

Plant Growth Regulating Activity of Some *N*-Substituted Chloroacetylanilines

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ABSTRACT: Purpose. The synthesis and physico-chemical characterization of six *N*-substituted chloroacetylanilines and testing their plant growth regulating activity. Material/Methods. The synthesis of the six *N*-substituted chloroacetylanilines was accomplished by condensation of *N*-substituted anilines, in an acidic medium, with chloroacetylchloride. Purified compounds obtained were physico-chemical characterized by elemental analysis and spectral analysis. Five different concentrations (0.1%, 0.5%, 0.75%, 1% and 5%) of the compounds solubilized in chloroform were used to analyze their effects on the germination and mainly on the radicular elongation of wheat caryopses, *Triticum aestivum* subsp. *aestivum* (Poaceae), Dropia variety. Results. The *N*-substituted chloroacetylanilines were solid, differently colored, with high melting temperatures and high yields. Their structure was confirmed both by elemental analysis and by the spectral methods (UV-Vis, FTIR, ¹H-NMR, ¹³C-NMR, GC-MS). Conclusions. For the six analyzed compounds, at five different concentrations (0.1%, 0.5%, 0.75%, 1% and 5%), the experimental data obtained by the method of linear measurement, in the *Triticum* assay, showed the inhibition of mean radicular elongation compared with the reference.

KEYWORDS: *N*-substituted chloroacetylanilines, melting point, yield, *Triticum* assay

Introduction

N-substituted chloroacetylanilines are an important class of herbicides used to control grass weeds in various crops [1, 2].

2-Chloro-*N*-2,6-diethylphenyl-*N*-(methoxymethyl) acetamide (Alachlor) is a well-known pre- and post-emergence herbicide from the *N*-substituted chloroacetylanilines family, and is commonly used to control the annual grasses and many broad-leaf weeds in crops [3, 4]. This compound was incriminated as carcinogen and was known to be a highly toxic endocrine disrupting chemical and the permissible maximum concentration in drinking water is 20 µg/L. Alachlor is a chlorine endocrine disruptor and its toxic and genotoxic properties may cause cancer and mutagenicity in laboratory animal or contribute to infertility [5–8].

The adverse effects of exposure to pesticides on the population, and specifically on the more susceptible groups such as infants and children, are a public health concern [9]. The population is exposed to pesticides mainly through diet and

through household use of pesticides [10]. Inhalation of polluted air could also be a relevant exposure pathway, mainly for those working or living near agricultural areas [11–13].

2-Chloro-*N*-(ethoxymethyl)-*N*-(2-ethyl-6-methylphenyl) acetamide (Acetochlor) is widely used for pre-emergent control of grasses and broadleaf weeds [14] is the third most frequently detected herbicide in agricultural watersheds [15]. Its main metabolite generates reactive oxygen species, which may have been crucial to apoptosis-mediated cytotoxicity [16, 17].

N-substituted chloroacetylanilines can also be used as intermediates in the synthesis of thermotropic nematic and smectic liquid crystals, which are used in surface thermography in the diagnosis of different types cancers [18].

According to data from the literature [19], we synthesized six *N*-substituted chloroacetylanilines by condensation of some *N*-substituted anilines with chloroacetylchloride, in an acidic medium as presented in Fig.1.

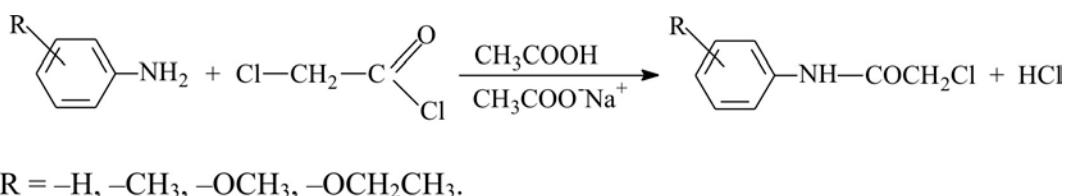


Fig.1. Synthesis reactions of the *N*-substituted chloroacetylanilines

Material and methods

Synthesis

Synthesis of *N*-substituted chloroacetylanilines was realized by stirring one hour, using glacial acetic acid to create an acidic medium, at cold to avoid possible secondary reactions.

Solid state reaction products, coloured from white to grey and yellow to light brown, were obtained.

The mixture of *o,m,p*-xylenes was used as solvent for purifying the reaction products. The presence of a single chromatographic peak confirmed their purity.

Synthesis of *N*-chloroacetylaniline

In a reaction flask equipped with a stirrer were introduced 45.607 ml (0.5 moles) of aniline and a minimum quantity of glacial acetic acid (held prior to cold) required for the dispersion of the amine (if the amine is solid dissolution it is not absolutely necessary beforehand).

Under continuous stirring was added drop by drop chloroacetyl chloride. If the addition of the chloroacetylchloride solidified the reaction mixture then was added glacial acetic acid for fluidization.

The reaction is highly exothermic so was necessary cooling the system in an ice bath, to avoid some secondary reactions.

After termination of the acylating agent was added 68.04 g (0.5 moles) of sodium acetate saturated solution and stirring was continued for another 30 minutes.

Solidification of the reaction mass was due to the formation of *N*-chloroacetylaniline and relatively low working temperature (0–4°C).

At the end of the reaction we added a large amount of water, followed by filtration through Büchner funnel and washing the precipitate with water in order to remove acetic acid and its sodium salt.

Handling precipitate was done very carefully because *N*-substituted chloroacetylanilines are

particularly irritating to skin and mucous membranes.

Towards the end of the filtering, the precipitate was treated with a small amount of absolute methanol to facilitate the removal of water therefrom.

The mixture of *o,m,p*-xylenes was used as solvent for purifying the reaction product.

The melting point: 123°C. Yield: 88%.

The synthesis was similar for all *N*-substituted chloroacetylaniline.

Triticum assay

Concerning the phytobiological analysis, the action of five different concentrations (0.1%, 0.5%, 0.75%, 1% and 5%) of the compounds (1)–(6), solubilized in chloroform, was studied on the germination and mainly on the radicular elongation of wheat caryopses, *Triticum aestivum* subsp. *aestivum* (*Poaceae*), Dropia variety. After filtration of the chloroform solutions, 1 mL of each sample was brought in five Petri dishes. The solvent was allowed to evaporate over 24 hours. The analysis was performed compared to a reference consisting, as samples, of 10 pre-germinated wheat caryopses with 10 mm radicle, coated at 12 hours interval in 2 mL freshly boiled and cooled tap water [20].

Reagents

Chloroacetylchloride, aniline, and *p*-methylaniline, *o*-methylaniline, *p*-methoxyaniline, *p*-ethoylaniline, *o*-ethoylaniline, acetic acid, sodium acetate, *o,m,p*-xylenes, methanol, chloroform used in the synthesis were Fluka or Merck commercially available products.

Equipments

The melting points were established with a Boetius apparatus and a Sanyo apparatus.

Elemental analysis was made on CHNOS Vario El analyzer.

Electronic spectra were recorded with a UV–Vis Jasco V–530 spectrophotometer, within 200–700 nm range. Dioxane solutions (4×10^{-5} M) were prepared one day before recording spectra and kept in a dark place.

FTIR spectra were recorded in potassium bromide pellets (KBr, Merck), with a Bio-Rad FTS 135 spectrophotometer, within the range 3500–400 cm^{-1} .

Mass spectra were obtained using a HPGC–MS 5890 MD 5971 spectrometer, at 70 eV, with carrier gas He at 2 mL/min.

^1H -NMR spectra were recorded with a Varian NMR-System 300 spectrometer, at 300 MHz, in DMSO-d_6 . The chemical shifts were referred to tetramethylsilane (TMS) as internal standard.

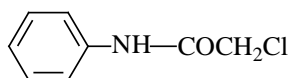
^{13}C -NMR spectra recorded with a Varian NMR-System 300 spectrometer, at 125,7 MHz in DMSO-d_6 .

Results and discussion

Synthesis of *N*-substituted chloroacetylanilines occurred with good reaction yields and it was dependent on their solubility in the chosen solvent.

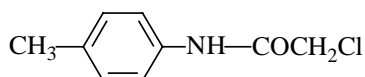
The melting points had high values due to the presence of the substituents with attracting electrons effect from the *para* or *ortho* position.

N-chloroacetylaniline (1)



mp. = 123°C, yield = 88%; yellow powder; (M^+ = 169.5); IR ν (cm^{-1}): 3267, 3096, 1560, 1306 (i, i, i–vi, m, NH); 1655 (i, C=O); 750, 690 (i–vi, i, C–Cl); 1291, 1252, 1193 (i, i–vi, m Ar–NH); 750, 650 (i–vi, i, CH aromatic ring); ^1H NMR ($\text{CDCl}_3/\text{DMSO-d}_6$): 7.98 (1H, s, NH); 4.33 (2H, s, CH_2); 7.00–7.64 (5H, m, CH aromatic ring); ^{13}C NMR (DMSO-d_6): δ 138.5 (1C); 121.6 (2C); 129 (2C); 124.4 (1C); 165.4 (1C); 42.8 (1C); calculated, %: C 56.63; H 4.72; N 8.26; Cl 20.94; found, %: C 56.73; H 4.70; N 8.36; Cl 20.96; $\text{C}_8\text{H}_8\text{NOCl}$; M = 169.5 g/mol.

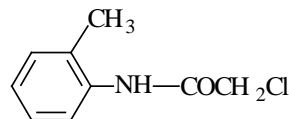
4-methyl-*N*-chloroacetylaniline (2)



mp. = 105°C, yield = 78%; white powder; (M^+ = 183.5); IR ν (cm^{-1}): 3274, 3091, 1552, 1306 (i, m–i, i, m, NH); 1670 (i–vi, C=O); 745, 696 (i, m–i, C–Cl); 1290, 1251, 1192 (i, i–vi, m Ar–NH); 2951, 2860, 1460, 1039 (w–m, w–m, m, w, CH_3); 745, 696 (i, m–i, CH aromatic ring); ^1H NMR ($\text{CDCl}_3/\text{DMSO-d}_6$): 8.00 (1H, s, NH); 4.32 (2H, s, CH_2); 2.36 (3H, s, CH_3); 7.04–7.52 (4H, m, CH aromatic ring); ^{13}C NMR (DMSO-d_6): δ 24.3 (1C); 135.5 (1C); 121.5

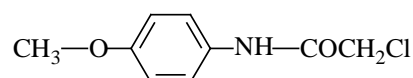
(2C); 129.3 (2C); 134 (1C); 165.4 (1C); 42.8 (1C); calculated, %: C 58.85; H 5.45; N 7.63; Cl 19.34; found, %: C 58.81; H 5.56; N 7.62; Cl 19.39; $\text{C}_9\text{H}_{10}\text{NOCl}$; M = 183.5 g/mol.

2-methyl-*N*-chloroacetylaniline (3)



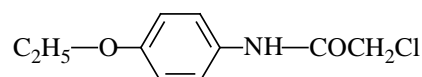
mp. = 104°C, yield = 75%; dirty white powder; (M^+ = 183.5); IR ν (cm^{-1}): 3269, 3092, 1553, 1305 (i, m–i, i, m, NH); 1669 (i–vi, C=O); 746, 694 (i, m–i, C–Cl); 1287, 1253, 1190 (i, i–vi, m Ar–NH); 2959, 2863, 1458, 1035 (m, m, m–i, w, CH_3); 746, 694 (i, m–i, CH aromatic ring); ^1H NMR ($\text{CDCl}_3/\text{DMSO-d}_6$): 7.99 (1H, s, NH); 4.31 (2H, s, CH_2); 2.35 (3H, s, CH_3); 6.88–7.52 (4H, m, CH aromatic ring); ^{13}C NMR (DMSO-d_6): δ 15.2 (1C); 137.4 (1C); 134.4 (1C); 129.3 (1C); 121.5 (1C); 124.3 (1C); 126.0 (1C); 165.4 (1C); 42.8 (1C); calculated, %: C 58.85; H 5.45; N 7.63; Cl 19.34; found, %: C 58.82; H 5.51; N 7.65; Cl 19.32; $\text{C}_9\text{H}_{10}\text{NOCl}$; M = 183.5 g/mol.

4-methoxy-*N*-chloroacetylaniline (4)



mp. = 106°C, yield = 78%; grey powder; (M^+ = 199.5); IR ν (cm^{-1}): 3295, 3097, 1548, 1304 (i, m–i, i, m, NH); 1663 (i–vi, C=O); 744, 689 (i, m–i, C–Cl); 1299, 1247, 1179 (i, i–vi, m Ar–NH); 2835, 1270, 1030 (m–i, vi, m, i–vi, CH_3); 747, 691 (i, m–i, CH aromatic ring); ^1H NMR ($\text{CDCl}_3/\text{DMSO-d}_6$): 8.00 (1H, s, NH); 4.32 (2H, s, CH_2); 3.73 (3H, s, CH_3); 6.75–7.53 (4H, m, CH aromatic ring); ^{13}C NMR (DMSO-d_6): δ 55.9 (1C); 130.8 (1C); 122.6 (2C); 114.5 (2C); 156.3 (1C); 165.4 (1C); 42.8 (1C); calculated, %: C 54.13; H 5.01; N 7.01; Cl 17.79; found, %: C 54.11; H 5.12; N 7.09; Cl 17.67; $\text{C}_9\text{H}_{10}\text{NO}_2\text{Cl}$; M = 199.5 g/mol.

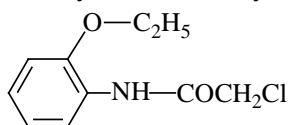
4-ethoxy-*N*-chloroacetylaniline (5)



mp. = 152°C, yield = 82%; grey powder; (M^+ = 213.5); IR ν (cm^{-1}): 3271, 3097, 1560, 1304 (i, m–i, i, m, NH); 1671 (i–vi, C=O); 750, 706 (m–i, m–i, C–Cl); 1289, 1247, 1197 (m–i, i–vi, m Ar–NH); 2866, 1289, 1044 (m–i, i–vi, i, C–O– $\text{C}_{\text{aromatic}}$); 750, 678 (i, m–i, CH aromatic ring); ^1H NMR ($\text{CDCl}_3/\text{DMSO-d}_6$): 7.99 (1H, s, NH); 4.32 (2H, s, CH_2); 1.33 (3H, t, CH_3); 3.98 (3H,

q, CH₂); 6.75–7.53 (4H, m, CH aromatic ring); ¹³C NMR (DMSO-d₆): δ 14.8 (1C); 64.7 (1C); 153.1 (1C); 114.6 (2C); 122.2 (2C); 130.1 (1C); 165.4 (1C); 42.8 (1C); calculated, %: C 56.21; H 5.62; N 6.55; Cl 16.63; found, %: C 56.18; H 5.67; N 6.61; Cl 16.59; C₁₀H₁₂NO₂Cl; M = 213.5 g/mol.

2-ethoxy-N-chloroacetylaniline (6)



mp. = 107°C, yield = 86%; light brown powder; (M⁺ = 213.5); IR ν (cm⁻¹): 3271, 3080, 1544, 1303 (i–vi, m–i, i, m, NH); 1672 (vi, C=O); 7690, 662 (i–vi, i, C–Cl); 1293, 1251, 11787 (i, i, m Ar–NH); 2865, 1293, 1041 (i, vi,

i, C–O–C_{aromatic}); 769, 689 (i, m–i, CH aromatic ring); ¹H NMR (CDCl₃/DMSO-d₆): 7.99 (1H, s, NH); 4.32 (2H, s, CH₂); 1.32 (3H, t, CH₃); 3.99 (3H, q, CH₂); 6.75–7.53 (4H, m, CH aromatic ring); ¹³C NMR (DMSO-d₆): δ 14.8 (1C); 64.7 (1C); 125.0 (1C); 120.6 (1C); 114.6 (1C); 149.6 (2C); 122.2 (2C); 126.9 (1C); 165.4 (1C); 42.8 (1C); calculated, %: C 56.21; H 5.62; N 6.55; Cl 16.63; found, %: C 56.19; H 5.52; N 6.58; Cl 16.67; C₁₀H₁₂NO₂Cl; M = 213.5 g/mol.

The experimental data obtained by *Triticum* assay, using the method of linear measurement, are highlighted in **Table 1** and in **Fig.2–6**. The inhibition of radicular elongation, compared with the reference, was observed for all five concentrations of the compounds (1)–(6).

Table 1 *Triticum* assay for different concentrations of the compounds (1)–(6)

No.	Time [hours]	Mean radicular elongation for 10 germinated caryopses [mm]						Reference
		Concentration of compound (1)						
		0.1%	0.5%	0.75%	1%	5%		
1.	24	12	12	12	11.5	12	20	
2.	48	15	14.5	14	15	14	38	
3.	72	18.5	18	17	16	17	56	
4.	96	23	23.5	24	22.5	23	82	
5.	120	29	28.5	27.5	26	28	110	
No.	Time [hours]	Mean radicular elongation for 10 germinated caryopses [mm]						Reference
		Concentration of compound (2)						
		0.1%	0.5%	0.75%	1%	5%		
1.	24	12.5	12.8	13	14	14.5	20	
2.	48	14.5	15	16	15	15	38	
3.	72	17.5	18	18	18.5	17	56	
4.	96	23.5	22.5	22	21.5	24	82	
5.	120	30.5	31	29.5	30	31.5	110	
No.	Time [hours]	Mean radicular elongation for 10 germinated caryopses [mm]						Reference
		Concentration of compound (3)						
		0.1%	0.5%	0.75%	1%	5%		
1.	24	13	14	13.5	15	15.5	20	
2.	48	15	13.5	14	14.5	16	38	
3.	72	19	17.5	18.5	18	17.5	56	
4.	96	24	23.5	23	24	23.5	82	
5.	120	31	30	29.5	30	31.5	110	
No.	Time [hours]	Mean radicular elongation for 10 germinated caryopses [mm]						Reference
		Concentration of compound (4)						
		0.1%	0.5%	0.75%	1%	5%		
1.	24	11.5	10.5	11.5	11	11.5	20	
2.	48	15.3	15.5	15.2	15	15.5	38	
3.	72	22.5	22.3	22	22.5	22	56	
4.	96	30.5	30.3	30	29.5	29.8	82	
5.	120	37.5	36.5	37	37.5	36	110	
No.	Time [hours]	Mean radicular elongation for 10 germinated caryopses [mm]						Reference
		Concentration of compound (5)						
		0.1%	0.5%	0.75%	1%	5%		
1.	24	11.7	12	12.5	11.5	11	20	
2.	48	16.5	16.3	16	16.5	16	38	
3.	72	23.5	23.5	23	23.3	23	56	
4.	96	31	30.5	30.5	31	31	82	
5.	120	38.5	38	38.7	39	39	110	

No.	Time [hours]	Mean radicular elongation for 10 germinated caryopses [mm]					
		Concentration of compound (6)					Reference
		0.1%	0.5%	0.75%	1%	5%	
1.	24	12	12.5	11.7	11.5	11.5	20
2.	48	17.5	17.5	17	17.5	17.3	38
3.	72	24	23.8	24	23.7	24.2	56
4.	96	33.5	33.2	33	33.5	33	82
5.	120	39	39.5	40	38.5	39	110

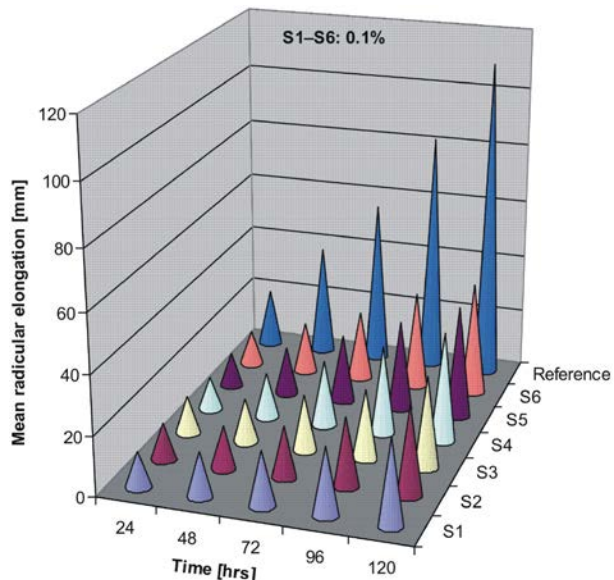


Fig.2. Mean radicular elongation [mm] compared with the reference (Triticum assay), for the compounds (1)–(6), at 0.1% concentration

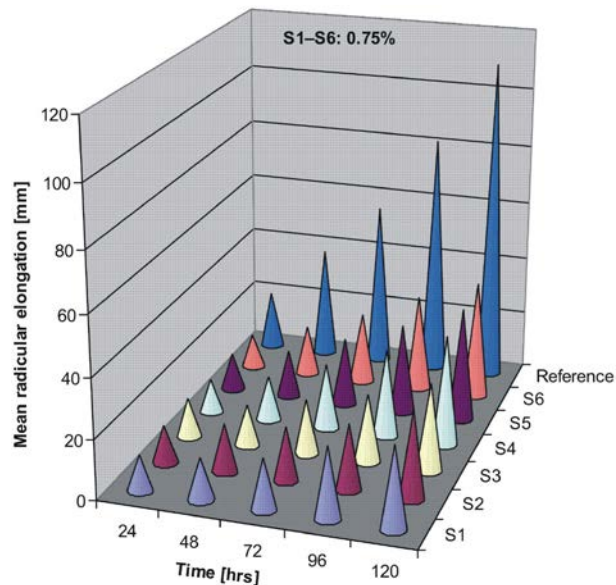


Fig.4. Mean radicular elongation [mm] compared with the reference (Triticum assay), for the compounds (1)–(6), at 0.75% concentration

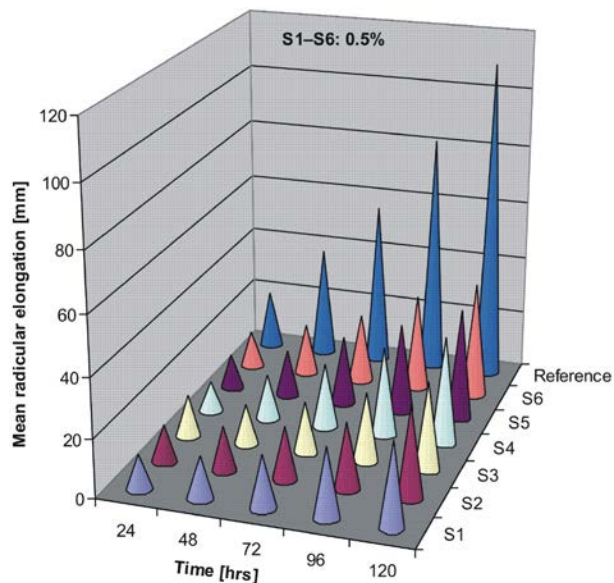


Fig.3. Mean radicular elongation [mm] compared with the reference (Triticum assay), for the compounds (1)–(6), at 0.5% concentration

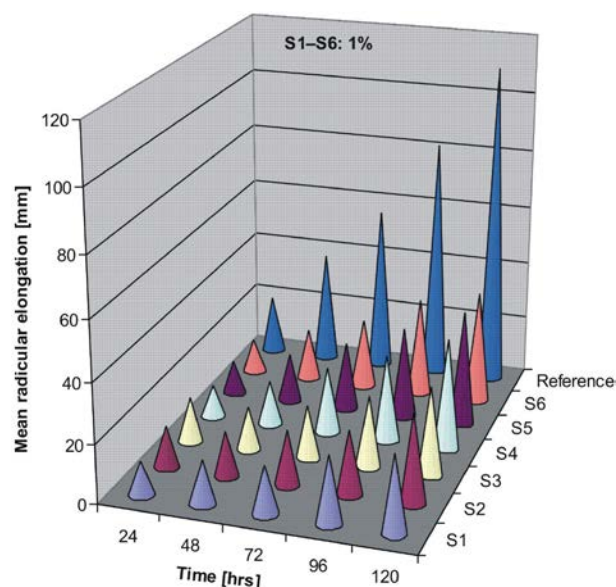


Fig.5. Mean radicular elongation [mm] compared with the reference (Triticum assay), for the compounds (1)–(6), at 1% concentration

Conclusion

N-substituted chloroacetylanilines were synthesized by condensation reactions and were

physico-chemical characterized (melting point, elemental analysis, spectral analysis).

The phytobiological action of these compounds at five different concentrations, was tested on chloroform solutions, in terms of

germination and radicular elongation of *Triticum aestivum* subsp. *aestivum* (Dropia variety) caryopses. For all samples, the observed data showed the inhibition of mean radicular elongation compared with the reference.

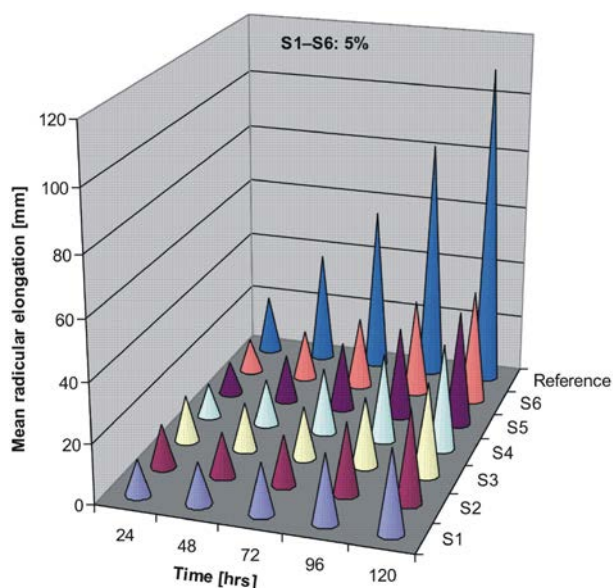


Fig.6. Mean radicular elongation [mm] compared with the reference (*Triticum* assay), for the compounds (1)–(6), at 5% concentration

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