Biol. Stud. 2021; 15(4): 17–24 • DOI: https://doi.org/10.30970/sbi.1504.667
www.http://publications.lnu.edu.ua/journals/index.php/biology

UDC: 579.69+58.04+57.033

ANTIBACTERIAL AND PHYTOTOXIC ACTIVITY OF THE SCHIFF'S BASES OF 5-PHENYL-4-AMINO-3-MERCAPTO-4*H*-1,2,4-TRIAZOLE WITH THE DONOR SUBSTITUENTS IN THE 4th POSITION OF HETEROSYSTEM

N. V. Tkachuk [1], ***V. O. Yanchenko*** [1], ***A. M. Demchenko*** [2]

[1] *T. H. Shevchenko National University "Chernihiv Colehium"*
53 Hetman Polubotok St., 14013 Chernihiv, Ukraine

[2] *Nizhyn Mykola Gogol State University, 2 Grafska St., 16600 Nizhyn, Chernihivska Oblast, Ukraine*

Tkachuk, N. V., Yanchenko, V. O., & Demchenko, A. M. (2021). Antibacterial and phytotoxic activity of the Schiff's bases of 5-phenyl-4-amino-3-mercapto-4*H*-1,2,4-triazole with the donor substituents in the 4th position on heterosystem. *Studia Biologica*, 15(4): 17–24 • DOI: https://doi.org/10.30970/sbi.1504.667

Background. Triazoles and Schiff's bases have a high biological activity. For the practical use of the derivatives, their low toxicity is important. The purpose of this work was to investigate the antibacterial and phytotoxic properties of Schiff's bases of 5-phenyl-4-amino-3-mercapto-4*H*-1,2,4-triazole with donor substituents in the 4th position of heterosystem.

Materials and methods. In the study of antibacterial activity of the derivatives, corrosion-active 4-day association cultures of ammonifying and sulfate-reducing bacteria were used as a test culture of microorganisms. Sensitivity of bacteria to derivatives was determined by diffusion method in agar using sterile paper disks according to the standard method. In the investigation of phytotoxic activity of the derivatives, *Lepidium sativum* of the "Ajour" cultivar was used as a test plant. Seed germination and biometric indices (length, weight of the aboveground part and roots) of 5-day sprouts were determined, the phytotoxic effect of the derivatives was calculated. Experimental data were processed using methods of mathematical statistics.

Results. The introduction of substituents does not provide for an increase in antibacterial properties of the studied compounds in relation to some corrosion active ammonifying and sulfate-reducing bacteria. Low activity was observed regarding the association culture of ammonifying bacteria to the compound without substituents in the phenyl fragment and the compound with fluor as a substituent in the phenyl fragment at

a concentration of 2.0%. Derivatives with the methoxyl substituent in the phenyl fragment and with the hydroxyl substituent in the phenyl fragment did not show any antibacterial activity against the association culture of ammonifying bacteria isolated from ferrosphere in meat-peptone broth. Antibacterial action against the association of sulfate-reducing bacteria *Desulfovibrio orizae* with organic acid-producing bacteria *Anaerotignum propionicum* for derivatives were not detected. Phytotoxic properties were observed for the compound with the hydroxyl substituent that influenced the processes of growth in the test plant.

Conclusion. The introduction of electron-donor substituents into the basic structure did not provide for an increase in antibacterial properties against corrosive bacteria. Phytotoxic properties were observed for the compound with the hydroxyl substituent in the phenyl fragment, which influenced the *L. sativum* growth processes by inhibiting growth of the above-ground part and roots. Other compounds either did not show any action, or demonstrated a weak stimulating effect on the growth and development of the test plant.

Keywords: ammonifying bacteria, bactericides, *Lepidium sativum* L., phytotest, sulfate-reducing bacteria, the base of Schiff of 5-phenyl-4-amino-3-mercapto-4*H*-1,2,4-triazole

INTRODUCTION

One of the effective methods to protect against microbial induced corrosion is the use of bactericides (Abd-Elaal, Aiad, Shaban, Tawfik, & Sayed, 2014; Andreyuk *et al.*, 2005; Hsu, Chen, Lo, & Lee, 2019; Kong, Zhang, & Fang, 2017). Triazoles and Schiff's bases have high biological activity (Abd-Elaal *et al.*, 2014; Celik, Maman, & Babagil, 2018; Da Silva *et al.*, 2011; Dehaen, & Bakulev, 2015; Orek *et al.*, 2018). Among triazole derivatives, antibacterial and antifungal agents have been introduced into practice (Dehaen, & Bakulev, 2015). Triazoles have high anti-corrosion properties, in particular, in case of microbial corrosion (Abd-Elaal *et al.*, 2014). Schiff's bases are also known to have antimicrobial properties (Da Silva *et al.*, 2011). We have synthesized Schiff's bases of 5-phenyl-4-amino-3-mercapto-4*H*-1,2,4-triazole whose biological activity has not been studied yet. For practical use of the derivatives, their low toxicity is important (Zain, Salleh, & Abdullah, 2018). The most readily available method for determining toxicity is phytotesting using *Lepidium sativum* L. as a test plant (Smolinska, & Cedzynska, 2010; Tongur, Yildiz, Ünal, Atalay, & Yeniköşker, 2017).

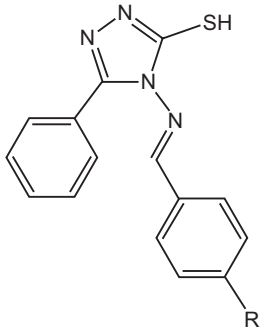
The purpose of this work was to investigate the antibacterial and phytotoxic properties of Schiff's bases of 5-phenyl-4-amino-3-mercapto-4*H*-1,2,4-triazole with donor substituents in the 4th position of heterosystem.

MATERIALS AND METHODS

Structure of derivatives. The formulas of the studied compounds are presented in **Fig. 1.** The structure of the synthesized compounds has been proved based on proton magnetic resonance spectroscopy data.

Investigation of antibacterial activity. In the investigation of antibacterial activity of the derivatives, corrosion-active 4-day association cultures of ammonifying and sulfate-reducing bacteria were used as a test culture of microorganisms. Association cultures were obtained from the ferrosphere of the corroding steel structure after five passages on meat-peptone broth and Postgate's B medium, respectively, under periodic cultivation

(Romanenko, & Kuznetsov, 1974). The association culture of sulfate-reducing bacteria included *Desulfovibrio oryzae* and their bacteria-satellite *Anaerotignum propionicum* (Tkachuk, Zelena, Mazur, & Lukash, 2020). Sensitivity of bacteria to the derivatives was determined by agar diffusion method using sterile paper disks according to the standard method (Egorov, 1969). The repetition of the experiment was 3-fold.



Compound	R
5-Phenyl-4-benzylidenamino-3-mercapto-4*H*-1,2,4-triazole – positive control (PC)	-H
I	$-OCH_3$
II	-F
III	-OH

Fig. 1. The general formula of derivatives of 5-phenyl-4-amino-3-mercapto-4*H*-1,2,4-triazole

Рис. 1. Загальна формула похідних 5-феніл-4-аміно-3-меркапто-4*H*-1,2,4-триазолу

Investigation of phytotoxic activity. In the investigation of phytotoxic activity of the derivatives, *L. sativum* of the "Ajour" cultivar was used as a test plant. Seed germination and biometric indices (length, weight of the aboveground part and roots) of 5-day sprouts were determined, the phytotoxic effect of derivatives was calculated. The concentration of compounds was 100 μg/mL. Distilled water was used as a negative control (NC). The scheme of the experiment was previously presented by the authors (Tsekhmister, Pinchuk, Tkachuk, Yanchenko, & Demchenko, 2012). The repetition of the experiment was 3-fold.

Statistical analysis of experimental data. Experimental data were processed using methods of mathematical statistics (Kalinin, & Yeliseyev, 2000). Statistical processing of the obtained results was performed using the statistical module of Microsoft Office Excel 2010. Methods of descriptive statistics were used to calculate the arithmetic mean (M) and the standard error of the arithmetic mean (m). The Student's significance criterion (*t*) was calculated, and the 95% probability of differences ($p<0.05$) was considered statistically significant.

RESULTS AND DISCUSSION

Antimicrobial activity of the new derivatives. In the study of antibacterial properties of the new derivatives, low activity regarding the association culture of ammonifying bacteria was observed for PC (without substituents in the phenyl fragment) and compound II (with fluor as a substituent in the phenyl fragment) at 2.0% concentration. In this case, the diameter of the zone of inhibitory growth of bacteria was 10.1 ± 0.2 mm and 9.0 ± 0.2 mm, respectively (**Fig. 2**). However, the test culture was heteroresistant to these derivatives and some colonies were in the growth inhibition zone. The derivatives of compound I (with methoxyl substituent in the phenyl fragment) and III (with hydroxyl substituent in the phenyl fragment) did not show antibacterial activity against the test culture of ammonifying bacteria. Antibacterial action against sulfate-reducing bacteria for the derivatives was not detected.

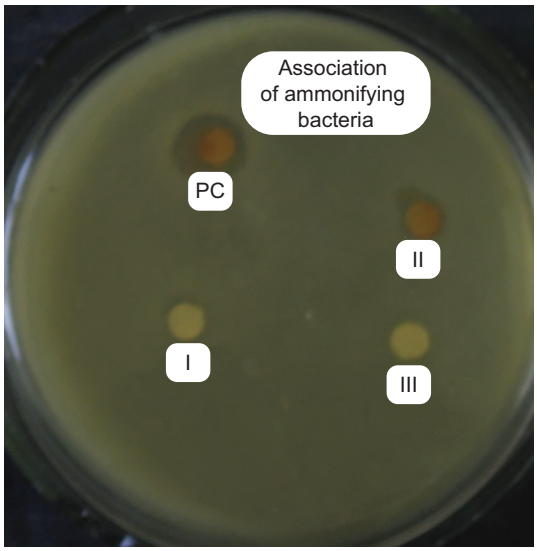


Fig. 2. Sensitivity of the association of ammonifying bacteria to the derivatives of 5-phenyl-4-amino-3-mercapto-4*H*-1,2,4-triazole (agar diffusion method)

Рис. 2. Чутливість асоціації амоніфікувальних бактерій до похідних 5-феніл-4-аміно-3-меркапто-4*H*-1,2,4-триазолу

Phytotoxic activity of the new derivatives. In the study of phytotoxicity of the new compounds, we observed the absence of influence on the energy of seed germination of the test plant – it was within the limits of control, marked changes in the indicator are unreliable.

The results of the study of the biometric indices of seedlings of the garden cress are shown in **Figures 3–4**.

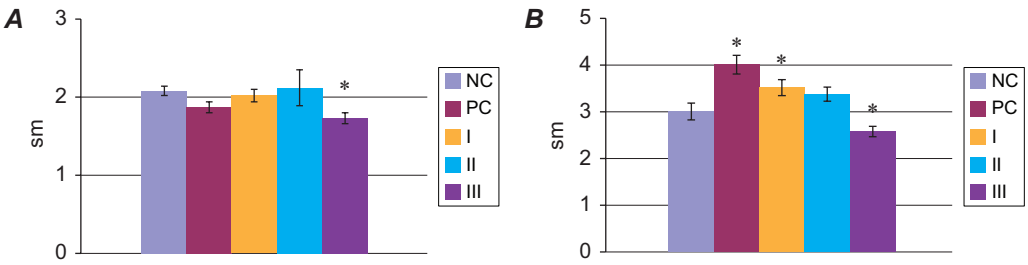


Fig. 3. An average length of the above-ground part (***A***) and roots (***B***) of *L. sativum* seedlings in the presence of Schiff's bases of 5-phenyl-4-amino-3-mercapto-4*H*-1,2,4-triazole with donor substituents in the 4th position of heterosystem
Comment: the differences from the NC are reliable at * $p \le 0.05$ (t_{st} = 2.0–2.6–3.4)

Рис. 3. Середня довжина надземної частини (***A***) та коренів (***B***) проростків *L. sativum* за наявності основ Шиффа 5-феніл-4-аміно-3-меркапто-4*H*-1,2,4-триазолу з донорними замісниками у 4-й позиції гетеросистеми
Примітка: відмінності від негативного контролю достовірні при * $p \le 0,05$ (t_{st} = 2,0–2,6–3,4)

According to our findings, only compound III affected growth of the aboveground part of the seedlings. At the same time, the length of the above-ground part of the seedlings was significantly (1.2 times) smaller than that in the negative control. It points to the property of the compound to inhibit growth (**Fig. 3*A***).

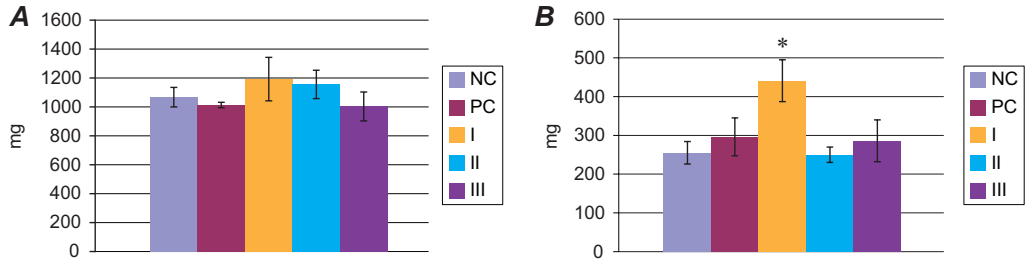


Fig. 4. An average weight of the above-ground part (***A***) and roots (***B***) of 100 *L. sativum* seedlings in the presence of Schiff's bases of 5-phenyl-4-amino-3-mercapto-4*H*-1,2,4-triazole with donor substituents in the 4th position of heterosystem
Comment: the differences from the NC are reliable at * p≤0.05 (t_{st} = 2.8-4.6-8.6)

Рис. 4. Середня вага надземної частини (***A***) та коренів (***B***) 100 проростків *L. sativum* за наявності основ Шиффа 5-феніл-4-аміно-3-меркапто-4*H*-1,2,4-триазолу з донорними замісниками у 4-й позиції гетеросистеми
Примітка: відмінності від негативного контролю достовірні при * p ≤ 0,05 (t_{st} = 2,8–4,6–8,6)

The longitudinal growth of the roots of the seedlings of garden cress was influenced by compounds PC, I and III. In this case, both weak inhibition of growth (compound III) and weak stimulation of it (PC and compound I) was observed. In particular, in the presence of compound III, the length of the roots was significantly (1.2 times) smaller than that of the negative control (**Fig. 3*B***), and the phytotoxic effect constituted 14.3%. In the presence of PC and derivative I, the length of the roots was significantly greater than that of the negative control – by 33 % and 17 %, respectively (**Fig. 3*B***).

We did bot observe any influence of triazole derivatives on the weight of the above-ground part and roots of seedlings (except for compound I) (**Fig. 4*A***). In the presence of derivative I, the weight of roots was significantly greater (1.7 times) than that of the negative control, indicating that the compound is weakly stimulating the development (**Fig. 4*B***).

CONCLUSION

An introduction of electron-donor substituents into the basic structure did not provide for the increase in antibacterial properties against corrosive bacteria. Phytotoxic properties were observed for the compound with the hydroxyl substituent in the phenyl fragment, which influenced growth processes by inhibiting the growth of the above-ground part and roots. Other compounds either did not show any action, or demonstrated a weak stimulating effect on the growth and development of the test plant.

COMPLIANCE WITH ETHICAL STANDARDS

Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Animal Rights: This article does not contain any studies with animal subjects performed by any of the authors.

AUTHOR CONTRIBUTIONS

Conceptualization, [N.V.T.; V.O.Y.; A.M.D.]; methodology, [N.V.T.; V.O.Y.; A.M.D.]; validation, [N.V.T.; V.O.Y.; A.M.D.]; formal analysis [N.V.T.; V.O.Y.; A.M.D.]; investigation, [N.V.T.; V.O.Y.; A.M.D.]; investigation, [N.V.T.; V.O.Y.]; resources, [N.V.T.; V.O.Y.; A.M.D.]; data curation, [N.V.T.]; writing – original draft preparation, [N.V.T.; V.O.Y.; A.M.D.]; writing – review and editing, [N.V.T.; V.O.Y.; A.M.D.]; visualization, [N.V.T.]; supervision, [N.V.T.; V.O.Y.; A.M.D.]; project administration, [NVT, VOY, AMD]; funding acquisition, [N.V.T.; V.O.Y.; A.M.D.]

REFERENCES

Abd-Elaal, A. A., Aiad, I., Shaban, S., Tawfik, S. M., & Sayed, A. (2014). Synthesis and evaluation of some triazole derivatives as corrosion inhibitors and biocides. *Journal of Surfactants and Detergents*, 17(3), 483–491. doi:10.1007/s11743-013-1547-0
Crossref ● Google Scholar

Andreyuk, K., Kozlova, I., Kopteva, Zh., Pilyashenko-Novokhatny, A., Zanina, V., & Purish, L. (2005). *Mikrobna koroziia pidzemnykh sporud* [*Microbial Corrosion of Underground Structures*]. Kyiv: Naukova Dumka. [In Ukrainian]
Google Scholar

Celik, H., Maman, M., & Babagil, A. (2018). HCA isoenzyme inhibitory activities of Schiff bases derived from cinnamaldehyde and p-methoxycinnamaldehyde. *FEB-Fresenius Environmental Bulletin*, 27(6), 4469–4482.
Google Scholar

Da Silva, C. M., Da Silva, D. L., Modolo, L. V., Alves, R. B., de Resende, M. A., Martins, C. V. B., & de Fátima, Â. (2011). Schiff bases: A short review of their antimicrobial activities. *Journal of Advanced Research,* 2, 1–8. doi:10.1016/j.jare.2010.05.004
Crossref ● Google Scholar

Dehaen, W., & Bakulev, V. A. (Eds.). (2015). Chemistry of 1,2,3-triazoles. *Topics in Heterocyclic Chemistry*. New York, Dordrecht, London: Springer. doi:10.1007/978-3-319-07962-2
Crossref ● Google Scholar

Egorov, N. S. (2004). *Osnovy ucheniya ob antibiotikah* [*Fundamentals of the study of antibiotics*]. Moscow: High School. [In Russian]
Google Scholar

Hsu, Ch.-W., Chen, T.-E., Lo, K.-Y., & Lee, Y.-L. (2019). Inhibitive properties of benzyldimethyldodecylammonium chloride on microbial corrosion of 304 stainless steel in a *Desulfovibrio desulfuricans*-inoculated medium. *Materials* (*Basel*), 12(2), 307–318. doi:10.3390/ma12020307
Crossref ● PubMed ● PMC ● Google Scholar

Kalinin, M. I., & Yeliseyev, V. V. (2000). *Biometriia: Pidruchnyk dlia studentiv vuziv biolohichnykh i ekolohichnykh napriamkiv.* [*Biometrics: Textbook for students of higher educational institutions in biological and ecological directions*]. Mykolaiv: Publisher of the MB NaUKMA. [In Ukrainian]
Google Scholar

Kong, L., Zhang, B., & Fang, J. (2017). Study on the applicability of bactericides to prevent concrete microbial corrosion. *Construction and Building Materials,* 149, 1–8. doi:10.1016/j.conbuildmat.2017.05.108.
Crossref ● Google Scholar

Orek, C., Sarac, K., Parlak, A. E., Koparir, P., Turkoglu, S., & Koparir, M. (2018). Synthesis, structure investigation, spectral properties and *in vitro* antioxidant evaluation of new 1-(3-methyl-3-mesityl)-cyclobutyl-2-(5-thiophen-4-ethyl-2h-[1,2,4]triazol-3-ylsulfanyl)etanone. *FEB-Fresenius Environmental Bulletin*, 27(5), 2992–3005.
Google Scholar

Romanenko, V. I., & Kuznetsov, S. I. (1974). *Ekologiya mikroorganizmov presnyh vodojomov* [*Ecology of freshwater microorganisms*]. Leningrad: Science. [In Russian]
Google Scholar

Smolinska, B., & Cedzynska, K. (2010). Iodide for the phytoextraction of mercury contaminated soil. *FEB-Fresenius Environmental Bulletin,* 19(12a), 3049–3054.
Google Scholar

Tkachuk, N., Zelena, L., Mazur, P., & Lukash, O. (2020). Genotypic, physiological and biochemical features of *Desulfovibrio* strains in a sulfidogenic microbial community isolated from the soil of ferrosphere. *Ecological questions*, 31(2), 79-88. doi:10.12775/EQ.2020.016.
Crossref ● Google Scholar

Tongur, S., Yildiz, S., Ünal, A., Atalay, K., & Yeniköşker, M. (2017). Toxicity assessment of analgesics by *Lepidium sativum* toxicity test method. 2nd International conference on civil and environmental engineering – ICOCEE 2017 (Nevsehir, Turkey, May 8–10, 2017). Sahinkaya, S. & Kalipci, E. (Eds.), 1–9.

Tsekhmister, A. V., Pinchuk, L. A., Tkachuk, N. V., Yanchenko, V. O., & Demchenko, A. M. (2012). Pokaznyky rostu *Lepidium sativum* L. za prysutnosti 1-aryltetrazolvmisnykh pokhidnykh 1-tetralin-6-il-etanonu [Indicators of growth of *Lepidium sativum* L. in the presence of 1-aryltetrazole-containing derivatives of 1-tetralin-6-yl-ethanone]. *Scientific notes of Ternopil National Pedagogical University named after V. Gnatyuk. Series: Biology*, 3(52), 84–88. [In Ukrainian]
Google Scholar

Zain, W. S. M., Salleh, N. I. H., & Abdullah, A. (2018). Natural Biocides for Mitigation of Sulphate Reducing Bacteria. *International Journal of Corrosion,* 2018, 1–7. doi:10.1155/2018/3567569
Crossref ● Google Scholar

АНТИБАКТЕРІАЛЬНА ТА ФІТОТОКСИЧНА АКТИВНІСТЬ ОСНОВ ШИФФА 5-ФЕНІЛ-4-АМІНО-3-МЕРКАПТО-4*H*-1,2,4-ТРИАЗОЛУ З ДОНОРНИМИ ЗАМІСНИКАМИ У 4-VE ПОЛОЖЕННІ ГЕТЕРОСИСТЕМИ

***Н. В. Ткачук*[1], *В. О. Янченко*[1], *А. М. Демченко*[2]**

[1] *Національний університет "Чернігівський колегіум" імені Т. Г. Шевченка*
вул. Гетьмана Полуботка, 53, Чернігів 14013, Україна

[2] *Ніжинський державний університет імені Миколи Гоголя*
вул. Графська, 2, Ніжин 16600, Чернігівська область, Україна

Вступ. Триазоли та основи Шиффа мають високу біологічну активність. Для практичного використання похідних важлива їхня низька токсичність. Мета роботи – дослідити антибактеріальні та фітотоксичні властивості основ Шиффа 5-феніл-4-аміно-3-меркапто-4*H*-1,2,4-триазолу з донорними замісниками у 4-му положенні гетеросистеми.

Матеріали та методи. Під час дослідження антибактеріальної активності похідних як тест-культури мікроорганізмів використано 4-денні корозійно активні асоціативні культури амоніфікувальних і сульфатвідновлювальних бактерій. Чутливість бактерій до похідних досліджували дифузійним методом в агар із застосуванням стерильних паперових дисків за стандартною методикою. У ході дослідження фітотоксичної активності похідних *Lepidium sativum* L. сорту "Ажур" був використаний як тест-рослина. Визначено схожість насіння та біометричні показники (довжина, вага надземної частини та коренів) 5-денних проростків, розраховано фітотоксичну дію похідних. Експериментальні дані оброблено з використанням методів математичної статистики.

Результати. З'ясовано, що введення замісників не забезпечує підвищення антибактеріальних властивостей досліджуваних сполук щодо корозійно активних амоніфікувальних і сульфатвідновлювальних бактерій. Низьку активність спостерігали щодо асоціативної культури амоніфікувальних бактерій у сполуки без замісників у фенільному фрагменті та сполуки з замісником флуором у фенільному фрагменті за концентрації 2,0%. Похідні з метоксильним замісником та з гідроксильним замісником у фенільному фрагменті не виявляли антибактеріальної активності щодо досліджуваної культури амоніфікувальних бактерій. Антибактеріальної дії щодо сульфатвідновлювальних бактерій для похідних не виявлено. Фітотоксичні властивості були відзначені для сполуки з гідроксильним заступником, яка впливала на показники процесу росту тест-рослини.

Висновки. Введення електроно-донорних замісників у базову структуру не забезпечило підвищення антибактеріальних властивостей щодо корозійно активних бактерій. Фітотоксичні властивості спостерігали для сполуки з гідроксильним замісником у фенільному фрагменті, яка впливала на процеси росту *L. sativum*, інгібуючи ріст надземної частини та коріння. Інші сполуки або не проявили дії, або мали слабке стимулювання росту й розвитку тест-рослини.

Ключові слова: амоніфікувальні бактерії, бактерициди, *Lepidium sativum* L., фітотест, сульфатвідновлювальні бактерії, основи Шиффа 5-феніл-4-аміно-3-меркапто-4*H*-1,2,4-триазолу

Received / Одержано 04 October, 2021 | Revision / Доопрацьовано 12 October, 2021 | Accepted / Прийнято 24 December, 2021 | Published / Опубліковано 29 December, 2021