Antifungal Activity, Synthesis and Structure of New Derivatives of 1,2,4-triasole, α-(*p*-chloro-phenoxy) - α - (1,2,4-triasole-1-il) Acetic Acid

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The paper presents the synthesis of a new derivative of 1,2,4-triasole, α-(p-chloro-phenoxy) -α-(1,2,4-triasole-1-il) acetic acid, which was characterized by elemental analysis, IR,DSC,UV-VIS, FT-IR, [1]H-RMN, and [13]C-RMN. With the purpose of determining the active biological potential of the compound, an environment was developed and optimized for breeding four different fungi species: Aspergillus flavus, Trichoderma viride, Alternaria alternata, and Penicillium crysogenum. When the antifungal action of α-(p-chloro-phenoxy) -α-(1,2,4-triasole-1-il) acetic acid was tested, 21 species of cellulosolitic fungi in an isolated pure culture on art objects (paper, textile materials, mural oil paintings, frescoes, glass, stone, and ceramic).

Keywords: 1,2,4-triasole, α-(p-chloro-phenoxy) -α- (1,2,4-triasole-1-il) acetic acid, synthesis, optimizing, fungicide, art

The compounds of the class of the triasoles exhibiting fungal action are used internationally, both in medicine and as a pesticide applicable in different fields. Triasolic fungicides stand out due to the fact that they have a large action spectrum, being used in small, unpolluting doses, with a systematic action. [6,7] The action of the triasolic fungicides consists of the inhibition of the biosynthesis of the ergosterol – which is the main compound of the cell membranes – which is why they are also called inhibitors of the biosynthesis of the ergosterol [1].

Due to the hypothesis that the asolitic compounds have the propriety of forming biologically active ions "in vivo", after laborious researches, there was established that the active derivatives are the ones that contain the non-substituted asolitic cycle, and the connection with the lateral catena is made from the azote 1, to which a mobile atom of hydrogen is related [2,3].

Using these data, a series of triasole derivatives with a specific activity against moulds were synthesized and will be presented in this study.

Experimental part

Synthesis of α-(*p*-chloro-phenoxy) -α- (1,2,4-triasole-1-il) acetic acid

The synthesis of the compound was made in a solid phase, using: phenol, o-chloro-phenol, p-chloro-phenol, dichloro acetic acid,1-*H*-1,2,4-triasole compounds as raw materials. A polar solvent – ethylic alcohol and as an acid acceptor, NaOH have been used. The synthesis consists in a condensation reaction between the dichloroacetic acid with phenols or chloro-phenols and 1*H*-1,2,4-triasole in a basic environment in reaction conditions specific to each compound.

In a flask of 250 mL with two necks, endowed with a reflux refrigerent and a thermometer, 0.08 moles (7.528 g) of phenol, 0.08 moles (6.57 mL) of dichloroacetic acid, 0.08 moles (5.525 g) of 1-*H*-1,2,4-triasole, 0.08 moles (80 mL) of ethylic alcohol, and 0.16 moles (6.4 g) of sodium dioxide. The reaction temperature was maintained at 85°C for 13 h. The final product is white solid crystals, that were characterized by physical-chemical methods. The yield was of 90% in useful product.

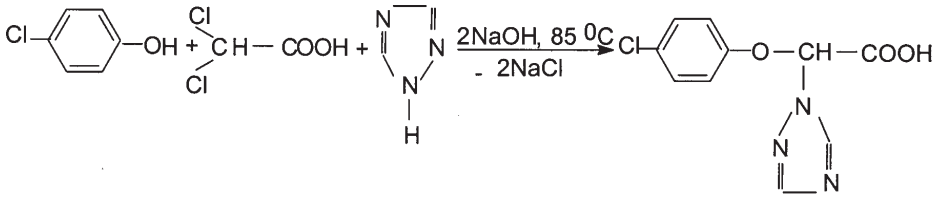
The characterization of the synthesized derivative 1-H-1,2,4-triasole, α-(p-chlorophenoxy) - α - (1,2,4-triasole-1-il) acetic acid, was made by IR, the elemental analysis, DSC, UV-VIS, FT-IR, [1]H-NMR and [13]C-NMR [5].

The IR spectrums were recorded on a Specord M80 Carl Zeiss Jena spectrophotometer, in KBr. The spectrums UV-VIS were recorded with a SPECORD M 42 spectrophotometer, with a spectral field of 190-900 nm, and methanol as a solvent, and dishes of 10 mm were used. The FT-IR specters were obtained with a JASCO-FF/IR 660 + device, in KBr. The spectra [1]H-NMR and [13]C-NMR were recorded on a Bruker apparatus with a frequency of 400 MHz, at room temperature, using the deuterated bimethyl-sulphoxide (DMSO) as a solvent.

Results and discussions

Establishing the biologic activity

A culture medium balanced have been developed and optimized for the growing of fungi in pure culture with the purpose of testing the effects produced by the biotic agent.



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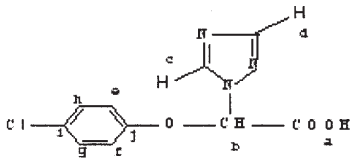

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^{1}H – NMR: 7.08-7.14 and 7.15-7.17 ppm 4H, m, H aromatic (e,f,g,h);

7.18 ppm 1H, s, CH (b);

8.88 ppm 1H, 1s H heterocyclic (c);

8.12 ppm 1H, 1s H heterocyclic (d);

^{13}C –NMR: 165.48 ppm COOH

80 ppm CH

152.36 ppm C-O

153.98 ppm C – heterocyclic

145.73 ppm C – heterocyclic

129.6-127.7 ppm 2C aromatic (g,h)

116.6-116.9 ppm 2C aromatic (e,f)

127.08 ppm 1C aromatic (i)

Table 1
VALUES OF α PARAMETER

Factorial plan	2^2	2^3	2^4	2^5	2^{5-1}	2^6	2^{6-1}	2^{7-1}
Values of α	1.414	1.682	2.000	2.378	2.000	2.828	2.378	2.828

Table 2
EXPERIMENTATION PLAN EXTENDED WITH 3 VARIABLES FOR THE SPECIES ASPERGILLUS FLAVUS

Variable	Code	Level of variables				
		-α-1	-1	0	1	α+1
Source C	X_1	36,68	35	37	39	40,68
Source N organic	X_2	1.74	3.422	3.57	3.768	5.4
Monopotasic phosphate	X_4	0.822	0.860	0.804	0.656	2.338

In order to conceive a good culture medium, the microorganisms that will be cultivated on it and their necessities for an optimum development must be taken into account.

The developed medium is based on the following components: glucose, soya bean peptone, sodium azotate, potassium phosphate, magnesium phosphate, iron sulphate, and copper sulphate. The value of the optimum concentration of the carbon and energy source, determined by means of the maximum descents, surpasses the chosen maximum value, and this value will be checked as well by means of the statistical method by means of a 2nd degree compound, centered, revolving experimental programme with extension in the field ($-\alpha$-1), 0, ($+1+\alpha$) [4].

Experimentally there were prepared eight media of culture whose composition is represented in table 2, and that refer to the maximum and minimum values and a culture medium with the basic composition. In each medium, according to the standard procedures four cellulosolitic fungi were cultivated: Aspergillus flavus, Trichoderma viride, Alternaria alternata, and Penicillium crysogenum, and the viable density for each case was determined.

For the analyzed case, that contains 3 factors, the value of the parameter α is 1.682,[4] and the experimentation plan and the experimental matrix are showed in tables 2 and 3.

Table 3
EXPERIMENTAL MATRIX EXTENDED FOR 3 FACTORS FOR A COMPOSED, CENTRAL, ROTATABLE PROGRAMME, APPLIED TO THE BREEDING OF THE FUNGUS ASPERGILLUS FLAVUS

x_0	x_1	x_2	x_4	x_1^2	x_2^2	x_4^2	x_1x_2	x_1x_4	x_2x_4	y (g/l)	$Y_{calculated}$
1	-1	-1	-1	1	1	1	1	1	1	47	46.41
1	1	-1	-1	1	1	1	-1	-1	1	59	60.31
1	-1	1	-1	1	1	1	-1	1	-1	57	58.05
1	1	1	-1	1	1	1	1	-1	-1	63	65.95
1	-1	-1	1	1	1	1	1	1	1	46	45.46
1	1	-1	1	1	1	1	-1	-1	1	55	54.35
1	-1	1	1	1	1	1	-1	1	-1	50	48.1
1	1	1	1	1	1	1	1	-1	-1	49	50.99
1	-1.682	0	0	2.829	0	1	0	0	0	48	49.15
1	1.682	0	0	2.829	0	1	0	0	0	64	63.27
1	0	-1.682	0	0	2.829	1	0	0	0	48	50.79
1	0	1.682	0	0	2.829	1	0	0	0	59	57.76
1	0	0	-1.682	0	0	1.000	0	0	0	59	57.39
1	0	0	1.682	0	0	1.000	0	0	0	43	44.02
1	0	0	0	0	0	0	0	0	0	55	54.28
1	0	0	0	0	0	0	0	0	0	54	54.28
1	0	0	0	0	0	0	0	0	0	53	54.28
1	0	0	0	0	0	0	0	0	0	54	54.28
1	0	0	0	0	0	0	0	0	0	55	54.28
1	0	0	0	0	0	0	0	0	0	55	54.28

For these experiments, the regression equation is:

$$y=54.28 + 4.197x_1 + 2.07x_2 - 3.974x_3 + 0.683x_1^2 - 1.262x_4^2 - 1.5x_1x_2 - 1.25x_1x_4 - 2.25x_2x_4 \quad (3)$$

According to the mathematic model obtained as follows to be validated, the R multiple correlation coefficient is calculated with:

$$R_{Yx_1x_2x_3} = \sqrt{1 - \frac{\sum_{i=1}^{n}(Y_{ei} - Y_{ci})^2}{\sum_{i=1}^{n}(Y_{ei} - \overline{Y}_e)^2}} \quad (4)$$

The following value was obtained:

$$R_{Yx_1x_2x_4} = 0.988 \quad (5)$$

The obtained value demonstrates that there is a high correlation between the dependent and the independent variables.

According to the *Fisher* test, if $F_{calculated} = 1315.48 > F_{critical} = 4.6$, the mathematical model was found adequate to the two independent variables X_1, X_2, and there are no errors.

The testing of the calculated coefficients the Student test was used. The dispersion of the coefficients (s^2) and the test statistics (t) are calculated, with:

$$s^2 = \frac{\sum_{i}^{n}(x_i - \overline{x})}{n-1} \quad (6)$$

In this case:

$$s^2 = 0.023333 \quad (7)$$

The information provided by the model are the following:

- the sense of each effect: the sign of each coefficient indicates the sens in that the parameter may influence the function-objective. The conclusion of the regression equation determined for the analyzed process is that the variables X_1 and X_2 advantage the process of growing of the fungi.

- the intensity of the effects is indicated by the means of the absolute values of each coefficient. In the analyzed process, the significance of the effects varies in the following order:

$$X_1 > X_2 > X_1X_2 > X_2X_4 \quad (8)$$

The variable X_1 (the carbon and energy-glucose source) influences the most the analyzed process.

After the confirmation of the appropriateness of the mathematical models for the regression equation, a classic method of optimization was applied, that consists of determining the existing stationary points by solving the equation system obtained by canceling the 1st degree derivatives.

Thus, it was obtained:

$$Y_{max,calc} = 68.02 \quad (9)$$

By optimizing the regression equation, the following coordinates of the maximum point were established:

$$x_1 = -3.126 \quad x_2 = 2.372 \quad x_4 = -1.578 \quad (10)$$

As these values are situated in encoded space, by being decoded lead to the real values for the concentration of the culture medium expressed depending on the 3 determining factors:

X_1 = 43.25 g/L - carbon and energy (glucose) source;
X_2 = 3.92 g/L - organic azote (soya peptone) source;
X_4 = 0.892 g/L - monopotasic phosphate source.

The results obtained through the development and optimization of the culture medium indicate the optimum conditions of growing of the cellulozolitic fungi.

In order to emphasize the accurateness of the obtained regression equation, graphic tri-dimensional

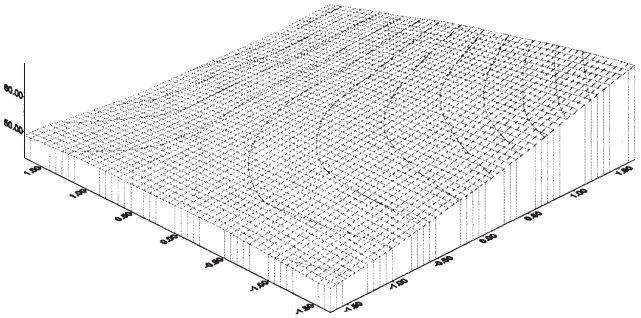

Fig.1. Spatial representation of the function $Y_{calc}=f(x_2,x_4)$ for $x_1=0$

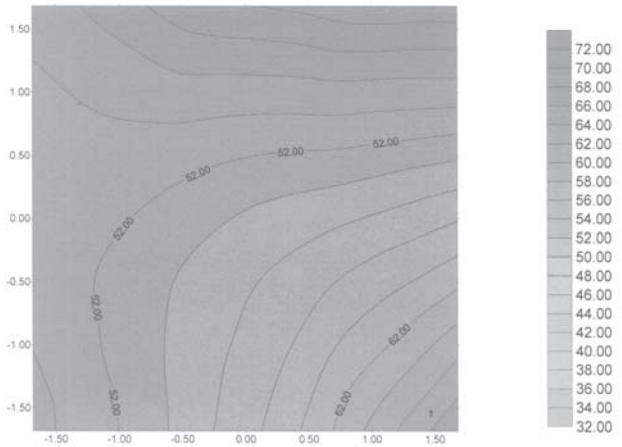

Fig.2 Representation through isolines of the function $Y_{calc}=f(x_2,x_4)$ for $x_1=0$

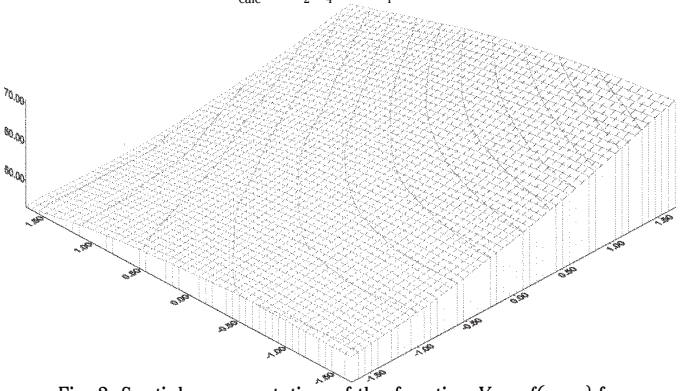

Fig. 3. Spatial representation of the function $Y_{calc}=f(x_1,x_4)$ for $x_2=0$

representations were made for the function $Y_{calc}=f(x_1,x_2,x_4)$ in the cases: $x_1=0$; $x_2=0$; $x_4=0$.

The graphic representations shaped as level curves make possible the accurate interpretation of the obtained results.

The necessary calculations for the determination of the coefficients, the establishment of the purpose function, as well as the estimation of the appropriateness of the model were carried out by using the programs MATLAB and WINSURF.

Testing the fungicide activity of the studied triasolic compounds

With the purpose of testing the biocide activity of the α-(*p*-chloro-phenoxy) – α- (1,2,4-triasole-1-il) acetic acid, 21 species of cellulosolitic fungi were used in pure culture isolated on art objects (paper, textile materials, mural oilpaintings, frescoes, glass, stone, ceramic).

Thus there was prepared a culture medium containing 40.25 g glucose, 3.92 g soya peptone, 0.892 g KH_2PO_4,

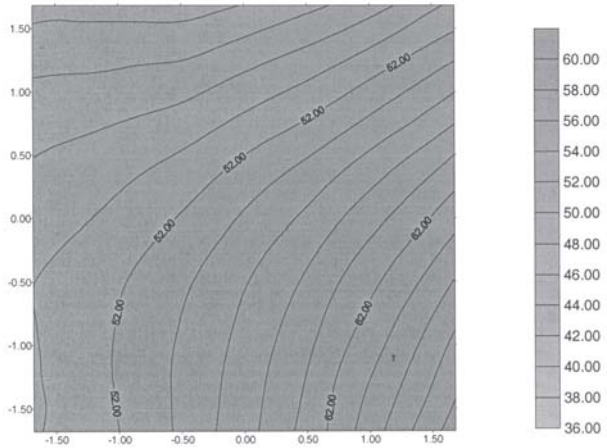

Fig. 4. Representation through isolines of the function $Y_{calc}=f(x_1,x_4)$ for $x_2=0$

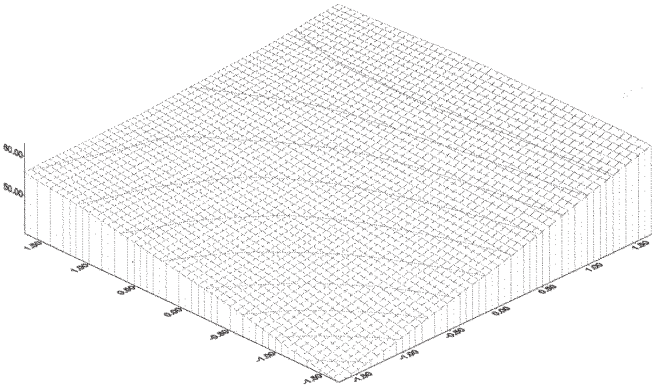

Fig.5 Spatial representation of the function $Y_{calc}=f(x_1,x_2)$ for $x_4=0$

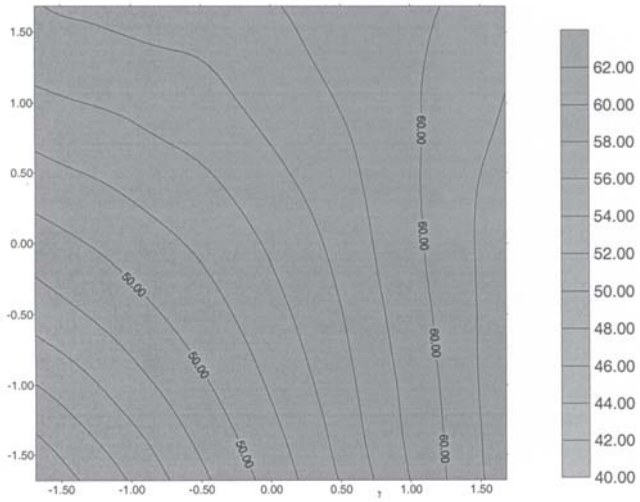

Fig. 6. Representation through isolines of the function $Y_{calc}=f(x_1,x_2)$ for $x_4=0$

agar 15 g, 1000 mL water. All components are dissolved, except for KH_2PO_4 that is dissolved separately. The two solutions are mixed, the agar is added and the whole mixture is sterilized at 121°C for 15 min.

105 solutions of biocides with the following concentrations: 0.0002, 0.0004, 0.001, 0.02, 0.2% for each compound are prepared. The sowing was carried out in sterile conditions on 23 lots of 3 Petri dishes each, with the diameter of 100 mm that contain 20 mL of optimized culture medium for each analyzed concentration of biocide.

Table 4
VARIATION OF THE GROWING OF THE FUNGI IN THE PRESENCE OF
α-(p-CHLORO-PHENOXY) - α - (1,2,4-TRIASOLE-1-IL) ACETIC ACID

Concentration of the biotic agent	Number of developed colonies					
Type of stems	Witness 0%	0.0002 %	0.0004 %	0.001 %	0.02 %	0.2 %
Aspergillus ustus	63	15	11	7	1	-
Aspergillus flavus	61	15	13	8	4	-
Aspergillus niger	63	16	10	9	3	-
Aspergillus penicilloides	62	13	10	8	1	-
Penicillium chrysogenum	56	18	15	10	3	-
Penicillium funiculosum	58	10	6	3	-	-
Penicillium frequentans	56	15	10	6	2	-
Cladosporium herbarum	49	17	9	6	1	-
Cladosporium cladasporoides	51	13	7	6	3	-
Chaetomidium globulosum	53	17	12	9	4	-
Chrysosporium panorum	47	36	32	27	12	-
Alternaria alternata	65	23	18	16	1	-
Trichoderma viride	59	17	10	9	3	-
Paecilomyces varioti	51	13	9	8	5	-
Aureobasidium pullulans	56	38	34	14	4	-
Ulocladium spp.	48	27	21	19	3	-
Myxotrichum chartarum	52	46	42	38	2	-
Stachybotrys sp.	54	16	13	9	1	-
Eurotium herbariorum	51	24	20	14	2	-
Neosartorya sp.	57	15	11	9	1	-

The observations regarding the obtained results are made by visual microscopic and photographic analysis.

Conclusions

In the case of α-(*p*-chloro-phenoxi) - α - (1,2,4-triasole-1-il) acetic acid, at a concentration of 0.2% the total inhibition of the growing of studied the 20 species the fungi occured.

The obtained results confirm the biologically active potential of the tested compound due to the fact that the presence of the microorganism.

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