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Temporal fluctuations of insecticides resistance in *Musca domestica* Linn (Diptera: Muscidae) in Malaysia

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Abstract. House flies were collected from April 2007-April 2008 from two poultry farms (Balik Pulau and Juru) in the state of Penang. The resistance level of the first generation offspring was evaluated against DDT, malathion, propoxur, and permethrin using the topical application method. The resistance ratio (RR) of the Balik Pulau strain house flies for propoxur, malathion and DDT ranged from 10.28 to 99.00, 7.83 to 47.01 and 6.05 to 31.10, respectively. Resistance to propoxur and malathion in house fly was attributed to cross resistance to organophosphate insecticides used in the farm. Increased metabolic detoxification might be the mechanism involved in DDT resistance due to excessive application of cypermethrin formulation. The RR of the Juru strain for propoxur, malathion and DDT was in a decreasing pattern throughout the study period, ranging from 5.58 to 83.38, 15.19 to 27.82, and 10.04 to 22.69, respectively. Permethrin appeared to be the most potent insecticide in controlling house fly in both the Balik Pulau (RR = 0.50 to 1.96) and Juru poultry farms (RR = 0.64 to 2.40). The fluctuations of insecticides resistance in house fly was also found to correlate with climatic factors due to its rapid breeding. Relative humidity exhibited positive correlation indices with the changes in the resistance level for DDT ($r = 0.481$, $p < 0.05$), malathion ($r = 0.698$, $p < 0.01$), and permethrin ($r = 0.580$, $p < 0.05$) in Balik Pulau. Similarly, relative humidity in Juru also showed strong correlation with the RR for DDT ($r = 0.900$, $p < 0.01$), malathion ($r = 0.762$, $p < 0.05$), permethrin ($r = 0.760$, $p < 0.05$), and propoxur ($r = 0.897$, $p < 0.01$).

INTRODUCTION

House flies greatly developed resistance to every insecticide used against them through encounters with selection pressure from frequent treatments, particularly with single products. Insecticide resistance in housefly is a global problem (Georghiou & Mellon, 1983; Scott *et al*., 1989). House fly resistance to organochlorine, organophosphate, carbamate and pyrethroid insecticides has been reported in several regions of peninsular Malaysia (Singh, 1973; Nazni *et al*., 1998, 2003).

Due to the high resistance of house flies to various insecticides, it is vital to assess the monthly resistance status of the insect for the successful implementation of fly control.

The alterations of resistance status of house fly are believed to be related with insecticide application profile. Immigration of susceptible individuals into a treated area and the presence of non-insecticidal treatment areas are believed to delay the development of insecticide resistance (Georghiou & Taylor, 1977; Tabashnik & Croft, 1982). Moreover, environmental factors may influence the expression of insecticide resistance in insects. Any alterations of resistance levels affected by environmental factors could provide an insight into the importance of these factors in the evolution of resistance in the field.

To date, limited information on temporal changes of house fly resistance status in Northwestern peninsular Malaysia is available.

MATERIALS AND METHODS

House flies sampling
House flies were collected monthly from April 2007 to April 2008 by using sweep net from two sites, a poultry farm in Balik Pulau located on the island, and the other poultry farm in Juru located on the mainland. Both sites are 21.68km apart.

Balik Pulau, a suburban area on the island of Penang, is famous for its fruit orchards and spice gardens. It is located approximately 8km from the capital, Georgetown. The egg-production poultry farm consists of one narrow-style (4.32m by 23.87m) poultry shed, with two rows of three-tiered cages (91 cages per tier with 2-3 birds per cage) suspended 0.80m above the ground. Removal of chicken dung is sporadic with dung accumulating on the ground. A durian plantation is situated a kilometer away from the poultry farm.

Juru is one of the industrial corridors in the northern region of peninsular Malaysia which has led to rapid housing development around the area. The meat-production poultry farm is located on mainland Penang, approximately 15km (altitude 15.0m) from Georgetown. It is approximately 5 km away from the housing and industrial areas, and situated in an oil palm plantation. The farm consists of four wide-span (6.50m by 75.00m) poultry sheds with approximately 4000-5000 birds per shed. The sheds are suspended 1.00m above the ground. Dung is removed regularly before a new batch of chickens is released into the poultry sheds. Fly sampling in Juru could only be done every two months when the chickens are 25-28 days old.

The collected house flies were reared in the insectarium of the Vector Control Research Unit (VCRU) of Universiti Sains Malaysia (USM), and maintained at temperature of 25.0 ± 2.0°C, relative humidity 60 ± 5%, and a photoperiod of 12:12 (L:D). The WHO susceptible strain, obtained from the Danish Pest Infestation Laboratory, Lyngby, Denmark, was used as a reference strain for comparison.

Insecticides
4 insecticides namely DDT 99% (Aldrich Chemical Company), malathion 92.8% (American Cyanamid Company), permethrin 95.6% (FMC Corporation) and propoxur 99.4% (Bayer CropScience) were each diluted into a series of concentrations with analytical butanone (Fisher Scientific).

Bioassay
The first generation of field-collected house flies were bioassayed using the topical application method (WHO, 1970) at a temperature of 26.4 ± 0.7°C, relative humidity of 45 ± 6%, and a photoperiod of 12:12 (L:D). Three replicates of 25 female house flies (3-6 days old) each were used for each dose level. House flies were anaesthetized by chilling. By using the micropipette (Eppendorf, AG, Hamburg, Germany), each fly was treated by applying 1.00µl of insecticide with required concentration on the dorsal part of the thorax. Immediately after treatment, the flies were kept in clean plastic containers (11.0cm diameter x 6.5cm height). Adequate and standardized food was given before and after the test. Mortality of the flies was recorded after 24 hours.

Scudder grill
Prior to the collection, the density of house flies was measured by using the Scudder grill (Scudder, 1947). The flies that landed on the grill within 30 seconds were counted; 4 to 6 counts were made in each locality, and the arithmetic means of these counts were recorded as the fly index (Bong & Zairi, 2009).

Meteorological data
Precipitation data from March 2007 to April 2008 was obtained from the Malaysian Meteorological Department (MMD), Bayan Lepas and Prai station, Penang, Malaysia. Temperature and relative humidity were

recorded using a hygro-thermometer (Ters Electronic Ltd., China).

Data analysis

The data obtained were subjected to probit analysis (SPSS version 11.0) to determine the LD_{50} value. Computation of resistance ratio (RR) value in comparison to the susceptible WHO strain was obtained to determine the resistance status of house fly (Keiding, 1976, 1977). Pearson correlation test (SPSS analysis version 11.0) was carried out to evaluate the relationship between the resistance status of house fly and environmental factors. Published data on the density of house flies (Bong & Zairi, 2009) was also correlated with the resistance status. The test was performed using a significance level of = 0.05. A three-dimensional plot of climatic factors and resistance status was generated using SigmaPlot (SPSS 2002) software.

RESULTS AND DISCUSSION

House fly resistance to insecticide in both poultry farms was in the descending order of propoxur > malathion > DDT > permethrin (Figure 1 and Figure 2). The resistance level for propoxur, malathion and DDT varied widely throughout the 13 months. In Balik Pulau, resistance ratios (RR) of housefly for propoxur, malathion and DDT ranged from 10.28 to 99.00, 7.83 to 47.01 and 6.05 to 31.10 (Figure 1) respectively, whereas in Juru, RR ranged from 5.58 to 83.38, 15.19 to 27.82, and 10.04 to 22.69 (Figure 2) respectively. The resistance level for permethrin was also variable, but with a narrower range of 0.50 to 1.96 in Balik Pulau and 0.64 to 2.40 in Juru, denoting a low degree of resistance (Table 1). Thus, permethrin appears to be the most potent among the insecticides mentioned above in both sites for the control of house flies.

In Balik Pulau, RR for propoxur considerably varied throughout the study period with apparent high resistance level recorded in July 2007 (99.00), September 2007 (62.06) and February 2008 (94.27) (Figure 1). Carbamate insecticides have never been applied by the farmers to control insect pests (personal communication). Thus, the high propoxur resistance found in house fly could be attributed to cross resistance to organophosphate insecticide (a.i: chlorpyrifos 38.7% w/w) used in fly larval control. In Denmark, organophosphorus- multiresistant strains of house flies showed high resistance to carbamates (Keiding, 1977).

The resistance level for malathion was classified as moderate (Table 1) in April 2007 (23.10) and May 2007 (13.97). Apart from the insecticide mentioned above, another pesticide (a.i: chlorpyrifos 45.9% w/w + cypermethrin 4.6% w/w) was applied weekly to control insect pests. This might have triggered the development of house fly resistance against malathion. The resistance level in June 2007 increased 3-fold to 47.01 which was classified as high as stated in WHO (1980). It could have been due to the frequent application of the pesticide and dimethoate (38.0% w/w) during the fruiting season (April to June). The resistance level decreased in July 2007 and remained moderate for 8 months (10.08 to 27.92) because of the less frequent insecticide treatment after the fruiting season. In March 2008 and April 2008, the resistance status was low, with values of 7.83 and 9.83 respectively.

The low level of house fly resistance to DDT from August 2007 to April 2008 (Figure 1) might be attributed to the absence of DDT insecticidal pressure. Surprisingly, the resistance level from April 2007 to July 2007 was elevated (19.96 to 31.10). The rise in kdr-type DDT-resistance is always paralleled by an increase in permethrin-resistance (Prapanthadara *et al.*, 2002). However, permethrin resistance was not observed in the present study. Also, Scott & Matsumura (1983) indicated that Geman cockroach of *kdr*-type DDT-resistant showed cross-resistance to type I pyrethroids, but not to type II pyrethroids. This strongly indicated that the involvement of *kdr* in resistance to DDT was not possible. However, some studies documented a rise in monooxygenase

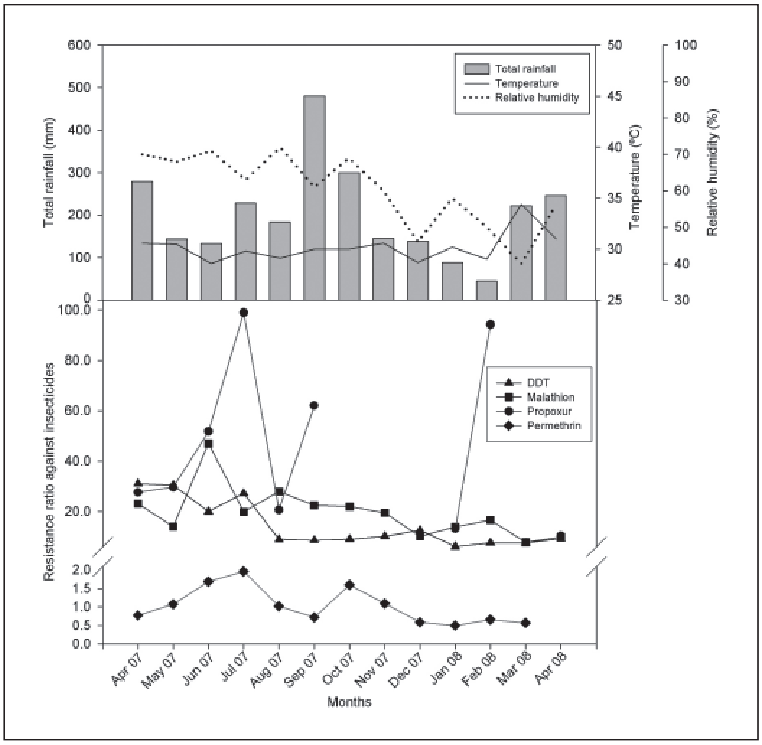

Figure 1. Monthly resistance of Balik Pulau strain female house fly against insecticides and its correlation with climatic factors

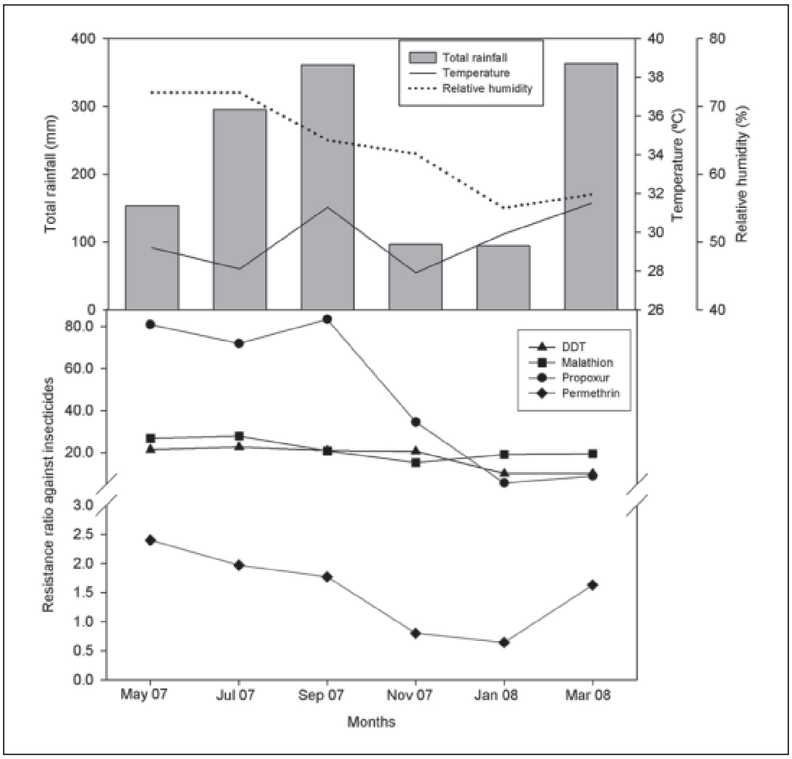

Figure 2. Monthly resistance ratio of Juru strain female house fly against insecticides and its correlation with climatic factors

Table 1. Resistance classification on house flies (WHO, 1980)

Resistance ratio (RR)	Resistance level
< 10	Low
10 to 40	Moderate
40 to 160	High
> 160	Very high

activity, as shown in cypermethrin treated insects (Kotze, 1994), in DDT resistance (Liu & Yue, 2000; Prapanthadara *et al.*, 2002). This showed that cypermethrin treated insects might show cross resistant to DDT. Thus, it is explained that the elevation in DDT resistance level from April 2007 to July 2007 might be due to the application profile of the cypermethrin formulation pesticide during the fruiting season. The resistance level for DDT was also shown to have a strong correlation with the density of house flies ($r = 0.631$, $p < 0.05$) (Table 2). Intensive cypermethrin applications might result in house fly cross resistance towards DDT. This promotes insecticide selection pressure and leads to the increase of fly density.

In Juru, the RR for propoxur, malathion and DDT were in decreasing pattern (Figure 2). The resistance levels for propoxur was classified as high (Table 1) in the months of May 2007 (80.86), July 2007 (71.89), and September 2007 (83.38). It declined to a moderate level in November 2007 (34.57) and to low levels in January 2008 (5.58) and March 2008 (8.94). The degrees of resistance for malathion and DDT were in moderate level although they showed alterations throughout the study period. The resistance status of house fly was decreasing because insecticides were not used to control house fly in the farm since November 2007 (insecticide used before November 2007 was uncertain). The practice of better sanitation was the mainstay of house fly control in the farm. Also, thermal fogging with organophosphate insecticide in mosquito control was replaced by synthetic pyrethroid in 2007. This might be the main reason for the gradual decrease in house fly resistance to malathion during the study period. Keiding (1967) stated that the high level of insecticide resistance normally decreases, at least partially, when the selection pressure is eliminated, although it may persist for a long time.

There was no particular insecticide to control house fly in Juru. However, thermal fogging with synthetic pyrethroid to control the outbreak of *Aedes* mosquito in the area (provided by the Penang Health Department) is believed to have affected the alterations of the house fly resistance ratio to permethrin although it had not reached the threshold of resistance. The resistance ratio of house fly was slightly increased in May (Figure 2). It was probably affected by the thermal fogging conducted on 19th and 28th April 2007. The level decreased until the month of January because the sampling in every respective month was carried out before thermal fogging (Table 3). The interval between the thermal fogging activities and the next

Table 2. Pearson correlation (r) for resistance ratio in relation to fly population and climatic factors (relative humidity, temperature, and total rainfall)

	Resistance ratio							
	Balik Pulau				Juru			
	DDT	Malathion	Permethrin	Propoxur	DDT	Malathion	Permethrin	Propoxur
Density of flies	0.631*	0.150	0.145	0.551	-0.298	0.285	0.488	-0.021
Relative humidity	0.481*	0.698**	0.580*	-0.310	0.900**	0.762*	0.760*	0.897**
Temperature	-0.109	-0.518*	-0.290	-0.486	-0.554	-0.176	0.107	-0.191
Total rainfall	0.024	0.047	0.097	0.001	0.023	0.262	0.516	0.264

* Correlation is significant at the 0.05 level
** Correlation is significant at the 0.01 level

Table 3. Day interval between thermo fogging activity and house fly sampling

Date of thermo fogging	Date of sampling	Day interval (d)
19 April 2007	–	–
28 April 2007	7 May 2007	9
–	16 July 2007	79
19 July 2007	12 September 2007	55
17 Sep 2007	12 November 2007	56
17 November 2007	–	–
6 December 2007	–	–
19 December 2007	18 January 2008	30
14 March 2008	–	–
16 March 2008	17 March 2008	1

sampling was long enough to eliminate the selection pressure. The increment level in March 2008 was triggered off by the thermal fogging activities on 14th and 16th March 2008.

Nazni *et al.* (2003) observed that the RR for DDT, malathion, permethrin and propoxur in Kundang and Cameron Highlands were varied and high throughout the year, whereas lambdacyhalothrin had not reached the threshold of resistance. The high resistance of house fly to insecticides in Cameron highlands may be due to the applications of agricultural insecticides to control agriculture pests in the vegetable farms.

In Turkey, the resistance level of house fly for pyrethroids and fenitrothion decreased from spring to fall due to the infrequent use of these insecticides. Apart from that, individuals that are to produce the next generation posses high vigor tolerance selected by the harsh winter, resulting in high levels of resistance in the spring (Akiner & Caglar, 2006). Pap & Farkas (1994) also reported that the high resistance of house fly to various organophosphates and pyrethroids from 24 farms in Hungary was correlated with insecticide usage profile in these farms.

Resistance level of house fly increased with insecticide usage. However, the fluctuations cannot be explained solely by insecticide usage profile and frequency. Hansens & Anderson (1970) noted that sanitation and climatic factors will also influence the resistance status of fly. In the present study, the resistance status of fly for insecticides was found to strongly correlate with relative humidity.

In Juru, relative humidity was shown to have positive correlations with the RR for DDT ($r = 0.900$, $p < 0.01$), malathion ($r = 0.762$, $p < 0.05$), permethrin ($r = 0.760$, $p < 0.05$) and propoxur ($r = 0.897$, $p < 0.01$). In Balik Pulau, relative humidity was also shown to have positive correlations with the RR for insecticides.

Figure 3 shows that relative humidity (RH) has more impact on the RR of Balik Pulau flies compared to total rainfall (TR) although the resistance level for malathion increased with high humidity and low total rainfall [$RR = (0.72 \pm 0.23)(RH) - (0.01 \pm 0.02)(TR) - 22.08 \pm 13.84$; $r^2 = 0.496$; Standard Error of Estimation = 7.916; $F = 4.925$; $p < 0.05$]. Humidity per se is not a selective factor, but moderate total rainfall with high humidity would provide optimum conditions for house flies to breed. The increase in the RR might be the result of selection pressure due to the frequent insecticide treatments by the farmers to control the fly density. In New Jersey, Hansens *et al.* (1970) reported that housefly resistance increases in the summer due to the presence of residual insecticide in the dairy which nearly eliminate susceptible flies.

Heavy rainfall is expected to encourage the development of high resistance in housefly due to its rapid breeding, as reported by Nazni *et al.* (2003). In Cameron Highlands, the RR of house fly

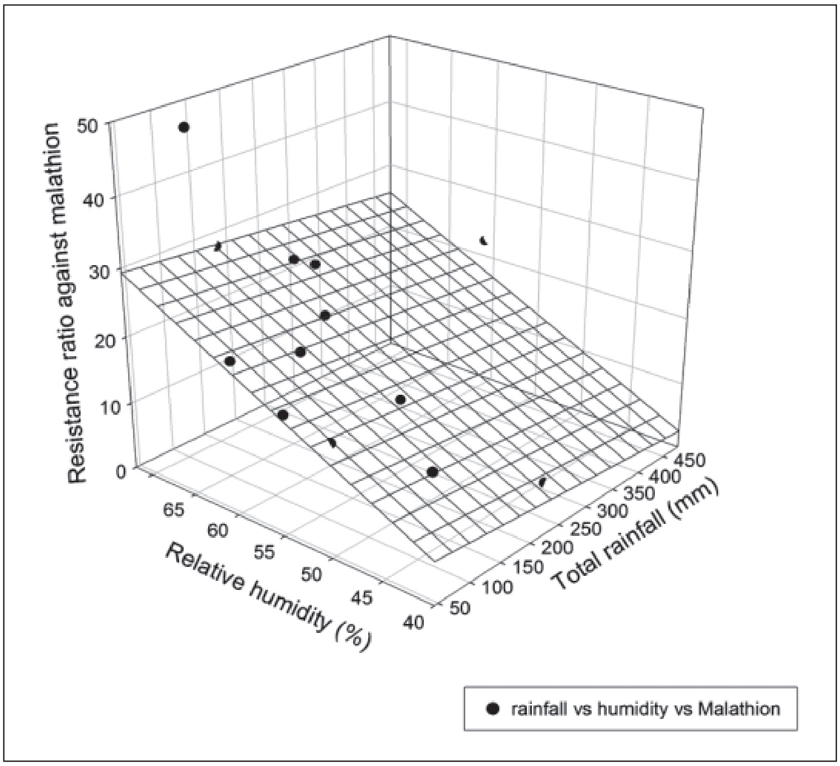

Figure 3. Scatter plot of resistance ratio of Balik Pulau house fly against malathion in relation to relative humidity and total rainfall

resistance towards various insecticides increased when the optimum temperature is around 30.0ºC with moderate rainfall of 200mm to 300mm (Nazni *et al.*, 2003). The finding further supports the present study in Juru, indicating that the RR for malathion was high when temperature (T) was 29.0ºC to 30.5ºC and the TR was 200mm to 300mm (Figure 4) [$RR = (0.25 \pm 0.02)(TR) + (100.42 \pm 11.98)(T) - (1.70 \pm 0.21)(T)^2 - 1482.33 \pm 173.14$; $r^2 = 1.000$; Standard Error of Estimation = 0.220; $F = 607.044$; $p < 0.05$]. Nevertheless, total rainfall per se showed no correlation with the resistance level of housefly in our study (Table 2).

The present study did not show any significant interaction of temperature with the expression of insecticide resistance in house fly.

From the present study, pyrethroid insecticides like permethrin appeared to be the most effective insecticide for the control of house fly in both poultry farms. The fluctuations in the level of house fly resistance to insecticides were not only correlated to insecticides application profile, but also closely related to climatic factors such as temperature. However, the effect of temperature on the potential resistance to insecticides in the field is not as obvious as in temperate countries due to the imperceptible changes in monthly temperature in tropical countries, such as Malaysia. Another important finding which has not been reported before is that the degree of resistance to insecticides is influenced by relative humidity. This is because high humidity with warm weather greatly affects breeding conditions and these in turn affect fly numbers. Thus, the fluctuation of house fly resistance mostly depends on the above mentioned conditions and also insecticide usage profile.

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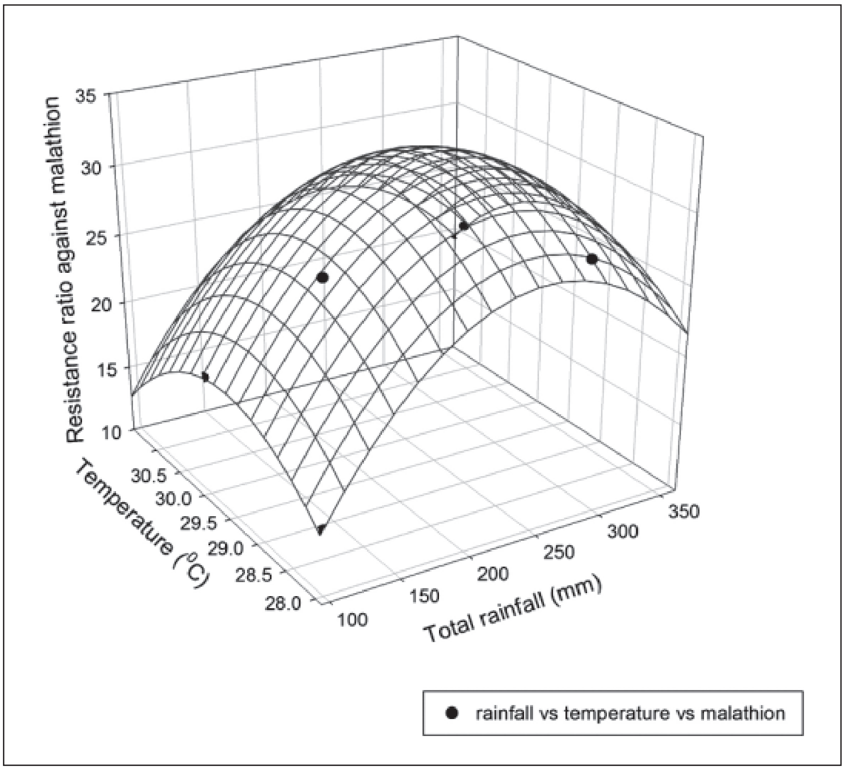

Figure 4. Scatter plot of resistance ratio of Juru house fly against malathion in relation to temperature and total rainfall

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