

## REVIEW

of the Official Opponent **Serhii Stankevych** Candidate of Agricultural Sciences, Associate Professor, Head of the Litvinov Department of Zoology, Entomology, Phytopathology, Integrated Plant Protection and Quarantine for PhD Thesis **Cao Zhishan** «*Biological Control of Distribution Grapholita Molesta Through Immunological Features*», Submitted for a Scientific Degree of a Doctor of Philosophy Field of Study: 20 – Agricultural Sciences and Food Specialty 202 – Plant Protection and Quarantine

**Relevance of the dissertation topic.** The *Grapholitha molesta*, oriental fruit moth has become one of the most destructive pests of rosaceous fruits throughout temperate regions of the world. The larvae not only harm the tender shoots of fruit trees, but also damage the fruits, causing a great loss of economy. Due to the concealment of its larvae, it is difficult to control, and traditional chemical control methods are rarely effective.

In this dissertation, the selection of infection mode and infection concentration of *Beauveria bassiana* were studied through laboratory experiments. In this dissertation, through the study of the molecular mechanism of *B. bassiana* and the molecular study of immune-related genes, all the results laid a foundation for the exploration of new biological control targets of *G. molesta* IPM system and the enhancement of *B. bassiana* for effectively biological control. Finally, the target immune genes were analyzed and functionally identified by a variety of molecular biological methods, and the target genes were silenced successfully by RNAi

interference technology. Nanotechnology-mediated RNAi interference efficiency was significantly improved. The results showed that the mortality rate of larvae infected with *Beauveria bassiana* was significantly higher than that of the control group after immune target gene interference. In addition, the better biopesticides were selected by field determination of 5 commonly used biopesticides.

Therefore, the research direction of Cao Zhishan's thesis provides reliable data support for improving the biological control effect of *B. bassiana*, and provides a new idea for developing new target pesticides, which has important significance in the biological control and comprehensive management of *G. molesta*.

**Connection with scientific programs, topics, plans.** This research was conducted in alignment with the overarching research focus of Sumy National Agrarian University and Henan Institute of Science and Technology within the framework of specific scientific topics: in accordance with the thematic plans of research works of Sumy National Agrarian University in the framework of the topic «Phytopathological monitoring and regulation of harmful organisms in agriculture» (state registration number 0123U104019); Key Scientific and Technological Research Project of Henan Province (NO. 202102110220); Key Scientific and Technological Research Project of Henan Province (NO. 222102110010), and the Graduate Education Innovation Training Base Project of Henan Province in 2021 (107020221005).

**Scientific novelty of the obtained results.**

(1) This dissertation firstly selected the optimal concentration and infection mode of *B. bassiana* on *G. molesta* larvae under laboratory conditions, these results

provided data support for the field control of *G. molesta*.

(2) This dissertation firstly performed the transcriptomics analysis of *G. molesta* larvae after infected by *B. bassiana*. The results made a significant contribution for better understanding the interactions between *G. molesta* and *B. bassiana*. In addition, it provides the comprehensive sequence resources of the immune-related genes of *G. molesta* larvae, presenting valuable information for deeply understanding the molecular mechanism of innate immunity process in *G. molesta* against *B. bassiana*

(3) This dissertation firstly identified the complete sequence of *GmPGRP-SC* gene, *GmBGRP*, *GmSerp1-2* and *GmSerp1-3* are obtained and characterized. They have been submitted to NCBI GenBank respectively, and the GenBank accession number is MW773839, ON055286, OQ359960 and OQ35996.

(4) This dissertation firstly get a good grasp of the occurrence rule of *G. molesta* in Xinxiang area by investigation of the damage of adult and larva in peach orchard. Meanwhile test five different biological pesticides in field.

**Theoretical significance of the dissertation.** The dissertation provides a theoretical generalization and a new solution to the scientific task of improving the biological control of *G. molesta* by using immune genes as target genes, based on the interaction and immune response of *B. bassiana* and *G. molesta*, the effects of *B. bassiana* on the infection concentration and infection mode of *G. molesta*, the immune response of *G. molesta* larvae to *B. bassiana* and the molecular identification and functional analysis of immune-related genes of *G. molesta*, and the investigation on the occurrence and dynamics of *G. molesta* in peach orchard

and evaluation on the control of five different biological pesticides.

**Practical significance of the results of the dissertation.** Transcriptome analysis can provide a deeper understanding of the immune interaction between entomogenous fungi and insects. It laid a foundation for the better use of entomogenous fungi for biological control of pests. Both RNAi technology and nanoparticle mediated termination are advanced and have broad application prospects in the biological control of pests, providing support for the screening of effective target genes and the development of new biological pesticides. All results could contribute to a better understanding of the resistance mechanism of *B. bassiana* on *G. molesta* and provided a foundation for designing the IPM strategy for the effectively controlling *G. molesta* in the field.

**Number of scientific publications.** Based on the results, a total of 10 academic articles have been published, including 1 Web of Science Core Collection, 4 Ukrainian professional journals, 5 other conference papers.

**Complete presentation of the dissertation material in scientific publications.** The main material of the dissertation is fully presented in 5 articles published in scientific publications and 5 papers published in international academic conferences with the applicant as the first or corresponding author. The published works effectively reflect and confirm the results of the studies conducted.

**The degree of validity of scientific provisions.** This dissertation is a detailed study independently completed by the applicant, aiming to provide a more effective solution for the biological control of the *Grapholita Molesta*. Taken together, these fully demonstrate the validity of the applicant's compliance with the scientific

provisions of the resolutions of the Cabinet of Ministers of Ukraine (Resolution 283 of April 3, 2019, Resolution 502 of May 19, 2023).

**The structure and content of the dissertation, its completeness and compliance with the established requirements for design.**

The dissertation consists of 5 sections, including Introduction: Section 1 – Biological Control of Distribution *Grapholita Molesta* (Literature Review); Section 2 – Materials and Methods; Section 3 – Laboratory Evaluation of the Effect of *Beauveria Bassiana* on the Vital Activity of *G. Molesta*; Section 4 – Characterization and Functional Analysis of Gene: *Gmpgrp*; *Gmbgrp*; *Gmserpin-2* And *Gmserpin-3*; Section 5 – Investigation on the Occurrence and Dynamics of *G. Molesta* in Peach Orchard and Evaluation on the Control of Five Different Biological Pesticides.

The structure of the dissertation is reasonable and scientific, consistent with standards, clear logical thinking, detailed, systematic and complete. It has a meaningful integrity, consistency and completeness that is entirely consistent with the requirements formulated in the design.

**Discussion clauses, comments and wishes regarding the content.**

There are a few points worth discussing regarding the content of the dissertation:

1. Clause 2.2.8 for Real time PCR, should add repetition times of sample and one-way ANOVA method.
2. Table 3 “Treatment group” text orientation needs to be changed to landscape.

3. Research methods are formed in a general way. It is desirable to indicate the used scientific research methods and to note exactly which phenomena and parameters were studied by each of the methods; to justify the choice of methods that ensure the reliability of the obtained results and conclusions.

4. What was the criterion for selecting biological products for research? It would be desirable to summarize this position.

5. There are typographical and stylistic errors in the text.

The above comments and suggestions are debatable and do not diminish the the scientific and practical value of the work, its relevance and practical significance. I hope that the wishes expressed will contribute to the further scientific growth and improvement of the dissertation.

**General conclusion.** The dissertation work of Jia PeiPei intitled «*Biological Control of Distrbution Grapholita Molesta Ththrough Immunological Features*», which was submitted for defense to the specialized academic council for obtaining the degree of Doctor of Philosophy in the field of knowledge 20 – «Agricultural sciences and food» in the specialty 202 – «Plant Protection and Quarantine» in terms of its relevance, scientific and theoretical level, main results of validity, main provisions and results published in professional publications, and novelty of the setting and practical significance meets the requirements of the order of the Ministry of Education and Science of Ukraine No.40 dated January 12, 2017 «On approval of requirements for registration dissertation» and the Decree of the Cabinet of Ministers of Ukraine dated January 12, 2022 No.44 “On approval of the Procedure for awarding the degree of Doctor of Philosophy and cancellation of the decision of

the one-time specialized academic council of the higher educational institution, scientific institution on awarding the degree of «Doctor of Philosophy» with amendments made according to the Resolution of the Cabinet of Ministers No.341 dated 03.21.2022. The PhD candidate Jia PeiPei deserves to be awarded the scientific degree of Doctor of Philosophy in specialty 202 - «Plant Protection and Quarantine».

Official opponent:  
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