

## Neuropathological studies of *Drosophila melanogaster* with sublethal doses of malathion

Mehdi SH, \*Qamar A

Department of Zoology, Aligarh Muslim University, Aligarh 202002, India.

\*Corresponding Author: ayesha.zoology@gmail.com

### Abstract

In the present work, we studied the neuropathological and behavioral effects on locomotion due to sub lethal chronic exposure to malathion in *Drosophila melanogaster*. To examine its effects we performed locomotion assay and brain histopathological analysis on treated flies. After 48 hrs of exposure, the treated flies exhibited characteristic locomotor impairments that increased with the dose of malathion. Histopathological observation demonstrated selective loss of nervous tissue in the various regions of brain. As compared to normal brain section, the affected regions exhibited disrupted neuro secretory cells and formation of vacuoles due to neuronal damage. Therefore, our study suggests that pesticide play possible role in neurodegeneration in *D. melanogaster* which provides *in vivo* model for studying the histological aspect of nervous tissue.

**Keywords:** Neuropathological; Malathion; Locomotor; *D. melanogaster*.

### Introduction

Pesticides used in agriculture are designed to protect crops against unwanted organisms. Many compounds target the nervous system of insects and because of the similarity in brain biochemistry; such pesticides may be neurotoxic to humans. A number of studies have investigated the presumed role of environmental toxin in the pathogenesis of neurological disease like symptoms in the experimental models. Malathion, an organophosphate insecticide, is commonly used to control variety of insects that attack fruits etc. According to US EPA, malathion is the fourth most used pesticide which is associated with chemical sensitivity (Miller *et al.*, 1995). Malathion kills insects by preventing their nervous system from working properly. Human beings and animals can be affected the same way as insects if they are exposed to enough malathion (National Pesticide Information Centre). Malathion toxicity causes permanent nerve damage with muscle, auditory and vestibular dysfunction, weakness and easy fatigability (Rosenthal *et al.*, 1991). Organophosphate can cause chronic brain damage (Broughton, 1990). Malathion exposure is associated with damage to multiple body organs, especially the brain and nervous system (Lotti, 1986). It has many structural similarities with natural occurring compounds and their primary target of action in insects is the nervous system; it also inhibits the release of the acetylcholinesterase at the synaptic junction (Cabello *et al.*, 2001).

In recent years, *D. melanogaster* has been used as a model of several neurodegenerative disease, including a genetic model of Parkinson's disease based on directed expression of human *a-synuclein* in the *Drosophila* brain (Feany and Bender, 2003; Auluck *et al.*, 2002). In the last decade, numerous researchers have focused their attention on understanding neurodegenerative diseases by utilizing this model system. Here we studied the neuropathological and behavioral effects on locomotion due to the sub lethal chronic exposure to malathion in *D. melanogaster*.

### Materials and Methods

**Chemicals:** Malathion (PESTANAL<sup>R</sup>) was purchased from Sigma-Aldrich (St. Louis, MO). All reagents were obtained from Merck, India.

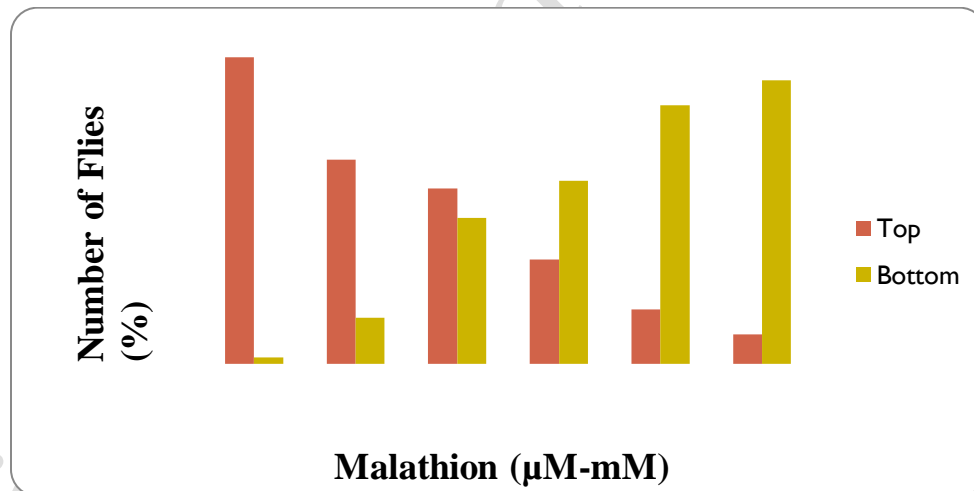
***Drosophila* culture and drug treatment:** Wild type Oregon R *D.melanogaster* stocks were maintained in a light/dark cycle of 12:12h at room temperature (25±2° C) in glass bottles and cages containing a standard cornmeal agar diet. From the stock, flies were transferred to glass vials containing 50µl of test food (Sucrose + Malathion). The flies were held in groups of 20 per vial and incubated at 25° C for 7 days at different doses with a solution containing 0-1mM malathion. We made sure that freshly made solutions were transparent.

**Climbing assay:** We determined locomotor ability with a negative geotaxis assay as described previously (Feany and Bender, 2000; Friggi-Grelin *et al.*, 2003; Rival *et al.*, 2004). Three trials were performed in each experiment at 1 min intervals. The scores are the mean of the numbers of flies at the top (*ntop*) and at the bottom (*nbot*), expressed as percentages of the total number of flies (*ntot*). Results are presented as mean SEM of the scores obtained in the three independent experiments which was calculated as  $1/2[(ntot + ntop - nbot)/ntot]$ .

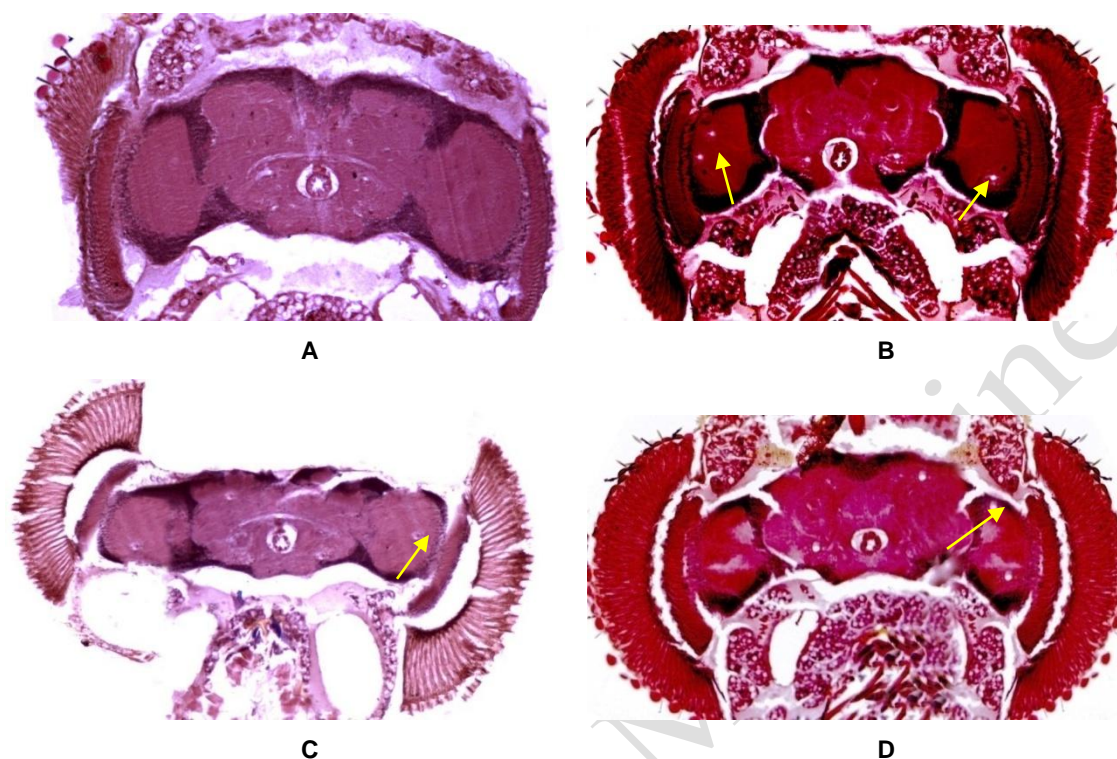
**Histological detection:** We anesthetized treated wild type Oregon R flies for histological studies. Tissues were incubated in 8% w/v paraformaldehyde for 8 hrs. After fixation, tissues were rinsed in PBS five times, each time for 10 min at room temperature. The tissue was

dehydrated through an ethanol series to xylene. Xylene was replaced with paraffin in increasing volume at 62°C in an oven. Tissue was placed in hard paper mold containing molten paraffin, and stored at 0°C for at least 24 hr. blocks were mounted and sectioned at 7 µm thickness on a Yorco rotary microtome and slides containing sections were stored upright overnight at 37°C. Slides were processed in xylene for 10 min, followed by decreased ethanol series (100-30%). The sections were stained with Ehlich's Haemotoxylin for 2 min followed by 0.5% hydrochloric solution (1 dip), 0.5% sodium bicarbonate (5 min) and Scott's solution (5 min). After that slides were stained with eosin for 10 min, rinsed with upgrade ethanol series (30-100%), followed by xylene for 10 min. Finally, the slides were mounted with DPX and observed under Leica microscope.

## Results



**Fig 1. Negative geotaxis assay of adult flies exposed previously for 7 days to various amounts of malathion. Red bars indicate the percentage of flies that climbed to the top of the column, and yellow bars indicate the percentage of flies that remained at the bottom after 1 min.**



**Fig 2.** Light micrographs of *Drosophila* nervous tissue section treated with different doses of malathion for 7days. Frontal sections at midbrain from adults. Large hole in the middle of each section is the oesophagus. (A) Control exhibit no pathology whereas (B-D) at 7 day exposure showed little to moderate neuropathology.

**Climbing activity:** Climbing is the most commonly assayed behavior with respect to fly neurodegeneration due to its behavior i.e. for most strains, 90% of flies will immediately start climbing the walls of a container after they have been tapped to the bottom. Drug feeding of malathion at the dose from 50 $\mu$ m-1mM produced effect of spending more time in mobility. In all cases, *Drosophila* seem immobile and less active than control and spend more time at the bottom level as the dose increased. The time spent in immobility was increased and the distance covered was reduced in treated flies as shown in Fig 1. We observed flies trembling on the floor, fallen backward and abnormal movement in the climbing.

**Histology:** Histopathology examination of *Drosophila* nervous tissue section treated with different doses of malathion showed different alterations. The most striking lesions in these histological sections are the vacuoles or holes in the central neuropile or outer ring where most neuron cells bodies reside as shown in Fig 2 in a *Drosophila* brain. Wild type Oregon R controls

exhibit little or no pathology (Fig 2A). Overall brain structure is preserved including the outer cellular cortex layer, central neuropil areas, and neurosecretory cells. Whereas, treated *Drosophila* brain sections showed widespread neuropathology including vacuolar-like lesions (arrows) throughout the neuropile and loss of cell bodies. This pathology is more extensive and severe than that seen in control.

### Discussion

Several studies have shown the possibility that environmental neurotoxicant such as pesticides may be related to the development of neuronal diseases (Ritz *et al.*, 2000). In the present work we report that the exposure of *Drosophila* to sublethal doses of malathion exhibit main feature of neurodegeneration, inducing locomotion deficits. Malathion, used as pesticide, is an environmental factor that may contribute to the appearance of Parkinson's disease in humans. We noted several differences between treated and non-treated *Drosophila* brain sections. The locomotor deficits in the treated flies clearly establish its toxic

effect to nervous system in comparison to the normal flies. Loss of neurons appearing as vacuolar like lesions was apparent in the malathion treated flies sections. Limited information was found regarding morphological changes in the nervous system after exposure to malathion. A single dose of up to 2,000 mg/kg of malathion (88% pure) caused no neuropathologic lesions in segments of the medulla, cervical and lumbar spinal cord, and branches of the tibial nerve, and cerebellum from rats sacrificed 21 days after dosing (Ehrich *et al.*, 1993). Helene *et al.* (2004) stated that chronic exposure of *Drosophila* to sublethal doses of rotenone recapitulates the main symptomatic feature of Parkinson's disease: a selective loss of dopaminergic neurons inducing locomotor deficits. Burgees *et al.* (1999) observed that an organophosphate insecticide reduced cholinesterase activity in birds. Taylor *et al.* (1999) stated that a sublethal dose of field grade malathion (0.01 mg/g) lowered brain cholinesterase levels by 22% and 175, respectively. Parson *et al.* (2000) observed effect of organophosphate and carbamate on non-target wild animals and these pesticides inhibited cholinesterase activity. However, brain congestion, neuronal degeneration, and gliosis were seen in the brain of rats during the first few days after administration of a single dose of 1,950 mg/kg of malathion (Piramanayagam *et al.*, 1996).

The findings of this study suggest that sublethal exposure of malathion to *Drosophila* exhibits key aspects of neurodegeneration. This study also reveals that various concentrations of malathion exhibit neuronal loss in *Drosophila*.

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