Statistical procedure

Throughout this paper, error bars indicate standard error of the mean (SEM = standard deviation / square root of n). Student's t-test was performed for pair wise comparisons.

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Silver nanoparticle affects flying ability of fruit flies.

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Introduction

With the growing knowledge and advancement of nanotechnology, nanoparticles are being ubiquitously utilized in a wide array of applications including consumer goods, construction, food industry, and medicine. The extraordinary feature that makes nanoparticles act differently than bulk counterparts is their relative size in the scale of nanometers, which offers larger surface area for enhanced reactivity (Nam *et al.*, 2008). Silver nanoparticle (SNP) is one of the commonly used nanoparticles that is known for its anti-microbial property (Sharma *et al.*, 2009; Chen *et al.*, 2008). However, several reports using *Drosophila* as a model system suggest that higher dose of SNP compromise behavioral activities such as climbing of *Drosophila* (Key *et al.*, 2011). Flight is the integral behavior found in insects and is vital for performing various activities such as mating, migrating in search of food, and so forth. The influence of SNP on the basic fundamental behavior of *Drosophila, i.e.*, flight is largely unknown at this stage. In view of this, the impact of silver nanoparticles on flight of *Drosophila melanogaster* has been successfully monitored by modifying the experimental set up from the existing ones (Sadaf *et al.*, 2012; Sherwood *et al.*, 2004; Wojtas *et al.*, 1997).

Methodology

The flight assay was performed by placing a set up where an empty vial containing flies was positioned in the center of the beaker (14.5 cm in diameter). The beaker was half filled with water to create a barrier between the vial containing flies and the wall of beaker. The inner side of the beaker was coated with yeast paste in order to attract the flies. The beaker containing the vial was placed in a large enclosure (23 cm in diameter) for 40 minutes. Flight ability was determined by releasing flies from the vial and counting the numbers of flies that flew and crossed the water barrier and sitting on the walls of beaker or enclosure were scored as flyers. However, the flies still wandering in the vial or drowned in the water surrounding the vial were considered as non-flyers. Flies were collected in batches of 30 and starved for 1 hour before conducting this assay (Figure 1).

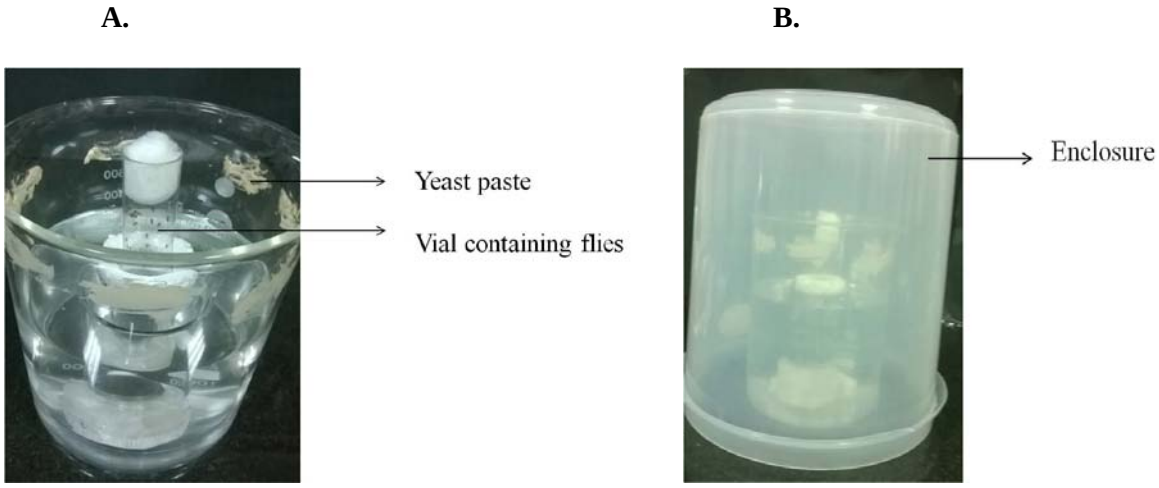

Figure 1. Experimental setup for flight assay. A, without enclosure; B, with enclosure.

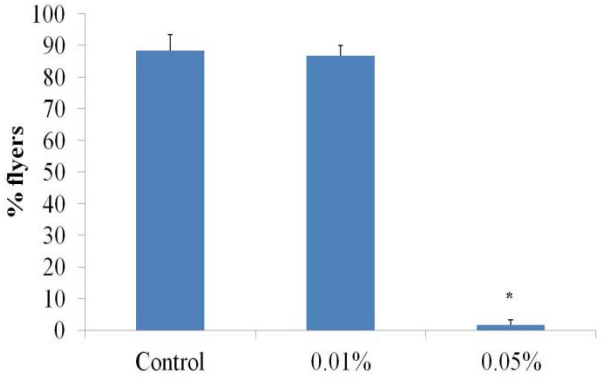

Figure 2. Dose-dependent effect of SNP on flying behavior of flies reared on SNP-supplemented food. *, $P < 0.02$.

Results

A significant decline in the flying ability was observed in the flies reared on SNP supplemented food (Figure 2). The percentage of flies reared at 0.01% SNP could cross the water barrier like the non-fed ones; however, at a higher concentration of SNP (0.05%), a significant decrease in flying ability was observed when compared with the control flies. At 0.05% SNP, 98% (approximately) of flies got either drowned in the water or they were wandering in the vial.

Discussion

Flight assay which is being modified by us is a good measure of flying ability of flies. Exposure to higher concentration of SNP throughout the development led to a significant loss in flying ability suggesting that silver nanoparticles might be interfering with the pathway responsible for flight. Flight deficit has been implicated to be caused due to loss of neurons and synaptic activity (Agrawal *et al.*, 2015; Sadaf *et al.*, 2012; Sherwood *et al.*, 2004) or intervention in the functioning of flight muscles (Wojtas *et al.*, 1997). Since nanoparticles may diffuse across the biological membranes (Chen *et al.*, 2008) and thereby opens a new gateway towards detailed understanding of the mechanism through which SNP might disrupt flight.

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Collection of drosophilids from the Font Groga site, Barcelona (Spain).

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Following a series of studies, another collection of drosophilids was obtained from Font Groga (Barcelona) on 6th October 2014. The detailed description of this site can be found in Araúz *et al.* (2009) and samples have been obtained in previous years (Canals *et al.*, 2013; Pineda *et al.*, 2014). A total of 12 baits containing fermenting bananas placed along a trail were used for attracting flies, and they were sampled by nets. Individuals were collected from 4 to 7 pm. In Table 1, the number of individuals of each species and sex is presented.

Table 1. Number and percentage of drosophilids obtained from Font Groga site (Barcelona, Spain) on 6 October 2014.

Species	Number	Percentage
D. subobscura (♂)	18	6.32
D. subobscura (♀)	69	24.21
D. simulans (♂)	37	12.98
D. melano/*simulans* (♀)	74	25.96
D. suzukii (♂)	7	2.46
D. suzukii (♀)	51	17.89
D. immigrans (♀)	19	6.67
D. phalerata (♂)	5	1.75
D. phalerata (♀)	4	1.40
D. cameraria (♂)	1	0.35
Total	285	100

It is worth to compare these data with those from the previous samples of 2012 (Canals *et al.*, 2013) and 2013 (Pineda *et al.*, 2014). In the present collection, the most abundant species is the *melano*/*simulans* group (38.94%), increasing with regard to last year (25.21%), but far from the percentage of 2012 sample (81.01%). In 2014, *D. subobscura* represents 30.53%, approximately half of the percentage detected in 2013 (62.60%), but clearly higher than that of 2012 (6.85%). The invasive species *D. suzukii* has increased reaching the 20.35%, clearly over the 7.98% (2013) and 9.20% (2012). This species seems to be well adapted to the Font Groga habitat.

Finally, we have estimated both the *H'* (Shannon diversity index) and *J* (Shannon uniformity index). The values obtained were 1.36 and 0.76, respectively. They are similar, but higher than those from 2012 and 2013 samples.

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