

REVIEW

of the Official Reviewer **Andrii Butenko** Candidate of Agricultural Sciences, Associate Professor of the Department of Agricultural Technology and Soil Science for PhD Thesis **Zhu Hongxia** «Biocontrol mechanisms of *Streptomyces* spp. HU2014 against *Rhizoctonia* spp. caused wheat sharp eyespot», 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. Wheat (*Triticum aestivum* L.) is an essential global crop. Soil-borne pathogens can cause extensive damage to wheat by limiting water and nutrient uptake or by reducing the quantity and quality of the grain. Wheat sharp eyespot (WSE) is a soil-borne disease that affects almost all temperate wheat-growing regions worldwide. In China, it is common in several main provinces for wheat production, such as Jiangsu, Anhui, Henan, Shandong, Shaanxi and Hubei, with the wheat losses from 10% to 40% totally. This disease is caused primarily by the soil-borne fungus *Rhizoctonia* spp. The most effective method of controlling the disease is the cultivation of resistant varieties, but the use of pesticides is common in China. An alternative method of wheat protection is biological, which includes application *Streptomyces* spp.

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: "Optimization of fermentation conditions of *Streptomyces* sp. HU2014 and study on antibacterial active substances" (the Key Science and Technology Program of Henan Province, China. Grant Number 162102210106), "Study on the active components and antibacterial lead compounds from the extracts of *Carpesium abrotanoides* L. (Asteraceae)

against phytopathogenic fungi" (the Key Science and Technology Program of Henan Province, China. Grant Number 212102110148), and "Study and application of a beneficial *Streptomyces* strain in disease prevention and growth promotion of wheat" (the Key Science and Technology Program of Henan Province, China. Grant number 232102111015).

3. Scientific novelty of the obtained results. 1) The author of the dissertation for the first time a new strain of *Streptomyces* sp. HU2014 was isolated and identified; effective antifungal effect of strain HU2014 against pathogens of wheat rhizoctoniosis has been proven; the mechanisms of induced resistance of wheat by treating *Streptomyces* sp. plants were studied. HU2014 proved that *Streptomyces* sp. HU2014 promotes the growth of wheat by stimulating growth processes, improving soil nutrition of plants by changing the microbial structure of the rhizosphere; optimal cultivation conditions of HU2014 were determined.

2) The improvement of biological protection of wheat by using a new strain of *Streptomyces* sp. HU2014, which will expand the range of existing bioagents.

4. Theoretical significance of the dissertation. The theoretical significance of the dissertation lies in the following: based on the results of the research, a novel strain, HU2014 was found, which is being researched and refined in scientific programs at Sumy National Agrarian University and Henan Institute of Science and Technology (Xinxiang, China). The mechanism of HU2014 against WSE was conducted. The strain showed a

strong antifungal activity against WSE caused by *R. solani* YL-3 and *R. cerealis* G11, as well as the ability of promoting wheat growth. Additionally, the optimization of fermentation process provided a basis

for the development and application of HU2014 as biocontrol agent. In field experiments, the abilities of HU2014 to suppress *R. cerealis* G11 and promote wheat growth were verified.

5. Practical significance of the results of the dissertation. The practical significance of the research results in this paper is to provide comprehensive technical suggestions, complete experimental programs and improvement measures for the biological control of *Rhizoctonia cerealis* Van der Hoeven and *R. solani* Kuhn. Research results show that a new strain HU2014 has been identified, which has proven to be effective in protecting wheat against *Rhizoctonia* spp. and can be used for the production of new biofungicides. The studied strain of *Streptomyces* sp. HU2014 showed a phytotoxic effect on seed germination by the green mouse, so it is promising in the fight against segetal vegetation. The stimulating effect of the new strain on the growth and development of wheat plants due to the improvement of soil nutrition and plant metabolism has been proven. The obtained results are included in the training programs of bachelors in the specialty 202 "Protection and quarantine of plant at the Sumy National Agrarian University.

6. Number of scientific publications. Based on the results, a total of 13 academic papers were published, including 2 indexed by Scopus or WoS core database, 4 in Ukrainian professional journals, and 7 in conference proceedings.

(1). Zhu H., Zhou F., Rozhkova T. Quantitative changes of enzyme activity in wheat induced by *Streptomyces* sp. strain HU2014. Bulletin of Sumy National Agrarian University. The series: Agronomy and Biology. 2020. Vol. 3. P. 57-62. DOI: 10.32845/agrobio.2020.3.7.

(2). Zhu H., Cao Z., Rozhkova T., Hu L. Study on antifungal activity of hyphae extract of a *Streptomyces* strain HU2014 against four phytopathogenic fungi. Bulletin of Sumy National Agrarian University.

The series: Agronomy and Biology. 2021. Vol. 3. P. 87-92. DOI: 10.32845/agrobio.2021.3.11.

(3). Zhu H., Rozhkova T. Induction of wheat resistance by *Streptomyces* sp. HU2014 strain. Quarantine and Plant Protection. 2023. P. 38-43. DOI: 10.36495/2312-0614.2023.1.38-43.

(4).Zhu H., Rozhkova T. Allelopathic effect of metabolites produced by *Streptomyces* sp. HU2014 on wheat and green bristlegrass. Bulletin of Sumy National Agrarian University. The series: Agronomy and Biology. 2023. Vol. 50. P. 8-13. DOI: 10.32845/agrobio.2022.4.2

(5). Zhu H., Hu L., Hu H.-Y., Zhou F., Wang S., Wu L., Rozhkova T., Li C. Identification of a novel *Streptomyces* sp. strain HU2014 showing growth promotion and biocontrol effect against *Rhizoctonia* spp. in wheat. Plant Disease. 2023. P. 1-15. DOI: 10.1094/pdis-06-22-1493-RE.

(6). Zhu H., Hu L., Rozhkova T., Wang X., Li C. Spectrophotometric analysis of bioactive metabolites and fermentation optimisation of *Streptomyces* sp. HU2014 with antifungal potential against *Rhizoctonia solani*. Biotechnology & Biotechnological Equipment. 2023. Vol. 37. P. 231-242. DOI: 10.1080/13102818.2023.2178822.

(7). Zhu H., Rozhkova T., Hu L., Li Ch. Biocontrol of main diseases and advance in antifungal mechanism of *Streptomyces* spp. in wheat. Problems of ecology and ecologically oriented plant protection: materials of the international science and practice conference of the Faculty of Plant Protection of the Kharkiv National Agrarian University named after V.V. Dokuchaeva, October 17-18, 2019. Kharkiv: Madrid Printing House, 2019. P. 122 –124.

(8). Zhu H., Rozhkova T., Li Ch. Interaction between plant and beneficial microorganisms in agriculture. Problems of ecology and ecologically oriented plant protection. Materials of the International scientific and practical conference of plant protection of the Kharkiv National Agrarian

University named after V.V. Dokuchaeva, dedicated to the 130th anniversary of the birth of academician VASGNIL, corresponding member of NASU, doctor of biological sciences, professor and first dean of the faculty T.D. Strahov (October 29-30, 2020). Kharkiv, "Planeta-print", 2020. P. 169-170.

(9). Zhu H., Rozhkova T. O., Zhu Y. The promoted effect of *Streptomyces* sp. in wheat planting. 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 (Kharkiv, October 21-22, 2021). Kharkiv: Ivanchenko I. Publishing House. P. 55–57.

(10). Zhu H., Rozhkova T. O., Zhu Y. 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.

(11). Zhu Hongxia, Wang Xinfu, Rozhkova T. Preliminary study on antifungal activity of a *Streptomyces* sp. strain HU2014 against phytopathogenic fungi. Topical issues of modern science, society and education. Proceedings of the 3rd International scientific and practical conference. SPC – Sci-conf.com.ua. Kharkiv, Ukraine. 2021. Pp. 21–25.

(12). Hongxia Zhu, Yinghui Zhu, Rozhkova T. O. Research progress on active components of metabolites of *Streptomyces* sp. Effectiveness of agricultural technologies of Zhytomyr region: materials of the All-Ukrainian scientific and practical conference (November 10–12, 2021). Zhytomyr: ZHATFK, 2021. P.130-133.

(13).Zhu H., Wang X., Rozhkova T. Study the allelopathy of the fermentation extracts from *Streptomyces* sp. HU2014 on cucumber. «Honcharivski chytannya»: Materials of the International Scientific and Practical Conference dedicated to the 93rd anniversary of the birth of the Doctor of Agricultural Sciences, Professor Mykola Goncharov (May 25, 2022). Sumy, 2022. P. 165-166.

7.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.

8. Discussion clauses, comments and wishes regarding the content. HU2014 could inhibit the normal growth of phytopathogenic fungi through active metabolites, resist the invasion of phytopathogenic fungi by inducing defense enzymes in wheat, and indirectly reduce the disease loss by promoting the growth of wheat. However, there are several problems need to be solved in future: i) The production process and quality control technology of microbial agents not in a mature level; ii) Instability in field application, which to some extent restricts their promotion and application. The next goal is devoted to solving these problems.

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

1. Some language errors and formatting errors need to be polished.
2. It would be important to understand and measure the viability of HU2014 overtime (during the first week for example). If the strain loses

completely its viability (>90%) after 2-3 days, as it happens to many *Streptomyces* strains, the effects observed later in the plants can be associated mostly to other bacteria present in the seeds.

3. Should not it be bacterial suspension of HU2014? Why is it mycelial suspension?

4. The dissertation lacks short conclusions after each chapter.

General conclusion. The dissertation work of Zhu Hongxia intitled «Biocontrol mechanisms of *Streptomyces* spp. HU2014 against *Rhizoctonia* spp. caused wheat sharp eyespot», 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 Hongxia deserves to be awarded the scientific degree of Doctor of Philosophy in specialty 202 - “Plant Protection and Quarantine”.

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