REVIEW

Official reviewer, **Andrii Butenko**

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 »**, on specialty **202 and food Plant Protection and Quarantine.**

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

Pesticides serve as a vital tool in enhancing agricultural productivity and contributing significantly to economic gains in society. However, recent surveys highlight a growing concern regarding the increasing levels of pesticide residues found over the past decade. Even when pesticides are used judiciously, crops are absorbing and transporting these chemicals, thereby presenting a latent threat of exceeding safety standards for pesticide residues. This not only jeopardizes the quality of crops but also poses serious risks to ecological systems and human health.

Leafy vegetables, distinguished by their distinct seasonality, economic significance, and shorter growth cycle, face a unique set of obstacles. The demanding environmental conditions these crops require, coupled with the prevalence of various pests and diseases, have led to extensive pesticide use in leafy vegetable cultivation. Unfortunately, this has resulted in frequent instances of ecological pollution, prompting a heightened societal awareness of the importance of safe leafy vegetable production. It's vital for effective control of pesticide pollution to research the intricate processes of pesticide absorption and transportation in leafy vegetables.

Conventional methods for detecting pesticide residues, such as High-Performance Liquid Chromatography and Gas Chromatography,

while effective, are hindered by the need for expensive equipment, lengthy analysis periods, high technical complexity, and specific detection conditions. Given these limitations, the development of an analytical method for the rapid detection of pesticide residues becomes of paramount importance. Electrochemical sensor technology emerges as a key player in this field, offering a swift, efficient, and highly sensitive approach to address these challenges.

Utilizing the advancements in nanotechnology, researchers are actively focusing their efforts on the development of specialized electrochemical sensors designed for the precise detection of pesticide residues. These cutting-edge sensors, which leverage the unique properties of nanomaterials, are intended to not only enhance detection effectiveness but also overcome the limitations associated with conventional detection techniques. The pursuit of such innovative approaches is of paramount importance in ensuring the safety and sustainability of agricultural practices, especially in light of the challenges posed by pesticide usage.

The primary objective of this dissertation was to investigate the variances in pesticide absorption and transfer among different types of leafy vegetables. Additionally, the study aimed to explore the dynamic patterns of pesticide absorption and accumulation in hydroponically grown lettuce. Furthermore, this research project encompassed the development and evaluation of four distinct electrochemical sensors that make use of carbon-based nanomaterials and composites. These sensors were specifically designed for the detection of three pesticides: carbendazim, imidacloprid, and methyl parathion.

The research findings from this study demonstrated that electrochemical sensing technology yielded satisfactory results when applied to a wide range of vegetable samples. To validate the accuracy of pesticide residue detection through electrochemical methods, High-Performance Liquid Chromatography (HPLC) was employed. The

results of the HPLC analysis confirmed that electrochemical sensors meet the precision requirements for detecting pesticide residues in real vegetable samples. Additionally, the ease of operation and rapid response times associated with electrochemical sensors open up new possibilities for on-site pesticide residue detection in agricultural products.

2. 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: 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 study found significant differences in pesticide IMI residue levels among different types of leafy vegetables. By detecting the IMI residues in different parts of these vegetables, high residue and low residue varieties were determined. In addition, the study also determined that the absorption, transportation, and accumulation of IMI pesticides in vegetables are influenced by the duration of IMI pesticides.

(2) A simple and efficient electrochemical detection of pesticide CBZ was carried out using MWCNT-COOH/GCE sensors. This innovative method utilizes the unique properties of hollow conductive carbon structure and carboxyl functionalization of MWCNT-COOH, combined to synergistically enhance the sensor's ability to detect CBZ. In order to ensure the accuracy of CBZ detection in vegetable samples, high-performance liquid chromatography was used to cross validate the results obtained from the MWCNT COOH/GCE sensor.

(3) An efficient electrochemical detection method for imidacloprid (IMI) was studied using SDPC/GCE sensors. This material uses expired

soybeans as the carbon source and has a three-dimensional interconnected porous structure. This sensing method combines the advantages of high conductivity and large specific surface area of carbon materials, achieving good results in IMI pesticide residue detection. The accuracy of SDPC/GCE sensor in detecting IMI was verified by high-performance liquid chromatography.

(4) By adopting SCB@ZrO_2/GCE sensor achieves high sensitivity electrochemical detection for methyl phosphorus (MP) detection. This innovative method relies on the special conductivity of SCB nanoparticles, promoting effective charge transfer. In addition, ZrO_2 nanoparticles have 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 improves SCB@ZrO_2 /The MP detection capability of GCE sensors. To ensure the accuracy of MP electrochemical detection, cross validation was conducted on the results obtained by the sensor using high-performance liquid chromatography.

(5) The SJPC@β-CD /GCE sensor was establish for high sensitivity detection of MP. SJPCS were prepared by hydrothermal method and modified with β-cyclodextrin (β-CD). SJPCS have good electrical conductivity, good adsorption property and high specific surface area. At the same time, β-CD with molecular recognition properties plays a crucial role in ensuring the uniform dispersion of SJPCS and promoting the recognition and adsorption of MP molecules.

4. Theoretical significance of the dissertation.

Through a series of experiments, we investigated the patterns of pesticide imidacloprid absorption and accumulation in various types of leafy vegetables. Additionally, we examined how imidacloprid is dynamically absorbed and transferred within different parts of lettuce plants. These investigations aimed to provide a comprehensive understanding of how imidacloprid is distributed in leafy vegetables and

to establish a theoretical foundation for assessing the risks associated with pesticide residues in these crops. By considering the unique characteristics of different vegetables and the properties of pesticides, our research seeks to guide the judicious use of agricultural practices, thereby minimizing the occurrence of excessive pesticide residues. This, in turn, serves as a vital groundwork for future studies involving other leafy vegetable species.

To enhance the methodology for detecting pesticide residues, we employed an electrochemical sensing approach based on carbon-based materials in our study. Specifically, we utilized this method to detect three pesticides: imidacloprid, carbendazim, and methyl parathion. The insights gained from this research provide crucial theoretical guidance for the application of composite nanomaterials in the field of sensing and detection.

5. Practical significance of the results of the dissertation.

The residue of imidacloprid in different types of leafy vegetables is different, mainly because of the difference in their ability to absorb and transport the compound. In this dissertation, the distribution, transfer differences and laws of IMI residues in leafy vegetables and surrounding water systems were preliminarily explored through hydroponic experiments on different leafy vegetables varieties, which provided practical guidance for the rational and safe use of common pesticides such as imidacloprid on leafy crops. At the same time, combined with the molecular structure of pesticides, functional nanomaterials and composite electrochemical sensing technology, we developed a new electrochemical sensor for rapid, highly sensitive and accurate detection of pesticide residues. These sensors are designed for three specific pesticides: carbendazim, imidacloprid and methyl parathion. This study provides a reliable method for trace detection of pesticide residues, and plays a vital role in ensuring the safety of plant products.

6. Number of scientific publications.

According to the research results of the dissertation work, 15 articles were published in scientific journals. There were 5 scientific articles indexed by Web of Science Core Collection(Q1,Q2); 4 scientific articles published in Ukrainian scientific professional journal (category “B”) （of which one was sole author），and the remaining 6 publications were international conference papers. Complete presentation of the dissertation material in scientific publications.

7. The degree of validity of scientific provisions.

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

The research results of this dissertation are not only based on professional scientific research methods and systematic theoretical research, but also verified by extensive experimental research and testing, and tested the pesticide residues in actual fruit and vegetable samples. The effectiveness of scientific laws and regulations is fully reflected in published scientific publications.

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

The thesis has clear logic, reasonable structure, prominent content, conciseness and completeness. It can fully display the tasks and main achievements of the research and reflect the whole research process. The structure, content and integrity of the dissertation fully meet the established design requirements.

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

The use of chemical pesticides has a positive effect on the protection of crop diseases and insect pests and improving the output of agricultural products. Therefore, chemical pesticides are still widely used in agricultural production. Chemical pesticides spread and accumulate in inorganic environment and organisms through food chain, which seriously harms human health and ecological stability. Therefore, reducing pesticide residues and accelerating pesticide degradation have

become the primary tasks of agricultural development. In the future research, the substances that can decompose, volatilize and transfer pesticides in leafy plants can be deeply studied and improved.

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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