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Effect of some new monoquaternary salts of diazine on germination and seedling growth of Norway spruce (*Picea abies* (L.) Karsten)

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Abstract

*Studies to test the effect of some new monoquaternary salts of diazine on germination and seedling growth of Norway spruce were conducted in controlled temperature room and results showed that germination percentage, shoot and root lengths and fresh weights varied as a function of concentration and structure of each investigated compound. Thus, lots of 50 seed samples of spruce were treated with $5\cdot10^{-5} - 1\cdot10^{-3}$ molar solutions of diazinium halides (**S_1-S_3, S_5**) or diazinium ylides (S_4) for 1 hour. A blank with bidistilled water was also carried out. After a 21 day period of germination in the presence of the investigated compounds, the spruce hypocotyls and radicles were cut from the seeds, and their length measured. Higher concentrated solutions of the investigated compounds exhibited highly inhibitory activity on spruce germination, while the lower concentrations resulted in a stimulating action, especially for the radicles length. All results are statistically validated.*

Keywords**:** monoquaternary salts; diazine; Norway spruce; germination rate.

Introduction

In the previous papers [1-3] new monoquaternary salts of 4,4'- bipyridyl, 1,10-phenanthroline or 2,2'-bipyridyl have been tested within germination experiments. The investigated compounds proved to be phytotoxic agents killing plant cells [4]. Such species are commercially available as herbicides and weed killers and may also be found within certain formulations for selective clearing of paths or in combination pesticides. Higher plants are recognized as excellent indicators of cytogenetic and mutagenic effects of some chemicals and are applicable for the detection of environmental noxious compounds [5-7]. The germination test determines the actual germination potential of normal seeds within a seed lot, which can be used to compare the quality of different lots, and also estimate the field planting value. The aim is to determine the optimum germination capabilities of the seeds under ideal conditions. Nevertheless, germination tests are very simple, little time consuming, cheap and, therefore, could be ideal methods for testing the biological activity of some new synthesized compounds, including the monoquaternary salts and diazinium ylides. Some new synthesized diazine derivatives were used in many research fields due to their structure, stability and reactivity as well as to their tendency to form stable ylides with important biological properties [8]. They have a rapid systemic effect on the plants and are active at very low concentration. Some pyridazine compounds have antimicrobial and antifungal activity as well [9,10]. In addition, similar chemical structures could be found in cells related to the biological reaction pathways. For example, phenoxyacetic or indolylacetic acids are potent stimulators of seed germination. Besides, some of the investigated diazine salts are similar structures to these phytohormones. On the other hand, as a rule, a cytotoxic effect of relatively high

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concentrations of monoquaternary salts is related to a stimulating effect of lower concentrations. Some monoquaternary diazinium salts were previously synthesized by I. Mangalagiu *et al.* [8] using Kröhnke method [11]. Therefore, this paper reports their biological activity on wheat germination and seedling growth.

Materials and Methods

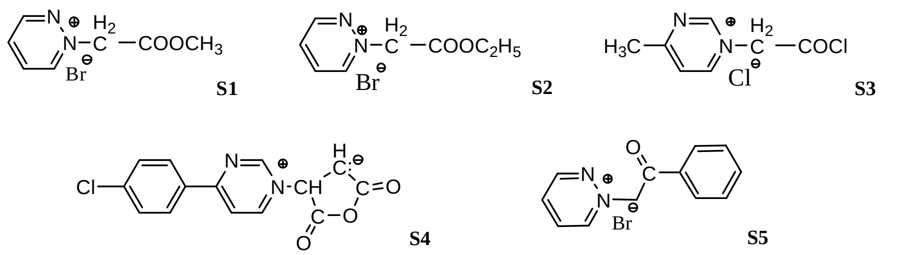
Apparatus. The germination test was performed in a growth chamber Conviron MP4030 model G30 with programmed temperature, humidity and light.

Biological material. Seed samples of Norway spruce (*Picea Abies* (L.) Karsten), 37.2 g / 1000 seeds, was purchased from the Moldovita area, Romania, (yield 2003) for which the germination rate was measured.

Treatment solutions. The diazinium monoquaternary salts were previously prepared by treatment of pyridazine (S1, S2 and S5) and pyrimidine (S3) with reactive halides derivatives according to Kröhnke's method [11] The pyrimidinium ylide (S4) was prepared by treatment of corresponding pyrimidine derivative with unsaturated anhydride (maleic anhydride) [12]. Elemental analysis and IR, ^{1}H-NMR spectra proved their structure.

First evaluations were conducted on three replicates of 50 seeds at six treatments. Seeds were collected from plants growing in forests, and were then stored in a dry chamber at 15°C. Each seed lot was characterized for dried weight (12%). Surface sterilized (commercial hypochlorite 67.2 mmol kg^{-1} for 10 min) seeds were treated with 5 mL of $5\cdot10^{-5}$-10^{-3} molar solution of diazinium derivatives (**S_1-S_5**) for 1 hour, and sown in Petri dishes on filter paper together with the treatment solutions. Seed imbibition was initiated in the test tubes by adding 5 mL of distilled water (Blank, **B**) or diazine salt solutions. Seeds 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. A seed with visible coleorhizae was considered germinated.

The following compounds were used in this study:



1-(methoxycarbonylmethyl)-pyridazinium bromide (**S_1**);
1-(ethoxycarbonylmethyl)-pyridazinium bromide (**S_2**);
1-(2-chloro-2-oxoethyl)-4-methylpyrimidinium chloride (**S_3**);
1-[4-(4-chlorophenyl)-pyrimidinium]-1,2-dicarboxyandydro-ethylide (**S_4**);
1-(phenacyl)-pyridazinium bromide (**S_5**).

Figure 1. Some monoquaternary salts of diazine derivatives tested on spruce seed

Procedure. Fifty seed samples of spruce were treated with 5 mL of each $5\cdot10^{-5}$ - $1\cdot10^{-3}$ molar solutions of **S_1-S_5** for 1 hour. A blank with bidistilled water was also carried out (**B**), and all the determinations were performed in triplicate. Then, the seeds were taken out and put into Petri dishes on double filter paper together with their treatment solutions. The seeds

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were periodically moistened and the percent of germinated seeds was reported after 21 days (the germination rate, **GR**). Young spruce plants were harvested from their seeds and measured (hypocotyls, H_H and radicles, H_R, expressed as cm).

Statistics. The data were validated by the poly-factorial variance analysis [13].

Results and Discussion

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 is the concentration, namely the increasing $\mathbf{S_1} – \mathbf{S_5}$ concentration inhibited the length of the radicles and hypocotyls and also the germination rate of spruce seeds (**Tables 1, 2 and 5**).

According to the variance analysis (**Tables 2 - 6**), the diazinium derivatives produce the elongation of the radicles, especially for the S_3 and S_5 compounds (**Table 6**). This effect can be very useful in order to allow a better and quicker fixation of the spruce plantlets in the soil.

As for the hypocotyls length and the germination rate, insignificant differences between the values were observed.

Table 1. Average values (hypocotyls, radicle length, and the germination rate, **GR**) for Norway spruce as an effect of the treatment with $\mathbf{S_1}$- $\mathbf{S_5}$ compounds ($\mathbf{C_1}$=1·10^{-3} M, C_2=5·10^{-4} M, C_3=1·10^{-4} M, C_4=5·10^{-5} M) against water (**B)**

Com-pound	Hypocotyls length (mm)				Radicles length (mm)				GR, %			
	C_1	C_2	C_3	C_4	C_1	C_2	C_3	C_4	C_1	C_2	C_3	C_4
S_1	29,86	37,93	27,06	30,63	12,36	25,43	16,23	28,13	50,33	54	45,32	63,32
S_2	29,53	33,33	28,70	39,23	12,36	29,9	23,06	26,40	62,66	64	58,66	57,32
S_3	29,70	35,36	31,16	38,46	16,03	33,03	23,00	31,23	48	60	62,66	64,66
S_4	31,80	36,33	36,50	31,1	8,43	26,76	22,33	26,30	36	55,32	48	56
S_5	29,06	36,10	30,20	31,60	10,36	33,50	20,56	29,53	48	52,66	64	62,66
B	33,16	33,16	33,16	33,16	20,73	20,73	20,73	20,73	56	56	56	56

The dispersion of the values proved to be high and we considered it as an effect of using many genotypes of the spruce seeds, although they were from the same year and location.

Table 2. Variance analysis of the hypocotyls

Variability source	Squares sum	Degrees of freedom	Variance	Value of F test	Significance
TOTAL	1226.24	71			
Repetitions	10.12	2			
A= Concentration	289.77	3	96.59	30.824	***
Error of A	18.80	6	3.13		
B= Compounds	64.05	5	12.81	1.196	-
AB	414.92	15	27.66	2.582	*
Error of B	428.55	40	10.71		

DL_A 5% = 1.44; DL_A 1% = 2.18; DL_A 0.1% = 3.51; DL_B 5% = 2.70; DL_B 1% = 3.61; DL_B 0.1% = 4.74

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Seed germination increased gradually as diazinium derivatives concentration decreased. (**Table 1**). Nevertheless, higher dilutions did not show any significant activity. Thus, we proved that $\mathbf{S_2}$, $\mathbf{S_3}$ and $\mathbf{S_5}$ show a stimulating effect on germination rate of the spruce seeds.

Table 3. Average values for the length of hypocotyls due to the treatments

Graduation	Average values (mm)	Differences(+/-)	Significance
B	33.16	-	-
S_1	31.37	- 1.79	-
S_2	32.70	- 0.46	-
S_3	33.67	+ 0.51	-
S_4	33.93	+ 0.77	-
S_5	31.74	- 1.42	-

Table 4. Average values of the hypocotyls length due to the concentration influence

Graduation	Average values (mm)	Differences(+/-)	Significance
B	33.16	-	-
C_1	30.52	- 2.64	00
C_2	35.37	+ 2.21	**
C_3	31.13	- 2.03	0
C_4	34.03	+ 0.87	-

There was found an interesting relationship between the structure of the investigated compounds and their biological activity as the data in this paper demonstrated. On contrast with some compounds having a p-nitrophenacyl group, which have toxic properties [5], the investigated substances in this work cannot act as metabolic inhibitors.

Table 5. Variance analysis of the radicles

Variability source	Squares sum	Degrees of freedom	Variance	Value of F test	Significance
TOTAL **Repetitions**	3699.80 13.68	71 2			
A= Concentration **Error of A**	2500.70 28.56	3 6	833.56 4.76	175.080	***
B= Compounds **AB** **Error of B**	258.18 687.61 211.05	5 15 40	51.63 45.84 5.27	9.786 8.688	* **

DL_A 5% = 1.77; DL_A 1% = 2.69; DL_A 0.1% = 4.33; DL_B 5% = 1.89; DL_B 1% = 2.53; DL_B 0.1% = 3.32.

Nevertheless, the investigated compounds acted by a completely different and unknown mechanism. We can only hypothesize that the investigated diazinium derivatives might create a non-specific stress, against which the living seedling reacts by intensifying its metabolism. At higher concentrations, the plantlets fail to release the alien compounds.

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Table 6. Average values of the radicle's length due to the compounds influence

Graduation	Average values (mm)	Differences(+/-)	Significance
B	20.73	-	-
S_1	20.54	- 0.19	-
S_2	22.93	+ 2.20	*
S_3	25.82	+ 5.09	***
S_4	20.95	+ 0.22	-
S_5	23.49	+ 2.76	**

Further investigation is necessary to establish the correct mechanism of action of diazinium derivatives studied here.

Conclusion

The effect of some new monoquaternary diazinium salts on germination and seedling growth of Norway spruce were investigated. Germination rate, the length of hypocotyls and radicles varied as a function of concentration and structure of each investigated compound. Some derivatives of 1-(methoxycarbonylmethyl)-pyridazinium bromide (**S_1**); 1-(ethoxycarbonylmethyl)-pyridazinium bromide (**S_2**); 1-(2-chloro-2-oxoethyl)-4-methylpyrimidinium chloride (**S_3**); 1-[4-(4-chlorophenyl)-pyrimidinium]-1,2-dicarboxyanhydro-ethylide (**S_4**); 1-(phenacyl)-pyridazinium bromide (**S_5**) that were investigated in this paper act specifically on Norway spruce germination causing also changes of the height and weight of the resulted plantlets, depending on the concentration and the type of compound. The strongest stimulatory effect is observed in the case of 1-(2-chloro-2-oxoethyl)-4-methylpyrimidinium chloride. All the obtained results are statistical validated.

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