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EFFECT OF DICHLORVOS ON THE BRAIN AND FAT BODY CELLS OF THE ADULT *MUSCA DOMESTICA* L. (MUSCIDAE : DIPTERA)

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Abstract: LD_{50} for dichlorvos was calculated to be 11.8 ppm after 24 hours of treatment. Of all the components of the brain the neurons were the most affected. They showed hypertrophy and pyknosis which ultimately led to the histolysis of the cells. Glial cells, although, not so severely affected, changed in their nuclear contents to some extent. Neural lamella thickened irregularly after treatment. The neuropile along with its glomerular bodies underwent vacuolization. The plasma membranes of the fat body cells ruptured after extensive vacuolization and the cytoplasmic content almost disappeared. The chromatin material darkened and became concentrated in the middle of the cells.

Key words: Insecticide toxicity, organophosphate, housefly, LD_{50}, cell morphology.

INTRODUCTION

Musca domestica (housefly) is a cosmopolitan non-biting muscid fly. Its abundance varies seasonally throughout the year. It oviposits on a variety of decomposing materials, such as fruits, vegetables, animal viscera, marine animals etc (D'Almedia and Mayio, 1993). Usually some 500-1000 eggs are deposited by a female during its lifetime, while its life cycle may be completed within 11 to 49 days depending upon the temperature. It can transmit a large number of diseases to man. Over 100 species of pathogens have been recorded as being carried by it (Service, 1980), some of them such as diarrhoea can be fatal especially for children.

In order to eliminate this pest, various methods can be undertaken; physical and mechanical control, environmental sanitation, biological and chemical control. The chemical control is the most popular method ranging from chlorinated hydrocarbons, organophosphates, carbamates and pyrethroids.

Generally, pesticides have a specific mode of action and toxicity (Corbett, 1974; Matsumura, 1975). The organophosphates can be categorized as stomach and systemic poisons, some also as abrasive compounds and contact piosons, while others as fumigants (Hassal, 1983). They primarily act upon the nervous system. An important feature of this group is that different members possess very different physicochemical

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properties, in particular, they have different vapour pressure at room temperature and different stabilities in water. They also vary considerably in the chemical stability and their toxicity to mammals (Hassall, 1983).

Dichlorvos which was used in the present study is an organophosphate compound. It is regarded as a low persistance, contact poison with a vapour pressure sufficiently high to enable it to act in the vapour phase, *e.g.* for protection of stored products. It decomposes rapidly in animals, and consequently has less harmful effects (Hassall, 1983).

present study its effect on the different components of the brain of *Musca domes* as been studied.

MATERIALS AND METHODS

Musca domestica, used for the present research work, were collected and reared as described previously (Sharif and Ali, 1996). The newly emerged flies were kept separately and fed on a diet of milk-sugar solution. This was to maintain the uniformity of the flies to be used for experimental purposes.

Insecticide and its treatment

Dichlorvos which is sold under the trade name of Nogos was obtained in commercial formulation of 50EC (Emulsifiable concentration) from Ciba Geigy. It is a contact and stomach poison with fumigant action, and is used specifically for the control of houseflies (Hassal, 1993). The insecticide was diluted five time in distilled water and was used as the stock solution. This was further diluted to get 5 ppm and 10 ppm solution. The adult flies were kept in batches of 10 each in glass jars. Insecticidal dose was given to the insects by pouring 1 ml of it over the sugar granules given as food. About 1 g of sugar was used for each batch. Control insects were given equal amount of distilled water mixed in 1 g of sugar. The flies were taken out at intervals of 1 hour (10 ppm) and 2 hour (5 ppm) after dichlorvos treatment for histological preparations. LD_{50} was calculated by probit analysis (Finney, 1952; Busvine, 1971).

Histological studies

The head was fixed in Bouin's fixative, dehydrated in ascending grades of alcohol, cleared in cedar wood oil and embedded in paraffin wax. Serial sections 8-10 μm thick were cut with a rotary microtome. Sections were stained in hematoxyline and counter stained with eosin.

RESULTS

The toxic effect of dichlorvos (5 ppm and 10 ppm) on the histology of the brain was studied after various time intervals. The earlier signs of the syndrome appeared

shortly after the treatment and the intensity increased with prolongation.

Cortex

The general sensory neurons and the few motor neurons found in the brain show hypertrophy and their chromatin contents start clumping thus forming irregular lumps near the periphery of the nuclei. The effects of treatment with 5 ppm after 6 hours and with 10 ppm after 3 hours respectively (Tables I and II), are almost the same but the brains of the insects treated with the higher dose show extensive cellular histolysis and ultimate disintegration.

Motor neurons

These cells show some increase in the size of nuclei after treatment. However, the nuclear contents undergo the most conspicuous changes, starting with the clumping of the chromatin material and irregular lump formation around the inner side of the nuclear membrane. Treatment with 5 ppm after 6 hours and 10 ppm after 3 hours results in intense histolysis and ultimately disintegration of the cellular contents.

Sensory neurons

Optic ganglion neurons

These cells show significant increase in size. Their cell boundaries become distinct and nuclei start undergoing pyknosis after 3 and 6 hours when treated with 10 ppm and 5 ppm of the insecticide respectively. The chromatin forms clumps in most of the optic neurons.

Globuli cells of corpora pedunculata

The globuli cells also show considerable hypertrophy and necrosis. The cell boundaries of these neurones also became very clearly defined after 2 and 4 hours of treatment with 10 and 5 ppm respectively. the chromatin material becomes scattered which later on becomes scarce, the nucleoli also became more conspicuous.

General sensory cells

These cells also become considerably hypertrophied with significant increase in the size of the nuclei which is accompanied by necrosis. However, unlike motor neurons none of the sensory type cells undergo complete histolysis and disintegration. The contents of the nuclei show similar charges as that of the other neurons.

Table I: **Dimensions of nuclei of different brain cells in control of *Musca domestica* treated with 5 ppm dichlorvos for 6 hours (each reading is a mean of 30 values)**

Cell types	Control		Dichlorvos treatment (hours)					
			2		4		6	
	L	W	L	W	L	W	L	W
Glial Type I	5.50[a] ±0.68	3.00 ±0.71	6.30 ±0.87	3.00 ±0.55	7.00 ±0.55	3.00 ±0.55	7.00 ±1.11	3.00 ±1.04
Glial Type II	3.00 ±0.88	3.00 ±0.68	3.00 ±0.68	3.00 ±0.32	5.00 ±0.88	4.00 ±0.68	4.00 ±0.96	3.00 ±0.31
Glial Type III	15.50 ±2.73	10.00 ±0.88	19.50 ±4.80	14.00 ±3.30	15.50 ±2.73	11.00 ±2.65	14.00 ±2.36	11.00 ±1.44
Glial Type IV	5.00 ±0.55	2.00 ±0.27	5.00 ±1.04	4.00 ±0.17	5.00 ±0.63	2.50 ±0.00	6.00 ±1.44	3.00 ±0.28
Motor neurons	10.50 ±3.60	8.00 ±1.60	12.00 ±1.85	9.50 ±2.09	11.00 ±2.85	9.50 ±2.09	12.00 ±1.89	9.90 ±1.53
Optic ganglion neuron	2.50 ±0.55	2.00 ±0.31	3.00 ±0.22	3.00 ±0.27	2.50 ±1.11	3.00 ±0.22	4.50 ±0.75	4.50 ±0.74
Globuli cells	3.00 ±0.36	3.00 ±0.55	4.00 ±0.29	3.00 ±0.62	5.00 ±0.35	3.50 ±0.41	6.00 ±0.88	5.00 ±0.55
General sensory cells	3.00 ±0.95	3.00 ±0.32	3.00 ±0.45	3.00 ±0.29	6.00 ±0.94	5.50 ±0.72	7.00 ±1.22	5.00 ±0.55
Neurosecretory cells	10.00 ±3.11	6.00 ±1.25	11.00 ±3.22	8.00 ±2.39	10.00 ±2.23	9.00 ±1.36	11.00 ±1.44	8.00 ±1.44
Fat body cell nuclei	8.50 ±2.85	7.60 ±2.50	11.00 ±1.25	9.00 ±1.44	11.50 ±1.36	10.00 ±1.76	16.00 ±3.75	14.50 ±3.60

[a]Mean ± SEM

Abbreviations used: L, length; W, weight.

Table II: **Dimensions of the nuclei of different brain cells in control of *Musca domestica* treated with 10 ppm dichlorvos for 3 hours (each value is a mean of 30)**

Cell types	Control		Dichlorvos treatment (hours)					
			1		2		3	
	L	W	L	W	L	W	L	W
Glial Type I	5.05[a] ±0.68	3.00 ±0.71	5.00 ±0.55	3.30 ±0.68	7.00 ±1.67	3.50 ±1.85	6.00 ±0.88	2.00 ±0.68
Glial Type II	3.00 ±0.88	3.00 ±0.68	7.00 ±0.72	6.00 ±0.72	6.00 ±0.88	5.50 ±0.68	8.00 ±1.42	6.50 ±2.23
Glial Type III	15.50 ±2.73	10.00 ±0.88	20.50 ±4.10	10.00 ±2.88	18.00 ±4.80	14.00 ±3.95	23.00 ±5.70	17.00 ±6.47
Glial Type IV	5.00 ±0.55	2.00 ±0.27	5.50 ±0.35	2.50 ±0.22	5.50 ±1.11	3.00 ±0.68	6.00 ±1.11	2.00 ±0.55
Motor neurons	10.50 ±3.60	8.00 ±1.60	9.00 ±2.09	8.00 ±1.21	9.00 ±0.88	8.00 ±0.55	10.00 ±1.76	7.00 ±1.62
Optic ganglion neuron	2.50 ±0.55	2.00 ±0.31	2.50 ±0.22	2.50 ±0.00	3.00 ±0.86	3.00 ±0.68	4.00 ±1.46	3.00 ±1.03
Globuli cells	3.00 ±0.36	3.00 ±0.55	5.57 ±0.68	5.00 ±0.87	5.50 ±0.68	4.50 ±0.78	6.00 ±1.04	5.50 ±1.21
General sensory cells	3.00 ±0.95	3.00 ±0.32	4.00 ±0.80	3.50 ±1.20	5.50 ±1.17	5.00 ±1.54	7.00 ±1.85	5.00 ±1.67
Neurosecretory cells	10.00 ±3.11	6.00 ±1.25	11.00 ±1.62	10.00 ±0.55	8.00 ±1.11	8.00 ±1.11	12.00 ±2.59	8.50 ±1.36
Fat body cell nuclei	8.50 ±2.85	7.60 ±2.50	14.00 ±2.36	9.00 ±1.44	13.50 ±1.36	12.00 ±1.86	13.00 ±3.11	11.50 ±2.05

[a]Mean ± SEM

Abbreviations used: L, length; W, weight.

Neurosecretory cells

The effect of intoxication on these cells is more like the motor neurons. General hypertrophy, scattering of the chromatin material, which is centrally placed in the control insects, and extensive vacuolization of the cytoplasm happens soon after treatment. All ends in more or less complete histolysis.

Glial cells

The glial type I cells also show slight increase in size while the neural lamella along which these cells are located becomes thickened unevenly. The few glial type II cells identified in the present study did not show any significant change in the size. The nuclei of the type III glial cells also show no significant increase in the size. However, their chromatin material became somewhat scattered. Apart from some scattering of the chromatin material glial type IV cells also do not undergo any significant change.

Neuropile

Upto two hours of the treatment no perceptible change in the neuropile was detected. But after 2 and 4 hours of treatment with 10 ppm and 5 ppm respectively vacuolization started which gradually intensified and became considerable towards the end of the experimental period.

Fig.1: **T.S. of brain of control *Musca domestica*, showing neurosecretory cells and some other features in the region of pars intercerebralis. a, axons; NC, neurosecretory cell; n1, neural lamella; N, neuropile. 40 x.**

Fig.2: **T.S. of brain of *Musca domestica*, showing motor neurones with scattered chromatin material (5 ppm dichlorvos, 2 hours after treatment). n, nucleus; cyt, cytoplasm; mn, motor neurones. 100 x.**

Fig.3: **T.S. of brain of *Musca domestica*, showing neurosecretory cell and pyknotic neurones (5 ppm dichlorvos, 4 hours after treatment). cyt, cytoplasm; n, nucleus; NS, neurosecretory cell; pn, pyknotic neurones.**

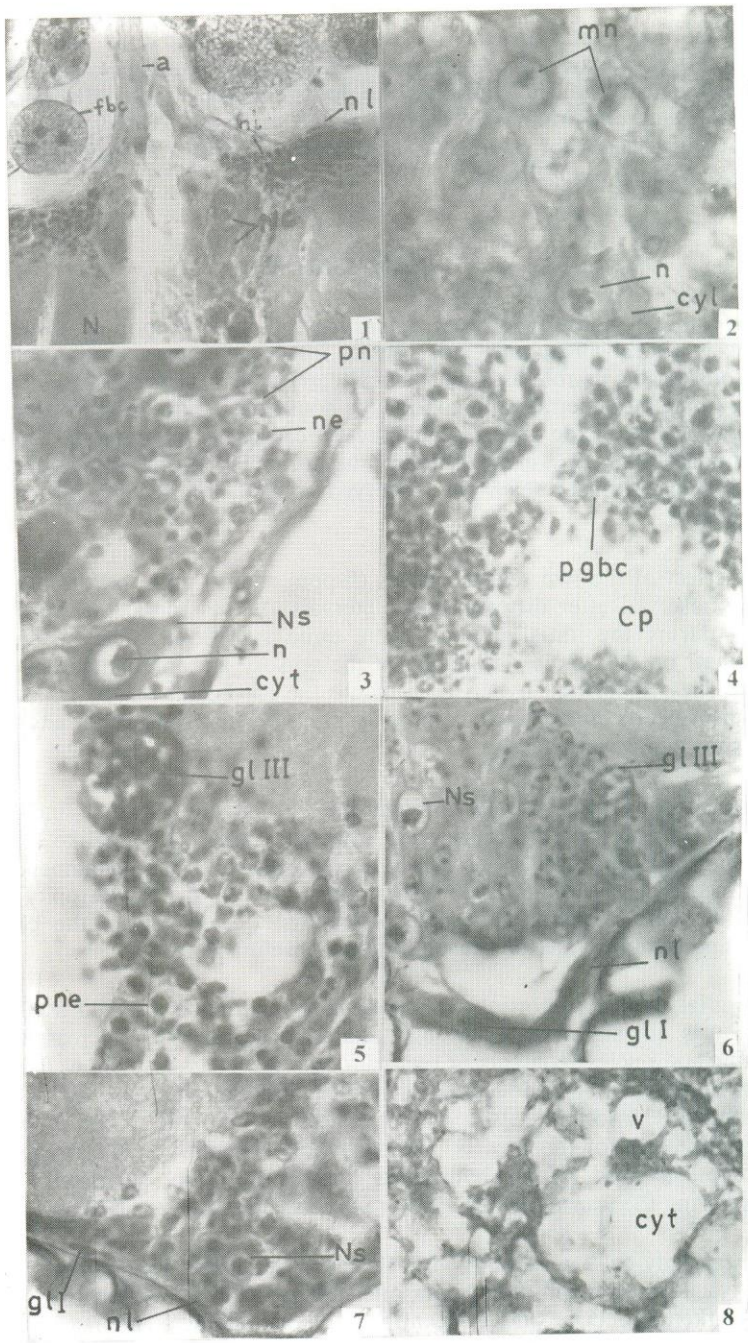
Fig.4: **Pyknotic globuli cells of experimental brain (5 ppm dichlorvos, 4 hours after treatment). cp, corpora pedunculata; gbc, globuli cells. 100 x.**

Fig.5: **Glial type III cell with clumped chromatin material (5 ppm dichlorvos, 4 hours after treatment). gl III, glial type III cells; pgsc, pyknotic general sensory cells. 100 x.**

Fig.6: **T.S. of experimental brain showing neurosecretory cells with dispersed chromatin material. Thickened perilema and glial type I cells can also be seen. (5 ppm dichlorvos, 4 hours after treatment).**

Fig.7: **T.S. of ventral portion of protocaebrum showing neurosecretory cells. Glial type II cells, Glial type I cells and reural lamella are also seen (5 ppm dichlorvos, 4 hours after treatment). NS, neurosecretory cells; gl I, glial type I cells; nl, neural lamella. 100 x.**

Fig.8: **Fat body cells of experimental insect showing vacuolated cytoplasm and ruptured cell membrane (5 ppm dichlorvos, 6 hours after treatment). cyt, cytoplasm; n, nucleus; v, vacuole. 100 x.**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Fat body cells

The fat body cells are the most pronouncely effected by the insecticide. The immediate effect was on the cytoplasm which became vacuolized at the very beginning of the treatment. Treatment with both the doses resulted in vacuolization and ruptured cell walls. The chromatin material became scarce and these cells which are multinucleate in the normal insects showed only a small mass of chromatin material thus giving the appearance of being uninucleate.

DISCUSSION

The common housefly (*Musca domestica*) has a cosmopolitan distribution and is important as a mechanical carrier of various infectious agents (Lapage, 1962; Service, 1980). Considering its role as a mechanical carrier of different diseases various control measures are being undertaken to control the houseflies. In the present study, dichlorvos was used as an insecticide to study the effects it caused on its brain and fat body. During the determination of LD_{50} after 24 hours, the normal syndrome of neural poisoning like tremors and the ultimate death was observed as also reported by earlier workers like O'Brien (1960).

The effect of the two selected doses (5 ppm and 10 ppm) on the brain and the fat body was observed after different time intervals. It was found that the results of the poisoning during the initial 6 hours were quite significant. The histological studies revealed that, on the whole, the nuclei of the sensory and motor neurons were effected the most. They underwent hypertrophy while the chromatin material became clumped. As the sensory cells are the most commonly present cells in the brain any adverse effect on them showed up very clearly.

The neuropile also showed considerable vacuolization, which indicated that the synapses and different nerve tracts were affected. Other workers like Ali and Ahmad (1982) using different insecticides have also reported the same phenomenon for various insects. According to Teleferd and Mastsomura (1970), the hypertrophy is due to the fact that the cell membranes became adversely effected, hence the changes in the membrane permeability are the cause of the increase in size.

The glial or the supporting cells were not very much affected as the increase in their size was not significant, although their nuclei somewhat increased in size. The cytoplasmic and nuclear contents of the neurosecretory cells also showed adverse effects indicating disturbance in their secretory activity, although their size did not significantly change. As this insecticide is a neural inhibitor, its mild form of action on the neurosecretory cells of the flies is understandable. Dichlorvos is particularly effective against cholinesterase which hydrolyses the acetylcholin generated in myoneural junctions during the transmission of motor commands (Hartley and West, 1969).

The fat body cells were generally affected. Their plasma membranes ruptured after extensive vacuolization and the cytoplasmic contents almost disappeared. The chromatin material darkened and became concentrated in the middle. The fat body which is a

general food reservoir (Richards and Davies, 1977) was thus badly damaged.

The present results suggest that, dichlorvos is an effective neurotoxin. It starts its action very quickly and leaves considerable effects on the brain tissue within hours, unlike dieldrin which damaged the nervous tissue of the cockroach after 24 hours of treatment with a much higher dose (Ali and Ahmad, 1982). The development of resistance to the various insecticides in insects when their cholinesterase activity rises significantly is a very common phenomenon as shown by various workers like Hirashima *et al.* (1989); Shakoori and Saleem (1991) and Mourya *et al.* (1993).

However, in the present studies this insecticide was quite effective, so it seems that the flies have not yet developed resistance against it.

REFERENCES

ALI, F.A. AND AHMAD, M.S., 1982. Some histological effects of dieldrin on the brain of the American cockroach (*Periplaneta americana*). *Pakistan J. Zool.*, **14**: 147-162.

BUSVINE, J.R., 1971. *Critical resume of the techniques for testing insecticides*. Cambridge, London.

CORBETT, J.R., 1974. *Biochemical mode of action of pesticides*. Academic Press, New York, pp.330.

D'ALMEDIA AND MARIO, J., 1993. Capture of caliptrate flies with different breeding substrates on beaches. *Mem. Inst. Oswaldo Cruz. Rio L.*, **88**: 215-220.

FINNEY, D.J., 1952. *Probit analysis*. Cambridge Univ. Press, London, pp.319.

HARTLEY, G.S. AND WEST, T.F., 1969. *Chemicals and pest control*. Pergamon Press, pp.65-70.

HASSAL, K.A., 1983. *The chemistry of pesticides*. The MacMillan Press Ltd., pp.67-90.

HIRASHIMA, A., UENO, R., OYAMA, K., ISHAAYA, I. AND ETO, M., 1989. Effect of 5 membered cyclic phosphorothionates on larval growth, trehalase, digestive, enzymes, acetylcholinesterase, and cyclic adenosine 3', 5'-monophosphate level of *Tribolium castaneum* and *Musca domestica*. *Pestic. Biochem. Physio.*, **35**: 127-137.

LAPAGE, G., 1962. *Monnig's veterinary helminthology and entomology*. 5th ed., London, Bailiere Tindal and Company, pp.600.

MATSUMURA, F., 1975. *Toxicology of insecticide*. Plenum Press, New York and London.

MOURYA, D.J., HEMIRGWAY, J. AND LEAKE, C.J., 1993. Changes in enzyme tirates with age in four geographical strain of *Aedes aegypti* and their association with insecticide resistance. *Med. Vet. Ent.*, **7**(1): 11-16.

O'BRIEN, D.R., 1960. *Toxic phosphorus esters. Chemistry, metabolism and biological effects*. Academic Press, New York, pp.241-244.

RICHARDS, O.W. AND DAVIES, R.G., 1977. *Imms general textbook of entomology*. Chapman & Hall, London. John Wiley and Sons, New York.

SERVICE, M.W., 1980. *A guide to medical entomology*. The MacMillan Press Ltd., London and Basingstock, pp.102-109.

SHAKOORI, A.R. AND SALEEM, M.A., 1991. Comparative biochemical composition of a susceptible (FSSII) and two malathion resistant (CTC 12 and Pak) strains of *T. castaneum* (Coleoptera : Tenebrionidae). *Pakistan J. Zool.*, **23**: 1-10.

TELFORD, J.N. AND MATSOMURA, F., 1970. Dieldrin binding in subcellular nerve components of cockroaches. An electron microscopic and autoradiographic study. *J. Econ. Entomol.*, **63**: 795-800.

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