Impact Of An Agrochemical On The Mitochondrial Function In Bee Drone Spermatozoa

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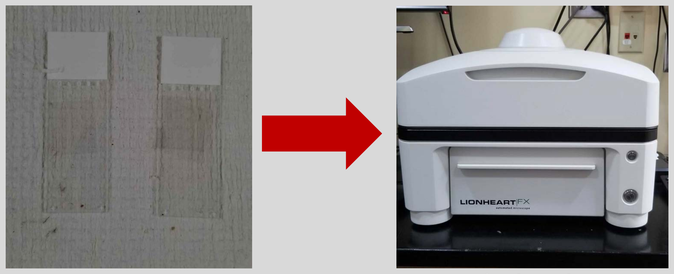
Introduction

- Agrochemicals are suspected to disrupt the physiology of the bees resulting in bees incapable of foraging or performing mating flights. Our study assays the mitochondrial status in the bee sperm cell exposed to *N*-(phosphonomethyl)glycine, *aka* glyphosate.
- If mitochondrial function is affected, spermatozoa's ability to swim to the spermatheca in the queen bee where it is stored through the queen's lifetime (~5-8 years) can be affected.
- In this study, the mitochondrial characteristics in the spermatozoa after exposure to near field-level doses were assayed using the dye, MitoTracker green (MTG).
- MTG measures mitochondrial function by assessing the mitochondrial mass **[1,5]**, cysteine abundant proteins (thiol proteins) **[2,3]** and certain glycoproteins [**4**] and most importantly Reactive Oxygen Species (ROS)[**7**].

Methods and Materials

- MTG working solution was prepared according to **https://tinyurl.com/y55mhprk**.
- 1.8 µl of semen was mixed with 300 µl of semen buffer to which appropriate amounts of glyphosate stock solution was added to obtain 3.2 - 320 nM treatment dilutions. After 24 hours, 3µl of MitoTracker Green working solution was added to the semen solution.



Loaded Slides **Cytoanalysis Microscope**

- Slides were analyzed using fluorescence microscopy with the Biotek® LIONHEART FX automated microscope (Biotek, Inc., USA). A GFP filter was used to observe green MTG stain and a Hoechst/DAPI filter to observe blue DAPI stain. Images were combined and the composites are shown in **Fig. 2**.

Results

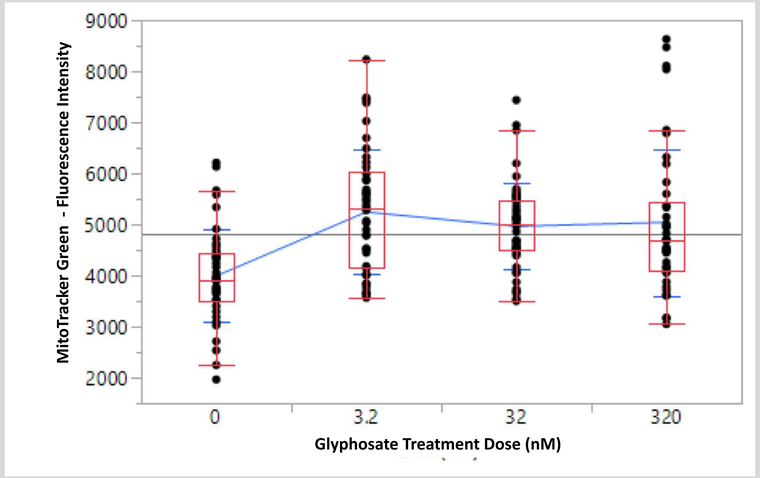


Fig. 1: Plot shows mitochondrial intensity in individual spermatozoa after each treatment. Significant differences was noted when comparing the control with each treatment where p=<0.0001 for each treatment. However, comparing within the treatment (3.2 nM - 32 nM, 3.2 nM - 320 nM, and 32 nM - 320 nM) no significant difference in concentration effect was found (p=0.6520, 0.3551, and 0.7332, respectively). Statistic adopted was Dunn's test using Kruskal-Wallis multiple pairwise comparison and Benjamini-Yekutieli adjustment with FDR set at 0.05.

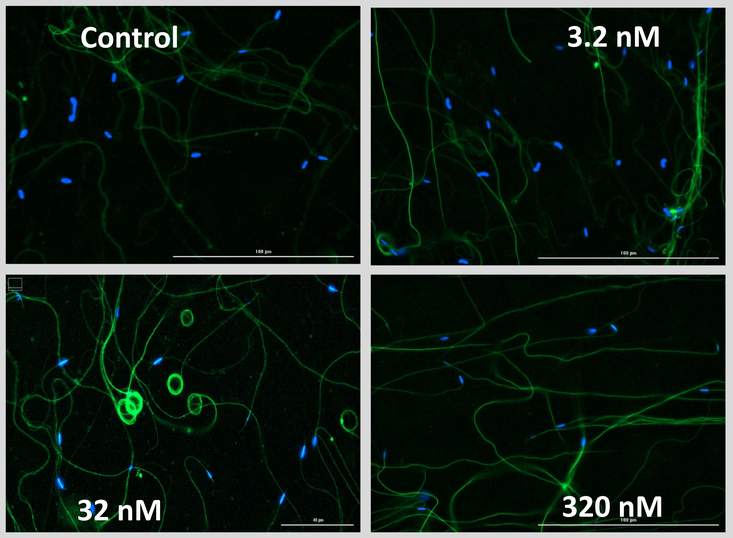


Fig. 2: Composite image of DAPI stained nuclei and MTG stained caudal mitochondria. In both treated and untreated bee semen suspended in semen buffer, DAPI was used as nuclear stain and MitoTracker Green (MTG) was used as a mitochondria specific stain. MTG passively diffuses across the plasma membrane and accumulates in active mitochondria binding to the free thiol- groups of certain proteins [4].

Conclusions

- Results indicate there is a marked effect on mitochondrial MTG staining intensity characteristics independent of glyphosate dosages in the range of 3.2 - 320 nM (**Fig. 1**).
- Since MTG accumulation is independent of the membrane potential, we believe ***that glyphosate treatment results in significant stress and elevated production of reactive oxygen species*** of which MTG is also an indicator [**6,7**](**Fig. 3**)

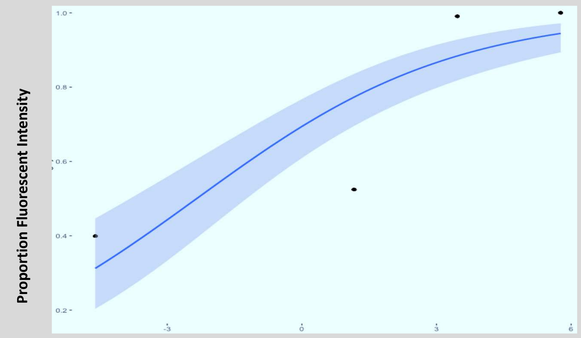


Fig. 3: Quasi-Poisson modelling of the proportion MTG binding/levels within the mitochondria of a spermatozoon. The LI_{50} (increase in 50% intensity) is estimated to be 0.005 nM.

Future Directions

- Further in-depth mitochondrial disfunction studies will be conducted.
- Role of glyphosate in oxidative stress leading to spermatozoa pathogenesis will be studied.
- We will also assess the effects of glyphosate on the membrane potential with other appropriate mitochondrial dyes such as Rhodamine 123.

References

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All results presented here are preliminary