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Zoology

EFFECT OF MALATHION ON CHROMOSOMAL PATTERN OF Drosophila kikkawai

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ABSTRACT

Drosophila has long been a favorite model system for studying the relationship between chromatin structure and gene regulation due to the cytological advantages provided by the giant salivary gland polytene chromosomes of third instar larvae. This study emphasize adverse effect of an organophosphate compound, Malathion, on chromosomal pattern in the form of puffing in D.kikkawai. Drosophila kikkawai was selected as model organism for biomonitoring of pesticides due to its genetic importance and coexistence with other insect pests in agriculture field. The pesticide was administered by feeding the larvae at 100ppm, 10ppm, 0.1ppm, 0.01ppm in every 30 ml of food medium. Through chronic toxicity test, the 2 days LC50 was evaluated. In all the concentrations, markable effect on chromosomal pattern was observed. Such an effect of Malathion, is discussed in the light of the effects caused by other insecticides.

Keywords: Chromosomal pattern, *Drosophila kikkawai*, genetic importance, Malathion.

INTRODUCTION

Malathion is a pesticide that is widely used in agriculture, residential landscaping, public recreation areas, and in public health pest control programs such as mosquito eradication. Malathion was used in the 1980s in California to combat the Mediterranean fruit fly. Malathion is approved by the United States Food and Drug Administration for treatment of pediculosis. Dulout(1983) worked on Malathion induced chromosomal aberrations in mice. Malathion itself is of low toxicity; however, absorption or ingestion into the human body readily results in its metabolism to malaoxon, which is substantially more toxic. In studies of the effects of long-term exposure to oral ingestion of malaoxon in rats, malaoxon has been shown to be 61 times more toxic than Malathion (Gayatri MV and Krishnamurty, 1981). According to the United States Environmental Protection Agency, there is currently no reliable information on adverse health effects of chronic exposure to Malathion. Possible symptoms include skin and eye irritation, cramps, nausea, diarrhea, excessive sweating, seizures and even death. Most symptoms tend to resolve within several weeks. Since Malathion is widely used in agricultural field, we were prompted to evaluate its toxic effect on choromosomal pattern of *Drosophila kikkawai*, a wild species inhabiting agricultural field.

Strickberger (1962) explained the genetics of *Drosophila*. We picked to perform genetic studies, but this organism also offers an outstanding opportunity to study chromosome structure due to the polytene nature of the chromosomes found in its salivary glands. In these glands, many multiple DNA replications have occurred without mitosis. The multiple DNA strands remain closely associated yielding large chromosome structutres with specific

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banding patterns along their length. Thus chromosomal alterations such as deletions, transpositions, duplications, etc can be visually verified using the unique banding as identifying landmark features.these changes can be considered by comparing with chromosomal map of *Drosophila melanogaster* prepared by Bridges (1935) and chromosomal map of *Drosophila kikkawai* by Diwedi and Gupta (1980). Charlesworth (1996) also worked on genetics of *Drosophila*. Daneholt B (1992), Dulout *et al*. (1983), Hartwell *et al*. (2011), Johnson *et al*. (2009), Prakash *et al*. (2010), Ristroph (2010) are some other scientists who worked on chromosomal structures of *Drosophila*, mice and some others.

MATERIALS AND METHODS

Slightly toxic compound of EPA toxicity class III Malathion (EC 50%)

TEST ORGANISMS

Flies and third instar larve of *Drosophila kikkawai* were used as test organism.

COLLECTION AND CULTURE OF THE *Drosophila kikkawai*

Drosophila kikkawai were collected from different eco-geographical areas and agricultural field from nearby places of central U.P. All culture was maintained in lab, on corn-sugar-agar *Drosophila* food medium in B.O.D incubator at 24±1°C.

Treatment Schedule: For each study, the test chemicals were dissolved in 0.3% DMSO (Dimethyl Sulfoxide) finely mixed with food after cooling. Larva and adult were fed on this treated food for experiment at different concentrations.

Determination of LC50 (by method of Bliss1935, Finney 1989) of *Drosophila kikkawai* :LC50 at 48 h of organophosphate test chemical Malathion was determined after feeding the 20 first instar larvae with 100 ppm,10 ppm,0.1ppm,0.01 ppm,0.001ppm,DMSO and control food medium. Ten replicates were used for each concentration. The LC50 concentration Experiment showed truly on 0.1ppm concentration 50% mortality was observed. (Figure 1, Table 1)

Preparation of polytene chromosome: Johnson *et.al*. prepared polytene chromosome by using antibodies. We prepared chromosomes at different concentrations viz., 1ppm, 0.1ppm (LC 50%) and control of Malathion.

Following are the summarized way to prepare polytene chromosome.

1. Take IIIrd instar larvae and pour them into PSS solution.
2. Dissect salivary gland of larvae.
3. Pour 45% acetic acid.
4. Wash with one drop HCL.
5. Stain with aceto-orecin for 4-5 minutes.
6. Wash with 60% acetic acid to remove excess stain.
7. Pour cover slip.
8. Squeeze the salivary gland.
9. See under microscope.
10. Take a photograph.

Table 1: Determination of LC50 of *Drosophila Kikkawai* at different concentration of Malathion

Concentration	Total larvae taken	TOTAL	MORTALITY (%)
100 ppm	200	200	100
10 ppm	200	198	99
1 ppm	200	142	71
0.1 ppm	200	99	49.5
0.01 ppm	200	62	31
0.001 ppm	200	31	15.5
0.0001 ppm	200	16	8.0
Dmso	200	8	4.0
Control	200	0	0

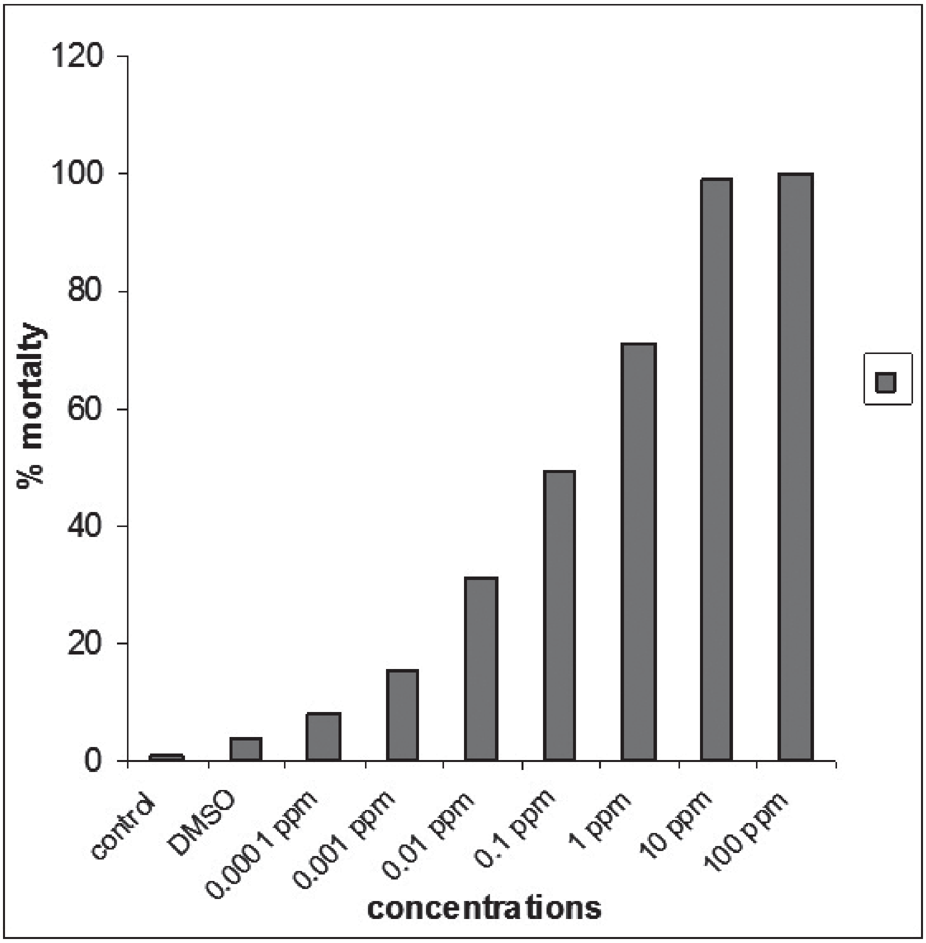

Figure 1: Determination of LC50 of *D.kikkawai*

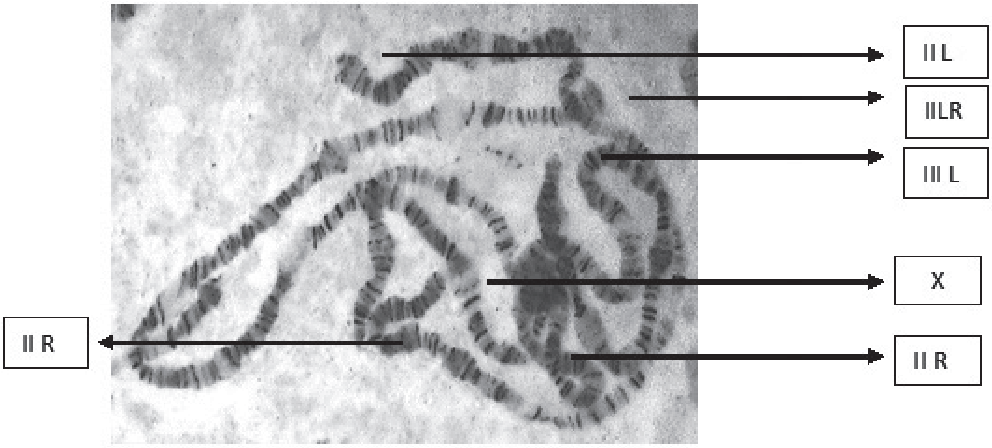

Figure 2: Control Chromosome of *D.kikkawai* (with arms)

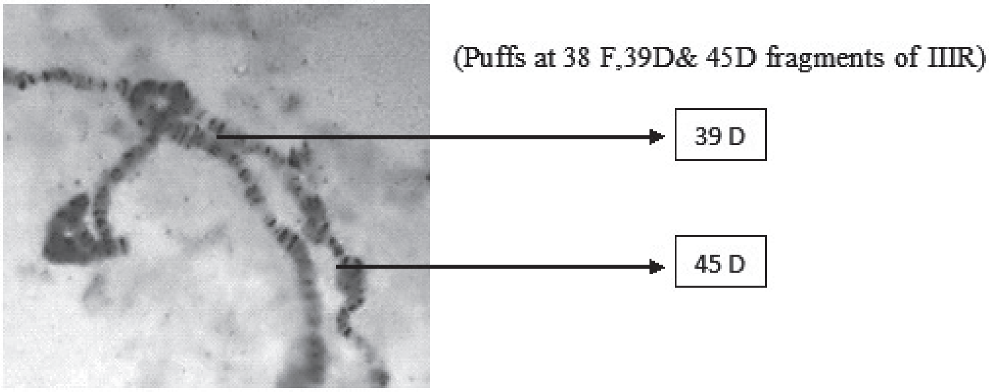

Figure 3: Changes in 1ppm concentration of Malathion

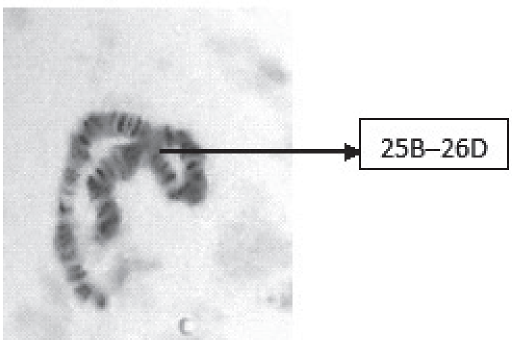

Figure 4.1

(Inversion at 25B-26D fragment of IIR)

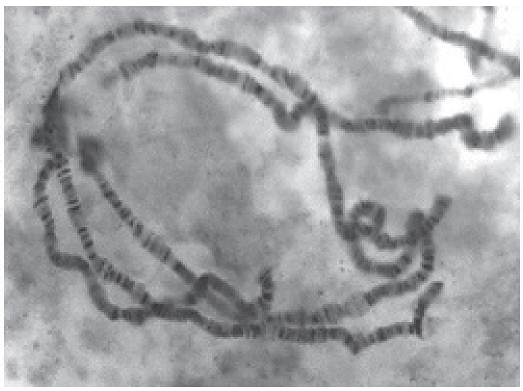

Figure 4.2

(Five arms with chromocentre)

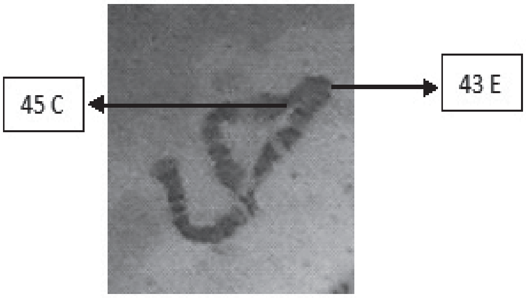

Figure 4.3

(Puff at 43E fragment of IIIR)

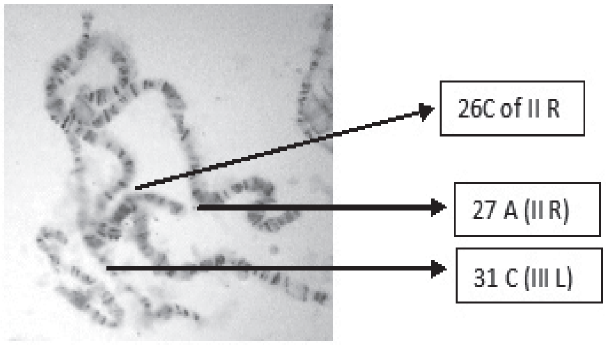

Figure 4.4

Puff at 27 A,26C of IIR & 31C fragment of IIIL)

Figure 4: Changes in 0.1ppm concentration of Malathion

RESULT AND DISCUSSION

The Polytene chromosomes prepared from salivary gland chromosomes of third instar larvae showed five euchromatin arms and one very short strand arising from common chromocentre. The most eminent way to describe the Polytene configurations, the arms were arbitrarily designed: I for X chromosome, II L,IIR,III R, III L for the left and right arms of two pairs of V shaped chromosome and IV for the small dot shape chromosome of metaphase.

The gene arrangements from natural populations of *Drosophila kikkawai* and its breakpoints over Polytene chromosomes were conveniently described by comparing it to a standard Polytene map published by Diwedi & Gupta(1980).The chromosomes X , II, III and IV and these chromosomal configurations in natural populations were chosen as standard. Larvae obtained from the treatment of different concentrations of malathion were examined for naturally occurring chromosomal polymorphism in form of inversions.

The cytological observations for effect of Malathion also revealed some minor changes in chromosomal rearrangement in the salivary gland chromosome and also the induction of new puffs in the fragment 38F & 39D & 49D fragment of IIIR arm of chromosome in 1ppm concentration and 45C, 43E fragment of IIIR, 27A, 26 C and 31 fragment of IIIL arm in 0.1ppm concentration of malathion which may be due to stress conditions. Inversion was observed at the region of 25B-26D of IIR arm of chromosome at 0.1 ppm concentration of malathion. The stress puffs require further molecular or radioisotopic confirmation for mRNA formation.

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