

REVIEW

of the Official Reviewer **Andrii Butenko** Candidate of Agricultural Sciences,
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for PhD Thesis **Zhu Yinghui**

«Insights into profiling of resistance mechanism of alfalfa to atrazine»,
Submitted for a Scientific Degree of a Doctor of Philosophy Field of Study: 20
– Agricultural Sciences and Food Specialty 202 – Plant Protection and
Quarantine

1. Relevance of the dissertation topic. Alfalfa has important ecological and economic value. Atrazine is one of the commonly used herbicides in corn fields, and its residual time in soil can last for about a year. The toxicity of atrazine residue to alfalfa is a common problem in the corn alfalfa planting model. In Henan, China, corn is an important economic crop, and nearly 100000 acres of alfalfa base have been planted, with one-third of the seedlings being affected by residual atrazine from the previous crop of corn. Therefore, the optimal method to reduce the harm of residual atrazine in soil to alfalfa production is to breed alfalfa varieties that are resistant to atrazine.

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 Academy of Agricultural Sciences within the framework of specific scientific topics: "Research and application of herbicide soil residue pest control technology" (the Key Science and Technology Program of Henan Province, China. Grant Number 221111112300).

3. Scientific novelty of the obtained results. *Practicality of the topic:* This topic closely focused on the urgent problems that need to be solved in current agricultural development, with the combination of agriculture and animal husbandry as the research entry point. Different alfalfa varieties had significant differences in their toxicity to atrazine. It not only solved the problem of alfalfa damage in the corn alfalfa rotation mode, but also improved the yield and quality of alfalfa, which met the requirements of sustainable development.

The comprehensiveness of the experimental design: Through the changed in physiological and biochemical indicators, residual levels, and recording levels of SF and J2 alfalfa under atrazine stress, as well as field experiments, the differences in phenotype and gene changed of SF and J2 alfalfa under different concentrations of atrazine stress were fully understood, ensuring the rationality and accuracy of the experimental results.

The benefits of the research findings: The research results had determined the mechanism of resistance of alfalfa to atrazine, confirmed the effects of atrazine resistant alfalfa varieties in the corn alfalfa rotation mode on agricultural production, such as yield increase and resistance. It increased the yield and quality of alfalfa, and reduced the pollution of large-scale atrazine residues on the environment, making people realize the economic, social, and environmental benefits of choosing alfalfa varieties in corn alfalfa rotation. In the future, the combination of atrazine resistant variety selection and cultivation for rotational planting of alfalfa in corn fields will be increasingly widely applied in achieving high yield and efficient agriculture of alfalfa.

For the first time, a preliminary study was conducted on the resistance mechanism of different alfalfa varieties to atrazine in Henan Province, China. The differences in alfalfa resistance to atrazine were determined, and the reasons for the differences were identified.

It was expanded the scope of alfalfa in the corn alfalfa rotation mode, improved the yield and quality of alfalfa, and provided theoretical support for the selection and cultivation of atrazine resistant alfalfa varieties.

It was improved to provide a theoretical basis for breeding atrazine resistant alfalfa varieties.

4. Theoretical significance of the dissertation. The theoretical significance of this article lies in the screening of resistant SF and sensitive J2 alfalfa based on the research results, which are currently being studied and refined in research projects at Sumy National Agricultural University in Ukraine and

Henan Academy of Agricultural Sciences (Henan Province, China). Studied the resistance mechanism of SF and J2 to atrazine. Conduct differential studies on SF and J2 to provide theoretical basis for the breeding and internal mechanism research of alfalfa varieties resistant to atrazine. In field experiments, it was confirmed that atrazine residues exist in soil, and it was also demonstrated that SF and J2 plants produce atrazine residues, with significant differences between the two.

5. Practical significance of the results of the dissertation. Based on the research results, alfalfa varieties resistant to atrazine were planted, and the toxic relationship between atrazine residues and alfalfa was clarified. The yield and quality of alfalfa were improved, and the soil pollution environment was improved. At the same time, the introduction of excellent varieties, the knockout of genes sensitive to atrazine, and continuous improvement of planting techniques have increased the yield and quality of alfalfa, reduced atrazine environmental pollution, and contributed to the sustainable development of agriculture. It has also played an important role in improving the ecological environment and alleviating the shortage of high-quality domestic forage. This is being studied and improved in the scientific project of the Henan Academy of Agricultural Sciences (Henan, China).

6. Number of scientific publications. Based on the results, a total of 7 academic papers were published, including 1 indexed by Scopus core database, 3 in Ukrainian professional journals, and 3 in conference proceedings.

1. **Zhu Y.**, Rozhkova T. Study on toxicological effects of herbicide atrazine on alfalfa seedlings. Bulletin of Sumy National Agrarian University. The Series: Agronomy and Biology. 2023. Vol. 53. Pp. 3-8. DOI: <https://doi.org/10.32782/agrobio.2023.3.1>

2. **Zhu Y.**, Rozhkova T.O. Determination of alfalfa resistance to atrazine. Bulletin of Sumy National Agrarian University. The series: Agronomy and Biology. 2024. Vol. 57 (3). Pp. 12-17. DOI: 10.32782/agrobio.2024.3.2

3. **Zhu Y.**, Rozhkova T.O. Determination of atrazine residues in alfalfa plant matrix by ultra-high performance liquid chromatography and tandem mass spectrometry. *Quarantine and Plant Protection*. 2024. № 4 (279). Pp. 40-44. DOI: 10.36495/2312-0614.2024.4.40-44

4. **Zhu Y. H.**, Rozhkova T. Phytotoxicity response of lucern to herbicide atrazine in soil. *Ecological Engineering & Environmental Technology*. 2024. Vol. 25. Pp. 344-351. DOI: 10.12912/27197050/187773

5. **Zhu Y.**, Rozhkova T., Zhu H. Common weed species in wheat fields in Henan province, China. *Fundamental and applied problems of modern ecology and plant protection. Materials of the International Scientific and Practical Conference dedicated to the 100th anniversary of the birth of doctor of biological sciences, professor B.M. Lytvynov (Kharkov, October 21-22, 2021)*. Kharkiv: Ivanchenko I. Publishing House. P. 57-59.

6. **Zhu Y.**, Rozhkova T. Research progress of atrazine herbicide residues. *Materials of the International Scientific and Practical Conference dedicated to the anniversary dates of the birth of outstanding scientists-phytopathologists, Doctors of Biological Sciences, Professors V. K. Panteleyev and M. M. Rodigin (Kharkiv, October 20-21, 2022)*. Kharkiv: 2022. P. 241-243.

7. **Zhu Y.** Rozhkova T.O. Transcript analysis and study of atrazine residues of alfalfa varieties SF8001 and Juneng2. The effectiveness of agricultural technologies in the Polissya zone of Ukraine: materials of the IV All-Ukrainian Scientific and Practical Conference (November 13-14, 2024). Zhytomyr, 2024. Pp. 36-38.

7. Comments and wishes on the content of the dissertation work. Positively assessing the dissertation work of Zhu Yinghui, its important scientific, theoretical and practical significance, the novelty and relevance of the research, it is necessary to dwell on the following comments and wishes:

1. It would be desirable to consider in more detail in the literature review the issue of the negative impact of atrazine on agricultural crops.

2. In our opinion, it is necessary to explain why 60 varieties of alfalfa were selected for analysis for their resistance to atrazine. What was the reason for the choice of these varieties.

3. Errors in the design of literary sources were noted - 36, 56 and 64.

8. General conclusion. The dissertation work of Zhu Yinghui intitled «Insights into profiling of resistance mechanism of alfalfa to atrazine», 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 Zhu Yinghui deserves to be awarded the scientific degree of Doctor of Philosophy in specialty 202 - “Plant Protection and Quarantine”.

Official Reviewer:

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