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Effects of Some Heavy Metals on the Life-Table Parameters of *Anopheles stephensi* (Liston) (Diptera: Culicidae)

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Abstract
The exposure sub lethal doses of three heavy metals, Cd, Fe and Mn as solutions of $CdCl_2$, $FeCl_3$ and $MnCl_2$ resulted a variable increase in mortalities of various life stages (L_1-L_4, pupa), adult emergence and generation morality (k-factor). On the other hand, the developmental duration of various larval stages was declined significantly in early or late instars. Beside toxic and adverse effects of Cd, Fe and Mn salts on survival and longevity, stress of these heavy metals was found as accelerating factor for the larval development.
Key words: *Anopheles, Cadmium, Iron, Manganese, Life table.*

Introduction

Because mosquitoes are close enemies of human being, immense studies have been centred on them. *A. stephensi* is major vector of malaria. Mosquitoes well adapt for terrestrial as well as aquatic mode of life in adult and larval forms respectively, hence affected by various environmental elements in both niches. Heavy metals are leached in environment naturally and by man activities. Toxicity of several heavy metals has been studied by many workers on different animals including mosquitoes but no record is available on sub lethal effects of heavy metals on mosquitoes. Therefore sub lethal doses of Cd, Fe and Mn were selected for Life table analysis.

Materials and method

Larvae of *A. stephensi* were collected from natural sites and reared in laboratory. Emerging adults were identified using keys by Roy and Brown (1987) and Das *et al.* (1990) as well as with the help of Scientists of the National Institute of Communicable Diseases, Delhi and pure culture was maintained using methods described by Ansari *et al.* (1977, 1978) and Roy and Brown (1987). The larvae were reared in glass and enamel trays filled with tap water. Larvae were allowed to feed on Yeast Tablets and dog biscuits (1:1. w/w). The pupae were separated by quick chilling process (Ramakrishnan *et al.*, 1963) and were kept in cages of wire frame (30x30x30cm) covered with muslin cloth and having windows made of mosquito net. The emerging adults were provided Glucose solution (1%w/v) soaked in cotton. Additionally females were fed with blood of rabbit, introduced in cages in the evening for 12 hrs. Subsequently, three day old male and females were allowed to mate in a sex ratio of 1:2 and lay eggs for two days (Gerberg *et al.*, 1968, 70). For egg laying water filled and filter paper lined trays were placed During experiments the room temperature was maintained between 25-30^{o}C and sufficient humidity was maintained by lining cages with wet cloth.

On the basis of upper permissible limits (WHO, 1971, 1984; IS: 10500-1983) following concentrations of Cd, Fe and Mn were prepared using molecular weight of metal ions present in salts $CdCl_2$, $MnCl_2.4H_2O$ and $FeCl_3$ for the treatment of larval stages.

Metal	Treatment (ppm)		
	1	2	3
Cadmium	0.001	0.005	0.01
Manganese	0.1	0.5	1.0
Iron	0.1	1.0	2.0

The experiment was initiated by allowing the females to lay eggs in these solutions separately. The eggs were sorted out and released in plastic cups in group of hundred eggs each to be reared. The eggs laid in freshwater constituted the controls. Five replicates of each treatment and controls were run.

The mean duration required by each immature stage (egg, L_1-L_4=larval instars and pupa) for transforming into the next stage of the development were determined visually up to L_2 stage and thereafter by counting the presence of exhuvia and measurements of head capsules of the larvae (Raina, 1970 and Lefroy, 1990) To determine the survival values, mortality rates and *k*-values, the average number of eggs hatching into L_1 stage and the number larvae changing into subsequent stages were recorded under various treatments and control conditions. Various life-table parameters were calculated using the methods described by Odum (1983) and Krebs (1985):

$$l_x = \frac{n_x}{n_0}$$

$$d_x = n_x - (n_{x+1})$$

$$q_x = \frac{d_x}{n_x}$$

Where,

x = Age interval

n_0 = Number of individuals at the beginning of the experiment.

n_x = Observed number of alive individuals at the start of age interval x, same as (n_{xi})

n_{x+1} = Observed number of alive individuals in next (x+1) stage, same as (n_{xi+1})

l_x = Proportion surviving to start of age interval x.

d_x = Number of organisms dying within age interval x to x+1.

q_{xi} = Rate of mortality during age interval x_i to x_{i+1}

To determine the absolute numbers of larvae and pupae each day (q_{xd}) the following equations (Service, 1973 and 1976) were used:

$$q_{xd} = 1 - \sqrt[d]{\frac{n_{xi}}{n_{xi-1}}}$$

Where,

d = duration of instars (xi+1-xi)

n_{xi} = Observed no of alive individual of i stage at start of age x.

n_{xi-1}= Observed number of alive individual of previous (i-1) instars.

q_{xd} = Proportion of individual dying per day (d) at age x in corresponding instars.

The '*k-values*' were calculated for all immature stages separately reared under various treatments and control conditions by the equations described Morris (1963), Varely *et al.* (1973) and Aniedu *et al.* (1993), as follows :

$$k = log\ (n_{xi}) - log\ (n_{xi+1})$$

Where,

K = Instantaneous mortality coefficient.

n_{xi} = Number of individual at start of i stage.

n_{xi+1}= Numbers of individual in start of i+1 stage.

Subsequently, the 'generation mortality' ('K') was calculated by summation of all 'K' values separately for all the life-tables:

$$K = \sum_{iL}^{iI} k$$

Where,

iI = Initial instars or stage in life-table.

iL = Last stage or instars in life-table.

Results

Durations of immature stages

The immature stages of *A. stephensi*, reared under exposures of $CdCl_2$ revealed that the developmental period of L_1 stage was significantly reduced to 1.4 days ($P<0.01$) in comparison to controls (2.0 d) at the highest concentration (0.01 ppm) of $CdCl_2$ only. The duration of other immature stages were identical to those of the controls (Table 1). The total developmental period, hence, completed in 13.5, 12.9 and 12.8 days at

three Cd-exposures (0.001, 0.005 and 0.01 ppm respectively), however, all were statistically identical to that of control.

The developmental stages of A. stephensi treated with $FeCl_3$ were also differentially affected (Table 1). The time required for egg hatching was not effected in comparison to controls. The duration of L_1 (1.9 d) was significantly reduced ($P<0.05$) at 0.1 ppm treatment. Incidentally, the duration of L_3 stage at 2.0 ppm was significantly prolonged ($P<0.05$) vis-à-vis that of control. A severe decline in the duration of the fourth instar larvae (L_4) ($P<0.01$) was noticed at all the three concentrations of $FeCl_3$, although the pupal duration was not affected. The total duration of development also reduced significantly ($P<0.01$) at all Fe-treatments, as the total larval developmental period (L_1-L_4) declined significantly ($P<0.01$ or $P<0.05$) at all three treatments when compared with those of control (Table-1, Fig 1).

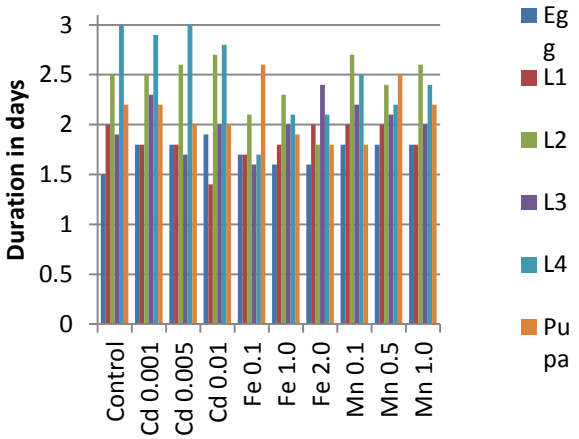
Table 1: Duration (in days) of developmental stages of *A. stephensi* under various treatments

Stage/ Treatment	Egg	L_1	L_2	L_3	L_4	Pupa	Total L_1-L_4	Total Days
Control	1.6 ±0.1	2.0 ±0.1	2.5 ±0.2	1.9 ±0.2	3.0 ±0.3	2.2 ±0.2	9.4 ±0.4	13.3 ±0.3
$CdCl_2$ 0.001 ppm	1.8 ±0.0	1.8 ±0.1	2.5 ±0.2	2.3 ±0.2	2.9 ±0.3	2.2 ±0.3	9.4 ±0.4	13.5 ±0.3
$CdCl_2$ 0.005 ppm	1.8 ±0.0	1.8 ±0.1	2.6 ±0.2	1.7 ±0.1	3.0 ±0.3	2.0 ±0.1	9.1 ±0.2	12.9 ±0.3
$CdCl_2$ 0.01 ppm	1.8 ±0.0	1.4# ±0.1	2.7 ±0.3	2.0 ±0.3	2.8 ±0.2	2.0 ±0.0	8.9 ±0.4	12.8 ±0.4
$FeCl_3$ 0.1 ppm	1.8 ±0.1	1.7* ±0.1	2.1 ±0.2	1.6 ±0.2	1.7# ±0.2	2.6 ±0.5	7.1# ±0.1	11.4# ±0.4
$FeCl_3$ 1.0 ppm	1.6 ±0.0	1.8 ±0.2	2.3 ±0.4	2.0 ±0.0	2.1# ±0.2	1.9 ±0.0	8.2# ±0.3	11.7# ±0.3
$FeCl_3$ 2.0 ppm	1.6 ±0.0	2.0 ±0.1	1.8 ±0.2	2.4* ±0.2	2.1# ±0.1	1.8 ±0.4	8.4 ±0.4	11.8# ±0.4
$MnCl_2$ 0.1 ppm	1.7 ±0.2	2.0 ±0.1	2.7 ±0.2	2.2 ±0.1	2.5# ±0.3	1.8 ±0.1	9.4 ±0.2	13.0 ±0.3
$MnCl_2$ 0.1 ppm	1.8 ±0.0	2.0 ±0.1	2.4 ±0.2	2.1 ±0.1	2.2# ±0.1	2.5 ±0.2	8.6 ±0.4	13.0 ±0.3
$MnCl_2$ 0.1 ppm	1.8 ±0.0	1.8 ±0.1	2.6 ±0.2	2.0 ±0.2	2.4# ±0.2	2.2 ±0.1	8.8 ±0.4	12.8 ±0.3
'F' value	1.581	2.159	1.4887	2.468	11.340	1.630	5.758	4.374
CD 1%	--	0.5	--	0.6	0.5	--	1.2	1.3
5%	--	0.3	--	0.5	0.4	--	0.9	1.0

* Significantly differ at 5% from control

\# Significantly differ at 1% from control

Fig 1: Duration of development at various treatments



The observations on the effect of various concentrations of $MnCl_2$ on the developmental duration of the various stages revealed that only the duration of L_4 stage severely declined ($P<0.01$) at all the concentrations, while the duration of other instars remained unchanged.

Stage-specific Survival, Mortality rates and *'k-values'* (Stage-specific Life-tables)

The life-tables of the immature stages of *A. stephensi* exposed to the three levels of $CdCl_2$ (Tables 3-5) revealed almost identical hatchability of eggs. The l_x values of the larval stages reared under all the three treatments of $CdCl_2$ were almost identical to those of controls (65.0%) (Table 2) The values of stage-specific mortality (q_{Xi}) of eggs (20% at 0.001 ppm of $CdCl_2$) (Tables 3-5) and pupae (28.2% at 0.005 ppm and 30.2% at 0.01 ppm concentration of $CdCl_2$ were significantly higher than those of controls (14.6%). The daily age-specific fractional mortalities-rates (q_{xd}) at all the concentrations of $CdCl_2$ revealed a trend identical to those of controls (Tables 2-5) with higher daily mortalities in pupae and eggs although there were slight variations in the extent of the mortality under various treatments. The daily pupal mortality at 0.01 ppm (16.5%) and 0.005 ppm (15.3%) treatments of $CdCl_2$ were higher than those recorded either in controls or at 0.001 ppm treatments of $CdCl_2$.

Table 2: Life-Table of *A. stephensi* under control conditions

Life stages (i)	Initial age (x)	n_x	l_x	d_x	q_x	q_{xd}	k-value
Egg	0	500	1.000	73	0.146	0.100	0.069
L_1	1.5	427	0.854	4	0.009	0.005	0.004
L_2	3.5	423	0.846	9	0.021	0.009	0.009
L_3	6	414	0.828	5	0.012	0.006	0.005
L_4	7.9	409	0.818	28	0.068	0.023	0.031
P	10.9	381	0.762	56	0.147	0.070	0.069
A	13.1	325	0.650			K=0.187	

The calculations of 'key-factors' (*k-values*) at various stages of *A. stephensi* reared under the three concentrations of $CdCl_2$ revealed the mortalities in the eggs and pupae as the important *'killer-factor'* which could determine the size of the emerging adult population. The sum-total of the k-values (Total generation mortality: K) of the immature stages exposed to 0.001, 0.005 and 0.01 ppm of $CdCl_2$ were significantly higher than those of the controls (K=0.187), indicating an overall killer-effect of $CdCl_2$ (Table 2-5).

Table 3: Life-Table of *A. stephensi* under exposure of Cd 0.001 ppm

Life stages (i)	Initial age (x)	n_x	l_x	d_x	q_x	q_{xd}	k-value
Egg	0	500	1.000	100	0.200	0.117	0.097
L_1	1.8	400	0.800	0	0.000	0.000	0.000
L_2	3.6	400	0.800	25	0.063	0.025	0.028
L_3	6.1	375	0.750	13	0.035	0.015	0.015
L_4	8.4	362	0.724	40	0.110	0.040	0.051
P	11.3	322	0.644	48	0.149	0.071	0.070
A	13.5	274	0.548			K=0.261	

Table 4: Life-Table of *A. stephensi* under exposure of Cd 0.005 ppm

Life stages (i)	Initial age (x)	n_x	l_x	d_x	q_x	q_{xd}	k-value
Egg	0	500	1.000	76	0.152	0.088	0.072
L_1	1.8	424	0.848	6	0.014	0.008	0.006
L_2	3.6	418	0.836	3	0.007	0.003	0.003
L_3	6.2	415	0.830	7	0.017	0.010	0.007
L_4	7.9	408	0.816	25	0.061	0.021	0.027
P	10.9	383	0.766	108	0.282	0.153	0.144
A	12.9	275	0.550			K=0.260	

Table 5: Life-Table of *A. stephensi* under exposure of Cd 0.01 ppm

Life stages (i)	Initial age (x)	n_x	l_x	d_x	q_x	q_{xd}	k-value
Egg	0	500	1.000	78	0.156	0.085	0.074
L_1	1.9	422	0.844	14	0.033	0.020	0.015
L_2	3.6	408	0.816	20	0.049	0.033	0.022
L_3	5.1	388	0.776	13	0.034	0.016	0.015
L_4	7.2	375	0.750	30	0.080	0.039	0.036
P	9.3	345	0.690	16	0.046	0.023	0.021
A	11.3	329	0.658			K=0.282	

The life-table parameter of *A. stephensi* exposed to $FeCl_3$ indicated (Tables 6-8) that the final survival (l_x) at 2.0 ppm of $FeCl_3$ (60.4%) declined in comparison to that of control (65.0%). In L_4 stage also the highest concentration of $FeCl_3$ considerably reduced the survival 76.0% *vis-a-vis* control (81.8%). (Table 2) The egg hatchability was not affected at all. Relative instar mortality (q_{xi}) during the course of development in *A. stephensi* exposed to 0.1 and 2.0 ppm level of $FeCl_3$ showed a considerable increase during L_3 stage (3.9 and 4.0% respectively) in comparison to controls (1.2%). At the highest concentration (2.0 ppm), however, the mortality (Table 8) was higher in eggs and L_4 stage also (18.0 and 9.5%) *vis-a-vis* their counterparts in controls. The expression of mortality in terms of proportion dying daily (q_{xd}) in different instars of *A. stephensi*, revealed an increase at L_3 and L_4 stags only at the lowest concentration (0.1 ppm) but in the cases of pupae this concentration lowered the daily mortality levels (4.1%; Table 6). At 1.0 ppm and 2.0 ppm concentrations of $FeCl_3$ the q_{xd} values for others stages were almost similar to those recorded under control conditions.

Table 6: Life-Table of *A. stephensi* under exposure of Fe 0.1 ppm

Life stages (i)	Initial age (x)	n_x	l_x	d_x	q_x	q_{xd}	k-value
Egg	0	500	1.000	77	0.154	0.094	0.073
L_1	1.7	423	0.846	4	0.009	0.006	0.004
L_2	3.4	419	0.838	5	0.012	0.006	0.005
L_3	5.5	414	0.828	16	0.039	0.024	0.017
L_4	7.1	398	0.796	31	0.078	0.047	0.035
P	8.8	367	0.734	38	0.104	0.041	0.047
A	11.4	329	0.658	-	-	- **K=0.182**	

The *'key-factor'* analyses for the immature stages of *A. stephensi* exposed to various concentrations of $FeCl_3$ revealed higher values for eggs at 0.1 and 2.0 ppm levels of $FeCl_3$ in comparison to those of controls. During L_4 stage the *k-value* was higher at 2.0 ppm level of $FeCl_3$ (0.043) only. In pupa, however, lower *k-values* were recorded at the lowest (0.1 ppm) and highest (2.0 ppm) concentrations of $FeCl_3$. The total generation mortality (*k*) was considerably higher at 2.0 ppm treatment with $FeCl_3$ (0.219) as compared to that of control (0.187)

Table 7: Life-Table of *A. stephensi* under exposure of Fe 1.0 ppm

Life stages (i)	Initial age (x)	nx	lx	dx	qx	qxd	k-value
Egg	0	500	1.000	69	0.138	0.089	0.064
L1	1.6	431	0.862	3	0.007	0.004	0.003
L2	3.4	428	0.856	11	0.026	0.011	0.011
L3	5.7	417	0.834	10	0.024	0.012	0.011
L4	7.7	407	0.814	24	0.059	0.029	0.026
P	9.8	383	0.766	59	0.154	0.084	0.073
A	11.7	324	0.648	-	-	-	**K=0.182**

Table 8: Life-Table of *A. stephensi* under exposure of Fe 2.0 ppm

Life stages (i)	Initial age (x)	n_x	l_x	d_x	q_x	q_{xd}	k-value
Egg	0	500	1.000	90	0.180	0.117	0.068
L_1	1.6	410	0.820	4	0.010	0.005	0.004
L_2	3.6	406	0.812	10	0.025	0.014	0.011
L_3	5.4	396	0.792	16	0.040	0.017	0.018
L_4	7.8	380	0.760	36	0.095	0.046	0.043
P	9.9	344	0.688	42	0.122	0.070	0.057
A	11.7	302	0.604	-	-	-	**K=0.219**

Table 9: Life-Table of *A. stephensi* under exposure of Mn 0.1 ppm

Life stages (i)	Initial age (x)	n_x	l_x	d_x	q_x	q_{xd}	k-value
Egg	0	500	1.000	77	0.154	0.106	0.073
L_1	1.8	423	0.846	0	0.000	0.000	0.000
L_2	3.8	423	0.846	21	0.050	0.020	0.022
L_3	6.5	402	0.804	7	0.017	0.009	0.008
L_4	9.0	395	0.790	18	0.046	0.015	0.020
P	10.8	377	0.754	61	0.162	0.077	0.077
A	13.1	316	0.632			K=0.199	

The life-tables characteristics of *A. stephensi* exposed to $MnCl_2$ revealed (Table 9-11) a feebly declining trend in l_x values from L_1 to adult stages exposed to 1.0 ppm concentration of $MnCl_2$ (Table 11) in comparison to controls (Table 2). The other concentrations of $MnCl_2$ did not significantly alter the survival or any other life-table parameter in comparison to controls. Individuals, exposed to 1.0 ppm concentration of $MnCl_2$ revealed higher mortality in eggs (q_{xi}=0.214) and lower mortality in pupae (q_{xi} = 0.090) *vis-a-vis* their counterparts in control. Consequently, the killer effect of eggs (*k*=0.105) and the total generation mortality (*k*=0.203) were higher than those observed either in controls or in treatments with lower concentrations of $MnCl_2$.

Table 10: Life-Table of *A. stephensi* under exposure of Mn 0.5 ppm

Life stages (i)	Initial age (x)	n_x	l_x	d_x	q_x	q_{xd}	k-value
Egg	0	500	1.000	53	0.106	0.060	0.049
L_1	1.8	447	0.894	3	0.007	0.003	0.003
L_2	3.8	444	0.888	24	0.054	0.023	0.024
L_3	6.2	420	0.840	11	0.026	0.013	0.012
L_4	8.3	409	0.818	14	0.034	0.016	0.015
P	10.5	395	0.790	71	0.180	0.076	0.086
A	13	324	0.648			K=0.188	

Table 11: Life-Table of *A. stephensi* under exposure of Mn 1.0 ppm

Life stages (i)	Initial age (x)	n_x	l_x	d_x	q_x	q_{xd}	k-value
Egg	0	500	1.000	107	0.214	0.125	0.105
L_1	1.8	393	0.786	2	0.005	0.003	0.002
L_2	3.6	391	0.782	4	0.010	0.004	0.004
L_3	6.2	387	0.774	8	0.021	0.010	0.009
L_4	8.2	379	0.758	35	0.092	0.040	0.042
P	10.6	344	0.688	31	0.090	0.042	0.041
A	12.8	313	0.626			K=0.203	

Discussion

The observations revealed that under exposure of $CdCl_2$ only early stages were differently affected but $FeCl_3$ caused significant decline in developmental period. The inhibition of feeding activity, if any, (Garhardt, 1995) did not retarded its development. In case of $MnCl_2$ treatment, the decline in the duration of early instar development may be due to any change in enzymatic activity (Furneisen and Carman, 2000) or activation of Juvenile hormone biosynthesis (Sen *et al*., 1996). The induction of MT gene in presence of heavy metals, may be a factor to tolerate the stress which was expressed as decline in developmental period of larval stages differently (Sarkar *et al*. 2004)

The life-table analysis shows that $CdCl_2$ exposure increased the juvenile mortality and total generation mortality. Lethal effects of $CdCl_2$ have been reported in *C. pipens pallens* (Jiang *et al*., 1991), *A. aegypti* (Rayms keller, 1998) and May fly (Garhardt, 1995 and Courtney and Clements, 2000). Obviously mortality of immature stages of *A. stephensi* treated with $CdCl_2$ would have resulted either by the disturbance of Cl^- or K^+ ions in their body (Gerhardt, 1995) or by improper development of peritrophic matrix (Rayms-keller,

1998) or by induction of hyperpolymerization and apoptosis (Raes *et al*., 2000).

When $FeCl_3$ treatment was given, only the higher concentration (2.0 ppm) increased mortality percentage during the L_1 and L_4, therefore higher generation mortality was obtained in comparision to control. Interestingly the increased iron contents in water accelerated the development significantly (Table 1) at all concentrations, the cause of mortality in L_1 and L_4 may be due to crust formation or adversely affected food consumption (Meena Kumari and Balakrishnan, 1993; Gerhardt, 1995)

The $MnCl_2$ treatment adversely affected the survival of eggs, pupae and overall generation mortality at all the three concentrations. The larval mortality was also seen at the highest treatment concentration. It has been reported that Mn^{++} reduced Tyrosine β-glycosyl transferase activity, required for the synthesis of pupal cuticle (Ahamad *et al*., 1996) and inhibited number of phosphatases in insect cell culture (Furneisen and Carman, 2000). It reveals that Mn^{++} plays a significant role in developmental process of *A. stephensi*.

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