STATISTICAL APPRECIATION OF EXPERIMENTAL DATA ON VARIATIONS IN THE MORTALITIES OF THE MOSQUITO CULEX FATIGANS WHEN EXPOSED TO DDT

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ABSTRACT

Variations in the mortality of the insect *Culex Fatigans* Wied when exposed to DDT have been reported by Koshi and Ranganathan[1,2] with the help of data collected every fortnight in the Defence Research Laboratory (Stores), Kanpur beginning September, 1949. The statistical analysis of the data is presented in the present paper. It appears from the analysis that over and above the significant influence of humidity and the influence, though not significant, of temperature on mortalities, there is a marked seasonal pattern exhibited by the mortalities.

Introduction

In biological assays, it is well known that mortalities of animals and insects exposed to the same concentration of the same poison in the same laboratory vary from day to day. This variation has led to the procedure whereby whenever the potency of an unknown concentration of a drug or poison has to be assayed, a standard known concentration is included in the biological assay along with the unknown.

Extensive literature exists in which the association of temperature and humidity with mortality has been studied[1–9]. However, a full explanation of the nature of relationship is yet to be obtained. An attempt to study the relationship was made in the Defence Research Laboratory (Stores), Kanpur by Koshi and Ranganathan [1,2] with the help of data collected systematically every fortnight starting from September, 1949. The statistical analysis of the data[2] is presented in this paper.

Here the author uses the data of LD-50 whereas Koshi and Ranganathan use the percentage mortality. It may be noted that LD-50 is inversely related to percentage mortality so that as percentage mortality increases, LD-50 decreases and *vice versa*.

The Data

Full details of experimental technique have been described by Koshi and Ranganathan [1, 2]. But in order to make this paper self contained, a summary of the above experimental details is given below.

The experiments were carried out with the insect (*C. fatigans*) drawn from a colony which was established from a single egg-raft on 17th September 1945[10]. The breeding of insects as well as the toxicity tests were carried out in a partially air conditioned room in which the variations in temperature and humidity during any one day were not appreciable. Over a period of eight years ending September, 1957, the temperature and relative humidity ranged between 80°F and 90°F, and 48 percent and 92 percent respectively. The diet was kept the same throughout.

Female mosquitoes, 40 $\pm$ 5 hours old, were exposed at fortnightly intervals to filter papers impregnated with solutions of pure pp'—DDT in liquid paraffin in varying concentrations. The exposure period was one hour and observations on mortality were recorded 24 hours thereafter. The temperature and relative humidity at the time of each experiment were recorded. There were no mortalities observed in the control sets. Two groups of 20 mosquitoes were exposed to each concentration at each experiment.

For each experiment, the dose-response relationship was obtained by plotting the probit of percentage mortality against logarithm of concentration. The relationships were found to be linear in every case. The LD-50 and the slope 'b' figures have been taken for this investigation.

Analysis and Results

Since the LD-50 figures or the 'b' figures showed no secular trend, it was concluded that the population of the insects did not undergo any gradual change as regards the distribution of susceptibilities to DDT.

Fortnightly averages were calculated by adding all figures corresponding to the same fortnight over different years, and dividing the total by the number of figures involved. This procedure leads to 24 average figures corresponding to the 24 fortnights in a year. Since there is no secular trend, these averages represent the pattern of variation during a year. The fortnightly averages in respect of LD-50, 'b' Temperature, and Relative Humidity are presented in Table I. An examination of the table reveals seasonal patttern, which is the same for LD-50 and 'b'.

In order to test whether the observed seasonal pattern is significant, an analysis of variance was carried out and the results are presented in Table 2. The variations of LD-50 and 'b' are found to be highly significant, and significant respectively.

To investigate the variations after allowing for the influences due to variations in temperature and relative humidity, an analysis of covariance was carried out on LD-50 figures and the 'b' figures, and the results are presented in Tables 3 and 4. It is found that the residual variation of LD-50 figures is still highly significant, and the residual variation of 'b' figures is still significant.

The partial regression equation of LD-50(u) on temperaure (x_1) and relative humidity (x_2) is found to be—

$$u=48\cdot47 + 0\cdot18\, x_1 + 0\cdot98\, x_2.$$

Testing the significance of the partial regression coefficients, we find that the regression coefficient of x_1 is not significant, but that for x_2 highly significant.

TABLE I

Fortnightly Averages

Month	Fortnight	LD-50	b	Temperature F	R. Humidity %
January ..	1st	122·8	8·27	81·1	66·7
	2nd	117·4	8·48	81·7	70·1
February ..	1st	117·7	7·26	82·8	66·4
	2nd	126·0	7·37	83·9	65·2
March ..	1st	123·1	7·22	84·3	65·1
	2nd	130·2	7·97	85·0	63·3
April ..	1st	134·2	8·56	83·7	67·6
	2nd	126·5	9·52	83·4	69·9
May ..	1st	137·2	8·54	85·8	68·9
	2nd	130·3	7·90	87·1	75·4
June ..	1st	137·1	9·92	88·0	76·3
	2nd	139·6	9·23	88·6	84·9
July ..	1st	133·9	8·77	87·3	87·1
	2nd	156·6	9·64	87·4	86·3
August ..	1st	156·5	10·13	86·3	88·2
	2nd	151·6	11·20	86·0	89·3
September ..	1st	163·6	8·94	86·1	89·1
	2nd	157·9	10·50	85·3	87·5
October ..	1st	156·1	10·09	83·8	82·0
	2nd	142·3	10·46	83·4	73·0
November ..	1st	133·5	9·12	83·7	70·5
	2nd	126·0	8·44	83·5	67·0
December ..	1st	125·3	7·02	83·2	68·5
	2nd	127·0	7·60	82·9	68·0

TABLE 2

Analysis of Variance

Source of variation	d.f.	LD-50			'b'			Temperature			R. Humidity		
		S.S.	M.S.	F	S.S.	M.S.	F	S.S.	M.S.	F	S.S.	M.S.	F
Between fortnights ..	23	32426	1410	5·9**	224·5	9·76	1·74*	684	29·7	17·2**	14378	625	13·6**
Within fortnights ..	153	36528	239	..	860·6	5·62	..	264	1·7	..	6964	46	..
Total ..	176	68954	..	..	1085·1	..	..	948	..	..	21342	..	..

TABLE 3

Analysis of covariance of LD-50 by taking temperature °F (x_1) and relative humidity % (x_2) as concoммittant variates.

Source of variation	d.f.	Σx_1^2	Σx_2^2	$\Sigma x_1 y$	$\Sigma x_2 y$	Σy^2	b_{yx_1}	b_{yx_2}	$b_{x_1x_2}$	$b_{x_2x_1x_2}$	$b_{yx_1 \cdot x_2}$	$b_{yx_2 \cdot x_1}$
Between fortnights	23	683·6	14378	2401·8	17862	32426	..	..	..	..	..	..
Within fortnights	153	264·4	6964	—500·3	3278	36528	—1·89	·47	—·036	—·95	—1·49	0·42
Total ..	176	948·0	21342	1901·5	21140	68954	2·01	·99	·083	1·88	0·18	0·98

Source of variation	d.f.	Σy^2	$b_{yx_1 \cdot x_2}\Sigma yx_1$	$b_{yx_2 \cdot x_1}\Sigma yx_2$	$\Sigma y'^2$	d.f.	F.
Between fortnights	23	32426·30	..	..	13488·81	23	2·57**
Within fortnights	153	36528·12	745·49	1376·74	34405·89	151	..
Total ..	176	68954·42	342·24	20717·45	47894·70	174	..

TABLE 4

Analysis of covariance of 'b' the regression coefficient by taking temperature °F (x_1) and R. Humidity-% (x_2) as concomittant variates

Source of variation	d.f.	Σx_1^2	Σx_2^2	$\Sigma x_1 y$	$\Sigma x_2 y$	Σy^2	b_{yx_1}	b_{yx_2}	$b_{x_1x_2}$	$b_{x_2x_1}$
Between fortnights	23	683·55	14378·45	138·32	1266·37	224·54	..	..	..	..
Within fortnights	153	264·35	6963	—9·56	—537·70	860·55	—0·036	—0·077	—0·036	—0·95
Total ..	176	947·90	21342·29	128·76	728·67	1085·09	0·136	0·034	0·083	1·88

Source of variation	d.f.	$b_{yx_1 \cdot x_2}$	$b_{yx_2 \cdot x_1}$	$b_{yx_1 \cdot x_2} \Sigma yx$	$b_{yx_2} \cdot \Sigma xyx_2$	Σyl^2	d.f.	
Between fortnights	23	..	..	..	..	229·16	23	1·80*
Within fortnights	153	—0·113	—0·081	1·08	43·55	815·92	151	..
Total ..	176	0·158	0·027	20·34	19·67	1045·08	174	

The partial regression equation of 'b' on x_1 and x_2 is found to be—

$$b=6\cdot57 + 0\cdot16\ x_1 + 0\cdot03\ x_2$$

Testing the significance of the partial regression coefficients of x_1 and x_2, we find that the coefficient of x_1, *i.e.*, temperature is significant, but the coefficient of x_2, *i.e.* Relative humidity is not significant. However, the coefficient of x_1, *i.e*, 0·16, though significant, is small.

We thus find that 'b' is affected but little by variations in temperature and humidity, but not so the LD-50. LD-50 is significantly affected by variations in relative humidity, and it is affected, though not significantly, by variations in temperature. The explanation of the failure of the variations in temperature to exert a significant influence on the variations in LD-50 is perhaps to be found in the fact that temperature fluctuated within a narrow range only.

Discussion

The analysis of covariance carried out on LD-50 where temperature and relative humidity were taken as concommittant variates establishes that there are causes, other than temperature and humidity, that affect mortality. These causes vary over the fortnights and cause the LD-50 figures to vary over the fortnights. These unknown causes that are significant in their combined action are lumped together as seasonal causes. In other words, there are seasonal variations in the mortality of *C. Fatigans* exposed to DDT.

It has been established by other workers[3—9] that factors, *e.g.*, surface of application, mode of application, the constancy or otherwise of temperature and humidity, and in the latter case whether the changed conditions are encountered before exposure or after exposure to the insecticide or poison, whether there is a rise or a fall in the temperature and humidity before exposure or after exposure, the type of insect, the insecticide, etc. greatly influence mortalities. Hence, the variations noted are strictly applicable to the conditions under which the experiments have been carried out. Without further experimentation, it is not possible to say whether the same conclusions are applicable when the conditions, *e.g.*, mode of application, surface of application, etc. are changed.

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