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THE USE OF TEBUCONAZOLE AS A FUNGICIDE TO PROTECT THE LIBRARY

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Chemical derivatives of 1,2,4-triazole are biologically active chemicals that were meant for use in plant protection for agricultural purposes, nevertheless those substances can be used as biocides and fungicides in book preservation. All chemical substances must be certified, i.e. their efficacy in the environment should be tested in advance, which in case of this study means place where books can be exposed to the impact of different biological factors (library and books' storage). It is also very important to check the influence of the tested chemicals on people, animals and the natural environment. Even though there are some controversies attached to how chemicals are used and how they negatively affect the environment, still they seem rather safe for plant cropping and by these means also for paper and human beings. The elementary issue that describes any chemical is its active substance, i. e. the substance biologically active to specified organisms, which causes physiological distemper by blocking the activity of their enzymes. The main aim of this study is to get new species of chemicals into the preservative practice of all kinds of books. This can help in eliminating the big part of dangerous and toxic substances that can cause distress and discomfort of the worker form the conservation process. What is more, this study may help to extend the range of triazole derivatives' usage, when their activity starts to fade, as a side effect of materials' resistance phenomena.

Keywords: tebuconazole, fungus, active substances, disinfection, book.

Introduction

Fungicides, i. e. all fungicidal and fungistatic chemical substances, used not only in agricultural and plant protecting practice, but also as disinfectants, can be divided in two following groups:

– surfactants active only on the surface without penetrating plant tissues. Their task is to block the cellular respiration and stop the metabolism of the cells. This group includes: copper- and sulphur fungicides, thiocarbonyl derivatives, ditiocarbamates, aromatic hydrocarbons derivatives, dicarboximide fungicides;

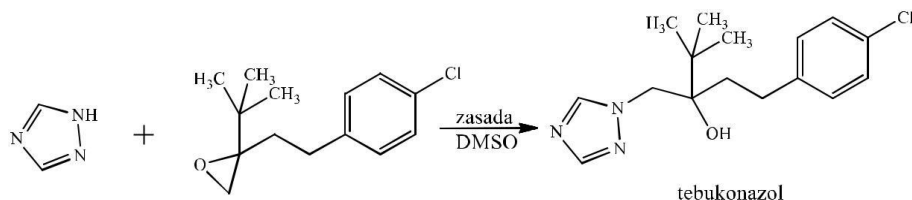
– inner acting fungicides with systemic performance, that causes disturbance in development of pathogens and stopping the destruction of paper. This group contains: benzimidazole compounds, triazol compounds, phenylamids and anilino-pyrimidines.

Tebuconazole (triazoles group), which shows high fungicide efficacy and simultaneously low damage to books and other printed materials was the main substance used for this particular examination. For that reason the tebuconazole is used in fast interventions. It is also non toxic for the environment and provides no carcinogenic activity. The mechanism of biological activity of this fungicides' group lies in their ability to inhibit the synthesis of sterolic alcohol called ergosterol, which is responsible for selectivity

of cytomembrane¹. This selectivity is broken by the lack or insufficiency of the sterol in the cell's environment².

Task of the study

The main aim of this study was to carry out an initial experiment to check, whether triazoles could be used to fight microbiological hazards of antique library collections and separate printed materials. The construction of old prints causes variety of microbiological dangers, mainly with viral and fungal infections. For this particular study tebuconazole was chosen as a model example of the active substance and pathogen called *Penicillium chrysogenum* was the tested fungal material. The tebuconazol is a racemic solid substance, soluble in polar organic solvents, photo- and thermal stable in alcohols. The examined substance is usually obtained by reaction of 2-*t*-butyl-2-(4-chlorophenylethyl)-oxirane with 1,2,4-triazole in dimethyl sulfoxide in the presence of a base (*Pic. 1*). On the market it is known as Tebu 250 EW and Horizon 250 EW.



For its high fungicidal activity the tebuconazol is very often used and added to other plant protection agents and what is more it is indeed the most popular of all triazole fungicides.

The experiment

To relate the impact of fungicidal activity of tebuconazol on old prints and other historic materials, that would need preservation in the library, the ED₅₀ factor was to be determined before the right investigation. The biological activity of the fungicides was referred in the ancient book preservation process, involving the appointment of the so-called ED₅₀ coefficients, i. e. how big amount of the substance is needed to destroy half of the fungal pathogens' population. The ED₅₀ ratio is used to determine the concentration of working solution, which should be sprayed on the surfaces of the protected material: paper, parchment, leather or fabric, providing the required protective efficacy.

To get the correct ratio a set of concentration series, consisting of isopropanolic solutions of tebuconazol was prepared. In the next stage, the solutions in the volume of 1 cm³ were mixed 50 cm³ of nutrient that consisted of agar-agar solved in wort and then put into Petri dish. On the set nutrient the test material, i.e. spawns of fungus *Fungicidal activity*, were placed. The concentration of the active substance in reference to 1 cm³ of nutrient were set in the range from 10⁵ to 10² ng/cm³. The linear growth of the fungus was measured every 24 h until the tested fungus totally covered the control dish, which included the set nutrient with 1 cm³ of isopropanol.

¹ Kraus P. Untersuchungen zum Wirkungsmechanismus von Baycor // *Pflanzenschutz-Nachrichten Bayer*. – 1976. – Nr 12. – S. 17–30.

² Kramer D. W. *Sterol biosynthesis inhibitors and Anti-Feeding Compounds*. – Berlin: Springer-Verlag, 1986. – P. 25–64.

Results and discussion

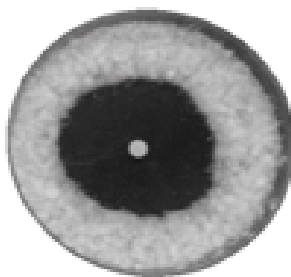
The fungicidal activity of tebuconazol on *Fungicidal activity* is given as ED₅₀ ratio and the results are presented below in the *Table 1*.

Table 1.

Fungicidal activity of examined substances

Dosis [ng/cm ³]	Log of the dosis	The medium size of the fungal spawn [mm]	Fungicidal efficacy [%]	Probit	The mortality of spawn	Values of the probit function patern according to the column 6''=NORMSINV (x) 5.S.ODW(x)+5''
80	1,903	42,5	15	3,964	0,15	3,964
160	2,204	32,5	35	4,615	0,35	4,615
280	2,447	25	50	5,000	0,50	5,000
560	2,748	12,5	75	5,674	0,75	5,674
1420	3,152	2,5	95	6645	0,95	6,645
2800	3,447	0	100	–	1,0	–

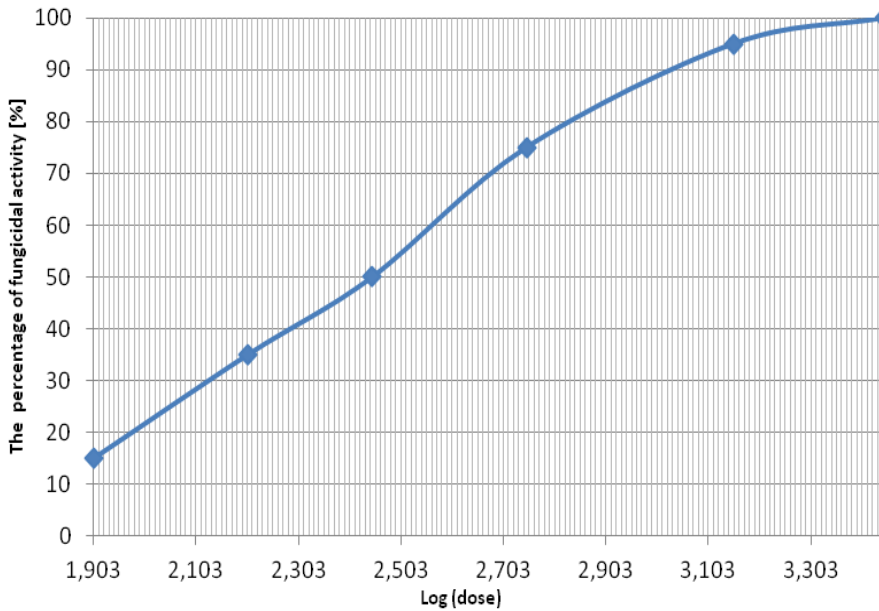
The model example of the dynamics of the linear growth of fungal test material presents *Pic. 1* and it refers to dose of tebuconazol in size of $0,56 \times 10^3$ ng/cm³ of nutrient and the incubation period of 7×24 h.



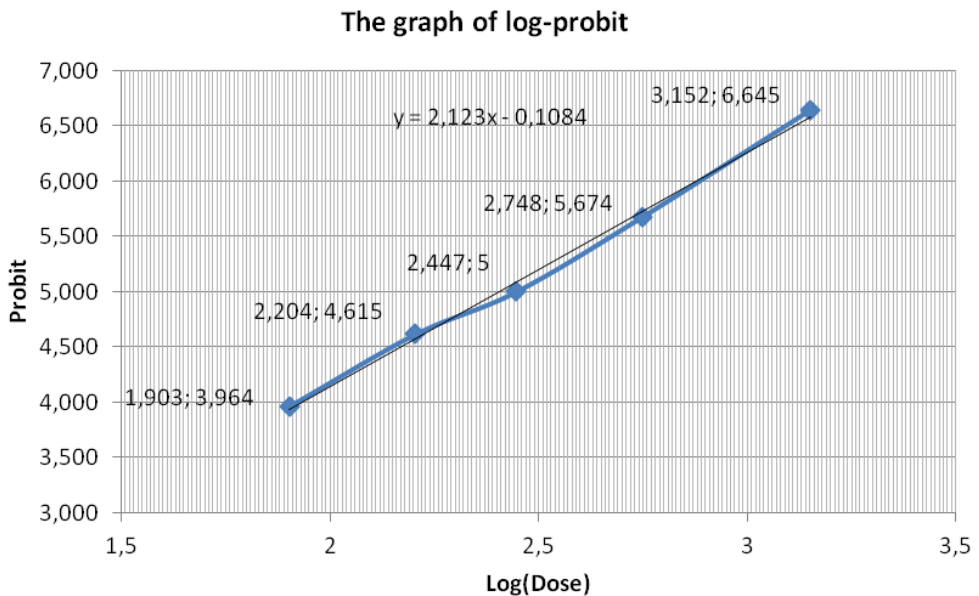
Pic. 1. The linear growth of fungus after the 7 d. incubation period

The dynamics of the growth of the test fungus was measured with the diameter of the zone of growth in the examined probe and control sample given in millimeters and the quantitative measure was set by the relation of the difference of the growth zones of the examined probe and control sample to the control sample given *per centum*. To determine the ED₅₀ ratio the Lietchfield and Wilcoxon³ method was used for simplified log-probit function, extrapolating the dose from the curve at the 50 % level of efficacy. The relation of efficacy of tebuconazol to the dose used was drawn in the *Pictures 2 and 3*.

³ Lietchfield J. T., Wilcoxon F. A simplified method of evaluating dose – effect experiments // *Journal of Pharmacology and Experimental Therapeutics*. – 1949. – Nr 96. – P. 99–113.



Pic. 2. The test of Fungicidal activity, the efficacy of tebuconazol depending on the dose



Pic. 3. The graph of log-probit function

According to the graphs presented above the size of the dose was given in logarithmic form, in which 50 % of the fungal population was deceased. All in all, it gives 2,447 and corresponds with the concentration of the substance $0,280 \times 10^3$ ng/cm³ of the nutrient, which is the ED₅₀ designated earlier.

This dose of tebuconazol in 50 cm³ of the nutrient can be obtained by getting into it 1 cm³ of substance solved in 0,32 % isopropanol. This is the base for further investigation on the effectiveness of tebuconazol put in isopropanol solution, which is the working liquid for spray or contact preservation of the protected material's surface.

Conclusion

The results presented above should be treated as a foreword for further investigation on tebuconazol as a representative of triazoles in preserving old print materials. The interest in tebuconazol as fungicide and preservative arises from its biological activity, but also from the physiochemical stability. What is more, the potential metabolites of tebuconazol are not acidic, which allows to use it as disinfectant for all kinds of old prints.

The further investigation should focus on examining wider scale of fungal material, that can tell the level of sensitivity of different pathogens and develop the interpretation of the results with all required elements of the statistic analysis. Moreover, to describe the level of toxicological danger for the reader of the book the time decay of the active substance in the paper should be noted with the variety of chromatography techniques. The research also provides chromatographic and spectroscopic testing of changes in the book under the influence of the test product.

Used literature

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3. Lietchfield J. T., Wilcoxon F. A simplified method of evaluating dose – effect experiments // *Journal of Pharmacology and Experimental Therapeutics*. – 1949. – Nr 96. – P. 99–113.

ВИКОРИСТАННЯ ТЕБУКОНАЗОЛУ ЯК ФУНГЦИДУ ДЛЯ ЗАХИСТУ БІБЛІОТЕКИ

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Хімічні похідні сполуки 1,2,4-триазолу – це біологічно активні речовини, призначені для захисту рослинних культур, які також можна використовувати як нове покоління біоцидів для захисту і охорони бібліотечних зібрань від мікроорганізмів. Хімічні засоби після попередньої реєстрації можна допускати до використання тільки після перевірки їхньої біологічної ефективності в кліматичних умовах, а в даному випадку – в умовах бібліотек і сховищ. Важливим елементом оцінки придатності хімічної сполуки також є її токсичність для людини, тварин і навколишнього середовища. Незважаючи на різні суперечки, пов'язані з негативним впливом на середовище, ці засоби багато років є дуже ефективним методом профілактики загроз врожаю рослин, а відповідно – безпечні для людей і паперу. Підставовим складником, який визначає специфіку дії засобу охорони рослин, є активна речовина, тобто біологічно активна для певних організмів, яка викликає розлад їхніх фізіологічних процесів унаслідок блокування активності відповідних ферментів. Мета цього дослідження полягає у впровадженні до реставраційної практики нових біологічно активних речовин, які використовують для дезінфекції бібліотечних пам'яток. Це дозволить відмовитися від використання в реставраційній практиці небезпечних і токсичних речовин, які можуть викликати дискомфорт у працівника в процесі роботи. Крім того, це дослідження може допомогти розширити діапазон використання похідних триазолу, коли їхня активність починає зменшуватись як побічний ефект явищ опору матеріалів.

Ключові слова: тебуконазол, грибок, активні речовини, дезінфекція, книга.

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