Utricularia reticulata* Smith.

The distribution of the most frequent dicotyledonous weeds in the two districts was found to be wide and generally uniform, except in the case of the second species of *Ludwigia* stated earlier. In the Colombo district this weed was found to be regarded as troublesome, but it was clear from the farmer interviews that they made very little distinction between the unidentified* *Ludwigia* spp. and the more widespread *L. hyssopifolia.* The farmer interviews also revealed that apart from the *Ludwigia* species, dicotyledonous weeds cause very little concern to them.

3.3 Weed Flora of Rice-field bunds

The weeds found on the rice-field bunds were mainly a fair number of the semi-aquatic species recorded from within the fields and other species which were more terrestrial in nature (Table 4).

The commonest monocotyledonous weeds found on the bunds were: *Isachne globosa, Eragrostis unioloides* (Retz.) Nees ex Steud, *Fimbristylis miliacea, Cyperus pilosus, Panicum repens* L., *Commelina diffusa, Paspalum commersonii* and *Cyperus iria.*

The occurrence of *E. unioloides* and *P. repens* was limited to the bund habitat only and their infestations were heavy with scores of 3 or above

*This plant species is in the process of being recorded by the author as a new species for Sri Lanka, in a later issue of the J. Natn. Sci. Coun. Sri Lanka.

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Table 4. The Relative abundance at five levels of the most frequent monocotyledonous and dicotyledonous weeds on the bunds of 256 rice-fields in the Gampaha and Colombo districts (1984)

Taxon	Relative abundance						Total fields infested	Frequency of occurrence	Frequency of occurrence	
	0	1	2	3	4	5			in Gampaha	in Colombo
1. *Isachne globosa*	2	9	77	94	74	–	254	98.8	98.6	100.00
2. *Eragrostis unioloides*	7	17	86	101	45	–	249	97.3	95.5	100.00
3. *Ludwigia hyssopifolia*	19	20	167	45	5	–	237	92.6	89.2	100.00
4. *Fimbristylis miliacea*	29	28	137	56	6	–	227	88.7	87.0	92.6
5. *Cyperus pilosus*	35	52	149	20	–	–	221	86.3	81.9	96.1
6. *Panicum repens*	59	33	73	59	32	–	197	76.9	76.7	8 80.1
7. *Commelina diffusa*	62	13	99	77	5	–	194	75.8	84.7	56.3
8. *Paspalum commersonii*	79	21	115	40	1	–	177	69.1	70.6	65.0
9. *Cyperus iria*	86	64	94	11	1	–	170	66.4	63.7	73.8
10. *Echinochloa colonum*	97	62	63	34	–	–	159	58.2	73.8	36.2
11. *Lindernia anagallis*	133	56	62	5	–	–	123	48.1	36.4	73.8
12. *Spermacoce latifolia*	135	19	78	24	–	–	121	47.3	48.9	43.8
13. *Lindernia rotundifolia*	136	37	64	19	–	–	120	47.1	43.5	49.2
14. *Eclipta prostrata*	141	58	49	8	–	–	115	44.9	42.6	50.0
15. *Sacciolepis indica*	144	22	79	11	–	–	112	43.7	30.1	73.8
16. *Kyllinga brevifolia*	146	19	79	12	–	–	110	42.9	43.8	41.3
17. *Scoparia dulcis*	160	32	60	4	–	–	96	37.5	42.1	26.3
18. *Pycreus polystachyos*	173	36	44	3	–	–	83	32.5	34.1	28.8
19. *Azonopus affinis*	179	5	44	23	5	–	77	30.1	29.8	31.3

0 = weed species not observed; 1 = a few plants; 2 = occasional patches; 3 = weed widespread throughout field; 4 = a dense and severe infestation; 5 = weed completely dominant and masking crop.

Gampaha District – out of 176 fields sampled
Colombo District – out of 80 fields sampled

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recorded for 58.6% and 46.2% of fields where they occurred, respectively. *Isachne globosa* was the most frequent weed found on bunds in both districts (Table 4) and its infestations were usually heavy with scores of 3 or higher being given to 65.6% of fields in which the weed occurred in both districts. Other monocotyledonous species found at high frequency on the bunds included: *Echinochloa colonum, Ischaemum rugosum, Ischaemum muticum* L. *(Schaemum indicum* Houtt.) Merr., *Sacciolepis indica, Kyllinga brevifolia, Pycreus polystachyos* (Rottb.) Beauv. and *Axonopus affinis* Chase.

Several dicotyledonous semi-aquatics were also commonly found as potential weeds on the bunds. *Ludwigia hyssopifolia* was the major dicotyledonous weed found growing on bunds, and was recorded from 92.6% of sampled fields. Infestations of *L. hyssopifolia* on bunds were usually heavy with scores of 3 or higher being given to 21% of fields in which it occurred. *Lindernia* spp., *Eclipta prostrata, Scoparia dulcis* L., *Alternanthera sessilis* (L.) DC., *Leucas zeylanica* (L.) R.Br., *Hedyotis auricularia* L. and *Sphaerathus indicus* L. were common dicotyledonous semi-aquatic weeds found on the bunds.

In addition, many cosmopoliton and terrestrial weeds were also recorded from the bund habitat. These appeared to have spread to the rice-fields from nearby habitats, roadsides and home-gardens. Such species included: *Ageratum conyzoides L., Vernonia cinerea* (L.) Less., *Euphorbia hirta* L., *Desmodium heterocarpum* (L.) DC., *Desmodium triflorum* (L.) DC., *Desmodium heterophyllum* (Willd.) DC., *Alyssicarpus vaginalis* DC., *Mitracarpus hirtus* (L.) DC., *Spermacoce latifolia* Aubl., *Knoxia zeylanica* L., *Hyptis suaveolens* (L.) Poit, *Melochia corchorifolia* L. and *Triumfetta rhomboidea* Jacq.

4. Conclusions

It was evident from the survey data that monocotyledonous weeds constitute the major rice-field weeds in the two districts studied. Presently they appear to be the most frequent and widespread. *Fimbristylis miliacea, Isachne globosa* and *Cyperus iria* were the most abundant rice-field weeds both in terms of frequency of occurrence and levels of infestation. *Ludwigia hyssopifolia* emerged as the biggest dicotyledonous weed problem which plagues rice-fields in the two districts studied. A second species of *Ludwigia* whose identity is yet to be confirmed, was found to occur at high frequency mainly in the Colombo district and spreading rapidly, threatening to become a major weed soon. Many other dicotyledonous weeds occurred in the sampled rice-fields, but these did not pose a major threat to rice culture, requiring control measures. In general, a very rich weed flora was found in

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the fields studied, occurring within the field habitat and on the raised earthen bunds, composed mainly of semi-aquatic and terrestrial plant species.

Acknowledgements

The author is grateful to the Natural Resources, Energy and Science Authority (NARESA) of Sri Lanka for a research grant (RG/84/B/8) which enabled the present study to be carried out, and to Professors I. Balasuriya, S. Balasubramaniam and R.N. de Fonseka for their encouragement and support.

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Journal of the
National Science Council
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Published by
The Natural Resources, Energy and Science Authority of Sri Lanka (NARESA) and
Printed at NARESA Printing Unit, 47/5, Maitland Place, Colombo 7.

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