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Organochlorine Residue Sequestration by *Anopheles culicifacies sensu lato* Mosquito Larvae

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Anopheles culicifacies sensu lato is the major vector of rural and peri-urban malaria in India (Pattanayak et al. 1994). Residual insecticides such as HCH and DDT have been the mainstay of malaria control to interrupt transmission (Anon 1991). Due to persistent nature of organochlorine insecticides in nature and extensive use for malaria control , *A. culicifacies* became resistant to these insecticides (Pattanayak et al. 1994). Very high bioamplification of DDT by invertebrates (Johnson et al. 1971) and mosquito larvae (Metcalf et al. 1971) from water have been reported earlier which may cause resistance to DDT and HCH at the larval stages due to uptake of these residues from contaminated water. We report sequestration of HCH and DDT residues by *A. culicifacies* larvae from contaminated water of different breeding habitats.

MATERIALS AND METHODS

Anopheline mosquito breeding source surveys were carried out in Distt. Hardwar (U.P.), India and habitats with more than 50 larvae per dip were selected for the study. About 50 to 500 third and fourth instar larvae collected from each habitat (WHO 1975), brought to the laboratory and were identified . Studies were confined to *A .culicifacies* breeding habitats. *A. culicifacies* larvae from seven different habitats such as river bedpools, rice field, pits, ditches, ponds, canal and hoof prints alongwith their corresponding water samples were collected to find the HCH and DDT contaminations. Samples from different habitats were stored and analysed separately.

A .culicifacies larvae were rinsed with distilled water, dried and weighed. The larvae were washed twice with 10 ml n-hexane (AR) and filtered. The combined extract was evaporated, weighed and termed as external extract. After hexane washing, the larvae were homogenized with equal amount of anhydrous sodium sulphate and mixed thrice with n-hexane (15 ml each) in vortex mixer for 20 min and filtered. The filtrate was pooled, dried in a vacuum evaporator and termed as internal extract.

500 ml water sample from each *A culicifacies* habitat was collected in a clean 1 liter glass bottle, filtered by using Whatman filter paper No.1 and stored in the

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refrigerator. 250 ml water sample was extracted three times with 50 ml n-hexane for 10 min. in a separating funnel and n-hexane layer was pooled and dried. The external, internal larvae and water extracts were dissolved in 1 ml n-hexane and cleaned with anhydrous sodium sulphate- alumina column eluted with n-hexane-benzene (40:60). The eluant was evaporated on a vortex evaporator and kept at 4 ^{o}C until analysis.

Samples were analysed for organochlorine insecticide residues on Hewlett-Packard 5890 Gas Chromatograph fitted with Ni ^{63} electron capture detector on fused silica capillary TM ^{5} column PTE (length 30 m, 0.25 mm, id) Supelco Inc. USA. Nitrogen was used as a carrier gas @ 2 ml min^{-1} (split ratio 1:100). The injector, oven and detector temperatures were set at 210, 190 and 220 ^{o}C respectively. All water samples were analysed separately and their mean values were calculated for the determination of insecticide residues in a particular sample. Level of detection for DDT was 0.1 ng while for HCH was 0.2 ng. Below this value was termed as N.D. (not detected). The correlation between two variables were calculated by Carl Pearson method. The identities of the residues were confirmed by studying the disappearance or shifts in peak patterns in alkali-derivatized samples as compared to underivatized samples (EPA 1980).

RESULTS AND DISCUSSION

The external and internal larval extracts of *A. culicifacies* collected from river bed pools, rice field, pits, ditches, ponds, canal and hoof prints were analysed for HCH and DDT contaminations and the results are presented in Table 1. Mean HCH concentrations in external extracts from river bed pools, rice field, pits, ditches, ponds, canal and hoof prints were 9.65, 11.94, 9.42, 4.69, 11.62, 10.52 and 0.54 mg kg^{-1} respectively while in internal extracts were 17.62, 17.48, 13.21, 7.09, 18.27, 14.77 and 0.66 mg kg^{-1} respectively. β–HCH contributed maximum amount HCH isomers in all internal extract samples while γ-HCH and β-HCH were higher in external extract as compared to others. δ –HCH was undetected in all samples.

Mean DDT concentrations in external extracts from *A. culicifacies* larvae collected from river bed pools, rice field, pits, ditches, ponds, canal and hoof prints were 4.32, 7.63, 2.34, 4.79, 9.19, 7.61 and 1.40 mg kg^{-1} respectively while in internal extracts were 7.45, 11.54, 7.34, 7.21, 16.21, 10.25 and 1.81 mg kg $^{-1}$ respectively. p,p'-DDT contributed maximum in external extract samples while p,p'-DDE was found highest of the total DDT present in internal extracts.

Water samples of different *A.culicifacies* breeding habitats were also analysed for HCH and DDT contamination and their results are given in Table 2. Mean HCH residue in water from river bed pools, rice field, pits, ditches, ponds, canal and hoof prints were 8.74, 11.71, 5.34, 3.89, 10.95, 6.50 and 0.50 ug L^{-1} respectively while the DDT residues were 0.95, 8.73, 1.06, 4.14, 12.09, 5.95 and 0.92 ug L^{-1} respectively. β–HCH contributed maximum in HCH isomers while p,p'-DDT followed by p,p'-DDE accounted mainly for DDT residues.

Table 1. Mean concentrations (mg kg^{-1}) of HCH and DDT residues in *A. culicifacies* larvae from different breeding habitats

Breeding Sites		α - HCH	β - HCH	γ - HCH	δ- HCH	Total HCH	o,p'- DDE	p,p'- DDE	o,p'- DDT	p,p'- DDT	p,p'- DDD	Total DDT
River Bed	Ext	2.73	5.66	1.25	nd	9.65	nd	2.71	nd	1.61	nd	4.32
Pools	Int	4.23	12.80	0.58	nd	17.62	nd	6.67	nd	0.67	nd	7.45
Paddy	Ext	1.91	3.91	6.12	nd	11.94	nd	1.86	nd	5.48	0.27	7.63
Field	Int	2.93	10.57	3.97	nd	17.48	nd	7.14	nd	4.15	0.25	11.54
Pits	Ext	2.64	5.77	1.00	nd	9.42	nd	2.34	nd	nd	nd	2.34
	Int	3.69	9.27	0.24	nd	13.21	nd	7.12	nd	nd	nd	7.34
Dit-	Ext	0.95	3.07	0.67	nd	4.69	nd	1.27	nd	3.43	0.08	4.79
Ches	Int	1.11	5.40	0.58	nd	7.09	nd	2.95	nd	4.16	0.10	7.21
Ponds	Ext	1.78	7.99	2.09	nd	11.62	0.07	1.43	nd	6.91	0.77	9.19
	Int	3.09	13.75	1.58	nd	18.27	nd	8.21	nd	6.54	1.46	16.21
Canal	Ext	1.78	7.00	1.74	nd	10.52	nd	2.24	nd	5.37	nd	7.61
	Int	1.33	11.42	2.00	nd	14.77	nd	5 .37	nd	4.88	nd	10.25
Hoof	Ext	0.37	0.08	0.08	nd	0.54	nd	1.40	nd	nd	nd	1.40
Prints	Int	0.39	0.20	0.07	nd	0.66	nd	1.81	nd	nd	nd	1.81

Ext = External extract of the Larvae; Int = Internal extract of the Larvae; nd = Not detected

Table 2. Mean concentrations (ug L^{-1}) of HCH and DDT residues in *A. culicifacies* larvae from different breeding habitats

Breeding Sites	α- HCH	β- HCH	γ- HCH	δ- HCH	Total HCH	o,p'- DDE	p,p'- DDE	o,p'- DDT	p,p'- DDT	p,p'- DDD	Total DDT
River Bed Pools	1.11	6.35	1.07	0.20	8.74	nd	0.52	nd	0.43	nd	0.95
Paddy	1.68	2.31	6.96	0.75	11.71	nd	2.98	0.85	4.86	0.03	8.73
Pits	0.68	3.90	0.76	nd	5.34	nd	1.06	nd	nd	nd	1.06
Ditches	0.82	1.67	1.38	nd	3.89	nd	1.11	nd	2.96	0.06	4.14
Ponds	1.11	8.56	1.27	nd	10.9	0.14	1.02	0.19	9.70	1.03	12.09
Canal	0.90	4.48	1.12	nd	6.50	nd	2.83	nd	3.12	nd	5.95
Hoof	0.17	0.15	0.17	nd	0.50	nd	0.92	nd	nd	nd	0.92

nd = Not detected

Contamination of HCH and DDT residues in internal extracts from all breeding sources were found higher than external extracts. Statistically significant correlation was recorded between external and internal extracts of HCH ($r = 0.97$; $P<0.001$) and DDT residues ($r=0.92$; $P<0.001$). Kalra (1974) has also found more DDT residues in internal than external extracts. Highly significant correlation of

HCH was recorded between external extract and water ($r=0.91$; $P<0.001$) and between external extract and water ($r=0.93$ $P<0.001$). Similarly, significant correlation of DDT was found between external extract and water ($r=0.92$; $P<0.001$) and internal extract and water ($r=0.90$; $P<0.001$). A good correlation between water and larvae extract clearly implied that *A. culicifacies* larvae sequestrate these residues from contaminated water.

The biomagnification factor (the ratio of compound in bioorganism to the surrounding media) for HCH and DDT in internal extract was 1961 and 1831. Agarwal et al. (1986) have reported a biomagnification factor of 7484 for DDT in chironomus larvae from Okhla stream Delhi. Metcalf et al. (1971) determined a factor of 8000 in mosquqito larvae. The magnitude of biomagnification factor may depend upon the initial concentration of these residues in surrounding media, along with the time of contact with the larvae. β–HCH contributed maximum among HCH isomers in mosquito larvae because of its higher persistence in living organism as compared to α- and γ-HCH (Bakre et al. 1990). Recently Conchello et al. (1993) reported that β-HCH bioaccumulated more in animal tissue than other HCH isomers due to its lipophilic nature. p,p'-DDT contributed maximum of total DDT contents while p,p'-DDE in the mosquqito larvae from same breeding was detected highest. Perry (1960) reported that p,p'-DDT converted into p,p'-DDE in bioorganism in the presence of an enzyme dehydrochlorinase. Moreover, p,p'-DDE is more persistent than p,p'-DDT or p,p'-DDD (Aquialar, 1984).

Present study revealed that when mosquito larvae of *A. culicifacies* come in direct contact with HCH and DDT contaminated breeding habitats, they may sequestrate large amounts of these pesticides. This may help in developing resistance in aquatic stages without coming in direct contact with residual spraying. It is to point out that about 80% *A.culicifacies* population from this area was found resistant to HCH and DDT (Dua et al, 1988). Beard (1952) stated that insecticidal exposure for longer time will eliminate all susceptible larvae and will permit only the resistant ones.

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