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ORIGINAL ARTICLE

Drosophila as a model for assessing nanopesticide toxicity

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ABSTRACT

One of the fastest-moving fields in today's world of applied science, nanotechnology allows the control and design of matter on an extremely small scale, so it has now become an integral part of various industries and scientific areas, such as agriculture, food sector, healthcare and engineering. Understanding the interactions between nanopesticides and edible plants, as well as non-target animals, is crucial in assessing the potential impact of nanotechnology products on the environment, agriculture and human health. The dramatic increase in efforts to use nanopesticides renders the risk assessment of their toxicity and genotoxicity highly crucial due to the potential adverse impact of this relatively uncharted territory. Such widespread use naturally increases our exposure to nanopesticides, raising concerns over their possible adverse effects on humans and non-target organisms, which might include severe impairment of both male and female reproductive capacity. We therefore need better insight into such effects to derive conclusive evidence on the safety or toxicity/genotoxicity of nanopesticides, and *Drosophila melanogaster* (fruit fly) can prove an ideal model organism for the risk assessment and toxicological classification of nanopesticides, as it bears striking similarities to various systems in human body. This editorial review attempts to summarize our current knowledge derived from previous *in vivo* studies to examine the impact of several nanomaterials on various species of mammals and non-target model organisms at the genetic, cellular, and molecular levels, attracting attention to the possible mechanisms and potential toxic/genotoxic effects of nanopesticides widely used in agriculture on *D. melanogaster* as a non-target organism.

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Issue with pesticides

The use of pesticides in agriculture, either from organic or synthetic sources, serves many crucial functions, such as securing crop yield against invasive organisms and enabling farmers to produce safe and quality foods at affordable prices. Without pesticide use, more than 50% of the global agricultural output would be lost to diseases and pests (OECD-FAO Agricultural Outlook 2012). However, conventional pesticides bring about some serious environmental drawbacks. For instance, most tend to be nonspecific, and thus, along with invasive species, also damage harmless or even beneficial organisms like bees. Indeed, it has been estimated that only about 0.1% of the pesticides reach the target species during aerial spraying, with the rest polluting the surrounding ecosystem (Carriger et al. 2006). Besides, pesticides are also known to contaminate soil and water resources, and although we have limited evidence to measure the total health impact of pesticides across the globe, they have been reported to cause about 100 000 human deaths worldwide are due to acute and chronic poisoning (WHO Global Health Statistics 2015), with children and women in developing countries being particularly vulnerable to the toxic effects of pesticides.

The aims of this review are to present a comprehensive overview of all apparent studies carried out with nanopesticides and *Drosophila melanogaster*, to attain a clear and comprehensive picture of the potential risk of nanopesticide exposure to health, and to demonstrate the advantages of using *Drosophila* with new technology (for example CRISPR/Cas9 for pesticide resistance) in this field.

Nanopesticides as a solution

In recent years, the development and application of nanomaterial-based formulations in pesticide

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Table 1. Categories of nanopesticides.

Type	Nanotechnology	Advantages	AI (Active Ingredient)	Target Pest	References
Nanoclays	NPs of layered mineral silicates	Efficient carriers of pesticides Controlled release of AI High absorption potential Better insecticidal activity than microparticles	Cypermethrin (insecticide) Metalaxyl (fungicide)	Mosquitoes Fleas Spiders Termites Fungi	Wanyika 2013 Xiang et al. 2017
Nanocapsules	Polymeric NPs or nanoscale shells made from polymers to encapsulate AI	Efficient uptake and effectiveness against pests Efficient targeting Sustained pesticide release	*Eucalyptus globulus* extract Garlic oil *Thymus herba-barona*	Greenfly Flour beetle Whitefly	Khoshraftar et al. 2019
Nanoemulsions	AI loaded into nanoscale oil droplets	High cellular uptake by organisms Improved AI distribution Low toxicity to non-target organisms	Neem oil (from *Azadirachta indica* tree) Permethrin	Whitefly Aphids Moth larvae Spider mites Yellow fewer mosquito	Mishra et al. 2018 Pascoli et al. 2019
Nanogels	Natural or synthetic polymers with a diameter of 10-100 nm. The pores in the gel can be loaded with AI	Long lasting residual activity Increased efficacy Long shelf life Low-cost Good safety profile	Methyl eugenol	Oriental fruit fly (*Bactrocera dorsalis*)	Bhagat, Samanta, and Bhattacharya 2013
Nanoliposomes	They are structures at nanoscale used for the encapsulation and delivery of AI	High biocompatibility Biodegradable Improved stability of sensitive materials Sustained release of AI	Clove oil (eugenol) Etofenprox Pyrifluquinazon	Mosquitoes Leaf hoppers Whitefly Rice water weevils	Hwang et al. 2011 Kang et al. 2012
Inorganic NPs	Elemental metals, metal oxides, and metal salts used as nanopesticide AI	High effectiveness with increased toxicity to pests Excellent AI distribution Highly stable as compared to organic NMs	Gold NPs Titanium dioxide NPs Silver NPs Copper NPs	*Aedes aegypti* Fungi Bacteria	Soni and Prakash 2012Kim et al. 2012

production has emerged as a potential solution to the unwanted effects of conventional pesticides. Nanopesticides include nanomaterials (NMs) used as carriers of pesticidal substances to enable their controlled release at more efficient doses and refer to a wide range of products combining various surfactants, capsules, metal oxides, particles, and polymers on the nanoscale (often measuring 1–100 Nm) (Table 1). These include nano form of pyrifluquinaz, which is designed to modify insect behavior by interfering with the insect's feeding activity (Kang et al. 2012), nanocapsules of botanical insecticides [(the major constituents of Eucalyptus extract: 1,8-cineole (70.94%) and 1,2-benzenedicarboxylic acid (6.08%)] (Khoshrafta et al. 2019), nano-size silver (Ag) colloidal solution as fungicidal (Kim et al. 2012), neem-oil loaded zein nanoparticles (NPs) (Pascoli et al. 2019), fungus Chrysosporium tropicum used for synthesizing the Ag and gold (Au) NPs as a larvicide against *Aedes aegypti* (Soni and Prakash 2012), *Mesoporous silica* NPs (MSN) for storage and controlled release of metalaxyl fungicide (Wanyika 2013). Another example is a nanogel produced from methyl eugenol (a pheromone) using a low-molecular mass gelator to prevent pests from harming a range of fruits such as guava (Bhagat, Samanta, and Bhattacharya 2013).

This editorial review aims to present a concise overview of previous studies that examine possible adverse effects of different nanomaterials on non-target model organisms, to attract attention to the potential mechanisms and toxic/genotoxic effects of nanopesticides widely used in agriculture on *Drosophila* as a non-target model organism, and to demonstrate the advantages of using it with new technology [such as CRISPR/Cas9 (Clustered Regularly Interspaced Short Palindromic Repeats)] for pesticide resistance.

Unlike conventional pesticides, formulations containing NMs as additives are often designed to improve solubility of the active ingredient (AI) and to release the chemical in a precise and efficient way, thus protecting it from early degradation (Kookana et al. 2014). Nanoparticles (NPs) can reduce the required amount of pesticide for pest control by enhancing the durability and efficacy of chemicals. Nanopesticides may also contain ultrafine particles of AI, such as Ag, Au, and titanium dioxide (TiO_2) that are toxic to pests (Bergeson 2010; Kah and Hofmann 2014; Sekhon 2014). For example, Ag NPs, known to have antimicrobial properties (Kim et al. 2012), have been successfully used as active ingredients against harmful plant pathogens such as rice blast fungus (*Magnaporthe grisea*). Thanks to today's advanced nanotechnology, NPs can be modified by altering their size, shape, surface area, and surface charge of particles to make them more specific to target organisms as compared to conventional pesticides, insecticides, and insect repellents (Sasson et al. 2007). For example, nanocapsules can slow the release of substance in liquid or solid forms to specific plants through release mechanisms such as dissolution, biodegradation, diffusion, and osmotic pressure (Vidyalakshmi, Bhakyaraj, and Subhasree 2009). Nanogels containing small pores loaded with the pheromone methyl eugenol can be applied to a number of fruit crops as a protection against *Bactrocera dorsalis*. They have been found to preserve the active ingredient during the whole period of insect growth, thus allowing an effective pest control (Bhagat, Samanta, and Bhattacharya 2013). Furthermore, Polyethylene glycol (PEG) coated NPs loaded with garlic essential oil have been reported to be highly effective against harmful pests like *Tribolium castaneum Herbst* (Yang et al. 2009). Finally, silica NPs (Si NPs) are known to improve plant tolerance to biotic stresses, and amorphous nanosilica has been used for agricultural pest control (Barik, Sahu, and Swain 2008).

Production of nanopesticides involves the use of NMs with pesticidal properties like Ag, Au, and TiO_2 NPs as active ingredients or as nanocapsules to improve delivery systems. In both cases, the main routes of exposure to nanopesticides are via inhalation, ingestion of the foods containing nanopesticides, and dermal contact. Considering the higher cellular uptake of NPs once they have reached several systems in the organism, as compared to bulk materials in conventional pesticides, they may prove even more toxic to non-target organisms that play a crucial role in the ecosystem, which might include common pollinators like honey bees and birds. Such an effect could indirectly cause a significant loss of crop yield due to insufficient population of pollinators (Fishel 2011). For that reason, future production of bioactive agent-based nanopesticides containing silver and other materials should take into account the correct size and concentration of NPs that would be effective against pests and invasive pathogens but less toxic to non-target organisms. Therefore, a detailed investigation of interactions between engineered NMs and living systems seems highly crucial to develop pesticidal nanoformulations that can modulate specific molecular pathways.

Altogether, nanopesticides have emerged as a potential solution to address the toxic effects of pesticides. However, before they can be used for agricultural application, we need to better understand their impact on non-target animal species and ecosystem (Kah et al. 2013).

Addressing the toxicity of nanopesticides

The recent interest toward the use of nanopesticides in agriculture raises concerns over their possible toxic/genotoxic impacts on humans as well as non-target organisms, Thus, effective risk assessment tools are needed to evaluate their biological interactions, ecotoxicity, and cytotoxicity. The risk assessment of pesticide product formulations (PPFs) have previously been performed by evaluating the toxicity of the active ingredient, neglecting the potential hazards of other components or possible combined effects of mixtures. Thus, it is imperative to assess the toxicity of various nanopesticide formulations more systematically to fully understand the risks associated with their use. In particular, interactions of NMs and coating materials should be examined (Nagy et al. 2020).

Unlike NMs, larger bulk materials exhibit certain physical properties independent of their size, but at the nanoscale such properties may show dramatic variations depending on the particle size and shape. This especially applies to metal oxide NPs that have a great surface area, since it plays a key role in its reactivity and other physicochemical properties (Golbamaki et al. 2015). NMs can be engineered to impart special functions such as enhanced strength, magnetic, thermal and electrical conductivity and also to produce structures with high surface-to-volume ratios (Sanvicens and Marco 2008; Sau et al. 2010). Such novel functionalities and properties may result in unexpected biological and chemical reactivity, increasing their toxicity to humans and animals. Features such as particle size, surface area, shape, and surface coating appear to regulate cytotoxicity of NPs (Macaroff et al. 2006). For example, small particles are known to be more toxic to living cells than large ones (Gliga et al. 2014), suggesting that NPs with large surface-to-volume ratio may be more toxic (Donaldson et al. 2004). Thus, it is crucial to fully assess the possible toxicity of NPs, which include cell toxicity and DNA damage (Snyder-Talkington et al. 2012). Although *in vitro* testing using various cell types yield valuable data, the use of tissue culture approaches often fail to reflect what really happens within a living organism upon exposure to specific materials (Lewinski, Colvin, and Drezek 2008). Therefore, researchers tend to conduct *in vivo* nanotoxicology studies often using target organisms (pests) to test potential effects of NMs like Ag NPs and Au NPs used in pest control products. For example, an *in vivo* study using Ag NPs and Au NPs as active ingredients tested their pesticidal capacity on the yellow fever mosquito (*Aedes aegypti*) (Soni and Prakash 2012; Kim et al. 2012). Exposure to NPs exerted highly toxic effects on larvae, with mortality rates as high as 100%, leading to the conclusion that the use of Au and Ag NPs could be an effective choice for safer and environmentally friendlier mosquito control (Soni and Prakash 2012). However, pesticide formulations containing NMs as additives or active ingredients should also be thoroughly tested for their possible toxic effects on non-target animal species that are harmless or beneficial to nature and humans, including in particular pollinators like bees and predators hunting insects categorized as pests. Considering that only 1% of all insects are considered as pests (Triplehorn and Johnson 2005), nanopesticides could cause serious damage to integral parts of the ecosystem by destroying benign organisms that regulate the population of invasive species and pests.

Drosophila as a model for assessing the toxicity of nanopesticides

A thorough investigation of nanopesticides formulations for possible toxic effects on *in vivo* animal models is critical. Several factors such as high operational costs and ethical issues tend to restrict the use of traditional *in vivo* testing (mammalian acute toxicity testing), thus simpler experimental models like roundworms, zebrafish, and fruit flies. Among them, *Drosophila melanogaster* stands out as an ideal *in vivo* model organism for assessing the cytotoxicity and genotoxicity of nanopesticides. *Drosophila*, a member of the family Drosophilidae, has for some time attracted serious scholarly attention and gained acceptance across diverse fields of biological and medical research, particularly including genetics, evolutionary biology, ecology, physiology, and microbial pathogenesis. The species has enabled scientists from many disciplines to gain profound insight into physiology of various organisms including humans. In fact, as of 2020, a total of six Nobel prizes have been awarded to scientists that conducted studies using *D. melanogaster* as a model organism. One of the most frequently examined species over the last decades by biological and genetic research, as well as ecotoxicology, *D. melanogaster* could be the best-known eukaryotic organism on planet Earth.

Although the toxicity of nanopesticides has been investigated in different model organisms, there is rather limited research to examine the impacts of nanopesticides using *Drosophila* as an experimental model. One relatively recent study, possibly the only one so far, investigated possible toxic effects of Ag NPs and sulfur (S) NPs on larval, pupal, and adults of *D. melanogaster*, reporting significantly high mortality and reduced longevity for both types of NPs (Araj et al. 2015). Kah and Hofmann broadly classified nano-based plant protection products into five groups according to their active ingredients, which include (1) nanoemulsions, (2) polymer-based nanopesticides, (3) hybrid nanoformulations, (4) inorganic NPs associated with an organic active ingredient, and (5) inorganic NPs as active ingredient (Kah and Hofmann 2014), but Table 1 presents a more detailed categorization of current nanopesticides. Some of the NMs like Si, TiO_2, Ag, Au, and Co, which are commonly used as active ingredients in nanopesticide formulations were already examined in toxicity and genotoxicity studies using *Drosophila*. Therefore, reviewing such studies may provide crucial insight into the effects of nanopesticides. The first study to examine the impacts of NM (cerium oxide-CeO_2 NPs) exposure on *D. melanogaster* was carried out by Strawn, Cohen, and Rzigalinski (2006). Since then a multitude of research into genotoxicity, cytotoxicity, and biocompatibility of various NMs of different shape, size, and matter has been using *Drosophila* as a model organism. Although the current knowledge is rather limited to establish a clear picture of toxicity of NMs, as there are contradicting findings about their effects on ROS generation, DNA damage, reproductive capacity, and viability, a significant portion has been demonstrated to cause toxic effects during *in vivo* testing on this model organism. For example, in their *in vivo* study, Demir (2020) found that exposure to TiO_2 NPs triggered cellular uptake, toxicity, DNA damage, and oxidative stress in *D. melanogaster*. Besides, detrimental effects of Au NPs, Ag NPs, cobalt (Co) NPs and Si NPs on *Drosophila*, including somatic mutation, gene mutation, toxicity, impaired fertility and longevity, have been reported by several studies (Demir et al. 2011; Vecchio et al. 2012; Armstrong et al. 2013; Pandey et al. 2013; Vales et al. 2013; Alaraby et al. 2020).

The growing interest in utilizing *Drosophila* in toxicity studies ultimately led to emergence of a research field called Drosophotoxicology (Rand 2010). This new field involves a range of methodological approaches using *Drosophila* as a model organism in toxicity and genotoxicity research (Chifiriuc et al. 2016). Toxicological assays designed to test NM exposure in *Drosophila* include components such as chemical toxicants, mode of delivery to the organism, developmental stage of the nervous system, and endpoints to be assessed for detecting biological and toxicological effects. In this context, the mode of delivery plays a crucial role in exposing the cells or organ systems in flies to NMs, and such modes may involve embryonic exposure through maternal feeding, delivery by direct injection into embryo, and direct incubation of *Drosophila* embryos. Larvae and adult flies can be easily exposed to different concentrations of NMs through food ingestion, injection, and vapor/aerosol in a controlled environment. Acute and chronic toxicity of NMs could then be assessed by means of a wide variety of assays designed to characterize several factors like survival, fecundity, DNA damage, morphological defects, and neurological health (Rand, Dao, and Clason 2009; Demir et al. 2011; Vales et al. 2013; Alaraby et al. 2020; Demir 2020).

Since the whole human genome was first mapped and sequenced in 2003, comparison of complete-genome sequences of various species has demonstrated that humans share a substantial number of genes with all other living organisms, including fruit flies. Comparative genomic research involving side-by-side analysis estimates that approximately 60% of *Drosophila* DNA is identical to that of humans, and almost 75% of the genes associated with human diseases, such as autism, diabetes, and cancer, have functional homologs in *D. melanogaster* (Lloyd and Taylor 2010). *Drosophila* has also been used as a simple and readily available genetic model organism by research into underlying mechanisms of immunity, aging, oxidative stress, neurodegenerative disorders (Bier 2005), spinocerebellar ataxia (Latouche et al. 2007), and Alzheimer's disease (Moloney et al. 2010).

Another compelling feature of *Drosophila* is that it shares various basic biological and physiological mechanisms and molecular pathways with mammals (Pandey and Nichols 2011; Wang et al. 2012), which makes this insect an excellent model organism for a wide range of fields, including pharmacological research (Pandey and Nichols 2011), genotoxicity studies (Pandey and Nichols 2011), and neurotoxicity screening (Rand 2010), where mammalian model organisms are traditionally deemed an indispensable part of animal testing. This model organism partly owes its success to a series of advantages over vertebrate animal models, including rapid life cycle, ease of culturing, low production costs, large offspring production per generation, high fecundity, and relatively simple genetics with only four pairs of chromosomes. Most importantly, ethical issues associated with vertebrate animals do not apply to fruit flies (Jennings 2011). Finally, the *Drosophila* toolbox that allows easy genetic manipulation is unprecedented among model systems (Mohr et al. 2014). Besides these features, *Drosophila* is not only sensitive to the toxic effects of traditional pesticides but also it has been considered as a good model to test these pesticides associated with the use of resistant strains. Chemical pesticides constitute a vital tool in controlling most of the world's destructive pests, yet utilization of such products in great amounts also cause pests to develop resistance, which leads to serious repercussions for sustainable pest control. In that regard, the mechanisms through which pests develop resistance should be well established. Such an effort involves identification and functional characterization of potential resistance genes or mutations. Using non-target model organisms such as *D. melanogaster*, as well as recent advances in genome modification technology, most notably CRISPR/Cas9, has considerably accelerated research into mechanisms of pest resistance (Perry and Batterham 2018; Douris et al. 2020). Indeed, previous work in the relevant literature contains hundreds of instances of pesticide resistance that is associated with variation in the overexpression of metabolic enzymes such as cytochrome P450s, glutathione-S-transferases, carboxylesterases, and UDP-glucuronosyltransferases. Therefore, overexpression of metabolic genes from pests also found in *D. melanogaster* has been demonstrated to be a powerful tool for elucidating the link between pest resistance and enzyme activity (Ibrahim et al. 2015).

Future prospects

Drosophila can be a highly valuable model in meeting today's demands of toxicity studies into nano-based pesticides by allowing optimization of pathway-specific screening, facilitating rapid testing of samples for biological activity at cellular or molecular levels, and rapid identification of genes responsible for interactions with NPs. *Drosophila* is an ideal model for high throughput screening as its fast generation time enables processing of thousands of flies for a given screen. Its ability to reflect the true interactions between DNA and environment might afford profound insights into the toxicity mechanisms of certain substances in humans – so much so that Collins, Gray, and Bucher (2008) has proposed a new model where *Drosophila* is expected to bring a paradigm shift in toxicity studies. Future efforts are geared toward better throughput screening with a high degree of pathway specificity using *in vivo* assays based on *Drosophila*. Although *in vivo* toxicity studies carried out with alternative non-mammalian models like zebrafish have been claimed to reflect vertebrate response, *Drosophila* appears to be superior in many ways since it enables researchers to process a large number of samples and to identify antibodies and genes regulating certain pathways at much lower costs, thus granting access to valuable data on various parameters, including survival, mortality, longevity, mutagenic and recombinogenic activity (Demir et al. 2011; Vales et al. 2013; Alaraby et al. 2020; Demir 2020).

In conclusion, *D. melanogaster* might prove to be an ideal model organism for the risk assessment and toxicological classification of nanopesticides. Valuable contributions from the brand new research field known as Drosophotoxicology will help provide better insight into possible impacts of nano-based pest control products, which are more complex by design, on the environment and human health. The ongoing collaboration between humans and *Drosophila* fruit fly since biologist Thomas Morgan first used it as a model organism for his research on heredity may once again help us unravel such complex biological and ecological processes.

Disclosure statement

The author reports no conflict of interest. The author alone is responsible for the content and writing of the paper.

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