Letter to Editor

Influence of New Wood Preservatives on the Plant *Sinapis alba*

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Received: July 20, 2004
Accepted: May 30, 2005

Abstract

The toxic effects of new quaternary ammonium chlorides on dicotyledonous plant *Sinapis alba* was evaluated. Investigated compounds differed either in hydrophobic chain length or hydrophilic part properties. The compounds used in the study are meant to be new wood preservatives because of their excellent fungicidal properties. It has been stated that the toxic effects of examined chlorides on *Sinapsis alba* depend on the compound structure. Alkyl(alkoxymethyl)dimethylammonium chlorides had the lowest phytotoxic properties. The presence of benzene subsituent in the compound molecule caused an increase in toxic effects of ammonium chlorides on the *Sinapis alba* roots.

Keywords: quaternary ammonium chlorides, *Sinapis alba*, phytotoxicity.

Introduction

The ability of fungi to destroy timber is an important property of these organisms because of their role in the carbon and other element's cycles. On the other hand, decomposition of timber by fungi causes economic losses due to degradation and depreciation of raw wood material when deposed in depots of pulp and paper plants. Fungi also destroy wooden parts of house buildings, monuments and others [1]. Because of the material losses caused by destructive action of fungi at the beginning of the 20th century, protective treatments started to be employed using chemical preservatives [2].

Quaternary ammonium salts which contain alkyl substituents are a new generation of fungicides. They are used to protect timberwood, wooden elements of mushroom-growing cellars as well as plasters and brickworks from mould fungi. These compounds show a particularly high toxic effect toward the fungi subphyla *Basidiomycotina, Ascomycotina Deueteromycotina* [1] as well as to fungi which cause surfical and deep wood discolour. Quaternary ammonium salts are also useful in destruction of mould [3, 4]. These compounds are able to fix within wood tissue through bonding with main wood components, which is considered to be a significant advantage [5, 6]. Becasue of their properties, quaternary ammonium salts are sparingly washable from timber. However, on the basis of recent reports [7] this statement cannot always be regarded as correct. The significant effect of pH of medium on a degree of the fixation and leaching of these salts from timber has been stated. As pH decreases, the ability of these compounds to bond with timber also decreases [8].

Consequently, timber-protecting agents pose a serious risk for soil environment when washed out by precipitation, especially by acid rains. Cumulated in soil these compounds, they have a detrimental effect on soil microflora and vegetation. That was a reason for examination of the quaternary ammonium salts [1,9,10,11]. Their influence on the growth of shoots and roots of the broad-leaved plant *Sinapis alba* was determined. This plant was selected for examination on the basis of OECD recommendations [12].

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Table 1. Selected physicochemical properties of quaternary ammonium salts used in toxicological tests.

Compound symbol	Compound	Molecular weight	State of aggregation	Colour
A-1	decyloxymethyldimethyloctylammonium chloride	363	solid	straw-coloured
A-2	decyloxymethyldimethyldodecylammonium chloride	419	solid	white
A-3	nonyloxymethyldimethyldodecylammonium chloride	405	solid	white
A-4	octyloxymethyldimethyldodecylammonium chloride	391	solid	white
A-5	benzalkonium chloride, a mixture of benzyltetradecyldimethylammonium and benzylhexadecyldimethylammonium chloride at ratio 40 : 60 (w/w)	729	solid	dark yellow
A-6	benzyl (butoxymethyl) dimethylammonium chloride	257	solid (greasy)	white
A-7	benzyl (octyloxymethyl) dimethylammonium chloride	314,	solid	white
A-8	benzyl (decyloxymethyl) dimethylammonium chloride	342	solid	white
A-9	benzyl (dodecyloxymethyl) dimethylammonium chloride	370	solid	white
A-10	1-decyl-3-hexyloxymethylimidazole chloride	358	solid	white
A-11	1-decyl-3-cyclohexyloxymethylimidazole chloride	356	solid	pale straw-coloured

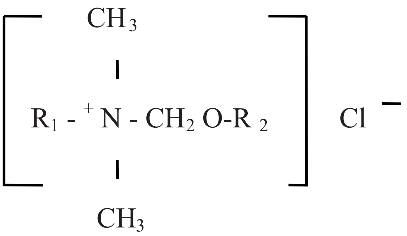

Fig. 1. General structural formula of alkyl (alkoxymethyl)dimethylammonium chlorides A-1 ÷ A-4.

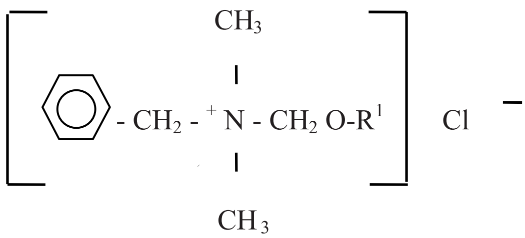

Fig. 2. General structural formula of benzyl (alkoxymethyl) dimethylammonium chlorides A-5 ÷ A-9.

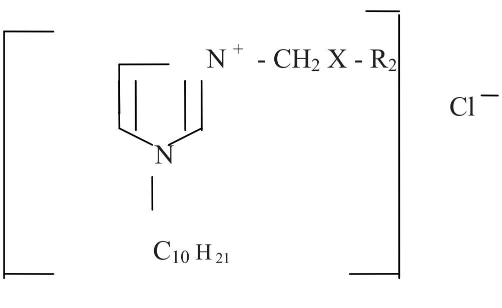

Fig. 3. General structural formula of imidazolium chlorides A-10 ÷ A-11.

Materials and Methods

Three groups of quaternary ammonium salts with different length of hydrophobic chain and various properties of hydrophilic part were investigated. The list of compounds used in the study is included in Table 1.

All compounds were readily soluble in aqueous solutions within the range of examined concentrations.

Determination of Inhibitory Effect of Quaternary Ammonium Salts on Growth of Shoot and Roots of *Sinapis alba*

Inhibition tests were carried out according to PN-ISO 11269-1, in cylindrical pots with perforated bottoms, 8 cm in diameter and 11 cm high. Untreated seeds of *Sinapis alba* (white mustard) were used [13]. Sand was used as a substratum due to its small absorbing capacity. Volumetric water capacity of employed substratum, determined using a scale-drier, was 17.34%. Sand was dried at 60 ± 5°C to air-dry condition, and then it was placed into the pots up to a height of about 9 cm. There was more or less 0.5 kg sand in each pot. Substratum was contaminated with the compounds in the concentrations of 1, 3, 9, 27 and 81mg/$kg_{d.m.}$. Solutions of the examined quaternary ammonium chlorides were introduced in the amount of 60 cm^3 into the each pot, which provided 70% determined volumetric water capacity. Such a quantity of liquid ensured that it was uniformly distributed in the assumed sand volume and prevented losses caused by dribbling. In each pot, 5 sprouted seeds of *Sinapis alba* (with roots of 2 to 5 mm length) were placed at a depth of 100 mm. The seeds were germinated on Petri dishes in a thermostat at 20°C for 48 hours.

Table 2. Comparison of the effect of alkyl(alkoxymethyl)dimethylammonium chlorides on the growth of *Sinapis alba* roots.

Compound	IC_{50} for root growth [mg/kg]	SD
A-1	105	2.0
A-2	110	3.8
A-3	102	1.3
A-4	108	2.5

SD- standard deviation

Next, the pots were placed in a growth chamber to be incubated for 120 hours. The day lasted 10 hours whereas the night lasted 14 hours. Diurnal and night temperatures were 23 ± 1°C and 20 ± 1°C, respectively. Investigations were conducted in three repetitions at the same time. Blank assays were running simultaneously planting three pots, where distilled water was added.

After a determined growth period, the lengths of the root and the stem were measured. A concentration that causes 50% inhibition of root and stem growth compared to reference test was used as a toxicity measure.

Results and Discussion

Toxicity tests were carried out for seeds when sowing in pots regarding three groups of quaternary ammonium salts. On the basis of these tests it was found that alkyl(alkoxymethyl)dimethylammonium chlorides were the compounds least toxic to the white mustard. Irrespective of preparation being examined within this group, IC_{50} was over 100 $mg/kg_{d.m.}$ (Table 2). However, in the case of benzyl(alkoxymethyl)dimethylammonium chlorides it was stated that the toxic effect on *Sinapis alba* was diverse depending on the hydrophobic chain structure.

The least toxic compound (with IC_{50} 50 ± 3.9 $mg/kg_{d.m}$ soil) was A- 5 - Fig.4. That was a compound without oxygen atom within a molecule. Oxygen attendance in the hydrophobic chain caused the increase in the toxicity of the tested compounds, at the same time chain length over 8 carbon atoms was of no importance. IC_{50} for A-7 ÷ A-9 chlorides was about 25 $mg/kg_{d.m.}$.

The most toxic effect was observed in case of A-6 preparation with IC_{50} equal to10 ± 0.8 $mg/kg_{d.m.}$. This compound contained an oxygen atom in its molecule and had the shortest hydrophobic chain (4 carbon atoms). Obtained results were similar to those observed by [14], where interesting reciprocal correlation was found between phytotoxicity in relation to the orchid seeds and chain length during investigations on phytotoxic action of non-ionic and cationic surface-active agents. The toxicity decreased as the molecular weight of the polyethyleneglycolic group increased, i.e. the increase in the number of ethylene oxide molecules. Compound A-6, due to its high phytotoxicity and low fungicidal action, should not be used in formulations commonly used as indicated by [15], the best fungicidal properties have compounds of 14 and 18 carbons in hydrophobic chain within a series of alkobenzyldimethylammonium chlorides.

The results show that benzene substituent increases the toxic effects of ammonium chlorides on the *Sinapis alba* root. When analyzing the IC_{50} values of the compounds having identical alkyl chain with and without benzene substituent (A-7, A-4 and A-8, A-2), fourfold larger susceptibility of root to ammonium chlorides with benzene substituent was noticed (Fig.5).

During the studies on the effect of two imidazolium chlorides A-10 and A-11 on *Sinapis alba*, measurements of both the root and the stem of the plant were made. First of the mentioned preparations seemed to be inconsiderably more toxic than the other, since the value of its IC_{50} as determined for *Sinapis alba* root was 43.2 ± 1.5 $mg/_{kgd.m.l}$ and that of the second was 58.2 ± 2.7 $mg/kg_{d.m.}$ (Table 3).

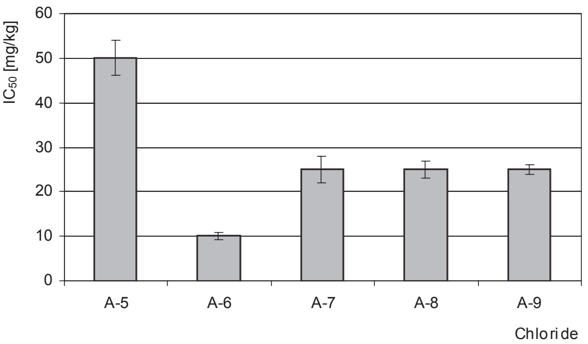

Fig. 4. Comparison of the effect of benzyl(alkoxymethyl) dimethylammonium on the growth of *Sinapis alba* roots.

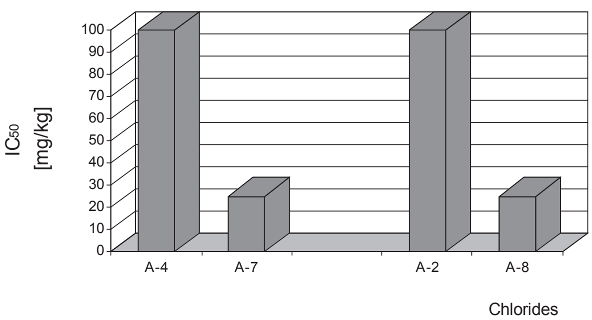

Fig. 5. Comparison of the effect of preparations having similar hydrophobic chains on the growth of *Sinapis alba* roots.

Table 3. Comparison of A-10 and A-11 compound effects on root and stem growth IC_{50} values.

Compound	IC_{50} for root growth [mg/kg]	SD	IC_{50} for stem growth [mg/kg]	SD
A-10	43.2	1.5	124.0	7.6
A-11	58.2	2.7	142.0	8.4

SD- standard deviation

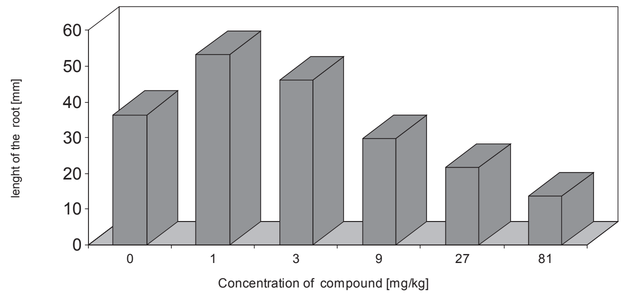

Fig. 6. Effect of compound concentration on root growth.

The IC_{50} values for stem growth inhibition were 124 ± 7.6 and 142 ± 8.4 $mg \cdot kg^{-1}_{soil}$ for compounds A-10 and A-11, respectively. It follows that the root has been proved to be more sensitive to examined compounds. Undoubtedly, it results from a fact that roots uptake the polar compounds more easily (which are preparations under examination) than cotyledons [16]. Furthermore, quaternary ammonium salts have the capacity for disturbance of aminoacid transport, which can result in reduction of their quantity in the roots, and the increase in the stems [17, 18].

Although phytotoxic action of the preparation A-10 has been indicated on the *Sinapis alba* root, however, with the lowest concentrations, stimulation of root growth of this plant was observed under the influence of the above-mentioned compound (Fig.6).

In all likelihood it results from simple structure of the l-decyl-3 hexyloxymethyl imidazole chloride and its similarity to histidine – an amino acid containing imidazole ring [19]. Histidine, apart from arginine, is one of two amino acids found in all proteins that occur in nature [20]. So, it seems that stimulating properties of these and similar compounds, in low concentrations, predominate over the inhibition and cause an increased rise of crops.

Conclusions

- It was stated that the effect of examined compounds on the dicotyledonous plant *Sinapis alba* varied and depended on the structure of the compounds.
- The most toxic compounds were alkyl(alkoxymethyl) dimethylammonium chlorides.
- The presence of benzene substituent in the compound molecule increased the toxic effect of ammonium chlorides on the *Sinapis alba* root.
- Compound A-6 (benzyl(butoxymethyl)dimethylammoni um chloride), containing only 4 carbon atoms in the hydrophobic chain, had the highest phytotoxic properties.

References

1. ZABIELSKA- MATEJUK J. Synthesis and fungicidal properties of quaternary imidazolium salts with alkoxymethyl substituent, Studies of Wood Tech. Inst., pp.3-72, **1999** (in Polish, summary, table and figures in English).
2. SANDERMANN W. Grundladen der Chemie und chemischen Technologie des Holzes. Akadem. Verlagsges. Geest and Portig Lepzig, **1956.**
3. URBANIK E., FOJUTOWSKI A. Proecological aspects of wood protecting using fungicides. Lect.: II *Symposium "Quaternary Ammonium salts and regions of their use in economy*". Publishing house ITD) Poznań pp. 6-23, **1996** (in Polish).
4. WAŻNY J. The use of quarternary ammonium compounds in the production of wood protecting agents against biodegradation. lect.: II Symposium „Quaternary ammonium salts and regions of their usage in economy" Publ. House ITD, Poznań pp. 37-46, **1996** (in Polish).
5. DOYLE K., RUDDICK J. The Microdistribution of Alkyloammonium Compouds in Ponderosa Pine Sapwood. Holzforschung, **48** (2), 106, **1994**.
6. LOUBINOUX B., MALEK H. Interactions of quaternary ammonium salts with wood: influence of cation and anion structure on fixation and leaching, Forest Products Journal, **42** (10), 55, **1992.**
7. ŁEBKOWSKA M., ZAŁĘSKA- RADZIWIŁŁ, Toxicity assessment of wood preservatives, *Secotox World Congress,* Kraków - Poland, pp. 173-175, **2001.**
8. WALKER L.E., LICHTENBERG F. Dialkyl Quats as wood preservatives., Lect.: III Symposium "Quaternary ammonium salts and regions of their use in economy" Publ. House ITD. Poznań pp. 57-71, **1997,** in Polish.
9. PERNAK J., ZABIELSKA- MATEJUK J. I URBANIK E. New Quaternary Ammonium Chlorides - Wood Preservatives, Holzforschung, **52 (**3), 249,**1998**.
10. URBANIK E., ZABIELSKA- MATEJUK J., SKRZYPCZAK A. Quantitative relation between surface active properties and antifungal activity of quaternary imidazolium chlorides (new wood preservatives), Forest Products Journal, **49** (10), 53, **1999**.
11. URBANIK E., ZABIELSKA- MATEJUK J., SKRZYPCZAKA., PERNAK J. Antifungal properties of new imid-

azolium chlorides against *Coniophora puteana, Trametes versicolor, Chaetomium globosum*., Material und Organismen, **31** (4), 247, **1997**.
12. OECD: *Guidelines Testing of Chemicals,*OECD, Paris, vol. 1, **1993.**
13. PN - ISO 11269-1. Quality of soil. Determination of the influence of impurities on soil flora (in Polish).
14. ERNST R, ARDITTI J., HEALEY P.L. Biological effects of surfactants I. Influence on the growth of orchid seedlings. New Phytol.*,* **70**, 457, **1971**.
15. TSUNODA K.T. Effect of alkyl chain length on the fungicidal efficacy of benzalkonium chloride. *Bokin Bobai.* **18**, 185, **1990**.
16. ŻURAWSKA M. GLABISZEWSKI J. The effect of different doses of 2,4-D on tomato seedlings and soil microflora in test experiments. Lect.: Conference "Bioindication of agricultural and industrial contaminatio*n*", PAN, Wrocław, **1983** (in Polish).
17. HOROWITZ M., GIVELBERG A. Toxic effect of surfactants applied to plant roots, Pestic.Sci. **10**, 547, **1979**.
18. WITEK S. Biological activity of quaternary ammonium salts and lysosomotropic amines. Lect.: IV Symposium "Quaternary ammonium salts and regions of their usage in economy*"* Publ. House ITD., Poznań pp. 9-20, **1998** (in Polish).
19. SHIRLEY D.A. Organic Chemistry, WNT, Warszawa, **1964** (in Polish).
20. FILIPOWICZ B, WIĘCKOWSKI W.,. Biochemistry, PWN, Warszawa, **1983** (in Polish).