SYNTHESIS AND THE EFECT OF SOME PYRIDAZINE DERIVATIVES IN GERMINATION AND SEEDLING GROWTH OF WHEAT

SINTEZA ŞI EFECTUL DERIVAŢILOR PIRIDAZINICI ASUPRA GERMINAŢIEI ŞI CREŞTERII PLANTELOR DE GRÂU

***TUCALIUC Roxana*[1], *COTEA V. V.*[1], *DROCHIOIU G.*[2], *MANGALAGIU I.*[2]**
e-mail: roxanatucaliuc@yahoo.com

Abstract. *1,3-Dipolar cycloaddition is one the most important methods of constructing the diazinic derivatives. Several studies to test the biologic effect of pyridazine derivatives have been done using simple experiments of wheat germination/ develop of the wheat plantlets. The results showed that the influence for tested compounds in germination percentage, shoot and root length, fresh weight varied as a function of structure of each investigated compound.*

Key words: pyridazine compounds, biological activity, wheat, germination, total height.

Rezumat. *Reacţiile de cicloadiţie 1,3-dipolare sunt cea mai convenabilă metodă pentru obţinerea derivaţilor diazinici. Pentru a testa efectul biologic al derivaţilor de piridazină, au fost efectuate diferite studii, folosind experimente simple de germinare a seminţelor de grâu / dezvoltare a plantulelor de grâu. Conform rezultatelor, activitatea compuşilor testaţi în procesul de germinare, lungimea rădăcinii şi greutatea plantulelor în stare proaspătă a variat în funcţie de structura fiecărui compus investigat.*

Cuvinte cheie: derivaţi piridazinici, activitate biologică, seminţe de grâu, germinare, înălţimea totală a plantelor.

INTRODUCTION

The bioactives substances are used in the treatment seeds, planting or on the field during the growing season. These substances are synthesis products and have chemical structure similar with hormones present in plant (auxine inhibitors, gibberellinic) excepting retardants, which are not synthesized in plants, but are produced by synthetic processes

Pyridazine compounds are commonly used as anticancer (Rodriguez-Ciria et al., 2003) antituberculosis (Butnariu et al., 2007) antihypertensive (Gokçe, 2001) antifungal (Dodge, 1989) or antimicrobial (Druţă et al., 2002) agents due to their intense biological activity. Also, they have a rapid systemic effect on the plants and are active at very low concentration (Butnariu (Tucaliuc) et al., 2008; Tucaliuc et al., 2010). The dates presented in the literature demonstrates the possibility to use

[1] University of Agricultural Sciences and Veterinary Medicine Iaş i, Romania
[2] "Alexandru Ioan Cuza" University of Iaşi, Romania

the pyridazine derivatives in growth and development of horticultural plants or as potential practical applications as insecticides, herbicides and pesticides (Rişcă et al., 2006; Mangalagiu et al., 2005).

In this manner, the work is focused to go on with the study (Butnariu (Tucaliuc) et al., 2008; Tucaliuc et al., 2010) and proposes to wide the products array used in treatment of wheat, specifically the use of azaheterocyclic compounds with 1,2 diazinic structure substituted with fluor. Targeted compounds are azaheterocycles with fluor, because since 1950 the interest for organic compounds with fluor is in accession, due to potential applications in medicine and biochemistry (Filler, 1986).

In 1970, only 2% from this derivatives were as pharmaceutically products, but nowadays the statistics are above 21%. Also, many compounds with fluor are used in complementary area, e.g. agro-chemistry where the diagram is increasing, furthermore 32%.

Therefore, we expect that the products we have obtained to present possible practical applications as biologically active compounds and for this reason we tested their effect on germination and growth plants.

MATERIAL AND METHOD

Synthesis of pyridazinic coumponds (fig. 1) was made in the classical method to obtain bicyclic adducts (cycloaddition reaction): quaternization of nuclear nitrogen, which in alkaline medium (triethylamine) generated the ylide in situ and cycloaddition of ylide to corresponding dipolarophile (Butnariu (Tucaliuc) et al., 2008; Tucaliuc, 2008; Zbancioc et al., 2010).

After purification, the structure of the compounds was proved by elemental and spectral analysis (FT-IR, MS, 1H NMR, 13C NMR, and two-dimensional experiments 2D-COSY, 2D-HETCOR (HMQC), long-range 2D-HETCOR (HMBC).

The following compounds were used in this study:

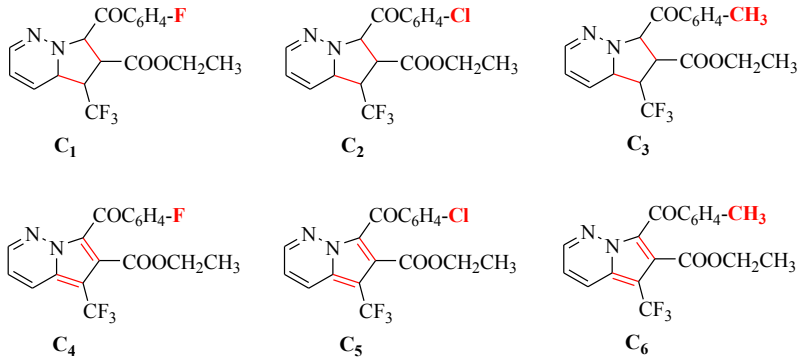

Fig. 1 - The structure of pyridizinic compounds tested.

Germination tests were performed in a growth chamber Conviron MP4030 model G30 with programmed temperature, humidity and light. We used seed samples of wheat (*Triticum aestivum*), with specific weight 37.2 g / 1000 seeds.[17] The procedures are simple, cheap and are considered a good way to test toxicity of new compounds.

For the experiment, the determinations were realized in triplicate or duplicate, with 20 seed samples of wheat. Each sample was treated with 5 mL of $5 \cdot 10^{-3}$ M solutions of pyridazine derivatives obtained, in paralleled with a redistilled water blank (**B**). Initially, the seed with analyzed solutions, were shaken in the tubes, at short time interval, for one hour. Then, the seeds with their treatment solutions, were taken out and put into Petri dishes on double filter paper together. The samples were maintained in the growth chamber at constant temperature and humidity regimes (21°C and 95%, respectively) and under illumination (12h/24h) until embryo elongation (hypocotyls and ridicules) was establish.

The seeds were periodically watered and the percent of germinated seeds were reported 3 days later (germination rate, **GR**). A seed with visible coleorhizae was considered germinated. On the 7-th day were harvest of young wheat plants, from their seeds, was measured height (**H**, expressed as cm) and weight (**W**, expressed as grams).

The dates were validated using the Tukey test, (Snedecor, 1994) with a probability of 95 %.

RESULTS AND DISCUSSIONS

The cycloadducts might be considered as classical bioisosteres due to the presence of F, Cl and CH_3 substituents. (Silverman, 1992) Considering the pyridazine – acetophenone skeleton (fig. 2) as the pharmacophoric active group, we tested the biological activity of some pyridazine derivatives on wheat germination and seedling growth, having in mind two structural modifications: introduction of a pyrrolo (I) ring, and a classical isosteres substitute R (R= F, Cl, CH_3) in the para- position of the benzoyl system.

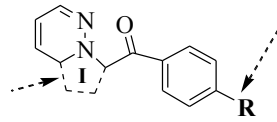

Fig. 2 - The pzridayine-acetophenone skeleton.

Experiments were conducted to determine the biological activity of the diazinium derivatives on the germination of spruce seed. The obtained results demonstrated that the main influence factor in germination percentage (the 3rd day of treatment), root length and weight of seedlings fresh (the7th day of treatment), is dependent with the structure for each investigated compound (table 1 and table 2).

Fig. 2 – The first day of treatment.

Fig. 3 - The 3rd day of treatment.

Fig. 4 - The 7rd day of treatment

Pyridazine derivatives tested did not show a toxic action in wheat seed germination and did not inhibit the growth / development of wheat seedlings.

Table 1

The effect of pyridazine derivatives on wheat germination (RG).

Comp.	Germination Rate (GR, %)	Number of plantlets	Comp.	Germination Rate (GR, %)	Number of plantlets
C1	14 ± 4	11 ± 1	**C4**	17± 6	15 ± 1
C2	13 ± 5	11 ± 1	**C5**	18 ± 4	15 ± 2
C3	14 ± 4	12 ± 1	**C6**	17 ± 3	15 ± 1
B	14 ± 2	10 ± 1	**B**	14 ± 2	10 ± 1

The structure of the tested piridazinic derivatives has an important role in wheat germination. Thus, for the azaheterocycles compounds **(C_1-C_3)**, with dehydrogenated pyrrolo ring, the values for germination rate are comparable with values for blank sample. Instead, the pyridazine derivatives **(C_4-C_6)**, with complete aromatized pyrrolo ring, influenced the germination process of the wheat seeds. According with results, this pyridazine derivatives presented relatively higher values for germination rate. Thus, the treatments with compound **C_5** slowly activated the number of plantlets obtained from the 20-seed lots (from 10 ± 1 (blank) to 15 ± 2 in the case of the compound **C_5)**. (Table 1)

In addition, both height and weight of the plantlets in the lots increased accordingly (table 2). The compound **C_5** induced the height of the lot from 82.5 cm (blank) to 87.5 cm, and the weight from 0.58 g to 0.67 g.

The total height of the plantlets, **H**, was a more effective parameter to show the effect of the investigated compounds on wheat germination. Whereas, the **H** value for the blank sample was 82.5 cm, the solution of compound **(C_4-C_6)** slowly influenced the height of the plantlets in the treated lots - have the total height of the lot more than 50% of the blank sample (table 2).

Table 2

The effect of pyridazine derivatives on wheat germination and seedling growth (the total height and the mean height of plantlets in the lot (H, Hmed), (W, Wmed))

Comp	H (cm)	H_{med} (cm)	W (g)	W_{med} (mg)
C1	83,8 ± 11,72	8,6 ± 0.01	0,64 ± 0,01	42.52 ± 13.30
C2	82,9 ± 10,36	7,9 ± 0.7	0,63 ± 0.30	43,60 ± 4.79
C3	83,4 ± 10,42	7,8 ± 0,5	0,63 ± 0,16	42,65 ± 4.35
C4	85,0 ± 10,87	8,0 ± 0.6	0.68 ± 0.15	45,84 ± 0,01
C5	87,5 ± 10,18	9,0 ± 0.9	0.67 ± 0.19	44,45 ± 2.14
C6	86,5 ± 10,18	8,0 ± 0.3	0,69 ± 0,14	45,86 ± 3,69
B	82,5 ± 10,93	7,8 ± 0.3	0.58 ± 0.08	40.32 ± 3.36

The investigated compounds with aromatized structure acted present a different and unknown mechanism in germination and development of wheat seedlings

The research will be continue on other azaheterocyales compounds with similar structure and functional groups with increase reactivity.

CONCLUSIONS

According to the obtained results, the tested pyridazine derivatives may influence germination rate, shoot and root length and fresh weights, as a function of structures on investigated compounds. The compounds **(C_4-C_6)** presented a stimulatory effect in the growth and development of wheat plants, while the compounds **(C_1-C_3)**, with values comparable with blank sample, were inerts.

The most active pyridazinic derivative of the series was compound (**C_5**). It presented benefic effect on plant growth and development.

Nevertheless, additional research is required to assess the impact of pyridazine derivatives on germination and development of agricultural plants.

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