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REVIEW OF THE DOCTORAL DISSERTATION
Jaya Sravanthi Mokkapati " Toxicokinetics and toxicity of plant protection products in the solitary red mason bee, *Osmia bicornis* (Hymenoptera: Megachilidae)" prepared under the supervision of prof. dr hab. Ryszard Laskowski

The basis for issuing this opinion is the letter of prof. dr hab. Andrzej Kozik - Chairman of the Biological Sciences Discipline Council of the Jagiellonian University in Krakow on June 26, 2021.

Jaya Sravanthi Mokkapati presented a doctoral dissertation for evaluation, which has a non-standard layout and consists of 6 chapters preceded by summaries in Polish and English. Chapters 2-5 are prepared like typical publications, and each includes: Introduction, Material and Methods, Results, Discussion and Conclusion.

In Chapter 1 - General Introduction, the PhD student discussed in detail the functions of pollinators and the economic value of their activities. She emphasised the fact that maintaining bee biodiversity and abundance is the key factor for global food security because without pollinators many plants will stop producing fruits, which in extreme events can bring famine, at least on a local scale, and in more moderate cases will lead to dramatic increases in food production costs, and, as a result, in food prices. Moreover, she presented the factors, including pesticides, influencing the depopulation of pollinators. Furthermore, she presented the biology and life cycle of *Osmia bicornis*. The chapter entitled "General Introduction" was completed by the PhD student with the aim and objectives of the research. This section seems to be one of the most important reference points in assessing the scientific value of the work. In my opinion, the goals are logically related, and their empirical verification enabled Jaya Sravanthi Mokkapati to carry out her research tasks. These are very well-written and supported by many items of literature, which increases their value and places them in the research on pollinators to date. It can be concluded that presenting this subchapter in such a way proves a thorough knowledge of the research issues and a good preparation the author did to undertake this research, also in terms of methodology.

In Chapter 2, the PhD student assessed the toxicity of three insecticide-based plant protection products containing chlorpyrifos, cypermethrin and acetamiprid to newly emerged adult female *O. bicornis* through different routes of exposure, namely oral feeding and typical contact. The estimated LC50s at infinite-time were lower than concentrations recommended for field application for Dursban (with chlorpyrifos) in both exposure routes, whereas in the case of Sherpa (with cypermethrin) and Mospilan (with acetamiprid) the values were lower

for oral exposure only. The author observed that regardless of the exposure route, high mortality in less than 24 hours was observed in Dursban-treated bees at a fraction of concentration actually used by farmers in the field, indicating high toxicity of this product to *O. bicornis*. Therefore, some commonly used insecticides may cause unacceptable effects to pollinators even when applied in the field according to recommendations, indicating the urgent need for revising current pesticide usage regulations.

In Chapter 3, Jaya Sravanthi Mokkapati examined the impact of the above-mentioned plant protection products on the development of the red mason bee. She determined larval mortality, body mass, altered larval development (e.g., time to pupation), overwintering mortality, time to emergence of adults or overall failure to emerge. It is worth noting that the PhD student proposed her own method of treating the larvae with insecticides in artificial - unnatural conditions. Based on the observations of the susceptibility of *O. bicornis* larvae to mechanical stress during transfer from native pollen to pollen in experimental tubes, three-day old larvae were found to well withstand the procedure. This method can be successfully used by researchers when testing the influence of various compounds on the development of *O. bicornis*. The PhD student used pellets of multi-floral pollen collected by honeybees in her experiment. My question is: Why was it not used in the experience with pollen from the bees nesting cells? In Discussion, the author wrote: "insecticides usually influence neuro-molecular interactions by altering neurohormonal secretions. For example, α-cypermethrin induced hypo-glycaemic and hypotrehalosaemic responses with decreased ATPase and acetylcholinesterase activities in emerging honeybees". Could the PhD student explain these neuro-molecular interactions in the context of biochemical changes in insects?

It is worth mentioning that the results presented in Chapter 2 had already been published in the *Polish Journal of Environmental Studies* (IF_{2020} 1.487), and those in Chapter 3 in *Science of the Total Environment* (IF_{2020} 7.963). This increases the scientific value of the doctorate even more. These publications were written by three or four authors, and the doctoral student is the first and corresponding author. This proves the significant contribution of the doctoral student to the creation of these publications and the dissertation at the same time. Having two publications published in journals with a very high Impact Factor, the PhD student could prepare, without much effort, a mixed-style dissertation, which is acceptable and well-regarded in Poland.

In Chapter 4, Jaya Sravanthi Mokkapati studied the toxicokinetics of chlorpyrifos (as Dursban 480 EC), cypermethrin (Sherpa 100 EC), and acetamiprid (Mospilan 20 SP) in *O. bicornis* females at sublethal concentrations (near LC20s). The toxicokinetics of the insecticides was analysed in bees continuously exposed to insecticide-contaminated food in the uptake phase followed by feeding with clean food in the decontamination phase. In Introduction, the PhD student wrote: "If an organism is exposed continuously for a prolonged time to trace dietary residues, the level of an insecticide and/or its metabolite can build up over time in its tissues and may become lethal or cause non-lethal yet biologically important negative effects". Could the PhD student give examples of these biologically important negative effects? In this Chapter, the student, comparing the concentrations of parent compounds and their metabolites from using the toxicokinetic model, revealed differences in metabolism of the insecticides. These parameters are important for further studies of

toxicodynamics for full understanding of the toxic effects induced by insecticides and organismal recovery. If the assimilation and elimination rate constants are known for an insecticide, the time course of toxicant effects and organism recovery can be simulated to assess and certain physiological and biochemical processes, what the doctoral student did given the experience described in Chapter 5.

Jaya Sravanthi Mokkapati, in Chapter 5, showed that these three tested insecticides altered the energetic budget of *O. bicornis* by the deprivation of energy derived from lipids and carbohydrates (but not proteins) and/or a decrease in respiration based on metabolic rate (energy consumption) compared to the controls. As most of the insecticides impair functioning of the nervous system, the PhD student determined the activity of acetylcholinesterase as considered to be a good biomarker for sub-lethal insecticidal effects in pollinators. Moreover, she determined the glutathione-S-transferase (GST) activities, as enzymes involved in the detoxification of pollutants, secondary metabolites and insecticides, as well as in the protection against oxidative stress. Jaya Sravanthi Mokkapati showed, by the decrease of metabolic rate and total energy available (derived from carbohydrates, lipids, and proteins) in the solitary bee when exposed orally to the insecticide formulations containing acetamiprid, cypermethrin or chlorpyrifos at sub-lethal concentrations. Moreover, she presented that the deprivation of total energy and its source compounds (specifically carbohydrates and lipids) may result in decreased longevity, long-term memory impairment, locomotor displacements, fecundity, and/or immune suppression in bees.

In the last chapter (Chapter 6) - General Discussion and Conclusion, the PhD student compared and discussed the results of her experiences with other authors at length. The conclusions seem to be one of the most important subchapters, as they summarise the results to date and set the directions for future research in several points. The last paragraph of this section is particularly important because it indicates the practical aspect of the doctoral student's research and also indicates the beneficiaries who should be interested in them.

It is worth emphasising that the results for this dissertation were financed from: 1) the OPUS 10 research project (2015/19/B/NZ8/01939; entitled "Beneficial insects in agricultural landscape: modelling effect of pesticides and landscape structure") of the National Science Centre (NCN) in Poland in which the Promoter was the Principal Investigator and 2) from the sources DS grant of the Jagiellonian University (DS/MND/WB/INoS/5/2018; entitled "Sub-lethal effects of pesticides in adults of the solitary bee *Osmia bicornis* - Metabolic and biochemical biomarkers"), in which the PhD student was the Principal Investigator. I consider this a distinction and prestige for such a young person who is just starting her scientific career. This also indicates an in-depth knowledge of the issues and "up-to-date" topics as well as a good methodological / analytical preparation.

Additional positive aspects of the dissertation that should be emphasised are:

- exceptional thoroughness in the presentation of figures and tables. All of them are legible, mostly coloured and with very well-prepared captions and descriptions,
- inserting a graphical abstract under each abstract in Chapters 2-5, which is an interesting form of illustrating the experiences,
- very large sample size / database,
- the use of many techniques and research methods from various branches of biology, mainly toxicology, biochemistry, physiology and ecology,

- a multi-faceted and comprehensive approach to the poisoning of solitary bees by pesticides that enables a more comprehensive risk assessment, more effective pesticide regulation, and pollinator protection,
- a very large number of used / cited publications - as many as 280 items, which proves a thorough knowledge of the literature, mainly that published after 2010. Undoubtedly, this increases the value of the dissertation and proves that the choice of the topic fits the trends of modern literature.

Critical comments include the repetition of information and sentences (e.g., descriptions of insecticides) in individual chapters (especially 2-5), which are the result of the dissertation system adopted by the PhD student. In addition, I would recommend putting the entire database and all analyses from Chapters 2-5 together in one whole / separate section, so that the reader has a picture of the workload that was put into carrying out this dissertation. With such complex experiments, I encourage the doctoral student to make a summary table / figure that will show the real number of analysed bees.

The above-mentioned comments and suggestions do not diminish the merits of this dissertation and a very high evaluation that I place with full responsibility for the doctoral dissertation of Jaya Sravanthi Mokkapati.

In the light of all the above arguments, I state that the doctoral dissertation by Jaya Sravanthi Mokkapati presented to me for review meets the requirements for doctoral dissertations specified in the Act on Academic Degrees and Academic Title and Degrees and Title in Art of March 14, 2003. (Journal of Laws of 2003, No. 65, item 595, as amended). In view of the above, I am asking the High Discipline Council of Biological Sciences of the Jagiellonian University in Krakow to admit Jaya Sravanthi Mokkapati to further stages of the doctoral dissertation.

The dissertation is characterised by high cognitive value, innovative character and a wide scope of research. This proves the PhD student's great scientific and research independence, freedom of movement in physiological, biochemical, toxicological, evolution, ecological and analytical issues, as well as her diligence and research reliability.

Considering the above, I submit an application to the High Discipline Council of Biological Sciences of the Jagiellonian University in Krakow to award this doctoral dissertation with an appropriate award.

Aneta Strachecka