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Insecticide Exposure May Pose a Threat to the Future Life Expectancy of Non-Targets: Inferred from Fly Research

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Abstract

The life span of organisms becomes very flexible and vulnerable due to environmental and climatic changes, which have both natural and man-made factors. One such example is the inappropriate use of insecticides and pesticides in the agriculture and health sectors, which cause environmental pollution, despite their agricultural and health benefits. Two of the most widely used organophosphate insecticides, chlorpyrifos (CP) and ethion (ET), are known to have neurotoxic properties and they target to inhibit the neurotransmitter function of acetylcholinesterase (AChE) in insects. Previous studies have shown that exposure to these insecticides brings detrimental health effects to non-targets including humans. The present work aims to estimate the effects of CP and ET on lifespan in two sibling species of *Drosophila, Drosophila melanogaster* and *Drosophila simulans*, after being exposed for 24 and 48 hrs to their sublethal concentrations (determined from LC_{50} and LC_{90}). To understand the molecular mechanism behind this, Differential Expression Gene (DEG) analysis of ET-exposed *Drosophila* flies was carried out through whole transcriptome sequencing, which shows 85 upregulated and 95 downregulated ageing-associated genes, involved in the regulation of cell cycle, growth and development. The flies were then continuously cultured in insecticide-treated and untreated media (Control) for ten generations, after which the lifespan was calculated (Control vs F_1 vs. F_{10}). In comparison, the results revealed a remarkable reduction of lifespan in F_1 treated, which increased in F_{10} treated flies, but still, it did not reach up to the average lifespan of the control. Thus, the long-term effect of insecticide exposure on the lifespan of non-targets cannot be overlooked and needs to be further addressed.

Keywords: Differential Gene Expression, *Drosophila*, Lifespan, Non-Targets, Organophosphates

1. Introduction

Life expectancy is a measure of the average living period that is associated with age standardization. The World Health Organization (WHO) finds life expectancy to be the most significant health indicator that represents the standard of living[1,2]. Although the life expectancy of humans has increased globally due to improved healthcare and treatments, it is affected measurably by environmental pollution[3]. Moreover, it is reported that only 40% of human life expectancy is inherited across generations, with the rest contributed by several environmental and physiological factors[4]. As a consequence, the individual's lifespan is observed to be highly variable and vulnerable

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as compared to their life expectancy. The aging and lifespan-associated pathways regulate an organism's growth, essential physiology, and reproduction. To distinguish the impact of environmental hazards from the genetic basis acting on longevity, it is useful to study lifespan variation among closely related or sibling species within a controlled environment. In the present study, the authors aim to understand mechanisms underlying variation in lifespan on exposure to the sublethal doses of two insecticides, chlorpyrifos (CP) and ethion (ET), of the organophosphate class using the *Drosophila* model representing non-target organisms, the result of which will lay the foundation for further molecular analysis.

Chlorpyrifos (CP) and ethion (ET) insecticides are widely used in agriculture and healthcare sectors to control insect populations by targeting their neurotransmission pathways through inhibition of the acetylcholinesterase (AChE) enzyme[5,6]. On the positive side, these insecticides are very effective, but their inappropriate or uncontrolled use results in the accumulation of their residues in the environment. According to 2022 estimates, only 0.1% of organophosphates reach their intended target[7,8]. Acute and chronic exposure to these insecticides and their residues can lead to various diseases by affecting various cellular and physiological activities of organisms inhabiting the earth, which are not targets. Literature has evidenced such effects on organ systems of the human body, such as the reproductive, immune, nervous, respiratory, and cardiovascular systems, and the life expectancy of humans as well[9]. Organophosphate insecticide poisoning leads to illness and death worldwide, particularly in underdeveloped countries[10,11]. Non-target organisms, including humans, are unfortunately exposed to these insecticides regularly.

Drosophila fruit fly is an ideal model organism to study longevity and aging because of its ease of handling and maintenance, a large number of progenies per adult, short life cycle, and lifespan. In the 21st century, Genome-Wide Analysis (GWA) has become a popular bioinformatics approach for identifying evolutionarily conserved genes involved in complex human diseases[12]. In this respect, the fruit fly *Drosophila* established itself as a human disease model, having ~75% of human disease genes homologs in its genome[13,14]. In the present study, a range of different concentrations of each CP and ET were used to determine their individual LC_{50} and LC_{90} and two sublethal doses of each insecticide were used to investigate the possible harmful acute and chronic effects on the lifespan of two sibling species of *Drosophila,* i.e., *Drosophila melanogaster and Drosophila simulans.* We have further identified ageing-associated upregulated and downregulated genes triggered due to low doses of ethion exposure, by performing RNA-Seq in *D. melanogaster.*

2. Materials and Methods

2.1 Fly Strain

Drosophila flies were collected from West Delhi, India, in 2022, and isofemale lines were established from the nature-caught inseminated females. After identification, two isofemale lines, one from *D. melanogaster* (DL 36) and the other from *D. simulans* (DL 42), were used in the present study. The flies were maintained on standard yeast-molasses media at 25°C, 60–70% relative humidity, and a 12-hour light/dark cycle in our laboratory before the setting up of experiments.

2.2 Organophosphate Insecticides

Chlorpyrifos ($C_9H_{11}Cl_3NO_3PS$) 20% EC and ethion ($C_9H_{22}O_4P_2S_4$) 50% EC (emulsifiable concentrate) were purchased from the commercial market. As per the instructions on the bottles, ethion and chlorpyrifos were produced by Ankur Industries Private Limited (Uttar Pradesh) and Insecticides India Limited (Rajasthan), respectively. The stock solution of both insecticides was prepared separately in a 1:1 ratio with acetone.

2.3 LC_{50} and LC_{90} Determination and Sublethal Doses

CP and ET of different amounts were mixed in 100 ml of standard food media, and the presence of these insecticides in 100 ml of media was calculated (as mentioned below in ppm). The 4-day-old 5 couples (virgin males and females) were acutely exposed to six concentrations of CP-treated media (0.5, 0.7 ppm, 0.9 ppm, 1 ppm, 3 ppm, and 5 ppm) and ET-treated media (2.5 ppm, 7.5 ppm, 12.5 ppm, 17.5 ppm, 22.5 ppm, and 27.5 ppm) for a time interval of 24 and 48 hrs, and the number of dead flies was noted. Six replicates were carried out for this experiment. LC_{50} and LC_{90} were calculated through probit analysis using the formula:

$$Log(c\%) = \frac{Test\ value - Intercept\ value}{Slope},$$

$$LC_{50} = 10\hat{}(Log(c\%).$$

Based on the survivability of flies, two sublethal concentrations of each insecticide, CP (0.3 ppm and 0.5 ppm) and ET (1.25 ppm and 1.88 ppm) were selected for the lifespan experiments. The flies were cultured consistently in these sublethal concentrations for 10 generations. The LC_{50} and LC_{90} values of F_{10} flies were again calculated following the same protocol as initially done with F_0 flies. The LT_{50} and LT_{90} were determined only with sublethal concentrations of both CP (0.3 and 0.5 ppm) and ET (1.25 and 1.88 ppm) using the probit analysis method as mentioned above.

2.4 Lifespan

The method of lifespan experiment is shown in Figure 1. Freshly emerged virgin males and females were kept on standard and insecticide-treated food vials (5 couples in each), and the number of dead flies was noted every day until the last fly died. For each experimental condition, the percentage of survivability was calculated from six replicates with 60 flies. The experiments were conducted with both sublethal concentrations of CP and ET as mentioned above.

2.5 Total RNA Extraction and Retrieval of Ageing Associated Functional Genes

Total RNA was extracted from control and ethion (1.88 ppm) exposed *D. melanogaster* flies (4 days old female; total: 20) using the conventional TRIzol method followed by Column purification using Quick RNA Miniprep Plus Kit (Zymo Research). The quality and quantity of the extracted total RNA samples were checked. The high-quality RNA was further used for sequencing and analysed for differential gene expression (Eurofins GS No: GS_2023_NGS-416 and Project Id: GA232729). From the upregulated and downregulated differential gene expression results, the ageing-associated functional genes were selected (literature-based).

2.6 Protein-Protein Interaction Network (PPI) and Hub Gene Identification

The Protein-Protein Interaction (PPI) enrichment network was constructed for both upregulated and downregulated ageing-associated genes using the STRING version 12.0 with the minimum necessary interaction score set at 0.4 (http://string-db.org/). The generated PPI networks were visualized by Cytoscape v.3.10.2. The top ten hub genes were extracted and identified by CytoHubba plugin from each network (upregulation and downregulation). The CytoHubba was used to determine the degree of every protein node which represents the strength of their interaction.

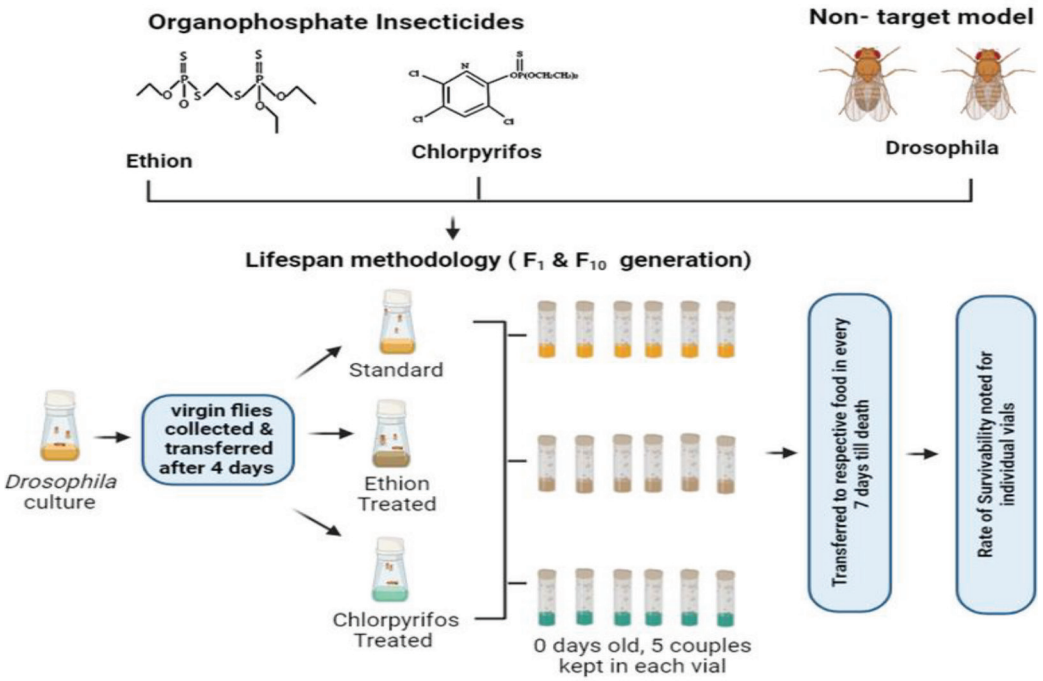


Figure 1. Overview of the lifespan methodology by exposing chlorpyrifos and ethion to a non-target *Drosophila* model.

2.7 Statistical Analysis

The LC_{50} and LC_{90} were calculated through the probit analysis method. The IBM SPSS statistics program version 29.0.1.0 was used for LT_{50} and LT_{90} calculations. All figures were generated with the help of Origin 202443b software.

3. Results and Discussion

Organophosphates are the most widely used broad-spectrum insecticides worldwide, which were initially introduced by German scientists in 1938[15,16]. It is evident from previous studies conducted using various models that their neurotoxicity affects the both behavioural and physical health of targets as well as non-targets, ultimately troubling the overall aging process[17-19]. Thus, there is a serious concern about the overuse and inappropriate application of these insecticides in agriculture and residential areas. The major focus of this study is to understand the long-term/transgenerational effect of two such organophosphates; CP and ET on the lifespan of non-targets using the *Drosophila* model.

Drosophila has been used as a eukaryotic model organism in various fields of research on account of its short lifecycle, well-defined biological system and short lifespan, i.e., within 2-3 months[20,21]. Thus, a transgenerational study could be possible for tracking the toxicological effects. The present study was conducted using *D. melanogaster* and its sibling species, *D. simulans*, for an in-depth interpretation of the study results. The isofemale lines of *D. melanogaster* (DL 36) and *D. simulans* (DL 42) were used in this study with the intention that we can proceed with the present study results for dissecting the molecular mechanism by transcriptomics analysis. These two isofemale lines were established in the laboratory using naturally inseminated female flies. The identification of both species was done through observation of morphological and anatomical features, e.g., size, shape, body pigmentation, male sexcomb, and genital region, as shown in Figure 2. The distinct male cuticular structures of the posterior lobe and a wedge-shaped outgrowth of the male genitalia confirmed their identification, as depicted in Figure 2.

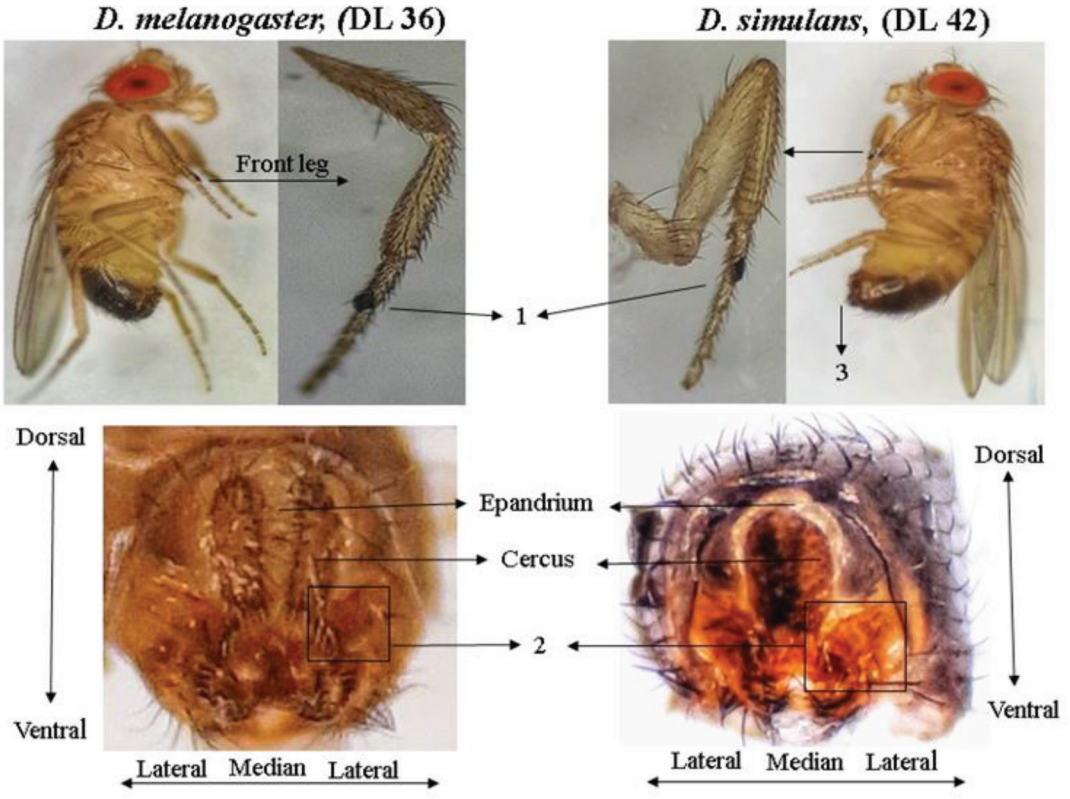


Figure 2. Stereomicroscopic images of species-specific traits in *D. melanogaster* and *D. simulans*;1. Sexcomb; 2. Posterior lobes; 3. Epandrium.

Initially, the flies (F_0) of *D. melanogaster* and *D. simulans* were exposed to six different concentrations of CP and ET-added media for 24 and 48 hours, respectively, and the survivability percentage was noted as shown in Figures 3a-h.

The LC_{50} and LC_{90} of both insecticides were calculated through the probit method using the slope and intercept values obtained from plotting the log value of insecticide concentration (ppm) against the number of flies' probit mortality. The plotted graphs of *D. melanogaster* have been shown in Figures 4(a)-(d) for more clarification. The remaining lethal concentrations were calculated similarly and mentioned in Table 1. The LT_{50} and LT_{90} values of sublethal concentrations of CP and ET were calculated through the probit method and are mentioned in Table 1.

The susceptibility to the impact of CP and ET was found to increase mortality with an increase in concentration as observed in both species. The LC_{50} of CP-treated *D. melanogaster* flies (F_0) was found to be 0.7 ppm and 0.6 ppm after an interval of 24 and 48 hours, respectively, which increased to 1.3 and 1.1 ppm after ten generations of CP exposure. The ET-treated LC_{50} values of

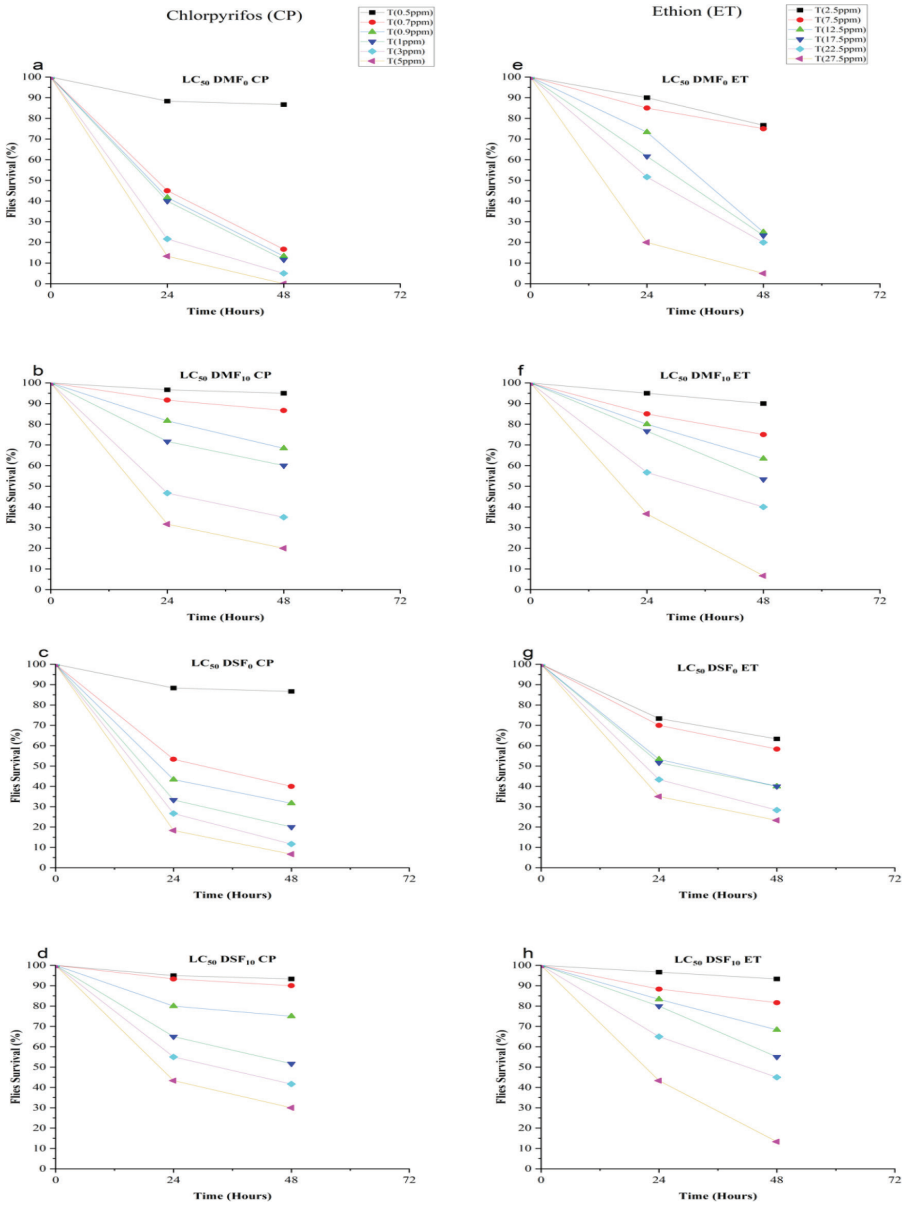


Figure 3. Percentage of flies' survivability on 24- and 48-hours of acute exposure to six different concentrations of CP and ET in F_1 and F_{10} generations of both studied species.

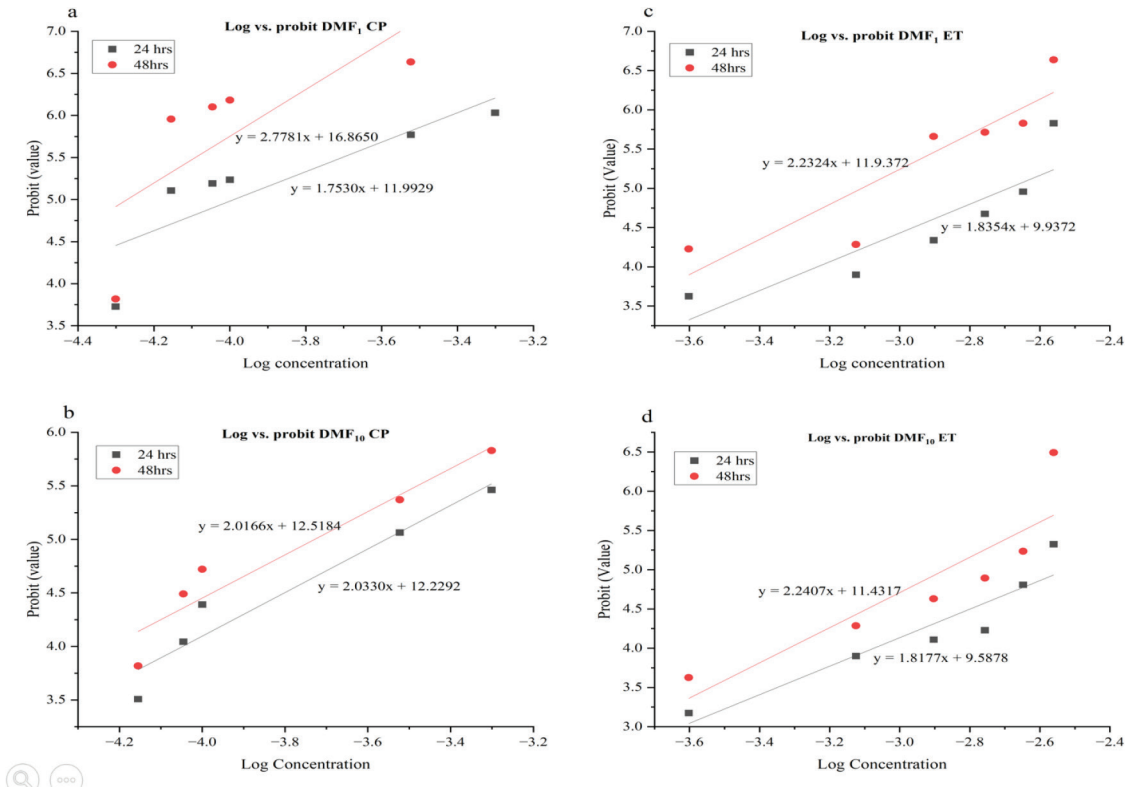


Figure 4. Shows the linear relationship of probit mortality vs log concentration in F_1 and F_{10} generations of CP and ET exposure (24 and 48 hours) *D. melanogaster* flies; six different concentrations of CP (a-b) and ET(c-d).

Table 1. Lethal concentrations and lethal time of chlorpyrifos and ethion insecticide evaluated in two species of *D. melanogaster* and *D. simulans*

Insecticides		**Chlorpyrifos**				**Ethion**			
Species		***D. melanogaster***		***D. simulans***		***D. melanogaster***		***D. simulans***	
LC_{50} and LC_{90} (ppm)									
Range of conc.		**0.5 – 5ppm**				**2.5 – 27.5 ppm**			
Generation		**F_0**	**F_{10}**	**F_0**	**F_{10}**	**F_0**	**F_{10}**	**F_0**	**F_{10}**
24 hrs.	LC_{50}	0.7	1.3	0.8	1.1	23.6	24.2	16.5	25.9
	LC_{90}	3.6	6.8	4.0	3.8	103.4	126.3	157.7	753.2
48hrs.	LC_{50}	0.6	1.1	0.7	1.0	10.4	18.4	7.7	19.3
	LC_{90}	1.7	5.3	2.8	3.3	98.1	60.3	87.5	1870.5
LT_{50} and LT_{90} (hours)									
Generation		**F_1**	**F_{10}**	**F_1**	**F_{10}**	**F_1**	**F_{10}**	**F_1**	**F_{10}**
Concentration		**0.3 ppm**				**1.25 ppm**			
LT_{50}		300.6	751.8	367.6	610.5	574.0	874.5	343.4	1039.9
LT_{90}		1133.8	1644.5	1031.6	1799.3	1614.3	1843.6	1464.9	1983.2
Concentration		**0.5 ppm**				**1.88 ppm**			
LT_{50}		300.4	485.7	281.7	495.7	534.7	541.7	307.3	725.8
LT_{90}		737.7	982.0	872.7	1364.6	1173.7	1620.8	1127.3	1694.0

F_0 generation were 23.6 and 10.4 ppm after 24 and 48 hours of exposure, which increased in F_{10} generation to 24.2 and 18.4 ppm respectively. Similar results were observed in *D. simulans* species, which reveal the development of resistance towards both insecticides on chronic exposure. By analysing the LC_{50} and LC_{90} values of both insecticides in both species, it is evident that 1) *D. simulans* is more susceptible to ET, and *D. melanogaster* is more susceptible to CP; 2) the higher LC_{50} and LC_{90} values in ET-treated experimental flies confirm ET to be less toxic than CP; 3) resistance towards both insecticides was developed faster in *D. simulans* species 4) the increased LT_{50} and LT_{90} values of sublethal concentrations of CP and ET were directly proportional with the generation and the LT values was found to be less in chlorpyrifos as compared to ethion in both sublethal concentrations.

The lifespan of *D. melanogaster* and *D. simulans* was found to be 1752 hrs. (73 days) and 1632 hrs. (68 days) respectively as estimated from the control groups. The CP-treated (0.3 ppm) *D. melanogaster flies* of F_1 and F_{10} generations survived till 744 hrs. (31 days) and 1440 hrs (60 days). In cases of 0.5 ppm concentration of F_1 and F_{10} generation, the flies survived for 600 hrs. (25 days) and 864 hrs (36 days). Thus, the mortality rate is high with the increase in concentration, even after exposure of 10 generations. Similarly, the *D. simulans* flies exposed to 0.3 ppm CP of F_1 and F_{10} generation, survived for 744 hrs. (31 days) and 1464 hrs. (61 days). In the case of 0.5 ppm, the survival duration of adult flies was 576 hrs. (24 days) and 984 hrs (41 days) as shown in Figures 5a-d.

The ET-treated (1.25 and 1.88 ppm) experimental groups of F_1 generation survived for 1248 hrs. (52 days)

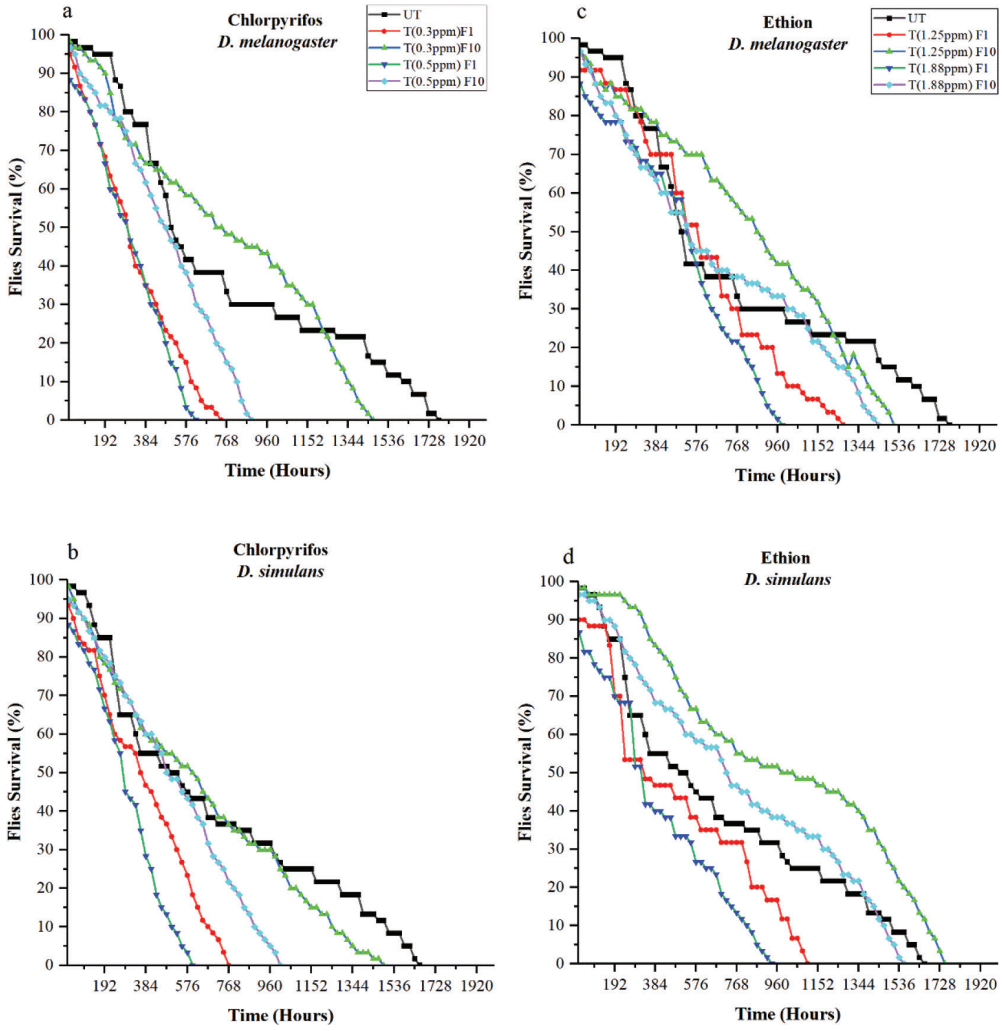


Figure 5. Effect of chlorpyrifos (a-b) and ethion (c-d) on the lifespan of both *Drosophila* species after exposure to different sub-lethal concentrations in F_1 and F_{10} generations

and 960 hrs. (40 days) in *D. melanogaster* and 1080 hrs. (45 days) and 912 hrs. (38 days) in *D. simulans* respectively. The flies exposed to both sublethal concentrations (1.25 and 1.88 ppm) survived more in F_{10} generation in both the study species. as shown in Figure 5a-d.

The result of lifespan showed that ET-exposed flies survival duration is higher than CP-exposed flies in both species. In both the insecticide-exposed F_1 flies, the lifespan has been reduced compared to the control ones. In the case of F_{10} generation, the lifespan is increased as compared to F_1 generation in both the ET and CP groups, but it's not similar as in the control groups, except in the case of 1.25 ppm concentration of ET. The Hormesis curve is seen in F_{10} generation in sublethal concentrations of both insecticides, which depicts the resistance and ecological backlashes[22]. Thus, there is no doubt, that the insecticide pollutants in the environment will affect the lifespan of non-targets in both acute and chronic exposure as inferred from *Drosophila* research.

To understand more about the genes involved in this process, ethion-exposed female *D. melanogaster* flies of the F_1 generation were used for the RNA profiling. Using the high-throughput Illumina RNA-sequencing method, in *D. melanogaster* 5528 differentially expressed genes (2515 upregulated and 3013 downregulated) were found in response to ethion exposure (1.88ppm). After exploring the functional data of DEGs available in NCBI, 85 upregulated and 95 downregulated genes associated with ageing were listed. The protein-protein interaction network was constructed separately for these upregulated and downregulated genes by using the STRING as depicted in Figure 6.

The top ten hub genes each from upregulated (foxo, elav, dpp, hh, Myc, puc, Stat92E, Pka-C1, htt, Mmp1) and

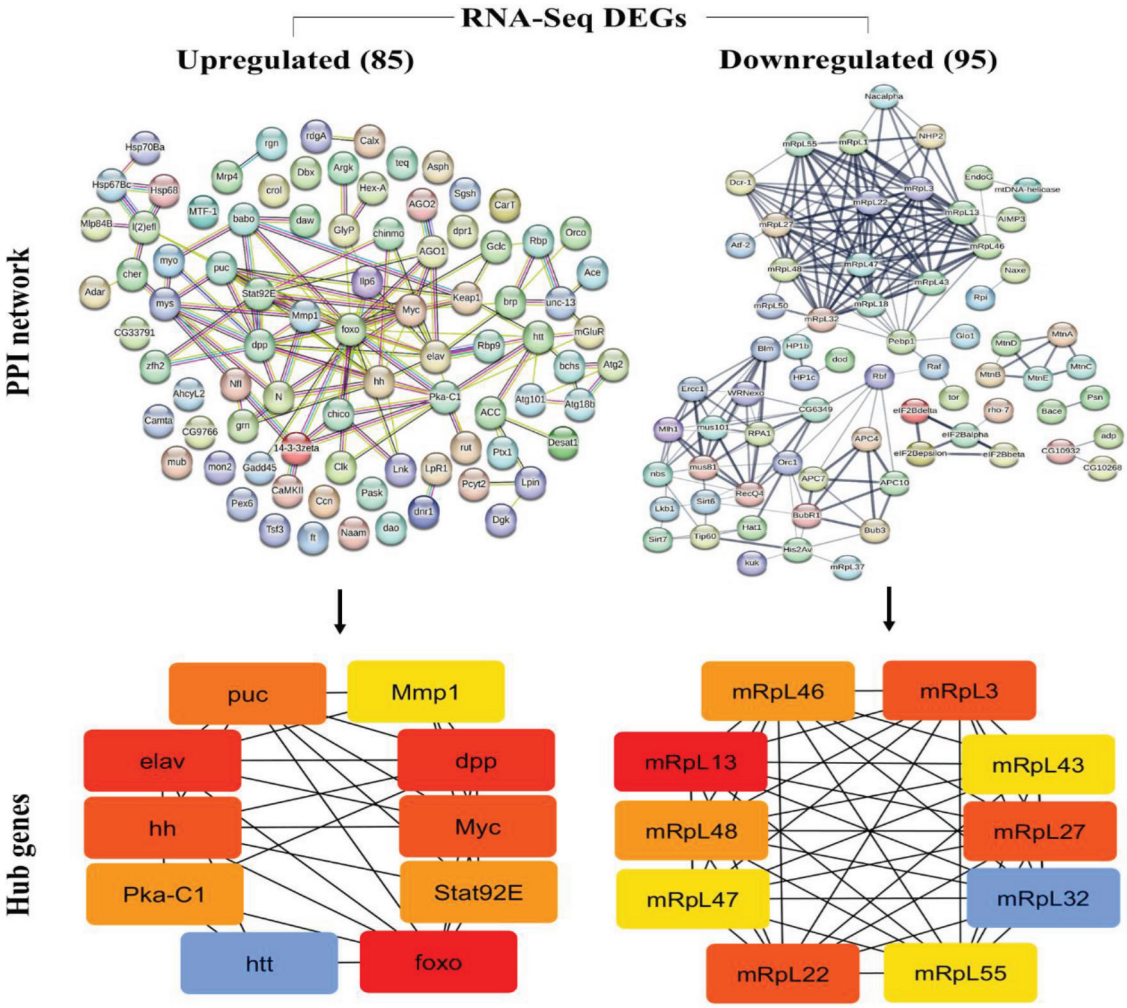


Figure 6. The PPI network of ageing-associated genes derived from STRING and Cytoscape.

downregulated (mRpL13, mRpL27, mRpL3, mRpL22, mRpL32, mRpL46, mRpL48, mRpL43, mRpL47, mRpL55) were ranked according to their score obtained in Cytoscape and studied for their associated function and human orthologue as shown in Table 2.

The functional aspects of this hub gene were further analysed and it is observed that these genes are mostly associated with the growth and development through the regulation of various gene expression. FOXOs are a subfamily of the Forkhead transcription factor family having over 100 members in humans, ranging from FOXA to FOXS. In mammals, this subfamily is engaged in a variety of critical physiological functions including stress tolerance, metabolism, cell cycle arrest, and apoptosis,

Table 2. Functional details of the top ten DEGs resulted from Ethion-treated RNA-Seq and their human orthologue

		Upregulated				
Rank	**Hub Genes**	**Gene ID**	**Score**	**Function**	**Used in studying**	**Human Orthologue**
1	foxo	FBgn0038197	20	Enables DNA-binding transcription activator activity and is involved in several processes, e.g. regulation of cellular catabolic process; signal transduction, and transcription by RNA polymerase II.	Parkinson disease	FOXO1, FOXO3 and FOXO4 (forkhead box O1, O3 and O4)
2	elav	FBgn0260400	13	Enables poly(U) RNA binding activity. Involved in central nervous system development and negative regulation of mRNA 3'-end processing	Cancer and neurodegenerative diseases	ELAVL3 (ELAV like RNA binding protein 3)
3	dpp	FBgn0000490	13	Heparin-binding activity; protein dimerization activity; and signalling receptor binding activity. Involved in several processes e.g., organ development; gamete generation; and open tracheal system development.	juvenile polyposis syndrome	BMP2 and BMP4 (bone morphogenetic proteins 2 and 4)
4	hh	FBgn0004644	11	Enables morphogen activity and patched binding activity. Involved in several processes, including animal organ development; nervous system development; and open tracheal system development.	bacterial infectious disease.	DHH (desert hedgehog signaling molecule)
5	Myc	FBgn0262656	11	Enables DNA binding activity; DNA-binding transcription factor activity; and ubiquitin-specific protease binding activity. Involved in several processes, including DNA endoreduplication; cell competition in a multicellular organism; and positive regulation of transcription, DNA-templated.	cancer	MYC (MYC proto-oncogene, bHLH transcription factor)
6	puc	FBgn0243512	10	Enables JUN kinase phosphatase activity. Involved in several processes, including eggshell chorion assembly; morphogenesis of an epithelium; and negative regulation of signal transduction.	Ageing	DUSP10 (dual specificity phosphatase 10)

Table 2. Continued...

		Upregulated				
Rank	**Hub Genes**	**Gene ID**	**Score**	**Function**	**Used in studying**	**Human Orthologue**
7	Stat92E	FBgn0016917	9	Enables several functions, including DNA-binding transcription activator activity, RNA polymerase II-specific; RNA polymerase II cis-regulatory region sequence-specific DNA binding activity; and histone binding activity. Involved in several processes, including oogenesis; post-embryonic animal organ development; and segmentation.	growth hormone insensitivity syndrome with immune dysregulation, lipoid nephrosis, prostate carcinoma and urinary schistosomiasis	STAT5A and STAT5B (signal transducer and activator of transcription 5A and 5B)
8	Pka-C1	FBgn0000273	9	Enables cAMP-dependent protein kinase activity. Involved in several processes, including oocyte axis specification; regulation of intracellular mRNA localization; and regulation of smoothened signalling pat.	alcohol use disorder	PRKACA and PRKACB (protein kinase cAMP-activated catalytic subunit alpha and beta)
9	htt	FBgn0027655	8	Involved in several processes, including establishment of mitotic spindle orientation; heterochromatin boundary formation; and synaptic vesicle transport.	Huntingtin disease	HTT (huntingtin)
10	Mmp1	FBgn0035049	7	Predicted to enable metallo-endopeptidase activity. Involved in several processes, including animal organ development; fasciculation of motor neuron axon; and open tracheal system development.	artery disease, female reproductive organ cancer, intrinsic cardiomyopathy, obstructive lung disease and urinary system cancer	MMP16 and MMP24 (matrix metallopeptidase 16 and 24)
		Downregulated				
1	mRpL13	FBgn0032720	16	Predicted to enable mRNA binding activity and structural constituent of ribosome. It is involved in the negative regulation of translation		MRPL13 (mitochondrial ribosomal protein L13)
2	mRpL27	FBgn0053002	15	Predicted to be a structural constituent of ribosomes and to be involved in mitochondrial translation		MRPL27 (mitochondrial ribosomal protein L27)
3	mRpL3	FBgn0030686	15	Predicted to be a structural constituent of the ribosome and to be involved in translation. Part of the mitochondrial large ribosomal subunit.		MRPL3 (mitochondrial ribosomal protein L3)

Table 2. Continued...

		Upregulated				
Rank	**Hub Genes**	**Gene ID**	**Score**	**Function**	**Used in studying**	**Human Orthologue**
4	mRpL22	FBgn0030786	15	Predicted to be a structural constituent of ribosomes and to be involved in ribosome assembly		MRPL22 (mitochondrial ribosomal protein L22)
5	mRpL32	FBgn0039835	15			MRPL32 (mitochondrial ribosomal protein L32)
6	mRpL46	FBgn0035272	14			MRPL46 (mitochondrial ribosomal protein L46)
7	mRpL48	FBgn0031357	14			MRPL48 (mitochondrial ribosomal protein L48)
8	mRpL43	FBgn0034893	13			MRPL43 (mitochondrial ribosomal protein L43)
9	mRpL47	FBgn0014023	13			MRPL47 (mitochondrial ribosomal protein L47)
10	mRpL55	FBgn0038678	13			MRPL55 (mitochondrial ribosomal protein L55)

although its significance in longevity is unknown[23]. The protein ELAV (embryonic lethal abnormal visual system) is a member of the family, which plays a vital role in RNA metabolism. This protein is found to be conserved throughout evolution, as studied in Xenopus, mouse, zebrafish, human, and rat[24]. In *D. melanogaster*, this gene is associated with the overall neural development[24]. Likely, Decapentaplegic (Dpp) plays a key role in all developmental stages, including stem cell maintenance to their regeneration. In the last few years, the role of Dpp has been explored primarily in the control of embryonic and imaginal disc patterning[25]. The hedgehog (hh) gene was first discovered in *Drosophila*, and its homologue Shh is the most researched ligand of the hedgehog signalling pathway found in vertebrates. It regulates vertebrate organogenesis and previous research has shown that it is vital during retinal development[26]. Myc plays an important function in increasing cell cycle progression and proliferation[27]. Puckered (puc), one of the Activator proteins (AP-1) target genes and its mutants have increased JNK signalling which plays a role in *Drosophila* ageing[28]. Furthermore, STAT proteins are transcription factors whose abnormal regulation in humans has been linked to cancer. There is one single STAT protein (STAT92E) in *Drosophila* and the JAK/STAT signalling affects multiple developmental processes like as sex specification, segmentation, organogenesis, and stem cell regulation[29]. Protein kinase A (PKA) is known to have multi-unit protein kinase which has numerous roles including regulation of carbohydrate and lipid metabolism. The

PKA pathway has aroused researchers' interest in the study of ageing in yeast, *Drosophila* and mice model[30]. HTT gene is responsible for causing Huntington's Disease (HD) which is an autosomal progressive neurological condition characterised by the amplification of CAG repeats[31]. Matrix Metalloproteinases (MMPs) are a group of zinc (Zn)-dependent endopeptidases that can cleave almost all Extracellular Matrix (ECM) substrates. MMP1, MMP-8, and MMP-9 are the most investigated potential genes, and their altered gene expression could be contributing to the cause of premature birth[32].

It has been evidenced from the literature that mitochondrial function plays a central role in ageing research[33-35]. Its dysfunction is linked to lifespan regulation, cognitive ageing, and Alzheimer's disease. Nuclear gene-encoded Mitochondrial Ribosomal Proteins (MRPs) are synthesised in the cytoplasm and then transported to the mitochondria to form mitochondrial ribosomes. MRPs play important roles in mitochondrial Oxidative Phosphorylation (OXPHOS) and act as apoptosis-inducing agents and cell state modulation. MRPs with abnormal expressions lead to mitochondrial metabolic disorder, cell malfunction, tumours etc[33,35]. Earlier studies in mice and *Caenorhabditis elegans* show that an imbalance in protein translation between mitochondrial and nuclear genes affects lifespan signalling pathways[34]. Thus, the downregulation of MRPs obtained from RNA-Seq evidenced the malfunction of mitochondria regulated through nuclear gene expression.

In the past, studies were conducted on ethion-exposed non-target pests such as *Procambarus clarkia* (crayfish) and *Apis mellifera* (honey bee), which showed high mortality rates in both species, especially affecting the honey bees[36,37]. The effect of CP was studied on female *D. melanogaster* and *Brachionus koreanus*, where the lifespan was significantly reduced in a dose-dependent manner[17,38]. As per the literature, the effect of CP causes harmful effects on the brain and disturbs motor activities in non-target species such as *Drosophila*, *Guppy Poecilia reticulata*, big brown bats (*Eptesicus fuscus*) and guinea pigs[39-42].

Thus, *Drosophila* warns about the toxic effects of insecticides on non-target aging, which needs to be taken seriously in the regulation and management of insecticide application. Along with the proper guidelines and restricting use of insecticides, it would be better to explore other possibilities, such as the usage of novel safe compounds, which may effectively control the insects of agricultural and medical importance. *Drosophila* being a human disease model with a 60% genome similarity and 75% homology in disease-causing genes proves itself a very useful model system to understand the molecular changes undergone in various associated metabolic pathways after exposure to various insecticides[43].

4. Conclusion

Through classical and molecular studies, it has been established that insecticide exposure affects the duration of lifespan and its associated various metabolic and signalling pathways. However, this work can be further extended in validating the DEGs (RT-PCR) and by their deep functional analysis, ageing-associated key genes/pathways can be identified. As the species used in this study have close genetic backgrounds, further work design and interpretation will provide a strong and significant outcome.

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6. References

1. Modig K, Rau R, Ahlbom A. Life expectancy: What does it measure? BMJ open. 2020; 10(7). http://dx.doi.org/10.1136/bmjopen-2019-035932 PMid: 34444306 PMCid: PMC8391297.
2. Chen Z, Ma Y, Hua J, Wang Y, Guo H. Impacts from economic development and environmental factors on life expectancy: A comparative study based on data from both developed and developing countries from 2004 to 2016. Int J of Environ Res Public Health. 2021; 18(16):8559. https://doi.org/10.3390/ijerph18168559 PMid: 34444306 PMCid: PMC8391297.
3. Rahman MM, Rana R, Khanam R. Determinants of life expectancy in most polluted countries: Exploring the effect of environmental degradation. PloS one. 2022; 17(1):e0262802. https://doi.org/10.1371/journal.pone.0262802 PMid: 35061838 PMCid: PMC8782287.
4. Bin-Jumah MN, Nadeem MS, Gilani SJ, Al-Abbasi FA, Ullah I, Alzarea SI, Ghoneim MM, Alshehri S, Uddin A, Murtaza BN, Kazmi I. Genes and longevity of lifespan. Int J Mol Sci. 2022; 23(3):1499. https://doi.org/10.3390/ijms23031499 PMid: 35163422 PMCid: PMC8836117.

5. Tefera YM, Gaskin S, Thredgold L, Pisaniello D. The role of formulation co-ingredients in skin and glove barrier protection against organophosphate insecticides. Pest Manag Sci. 2022; 78(1):177- 83. https://doi.org/10.1002/ps.6621 PMid: 34464493.
6. Cabrera M, Capparelli MV, Nacato-Ch C, Moulatlet GM, Lopez-Heras I, Gonzalez MD, Alvear-S D, Rico A. Effects of intensive agriculture and urbanization on water quality and pesticide risks in freshwater ecosystems of the Ecuadorian Amazon. Chemosphere. 2023; 139286. https://doi.org/10.1016/j.chemosphere.2023.139286 PMid: 37379974.
7. Ore OT, Adeola AO, Bayode AA, Adedipe DT, Nomngongo PN. Organophosphate pesticide residues in environmental and biological matrices: Occurrence, distribution and potential remedial approaches. J Environ Chem Ecotoxicol. 2023; 5: 9-23. https://doi.org/10.1016/j.enceco.2022.10.004
8. Neylon J, Fuller JN, van der Poel C, Church JE, Dworkin S. Organophosphate insecticide toxicity in neural development, cognition, behaviour and degeneration: Insights from Zebrafish. J Dev Biol. 2022; 10(4):49. https://doi.org/10.3390/jdb10040049 PMid: 36412643 PMCid: PMC9680476.
9. Kaur K, Kaur R. Occupational pesticide exposure, impaired DNA repair, and diseases. Indian J Occup Environ Med. 2018; 22(2):74. https://doi.org/10.4103/ijoem.IJOEM_45_18 PMid: 30319227 PMCid: PMC6176703.
10. Dar MA, Hamid B, Kaushik G. Temporal trends in the use and concentration of organophosphorus pesticides in Indian riverine water, toxicity, and their risk assessment. Reg Stud Mar Sci. 2023; 102814. https://doi.org/10.1016/j.rsma.2023.102814
11. Dawson AH, Eddleston M, Senarathna L, Mohamed F, Gawarammana I, Bowe SJ, *et al*. Acute human lethal toxicity of agricultural pesticides: A prospective cohort study. PLoS Med. 2010; 7(10):e1000357. https://doi.org/10.1371/journal.pmed.1000357 PMid: 21048990 PMCid: PMC2964340.
12. Mohanty S, Khanna R. Genome-wide comparative analysis of four Indian *Drosophila* species. Mol Genet Genom. 2017; 292:1197-208. https://doi.org/10.1007/s00438-017-1339-8 PMid: 28660309.
13. Ogienko AA, Omelina ES, Bylino OV, Batin MA, Georgiev PG, Pindyurin AV. *Drosophila* as a model organism to study basic mechanisms of longevity. Int J Mol Sci. 2022; 23(19):11244. https://doi.org/10.3390/ijms231911244 PMid: 36232546 PMCid: PMC9569508.
14. Clancy D, Chtarbanova S, Broughton S. Model organisms in aging research: *Drosophila melanogaster*. Front Aging. 2023; 3:1118299. https://doi.org/10.3389/fragi.2022.1118299 PMid: 36704580 PMCid: PMC9871377.
15. Singh BK. Organophosphorus-degrading bacteria: Ecology and industrial applications. Nat Rev Microbiol. 2009; 7(2):156-64. https://doi.org/10.1038/nrmicro2050 PMid: 19098922
16. Kushwaha M, Verma S, Chatterjee S. Profenofos, an acetylcholinesterase-inhibiting organophosphorus pesticide: A short review of its usage, toxicity, and biodegradation. J Environ Qual. 2016;45(5):1478-89. https://doi.org/10.2134/jeq2016.03.0100 PMid: 27695768.
17. Nazir A, Mukhopadhyay I, Saxena DK, Chowdhuri DK. Chlorpyrifos-induced hsp70 expression and effect on reproductive performance in transgenic Drosophila melanogaster (hsp70-lacZ) Bg 9. Arch Environ Contam Toxicol. 2001; 41:443-9. https://doi.org/10.1007/s002440010270 PMid: 11598781.
18. Gregorc A, Alburaki M, Rinderer N, Sampson B, Knight PR, Karim S, *et al*. Effects of coumaphos and imidacloprid on honey bee (Hymenoptera: Apidae) lifespan and antioxidant gene regulations in laboratory experiments. Sci Rep. 2018; 8(1):15003. https://doi.org/10.1038/s41598-018-33348-4 PMid: 30301926 PMCid: PMC6177410.
19. Boyda J, Hawkey AB, Holloway ZR, Trevisan R, Di Giulio RT, Levin ED. The organophosphate insecticide diazinon and aging: Neurobehavioral and mitochondrial effects in zebrafish exposed as embryos or during aging. Neurotoxicol Teratol. 2021; 87:107011. https://doi.org/10.1016/j.ntt.2021.107011 PMid: 34224825 PMCid: PMC8440393.
20. Piper MD, Partridge L. *Drosophila* as a model for ageing. Biochim Biophys Acta Mol Basis Dis. 2018; 1864(9):2707-17. https://doi.org/10.1016/j.bbadis.2017.09.016 PMid: 28964875
21. Li X, Liu J, Wang X. Exploring the multilevel hazards of thiamethoxam using *Drosophila melanogaster*. J Hazard Mater. 2020; 384:121419. https://doi.org/10.1016/j.jhazmat.2019.121419 PMid: 31630861.
22. Guedes RN, Cutler GC. Insecticide-induced hormesis and arthropod pest management. Pest Manag Sci. 2014; 70(5):690-7. https://doi.org/10.1002/ps.3669 PMID: 24155227.
23. Martins R, Lithgow GJ, Link W. Long live FOXO: Unraveling the role of FOXO proteins in aging and longevity. Aging cell. 2016; 15(2):196-207. https://doi.org/10.1111/acel.12427 PMID: 26643314.
24. Koushika SP, Lisbin MJ, White K. ELAV, a *Drosophila* neuron-specific protein, mediates the generation of an alternatively spliced neural protein isoform. *Curr Biol*. 1996; 6(12):1634-41. https://doi.org/10.1016/S0960-9822(02)70787-2 PMID: 8994828.
25. Hamaratoglu F, Affolter M, Pyrowolakis G. Dpp/BMP signaling in flies: From molecules to biology. Semin Cell Dev Biol. 2014; 32:128-136. https://doi.org/10.1016/j.semcdb.2014.04.036 PMID: 24813173.
26. Choy SW, Cheng SH. Hedgehog signaling. Vitamins Hormones. 2012; 88:1-23. https://doi.org/10.1016/B978-0-12-394622-5.00001-8.

27. Bretones G, Delgado MD, Leon J. Myc and cell cycle control. Biochim Biophys Acta. 2015; 1849(5):506-16. https://doi.org/10.1016/j.bbagrm.2014.03.013 PMID: 24704206.
28. Gan T, Fan L, Zhao L, Misra M, Liu M, Zhang M, Su Y. JNK signaling in *Drosophila* aging and longevity. Int J Mol Sci. 2021; 22(17):9649. https://doi.org/10.3390/ijms22179649 PMID: 34502551.
29. Sotillos S, Espinosa-Vazquez JM, Foglia F, Hu N, Hombria JC. An efficient approach to isolate STAT regulated enhancers uncovers STAT92E fundamental role in *Drosophila* tracheal development. *Dev Biol*. 2010; 340(2):571-82. https://doi.org/10.1016/j.ydbio.2010.02.015 PMID: 2017120.
30. Enns LC, Ladiges W. Protein kinase A signaling as an anti-aging target. Ageing Res Rev. 2010; 9(3):269-72. https://doi.org/10.1016/j.arr.2010.02.004 PMID: 20188216.
31. Heinz A, Nabariya DK, Krauss S. Huntingtin and its role in mechanisms of RNA-mediated toxicity. Toxins. 2021 Jul 14;13(7):487. https://doi.org/10.3390/toxins13070487 PMID: 34357961.
32. Pandey M, Awasthi S. Role of MMP-1, MMP-8 and MMP-9 gene polymorphisms in preterm birth. J Genet. 2020; 99(1):2. https://doi.org/10.1007/s12041-019-1161-7 PMID: 32089521.
33. Huang G, Li H, Zhang H. Abnormal expression of mitochondrial ribosomal proteins and their encoding genes with cell apoptosis and diseases. Int J Mol Sci. 2020; 21(22):8879. https://doi.org/10.3390/ijms21228879 PMID: 33238645.
34. Mozhui K, Snively BM, Rapp SR, Wallace RB, Williams RW, Johnson KC. Genetic analysis of mitochondrial ribosomal proteins and cognitive aging in postmenopausal women. Front Genet. 2017; 8:127. https://doi.org/10.3389/fgene.2017.00127 PMID: 28983317.
35. Li X, Liu J, Wang X. Exploring the multilevel hazards of thiamethoxam using *Drosophila melanogaster*. J Hazard Mater. 2020; 384:121419.
36. Desouky MM, Abdel-Gawad H, Hegazi B. Distribution, fate and histopathological effects of ethion insecticide on selected organs of the crayfish, *Procambarus clarkii*. Food Chem Toxicol. 2013; 52:42-52. https://doi.org/10.1016/j.fct.2012.10.029 PMid: 23127602.
37. Delkash-Roudsari S, Chicas-Mosier AM, Goldansaz SH, Talebi-Jahromi K, Ashouri A, Abramson CI. Assessment of lethal and sublethal effects of imidacloprid, ethion, and glyphosate on aversive conditioning, motility, and lifespan in honey bees (*Apis mellifera* L.). Ecotoxicol Environ Saf. 2020; 204:111108. https://doi.org/10.1016/j.ecoenv.2020.111108 PMid: 32798750.
38. Kim RO, Kim BM, Jeong CB, Lee JS, Rhee JS. Effects of chlorpyrifos on life cycle parameters, cytochrome P450S expression, and antioxidant systems in the monogonont rotifer Brachionus koreanus. Environ Toxicol Chem. 2016; 35(6):1449-57. https://doi.org/10.1002/etc.3288 PMid: 26496856.
39. Selvi M, Sarikaya R, Erkoc F, Kocak O. Investigation of acute toxicity of chlorpyrifos-methyl on guppy *Poecilia reticulata*. Chemosphere. 2005; 60(1):93-6. https://doi.org/10.1016/j.chemosphere.2004.11.093 PMid: 15910907.
40. Gomes KK, Macedo GE, Rodrigues NR, Ziech CC, Martins IK, Rodrigues JF, *et al*. *Croton campestris A*. St.-Hill methanolic fraction in a chlorpyrifos-induced toxicity model in *Drosophila melanogaster*: Protective role of gallic acid. Oxid Med Cell Longev. 2020; 2020. https://doi.org/10.1155/2020/3960170 PMid: 32273942 PMCid: PMC7121785.
41. Eidels RR, Sparks DW, Whitaker JO, Sprague CA. Sub-lethal effects of chlorpyrifos on big brown bats (*Eptesicus fuscus*). Arch Environ Contam Toxicol. 2016; 71:322-35. https://doi.org/10.1007/s00244-016-0307-3 PMid: 27491870.
42. Mullins RJ, Xu S, Pereira EF, Mamczarz J, Albuquerque EX, Gullapalli RP. Delayed hippocampal effects from a single exposure of prepubertal guinea pigs to sub-lethal dose of chlorpyrifos: A magnetic resonance imaging and spectroscopy study. Neurotoxicol. 2013; 36:42-8. https://doi.org/10.1016/j.neuro.2013.02.002 PMid: 23411083 PMCid: PMC3669662.
43. Pandey UB, Nichols CD. Human disease models in *Drosophila melanogaster* and the role of the fly in therapeutic drug discovery. Pharmacol Rev. 2011; 63(2):411-36. https://doi.org/10.1124/pr.110.003293 PMID: 21415126.