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A MULTIFACED MODEL FOR VIABILITY ASSESMENT: EFFECTS OF NATURAL AND SYNTHETIC STRESSORS ON *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.*

Introduction

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. Model organisms are used for this purpose. Among the most common model organisms are *Daphnia magna*, *Danio rerio*, *Caenorhabditis elegans* and *Drosophila melanogaster*.

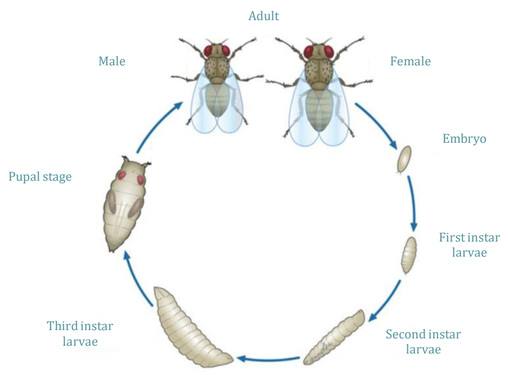


FIG. 1. Life cycle of *D. melanogaster*

Material and method

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.

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 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. We have tested the chemicals presented in table 1.

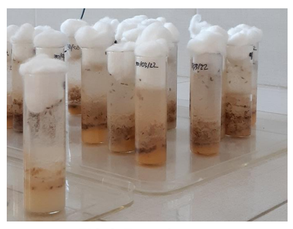


FIG. 2. Test tubes

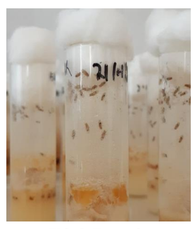


FIG. 3. Test tubes

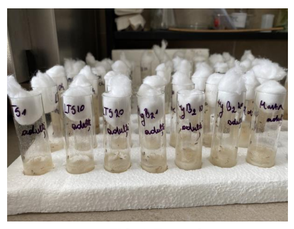


FIG. 4. Test tubes

TABLE 1. Used chemicals for diet altering of fruit flies

NATURAL COMPOUNDS	
Phytoestrogens and isoflavones	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.
Water (alkaline, tap)	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.
SYNTHETIC COMPOUNDS	
Captan	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.
Chlorantraniliprole	Synthetic insecticide from a class of pesticides called anthranilic diamides. It controls both adults and caterpillars (larvae) of moths and butterflies. 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.
Boron nanoparticles	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

FIG. 5. Female fruit fly (dorsal view)

FIG. 6. Female fruit fly (ventral view)

FIG. 7. Male fruit fly (dorsal view)

FIG. 8. Male fruit fly (ventral view)

Results and discussions

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.*

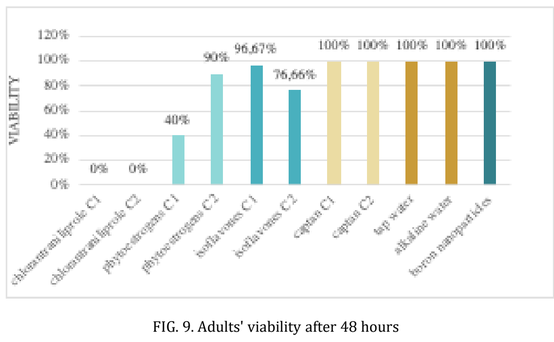


FIG. 9. Adults' viability after 48 hours

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 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.

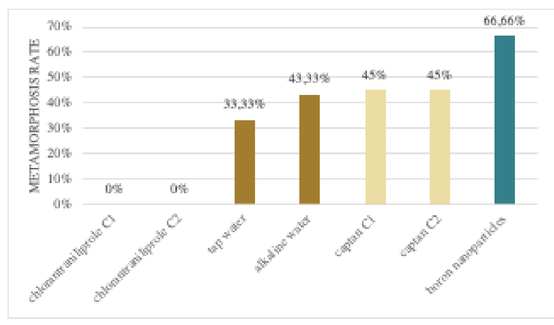
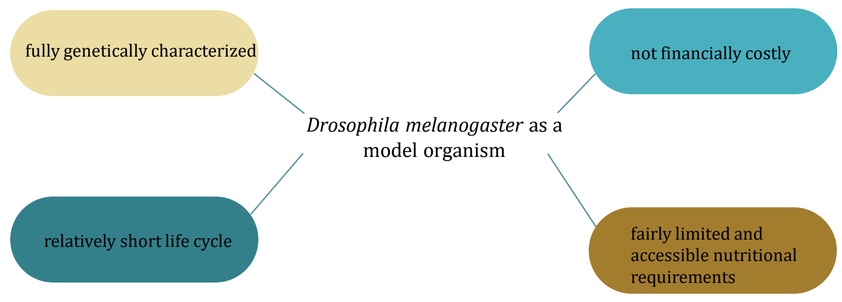


FIG. 10. Larvae metamorphosis rate after 14 days

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.



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