REVIEW

Official reviewer, Vladyslav Kovalenko candidate of agricultural sciences, associate professor

for **Li Fang**'s dissertation work « **Comparative evaluation of different methods of determining pesticide residues in plant products»**, which was submitted for obtaining the scientific degree of Doctor of Philosophy at the one-time special council at Sumy National Agrarian University, branch of knowledge **20 - « Agricultural sciences and food »**, on specialty **202 Plant Protection and Quarantine.**

1. **Relevance of the dissertation topic.**

Pesticides play a crucial role in boosting agricultural productivity and contributing significantly to societal economic gains. However, recent surveys indicate a growing concern over the escalation of pesticide residues in soil and water over the past decade. Crops, irrespective of the judicious use of pesticides, are absorbing and transporting these chemicals, posing a latent threat of surpassing safety standards for pesticide residues. This not only endangers crop quality but also poses severe risks to both ecological systems and human health.

While staple crops like rice and wheat face such challenges, leafy vegetables, characterized by distinct seasonality, economic importance, and a shorter growth cycle, have their unique set of hurdles. The stringent requirements of external environmental conditions, coupled with the prevalence of numerous pests and diseases, result in widespread pesticide application in leafy vegetable production. This, unfortunately, has led to frequent instances of ecological pollution, prompting heightened societal awareness regarding the safe cultivation of leafy vegetables. Understanding the intricacies and patterns of pesticide absorption and transportation in leafy vegetables becomes imperative for effective pesticide pollution control.

Conventional methods for detecting pesticide residues, such as

High-Performance Liquid Chromatography and Gas Chromatography, though effective, are encumbered by expensive equipment, prolonged analysis periods, high technical complexity, and specific detection conditions. In response to these limitations, the development of an analytical method for the swift detection of pesticide residues gains paramount importance. Electrochemical sensor technology emerges as a key player in this realm, offering a rapid, efficient, and sensitive approach. Leveraging the advancements in nanotechnology, researchers are directing their efforts toward constructing electrochemical sensors tailored for pesticide residue detection. These sensors, based on nanomaterial characteristics, aim to provide a solution that is not only effective but also addresses the limitations associated with traditional detection methods. The pursuit of such innovative approaches holds great significance in ensuring the safety and sustainability of agricultural practices in the face of pesticide-related challenges.

This dissertation aimed to investigate variations in pesticide absorption and transfer among different leafy vegetables and to explore the dynamic absorption and accumulation patterns in hydroponic lettuce. Furthermore, the study involved the development of four electrochemical sensors utilizing carbon-based nanomaterials/composites for the detection of three pesticides: carbendazim, imidacloprid, and methyl parathion. The outcomes of this research indicated that electrochemical sensing technology yielded satisfactory results when applied to samples of various vegetables. Additionally, High-Performance Liquid Chromatography (HPLC) was employed to verify the precision of pesticide residue detection through electrochemical methods, confirming that electrochemical sensors can meet the requirements for detecting pesticide residues in real vegetable samples. Moreover, the simplicity of operation and rapid response of electrochemical sensors opens up possibilities for on-site pesticide residue detection in agricultural products.

2. Connection with scientific programs, topics, plans.

The research work was carried out in accordance with the main direction of scientific research of Sumy National Agrarian University and Henan Institute of Science and Technology within the framework of scientific topics: National Key R&D Program of China (No. 2017YFD0301104), Project of Plant Protection Key Discipline of Henan Province (1070202190011005), and Zhongyuan Thousand Tal-ents Program of Henan Province (ZYQR201810142).

3. Scientific novelty of the obtained results.

(1) This research revealed significant disparities in the residue levels of the pesticide IMI across different types of leafy vegetables. By examining IMI residues in various parts of these vegetables, both high and low residue varieties were identified. Additionally, the study established that the absorption, transport, and accumulation of IMI pesticides in vegetables were influenced by the duration of exposure to IMI.

(2) A sensor based on MWCNT-COOH/GCE was used to detect traces of pesticide residues in CBZ. This innovative method makes use of the unique properties of hollow conductive carbon structure in MWCNT and carboxyl functionalization of MWCNT-COOH, and the combination of the two enhances the ability of the sensor to detect CBZ. In order to ensure the accuracy of CBZ detection in vegetable samples, the results obtained by MWCNT-COOH/GCE sensor were cross-verified by high performance liquid chromatography.

(3) A cost-effective method for electrochemical detection of Imidacloprid (IMI) was successfully implemented through the use of the SDPC/GCE sensor. The SDPC, characterized by its 3D interconnected porous structure, was derived from expired soybean as a carbon source. This sensor leveraged the benefits of a highly conductive porous carbon network and a substantial specific surface area, resulting in excellent IMI detection performance. The accuracy

of the IMI detection achieved by the SDPC/GCE sensor was confirmed by validating the results with HPLC.

(4) A highly sensitive electrochemical detection of Methidathion (MP) was accomplished using the SCB@ ZrO_2/GCE sensor. This innovative approach hinged on the exceptional electrical conductivity of SCB nanoparticles, facilitating efficient charge transport. Additionally, ZrO_2 nanoparticles exhibited a strong affinity for the phosphorus groups of MP, enhancing the sensor's ability to accumulate MP. The combination of SCB nanoparticles and ZrO_2 nanoparticles synergistically elevated the MP detection capabilities of the SCB@ ZrO_2/GCE sensor. To ensure the accuracy of MP electrochemical detection, the results obtained from this sensor were cross-validated using HPLC.

(5) Another highly sensitive electrochemical detection method for Methidathion (MP) was successfully developed, this time employing the SJPCS@β-CD/GCE sensor. SJPCS was produced via a hydrothermal method and further modified with β-cyclodextrin (β-CD). SJPCS, characterized by its interconnected porous structure, demonstrated excellent electrical conductivity, robust adsorption properties, and a high specific surface area. Meanwhile, β-CD, with its molecular recognition properties, played a crucial role in ensuring the uniform dispersion of SJPCS and facilitating the recognition and adsorption of MP molecules.

4. Theoretical significance of the dissertation.

The absorption and accumulation rules of pesticide imidacloprid on different varieties of leafy vegetables and the dynamic absorption and transfer rules of imidacloprid in different parts of lettuce plants were investigated through experiments, so as to comprehensively analyze the enrichment and transfer of imidacloprid in leafy vegetables and provide theoretical basis for risk assessment of the harm of residual pesticides in leafy vegetables. And according to the characteristics of different vegetables and pesticide properties to guide the rational application of

agricultural production activities, in order to effectively avoid the occurrence of excessive pesticide residues, for the follow-up study of other species of leafy vegetables to lay the theoretical foundation.

In order to optimize the detection method of pesticide residues, the electrochemical sensing detection method based on carbon-based materials was used in this dissertation to detect three pesticides, imidacloprid, carbendazim and methyl parathion, which provided important theoretical guidance for composite nanomaterials in the field of sensing detection.

5. Practical significance of the results of the dissertation.

Differences in the presence of Imidacloprid residues in various types of leafy vegetables can be attributed to variations in their capacity to absorb and transport this compound. By conducting hydroponic experiments with different leafy vegetable varieties, we were able to preliminarily establish an understanding of the distribution, transfer discrepancies, and patterns of IMI residues within the leafy vegetables and the surrounding water system. This research serves as a fundamental basis for the informed and safe utilization of imidacloprid and other common pesticides in leafy crops.

Leveraging the molecular structure of pesticides and the capabilities of functional nanomaterials and composite electrochemical sensing technology, we developed innovative electrochemical sensors tailored for the swift, highly sensitive, and precise detection of pesticide residues. These sensors were designed to target three specific pesticides: Carbendazim, Imidacloprid, and Methyl Parathion. This technological advancement plays a crucial role in ensuring the safety of plant products by providing a reliable means of detecting and monitoring pesticide residues.

6. Number of scientific publications.

Based on these results, a total of 15 academic articles have been published, including 5 Web of Science Core Collection, 4 Ukrainian

professional journals, 6 other conference papers.

7. Complete presentation of the dissertation material in scientific publications.

The main material of the dissertation is fully presented in 9 articles published in scientific publications and 6 papers published in international academic conferences with the applicant as the first or corresponding author.

1. Fang Li, Runqiang Liu, Volodymyr Dubovyk, Qiwen Ran,Bo Li , etc.. Three-dimensional hierarchical porous carbon coupled with chitosan based electrochemical sensor for sensitive determination of niclosamide, Food Chemistry. 2022,366:130563. (Web of Science Core Collection, Q1). (The applicant participated in research, analysis of the results and writing the article).

2. Fang Li, Runqiang Liu, Volodymyr Dubovyk, Qiwen Ran, etc.. Rapid determination of methyl parathion in vegetables using electrochemical sensor fabricated from biomass-derived and beta-cyclodextrin functionalized porous carbon spheres. Food Chemistry, 2022,384: 132573. (Web of Science Core Collection, Q1). (The applicant participated in research, analysis of the results and writing the article).

3. Runqiang Liu, Bo Li, Fang Li,Volodymyr Dubovyk, etc.. A novel electrochemical sensor based on beta-cyclodextrin functionalized carbon nanosheets@carbon nanotubes for sensitive detection of bactericide carbendazim in apple juice. Food Chemistry. 2022,384:132573. (Web of Science Core Collection, Q1). (The applicant participated in research, analysis of the results, writing the article and as corresponding author).

4. Liu Runqiang, Chang Yuqi, Li Fang, Dubovyk Volodymyr, Li Dongdong, Ran Qiwen, Zhao Hongyuan. Highly sensitive detection of carbendazim in juices based on mung bean-derived porous carbon@ chitosan composite modified electrochemical sensor. Food Chemistry, 2022,133301. (Web of Science Core Collection, Q1). (The applicant participated in research, analysis of the results, writing the article and as

corresponding author).

5. Wang Zhankui, Liu Yunhang, Li Fang, Dubovyk Volodymyr, etc.. Electrochemical sensing platform based on graphitized and carboxylated multi-walled carbon nanotubes decorated with cerium oxide nanoparticles for sensitive detection of methyl parathion. Journal of Materials Research and Technology,2022,19,3738-3748. (Web of Science Core Collection, Q2). (The applicant participated in research, analysis of the results, writing the article and as corresponding author).

6. Li Fang, Dubovyk Volodymyr, Liu Runqiang. Study of mathematical methods and models usage in the pesticide degradation and residue prediction. Bulletin of Sumy National Agrarian University，2019，35-36(1-2):67-71. (The applicant participated in research, analysis of the results and writing the article).

7. Li Fang, Dubovyk Volodymyr, Liu Runqiang. A review of rapid pesticide residues determination in vegetables and fruits. Bulletin of Sumy National Agrarian University.2020,42(4):40-47.(The applicant participated in research, analysis of the results and writing the article).

8. Li Fang, Dubovyk Volodymyr, Liu Runqiang. Rapid Electrochemical Detection of Carbendazim in Vegetables Based on Carboxyl Functionalized Multi-Walled Carbon Nanotubes. Bulletin of Sumy National Agrarian University. 2021,4(46),76-82. (The applicant participated in research, analysis of the results and writing the article).

9. Li Fang. Determination of methyl parathion in vegetables by high performance liquid chromatography. Bulletin of Sumy National Agrarian University. 2022,3(49),3-8. (The applicant participated in research, analysis of the results and writing the article, Sole author).

10.Li Fang, Dubovyk Volodymyr, Liu Runqiang. Progress electrochemical sensor based on carbon nanotubes for pesticide residual detection. The 4th International scientific and practical conference “Fundamental and applied research in the modern world”,2020,11-18~19. (PhD participant in carrying out of experimental research, processing of

results, and writing the article).

11. Li Fang, Dubovyk Volodymyr, Liu Runqiang. Present situation of pesticides uses and pesticides residue problems, Proceedings of the International Scientific and Practical CONFERENCE «HONCHARIVSKI CHYTANNYA» dedicated to the 92th anniversary of Doctor of Agricultural Sciences professor Mykolay Dem'yanovych Honcharov, 2021-5-25. (PhD participant in carrying out of experimental research, processing of results, and writing the article).

12.Li Fang, Dubovyk Volodymyr, Liu Runqiang. The use of pesticides and the hazards caused by pesticide residues. fundamental and applied problems of modern ecology and plant protection, International scientific-practical conference, 2021-10-21~22. (PhD participant in carrying out of experimental research, processing of results, and writing the article).

13. Li Fang, Dubovyk Volodymyr, Liu Runqiang. The principle of gas chromatography and its application in the analysis of pesticide residues. The 2nd International scientific and practical conference “Modern science: innovations and prospects”.2021-11-7~9. (PhD participant in carrying out of experimental research, processing of results, and writing the article).

14. Li Fang, Dubovyk Volodymyr, Liu Runqiang. A review about the application of high performance liquid chromatography in pesticide residue detection. The 4th International scientific and practical conference “Science, innovations and education: problems and prospects”.2021-11-10~12. (PhD participant in carrying out of experimental research, processing of results, and writing the article).

15. Li Fang, Wang Xinfa, Liu Dongmei, Dubovyk Volodymyr. A review of purified materials in QuEChERS pre-treatment method for pesticide residue detection. Proceedings of the International Scientific and Practical CONFERENCE «HONCHARIVSKI CHYTANNYA» dedicated to the 93rd anniversary of Doctor of Agricultural Sciences

professor Mykolay Dem'yanovych Honcharov, 2022-5-25. (PhD participant in carrying out of experimental research, processing of results, and writing the article).

8. **The degree of validity of scientific provisions.**

The research results of this dissertation are not only based on professional scientific research methods, systematic theoretical research, but also through extensive experimental research and test verification, and the actual fruit and vegetable samples were tested. The validity of scientific regulations is fully reflected in published scientific publications.

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

The structure and logic of the dissertation are clear, the construction is reasonable, the content is prominent, concise and clear, and the system is complete. It can fully demonstrate the work tasks and main achievements of the research and can reflect the entire research process. The structure, content and completeness of the dissertation fully comply with the established design requirements.

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

Due to various influences, chemical pesticides are still widely applied in agricultural production. Chemical pesticides are continuously transmitted and accumulated in inorganic environment and living organisms through the food chain, which seriously endangers human health and ecological stability. Therefore, reducing pesticide residues and accelerating the degradation of pesticides have become the primary task in agricultural development. In the future research, the substances that can decompose, volatilize and transfer pesticides in leafy vegetable plants can be deeply studied, and the improvement of pesticide residue detection technology can guarantee the safety supervision of agricultural products.

General conclusion

Li Fang's dissertation on the topic: «Comparative evaluation of different methods of determining pesticide residues in plant products», which is presented for obtaining a doctor of philosophy, is the independent study containing scientifically based results in the field of 202-Plant Protection and Quarantine. In terms of content and formal features, the dissertation meets the requirements for the design of dissertations and the Procedure for awarding the degree of Doctor of Philosophy approved by the Resolution of the Cabinet of Ministers of Ukraine dated 12.01.2022 No. 44, which cancels the previous orders of the Ministry of Education and Culture of Ukraine dated January 12, 2017 No. 40 and Ministry of Education and Culture of Ukraine dated May 31, 2019 No. 759 with changes and additions.

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