THE PHYTOBIOLOGICAL TESTING OF SOME NEW THIOUREA DERIVATIVES

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Abstract

Many thioureas, some of therapeutic use, have plant-growth inhibitory activity, therefore we have considered useful the phytobiological testing of some new compounds having thiourea scaffold, synthesized from 2-thiophenecarboxylic, 3-thiophenecarboxylic and 2-thiopheneacetic acids, using Constantinescu method, *Triticum* test. It has been shown that the effect on mitosis, of the nine tested compounds, is directly proportional to the concentration of solutions and does not depend substantially on the nature and position of the substitutes on the aromatic ring. It was found also, that the most pronounced inhibitory effect was for 2-thiopheneacetic acid thioureas and the effect is decreasing in intensity for 3-thiophenecarboxilic and 2-thiophenecarboxilic derivatives.

Rezumat

Multe tioureide, unele cu utilizare terapeutică, prezintă activitate inhibitoare asupra creşterii plantelor; de aceea am considerat utilă testarea fitobiologică a unor noi tioureide provenite de la acizii 2-tiofencarboxilic, 3-tiofencarboxilic şi 2-tiofenacetic, folosind metoda Constantinescu, testul *Triticum*. S-a evidenţiat faptul că efectul asupra mitozei, al celor nouă tioureide testate, este direct proporţional cu concentraţia soluţiilor şi nu depinde semnificativ de natura substituenţilor şi de poziţia acestora pe nucleul benzenic. De asemenea, s-a pus în evidenţă faptul că efectul inhibitor cel mai intens l-au manifestat tioureidele obţinute de la acidul 2-tiofenacetic, efectul scăzând în intensitate la derivaţii acizilor 3-tiofencarboxilic şi 2-tiofencarboxilic.

Keywords: thioureas, phytobiological testing, *Triticum* test

Introduction

Knowing that in the current scientific literature the pharmacological activity has been studied (anticonvulsant [9], analgesic [20], antiviral [14], antiaggregating, antiproliferative, antiarrythmic, antihyperlipidemic and local anaesthetic [15], antitubercular [10], antibacterial [18], fungicidal [16, 17], anticancer [13]**)** of thiourea

derivatives [12, 19] and that the activity evaluation of new synthesized substances can be performed on plant cell, we have accomplished a phytobiological study, because plant material has proved many times to be useful for natural or synthesis compounds investigations [1].

Plant meristematic cells have the following advantages over those of animal origin: they are less differentiated, the functioning of plant cells is less dependent on hormonal variations and plant cell membrane is more lenient to transport various substances with which it comes in contact, observing the various cytological changes being more accurate [8].

In this article we attend to establish a relationship between the chemical structure of the studied substances and macro and microscopic changes produced by them, depending on time and concentration.

Materials and Methods

As a biological reagent embryonic roots of wheat karyopses (*Triticum vulgare* Mill, Dropia variety 2008), germinated and treated in laboratory conditions were used.

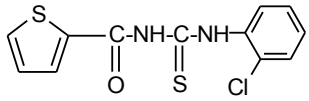
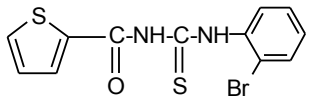
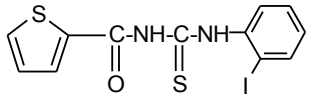
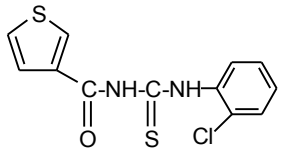
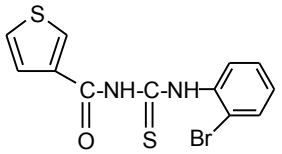
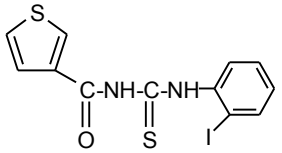
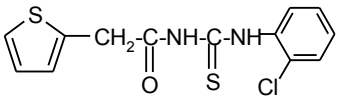
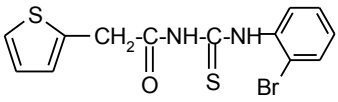
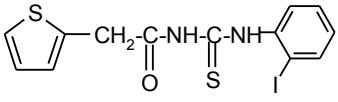
The phytobiological testing was performed using Constantinescu method, *Triticum* test. The method, devised by professor Grigore Constantinescu [11, 12] is based on the study of the influence of substances in various dilutions, on the root elongation and karyokinetic film, depending on their duration of action on the wheat (*Triticum* sp., *Poaceae*) karyopses germinated in laboratory conditions.

This testing method on wheat is convenient because wheat caryopses react immediately to the substances, allowing research in a short time (five days), while ensuring accuracy and reproducibility of the results. Also, the diploid caryotype is of 2n = 42 chromosomes, the number of chromosomes permitting to easily notice all the alterations of the mitotic film.

The structure and the chemical name of the thiourea derivatives tested, denoted S_1-S_9, are listed in table I. Synthesis and characterization of these compounds were presented previously [2-7].

Table I

Compounds studied in the phytobiological tests

Compd.	Chemical Structure	Chemical Name
2-thiophenecarboxilic acid thioureas		
S_1		N-(o-chlorophenyl)-N'-(2-thenoyl)-thiourea
S_2		N-(o-bromophenyl)-N'-(2-thenoyl)-thiourea
S_3		N-(o-iodophenyl)-N'-(2-thenoyl)-thiourea
3-thiophenecarboxilic acid thioureas		
S_4		N-(o-chlorophenyl)-N'-(3-thenoyl)-thiourea
S_5		N-(o-bromophenyl)-N'-(3-thenoyl)-thiourea
S_6		N-(o-iodophenyl)-N'-(3-thenoyl)-thiourea
2-thiopheneacetic acid thioureas		
S_7		N-(o-chlorophenyl)-N'-(2-thienylacetyl)-thiourea
S_8		N-(o-bromophenyl)-N'-(2-thienylacetyl)-thiourea
S_9		N-(o-iodophenyl)-N'-(2-thienylacetyl)-thiourea

Seed germination

Wheat caryopses selected to be as homogenous as possible were soaked with distilled water for 24 hours at room temperature (18-25^{0}C) and then were subjected to germination in Linhart pots on filter paper substrate mixed with common water until the length of main roots reached approximately 1 cm, being considered appropriate to be used in the experiment.

Sample preparation

All the compounds were tested at 10^{-3}, $0.66 \cdot 10^{-3}$, $5 \cdot 10^{-4}$, $3.33 \cdot 10^{-4}$, and $0.66 \cdot 10^{-4}$ mol/L concentrations.

Because the substances are not water soluble, the primary solutions were made by dissolving the substances in chloroform. Thus, the 10^{-3}M solutions were prepared by dissolving the substances in chloroform in volumetric flasks.

In Petri dishes with a diameter of 10 cm were added 15 mL, 10 mL, 7.5 mL, 5 mL and respectively 1 mL of the prepared chloroform solution of each compound. After evaporation to dryness on a water-bath, a volume of 15 mL of distilled water was added.

In parallel, a control sample (M) was prepared, in which the test solution was replaced by distilled water.

In each Petri box 8 caryopses of germinated wheat were placed, with the main root length of 1 cm. The dishes were covered with a lid and then the wheat karyopses were left in contact with the solutions for 5 days.

The root elongation was evaluated at the same time of the day, for 5 days. The experiments were performed in duplicate.

Macroscopic examination

To assess the roots growth in relation to the control sample, we used a linear measurement technique, estimating globally the axial growth of the main root, from the initial moment.

Microscopic examination

Microscopic examination was performed on embryonic roots of wheat, in the main meristematic roots zone, sectioned at a distance of 5 mm from the tip, after 24 hours of contact with the tested solutions. The preparations were obtained by the method of staining with diluted acetic orcein, a dye with great affinity for chromatin in acetic medium. The stained preparation were examined using a Nikon microscope Labophot II (ocular 10x, object glass 100x) by immersion in cedar oil.

Results and Discussion

The influence on the wheat caryopsis germination of the thioureas compounds is presented in figures 1-5. The data represent the average values from the two independent experiments. These data were statistically processed with *t-Student test* and all the results showed there is no statistically significant difference between the two independent experiments.

Figures 1-5 show the values of root elongation in centimeters and time of action expressed in hours for each of the five concentrations studied.

The *inhibition index* (I) on root elongation was calculated, on the measurements carried out in the 5th day, as:

$$I\,[\,\%\,] = \frac{(L_m - 1) - (L_t - 1)}{L_m - 1} \cdot 100$$

where: L_m is the average value of primary root elongation, in the 5th day, for the control sample and L_t is the average value of primary root elongation, in the 5th day, for the treated sample.

It was found that at 10^{-3}M concentration, the effect on root growth was inhibitory, with an *inhibition index* (I) in the range of 23.6 - 84.9%. Compound S_1 did not produce significant modifications compared to the control sample.

Results are presented in figure 1.

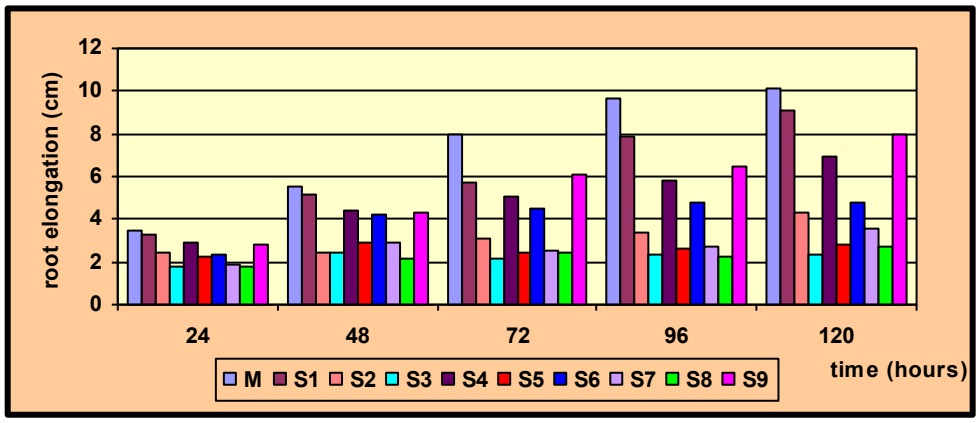


Figure 1
Daily root elongation in *Triticum* test under the treatment with S_1-S_9 compounds at 10^{-3}M concentration

At the $0.66 \cdot 10^{-3}$ M concentration the effect on root growth was also inhibitory, with an *inhibition index* (I) of 13.3 - 55%. Results are presented in figure 2.

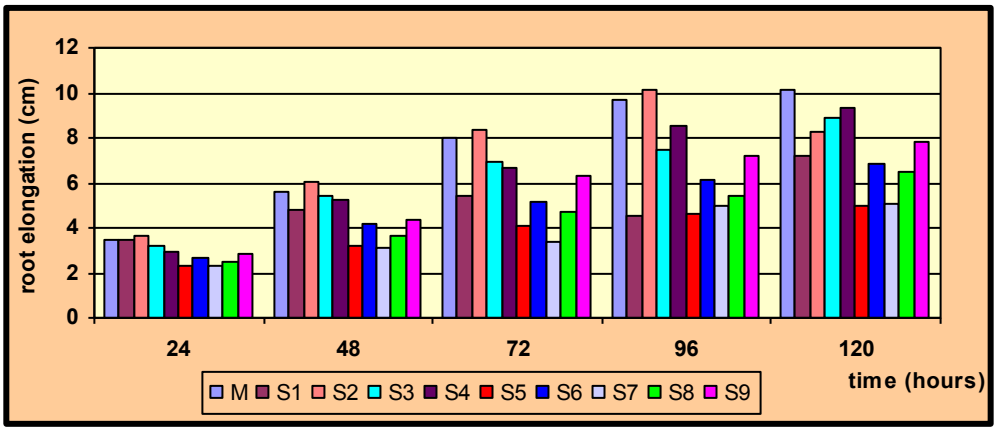


Figure 2
Daily root elongation in *Triticum* test under the treatment with S_1-S_9 compounds at $0.66 \cdot 10^{-3}$M concentration

At the $5 \cdot 10^{-4}$ M concentration the effect on root growth was inhibitory (around 38%), compared with the control sample. Substances S_1, S_2, S_4 and S_6 showed no significant changes compared to control sample. figure 3 shows these results.

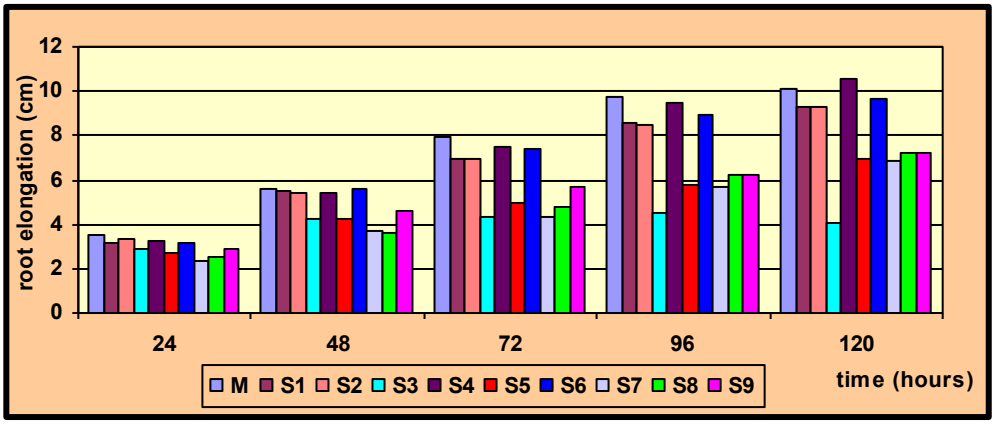


Figure 3
Daily root elongation in *Triticum* test under the treatment with S_1-S_9 compounds at $5 \cdot 10^{-4}$M concentration

At the $3.33 \cdot 10^{-4}$M concentration the effect on root growth was inhibitory, except substances S_2, S_4, S_5 and S_6 whose effects were similar to those of the control sample. These results can be seen in figure 4.

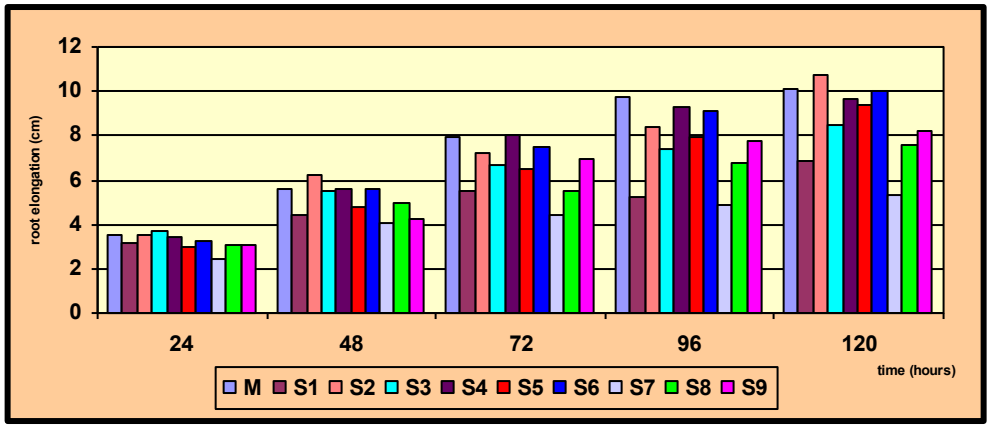


Figure 4
Daily root elongation in *Triticum* test under the treatment with S_1-S_9 compounds at $3.33 \cdot 10^{-4}$M concentration

At the $0.66 \cdot 10^{-4}$M concentration substances S_1, S_2, S_3, S_4 and S_8 did not produce significant changes compared to control sample. The other substances had inhibitory effects on root growth, higher in the case of S_6 (Figure 5).

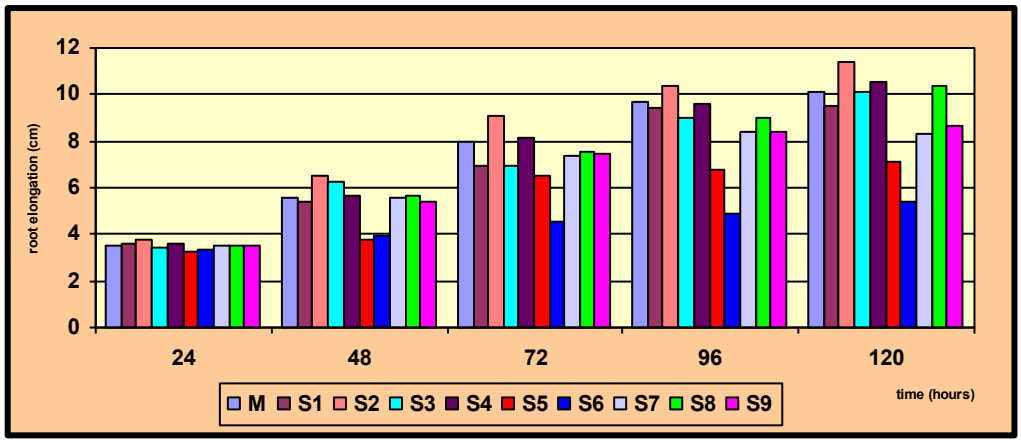


Figure 5
Daily root elongation in *Triticum* test under the treatment with S_1-S_9 compounds at $0.66 \cdot 10^{-4}$M concentration

As can be seen from the results presented above, that the inhibitory effect on root growth of the thiourea derivatives is the most intense at the highest concentration (10^{-3} M).

For lower concentrations ($5•10^{-4}$M, $3.33•10^{-4}$M and $0.66•10^{-4}$M) some substances determined a similar elongation with the control sample. In some cases it can be seen even a slightly stimulating effect of growth, evident in the presented figures.

As for the action in the three series of used acids (2-thiophenecarboxylic, 3-thiophenecarboxylic and 2-thiopheneacetic), it was found that the most pronounced inhibitory effect on root growth, at all concentrations, occur for the compounds of the 2-thiopheneacetic acid series, especially for substances S_7 and S_8.

In the case of 2-thiophenecarboxylic acid thioureas, it was found that the most inhibitory effect on the root growth is presented for the compound S_3 (N-(o-iodophenyl)-N'-(2-thenoyl)-thiourea), with an inhibitory index of 85%. All the compounds in this series present a root elongation similar to that of the control sample at $0.66 • 10^{-4}$M concentration, even a slightly stimulating effect of growth for the S_2 (N-(o-bromophenyl)-N'-(2-thenoyl)-thiourea).

For 3-thiophenecarboxylic acid thioureas series, the S_5 (N-(o-bromophenyl)-N'-(3-thenoyl)-thiourea) substance presented the highest inhibitory effect at all concentrations. The lowest inhibitory effect on cell division was shown by S_4 (N-(o-chlorophenyl)-N'-(3-thenoyl)-thiourea) which at $5 • 10^{-4}$ M and $0.66 • 10^{-4}$M concentration presented a slightly stimulating effect on growth. It was noted that in this series all the compounds have shown at the concentration of $3.33 • 10^{-4}$M, a similar effect to that of the control sample.

From 2-thiopheneacetic acid series the most active thioureas were S_7 (N-(o-chlorophenyl)-N'-(2-thienylacetyl)-thiourea) and S_8 (N-(o-bromophenyl)-N'-(2-thienylacetyl)-thiourea) in all concentration. The inhibitory index at 10^{-3}M was 80.98% for S_8 and 72.08% for S_7.

The microscopic examination of the wheat embryonic roots confirmed a mito-depressant effect of the substances at high concentrations. We found an inhibitory effect induced by most solutions of the studied substances upon kinesis and changes in the mitotic film of type: wavy membranes, hypertrophied nucleoli, pseudometaphases, lobed nuclei, telophasic bridges, tropokinesis in telophase and in metaphase. These results are depicted in figures 6-11.

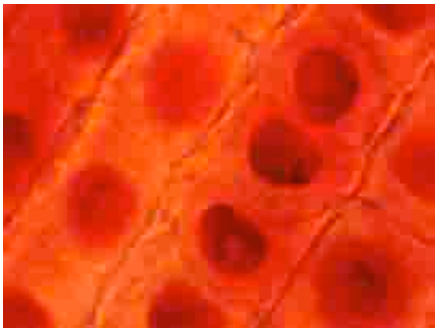


Figure 6
Wavy membranes, hypertrophied nuclei (interphase)

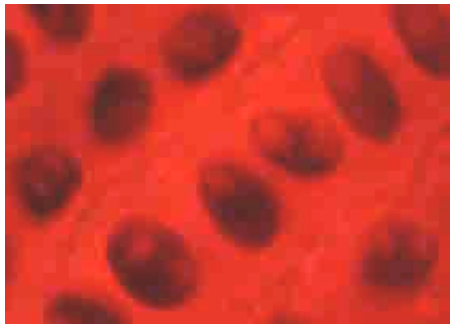


Figure 7
Hypertrophied nuclei

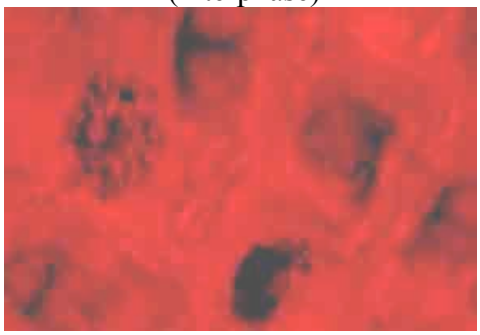


Figure 8
Disorganized methaphase

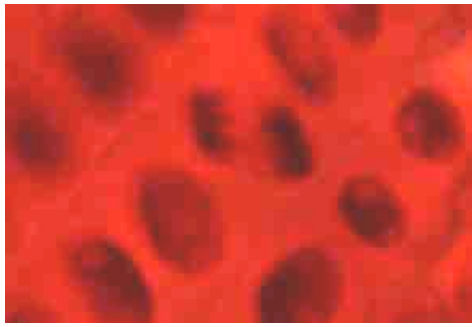


Figure 9
Tropokinesis in anaphase

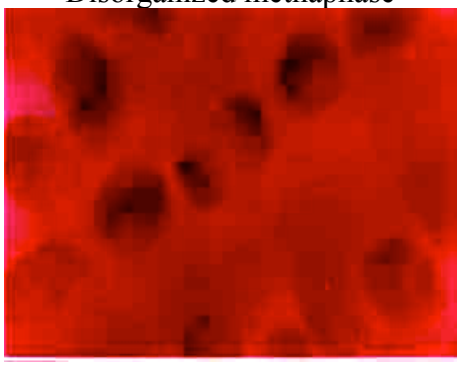


Figure 10
Thelophasic bridges

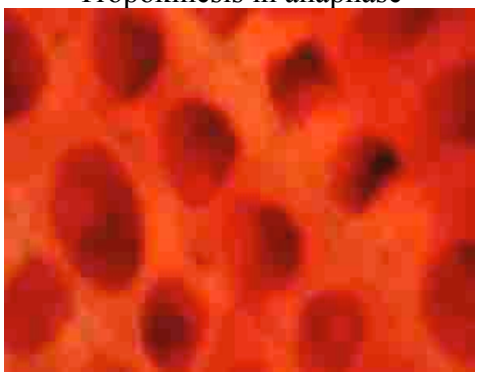


Figure 11
Tropokinesis in telophase

Conclusions

We have performed a phytobiological testing of nine newly synthesized thiourea derivatives using *Triticum* test, Constantinescu method.

All the compounds were tested at 10^{-3}, $0.66 \cdot 10^{-3}$, $5 \cdot 10^{-4}$, $3.33 \cdot 10^{-4}$, and $0.66 \cdot 10^{-4}$ mol/L concentrations. The substances have an inhibitory effect on root growth, the most intense being at the concentration of 10^{-3}M. It was found that the intensity of action of the studied compounds on the root elongation is dependent on solution concentration.

The microscopic examination confirmed all results of the macroscopic tests, showing cytotoxic or mito-depressant effect of the studied thiourea derivatives, less obvious for solutions with a concentration of $0.66 \cdot 10^{-4}$M.

Correlating the occurrence of inhibitory effects on cell division to the structure of the studied substances, it was found that the most pronounced inhibitory effect on root growth at all concentrations (except at $0.66 \cdot 10^{-4}$M) was for 2-thiophenacetic acid thioureas, decreasing in intensity for 3-thiophenecarboxilic and 2-thiophenecarboxilic derivatives.

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Manuscript received: November 14th 2012