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Background/Purpose

Colony collapse disorder (CCD) of honey bees is a major problem of modern agriculture, which may be caused by a number of factors including the increased use of pesticides. This is the reason why research on the impact of plant protection products on pollinator species, such as the widely used azole fungicides, is particularly importante.

Problem Description

Tebuconazole, a prominent member of the class of azole fungicides, is known to cause both redox homeostasis changes in the bee brain and altered fatty acid metabolism, which may be linked to certain cognitive changes leading to navigational as well as behavioral disturbances. However, there is lack of knowledge on how these agents act in other organs, such as the flight muscle.

Methodology

The aims of our study were to assess the effects of acute sublethal exposure to tebuconazole, with a focus on the redox homeostasis of honeybee flight muscles. Tebuconazole was applied per os mixed to saccharose containing feeding solution in lethal dose 50 (LD50)/10, LD50/20 and LD50/40 concentrations for 48 hours. Following the dissection and homogenization of flight muscles, colorimetric and fluorometric tests were performed to measure redox parameters such as malondialdehyde (MDA), hydrogen peroxide (H2O2), glutathione (GSH and GSSG) concentrations, as well as antioxidant enzyme activities.

Results and CONCLUSION: Our results showed detrimental effects considering GSH and GSSG concentrations as well as their ratio. On the other hand, flight muscles exhibited potential adaptive responses to acute oral tebuconazole exposure, contrasting with the vulnerability, previously observed in other organs, such as the brain. This was highlighted by decreased H2O2 and MDA levels together with unchanged redox enzyme activities.

Practical Implications for Beekeepers

Investigating the cellular effects of agricultural pesticides in bees is a high priority area in the context of sustainable agriculture. Our results show that although tebuconazole adversely affected defense mechanisms such as glutathione balance, negative effects for several other antioxidant parameters were not confirmed. Thus, compared to other organs such as the brain, flight muscles showed increased resistance and activation of efficient adaptive mechanisms.

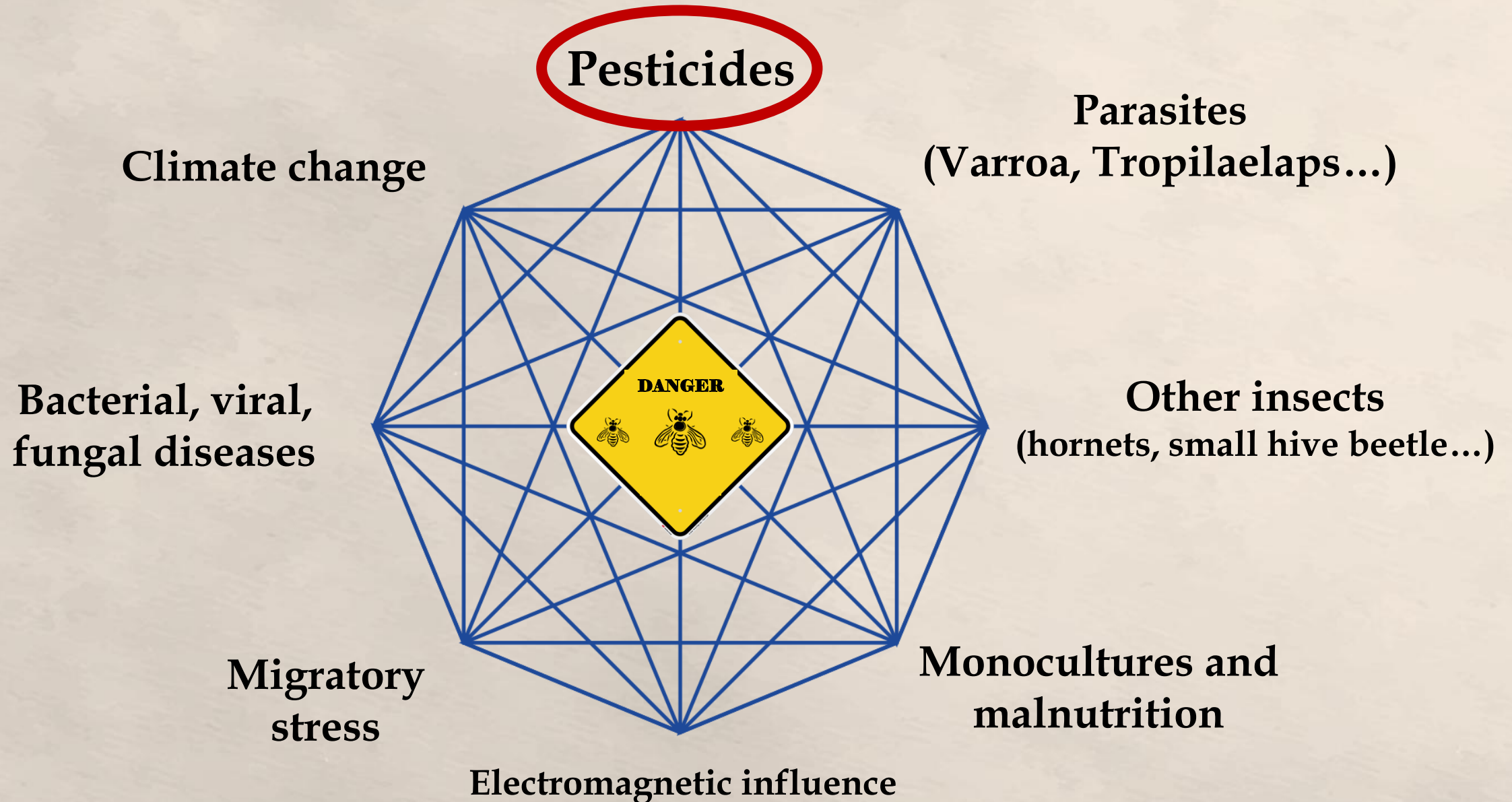
Tebuconazole-induced oxidative stress in honeybee flight muscles

„Another part of the mosaic”

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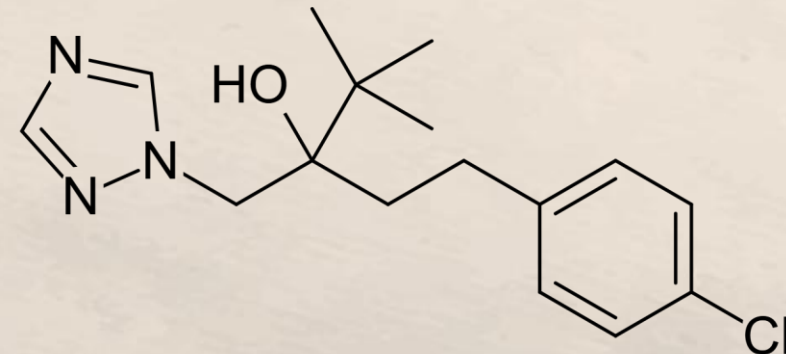


Major threats and stressors



Tebuconazole

- **Agricultural context:** widely applied triazole fungicide
- **Mode of action:** inhibits fungal **CYP450-dependent lanosterol 14 α -demethylase**, blocking ergosterol synthesis
- **Environmental exposure:** detected in **pollen, nectar**, especially during blooming
- **Toxicological concerns:**
 - Impairs **CYP450 enzymes** in insects (CYP4G11, CYP9Q2, CYP6AS14, CYP306A1)
 - Classified as an **endocrine disruptor**, altering hormone metabolism (ecdysteroids, juvenile hormone)
 - Reported to induce **oxidative stress** in multiple animal species



Results of previous studies and hypothesis

- Affects foraging, pollination, and learning
- Disrupts antioxidant defense and redox balance
- In brain: severe oxidative imbalance
- Does it affect metabolism of flight muscles as well?

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Detrimental consequences of tebuconazole on redox homeostasis and fatty acid profile of honeybee brain

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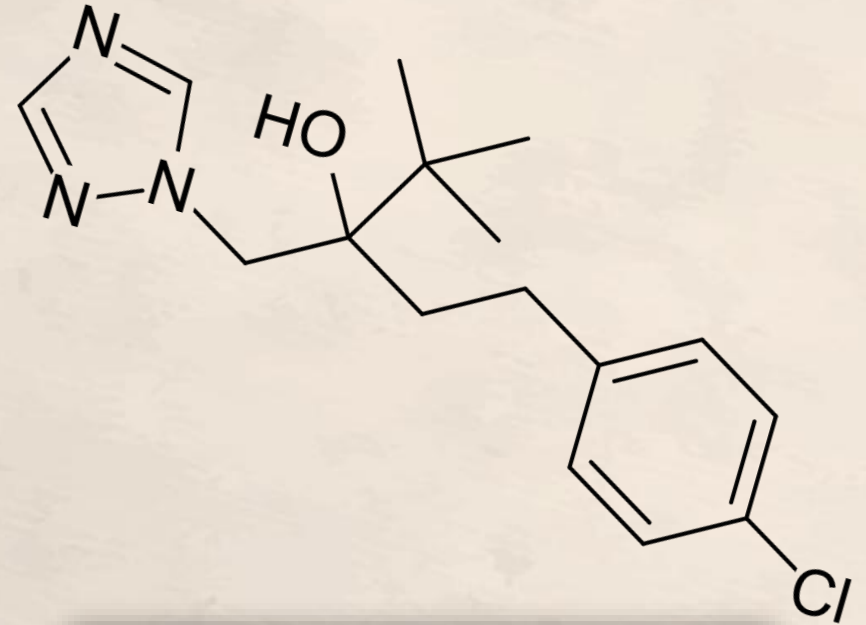
Materials and Methods

- Apiary in Ugod (Veszprém county)
- There was no chemical treatment in the 3 months prior to the experiment
- ~250-300 bees/treatment groups



Materials and Methods

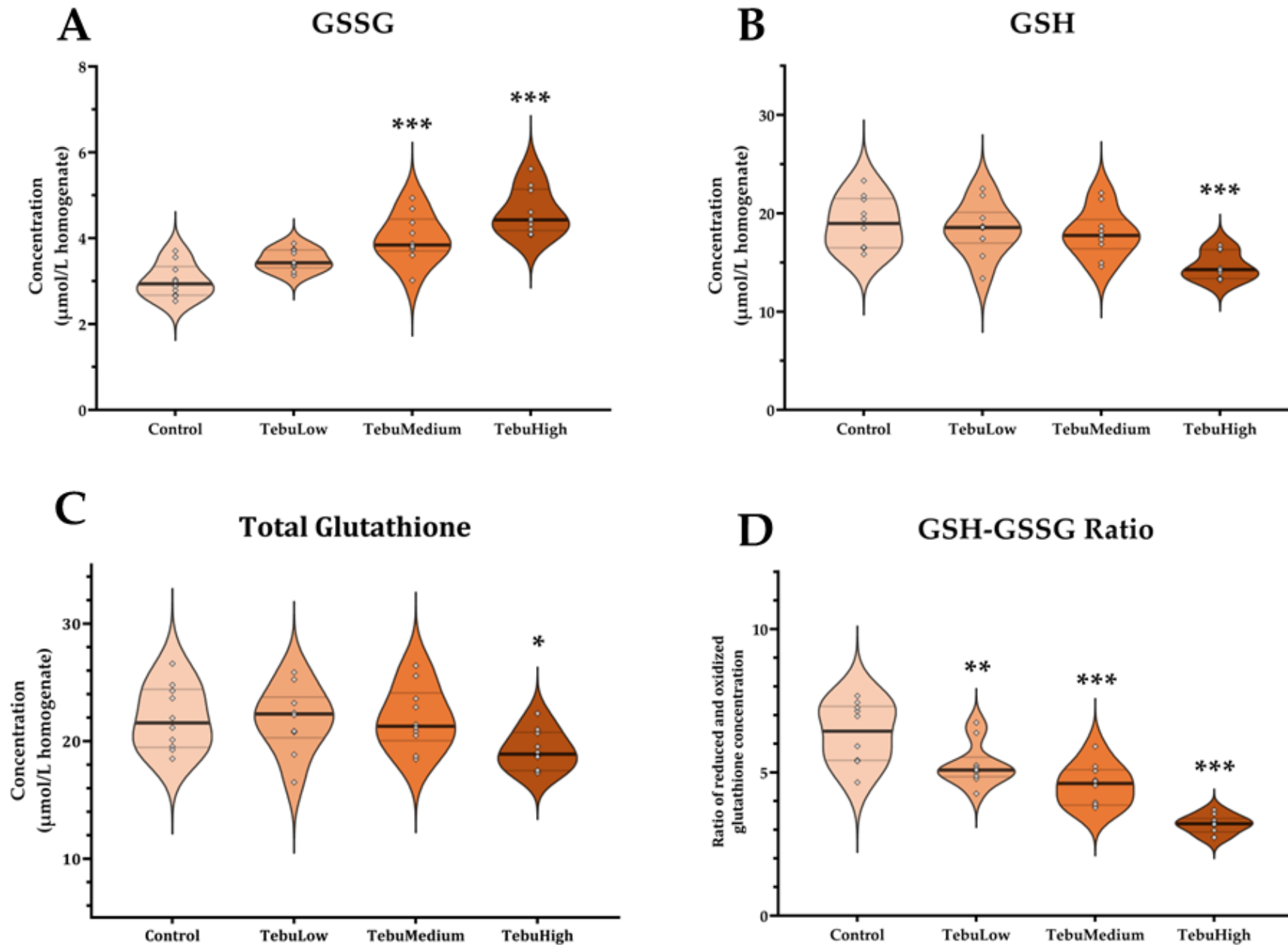
- Tebuconazole *per os* treatment
 - Mixed in sugar syrup feeding solution
 - LD50/10 = 8.505 µg/bee/day
 - LD50/20 = 4.253 µg/bee/day
 - LD50/40 = 2.123 µg/bee/day
- Shock freezing in dry ice
- Dissection of examined organs



Dissecting the flight muscles

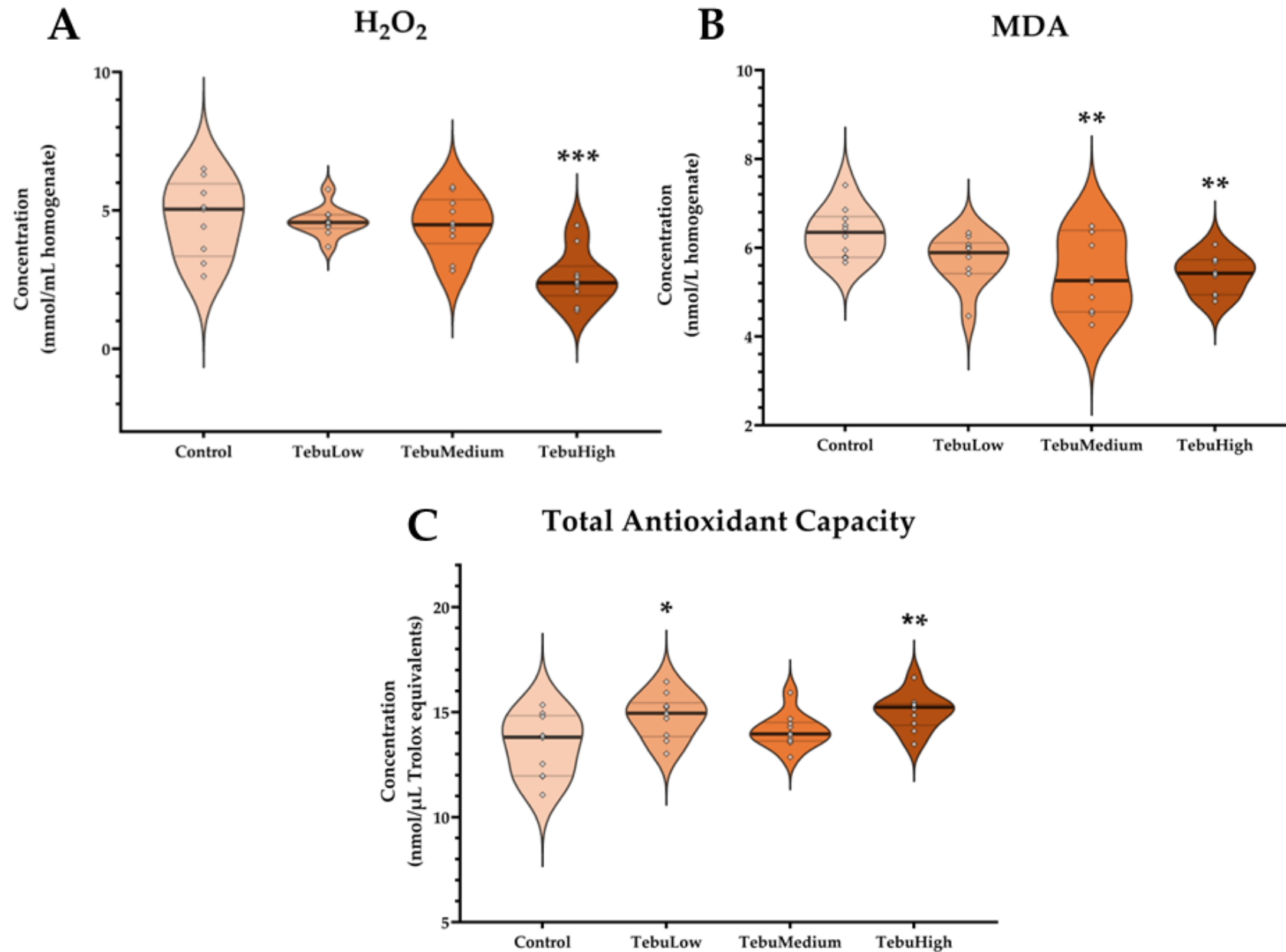


Results



* $P < 0.05$
** $P < 0.01$
*** $P < 0.001$

Results

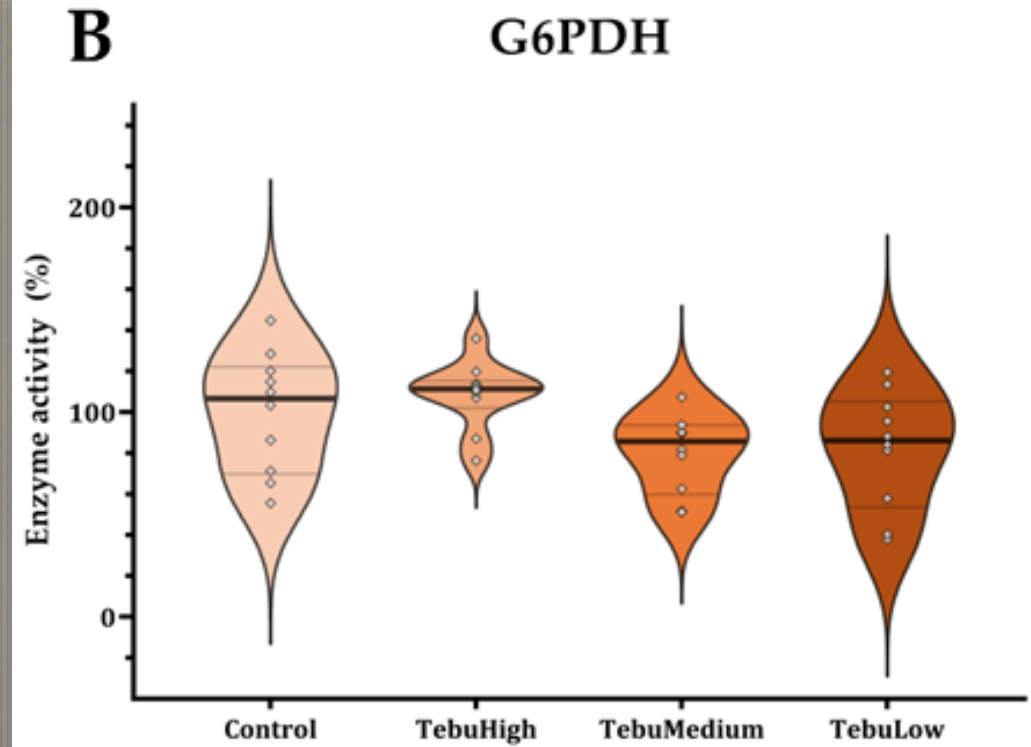
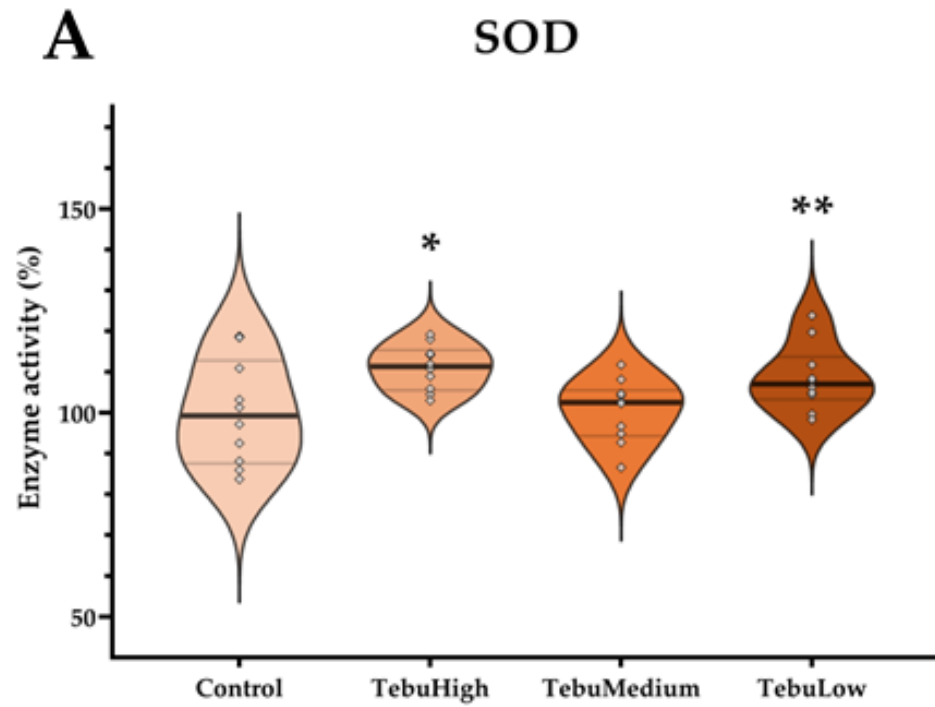


* $P < 0.05$

** $P < 0.01$

*** $P < 0.001$

Results



* $P < 0.05$
** $P < 0.01$

Conclusion

- Tebuconazole **significantly impairs the glutathione system** in honeybee flight muscles
- Flight muscles show **effective adaptation and possible overcompensation**
 - H_2O_2 and **MDA** ↓ ↓ ↓
 - **SOD** activity and **total antioxidant capacity** ↑ ↑ ↑
- Adaptive response of flight muscles **differs from the brain**, which is more vulnerable
- H_2O_2 and MDA may reflect **autophagy-mediated clearance** of damaged organelles
- Findings suggest **non-monotonic dose-response effects**, consistent with endocrine disruptor properties



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Thank you very much for your kind attention!

