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Testing effects of field realistic pesticide tank mixtures used in organic orchard farming on adult honey bees and bee colonies

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Abstract

In Germany, tank mixtures consisting of several insecticides do not comply with good agricultural practice, even if the individual mixing partners are classified as not dangerous to bees. These mixtures are considered as harmful to bees and should therefore not be used on plants which are in flower or which are visited by bees as a precautionary measure. Therefore, this project aimed to investigate if tank mixtures of insecticides used in organic orchard farming actually affect honey bees under realistic field exposure scenarios. Accordingly, different insecticide mixtures were tested in the laboratory and under semi-field conditions to evaluate their risk for honey bees. Tested insecticides included tank mixtures of the products Neudosan NEU (fatty acid potassium salt 515 g/L) and Micula (rapeseed oil 785.75 g/L), Neudosan NEU and Netzschwefel Stulln (sulphur 796 g/kg), Neudosan NEU and Kumulus WG (sulphur 800 g/kg) as well as the tank mixture of Kumar (potassium hydrogen carbonate 850 g/kg) and Netzschwefel Stulln. The first level of testing considered a contact exposure of honey bees to a spray solution at field realistic exposure rates in the laboratory using a spray chamber. In case of biologically relevant effects at the laboratory level, additional tests were conducted under semi-field conditions with whole bee colonies. The results obtained for all tested tank mixtures did not indicate an increased risk for honey bees and honey bee colonies when exposed up to the maximum authorized field realistic application rates.

Keywords: honey bee, tank mixtures, insecticide, organic farming

Introduction

Organic farmers depend only on plant protection products (PPPs) approved for use in organic production in accordance with Annex I of Regulation (EU) No. 2021/1165 to control pests in their fields (European Commission, 2021). In most cases, tank mixtures are used consisting of two different PPPs, which are mixed directly in the tank and applied to the crop. Tank mixtures are increasingly being used to counteract pest resistance and reduce working time and costs (Shannon et al., 2023; Wernecke & Castle, 2020). In Germany, the use of certain tank mixtures is regulated by the BVL (Federal Office of Consumer Protection and Food Safety). Some mixtures (e.g. several combinations of pyrethroids or neonicotinoids with azole fungicides) are known to cause a synergistically increase in toxicity to honey bees (Schuhmann et al., 2022). Therefore, these tank mixtures are associated with risk mitigation measures limiting or excluding the exposure of honey bees. Due to the wide range of commercial PPPs and the large number of possible tank mixtures, mixing effects have not been fully researched yet. While some tank mixtures show synergistic or additive effects, others are harmless to honey bees (Wernecke et al., 2019). Nevertheless, in Germany good agricultural practice does not recommend the use of tank mixtures consisting of several insecticides, as an increase in toxicity to honey bees cannot be excluded and is not regularly evaluated in the risk assessment of individual PPPs. In accordance with Regulation (EU) No. 1107/2009 the risk evaluation for bees is mandatory, since organic

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farming depends also to a certain degree on tank mixtures used on bee attractive crops (European Commission, 2009).

The first tested tank mixture consists of the PPPs Neudosan NEU (fatty acid potassium salt 515 g/L) and Micula (rapeseed oil 785.75 g/L). This combination acts as an alternative to pyrethrum preparations, which have known side effects on beneficial insects and non-target species (Schleier & Petterson, 2011; Diogo et al., 2023). Other insecticidal mixtures tested were the combinations of Neudosan NEU and Netzschwefel Stulln (sulphur 796 g/kg), Neudosan NEU and Kumulus WG (sulphur 800 g/kg) as well as the tank mixture of Kumar (potassium hydrogen carbonate 850 g/kg) and Netzschwefel Stulln partly serving as possible alternatives to copper applications in organic apple farming. In this study, we tested these four tank mixtures for their possible increase in toxicity to honey bees in order to evaluate if they are harmful to bees when used at field realistic application rates.

Material and Methods

In the first part of the study, a modified acute contact test was conducted, which is based on OECD Guideline 214 (OECD, 1998). The study design considered adult honey bees *Apis mellifera* L. (Buckfast) which were taken from the institute's own apiary. Immobilized bees were sprayed with spray solutions consisting of the individual PPPs and the corresponding tank mixtures in a spray chamber at a standard application volume of 300 L water/ha. Four different tank mixtures where tested, a tank mixture of Neudosan NEU and Micula (TM Neu+Mic), a tank mixture of Neudosan NEU and Netzschwefel Stulln (TM Neu+Net), a tank mixture of Neudosan NEU and Kumulus WG (TM Neu+KuWG) and a tank mixture of Kumar and Netzschwefel Stulln (TM Kum+Net). The tank mixtures were tested at concentrations equivalent to their maximum authorized application rates per hectare and realistic field application rates derived from organic orchard farming. Further application rates were additionally considered taking into account the unreduced maximum application rates in a water volume of 300 L/ha to represent a worst-case scenario. A toxic reference substance was not considered in the trials, as a possible increase in toxicity of the tank mixture compared to the individual PPPs was examined. One day before application, the bees where transferred into standard stainless steel cages and randomly placed in a climate chamber (5 cages per treatment, 10 bees per cage at 24°C and 60 % rel. humidity). Feeding was *ad libitum* with 50 % sugar solution (w/v). Mortality and abnormal behaviour were recorded for four days after application. When tank mixtures treatments resulted in increased mortalities compared to the individual mixing partners, these combinations were tested under higher tier semi-field conditions providing a more field realistic exposure scenario of whole bee colonies. The conduct of semi-field trials was based on the OECD Guidance Document 75 (OECD, 2007). Mortality and Foraging activity were assessed daily, from three days before application until seven days afterwards. Colony development was recorded once before application and two times afterwards, within a period of 24 days. The application of the tank mixture and water control was performed with a calibrated portable boom sprayer in flowering *Phacelia tanacetifolia* (BBCH 65). Each treatment consisted of four tents containing each one bee colony (approx. 7000-7500 bees). Statistical analysis was carried out using the statistical software R considering a significance level of $p<0.05$. Laboratory data was analysed using a survival analysis and cox proportional hazard model. Semi-field data was analysed using ANOVA and post-hoc tests.

Results and Discussion

Laboratory studies provide a worst case exposure scenario by testing individual bees in a highly artificial environment. At colony level, effects can be less evident due to the high buffering capacity of the superorganism (Henry et al., 2015). Thus, when under worst case laboratory conditions no effects are observed, no further higher tier testing under field realistic conditions is foreseen. None of the worst-case concentration of TM Neu+Mic (20 L Neudosan NEU/ha + 10 L Micula/ha), TM Neu+Net (10 L Neudosan NEU/ha + 3,5 kg Netzschwefel Stulln/ha) and TM Neu+KuWG (10 L Neudosan NEU/ha + 2 kg Kumulus WG/ha) resulted in an increase in toxicity incl. abnormal behaviour in the laboratory, indicating a safe use when applied in the field.

A statistically significant increase in mortality occurred when compared to the control group and Netzschwefel Stulln in the concentration of the maximum authorized application rate (3.75 kg Kumar /ha + 2.25 kg Netzschwefel Stulln/ha) and the one representing the worst-case scenario (3.75 kg Kumar /ha + 3 kg Netzschwefel Stulln/ha).

Therefore, the concentration of the worst case scenario of the TM Kum+Net was further tested in a semi-field trial. In the tunnel experiment, mortality and colony development of the TM Kum+Net showed no differences compared to the control. In the first 30 minutes after application, a reduced foraging activity was observed. The active substances of the TM Kum+Net are known to have a repellent effect on bees (Afik et al., 2006; Hagler, 1990). Presumably, this explains the period in which reduced foraging activity was observed reducing the exposure of bees to the freshly sprayed crop. Due to the short period, this effect was regarded as not biologically relevant. Accordingly, the TM Kum+Net did not result in any relevant effects on bees when applied under semi-field conditions (Fig. 1).

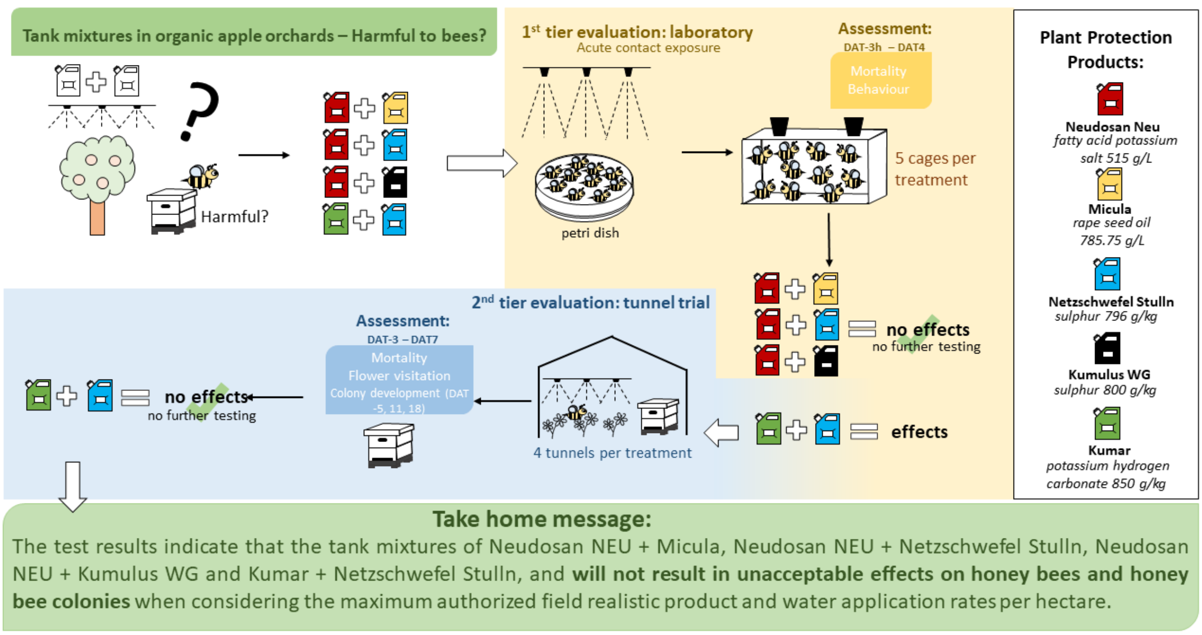

Figure1: Schematic representation of the phases of the experiment with the associated results. No effects denotes no statistically significant effects compared to the control and the individual products.

The test results indicate that the TM Neu+Mic, TM Neu+Net, TM Neu+KuWG and the TM Kum+Net will not result in unacceptable effects on honey bees and honey bee colonies when considering the maximum authorized field realistic product and water application rates per hectare.
In view of a potential hazard to honey bees, the tested TM Neu+Mic can be used as alternative to pyrethrum preparations and the TM Kum+Net can be used to reduce the use of copper.

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