Martina Gáliková, PhD
Institute of Zoology
Slovak Academy of Sciences
Dúbravská cesta 9
845 06 Bratislava
Slovakia

Referee's report on PhD Dissertation Thesis

Thesis: Effect of the entomopathogenic fungus *Isaria fumosorosea* on physiological processes in insects

By: Umesh Kumar Gautam

School of Doctoral Studies in Biological Sciences, University of South Bohemia in České Budějovice, Faculty of Science

The article-based doctoral thesis investigated processes associated with the infection of insects by entomopathogenic fungus *Isaria fumosorosea.* The author used two models – the firebug *Pyrrhocoris apterus* and the cockroach *Periplaneta americana,* and focused on the role of two factors involved in the host response: Adipokinetic hormone (AKH) and vitellogenins. Altogether, the thesis is well written, clear, interesting, and undoubtedly contributes to the research field. The thesis encompasses three highly interesting publications; the PhD candidate is the first author on one of them. All three manuscripts have been published in respected peer-reviewed journals. I will thus focus in this report on the remaining parts of the thesis (Introduction, Results, Objectives, Conclusions), which provide an opportunity to demonstrate the knowledge of the field, scientific independence and analytical thinking of the PhD candidate.

Introduction to the thesis is clear, understandable, and well written. Although I am not an expert in this field, I especially appreciate the comprehensive introduction to the life cycle and pathogenicity of entomopathogenic fungi and its usage in pest control.

Similarly, the last chapters on the role of AKH in the host defence, oxidative stress and digestive processes provide an excellent introduction to the publications included in the thesis.

Nevertheless, the Introduction also encompasses somehow weaker chapters - those on the roles of insect hormones and neuropeptides in the stress response. On one hand, it is great that the author tries to give a broader overview of the topic, but these chapters are somewhat superficial. The rationale behind including the selected list of neurohormones in the Table 2 Chapter 1.2.2 is not clear – the chapter is called 'Insect defence reactions against stress', but how the functions listed in the table relate to the stress response is not apparent (without knowing the literature). The author lists here few insect neuropeptides and mentions some of their functions, however, without giving proper information on the exact species he writes about. It is important to note that the functions of some of these neuropeptides are species- and ontogenetic stage-specific. It would be advisable to use proper names of the neuropeptides, not just generic names describing their function. For example, the author uses terms such as 'cardio peptides', 'hyperglycemic hormone', 'hypoglycemic hormone', etc. In many insects, these hormones have their proper scientific names that are worth mentioning. When listing a selection of insect neuropeptides, I recommend listing individual neuropeptides with their appropriate names, functions, insect species where the function was tested, and references. There is a tremendous amount of recent literature on this topic – it is therefore pity that the author cites in this table only papers that are over ten years old. Thus, information on many recently characterized neuropeptides related to the stress response is missing. Alternatively, one could have focused only on the two insect species used in the included publications.

Table 3, listing functions of AKH, is unfortunately rather superficial as well. As in Table 2, the author ignores recent and 'classical' literature, insect specificity, etc. For example, the author includes some of the key function of AKH, such as its roles in lipid,

carbohydrate and proline mobilization, however, does not provide any references – instead, in the reference fields, inserts the symbols '-'. These functions of AKH were described over 40 years ago (AKH as a hyperlipidaemic hormone in locusts - e.g. Beenakkers 1969, Mayer and Candy 1969; AKH as a hyperglycemic hormone in cockroaches – e.g. Steel 1961; AKH as a hyperprolinemic hormone in the fruit beetle – e.g. Gäde 1991). In addition, several of the functions that the author claims to be AKH-dependent have meanwhile been shown to be AKH independent in some insects, e.g. in *D. melanogaster*. Therefore, when writing about the roles of AKH, one should mention species or insect orders where this function has been described. This could be easily done by including another column between the function and the reference fields.

Several other statements in these introductory chapters lack relevant references, for example, statements where the author claims that most of the secondary metabolites produced by *I. fumosorosea* are considered to be used as natural pesticides (page 5), or when mentioning functions of JHs in processes such as diapause, phase polymorphism, pheromone production, etc. (page 18). There are also some small inconsistencies, e.g. reference Zhu et al., 1986, mentioned on page 13, is missing in the list of references. I have a few additional comments on the chapters on hormones and neuropeptides. For example, p.17 – the author claims that ecdysteroids are membrane permeant – however, according to the current literature, ecdysteroids are transported across cell membranes via transporters (Yamanaka et al., 2015; Okamoto et al., 2018). I also cannot entirely agree with the statement that neurohormones are secreted only by the brain, corpora cardiaca and ventral nerve cord (page 18). There are peripheral neurons and cells producing neuropeptides as well, e.g. Inka cells (ETH), lateral bipolar neurons (ITP), endocrine cells of the gut, etc.

Taken together, there is some space for improvements in the introductory parts, but nevertheless, the author provided a nice general overview of insect response to the fungus infection, and to the role of AKH and vitellogenin signalling in this process.

Chapters Objectives, Results and Conclusions are clear and well written, but rather brief (the conclusion section is limited to less than a page). Given the topic and quality of the manuscripts included in the thesis, one could draw much broader conclusions from the results of these three publications. Moreover, this section of the thesis provides a nice opportunity to show analytical thinking, for example, by putting the published results into a broader perspective. I recommend devoting sufficient time to this section during the defence talk. I also encourage the candidate to clarify his contribution to individual publications in more detail during the defence.

Altogether, the thesis is well written and includes interesting publications that significantly contribute to the research field. The thesis also documents author's knowledge of the research area and very good writing skills. Thus, I am convinced that the candidate fulfils all requirements for obtaining a PhD degree. It was my pleasure to read the thesis, and I wish the author all the best in his future scientific career.

Bratislava, 31st of January 2020

Martina Gáliková, PhD.

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Mgr. Pavel Dobeš, Ph.D.
Masaryk University | Faculty of Science
Department of Experimental Biology
Section of Animal Physiology and Immunology
Address: Kamenice 753/5 | 625 00 Brno - Bohunice
Phone: +420 549 49 3419
E-mail: pavel.dobes@mail.muni.cz

The review of Ph.D. thesis *"Effect of the entomopathogenic fungus Isaria fumosorosea on physiological processes in insects"* by Umesh Kumar Gautam, M.Sc.

The doctoral thesis of Umesh K. Gautam focuses on insect immune response against entomopathogenic fungi *Isaria fumosorosea* which is an important biocontrol agent used in agriculture to protect crops against many insect pest species. Results included in the thesis provide a detailed insight into the reaction of insect organism to the fungal infection and brings new knowledge that could be applied to improve the efficacy of fungi-based biocontrol.

The thesis includes 26 pages of review which covers basic aspects of *Isaria* biology, its practical use and especially very well explains mechanisms of insect immunity that participates, or are considered to be participating, in antifungal response. The introduction is written in good English, with a minimum of grammatical errors and has a good continuity. However, some parts of it are taken almost exactly from the publications of which Umesh is author or co-author; for instance, at least a scheme of *Isaria* life cycle or summary of immune response against its infection would be a nice added value which could distinguish the thesis from papers. The introductory part of the thesis is completed with a list of references, objectives of Ph.D. project and to my appreciation also with chapters summarising the results and conclusions of published studies whose full texts follow.

In one publication Umesh is the first author and in other two the co-author. All included articles are published in well respected scientific journals with impact factor and ranked in first or second quartile of their field. Since Umesh participated on experimental work in all these three articles, it is obvious and commendable that he handled a variety of methods that at the end helped to characterise the role of adipokinectic hormone and vitellogenin not only in response to *I. fumosorosea* but also in other types of infection.

According to my opinion, the Ph.D. thesis of Umesh K. Gautam summarises very well the current knowledge of the interaction between *Isaria* and insect hosts and moreover significantly helped to identify the mechanisms of physiological and immune response of insect towards the infection. I fully recommend this thesis for the defence.

Further, I would like the author to answer following questions and refine some of the claims involved in the thesis:

1. At the beginning of the introduction, you mention that entomopathogen is "any microorganism which develops in insects" and you refer to Vega et al., 2008. I believe, this is an error, since not all microorganisms found in insects could cause the infection.

2. Do you think that the same protective mechanisms are applied in response against *Isaria* and other entomopathogenic fungi, such as *Beauveria* or *Metarhizium*, or are there any hints of a specific reaction? I am aware of that the effect of destruxin A produced by *Metarhizium* is well characterised in one of your publication, however, it is not easy to compare it to the effect of *Isaria* which produces many active compounds during the infection.

3. Is it known how the fungal blastospores release produced toxins during an infection? If so, are there any products of insect host that could be used to respond at this stage of infection?

In Brno, 31st January 2020

Dobeš

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Mgr. Pavel Dobeš, Ph.D.
opponent

Zemědělská fakulta
Faculty of Agriculture

Jihočeská univerzita v Českých Budějovicích
University of South Bohemia in České Budějovice

PhD Thesis Review:

Effect of the entomopathogenic fungus *Isaria fumosorosea* on physiological processes in insects

by Umesh Kumar Gautam

As a general synthesis, this thesis stresses the usefulness of combining certain methodological approaches to investigate a role of the adipokinetic hormone and vitellogenin in infections elicited by entomopathogenic fungi and nematode. In order to illustrate this methodological approach, entomopathogenic fungi *Isaria fumosorosea*, *Metarhizium anisopliae*, nematode *Steinernema carpocapsae*, and two insect species (*Periplaneta americana* and *Pyrrhocoris apterus*) were treated as model systems for investigations ranging from investigation of mortality of insects after application of entomopathogenic fungi, analysis of levels of basic nutrients, activity of digestive enzymes, carbon dioxide production, changes of anti-oxidative stress markers, *AKH* gene expression, expression of *Vg* gene and level of vitellogenin proteins. Experimental studies resulted into publication of three paper, in (from the point of view of thesis topic focused on entomology) in prestigious international journals with high IF and quartile ranking between Q1 and Q2.

Overall, the synthesis presented at the beginning of the thesis is very thorough, and demonstrates an impressive depth of understanding of studied topics, analytical and molecular methods utilized in the various investigations. The papers presented are of high quality, were already published in peer-reviewed journals that are international in scope. Thus, I accept the work presented in this thesis as being of sufficient depth and quality for the granting of the PhD degree, and consider it ready for defense.

Specific comments related to each of the (unpublished) sections presented in this thesis appear below:

Katedra Genetiky a speciální produkce rostlinné
Zemědělská fakulta
Jihočeská univerzita v Českých Budějovicích
Na Sádkách 1780, 370 05 České Budějovice

prof. Ing. Vladislav Čurn, Ph.D.
vedoucí katedry
curn@zf.jcu.cz

+420 387 772 934
+420 387 772 935
www.zf.jcu.cz
http://biocentrum.zf.jcu.cz

- Chapter 3 – Objectives of the thesis – I suppose than not only aim of the study but also research hypotheses should be presented.
- Objectives are restricted only to role of AKH a Vg in insects infected by EPF *I. fumosorosea*, but in presented papers results of studies done of different insects and fungal pathogens and nematodes are presented.
- The same mistake is in chapter Conclusion – where are not mentioned all outputs of presented papers.
- On the page 51 is for the first time mentioned EPF *Metarhizium anisopliae*, it should be written in "full name" as in other organisms mentioned here.
- *AKH* gene and *Vg* gene should be written in italics.
- Question – why expression of vitellogenin was studied only in the firebug *Pyrrhocoris apterus* and not in the second model insect species cockroach *Periplaneta americana*? Do you plan to conduct these studies?

prof. Ing. Vladislav Čurn, Ph.D.
Department of Genetics and Crop Production
Faculty of Agriculture, České Budějovice