DIFFERENTIAL EFFECTS OF ZnO AND Ag NANOPARTICLES, AND DIAZINON ON THE ACTIVITY OF MEMBRANE AND SOLUBLE FORM OF ACETYLCHOLINESTERASE IN HONEY BEE HEAD AND THORAX

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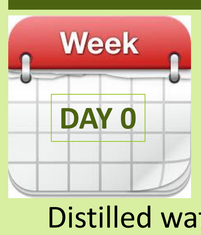
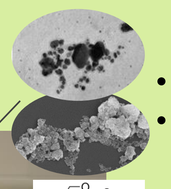
Introduction

Honey bee is an important pollinator threatened by diverse environmental factors, potentially also by products of nanotechnologies. Deliberate application of nanopesticides will result in inputs of engineered nanoparticles (NPs) into the environment, entering both soil and freshwater environments. It is of outmost importance to assess their potential hazards on honey bees. The activity of soluble form of acetylcholinesterase (AChE) is often used as an important biomarker of neurotoxicity after exposure to xenobiotics. In previous study we have demonstrated the alteration in the activity of brain soluble form of AChE, in honeybees orally and chronically exposed to ZnO NPs[1]. Recently, *in vitro* experiments suggested that the membrane form of AChE is mainly neuronal whereas the role of soluble form is largely unknown, but some suggestions of their protective role against xenobiotics have been given. Therefore, in regard to neurotoxicity, monitoring of only soluble AChE is not sufficient. Therefore, the aim of this study was to investigate *in vivo* effects of ZnO NPs , Ag NPs and AChE inhibitor diazinon not only on the activity of soluble AChE, but also membrane AChE in honey bee head and thorax.

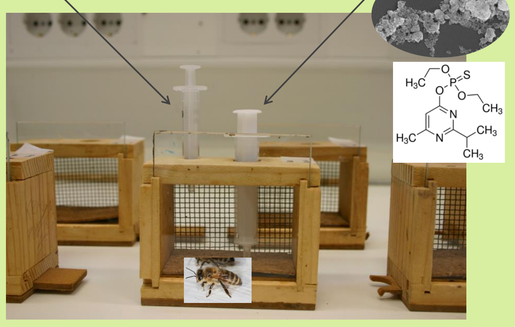
Methods & Materials



Distilled water

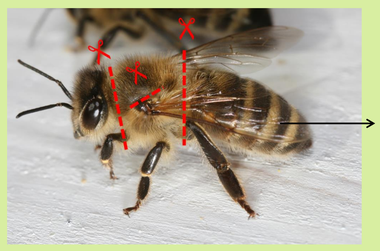


- **Nanomaterials:**
  Ag NPs (50 mg/L)
  ZnO NPs (500 mg/L
- **Organophosphate diazinon (**1.5 mg/L)
- **control**: sucrose feeding (1.5 M)

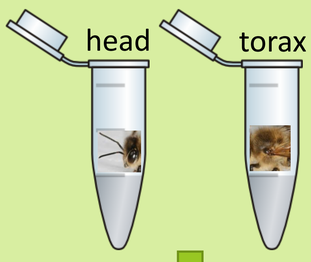


Winter honeybees,
Number of bees per treatment: Control N=45, ZnO NPs= 26, Ag NP N= 59, diazinon N= 26; Average 22-26 bees/cage

10 days incubation at 35°C
Daily inspection of feeding and survival

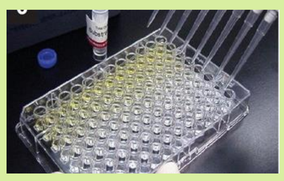


Abdomen-discharged



- Homogenisation,
- Separation of membrane and soluble fraction
- centrifugation (15 min, 4° C, 16000 g)

Acetylcholinesterase measurement[2]



MORTALITY AND FEEDING RATE

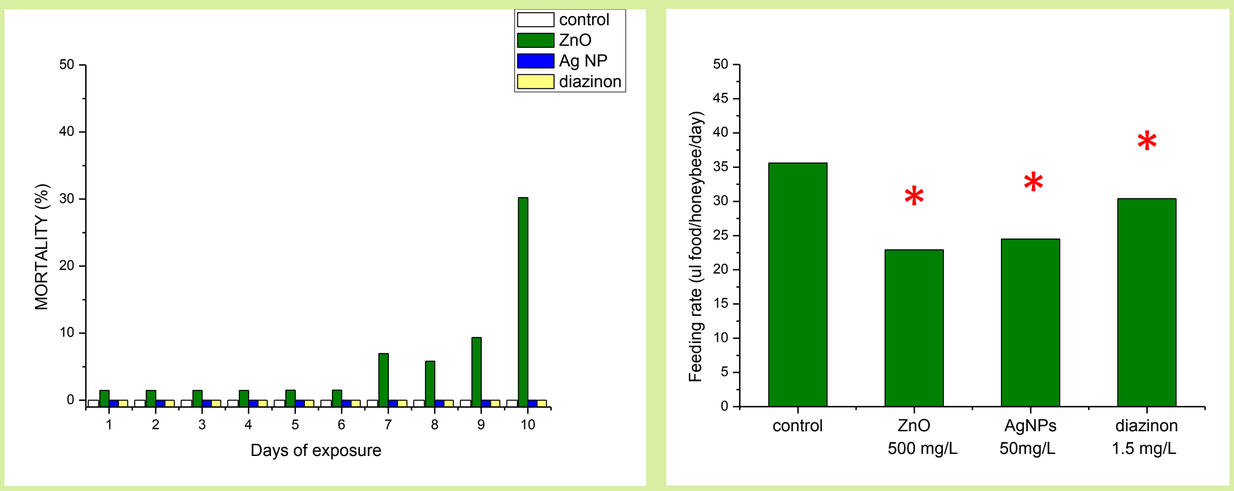


Fig. 1. Mortality and daily feeding rate of of honeybees exposed to ZnO NPs, Ag NPs and diazinon for 10 days.

ACETYLCHOLINESTERASE ACTIVITIES

MEMBRANE BOUND

SOLUBLE FRACTION

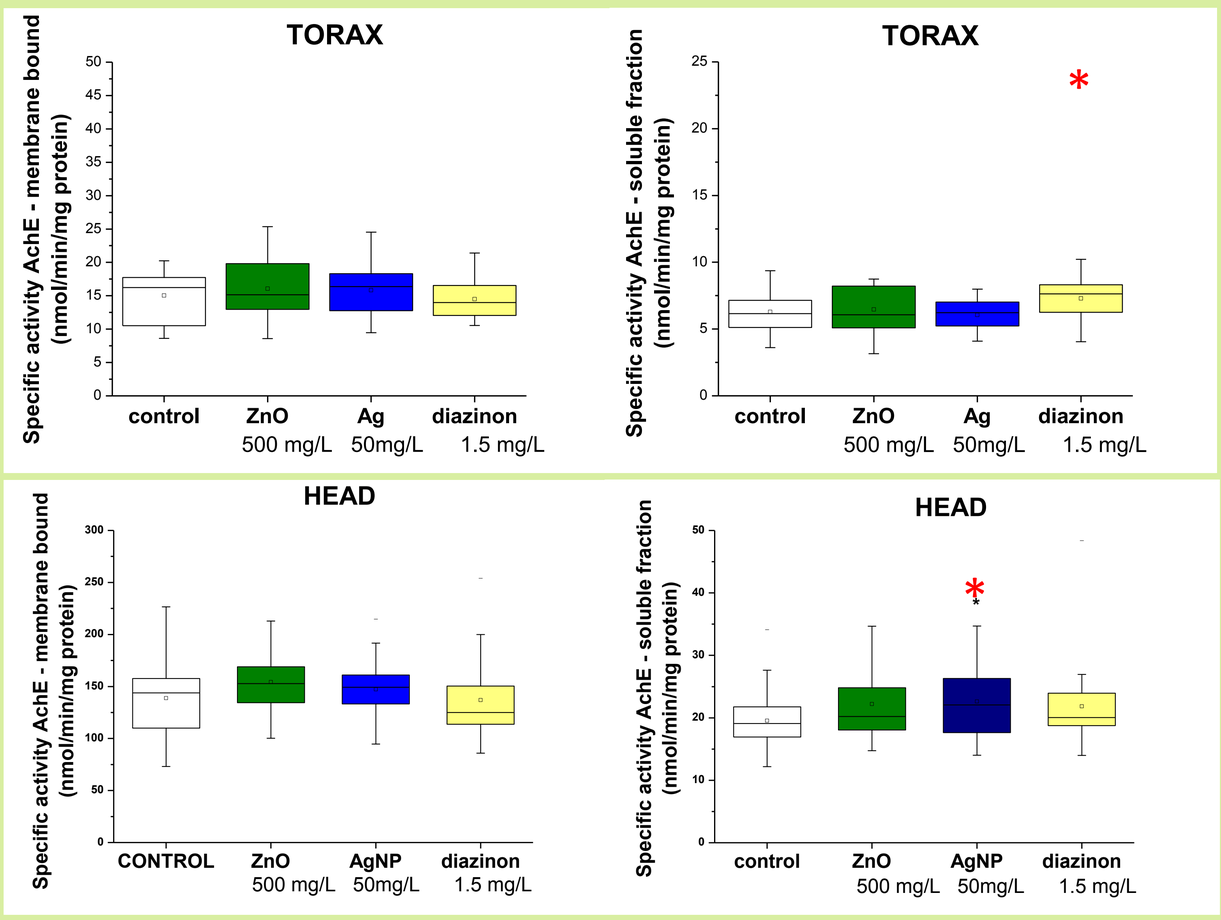


Fig. 2. Different fractions of acetylcholinesterase activity (membrane, soluble) in head and torax of honeybees exposed to ZnO NPs, Ag NPs and diazinon.

Results & Discussion

Chronic 10 days exposure to ZnO NMs, Ag NPs and diazinon caused a significant decrease in feeding rate, but the mortality increased only in group treated with ZnO NMs. The activity of membrane AChE in the head of untreated honey bees was much higher that the soluble confirming results of *in vitro* experiments showing that the membrane form is probably neuronal. In the thorax this ratio was much lower. Chronic exposure to ZnO NPs and Ag NPs elevated the activity of the soluble, but not the membrane AChE in the head. However, the same treatment to NPs had no effect of any form of AChE in the thorax. On contrary, the chronic exposure to AChE inhibitor diazinon diminished only the activity of soluble AChE in the head, but unexpectedly elevated the soluble AChE in the thorax.

Our results indirectly confirm the difference in the function of two forms of AChE. The elevation of the activity of soluble AChE might be predictable for its detoxifying function whereas the changes in the activity of membrane AChE could be the result of the compensatory effect of nervous system or direct inhibition by diazinon. However, the role of the soluble AChE needs to be further investigated. We show that the mechanism of ZnO NPs and Ag NPs action on AChE is similar but other than the mechanism of diazinon suggesting that ZnO and Ag NPs don't act directly inhibitory on AChE at the exposure set-up (doses, and duration) used in our experiment.

References:
1 Milivojević et al., 2015. Chemosphere 120: 547–554
2 Ellman et al. 1961. Biochem. Pharmacol. 7: 88-95.

Acknowledgements
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