Current resistance status of the house fly, *Musca domestica* (L.) in Cameron Highlands, Malaysia

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Abstract

Field strains of adult house flies (*Musca domestica*) collected from the Cameron Highlands, Pahang were tested against insecticides commonly used in Malaysia. The adult resistance status was determined using WHO standard bioassay with slight modifications and rapid enzyme microassay test. The WHO susceptible strain was used as a standard strain for comparison purpose. The LT_{50} value was determined, and based on this, the resistance ratio was calculated. The adult female flies have developed high resistance to malarhion with estimated resistance ratios of > 86.6, 32, 86.6 and 3x for flies collected from Ringlet, Kuala Terla, Brincang and Kampung Raja, respectively. These strains also showed development of resistance to pyrethroids. The rapid development of resistance could be due to the intensive agricultural usage of insecticides. Non-specific esterase microassay indicated no significant differences between the control and the field populations. Hence, this indicates that other enzymes are involved in development of resistance and esterases could not be playing a major role in the resistance of house flies. On electrophoresis analysis of esterase patterns, the Ringlet strain showed intense stained bands compared to all the other strains. As such the actual role of esterases in house fly resistance in this strain cannot be conclusively determined at this point of time.

Key Words: *Musca domestica*; insecticides; esterase-based mechanism

Introduction

As in many other tropical countries, the common house fly, *Musca domestica* (L.), is widely distributed throughout Malaysia. As house flies are mechanical vectors of a number of pathogenic viruses, bacteria, protozoa and helminth ova (Gordon & Lavoipierre, 1962), their control is important in diseases prevention. Although chemical insecticides were successful in controlling flies in the past decades, the development of resistance, has hampered their use. The house fly is an insect species that has shown the greatest ability to develop resistance to insecricides (Brown & Pal, 1971; Keiding, 1977). Resistance is due to genetic factors , usually one or a few major genes for each type of resistance (WHO, 1976). There are few reports on insecticide resistance in house flies in Malaysia. Previous findings in the Cameron Highlands showed the resistance spectrum of house flies covering all major groups of insecticides (Keiding, 1968; Cheong *et al.*, 1970; Singh, 1973; Oh & Sudderuddin, 1975). Twenty years has since rhen elapsed and these data have not been updated. Recently, there have been reports of house flies swarms invading settlements and becoming a nuisance to the public besides being the potential carriers of diseases (Jaffry, 1993). These ourbreaks have indicated the importance of information on the fly resistance status and mechanism. Hence , this study was conducted to re-evaluate the resistance status of house flies in the Cameron Highlands as intensive use of agrochemicals occur in this area.

Materials and Methods

Wild populations of house flies, *Musca domestica* were collected from Ringlet, Brincang, Kuala Terla and Kampung Raja, towns in Cameron Highlands. The major activity in these villages is agricultural farming. A wide range of insecticides, especially from organophosphate and pyrethroid groups are being used against pests of agriculturual importance. The adult flies were caught using sweep net and maintained in the insectary of Division of Medical Entomology, Institute For Medical Research. They were reared in aluminium cages , on larval food consisting of damp ground mice chow with a photoperiod of 12:12. The adults were fed on a diet of sugar and soaked corton wool served as water source. Fermented mice chow served as breeding media for the adults. Six insecticides from various groups were used: organophosphate (malathion and fenetrothion), pyrethroid (permethrin, deltamethrin and lambdacyhalothrin) and carbamate (bendiocarb).

The modified WHO bioassay test (WHO, 1981) was used to determine the resistance level. A concentration for each insecticide i.e, malathion 10%, fenitrothion 2%, permethrin 3%, deltamethrin 2%, lambdacyhalothrin 1.0% and bendiocarb 1.5% were impregnated on filter papers (140mm x 20mm) with ethanol as the diluent. Control filter papers were similarly impregnated with ethanol only. The papers were left to dry overnight at room temperature.

Less than six days old female and male flies were used throughout the study. For a complete test, three repli-

cates each with twenty adult female or male flies were exposed to each insecticide. The mortality was recorded at five minutes intervals for 1-6 hours in order to determine the lethal time (LT_{50}). Control flies were only exposed to ethanol - treated papers. Flies that survived the six hour period were then kept in holding tubes to observe the effect of post - treatment and mortality was recorded after 24 hours. Cotton pads soaked in 10% sugar solution were provided during the 24 hours holding period.

Data was analysed using the Probit Analysis Program (Raymond, 1985). Based on 50% lethal time (LT_{50}), the resistance ratio was determined by the ratio of LT_{50} of field to LT_{50} of the susceptible strain.

In addition to bioassay tests, rapid enzyme microassay was used to elucidate the possible resistance mechanism involved in adult house flies. *In vitro* preparations of enzymes derived from the field and laboratory strains were assayed for esterase.

The head and thorax of adult house flies were homogenised in wells of a porceline plate with 100 µl of 0.02M (pH 7.0) potassium phosphate buffer. Each homogenate was further diluted to a final volume of 500 µl and centrifuged at 12,045g for 15 minutes. The supernatant served as the enzyme source.

Fifty µl of homogenate was transferred to each well

Table 1. Susceptibility of *Musca domestica* adults, expressed in LT_{50} values against organophosphate and carbamate insecticides.

Strain/Insecticides/ Sex		LT_{50} (Min)	95% C.L[a]	Regression	R.Ratio[b]
Malathion 10%					
WHO	F	115.5	108.26-123.18	y=2.73x -0.63	-
	M	53.2	47.82- 58.43	y=2.18x+1.24	-
Ringlet	F	>10000	-	y=0.41x+2.51	>86.6
	M	720.5	-	y=1.89x -0.41	13.5
Kuala	F	>3000	-	y=0.82x+2.06	32.0
Terla	M	>1000	-	y=1.31x+0.94	18.8
Brincang	F	>10000	-	y=0.38x+3.09	>86.6
	M	440.3	-	y=1.90x -0.03	8.3
Kampung	F	361.2	291.14605.80	y=4.76x -7.18	3.1
Raja	M	>1000	-	y=0.61x+1.63	>18.8
Fenitrothion 2%					
WHO	F	44.7	41.04-49.29	y=4.64x-2.65	-
	M	40.5	35.97-44.28	y=6.65x-5.70	-
Ringlet	F	233.2	216.38-259.30	y=6.25x-9.97	5.3
	M	175.5	166.85-186.16	y=5.20x-6.66	4.3
Kuala	F	168.9	163.00-176.99	y=11.1x-19.7	3.8
Terla	M	145.6	136.65-157.62	y=4.64x-5.04	3.6
Bendiocarb 1.5%					
WHO	F	55.1	49.21-61.48	y=3.23x-0.62	-
	M	49.5	45.26-54.94	y=3.69x-1.27	-
Ringlet	F	819.5	455.65-3892.2	y=1.37x+1.01	14.9
	M	117.5	158.96-203.88	y=2.47x-0.56	2.4
Kuala	F	123.3	113.04-135.66	y=2.86x-0.98	2.2
Terla	M	98.8	80.75-114.63	y=3.36x-1.70	2.0

[a] 95% confidence limit. [b] Resistance ratio.

LT_{50} values of malathion towards Ringlet and Brincang strains are based on response - points below 50% knock-down.

in a microtiter plate. Using this technique, 8 replicate aliquots from a single adult were available for assays. Fifty µl of ∝-naphthyl acetate (substrate) was pipetted into each well and left for 60 seconds and then 50 µl of Fast Blue Salt and sodium dodecyl sulphate (coupling reagent) was then added. The intensity of the final colour which is an indication of esterase activity was read using an immunoassay reader (Dynatech, Model MR 5000) at 450 nm.

To observe the presence of a particular esterase or changes in intensity of the same esterase, agarose gel electrophoresis run was also conducted. Esterase electrophoretic patterns of individual head and thorax from female fly less then 6 days old were analysed using the method of Tadano (1986). The band intensities of assessed individuals were compared to standard female house flies, which were run parallel to the test samples in each agarose gel. Individual head and thorax was homogenized in 1 drop of 1% Tween solution, and a small piece of filter paper (1 mm x 2 mm) was used to absorb the crude house fly homogenate. The filter papers with the homogenate were placed on the gel origin for absorption of the homogenate into the gel.

Electrophoretic separation was performed horizontally at 4°C, 25mA/18 cm for 2.5 hours using 0.063M potassium phosphate buffer pH 6.8. Separation was stopped after approximately 2.5 hours and esterases were detected by a substrate consisting of 0.75g ∝-napthyl acetate and 0.75 g ß-naphthyl acetate and incubated at 37°C for 30 minutes. The 1% Fast Blue B salt was then poured onto the gel which was then incubated for another 15 minutes at 37°C. The intensity of stained bands were compared visually and the gels were photographed for records.

Results

Table 1 and 2 presents the LT_{50} data obtained from adults of various strains expose to 6 kinds of insecticides. The resistance ratio was calculated by dividing the LT_{50} values of field-strain by the LT_{50} value of the susceptible strain (WHO-strain). Of the 4 strains tested against malathion (Table 1), the Ringlet and Brincang strains indicates very high resistance (>86.6 times) in comparison with the standard WHO strain. The LT_{50}

Table 2. Susceptibility of *Musca domestica* adults, expressed in LT_{50} values against pyrethroid insecticides.

Strain/Insecticides/Sex		LT_{50} (Min)	95% C.L[a]	Regression	R.Ratio[b]
Permethrin 3%					
WHO	F	19.2	15.33-22.40	y=3.83x+0.09	-
	M	18.4	16.45-20.29	y=6.18x -2.82	-
Ringlet	F	1015.9	454.90-8208.5	y=0.69x+2.94	52.9
	M	2002.9	668.10-5952.6	y=0.63x+2.93	109.0
Kuala	F	42.6	34.93-49.91	y=1.81x+2.05	2.2
Terla	M	186.5	151.76-258.7	y=1.32x+2.01	10.1
Deltamethrin 2%					
WHO	F	5.5	0.10-11.94	y=1.26x+4.07	-
	M	0.01	2.10-3.53	y=0.53x+6.17	-
Ringlet	F	372.7	178.77-441.65	y=0.43x+3.90	67.0
	M	167.6	120.28-379.40	y=0.70x+3.40	>1000
Kuala	F	>1000	-	y=0.23x+3.18	>1000
Terla	M	>1000	-	y=0.83x+2.36	>1000
Lambdacyhalothrin 1%					
WHO	F	2.1	0.00-7.63	y=1.37x+4.57	-
	M	6.1	0.58-10.41	y=2.02x+3.42	-
Ringlet	F	183.4	120.23-496.23	y=0.60x+3.64	88.0
	M	19.1	9.13-28.84	y=0.97x+3.76	3.2
Kuala	F	40.7	29.59-50.90	y=1.20x+3.06	19.6
Terla	M	18.3	9.77-26.67	y=1.12x+3.58	3.0

[a] 95% confidence limit. [b] Resistance ratio.

value is unusually high i.e 166.6 hours. It is also interesting to note that the male house flies of most strains showed lower susceptibility towards malathion than the females with the exception of Kampung Raja strain. Ringlet strain seems to be the most resistant strain tested with the six insecticides (Tables 1 & 2) and the order of resistance in females (descending order) is : malathion > lambdacyhalothrin > deltamethrin > permethrin > bendiocarb > fenitrothion .

The mortality data of post-exposure treatments are shown in Table 3. Malathion at 10% concentration caused the least mortality . The pyrethroids including permethrin 3%, deltamethrin 2% and lambdacyhalothrin 1.0% also induced lower percentage of mortality in these strains when compared to the WHO strain. On the other hand, fenitrothion at 2% concentration was the most potent insecticide exhibiting mortality of 73.3% and 96.7% to female and male houseflies of Ringlet strain, respectively.

Esterase microassays conducted to confirm the bioassay results are presented in Tables 4. Comparisons were made using t-test at 95% confidence level (p=0.05). Esterase levels of the non-exposed F_1 of WHO and field strains were not significantly different based on mean

Table 3. Percentage mortality of *Musca domestica* adults 24 hr post-exposure

Insecticide/Strain/Sex		Malathion	Fenitrothion	Permethrin	Deltamethrin	Lambda cyhalothrin	Bendiocarb
WHO	F	100	100	78.3	70.0	80.0	76.7
	M	82.2	100	100	98.3	85.0	93.3
Ringlet	F	10.0	73.3	13.3	18.3	1.7	13.3
	M	33.3	96.7	10.0	46.7	46.7	51.7
Kuala Terla	F	25.0	81.7	28.3	3.3	55.0	78.3
	M	31.7	90.0	20.0	16.7	76.7	70.0
Brincang	F	20.0	-	-	-	-	-
	M	31.7	-	-	-	-	-
Kampung Raja	F	23.3	-	-	-	-	-
	M	5.0	-	-	-	-	-

Table 4. Comparison of esterase level between the standard WHO strain and field strains of adults *Musca domestica* using t-test.

Strain	Sex	N	Mean ± SD	t-value	p-value
WHO	F	80	0.32 ± 0.1185		
	M	80	0.35 ± 0.0900		
	#			- 1.381	0.169
Ringlet	F	80	0.30 ± 0.0753	1.808	0.072
	M	80	0.35 ± 0.0526	- 0.515	0.607
	#			- 5.452	<0.001
Kuala Terla	F	64	0.31 ± 0.0509	1.070	0.287
	M	64	0.31 ± 0.0996	2.500	0.014
	#			0.054	0.957
Brincang	F	73	0.29 ± 0.0911	1.824	0.070
	M	80	0.30 ± 0.0770	3.628	<0.001
	#			- 0.368	0.714
Kampung Raja	F	80	0.32 ± 0.0748	0.197	0.844
	M	80	0.37 ± 0.1191	- 1.738	0.084
	#			- 3.459	<0.001

t-test against female and male in each strain

optical density readings and standard deviation for the female and male house flies.

Further comparisons were conducted on esterase levels between pre-and post- exposure of the standard WHO strain and field strains as tabulated in Tables 5 and 6. It is interesting to note that there is a significant difference between pre- and post-exposure to malathion (Table 5) in all the strains. The mean optical.density reading clearly indicates a decrease in esterase levels though the bioassay results proved significant resistance to malathion. This phenomenon is exhibited in both female and male flies. However, the other insecticides generally demonstrated significant levels irrespective of negative and positive t-values.

Comparison of esterase patterns in *Musca domestica* in all the strains including the standard WHO strain is shown in Figure 1. Three loci are determined in the esterase system of house flies by electrophoretic run of 2.5 hours at 25mA. The three loci for convenience are named as locus 1 (L1), locus 2 (L2) and locus 3 (L3). Loci L1 and L2 can be clearly distinguished but the locus 3 is not sufficiently clear for analysis at this stage.

Table 5. Comparison of esterase level between pre- and post exposure of each strains of adults *Musca domestica* to organophosphate and carbamate insecticides.

Insecticide/Strain	Sex	N	Mean ± SD	t-value	p-value (p=0.05)
Pre-exposed					
WHO	F	80	0.32 ± 0.1139		
	M	80	0.34 ± 0.0899		
Ringlet	F	80	0.30 ± 0.0753		
	M	80	0.35 ± 0.0526		
Kuala Terla	F	64	0.30 ± 0.0510		
	M	64	0.31 ± 0.0995		
Brincang	F	73	0.29 ± 0.0912		
	M	80	0.30 ± 0.0770		
Kampung Raja	F	80	0.32 ± 0.0748		
	M	80	0.37 ± 0.1191		
Post-exposed					
Malathion 10%					
WHO	F	80	0.15 ± 0.0265	13.596	<0.001
	M	78	0.13 ± 0.0166	14.870	<0.001
Ringlet	F	77	0.14 ± 0.0342	16.244	<0.001
	M	79	0.14 ± 0.0562	24.469	<0.001
Kuala Terla	F	80	0.11 ± 0.0282	28.301	<0.001
	M	76	0.11 ± 0.0476	15.480	<0.001
Brincang	F	76	0.15 ± 0.0492	12.314	<0.001
	M	60	0.13 ± 0.0100	16.386	<0.001
Kampung Raja	F	56	0.19 ± 0.0561	11.186	<0.001
	M	80	0.26 ± 0.0861	7.243	<0.001
Fenitrothion 2%					
Ringlet	F	79	0.26 ± 0.1106	2.015	0.046
	M	79	0.25 ± 0.1148	7.288	<0.001
Kuala Terla	F	79	0.38 ± 0.0902	-5.897	<0.001
	M	80	0.42 ± 0.0908	-7.337	<0.001
Bendiocarb 1.5%					
WHO	F	80	0.45 ± 0.1343	-6.512	<0.001
	M	32	0.42 ± 0.1215	-3.751	<0.001
Ringlet	F	80	0.31 ± 0.1225	-0.721	0.427
	M	80	0.36 ± 0.0869	-0.924	0.357
Kuala Terla	F	80	0.43 ± 0.1236	-7.710	<0.001
	M	48	0.52 ± 0.0769	-7.223	<0.001

Table 6. Comparison of esterase level between pre- and post exposure of each strains of adults *Musca domestica* to pyrethroid insecticides.

Insecticide/Strain	Sex	N	Mean ± SD	t-value	p-value (p=0.05)
Pre-exposed					
WHO	F	80	0.32± 0.1139		
	M	80	0.34 ± 0.0899		
Ringlet	F	80	0.30 ± 0.0753		
	M	80	0.35 ± 0.0526		
Kuala Terla	F	64	0.30± 0.0510		
	M	64	0.31 ± 0.0995		
Brinchang	F	73	0.29 ± 0.0912		
	M	80	0.30 ± 0.0770		
Kampung Raja	F	80	0.32 ± 0.0748		
	M	80	0.37± 0.1191		
Post-exposed Permethrin 3%					
WHO	F	78	0.33 ± 0.1394	-0.143	0.866
	M	-	-	-	
Ringlet	F	80	0.28 ± 0.0976	0.798	0.426
	M	80	0.30 ± 0.1140	3.491	<0.001
Kuala Terla	F	65	0.24 ± 0.0408	7.914	<0.001
	M	80	0.38 ± 0.1567	6.875	<0.001
Deltamethrin 2%					
WHO	F	80	0.29 ± 0.1237	4.448	<0.001
	M	-	-	-	
Ringlet	F	72	0.30 ± 0.0992	0.317	0.752
	M	80	0.27 ± 0.0535	10.255	<0.001
Kuala Terla	F	80	0.41 ± 0.1054	-7.223	<0.001
	M	75	0.46 ± 0.1679	-6.281	<0.001
Lambdacyhalothrin 1%					
WHO	F	72	0.54 ± 0.0751	-13.997	<0.001
	M	72	0.35 ± 0.0899	- 6.953	<0.001
Ringlet	F	80	0.58 ± 0.1319	-16.652	<0.001
	M	72	0.51 ± 0.1074	-11.768	<0.001
Kuala Terla	F	80	0.51 ± 0.1236	-12.450	<0.001
	M	50	0.30 ± 0.2337	0.242	0.890

In this electrophoretic run, the substrate used was a mixture of ∝-naphthyl acetate and ß-naphthyl acetate and it is clear that four alleles are seen. The locus 1 has 4 kinds of alleles named as allele a, b, c and d. Allele a was the most common allele in locus 1. Locus 2 has 2 kinds of alleles, i.e allele a and b and allele b was the common allele for locus 2. The esterase band which denotes allele a in locus 1 in all strains have identical electrophoretic mobility (Rf) . Similar electrophoretic mobility was observed for allele b in locus 2 in all strains. The Ringlet strain showed densely stained bands at locus 1 as in slots 3 to 5. The densely stained bands might denote a high specific activity of esterases or a higher titer of the enzyme suggesting that in the Ringlet strain some variant esterase system may be involved in the resistance mechanism.

In the single substrate staining systems, the only difference which was observed using ∝-naphthyl acetate and ß-naphthyl acetate was the colour difference i.e, purplish and pinkish bands, respectively.

Discussion

The widespread use of agrochemicals in Malaysia especially in Cameron Highlands resulted in the emergence

of resistance in the house fly (Cheong *et al.*, 1970; Singh, 1973). The resistance status of house flies from Cameron Highlands had gained much interest during the 1970's and reports on the resistance have been documented (Cheong *et al.*, 1970; Singh, 1973 and Hayashi *et al.*, 1978). Our study indicates that the most resistant strain was from Ringlet which had high LT_{50} values against all the test insecticides. In fact, against malathion, apptoximately more than 166.6 hours is needed to kill fifty percent of the population. On the other hand, fenitrothion exhibited high toxicity, indicating that cross-resistance is not involved. The potency of fenitrothion could be due to the chemical structure i.e, the carbon cyclic derivatives unlike malathion which is aliphatic. Pyrethroid resistance was not really pronounced in the 1970' s as reported by Singh (1973) with resistance ratio of only 1.0 to 2.0 fold. In contrast, in this study resistance to pyrethroids is severe with resistance ratio ranging from 50-1000 folds as compared to the standard WHO strain. This may indicate that the use of pyrethroids in agricultural pracrice has increased tremendously in the past decades.

Though bioassay results showed high resistance level towards malathion, no significant difference is observed in the esterase levels of field strain and the standard WHO strain in the F_1 generation of *Musca domestica*. Hemingway (1982) reported similar findings with *An. stephensi*. Other enzymes are known to be involved in the breakdown of malathion, e.g., phosphatases, which may contribute to resistance mechanisms that restrict the uptake of malathion or hasten its excretion (Field *et al.*, 1984). Glutathione *S* - transferase is also active in rhe metabolism of organophosphorous compounds and the mechanism of resistance against malathion has been reported for house fly (Niwa *et al.*, 1977). Significant resistance to pyrethroids could be assiociated with the mechanism of knockdown resistance.

In the electrophoretic runit was apparent from visual examination of gels that the band intensity in the Ringlet strain was consistently and markedly stronger than thar in the other field and WHO strains. The Brincang strain also showed high resistance to malathion but surprisingly very faint bands were observed and the intensity was close to the WHO strain. This strongly suggests that the resistance mechanism in the house flies from the Cameron Highlands appear to be complex and several other enzymes could be involved. Clearly, a wider range of enzymes need to be studied to conclusively determine the resistance mechanism in house flies in the Cameron Highlands.

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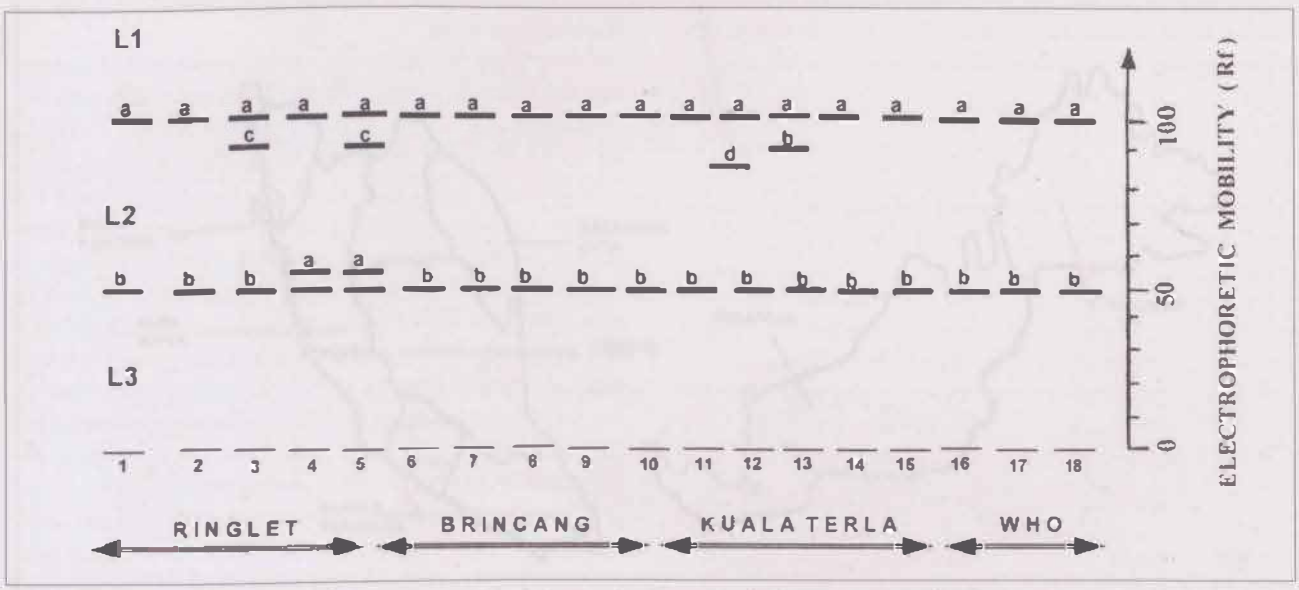

Fig. 1. Esterase patterns observed in single houseflies of Ringlet, Brincang, Kuala Terla and standard WHO strains. L1: Locus 1; L2: Locus 2; L3: Locus 3; a: allele a; b: allele b; c: allele c; d: allele d. Rf value of 100 given arbitrarily to fastest esterase band of allele a, Locus 1 WHO strain.

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