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STUDY ON CHEMICALLY-INDUCED DIET ALTERATION OF *DROSOPHILA MELANOGASTER*

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Abstract. *Toxicity assesment of chemical compounds that can end up into the environment is extremely important as chemicals have the ability to enter in biological organisms via different pathways and therefore reshape organisms' development. In vitro tests are successfully used to test chemical compounds, but do not provide an overall insight into the toxic potential they may have once reach a whole organism. Study models validated by repeated research, such as Drosophila melanogaster, are used to perform in vivo tests. One of the most significant parameters for testing the toxicity of compounds on the fruit fly is viability. An effective method to study viability in relation to various exogenous substances is by altering the diet of the test organisms. The main aim of the study is to better understand the impact that some chemicals have on animal organisms and to observe the response of fruit fly to different classes of compounds as seen from the viability perspective. Thus, in order to investigate the effects of chemicals we have altered the culture media by adding either natural compounds (different types of water, phytoestrogens) or synthetic compounds (nanoparticles, pesticides). Our findings suggest that the different types of water do not affect the viability of fuit flies and this has also been proven for nanoparticles. Regarding pesticides and phytoestrogens, there is a decrease in viability percentages in a dose-dependent manner.*

Keywords: *Drosophila melanogaster, natural compounds, synthetic compounds, viability*

INTRODUCTION

The importance of studying chemical exposure of organisms

The quantity of anthropogenic chemicals is increasingly in ecosystems, exposing both humans and other organisms to potentially toxic or destructive factors. It is imperative to develop methods for monitoring the exposure of organisms to chemicals in order to predict a potential environmental hazard and to circumvent the toxic effects that these substances may have on organisms. Although significant scientific progress has been made in recent years in understanding how chemicals (singly or in mixtures) act on organisms, there is room for optimization and improvement of monitoring and prevention strategies, all with the aim of ensuring a qualitatively superior living environment (DRAKVIK et al., 2020). Anthropogenic chemicals can enter animal organisms by various routes, such as food or water intake, inhalation or direct contact, and can cause profound disturbance. It is well known that chemicals have endocrine disrupting effects, which have a massive negative impact on the reproductive function of animals. Chemicals that produce endocrine dysregulation are called endocrine disruptors (MARLATT et al., 2022). In humans, there are many substances that can function as hormone disruptors, including nanoparticles, pesticides and phytoestrogens (CASERTA el al., 2008; CHARBONNIER et al., 2024).

The need to find model organisms for toxicity assesment emerge from the ethical issues raised by human testing. The use of non-mammalian organisms may be an alternative and efficient option. Commonly used model organisms are organisms with a simple life cycle and which in terms of environmental requirements are not very sophisticated. Among the most

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common model organisms are *Daphnia magna*, *Danio rerio*, *Caenorhabditis elegans* and *Drosophila melanogaster* (TKACZYK et al., 2021, PROKIC et al., 2021, TORTELLA et al., 2020).

Drosophila melanogaster as a model organism

Due to the fact that in vitro studies may be incomplete and may not give a comprehensive view of the potential effects that certain compounds may have at the moment when they enter a living organism, invertebrate animals, such as the fruit fly, are used to highlight the toxic potential of these compounds. *Drosophila melanogaster* is characterized very well genetically, has a relatively short life cycle, is not financially costly and has fairly limited and accessible nutritional requirements. Despite being an invertebrate organism, the fruit fly, has been a key tool in understanding the molecular genetics behind pathological conditions such as Alzheimer's, Parkinson's and cancer (PARVATHI et al., 2016). From its first use in the laboratory to the present day, *Drosophila melanogaster* is a benchmark in basic research. Fruit flies were first used in the early 1900s by Thomas Hunt Morgan, who was awarded the Nobel Prize in 1933 for his discoveries on the role chromosomes play in heredity (MARKOW, 1988). More recently, in 2017, Jeffrey C. Hall, Michael Rosbash and Michael W. Young were awarded for their discoveries on the mechanisms involved in circadian rhythm, research also carried out using fruit flies (DAS, 2017). Thus, for almost 100 years, *Drosophila melanogaster*, has been contributing to fundamental knowledge in extremely diverse fields, representing a pragmatic decision that does not raise cost/utility questions, while being an easy organism to maintain and manipulate (TOLWINSKI, 2017).

During its development *Drosophila melanogaster* passes through different stages, but in a relatively short time. Embryonic development starts with fertilization and lasts about a day, at the end of which the embryo becomes a larva. The larva goes through three stages, which are different in size. At the end of the third stage, pupation occurs. Inside the pupa the process of metamorphosis takes place, characterized by profound structural remodelling. After about nine days the adult is formed. Anatomically, the adult consists of a head, thorax and abdomen (ALBERTS et al., 2002). *Drosophila melanogaster* is characterized by a profound sexual dimorphism that goes beyond anatomical features, including some aspects that are physiological (BELMONTE et al., 2020).

The protocol by which this test can be performed can be modified due to the versatility of this model organism, thus according to RAND et al. (2023), several parameters can be taken into account, according to the developmental stage of the organism to be investigated. These parameters are related to behaviors or the morphobiological development of the individuals. In larvae, the most common parameters that could be considered are: viability, life cycle extension, locomotor dysfunctions, light attraction/avoidance, metamorphosis rate. In adults the most common test parameters are: viability, longevity, motor function (expressed by climbing and fighting), light attraction/avoidance. Chemicals can be administered to larvae by diet and injection and to adults by diet and air suspension of chemicals.

Types of chemicals

1. Phytoestrogens

In the last decades, research on the beneficial or adverse effects of phytoestrogens present in the human diet has intensified due to their estrogenic or estrogenic-inhibiting potential in humans and animals (STECK et al., 2020). Phytoestrogens have multiple benefits for human health, including a reduced risk of osteoporosis, cardiovascular disease, brain disorders and decreased menopausal symptoms. However, they can act as endocrine disruptors and may also induce adverse health effects (KOUDOUFIO et al., 2020). Phytoestrogens in the

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human diet function bidirectionally, for example, on one hand, the composition of the gut microbiota can influence phytoestrogen metabolism, and on the other hand, phytoestrogens and phytoestrogen metabolites can modulate and remodel the gut microbial composition (SEYED et al., 2020). Phytoestrogens are non-steroidal polyphenols with a structure that is very similar to endogenous estradiol and are able to bind to both estrogen receptors alpha and beta (PATERNI et al., 2014). Estrogen receptors alpha and beta have different functions. Estrogen alpha receptors act in cell proliferation, while beta receptors are responsible for cell apoptosis (RIETJENS et al., 2013).

Phytoestrogens are synthesized in plants as secondary metabolites under stress, UV-irradiation conditions and in response to pathogen attack, so the amounts of phytoestrogens produced by a plant increase significantly under extreme growing conditions (BOURGAUD et al., 2001). They have antibacterial, antifungal, antiviral and antioxidant properties in plants (VUORELAA et al., 2004). The most common phytoestrogens types are: isoflavones, prenylflavonoids, coumestans, lignans, stilbenes.

2. Pesticides

Chlorantraniliprole (commercially sold as Coragen) is a synthetic insecticide from a class of pesticides called anthranilic diamides. It controls both adults and caterpillars (larvae) of moths and butterflies. It also controls some beetles and "true" insects such as aphids and lice (GERVAIS et al., 2022). Chlorantraniliprol binds to a specific receptor in muscle called the ryanodine receptor. Anthranilic diamides, the insecticidal class of chlorantraniliprole, target insect muscle, stimulating the release of intracellular calcium, which leads to depletion of calcium stored in the sarcoplasmic reticulum, promoting muscle paralysis, cessation of feeding, lethargy and death (CASTRO et al., 2021).

Captan has been one of the most widely used broad-spectrum fungicides. Particularly for a wide variety of fruits, captan has been commonly used as an effective preservative, demonstrating remarkable efficacy in maintaining fruits' bright and healthy external appearance during storage. However, the misuse of captan has been an emerging problem and consequently has posed a serious challenge to human health (CHEN et al., 2020). The action mechanism of captan in fungal death involves inhibition of fungal respiration and metabolic processes by reacting with sulfhydryl groups to generate nonspecific sulfhydryl reagents (ZHOU et al., 2019).

3. Nanoparticles

Nanoparticles can be categorized into: carbon-based nanoparticles, inorganic compound (metal)-based nanoparticles, organic compound-based nanoparticles and composite-based nanoparticles (JEEVANANDAM et al., 2018). Among inorganic compound-based nanoparticles, boron-based nanoparticles are very commonly used in various fields. The main types of boron-based nanoparticles are: boron carbide, boron nitride, boron-based nanoparticles linked with metal ions, boron-based nanoparticles linked with organic compounds (KOZIEN et al., 2021, TATIYA et al., 2020, SANTHOSH, 2019, EFREMENKO et al., 2012). There is no universal answer in terms of finding a central mechanism leading to the occurrence of toxicity under the influence of nanoparticles, but the formation of reactive oxygen species seems to be a key element in the development of such phenomena (SAIFI et al., 2018). Boron-based nanoparticles can enter the food chain through various pathways, one of the most common being agriculture. Regarding the use of this type of nanoparticles in agriculture, the applications are multiple: increasing fruit quality, protection against pathogens, improving the content of minerals and pigments in plants (ABD EL-WAHED et al., 2023, GENAIDY et al., 2020, EL BATAL et al., 2019).

4. Water

Another important factor to consider regarding the interaction of the environment with organisms is water. Although it is not an exogenous chemical, because of the changes it can undergo, water can become a potential disruptor for organisms, as few organisms can survive under physiological conditions in an alkaline water environment, for example. Increased salinity and alkalinity are characteristics of an alkaline water environment. Environmental changes of these factors cause stress to organisms by affecting their physiological homeostasis (SONG et al., 2021). Carbonate in alkaline water is an important buffer system for maintaining an optimal pH. It has been shown that pH fluctuates in aquatic environments with low alkalinity. In low alkalinity conditions, effects that are not favorable for fishes survival occur and thus their metabolism will be modulated as to maintain homeostasis, this leading to impaired nitrogen removal, which can have a negative effect on fishes and on the entire ecosystem (LOGOZZI et al., 2020).

In this study we have investigated the effects of some chemical compounds introduced into the diet of fruit flies (adults and larvae). The tested compounds are either natural (water, phytoestrogens) or synthetic (pesticides, nanoparticles). The main aim of the study is to better understand the impact that the tested compounds have on animal organisms and to observe the response of *D. melanogaster* to different classes of chemicals.

MATERIAL AND METHODS

For this assay, we used 420 individuals of *Drosophila melanogaster* (2n=8), wild type, Oregon, at the adult stage and 240 individuals of the same species at the larval stage. All the subjects were randomly selected from the faculty's own collection. Both, males and females, were transferred into tubes. For easier transfer, the individuals were anesthetized with a small amount of ether for a few seconds. Of all individuals, some tubes were left intentionally untreated to provide a control sample.

Larvae were removed from the tubes in 5% sucrose solution. Larvae were randomly extracted at various stages of development and subsequently positioned on the surface of the medium. The individuals were divided in such a way that for each test tube there were 10 test individuals.

The medium into which the individuals were transferred consisted of: semolina, yeast, sugar, distilled water and propionic acid to which we added the test chemicals. The resulting medium was left overnight to solidify. The viability of adults was monitored after 24 and 48 hours, respectively. In the case of larvae, the number of adult individuals that emerged after 10 and 14 days of testing, respectively, was noted, this parameter being generically referred to as the metamorphosis rate. Assuming that the developmental cycle in *Drosophila melanogaster* takes approximately 10 days (FERNANDEZ-MORENO et al., 2007), all individuals that did not metamorphose within the above proposed time frame were considered non-viable.

Several experiments in which the following chemicals were tested individually on adults of *Drosohpila melanogaster* were therefore performed: phytoestrogens extracted from *Angelica sinensis* (from commercially available capsules), isoflavones extracted from *Glycine max* (from commercially available capsules), captan (sold commercially as Merpan), chlorantraniliprole (sold commercially as Coragen), alkaline water, tap water, boron-based

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nanoparticles. Tests on larval stage subjects were performed only for the following compounds: captan, chlorantraniliprole, alkaline water, tap water, boron-based nanoparticles.

RESULTS AND DISCUSSIONS

In the case of adult individuals we have calculated the viability rate as the number of living individuals out of the total number of individuals after 24 hours and 48 hours, respectively. The viability of adults after 48 hours is graphically shown in figure 1, with C1 concentration being the lower used concentration and C2 being the higher used concentration for each chemical. In the case of synthetic compounds (captan, chlorantraniliprole and nanoparticles), chlorantraniliprole showed zero viability after 48 hours, which is expected since it is an insecticide. However, after 24 hours, at low concentrations, the same compound had no profound effect on viability, with individuals managing to survive. As for captan and boron-based nanoparticles, both after 24 hours and after 48 hours the recorded viability was maximum. For natural estrogens, a profound change in viability (less than 50% after 48 hours) was observed in a dose-dependent manner. In the case of isoflavones, a higher viability is observed in samples treated with a higher concentration of isoflavones. This can be explained by the fact that isoflavones have the ability to modulate the metabolism of fruit flies but also by the fact that isoflavones may have a significant protective role on the epithelial barrier and general health of *D. melanogaster* (LUERSEN et al., 2023, PIEGHOLDT et al., 2016). As for water, both alkaline and tap water did not imprint a negative effect, the viability being maximal in both cases.

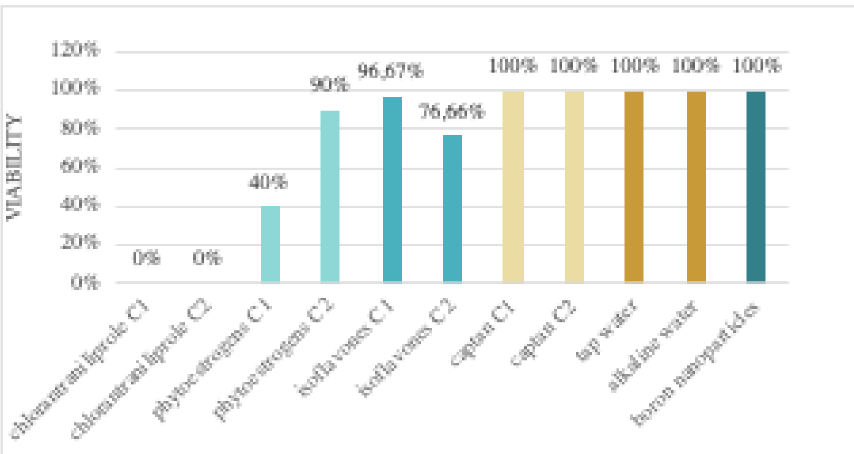

Figure 1. Adults' viability after 48 hours

For the larvae, we have calculated the metamorphosis rate as the percentage of viable pupae transforming into adults after 10 and 14 days, respectively. The metamorphosis rate of larvae after 14 days is graphically shown in figure 2, with C1 concentration being the lower used concentration and C2 being the higher used concentration for each chemical. In the case of chlorantraniliprole the final metamorphosis rate was 0, which confirms the insecticidal effects of the compound. In the case of captan the metamorphosis rate was below 50% suggesting a potential toxic effect of this compound. The literature suggests that when using in

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vivo study models captan does not show toxic effects, however in in vitro cultures it may show cytotoxic effects (OSABA et al., 2002), this may partly explain why in adults no change in viability was recorded but in larvae, which are more dependent on the culture medium and are more sensitive, this chemical compound may still have negative effects. Regarding water, an increase in metamorphosis rate was observed in alkaline water. It has been shown in previous experiments that environmental pH can affect the growth and development of *D. melanogaster* larvae (HODGE, 2001).

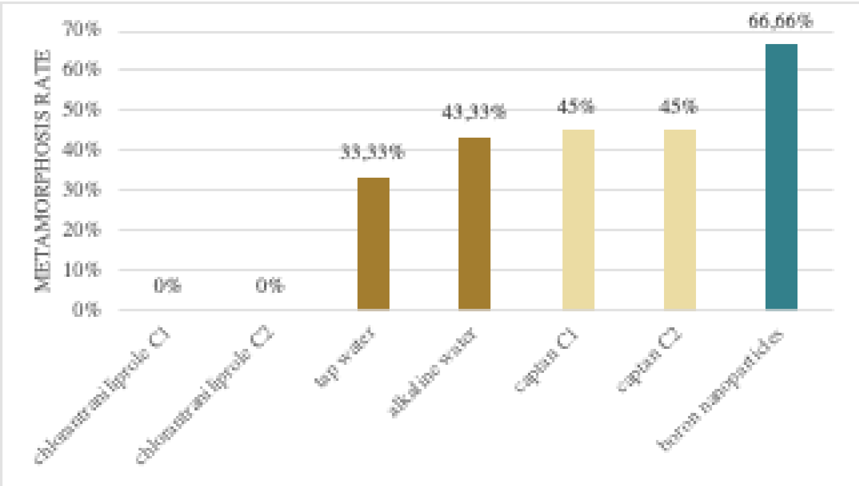

Figure 2. Larvae metamorphosis rate after 14 days

CONCLUSIONS

The experiments can and should be replicated using the same model or extrapolated to other study models to provide a complete perspective on the potential effects of particular chemicals. Vibility testing in adults and metamorphosis rate in larvae are very relevant and precise parameters to consider when performing in vivo tests. Our results reaffirm the suitability of this assay for testing a very wide range of compounds, whether natural of synthetic.

BIBLIOGRAPHY

1.ABD EL-WAHED, M. H., EISSA, M. A., ALMASOUDI, N. M., ABO-ELYOUSR, K. A., 2023- Macronutrient-rich biochar induces boron nanoparticles in improving the salt tolerance of pomegranate (Punica granatum L.) in arid degraded soils. Scientia Horticulturae, 313:111908.

2.ALBERTS, B., JOHNSON, A., LEWIS, J., RAFF, M., ROBERTS, K., WALTER, P., 2002- Molecular biology of the cell. New York: Garland Science, 3786 pp.

3.BELMONTE, R.L., CORBALLY, M.K., DUNEAU, D.F., REGAN, J.C., 2020- Sexual Dimorphisms in Innate Immunity and Responses to Infection in Drosophila melanogaster. Frontiers in Immunology, 10:3075.

4.BOURGAUD, F., GRAVOT, A., MILESI, S., GONTIER, E., 2001- Production of plant secondary metabolites: A historical perspective. Plant Sci., 161:839–851.

5.CASERTA, D., MARANGHI, L., MANTOVANI, A., MARCI, R., MARANGHI, F., MOSCARINI, M., 2008-Impact of endocrine disruptor chemicals in gynaecology. Human reproduction update, 14(1):59-72.

6.CHARBONNIER, P., JOUNEAU, P. H., DENIAUD, A., 2024- The endocrine disruptor effect of metal nanoparticles mainly depends on their capacity to release metal ions. Environmental Science: Nano, 11(7):3192-3201.

7.DAS, S., 2017- Nobel Prize for the Fruit Fly. Journal of the Practice of Cardiovascular Sciences, 3(2).

8.DRAKVIK, E., ALTENBURGER, R., AOKI, Y., BACKHAUS, T., BAHADORI, T., BAROUKI, R., BRACK, CRONIN, M.T.D., DEMENEIX, B., HOUGAARD BENNEKOU, S., VAN KLAVEREN, J., KNEUER, C., KOLOSSA-GEHRING, M., LEBRET, E., POSTHUMA, L., REIBER, L., RIDER, C., RÜEGG, J., TESTA, G., VAN DER BURG, B., VAN DER VOET,H., WARHURST, A.M., VAN DE WATER, B., YAMAZAKI, K., ÖBERG, M., BERGMAN, A., 2020- Statement on advancing the assessment of chemical mixtures and their risks for human health and the environment. Environment International, 134: 105267.

9.E CASTRO, B. M. D. C., MARTÍNEZ, L. C., PLATA-RUEDA, A., SOARES, M. A., WILCKEN, C. F., ZANUNCIO, A. J. V., FIAZ, M., ZANNUNCIO, J.C., SERRÃO, J. E., 2021- EXPOSURE TO CHLORANTRANILIPROLE REDUCES LOCOMOTION, RESPIRATION, AND CAUSES HISTOLOGICAL CHANGES IN THE MIDGUT OF VELVETBEAN CATERPILLAR ANTICARSIA GEMMATALIS (LEPIDOPTERA: NOCTUIDAE). CHEMOSPHERE, 263, 128008. CHEN Y., HUANG C., HELLMANN B., JIN Z., XU X., XIAO G. 2020. A new HPTLC platformed luminescent biosensor system for facile screening of captan residue in fruits. Food Chem., 309:125691.

10.EFREMENKO, A.V., IGNATOVA, A.A., BORSHEVA, A.A., GRIN, M.A., BREGADZE, V.I., SIVAEV, I.B., MIRONOVB, A.F., FEOFANOV, A.V., 2012- Cobalt bis(dicarbollide) versus closo-dodecaborate in boronated chlorin e6 conjugates: implications for photodynamic and boron-neutron capture therapy. Photochemical & Photobiological Sciences, 11:645-652.

11.EL-BATAL, A. I., ATTIA, M. S., NOFEL, M. M., EL-SAYYAD, G. S., 2019- Potential nematicidal properties of silver boron nanoparticles: synthesis, characterization, in vitro and in vivo root-knot nematode (Meloidogyne incognita) treatments. Journal of Cluster Science, 30:687-705.

12.ELSABAHY, M., WOOLEY, K.L., 2013- Cytokines as biomarkers of nanoparticle immunotoxicity. Chemical Society Reviews, 42:5552-5576.

FERNÁNDEZ-MORENO, M.A., FARR, C.L., KAGUNI, L.S., GARESSE, R., 2007- Drosophila melanogaster as a model system to study mitochondrial biology. Methods in Molecular Biology, 372:33-49.

13.GENAIDY, E. A., ABD-ALHAMID, N., HASSAN, H. S., HASSAN, A. M., HAGAGG, L. F., 2020- Effect of foliar application of boron trioxide and zinc oxide nanoparticles on leaves chemical composition, yield and fruit quality of Olea europaea L. cv. Picual. Bulletin of the National Research Centre, 44:1-12.

14.GERVAIS, J., HANSON, W., CROSS, A., JENKINS, J., 2022- Chlorantraniliprole Fact Sheet; National Pesticide Information Center, Oregon State University Extension Services. npic.orst.edu/factsheets/chlorantraniliprole.html.

15.HODGE, S., 2001- The effect of p H and water content of natural resources on the development of Drosophila melanogaster larvae. Drosoph Inf Serv, 84:38–43.

16.JEEVANANDAM, J., BARHOUM, A., CHAN, Y.S., DUFRESNE, A., DANQUAH, M.K., 2018- Review on nanoparticles and nanostructured materials: history, sources, toxicity and regulations. Beilstein Journal of Nanotechnololgy, 9:1050-1074.

17.KARLSSON, H.L., CRONHOLM, P., HEDBERG, Y., TORNBERG, M., DE BATTICE, L., SVEDHEM, S., WALLINDER, I.O., 2013- Cell membrane damage and protein interaction induced by copper containing nanoparticles - importance of the metal release process. Toxicology, 313(1):56-69.

18.KOUDOUFIO, M., DESJARDINS,Y., FELDMAN, F., SPAHIS, S., DELVIN, E., LEVY, E., 2020- Insight into Polyphenol and Gut Microbiota Crosstalk: Are Their Metabolites the Key to Understand Protective Effects against Metabolic Disorders?. Antioxidants, 9:982.

19.KOZIEN, ́D., SZERMER-OLEARNIK, B., RAPAK, A., SZCZYGIEŁ, A., ANGER-GÓRA, N., BORATYN ́SKI, J., PAJTASZ-PIASECKA, E., BUC ́KO, M.M., PEDZICH, Z., 2021- Boron-Rich Boron Carbide Nanoparticles as a Carrier in Boron Neutron Capture Therapy: Their Influence on Tumor and Immune Phagocytic Cells. Materials, 14(11).

20.LOGOZZI, M., MIZZONI, D., DI RAIMO, R., ANDREOTTI, M., MACCHIA, D., SPADA, M., FAIS, S., 2020- In vivo antiaging effects of alkaline water supplementation. J Enzyme Inhib Med Chem, 35:657-664.

21.LÜERSEN, K., FISCHER, A., BAUER, I., HUEBBE, P., UEKAJI, Y., CHIKAMOTO, K., NAKATA, D., HIRAMATSU, N., TERAO, K., RIMBACH, G., 2023- Soy Extract, Rich in Hydroxylated Isoflavones, Exhibits Antidiabetic Properties In Vitro and in Drosophila melanogaster In Vivo. Nutrients, 15(6):1392.

22.MARKOW, T.A., 1988- Reproductive Behavior of Drosophila melanogaster and D. nigrospiracula in the Field and in the Laboratory. Journal of Comparative psychology, 102(2):169-173.

23.MARLATT, V.L., BAYEN, S., CASTANEDA-CORTÈS, D., DELBÈS, G., GRIGOROVA, P., LANGLOIS, V.S., MARTYNIUK, C.J., METCALFE, C.D., PARENT, L., RWIGEMERA, A., THOMSON, P., VAN DER KRAAK, G., 2022- Impacts of endocrine disrupting chemicals on reproduction in wildlife and humans. Environmental Research, 208: 112584.

24.OSABA, L., REY, M. J., AGUIRRE, A., ALONSO, A., GRAF, U., 2002- Evaluation of genotoxicity of captan, maneb and zineb in the wing spot test of Drosophila melanogaster: role of nitrosation. Mutation Research/Genetic Toxicology and Environmental Mutagenesis, 518(1):95-106.

25.PARVATHI, V.D., RAJAGOPAL, K., SUMITHA, R., 2016- Standardization of Alternative Methods for Nanogenotoxicity Testing in Drosophila melanogaster Using Iron Nanoparticles: A Promising Link to Nanodosimetry. Journal of Nanotechnology, 2016(1):2547467.

26.PATERNI, I., GRANCHI, C., KATZENELLENBOGEN, J.A., MINUTOLO, F., 2014- Estrogen receptors alpha (ERα) and beta (ERβ): subtype-selective ligands and clinical potential. Steroids, 90:13-29.

27.PIEGHOLDT, S., RIMBACH, G., WAGNER, A. E. 2016- Effects of the isoflavone prunetin on gut health and stress response in male Drosophila melanogaster. Redox biology, 8:119-126.

28.PROKIĆ, M. D., GAVRILOVIĆ, B. R., RADOVANOVIĆ, T. B., GAVRIĆ, J. P., PETROVIĆ, T. G., DESPOTOVIĆ, S. G., FAGGIO, C., 2021- Studying microplastics: Lessons from evaluated literature on animal model organisms and experimental approaches. Journal of Hazardous Materials, 414: 125476.

29.RAND, M. D., TENNESSEN, J. M., MACKAY, T. F., ANHOLT, R. R., 2023- Perspectives on the Drosophila melanogaster model for advances in toxicological science. Current protocols, 3(8):e870.

30.RIETJENS, I., LOUISSE, J., BEEKMANN, K., 2017- The potential health effects of dietary phytoestrogens. Br. J. Pharmacol, 174:1263–1280.

31.SAIFI, M.A., KHURANA, A., GODUGU, C., 2018- Nanotoxicology: Toxicity and Risk Assessmens of Nanomaterials, In Nanomaterials in Chromatography- Current Trends in Chromatographic Research Technology and Techniques, Elsevier, 437-465pp.

32.SANTHOSH, M.S., 2019. Significance of Boron Nitride in Composites and Its Applications. Recent Advances in Boron Containing Materials, 63.

33.SEYED HAMEED, A., RAWAT, P., MENG, X., LIU, W., 2020- Biotransformation of dietary phytoestrogens by gut microbes: A review on bidirectional interaction between phytoestrogen metabolism and gut microbiota. Biotechnol. Adv., 43:107576.

34.SONG, L., ZHAO, Y., SONG, Y., ZHAO, L., MA, C., ZHAO, J., 2021- Effects of saline-alkaline water on growth performance, nutritional processing, and immunity in Nile tilapia (Oreochromis niloticus). Aquaculture, 544: 44-8486.

35.STECK, S., MURPHY, E., 2020- Dietary patterns and cancer risk. Nat. Rev. Cancer, 20:125–138.

36.TATIYA, T., PANDEY, M., BHATTACHARYA, S., 2020- Nanoparticles containing boron and its compounds—synthesis and applications: A review. Journal of Micromanufacturing, 3(2):159–173.

37.TKACZYK, A., BOWNIK, A., DUDKA, J., KOWAL, K., ŚLASKA, B., 2021- Daphnia magna model in the toxicity assessment of pharmaceuticals: A review. Science of the Total Environment, 763:143038.

38.TOLWINSKI, N.S., 2017- Introduction: Drosophila- A model System for Developmental Biology. Journal of Developmental Biology, 5(3).

39.TORTELLA, G. R., RUBILAR, O., DURÁN, N., DIEZ, M. C., MARTÍNEZ, M., PARADA, J., SEABRA, A. B., 2020- Silver nanoparticles: Toxicity in model organisms as an overview of its hazard for human health and the environment. Journal of hazardous materials, 390:121974.

http://doi.org/10.59463/RJAS.2025.2.02

40.VUORELAA,P., LEINONENB, M., SAIKKUC, P., TAMMELAA, P., RAUHAD, P., WENNBERGE, T., VUORELA, H., 2004- Natural products in the process of finding new drug candidates. Curr. Med. Chem, 11:1375–1389.

41.ZHOU, Y., CHEN, X., TENG, M., ZHANG, J., WANG, C., 2019- Toxicity effects of captan on different life stages of zebrafish (Danio rerio). Environ. Toxicol. Pharmacol., 69:80-85.