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1. INTRODUCTION

Pyrethrum flowers are grown commercially in Enga and Western Highlands and represent the second largest cash crop in the former Province. In 1983, 279 tonnes of flowers were processed at the Kagamuga Natural Products factory, thus generating an income of approximately K1,500, for the village growers. In 1984 274.6 tonnes, value K343,248.00 Enga alone, 1985 323.9 tonnes, value K375,000.00 Enga alone. Overall factory intake was 352 tonnes in 1985, thus Enga is producing 92% of total.

The insecticidally active ingredients in pyrethrum flowers are the pyrethrins (two groups of compounds referred to as pyrethrins I and pyrethrins II). The price obtained for the pyrethrum extract is dependent on, among other things, the pyrethrins content of the flowers and the ratio, pyrethrins I/II. This ratio is important, as for maximum biological activity the two groups of pyrethrins should be present in approximately equal proportions.

Research to select suitable planting material for distribution to farmers commenced at Aiyura in the late 1950's. The work was transfered to the high altitude station at Tambul, but is now carried out at the Sirunki base camp in Enga Province. The material grown at Sirunki consists of thirty clones for trial purposes and some polycross plants for multiplication and distribution. The material on trial comes from a number of sources. The oldest clones, designated AP 50 and AP 78/65 originate from the Aiyura trials. The bulk of the trial material originated from selections made at Aiyura but recorded at Tambul, and all have the prefix TP. The prefix K denotes material from Southern Highlands. Clone Ten 1 also originates from this Province.

The aim of the trial work is to identify superior clones which will then be used as parent material in the production of more polycross seed. Plants grown from this seed should have wide genetic variation but should also combine high flower yield with high pyrethrins content.

Once this material is in production, selections will be made of vigorously growing, early flowering and high yielding plants. Plants with high pyrethrins content will be cloned then subjected to further testing to eventually form the parents for a new polycross generation. In this way, it should be possible to gradually increase the quality of planting material available to farmers, leading to better returns and greater profitability of the industry as a whole.

In early 1985, the Cash Crops Support Services Unit of Enga Yaaka Lasemana assumed reponsibility fr the Sirunki pyrethrum research. The Unit commenced recording yield levels of the trial clones and samples were dispatched to the Agricultural Chemistry Section at Konedobu for determination of pyrethrin levels in the flowers.

At the same time, samples were taken from Enga gardens in an altitude range of 2,440 - 2,800m to provide data on the levels of pyrethrins in the gardens. This information may then be used to identify priority areas for replanting.

This report presents data obtained from the chemical analysis of pyrethrum from the Sirunki clonal trials and Enga gardens.

2. MATERIALS AND METHODS

2.1 Reference Material and Reagents

(a) A sample of crude pyrethrum extract (reference 3/85) was obtained from Kagamuga Natural Products Pty, Ltd., for use as a reference. A similar sample of this extract was analysed using the AOAC method (9th Ed.) by Salamon and Seabar Ltd., London, England. The pyrethrins content of this sample was found to be

Pyrethrins I : 15.2% w/w

Pyrethrins II: 16.6% w/w

Freshly prepared hexane solutions of the reference material were used for determination of the pyrethrins content of the flowers by high performance liquid chromatography (HPLC).

(b) Hexane and dichloromethane were both HPLC (Hi Per Solv) grade and were supplied by BDH Chemicals, Poole, England.

(c) Water was double distilled in glass.

2.2 Apparatus

(a) High performance liquid chromatograph:-

Model 510 solvent delivery system,

U6K injector,

481 variable wavelength spectrophotometer,

u-Porasil column (30cm).

BBC SE120 millivolt recorder.,

0.45 um solvent filtration apparatus.

Supplied by Waters Associates, Sydney, Australia.

(b) Volumetric flasks; 50mL and 100mL.

(c) Wiley mill with 30 mesh screen.

(d) Oven capable of maintaining a temperature of 60 $\pm$ 1°C, Blue M, Illinois, U.S.A.

2.3 Analytical Procedure

Flower samples were despatched to the laboratory after harvest and were received within 36 hours. Samples were transfered to a forced draught oven and dried at 60°C until "crisp" to the touch, at which stage they could be milled satisfactorily. The drying process was completed within 48 hours.

After milling, samples were mixed thoroughly and duplicate portions (1.00g) weighed into 50mL volumetric flasks. Each flask was adjusted to volume with hexane and the pyrethrins extracted, by shaking the flasks for a few seconds every 15 minutes, for at least 90 minutes. The flower particles were allowed to settle prior to analysis by HPLC. Samples were refrigated overnight if necessary.

Liquid chromatography was used to separate the pyrethrin esters in sample extracts prior to their determination by absorbance of light at 254nm (Otieno, et al. 1983). In this way, pyrethrins (Py) I and II could both be quantified for calculation of the Py I/Py II ratio. Conditions used for the HPLC determination were as follows:-

Mobile Phase : 1:1, anhydrous dichloromethane/water-saturated dichloromethane, at 2.ØmL/min.

Spectrophotometer: 254nm, Ø.1Ø Absorbance Units Full Scale (AUFS).

Injection volume : 1Ø.Ø uL.

Using these conditions, retention volumes for Py I and Py II were 4.2 and 11.ØmL respectively, relative to the solvent front.

Recorder responses (peak heights) obtained for Py I and Py II in sample extracts were compared with those of a standard solution prepared from crude extract 3/85. This standard solution was prepared by weighing accurately 1ØØmg of extract into a 1ØØmL volumetric flask and adjusting to volume with hexane. The percentage concentrations of pyrethrins in the flowers were calculated as follows:-

$$\%\text{Py I in flowers} = \frac{*\ P1 \times C \times V \times 1ØØ}{P2 \times W}$$

Where P1 = Sample peak height (sum of Py I peaks), (cm),

P2 = Standard peak height (as above), (cm),

C = Concentration of Py I in standard (mg/mL)

V = Volume of sample extract (mL)

W = Weight of sample taken (mg)

* Similarly, for Py II.

Moisture content was determined as the weight loss on drying portions (5.Øg) of ground flowers overnight in a forced draught oven at 1Ø5°C.

3. RESULTS AND DISCUSSION

3.1 Sirunki Clones

The thirty pyrethrum clone plots at Sirunki, Enga Province were harvested on 3/7/85 and 29/10/85. The fresh flowers were dispatched on the same day as harvest to Chemistry Section, Konedobu for drying and chemical assay of pyrethrins I and II.

The pyrethrin contents of each clone at each harvest and the ratio Py I/Py II are presented in Table I.

Table 1. Pyrethrin Levels: Sirunki Clonal Trials

% Pyrethrins, w/w expressed on a moisture-free basis for samples Collected in July and October 1985.

Clone	Py I		Py II		Total Py		Py I/Py II Ratio	
	JULY	OCTOBER	JULY	OCTOBER	JULY	OCTOBER	JULY	OCTOBER
AP50	0.71	0.64	0.78	0.94	1.49	1.58	0.91	0.68
AP78/65	0.41	1.08	0.50	0.65	0.91	1.73	0.82	1.66
TEN1	1.01	0.08	1.11	0.92	2.12	1.72	0.91	0.87
F1	0.47	0.35	0.82	0.56	1.29	0.91	0.57	0.62
K1	1.11	0.90	1.20	1.04	2.31	1.94	0.95	0.86
K4	1.18	0.94	0.91	0.75	2.09	1.69	1.30	1.25
K15	0.64	0.62	0.91	0.85	1.55	1.47	0.70	0.73
K17	0.71	0.82	0.67	0.90	1.38	1.72	1.06	0.91
K29	1.17	1.21	0.70	0.92	1.87	2.13	1.67	1.31
K45	0.88	0.65	0.93	0.97	1.81	1.62	0.95	0.67
K57	1.17	0.89	0.54	0.45	1.71	1.34	2.17	1.98
K68	1.09	0.36	0.64	0.52	1.73	0.88	1.70	0.69
TP1	1.36	1.06	0.80	0.65	2.06	1.71	1.70	1.63
TP2	1.02	0.86	0.58	0.72	1.60	1.58	1.76	1.19
TP3	1.12	0.98	0.90	0.74	2.02	1.72	1.24	1.32
TP4	0.39	0.40	1.22	1.74	1.61	2.14	0.32	0.23
TP5	0.55	0.35	0.91	0.63	1.46	0.98	0.60	0.56
TP6	0.91	0.67	1.19	0.81	2.10	1.48	0.76	0.83
TP10	0.63	0.36	0.89	0.60	1.52	0.96	0.71	0.60
TP11	0.69	0.59	0.74	0.59	1.43	1.08	0.93	1.00
TP12	1.05	0.98	1.36	1.22	2.41	2.20	0.77	0.80
TP13	1.27	1.23	0.66	0.69	1.93	1.92	1.92	1.78
TP14	0.87	0.77	0.81	0.72	1.68	1.49	1.07	1.07
TP15	0.86	0.66	0.89	0.63	1.75	1.29	0.97	1.05
TP17	1.25	1.02	0.89	0.75	2.14	1.77	1.40	1.36
TP23	1.20	0.95	0.87	0.84	2.07	1.79	1.38	1.13
TP25	1.16	1.06	1.09	1.08	2.25	2.14	1.06	1.36
TP26	0.95	0.78	0.98	0.91	1.93	1.69	0.97	0.85
TP27	0.69	0.69	1.09	1.31	1.78	2.00	0.63	0.53
TP28	0.50	0.43	1.09	0.87	1.59	1.30	0.46	0.49

3.2 Enga Gardens

Pyrethrum samples were taken from 46 gardens in Enga Province during the period 1st August - 1st October 1985. Fresh flowers were dispatched to Chemistry Section for drying and chemical assay for pyrethrins I and II.

Results for % pyrethrins and PyI/PyII ratios are shown in Table 2.

Table 2. Pyrethrin Levels: Enga Pyrethrum Gardens

Lab. No.	Garden No.	Village	Approx Altitude,M	% Pyrethrins, w/w * Py I	PyII	Total Py	PyI/PyII Ratio
85/2123	1	Lakolam	2440	0.70	0.94	1.64	0.74
2124	2	"	2440	0.70	0.68	1.38	1.03
2125	3	"	2480	0.72	1.00	1.72	0.72
2126	4	"	2520	0.66	0.88	1.54	0.75
2127	5	Aipanda	2560	0.88	0.72	1.60	1.22
2128	6	Tambitanis	2480	0.74	0.67	1.41	1.10
2129	7	"	2480	0.64	0.74	1.38	0.86
2130	8	"	2480	0.51	0.84	0.35	0.61
2131	9	"	2480	0.56	0.75	1.31	0.75
2132	10	"	2520	0.93	0.92	1.85	1.01
2133	11	"	2520	0.56	0.65	1.21	0.86
2134	12	"	2600	0.60	0.81	1.41	0.74
2140	13	Sirunki	2660	0.79	0.80	1.59	0.99
2141	14	"	2660	0.97	1.01	1.98	0.96
2142	15	"	2600	0.98	0.70	1.68	0.71
2143	16	"	2640	0.64	0.77	1.41	0.83
2144	17	"	2680	0.50	0.93	1.43	0.54
2145	18	"	2680	0.60	0.82	1.42	0.73
2146	19	"	2640	0.72	0.97	1.69	0.74
2147	20	"	2640	0.58	0.81	1.39	0.72
2148	21	"	2640	0.62	1.16	1.78	0.53
2149	22	"	2640	0.77	0.53	1.30	1.45
2150	23	"	2640	0.70	1.05	1.75	0.67
2151	24	"	2680	0.80	0.70	1.50	1.14
2153	25	Kaipal	2600	0.97	0.92	1.89	1.05
2154	26	"	2600	0.74	0.77	1.51	0.96
2155	27	"	2600	1.06	0.88	1.94	1.20
2156	28	"	2600	1.07	0.43	1.50	2.49
2156	29	"	2640	0.76	1.13	1.89	0.67
2158	30	Yamala	2640	0.92	0.87	1.79	1.06
2159	31	"	2660	0.76	0.81	1.57	0.94
2160	32	"	2680	0.83	0.83	1.66	1.00
2161	33	"	2700	0.95	0.76	1.71	1.25
2162	34	"	2700	1.10	1.08	2.18	1.02
2163	35	"	2700	0.95	0.98	1.93	0.97
2327	36	Kalan	2600	0.93	1.07	2.00	0.87
2328	37	"	2640	0.90	1.03	1.93	0.87
2329	38	"	2700	0.48	0.96	1.44	0.50
2330	39	"	2800	0.37	0.95	1.32	0.39
2331	40	"	2800	0.38	1.05	1.43	0.36
2332	41	"	2800	0.59	1.00	1.59	0.59
2333	42	Yokonda	2700	0.93	0.79	1.72	1.18
2334	43	"	2700	0.61	0.82	1.43	0.74
2335	44	"	2680	0.81	0.83	1.64	0.98
2336	45	Waiep	2680	0.63	0.80	1.43	0.79
2337	46	"	2680	0.72	0.89	1.61	0.81

Sampling Dates

Gardens	
1 - 12	1/8/85
13 - 24	12/8/85
25 - 36	19/8/85
36 - 46	1/10/85

* Expressed on a moisture-free basis.

Total pyrethrins content ranged from 1.21 to 2.18%, w/w (mean 1.60%) and Py I/Py II ratios varying from 0.36 to 2.49 (mean 0.89) were recorded. Pyrethrum flowers from Kenga and other sources contained total pyrethrins in the range 0.63 - 2.18%, w/w. Py I/Py II ratios were 0.65 to 2.42. Of the 46 Enga gardens sampled, only 13 bore flowers containing above average levels of pyrethrins and with a Py I/Py II ratio within the desirable biological activity limits of 0.8 - 1.2. However, as a whole, the gardens gave a satisfactory mean ratio of 0.89.

Table 3 shows the mean pyrethrins content of flowers grown at various altitudes with % total Py reaching a maximum in the 2,600 - 2,749m range and then declining at higher altitudes. 70 percent of the gardens sampled fell within this altitude range.

Table 3. Change in Pyrethrins Content with Altitude.

Altitude Range, m	Total Py, % w/w	No. in Group
2,400 - 2,449	1.51	2
2,450 - 2,499	1.43	5
2,500 - 2,549	1.53	3
2,550 - 2,599	1.60	1
2,600 - 2,649	1.68	16
2,650 - 2,699	1.58	10
2,700 - 2,749	1.74	6
2,750 - 2,799	-	0
2,800 - 2,849	1.45	3

CONCLUSION

On the basis of the analyses outlined above, plus other available date, a number of steps were taken in Enga to improve flower quality with regard to pyrethrin content. In conjuction with data on yield levels of individual clones, a number of clones were selected as ideal parent stock for a new generation of plants. Clones selected were as follows:-

TP12	Excellent Py content, but low yield.
K1	Excellent Py content, but low yield
TP25	Good Py content, but low yield.
TP1	Good Py content, but low yield.
TP17	Good Py content, but very good yield.
TP23	Good Py content, but good yield.
K29	Moderate Py content, but very good yield.

These clones were interplanted in a seed garden at Sirunki on 20th February 1986. It is anticipated that the polycross seed collected from these parents will give rise to progency continuing high average Pyrethrum content with a good Py I/Py II ratio, and an above average yield of flowers. Providing the pyrethrin and yield levels are found to be satisfactory these plants will be multiplied and distributed to growers.

Visits will also be made to those village gardens from which flowers of high chemical content were obtained. It may be possible to select a number of good clones from these gardens for future yield and pyrethrum assessment. Superior clones, if out-performing existing ones at Sirunki, could then be used as parents for a future generation of polycross progeny.

By these methods, it is hoped to improve the quality of material grown by producers, leading to a more profitable industry, to the benefit of growers, factory, and the national economy.

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