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GROSS BIOLOGICAL EFFECTS OF ACRIDINE ORANGE ON THE LABORATORY-REARED HOUSEFLY, *MUSCA DOMESTICA* (L)

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The oral administration of acridine orange (AO) seems to disrupt growth and development of the larvae and pupae of laboratory-reared houseflies. Higher doses (0.75 – 1%) proved toxic and most of the larvae died during two days following the treatment.. Midrange doses (0.25 – 0.5%) resulted in reduced growth, light weight larvae, delayed pupation, curtailment of normal pigment and darkening of puparia. Temporary depression in the size of gonads with decreased reproduction was also observed at midrange doses. Larvae treated at lower doses (0.0625 – 0.125%) that eventually pupated all failed to show toxic responses and the resulting adults appeared normal.

The morphological changes induced by the chemical at midrange doses are familiar to the symptoms that follow due to nutritional deficiencies. Acridine orange seems to inhibit the protein synthesis.

INTRODUCTION

Acridines are known to be antibacterial, carcinostatic and antimalarial drugs and a number of reviews dealing with the medical uses of acridine derivatives in vertebrates are available Acridine orange is also known to be mutagenic in bacteria and to bind with protein and with DNA [1]. The effects of acridines as mitotic poisons have also been thoroughly reviewed. Some related compounds like acriflavine and 3-amino-*N*-methyl acridine have been reported to intercalate DNA in lower organism [2,3]. The mutagenic properties of acridine orange and related compounds in silkworm, *Bombyx mori* have been reported by Murakami [4,5].

In insect tissues, oral feeding of acriflavine (2,8-diamino-10-methylacridinium chloride and 2,8-diaminoacridine) is known to disrupt the replication and transcription of closed circular mitochondrial DNA (m-DNA) and disturb the growth and development of *Heliothis zea* (Coddie) and *A. aegypti* (L) [6]. Rodriguez *et al.* [7] have reported the effects of acridine orange on the genetic fitness of *A. aegypti* (L) when the adult males were treated orally with the chemical.

No work on the inhibition of growth and development of houseflies by acridine orange has been reported. Therefore, the purpose of this work is to study the gross biological effects of this chemical during the active growth period of the housefly, *Musca domestica* (L) when the protein and amino acid requirement of the body is known to be the maximum.

MATERIAL AND METHODS

The effects of acridine orange (AO) on the laboratory-reared *Musca domestica* (L) were determined by administering the chemical via the rearing medium in plastic cups at 30±2 and 70% R.H. In each screening test 100 newly hatched first instar larvae of houseflies were treated orally with acridine orange by allowing them to feed freely in treated wheat bran till pupation. Dilutions varying from 0.0625–1% were made in 100 ml distilled water, and for each test 50 g wheat-bran was saturated with 100 ml aqueous solution containing specific concentrations of the chemical.

Larvae in the treated medium were observed to feed directly on treated bran. The imbibed quantity of AO was evident from the yellow coloration of the treated larvae, pupae and the adults. The presence of AO was, however, confirmed in the homogenates of treated larvae by TLC.

The weight of the larvae and pupae and the percentage of pupation and emergence of flies from treated medium were compared with the corresponding control. Treated and control population was examined daily for development progress and mortality. Pupae formed in the treated medium were removed to study the morphological changes and were placed in separate cages for emergence. The experiments were repreated three times.

Surviving adults from the treated group were provided with oviposition medium (powdered milk and glucose soaked in cotton) three days after emergence. The eggs obtained from the treated flies, at sublethal level, were observed for their hatchability. The biology of F1 progeny was observed

in untreated breeding medium.

The morphometric studies of the gonads in treated and control flies were undertaken at 1st, 3rd and 5th day following emergence. Mean length and width of gonads in treated and control flies were compared by applying t-test.

RESULTS

The treatment of larvae at midrange doses of 0.25 – 0.5% revealed significant retardency of their growth while doses ranging from 0.75 to 1% proved toxic and all the treated larvae died within two days following the treatment. The lower doses (0.0625 – 0.125%) showed slight to moderate degree of physiological effects which were observed to be temporary. The larvae that eventually pupated (93–95%) at this range of treatment failed to show toxic responses and the resulting adults appeared normal. The emergence of flies from the treated medium was 78 and 7% at 0.25 and 0.5% concentrations respectively. There was marked loss of weight of larvae and pupae at this range of treatment. Fig. 1 shows the inhibition and per cent loss of weight in treated larvae at various concentrations. The decrease of weight was observed to be directly proportional to the dose level.

The effect of AO seems to be linked not only to weight but also to the developmental rate of larvae. The data presented in Table 1 show that the larval period was nearly doubled at 0.5% concentration and the larvae resulted in characteristically misshaped pupae (Fig. 2E) and when dissected such pupae indicated arrested growth. The rate of pupation was also appreciably decreased at the above-mentioned dose, and only 17% larvae could pupate in treated medium. The size of the pupae was statistically very significant at 0.25 and 0.5% concentrations (Table 1).

Acridine orange at midrange doses seemed to curtail chemical tanning and normal darkening of cuticle in pupae. Dissection of adult males up to 5 days, following the emergence, showed that it interfered with the normal pigmentation of the testicular sheath as well. Besides the curtailment of pigmentation, an appreciable depression in the size of testes was observed in the emerged flies (Table 2). The frequency of the decrease of size of gonads was severe and statistically significant during 5 days postemergence when compared to corresponding controls, while the decrease in the size of ovaries was significant on 3rd and 5th

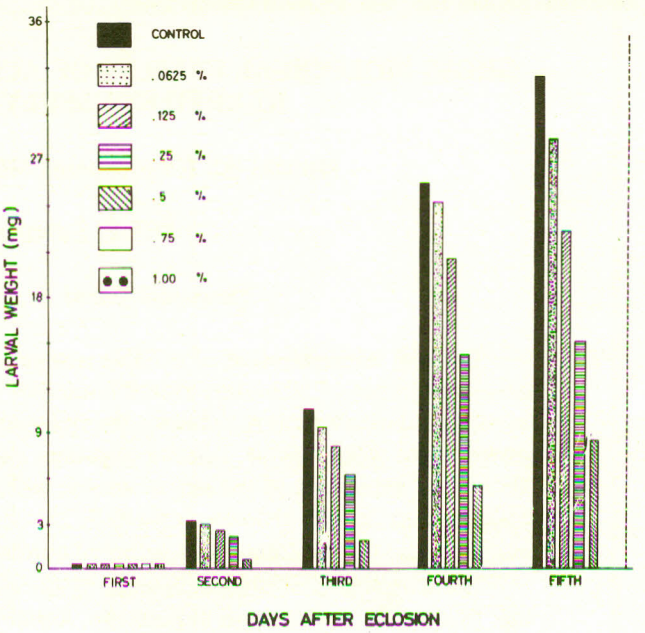

Fig. 1. Inhibition of larval weight induced by AO during growth. The vertical dotted-line shows the time when pupation occurred in control group of experiment.

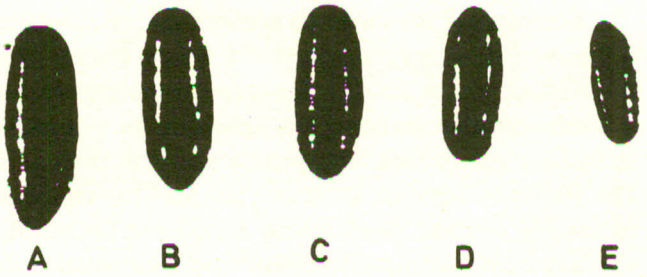

Fig. 2. The growth inhibition of pupae of *M. domestica* treated with AO at various concentrations: (A) control, (B)–(E) treated pupae.

Table 1. Effect of AO on the growth and development of housefly.

Dose (%)	No. tested	Average larval period	Pupation (%)	Size of pupae mean ± SE		Mean wt of pupae (mg) –	Decrease in wt (%)	Emergence (%)
				Length	Width			
Control	100	5	98	6.1±.129	2.6±.091	27.5	–	96.6
0.0625 (AO)	100	7	95	5.8±.108	2.5±.041	25.0	10	94.6
0.125 (AO)	100	7	93	5.8±.091	2.4±.041	25.5	15	90
0.25 (AO)	100	9	89	4.8±.227	1.9±.041	18.0	35	78
0.5 (AO)	100	11	17	3.6±.108	1.6±.183	9.0	67	7
0.75 (AO)	100	(No larva could survive)	–	–	–	–	–	–
1.00 (AO)		"	–	–	–	–	–	–

Table 2. Acridine orange-induced decrease in size of gonads of adult housefly (treated during larval and pupal period) at indicated days postemergence.

Test compound %	First day		Third day		Fifth day	
	Length	Width	Length	Width	Length	Width
			Size of ovaries (mm) mean ± SE			
Water control	0.792 ±.077	0.528 ±.035	1.485 ±.076	1.287 ±.171	2.31 ±.076	2.178 ±038
0.0625	0.693 ±.019	0.462 ±.078	0.99‡ ±.038	0.693* ±.061	2.31 ±.038	2.112 ±.038
0.125	0.66 ±.043	0.462 ±.038	0.825** ±.019	0.561† ±.057	1.947‡ ±.019	1.727*±.124
0.25	0.627 ±.076	0.429 ±.056	0.782 ±.01	0.603† ±.035	1.683† ±.133	1.32* ±.019
0.5	0.594† ±.028	0.396 ±.076	0.66* ±.043	0.66** ±.057	0.991±.037	0.793*±.077
			Size of testes (mm) mean±SE			
Water control	0.38 ‡±.007	0.275 ±.007	0.424* ±.013	0.303‡ ±.004	0.435±.013	0.303 ±.004
0.0625	0.325† ±.01	0.243‡ ±.01	0.38 ±.011	0.257† ±.016	0.42 ±.008	0.288† ±.008
0.125	0.305† ±.011	0.235** ±.009	0.347* ±.014	0.269*±.005	0.363±.005	0.265† ±.011
0.25	0.305*±.007	0.22† ±.007	0.336‡ ±.013	0.245* ±.007	0.355±.016	0.248‡ ±.021
0.5	0.297*±.006	0.187* ±.005	0.33† .016	0.199† ±.012	0.347±.016	0.21† ±.019

Level of significance on comparison of the values with corresponding controls: *–P>0.001, †–P>0.01, **–P>0.02, ‡–P>0.025

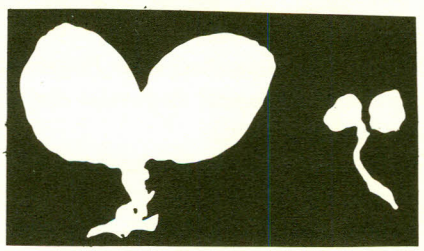

Fig. 3. Female reproductive system of 3-day old *M. domestica* showing fully grown untreated ovary (left) and suppressed ovary (right) from treated adult female at 0.5% concn.

day (Fig. 3). The growth inhibition of the gonads, however, proved to be temporary, and when the flies were offered normal food they started regaining the sizes.

Effects on the Reproductive Potential. Oral administration of AO at 0.25 – 0.5% concentrations inhibited the oviposition of the flies temporarily. The lower doses caused slight decrease of reproductive potential and hatchability of eggs. Larvae from the treated parents developed normally. No appreciable sterility was noticed in the F_1 female having an acridine history of 0.25 – 0.5%.

DISCUSSION

Midrange doses of AO ranging from 0.25 to 0.5% resulted in mortality of immature stages, slow or arrested growth and development, light-weight larvae, delayed pupation, curtailment of pigmentation, depression in the size of gonads and decreased reproduction. All these symptoms indicate that the larvae probably cannot meet the requirement of adequate level of protein needed for the above-mentioned physiological activities. Previous work on acridine orange and some of its derivatives also demonstrate that it binds with protein, DNA [1,8] as well as RNA and subsequently inhibited protein synthesis [9].

The changes connected with the cuticle also suggest that the proteins tyrosine and 'sclerotin' which are reported to be responsible for tanning and sclerotization of insect cuticle may be inhibited by AO. Such lack of pigmentation in *Aedes* larvae and pupae has been reported to be due to insufficient tyrosine and phenyl analine in the diet [10].

Larvae treated at lower doses (0.0625 – 0.125%) that eventually pupated all failed to show toxic responses and the resulting adults appeared normal.

Acridine orange, like some other alkylating agents, decreased the size of the ovaries and testes in the housefly. The reduced size of the ovarioles seems to contribute the reduction of fecundity. Besides the alkylating action, the reduction in the size of gonads may be due to the decreased protein formation in gonads.

Morphological changes observed in the gonads were, however, temporary as they disappeared when treated adult flies were given normal diet. The eggs obtained from flies 5 days after emergence were viable and the offspring of the treated parents was perfectly normal. No appreciable decrease in reproductive potential of female of F_1 generation was noticed. It seems very probable that AO is excreted by the surviving adults and the genetic damage is produced only at toxic concentration and the mutants thus die. The biological action of acridine dye is known to be enhanced by light (photodynamic effect). In the present experiment, performed in the absence of light, it is possible that the early stages of oocytes and sperms in larvae and pupae could not receive sufficient amount of light to induce mutations due to photodynamic action on AO–DNA complexes. Keeley and Olson [6] reported that acriflavine was fatal to mosquito larvae at lower doses (0.1 ppm) and larvae exposed to the higher dose level (1 ppm) died at the first larval–larval ecdysis. They found that acriflavine-treated larvae that eventually pupated all failed to show toxic responses and the resulting adults appeared normal.

Rodriguez *et al.* [7] reported significant genetic damage in *A. aegypti* (L) only at higher concentration of acridine orange (0.075%). According to them AO induced fewer lethal mutagenic damage which contributed towards decreased productivity rate in *A. aegypti* (L) during the 2nd week.

The data presented in this paper do not provide any direct evidence that the test compound inhibited protein formation in the treated insects. Nevertheless, it can be speculated that AO inhibited protein formation to some extent, as most of the responses observed agree with the predicted responses based on the inhibition of protein formation namely, reduced growth, loss of weight, delayed pupation, curtailment of normal pigmentation, retardation of ovarian development and decreased reproduction.

It is also possible that AO affects certain metabolic processes leading to the above-mentioned semistarvation symptoms. On the other hand, acridine orange may be exerting cytotoxic action on the intestinal cells thus leading to the reduction of protease (a specific aspect of protein synthesis) and subsequently the inhibition of protein formation.

The exact physiochemical cause of gross pathological symptoms induced by AO or the mechanism of its growth inhibition effects in insects can form an interesting study for the future research.

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