ENVIRONMENTAL SCIENCE ARCHIVES
ISSN: 2583-5092
Volume III Special Thematic Issue 1, 2024
www.envsciarch.com

RESEARCH PAPER OPEN ACCESS

Monocrotophos Induced Changes in the Life Cycle Parameters of Fruit Fly

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Received: 2024/02/15
Accepted: 2024/03/12
Published: 2024/03/19

Abstract

Current study investigated the chronic effect of monocrotophos exposure, a globally used pesticide on 3rd instar larvae and pupae of fruit fly *Drosophila melanogaster*. Study commenced with the investigation of chronic median lethal concentration (LC50) which was found as 0.68 µg/mL for 3rd instar larvae and 0.56 µg/mL for pupae. Accordingly five sub-lethal concentrations such as 0.2, 0.25, 0.3, 0.35 and 0.4 µg/mL were selected for experimental set up. Treated larvae manifested altered feeding habit, changed life cycle duration and reduced body weight and length in both larval and pupal stage. Interestingly alteration in sex-ratio and gender biased population was encountered after exposure to monocrotophos. Since *D. melanogaster* shares significant molecular and physiological homologies with higher vertebrates, hence the present findings could be alarming for other non-targeted organisms who are getting direct or indirect chronic exposure to this chemical.

Keywords: *Drosophila melanogaster*; Monocrotophos; LC50; Feeding assay; Sex biasness

Introduction

Pesticides are the chemical formulations that are popularly used in both developed and developing countries around the globe (Bhatt et al., 2024). Demand for pesticide usage is rising as a result of increasing global population, which prioritizes high agricultural yields (Kumar et al., 2024). Farmers of developing countries consider pesticides as the simplest way to safeguard their crops against a broad spectrum of pests (Kaushal and Singh, 2022). However, in reality extensive use of pesticides is the major cause of unintentional exposure (Jasim and Hariz, 2023). These chemical formulations can accumulate overtime in crops, soil, water, air, and eventually infiltrate the food chain thereby posing threats to human and other animals. Organophosphate pesticides (OPs) are the organic molecules having one or more phosphate ester groups. OPs are widely used in farming, secretarial for more than 45% cosmopolitan use insecticide sales (Mali et al., 2022). Hence, it is assumed that during the forecast period of 2018–2023, the global market for OPs will be increased yearly by 5.5 %. Monocrotophos [3hydroxy-N-methyl-cis-crotonamide dimethylphosphate] is an OP which is used against a wide range of insect pests including piercing and sucking insects, leaf eater beetles, bollworms and caterpillars (Kumar et al., 2020). Higher efficacy against insect pests made monocrotophos a popular pesticide. Field application dose of monocrotophos is 0.25–1.5 kg/ ha and the half-life is approximately 17–96 days in environment (Kaur and Goyal., 2019). It is reported that monocrotophos showed its half-life around 2500 days in 38º C in closed environment (Rajan et al., 2023). Several studies have revealed toxic potential of monocrotophos in non-target organisms. Structural alteration of neurons was observed after exposure to monocrotophos in low doses among rats (Karumuri et al., 2019). This study could help to unravel the potential hazards of monocrotophos exposure by using fruit fly *Drosophila* as a model because genetic homology between human and *Drosophila melanogaster* is over 70%.

Where, human disease-causing genes have 75% homologs in *D. melanogaster* among which some shares more than 90% nucleotide sequence identity (Bier, 2005). Toxicity of xenobiotics can be easily assessed in embryonic as well as in any other life cycle stages of the fly. The developmental, cellular and molecular mechanisms in *Drosophila* and higher vertebrates are well conserved. Hence, *D. melanogaster* is a promising alternative to vertebrate models and is important to 3R (Replacement, Reduction and Refinement) research.

Materials and methods

Model organism and the test chemical

Drosophila melanogaster of Oregon "R" strain was used for toxicological assessment of monocrotophos in non-target organism. Flies were reared at 23°± 1° C temperature, ~ 60 % relative humidity within environmental chamber maintain 12h:12h light and dark cycle.

Monocrotophos, (CAS number: 6923-22-4; molecular formula: $C_7H_{14}NO_5P$) was used in the present study as a test chemical. Water-based stock solution of 100µg/mL concentration was prepared for the study.

Determination of median lethal concentration

For determination of chronic LC_{30}, LC_{40}, LC_{50} of monocrotophos in *D. melanogaster* 3rd instar larvae and LC_{40}, LC_{50} and LC_{60} in pupal stage. Three replicate sets of thirty 1st instar larvae in each petri dish. The nascent larvae were provided for Standard Drosophila Medium mixed with 0.4, 0.5, 0.6, 0.7 and 0.8 µg/mL concentrations of commercially available monocrotophos solution that is directly used in agricultural fields. Larvae of *D. melanogaster* were commenced in each culture dish and waited. Then observed the number of pupae and adults were formed in each plate. The numbers were counted for determination of larval and pupal mortality percentage respectively. The data was recorded and used for probit analysis.

Preparation of experimental concentration in food media

After calculating different lethal concentration values, five sub-lethal concentrations were selected viz., 0.2, 0.25, 0.3, 0.35 and 0.4µg/mL that were lower than the LC_{30} value. Freshly hatched 1st instar larvae were introduced in the food media with selected concentrations of monocrotophos along with control set and allowed to grow till their adulthood. Different life cycle parameters were observed using the experimental sets.

Feeding assay

2% brilliant blue dye solution was prepared and mixed with the food media to colour the food where the 1st instar larvae were introduced and allowed to grow until they grew up as 3rd instar larvae. Afterward, 30 numbers of larvae were collected in phosphate-buffer-saline (PBS) and 10% tissue homogenate was prepared. The homogenate was collected through a fine mesh to avoid any debris and the absorbance was measured using a UV-Vis spectrophotometer in 580 nm wavelength (absorbance frequency of brilliant blue dye) following the protocol of Nguyen et al. (2018).

Study on Life cycle parameters

To investigate any alteration in the duration of life cycle, 1st instar larvae were allowed to grow in different treated concentrations and measured the time taken in days to attain their 3rd instar stage, pupal stage and the total days required for adult emergence.

Bodyweight measurement

For each concentration, treated 3rd instar larvae and pupae including control group were taken thirty in number for each concentration group. Test was maintained in three replicate sets. So, the individuals were considered for each experiment and the total individuals were 120 in number. The body weight was taken with the help of electronic weight machine (Weinser).

Larval and pupal size measurement

To evaluate any change in size of 3rd instar larvae and pupae for all concentrations along with control has done by taking 30 larvae and pupae with triplicate set. The length was measured in mm scale and the alteration over different concentrations was investigated.

Alteration in sex-ratio

To assess any alteration in sex ratio in MCP exposed flies in compared to control by feeding adults on different concentrations of MCP along with control and allowed to lay eggs and grew them till adults emerged. The number of newly hatched males and females were counted to determine the sex ratio.

Statistical analysis

The variations were tested, whether they were significant or not, between the treatment categories (0.2, 0.25, 0.3, 0.35 and 0.4 µg/mL) and control by the help of One-way ANOVA. The results of various experiments were measured as statistically significant if p values were less than 0.01 and 0.05 were designated as (**) and (*) respectively.

Results

Determination of chronic lethal concentration

Larvae got chronic exposure to 0.4, 0.5, 0.6, 0.7 and 0.8 µg/mL concentrations of monocrotophos observed a mean mortality percentage of 1.66 ± 0.33%, 6.66± 1.66%, 18.33± 3.33%, 40 ± 8.66% and 88.33 ± 9.27%, respectively. By the help of probit analysis the median lethal concentration (LC_{50}) of 3rd instar larvae was found 0.68 µg/mL. The LC_{30} and LC_{40} values were 0.42µg/mL and 0.54 µg/mL respectively.

In case of mean pupal mortality percentage for respective concentrations were 11.66 ± 1.66, 41.66 ± 9.27, 63.33 ± 8.81, 58.33 ± 1.66 and 90 ± 10. The median lethal concentration (LC_{50}) value determined was 0.56 µg/mL. The LC_{40} and LC_{60} were estimated at 0.40 µg/mL and 0.78 µg/mL respectively which indicated that 40% and 60% pupal mortality.

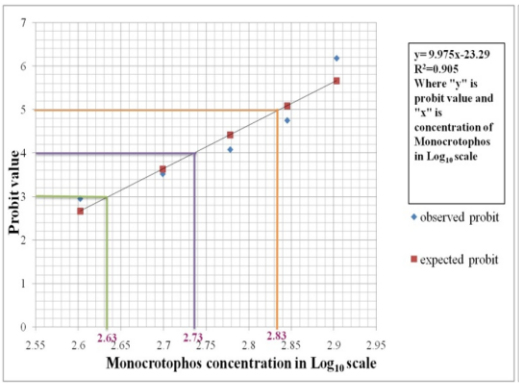

Fig. 1. Probit analysis. Graph represents LC_{30}, LC_{40} and LC_{50} value of monocrotophos exposed *Drosophila melanogaster* 3rd instar larvae

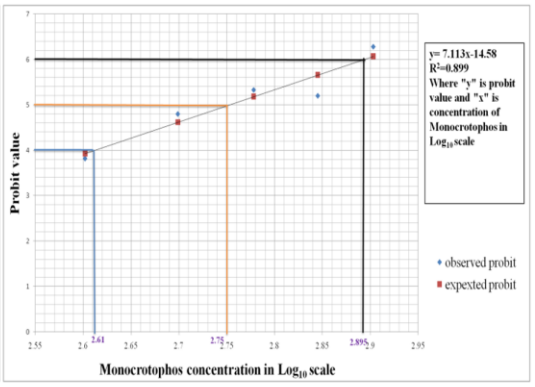

Fig. 2. Probit analysis. Graph represents LC_{40}, LC_{50} and LC_{60} value of monocrotophos exposed *Drosophila melanogaster* pupae.

Further study was carried out with some selected sub-lethal concentrations which were much lower than the LC_{50} value.

Feeding assay

The feeding assay reflects differences in palatability of different MCP concentration treatment categories. The concentration 0.3µg/mL showed significantly higher feeding value which depicts maximum palatability among 3rd instar larvae which makes the moderate concentration to exert more harmful effects than other higher concentrations (0.35 and 0.4 µg/mL).

Life cycle parameters

The larval duration is considered as the total days taken to reach pupal stage from the one-day old larva. Generally, it takes 7 to 8 days to pupate in case of control but in case of all treated groups the numbers of days were found to be reduced. In 0.25, 0.3 and 0.35 µg/mL concentration it reduced approximately 24%, 21% and 17.6% respectively in compared to control. It might possible that the toxic food medium provides unfavorable condition to continue the 7 to 8 days as normal

larval duration and instigate to pupate early. Likewise, the number of days took from 1st day of pupation till adult flies' emergence is called pupal duration. Usually, it takes 5 to 6 days in untreated groups. Contrastingly the MCP exposed groups showed dose dependent decrease in pupal duration. The 0.35 and 0.4 µg/mL treatment groups showed 34% and 62% decrease respectively in pupal duration which are significantly low.

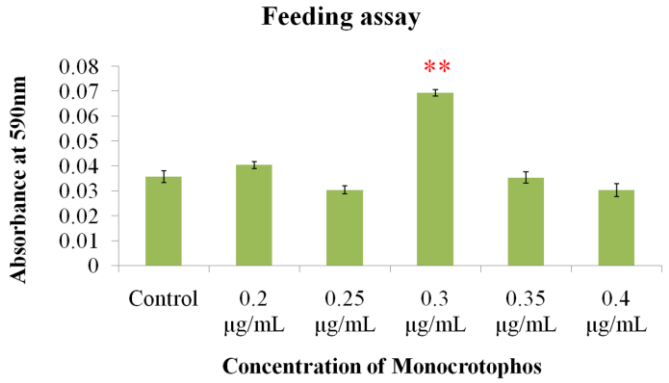

Fig. 3. Figure represents different absorbance of brilliant blue dye in different treatment categories. The intensity of absorbance indicates to the amount of food eaten by larvae.

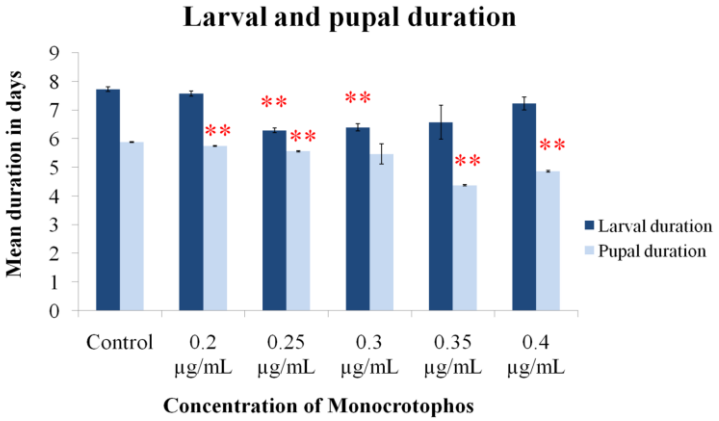

Fig. 4. Graph presenting relative trend of larval and pupal duration in days when exposed to different concentrations of MCP in compared to control.

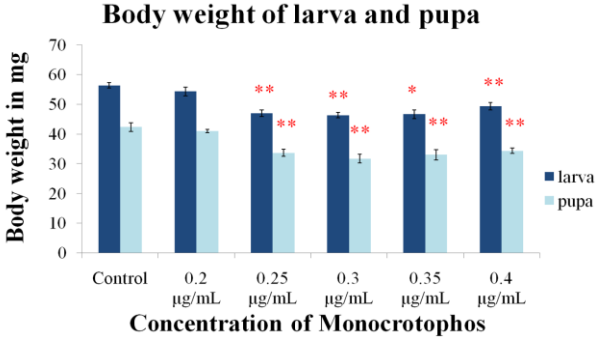

Fig. 5. Graph showing comparative trend of larval and pupal mean body weight when exposed to different concentrations of MCP in comparison to control.

Body weight measurement

The mean wet weight of treated 3rd instar larvae as well as the pupae was found to be significantly decreased when compared to control. Body weight of MCP treated larvae groups from 0.25, 0.3, 0.35 and 0.4µg/mL concentration was observed to be reduced by ~16.50%, ~18%, ~17% and ~12% in compared to control. Approximately 20%, ~25%, ~22% and ~18% decrease in pupal weight was observed in above mentioned MCP concentrations when compared to their control counterparts.

Table 1. Table data showing mean wet weight (mg) of 3rd instar larva and pupa.

Concentrations	Control	0.2µg/mL	0.25 µg/mL	0.3 µg/mL	0.35 µg/mL	0.4 µg/mL
Mean larval weight ± SE	56.33±0.88	54.33±1.45	47±1.15**	46.33±0.88**	46.66±1.45*	49.33±1.20**
Mean pupal weight ± SE	42.33±1.45	41±0.57	33.66±1.20**	31.66±1.45**	33±0.73**	34.33±1.20**

*p<0.05 and **p<0.01 are considered as statistically significant

Fig 6. Picture representing comparative account of 3rd instar larval and pupal size where A=Control, B= 0.2µg/mL, C= 0.25 µg/mL, D= 0.3 µg/mL E= 0.35 µg/mL and F= 0.4 µg/mL monocrotophos concentration.

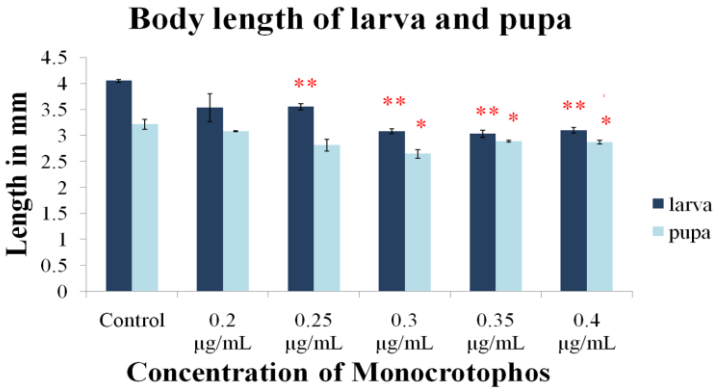

Fig. 7. Graph showing alterations in larval and pupal mean body length when exposed to different concentrations of MCP in comparison to control.

Table 2. Table data showing mean body length (mm) of 3rd instar larva and pupa.

Concentrations	Control	0.2µg/mL	0.25 µg/mL	0.3 µg/mL	0.35 µg/mL	0.4 µg/mL
Mean Larval length ± SE	4.05±0.02	3.53±0.26	3.55±0.05**	3.08±0.04**	3.03±0.06**	3.1±0.05**
Mean Pupal length ± SE	3.21±0.09	3.07±.008	2.81±0.11	2.64±0.08*	2.89±0.01*	2.87±0.03*

*p<0.05 and **p<0.01 are considered as statistically significant

Alteration in the Sex-Ratio

Significant alteration in sex-ratio was found in treated groups in compared to control. The higher concentrations were found to be a tendency towards increase percentage of male fly emergence. The percentage of male fly emergence has been observed in 0.25 µg/mL,0.3 µg/mL and 0.35 µg/mL groups viz., 63.33 ± 1.92, 58.88 ± 1.11 and 64.44 ± 1.11 respectively unlike control 51.10±2.22. Interestingly in 0.2 µg/mL and 0.4 µg/mL the male emergence flies are not significantly increased. The possible reason is in 0.2 µg/mL the concentration is quite less to provoke such change. In contrast the result of feeding assay revealed that amount of food uptake in the highest concentration in less. So, it might be for lower intake of toxic food, there is increase in male flies but not significantly increased.

Discussion

Current study explored deleterious effects of chronic exposure of an organophosphate pesticide, monocrotophos, at organism and sub-organism level on *D. melanogaster*. Present study determined the chronic LC_{30}, LC_{40} and LC_{50} value of monocrotophos of in 3rd instar larvae of *D.*

melanogaster. The data of probit analysis indicated that the values are 0.42, 0.54 and 0.68µg/mL respectively in case of 3rd instar larvae. Similarly in case of pupae the value of LC_{40}, LC_{50} and LC_{60} are 0.40, 0.56 and 0.78 µg/mL. Based on this observation, five sub-lethal concentrations (0.2, 0.25, 0.3, 0.35 and 0.4 µg/mL) were selected that were much lower than the determined LC_{50} value for the toxicity assessment. Physical growth and activity of living organism is considered as a parameter to assess the physiological condition of the organism.

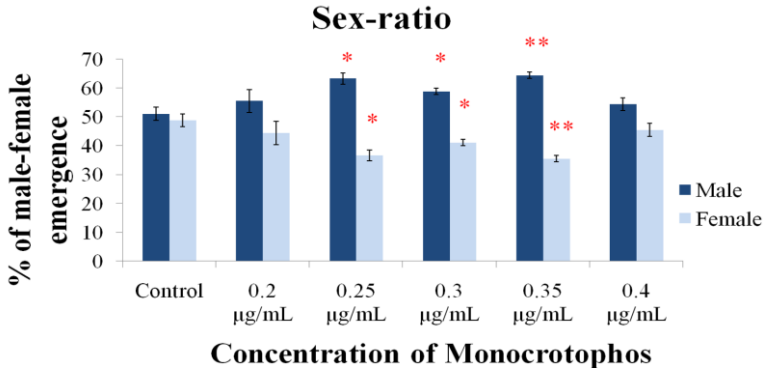

Fig 8. Graph representing the comparison of altered percentage of male and female fly emergence among the treated groups in respect to control.

In the present study it was observed that monocrotophos significantly affected the amount of food intake among exposed larvae. The group of larvae, who were exposed to 0.3 µg/mL of monocrotophos through their food medium showed maximum food intake which in turn negatively interfered with their body weight, body length and duration of their life cycle. Body weight of the larvae were found to be gradually decreased with increased concentration of monocrotophos. Interestingly, 0.3 µg/mL concentration showed maximum depletion in both body weight and size in case of larvae and pupae where feeding was maximum. This observation indicates feeding habit negatively impacted the body weight.

Similar result was observed in a previous study where significant weight loss in male fruit flies was found after chronic sub-lethal exposure to acephate (Mandi et al., 2019). Larval duration was found to be significantly reduced in the present study. Where, the higher monocrotophos concentrations larva took lowest days for pupation. Similar reduction in life cycle parameter was observed after exposure to another organophosphate pesticide acephate in *Drosophila melanogaster* (Rajak et al., 2013). Interestingly an alteration in sex-ratio has been observed. In treated groups there was biasness towards emergence of male flies. In 0.25, 0.3 and 0.35 µg/mL concentration groups showed significant increase in newly emerged male flies which indicates a stress induced alteration in sex determinant genes and their products. Similar type of result was observed in aquatic mollusks where starvation induced stress provoked upregulation of male specific genes like Dmrt1 (Sun et al., 2023). Another experiment performed by Cheryl et al., 2004 illustrated that specific diet induced altered sex-ratio in rats. This fact confirmed that specific diet or stress condition can led to change in sex determination.

Conclusion

Drosophila melanogaster is widely used as study model in the field of genetics, disease biology, developmental biology etc. Moreover, it provides wide range screening of potential ameliorative properties of various agents against any induced disease and toxicity. Chronic sub-lethal exposure of monocrotophos resulted into compromised physical condition which includes shortening of life cycle, reduced body weight and body length and alteration in sex ratio. As a consequence, outcomes of the current study can be extrapolated to other non-target organisms including birds, other invertebrates, mammals and even human beings as because of their developmental, physiological and genetic homologies. In addition, the work also establishes *Drosophila melanogaster* as an alternative model in the toxicological study for commercially available chemicals like food preservatives and other environmental contaminants.

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Author Contributions

Kanchana Das: Investigation, Data Curation, Writing, Original draft preparation.
Shanta Pramanik: Data curation, Writing
Prem Rajak: Writing, Reviewing and Editing
Gopal Biswas: Writing, Reviewing and Editing
Moutushi Mandi: Conceptualization, Reviewing and Editing

Acknowledgements
Authors are thankful to the Department of Zoology (DST-FIST sponsored), The University of Burdwan (BU) for providing infrastructural facilities to carry out the present work. We are grateful to Dr. Abhijit Mazumdar for providing environmental chamber.

Funding
Authors are grateful to Swami Vivekananda Merit-cum-Means scholarship, Department of Higher Education, Government of West Bengal, India, which was awarded to the author Kanchana Das (WBP231693899559).

Availability of data and materials
Not applicable.

Competing interest
The authors declare no competing interests.

Ethics approval
Not applicable.

Citation: Das K, Pramanik S, Rajak P, Biswas G and Mandi M (2024) Monocrotophos induced changes in the life cycle parameters of fruit fly. Environ Sci Arch 3(STI-1): 6-13.