Toxicity of nanoparticles on pyrethroid-susceptible and pyrethroid-resistant larvae of the yellow fever mosquito *Aedes aegypti*

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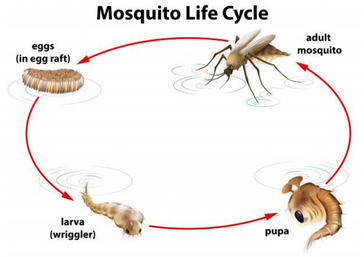
INTRODUCTION

- Chemical insecticides, such as pyrethroids, have been key components in the vector control of *Ae. aegypti* mosquitoes for many years. However, the widespread use of these insecticides has led to resistance.
- Nano particles (NPs) are being used as alternatives to chemical insecticides.
- NPs are cheap to produce and environmentally friendly, having minimal effects on non target plants, animals and aquatic organisms[1,2].
- Recent studies have shown that NPs have insecticidal activity on mosquito larvae, however, their mosquitocidal potency against pyrethroid-resistant larvae has been scarcely investigated[3].
- Therefore, we **aim** to elucidate the effects of a commercially-available NP on acute larval mortality and life cycle development of pyrethroid-susceptible (PS) and pyrethroid-resistant (PR) strains of *Ae. aegypti*.
- We **hypothesize** that NPs will have concentration-dependent larvicidal activity and/or delay larval development in PS and PR strains of *Ae. aegypti*.
- We **evaluated** the effects of NPs by generating a concentration-response curve that would determine the median lethal concentration (LC_{50}) within 48 h. Based on these results, we developed three different concentrations of NP (0.25, 0.1, and 0.01 mg/ml), that were used to daily evaluate larval mortality and development over the course of 14 days.



MATERIALS AND METHODS

Acute larval toxicity assay

- A NP stock solution (10mg/ml) was made by mixing NP powder (Vaylenx LLC., Washington, D.C.) and water. The stock was further diluted to the desired concentrations. (***Figure 1.a,b***)
- Six 1st instar *Ae.aegypti* larvae were placed in wells of tissue culture multiwell Falcon® plates at different concentrations in a controlled rearing chamber (28˚C, 80% HR, 12 hours of light/day). Each well contained 995 µl of NP solution and 5 µl of food (control contained 995 µl of water)(***Figure 1.c***) Larval mortality was evaluated after 48h.

Chronic larval toxicity assay

- 30 1st instar *Ae. aegypti* larvae were placed in 150x25 mm Falcon® petri dishes at 0.25, 0.1, and 0.01 mg/ml of NP concentration and reared under the same conditions as the acute mortality assay.
- Each plate contained 100 ml of NP solution or water (control) and provided daily with 0.01 g of food.
- Larval mortality and development was evaluated daily for 14 days.

- Graphs and statistical analysis was performed with GraphPad Prism (version 6.07) software.

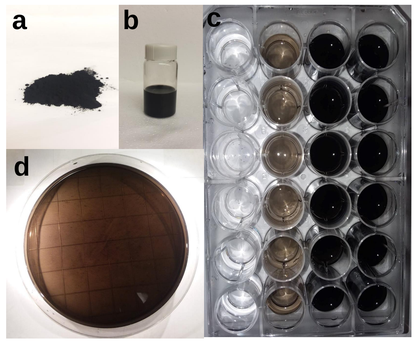


Figure 1. ***a.*** *Powder Nano Particles,* ***b.*** *NP stock solution,* ***c.*** *Acute larvae toxicity assay, from left to right : Control, 0.01, 1, 5 mg/ml of NP concentration* ***d.*** *Chronic toxicity assay, 1 mg/ml of NP concentration*

RESULTS

Acute larval toxicity assay

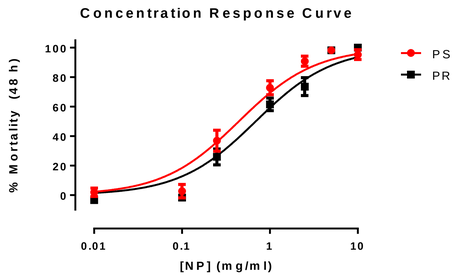


Figure 2*. Concentration response curve of larval mortality 48 hours after NP treatment exposure. The* ***red*** *and* ***black*** *curves represent mortality within the PS and PR strains respectively. 7 different concentrations (10, 5, 2.5, 1, 0.5, 0.1, and 0.01 mg/ml) were used to determine NP median lethal concentrations (LC_{50}) on larvae. LC_{50} of PS strain = 0.44 mg/ml; LC_{50} of PR strain = 0.69 mg/ml. There was NS difference between PS and PR strains (P>0.05, n=12)*

Chronic larval toxicity assay

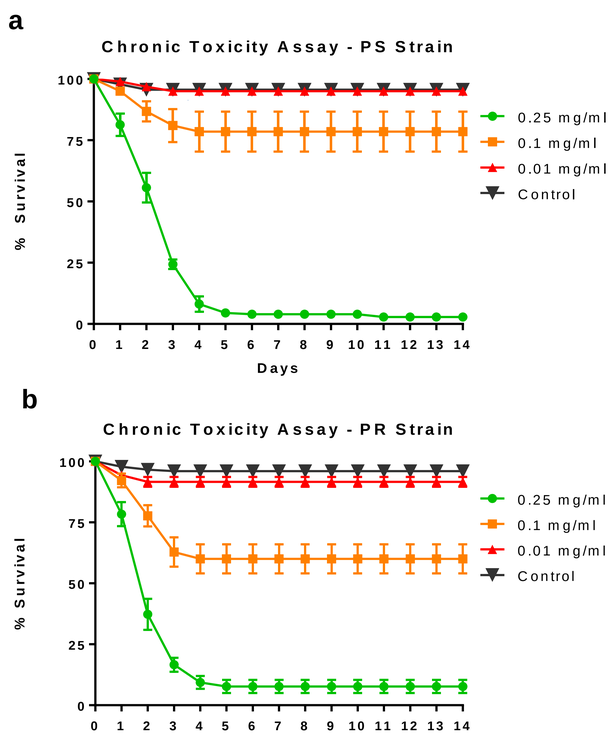


Figure 3. *Chronic larval assay 14 days after NP treatment exposure. In both figures (****a*** *and* ***b****) the following NP concentrations (mg/ml) are represented by:* ***green*** *(0.25),* ***orange*** *(0.1),* ***red*** *(0.01), and* ***black*** *(control) curves. 0.25 mg/ml concentration was lethal on both strains, with less than 20% of survival rate. On average, PR larvae appear to be more susceptible than PS larvae when exposed to 0.1 mg/ml however statistically there is no significant difference (P>0.05, ANOVA). Five days after NP exposure, % survival of larvae exposed to 0.25 and 0.1 mg/ml (concentrations with lowest % survival rates) stabilized on both strains. The remaining surviving larvae on both PS and PR strains (throughout all concentrations) did not show any significant difference in development (P=0.877 PS, P=0.9179 PR, ANOVA), completing their life cycle around the same time (approximately 10 days in PS, and 9 days in PR)*

DISCUSSION AND CONCLUSIONS

- NPs had concentration-dependent larvicidal activity in both PS and PR strains of *Ae. aegypti*, confirming our initial hypothesis. Similar results were observed in larvae of *Culex quinquefaciatus* (Murugan *et al*. 2015).
- Saxena et al. (2013) also showed toxic activity on 1st instar larvae of *Anopheles* sp., *Aedes* sp. and *Culex* sp. when exposed to NPs. However, in that study, larvae exposed to NP had a progressive delay in development, and after 25 days, larvae died without having reached pupal stage. In contrast, we found that surviving larvae reached adulthood without delay.
- The mode of insecticidal action is still unknown. Interestingly, dead larvae were often found with NP suspended near the mouth parts and anus (***Figure 4.a-d***), suggesting NPs might physically occlude the alimentary canal and/or respiratory siphon.

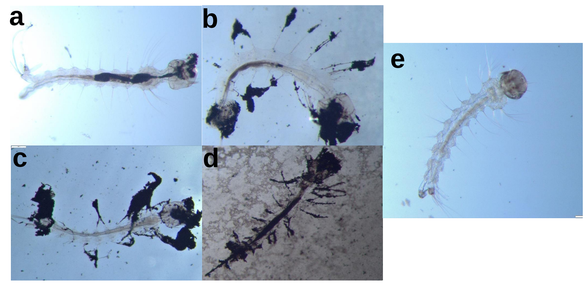


Figure 4. ***a***, ***b***, *and* ***c.*** *3rd instar dead mosquito larvae exposed to 0.1 mg/ml (****a****) and 0.25 mg/ml (****b*** *and* ***c****) of NP concentrations.* ***d.*** *3rd instar larvae in 0.25 mg/ml solution.* ***e.*** *3rd instar larvae control.*

- This present study demonstrates that NPs can be a potential alternative to chemical insecticides used in the control of PS and PR mosquitoes. Further studies are needed to elucidate the mode/mechanism of insecticidal action of NPs and confirm their safety against non-target organisms.

ACKNOWLEDGMENTS

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