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Commentary

Pollination and Pesticides in runner beans in Poland - a commentary on Kot et al. (2023) in *Agriculture* 13: 2138

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Abstract

Pollination of crop plants is highly valued as it contributes to productivity in terms of quality and quantity. Globally, pollination is valued at more than USD 500 billion. The primary pollinators are insects and amongst them, bees. The Western honeybee (*Apis mellifera*) is a very generalist pollinator that is managed. Honeybees contribute up to 50% of the pollination of various crop plants. Pollinators are at risk due to land-use/land-cover changes and agricultural pesticide input.

In a recently published study, research on honeybees in runner bean (*Phaseolus coccineus* L.) in Poland is presented. In one part of the study, the actual foraging of honeybees in beans is recorded, along with the time of day, abundance and time spent on flowers. The second part of the study investigated several apiaries concerning the potential poisoning of bees by means of pesticide exposure. The authors recorded the fitness of colonies, flight activity, behaviour, productivity and pesticide residues in bees.

The manuscript, unfortunately, has several scientific flaws that are outlined in this commentary. These flaws, particularly those related to experimental planning and data collection and analysis, have the potential to compromise the conservation of pollinators. The misguidance in the implementation of measures to protect pollinators and pollination services is a cause for concern and should motivate us to address these issues.

Keywords

good scientific practice, replicate, reproducibility, *Apis mellifera*, ecosystem service, research integrity, pesticide residue analysis

Climate change, land-use/land-cover (LULC) change and overuse of agrochemicals, especially pesticides, are the reason for declining pollinator populations (Dicks et al. 2021). Honeybees (*Apis mellifera*) are a widespread pollinator species occurring globally and a highly generalist species foraging for nectar and pollen on a vast number of plant species - crops and wild plants - is thought to be a primary contributor to pollination (Hung et al. 2018), its global stock steadily increasing over the past 50 years (FAO 1997). As honeybees are a managed species, beekeeping methods often replace dwindling colonies and the stock can be maintained. Wild bees, on the other hand, will not be replaced and, hence, might be lost forever if exposed to adverse conditions, which can be even more dramatic if these species are specialist pollinators of certain plants that cannot be pollinated by honeybees (Garibaldi et al. 2013). However, as climate change, LULC change and pesticide usage are highly politicised topics, it is necessary to ensure that the science behind it is reliable and replicable. Otherwise, shortcomings in research design, analysis and interpretation of data, as well as the conclusion drawn from the results, will fire back at the scientists and other researchers in the field. In the worst case, science's credibility might be affected.

Recently, a paper published in *Agriculture* (MDPI) (Kot et al. 2023) presents a study conducted in Poland on the foraging of *A. mellifera* in runner beans (*Phaseolus coccineus* L.), including an analysis of the bees' exposure to seasonal agrochemicals. The study was laid out to assess the foraging pattern in runner beans over the phenological period. A runner bean plantation was used with two apiaries close by (a few hundred metres away) and data collections on honeybee foraging were done daily at four time points (9 am, 12 pm, 3 pm and 6 pm) for a month. Abundance of bees, number of visited flowers and foraging time per flower were recorded, as well as primary weather data. In the second part of the study, the authors monitored seven apiaries (commercial) for the risk of pesticide exposure. The authors studied colonies' fitness, flight activity and behaviour (aggressive/gentle) and productivity. In addition, pesticide residue analysis was done on samples of bees from apiaries that showed signs of poisoning in the form of dead bees found outside of hives.

The authors report that more bees were foraging during the main flowering period; they visited fewer flowers during a foraging trip and spent more time visiting individual flowers. Whether differences are significant is difficult to determine as the authors did not analyse this, similar to the influence of the weather conditions (temperature, humidity) was not statistically assessed to reveal their influence on the foraging behaviour of bees. The authors leave us with three convoluted figures that contain all information at once, whether important or not. Overall, this experiment was conducted once; there are no replicates and no information on which parts of the runner bean plantation were monitored (core area vs.

edges will most likely reveal different results). Thus, this was an assessment of data without any proper research design. In addition, a statistical data analysis is needed to make these data, which might have some value, useful. It is also not understandable why the foraging was not assessed early in the morning, say at 6-7 am, as honeybees are known to start foraging very early (Abou-Shaara 2014). Furthermore, the authors state that, within the flight range of the bees, just one garden with ornamental crops was present, but otherwise, no other attractive flowering plants could have influenced the bees. This is astonishing to read as the authors do not present an analysis of the same. Honeybees fly 2 km foraging for nectar (Abou-Shaara 2014), which means they use an area of approx. 12.5 km^2. As two apiaries were present here, the area is most likely larger. It is possible to assess this using a large workforce or remote sensing tools like geoinformatics.

Of the seven apiaries that were used to assess pesticide exposure, two were so close that they cannot be considered independent data points. Thus, the authors monitored six apiaries; two showed dead bees, which were taken as a sign of pesticide poisoning. These two apiaries also showed increased aggressiveness, as the authors stated: "*The aggressiveness of surviving honeybees towards siblings and depopulation was also recorded from the bees of Site 4 and Site 7*". The depopulation was recorded by checking the number of occupied frames within the colonies. This method is not recommended. Instead, a proper assessment of the colony population (adult bees, capped brood, open brood) should be done using the Liebefelder method as outlined in the Bee Book, the gold standard for bee research (Delaplane et al. 2013). A statistical data analysis has not been performed. However, data for aggressiveness and colony fitness were assessed on an apiary level and not a colony level and, thus, most likely will not show any meaningful statistical differences.

Why diseases and pests of bees could not have caused depopulation and dead bees is unclear. The authors have not considered this and did not perform a rigorous check of the colonies for diseases. This is a significant shortcoming. The affected apiaries showed increased aggressiveness in subsequent weeks, but mortality was reduced. Bees from the apiaries showing signs of poisoning were collected, although it is unknown what kind of bees were collected: dead or alive, from one or all colonies, how many per colony and at what time point after detection of poisoning. Colonies placed on apiaries that showed no signs of poisoning were not sampled and analysed for pesticide residues. This does not allow us to draw any conclusions about background levels of pesticides. Due to the lack of an analysis of pests and pathogens, it is also impossible to understand whether detrimental effects were caused by the adverse synergistic effects of different stressors affecting the colonies.

Honey was harvested from all apiaries, except those showing signs of poisoning. Why the potentially poisoned apiaries were left out is unclear. Such an analysis could have shown whether putative poisoning affects productivity. Furthermore, an excellent scientific study would have taken samples of pollen, nectar and honey for pesticide residue analysis to understand whether it is possible to trace back the source of the pesticide. In addition, it would have told us about the relationship between pesticide residues found in bees and those found in the product. The authors have not followed best practices for answering scientific questions or testing hypotheses. Instead, they followed the guidelines of government authorities. However, science should inform the latter, not *vice versa*.

The authors did not replicate the pesticide residue analysis; neither biological nor technical replicates were used. Most likely, although not mentioned in the manuscript, pesticide residue analysis was done on pooled samples of approximately 50 bees. Several pesticides showed levels above the level of quantification (LOQ) and were considered for hazard quotient analysis. However, seven out of 200 pesticides showed quantifiable traces. The authors calculated the expanded uncertainty as a measure of variance as they lack replicates. However, as it is not explained how it was calculated, it is useless. If taken into consideration, only one pesticide (Azoxystrobin) can be detected with certainty, while five others are precisely at or below their LOQ and for one (p,p'-DDT), no LOQ was given. This is why replicate measurements are helpful, as they help improve the precision and accuracy of estimates.

Thus, the pesticide part of the study has no value as replicates were missing, necessary samples were not analysed and other stressors that might impact bees negatively or in synergy with pesticide exposure were not assessed. The source of the pesticide could not be evaluated. However, this is simple as pollen collected by foraging bees can be analysed for pesticide residues and palynological analysis of pollen can help trace back the botanical origin (Koech et al. 2023).

This study should not have been published as it undermines the efforts of many other researchers who develop testable hypotheses and corresponding experimental designs for testing the hypotheses, using statistical data analysis to measure factors in a known multifactorial setup. This is done to understand threats to pollinators and ecosystem services better so that helpful mitigation strategies and policies can be developed to implement pollinator protection to ensure the delivery of the ecosystem service of pollination. Integrity in science is based on good scientific practices and reproduceability amongst them (Diaba-Nuhoho and Amponsah-Offeh 2014). Poor science will not lead to optimal interventions, undermining the work of many others and finally setting pollinators and pollination at risk.

Conflicts of interest

The authors have declared that no competing interests exist.

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