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Sweet ionic liquids-cyclamates: Synthesis, properties, and application as feeding deterrents

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In this paper, we show that sweet quaternary ammonium, pyridinium, and imidazolium cyclamates are cheap, thermally stable, surface and biologically active ionic liquids, with potential application as feeding deterrents.

ionic liquids, cyclamates, deterrents, biological activity

1 Introduction

Ionic liquids can be used in a great variety of applications, e.g. as solvents, catalysts or electrolytes in different industry segments. Their unique properties allow making wholly new compounds for multifunctional use [1–3]. One of the most interesting applications could be ionic liquids as feeding deterrents. The best known antifeedants belong to different chemical groups and come from natural sources [4]. However, given the costs and their low content in plants, natural antifeedants are difficult to apply on a large scale. For these reasons, an increasing interest in the synthesis of more useful chemical analogues of natural compounds is observed. Additionally, the structural elements that evoke insect antifeedant activity are also investigated. In literature was described that choline derivative acesulfamates, didecyldimethylammonium lactates and benzalkonium lactates based ILs had high deterrent activities [5, 6]. ILs with organic anions, which are artificial sweetener derivatives, were described previously [7–9]. These compounds include non-nutritive sweeteners, such as saccharinate and acesulfame. Burgard [10] patented a process for preparing hexadecylpyridinium acesulfamate and using in the oral hygiene sector. Jodynis-Liebert *et al.* [11, 12] studied the cytotoxicity, acute, and subchronic oral toxicity of didecyldimethylammonium saccharinate and acesulfamate in rats.

Cyclamates, the well-known alternative sweeteners, have been around now for over 50 years, and they still enjoy widespread usage in over 50 countries [13]. Extensive animal studies did not confirm any coherence between cyclamate and cancer. The complete human body evidence proved that cyclamates are not carcinogenic [13–15]. Studies of the thermal behavior of sweeteners indicated that sodium cyclamate is highly thermally and hydrolytically stable [16].

Here we describe synthesis, physical and anti-microbial properties, phytotoxicity, and deterrent activity of sweet ionic liquids–cyclamates.

2 Results and discussion

New pyridinium cyclamates (**1–12**), quaternary ammonium cyclamates (**13**, **14**) and imidazolium cyclamates (**15**, **16**)

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were obtained by the metathesis reaction. Structures of obtained salts are showed in Scheme 1 and summarized in Table 1.

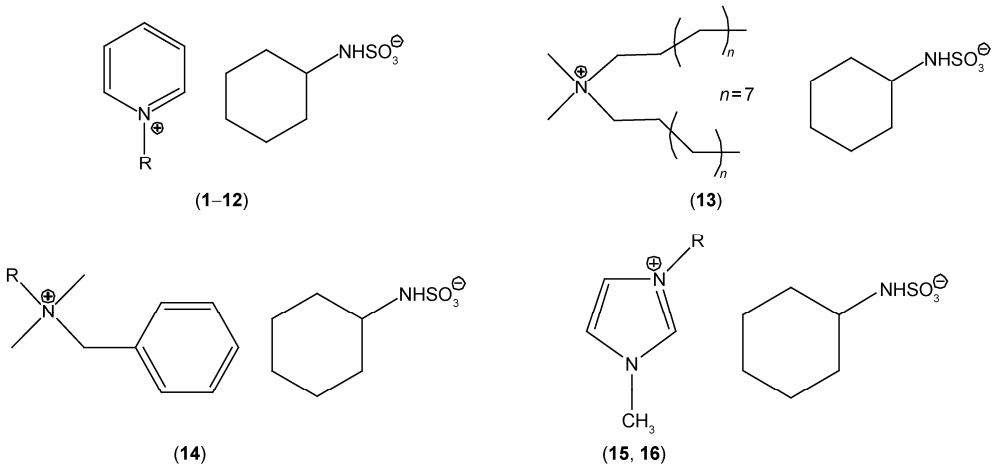
The synthesized salts are hygroscopic, highly viscous waxes. Only two pyridinium cyclamates (**11**, **12**) are crystalline solids with melting point up to 100 °C. Salts **11–15** were obtained from commercially available, inexpensive and widely used cationic surfactants, like 1-dodecylpyridinium chloride, hexadecylpyridinium chloride, didecyldimethylammonium chloride, and benzalkonium chloride.

1-Alkoxymethylpyridinium chlorides were prepared in specific type of Menschutkin reaction involving an S_N1 mechanism (Scheme 2).

Chloromethyl alkyl ether is an excellent reagent but very easily hydrolyzed. In this situation, quaternisation should be conducted under strictly anhydrous conditions. The synthesized 1-alkoxymethylpyridinium chlorides were very hygroscopic.

The cyclamates were prepared by simple, stoichiometric metathesis reactions (at room temperature, in distilled water) from the corresponding chlorides using aqueous sodium cyclamate. All cyclamates were obtained in high yields as non-hydrophobic salts with efficiencies over 82%. Sodium chloride as a byproduct was successfully removed from solution by one of two methods. Nature of these salts allowed extraction from the aqueous phase by water evaporation, drying, leaching by dry organic solvent, and evaporation. In other side, the crude product was extracted by chloroform from water solution. Finally, the products were dried in vacuum at 80 °C for 12 h and stored over P_4O_{10}. They quickly absorbed water in anhydrous form upon air exposure.

Our results allow classifying the synthesized fourteen

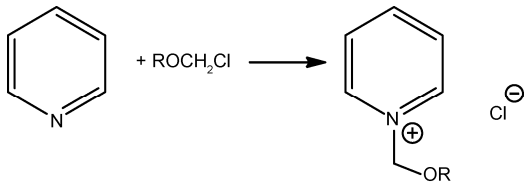


Scheme 1 Structures of the prepared cyclamates.

Table 1 Prepared cyclamates

Entry [a]	R	Yield (%)	Melting point [b] (°C)	Surfactant content [d] (%)
1	$C_4H_9OCH_2$	87	RTIL[e]	–
2	$C_5H_{11}OCH_2$	93	RTIL	–
3	$C_6H_{13}OCH_2$	96	RTIL	–
4	$C_7H_{15}OCH_2$	85	RTIL	–
5	$C_8H_{17}OCH_2$	84	RTIL	–
6	$C_9H_{19}OCH_2$	90	RTIL	95
7	$C_{10}H_{21}OCH_2$	89	RTIL	91
8	$C_{11}H_{23}OCH_2$	94	RTIL	93
9	$C_{12}H_{25}OCH_2$	96	RTIL	96
10	$C_{14}H_{29}OCH_2$	92	RTIL	94
11	$C_{12}H_{25}$	99	101–103 [c]	98
12	$C_{16}H_{33}$	82	117–119 [c]	99
13	–	95	RTIL	95
14	40% $C_{14}H_{29}$ 60% $C_{12}H_{25}$	92	RTIL	96
15	C_4H_9	95	RTIL	–
16	$C_{14}H_{29}OCH_2$	93	RTIL	98

a) For **1–10** isolation method B, for **11–16** isolation method A; b) visual melting point range via hot-plate apparatus; c) crystallized from ethyl acetate; d) determined by two-phase titration; e) RTIL-room temperature ionic liquids with high viscosity.



Scheme 2 Scheme of 1-alkoxymethylpyridinium chlorides synthesis.

salts (**1–10** and **13–16**) to ILs. Aqueous solutions of all obtained cyclamates preserved sweet taste. In aqueous solutions, the surfactant contents of the cyclamates **6–16,** excluding **15,** were assayed by a direct two-phase titration technique EN ISO 2871-2: 1990. These values ranged from 91% to 99% (Table 1).

Synthesized sweet ILs are air- and moisture stable and soluble in water, methanol, dichloromethane and acetone. They are immiscible with hexane and toluene. Ethyl acetate partially soluble pyridinium cyclamates with alkoxymethyl chains **1–10** and 1-butyl-3-methylimidazolium cyclamate **15**. However ILs **13** and **14** are only partially miscible with DMSO.

The water content of the obtained compounds, determined by Karl-Fischer measurements, was found to be less than 500 ppm. The prepared salts were characterized by ^{1}H and ^{13}C NMR spectroscopy and elemental analysis. Generally, in ^{1}H-NMR spectra were observed specific signals from anion and cation. The chemical shifts of the N–C group in cyclamate anion were observed between 52.6 and 52.9 ppm in ^{13}C-NMR spectra.

The obtained 1-alkoxymethylpyridinium ILs are thermal stable from 140 to 180 °C (Table 2). Thermal stability generally increased with alkyl substituent length. Replacing the alkoxymethyl group with alkyl substituent makes the salts more thermally stable. In the case of 1-dodecylpyridinium (**11**) and 1-hexadecylpyridinium (**12**) cyclamates decomposition begins at 223 and 235 °C, respectively. The thermal stability is dependent on the cation and increases in order of 1-alkoxymethylpyridinium (**3–10**) < tetraalkylammonium (**13**; **14**) < 1-alkylpyridinium (**11**; **12**) < 1,3-dialkylimidazolium (**15**) cyclamates.

Table 2 Thermal properties of synthesized cyclamates

Entry	T_g [a]	T_c [b]	T_m [c]	$T_{onset5\%}$ [d]	T_{onset} [e]
3	–	–40.9	–35	147	279
4	–57.7	–40.3	–14.1	140	273
5	–	–	–	153	293
6	–	–2	8.6	168	320
7	–	–	–	160	282
8	–	14.2	24.2	155	273
9	–	24.6	32.5	145	278
10	–	–	–	180	295
11	–	93	115.3	223	328
12	–45.3	74.1	104.5	235	292
13	–	24.7	50.4	203	297
14	–	–	–	205	298
15	–	47.2	72.4	248	338

a) Glass transition temperature determined by DSC; b) crystallization temperature determined by DSC; c) melting point on heating determined by DSC; d) decomposition temperature determined from onset to 5 wt% mass loss; e) decomposition temperature determined from onset to 50 wt% mass loss.

For prepared cyclamates only 1-heptyloxymethylpyridinium (**4**) and 1-hexadecylpyridinium (**12**) show glass transition at –57.7 and –45.3 °C, respectively. For 1-alkoxymethylpyridinium the melting point increases with alkoxy chain elongation from –35 to 32.5 °C for ILs (**3**) and (**9**).

The surface-active properties of the selected cyclamates are summarized in Table 3. The choice of salts was made due to the inexpensive chloride as a substratum (except **16**). Surface excess concentrations Γ_{max} were calculated from the slope of the linear portion of the γ-log C plots (Figure 1) using the Gibbs isotherm: $\Gamma_{max} = -\frac{1}{RT}\cdot\frac{d\gamma}{d(\ln C)}$, where Γ_{max} is the surface excess concentration at the saturated interface, R is gas constant and T the absolute temperature, C is concentration of salt.

From Γ_{max}, the minimum surface occupied by a molecule at the interface A_{min} can be calculated from equation: $A_{min} = \frac{1}{\Gamma_{max} N_A}$, where N_A is the Avogadro number.

Surface activity of surfactants can be judged by γ_{CMC}, the effectiveness of surface tension reduction. For studied ILs aqueous solutions, the surface tension decreased from the water value to a minimum located between 25.2 and 33.5 mN m^{-1}. After this point the surface tension reached the plateau region. In the case of 1-hexadecylpyridinium (**12**) and benzalkonium (**14**) cyclamates the solutions manifested the highest γ_{CMC} values equal to 33.4 and 33.5 mN m^{-1}, respectively. This demonstrated that 1-dodecylpyridinium (**11**), didecyldimethylammonium (**13**), and 1-methyl-3-tetradecyloxymethylimidazolium (**16**) cyclamates exhibited more pronounced intermolecular hydrophobic interactions, making it easier to form aggregates in water than it was in the case of (**12**) and (**14**).

From the surface tension plots, one can obtain two additional parameters, i.e., the adsorption efficiency, pC_{20}, and the effectiveness of surface tension reduction, Π_{CMC}. The pC_{20} is defined as the negative logarithm of the surfactant concentration in the bulk phase required to reduce the surface tension of the water by 20 mN m^{-1}, which represents efficiency of surface adsorption on an air-water interface. Then, the greater the pC_{20} value means the higher the adsorption efficiency of the surfactant. The other parameter, Π_{CMC} is the surface pressure at the *CMC*, being defined by: $\Pi_{CMC} = \gamma_0 - \gamma_{CMC}$, where γ_0 is the surface tension of pure solvent and γ_{CMC} is the surface tension of the solution at the *CMC*. This parameter indicates the maximum reduc-

tion of surface tension caused by the dissolution of surfactant molecules and, therefore, becomes a measure for the effectiveness of the surfactant to lower the surface tension of the solvent [17].

It can be seen that the highest values both pC_{20} and Π_{CMC} were obtained for didecyldimethylammonium cyclamate (**13**), which indicates that (**13**) is superior to the other two surface active ILs in both the adsorption efficiency (pC_{20}) and the effectiveness of surface tension reduction (Π_{CMC}).

The values of *CMC* determined from the break points in the γ versus log *C* plot, shown in Figure 1, were 5.01, 0.32, 1.00, 1.26, and 0.59 mmol L^{-1} for (**11–14**), and (**16**), respectively. In the case of 1-alkylpyridinium cyclamates the *CMC* value decreases from (**11**) to (**12**) as expected from the increased hydrophobicity due to the elongation of hydrocarbon chain.

The values of area per molecule A_{min} for quaternary ammonium and imidazolium cyclamates were higher than that of the corresponding pyridinium form, indicating that the molecules of the ILs (**11**) and (**12**) are more tightly packed at the water-air interface. *CMC* values of aqueous solutions of synthesized cyclamates are lower or of the same order as the *CMC* of cationic surfactants [18]. These results indicate that ILs with cyclamate self-assemble easily in the aqueous solutions.

The deterrent activity of synthesized cyclamate toward *Tribolium confusum* (larvae and beetles), *Sitophilus granarius* (beetles) and *Trogoderma granarium* (larvae) was determined by using a known method, in which the amount of food consumed is monitored over a specific time interval. The deterrent activities were estimated by the criteria listed in Table 4 and described previously [19].

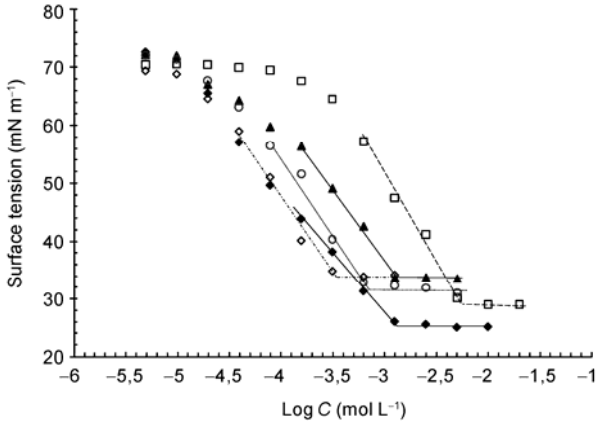


Figure 1 Surface tension as a function of concentration (log *C*) of cyclamates at 25 °C in water; □, (**11**); ◊, (**12**); ♦, (**13**); ▲, (**14**); ○, (**16**).

In Tables 5 and 6 are presented the relative, absolute, and total coefficients for tested cyclamates and for the natural deterrent azadirachtin as a standard. Azadirachtin is a triterpenoid of the class of limonoids, found in three species, the trees *Azadirachta indica* (Rutales:Meliaceae), *A. excelsa*, and *A. siamensis*. Chemical structure of azadirachtin required 18 years to solve. Furthermore, its synthesis required another 22 years. The total synthesis of azadirachtin was finally described by Ley's group [9]. Azadirachtin is a valuable natural pesticide with very low toxicity for vertebrates. However, in many countries it is not yet licensed for use [20]. Our results indicate that the tested cyclamates exhibit feeding deterrent activities. Number of carbon atoms in substituent has an impact on activity. Cyclamates: 1-hexadecylpyridynium (**12**) and didecyldimethylammonium (**13**) operate at the same level and sometimes slightly better in comparison with the standard azadirachtin.

The minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) or minimum fungicidal concentration (MFC) were established. The studies were conducted on ten strains of bacteria and two strains of fungi. The MIC and MBC or MFC values of the prepared cyclamates and the commercially available benzalkonium chloride are compared in Table 7. The results demonstrate that the cyclamates with cation including long alkyl group are effective agents against bacteria and fungi. The results obtained for 1-alkoxymethylpyridinium cyclamates indicate that butyl, pentyl and hexyl substituents make these salts not active against microorganisms. The presence of seven or more carbon atoms in alkoxy group decides that these ILs are biologically active. Their biological activity increases with the increase the number of carbon atoms in the alkoxy group. Figure 2 shows the mean value of the MIC, MBC and MFC of cyclamates **5–10**, **12,** and **13**. Exceptionally high activities against the tested microorganisms have two cyclamates: 1-hexadecylpyridynium (**12**) and didecyldimethylammonium (**13**). Their activities are more effective than for benzalkonium chloride. The very low values obtained for MIC, MBC and MFC show the high potential of 1-

Table 3 Surface properties of cyclamates (**11–14**, **16**) in aqueous solution at 25 °C

Entry	*CMC* (mmol L^{-1})	γ_{CMC} (mN m^{-1})	pC_{20}	Π_{CMC} (mN m^{-1})	Γ_{max} (μmol m^{-2})	A_{min} (10^{-19} m^2)
11	5.01	29.0	3.06	43.8	6.81	2.44
12	0.32	33.4	4.19	39.4	6.26	2.66
13	1.00	25.2	4.22	47.6	5.53	3.00
14	1.26	33.5	3.67	39.1	4.41	3.76
16	0.59	31.0	3.93	41.8	6.22	2.67

Table 4 Criteria for the estimation of deterrent activity based on the total coefficient

Total coefficient	Deterrent activity
200–151	very good
150–101	good
100–51	medium
0–50	weak

Table 5 Feeding deterrent activities of the prepared cyclamates against insects (beetles)

Entry	*Sitophilus granarius* (beetles)				*Tribolium confusum* (beetles)			
	R	*A*	total coefficient	deterrent activity	*R*	*A*	total coefficient	deterrent activity
2	86.0	–28.4	57.6	medium	96.4	6.1	102.5	good
3	91.8	–8.8	83	medium	93.2	24.5	117.7	good
4	53.7	55	108.7	good	95.5	36.4	131.9	good
5	95.7	56	151.7	very good	93.9	72.2	166.1	very good
6	75.1	1.7	76.8	medium	95.6	69.3	164.9	very good
7	87.3	17.6	104.9	good	95	67.9	162.9	very good
8	91.4	–3.2	88.2	medium	94.3	58.6	152.9	very good
9	92.4	2.2	94.6	medium	95.1	56.2	151.3	very good
10	95.6	4.1	99.7	medium	93.5	80.3	173.8	very good
11	93.8	20.8	114.6	good	90	92.7	182.7	very good
12	74.3	83.5	157.8	very good	92.9	92.7	185.6	very good
13	97.9	91.6	189.5	very good	97.1	81.4	178.5	very good
14	63.3	17.5	80.8	medium	97.6	–8.5	89.1	medium
15	97.1	–5.2	91.9	medium	91.5	8.2	99.7	medium
16	98	34.8	132.8	good	92.1	92	184.1	very good
Azadirachtin	100	74.3	174.3	very good	100	85	185	very good
LSD	39.1	19.3	43.6		6.1	29.3	29.8	

Table 6 Feeding deterrent activities of the prepared cyclamates against insects (larvae)

Entry	*Tribolium confusum* (larvae)				*Trogoderma granarium* (larvae)			
	R	*A*	total coefficient	deterrent activity	*R*	*A*	total coefficient	deterrent activity
2	88.2	15	103.2	good	82.3	36.4	118.7	good
3	94.4	36	130.4	good	48.3	21.5	69.8	medium
4	85.6	68.2	153.8	very good	88	45.4	133.4	good
5	79.5	78.3	157.8	very good	93.9	70.8	164.7	very good
6	68.3	56.7	125	good	68.9	40.5	109.4	good
7	96.1	16.5	112.6	good	96.3	90.5	186.8	very good
8	80.5	88.7	169.2	very good	96.7	91.2	187.9	very good
9	93.7	67.9	161.6	very good	97.8	86.6	184.8	very good
10	82.1	93.7	175.8	very good	95.6	82.4	178	very good
11	93.1	93.7	186.8	very good	96.4	95.1	191.5	very good
12	86.1	94.2	180.3	very good	93.6	95.1	188.7	very good
13	97.6	95.1	192.7	very good	95.4	89.3	190.5	very good
14	96.2	60	156.2	very good	95.3	93.3	188.6	very good
15	91.1	34.8	125.9	good	74.9	42.5	117.4	good
16	94.2	95.1	189.3	very good	74.9	83.7	158.6	very good
Azadirachtin	100	88.4	188.4	very good	100	94.2	194.2	very good
LSD	21	25.3	31.5		22.3	26.5	36.5	

Table 7 MIC, MBC, and MFC values[a] for prepared cyclamates

Strain		**5**	**9**	**10**	**11**	**12**	**13**	**15**	**16**	BA[b]
M.luteus	MIC	40	2.2	2.1	4.7	2.1	< 0.2	195	1.0	1.4
	MBC	40	4.4	2.1	38	2.1	< 0.2	394	2.1	11
S.aureus	MIC	624	18	2.1	19	4.1	0.4	1575	2.1	2.8
	MBC	1248	18	17	38	4.1	4.0	> 1575	2.1	23
S.epidermidis	MIC	624	2.2	2.1	1.2	0.4	0.4	394	1.0	1.4
	MBC	> 1248	2.2	4.1	9.4	2.1	2.0	788	2.1	5.6
E.faecium	MIC	1248	8.8	4.1	19	4.1	1.0	> 1575	2.1	5.6
	MBC	1248	8.8	33	38	4.1	1.0	> 1575	16	23
M.catarhalis	MIC	624	4.4	2.1	2.3	1.0	< 0.2	788	2.1	0.6
	MBC	1248	4.4	2.1	9.4	2.1	1.0	> 1575	4.1	1.4
E.coli	MIC	1248	4.4	2.1	4.7	2.1	0.2	788	2.1	2.8
	MBC	> 1248	4.4	4.1	9.4	4.1	1.0	1575	2.1	2.8
S.marcescens	MIC	624	274	516	293	64	38	1575	127	175
	MBC	> 1248	547	516	293	128	38	> 1575	256	175
P.vulgaris	MIC	> 1248	547	258	293	33	61	> 1575	64	88
	MBC	> 1248	547	258	293	33	61	> 1575	127	88
P.aeruginosa	MIC	> 1248	1095	516	586	518	61	> 1575	513	175
	MBC	> 1248	1095	1032	1172	1036	61	> 1575	1025	175
B.subtilis	MIC	624	8.8	4.1	19	2.1	0.4	> 1575	4.1	2.8
	MBC	1248	18	4.1	19	4.1	0.4	> 1575	4.1	2.8
C.albicans	MIC	624	18	8.3	38	4.1	16	1575	4.1	11
	MFC	> 1248	35	17	73	17	32	> 1575	8.2	88
R.rubra	MIC	624	4.4	2.1	19	8.3	32	> 1575	1.0	23
	MFC	1248	8.8	2.1	19	8.3	32	1575	2.1	88

a) in μM; b) benzalkonium chloride.

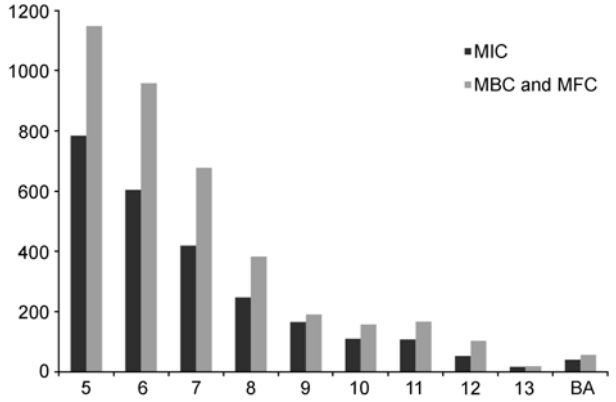


Figure 2 Mean value of MIC, MBC and MFC in μM of prepared cyclamates.

hexadecylpyridynium and didecyldimethylammonium cyclamates in disinfection.

Plant response to tested ILs was determined on the basis of cress garden (*Lepidium sativum*) seedling growth inhibition using effective dose (ED_{50}) values. The results of six cyclamates are presented in Table 8. Low ED_{50} indicates the strong phytotoxic effect. The tested ILs demonstrated a broad spectrum of ED_{50} values. The cyclamates benzalkonium (**14**), 1-methyl-3-tetradecyloxymethylimidazolium (**16**), 1-dodecylpyridinium (**11**) and didecyldimethylammonium (**13**) were the most phytotoxic to cress garden seedlings. These compounds demonstrated activity comparable to some herbicides. However, 1-hexadecylpyridinium (**12**) and 1-butyl-3-methylimidazolium (**15**) were significantly less toxic. The results show that it is very difficult to determine the relationships between chemical structure of tested cyclamates and their phytotoxic properties. It is interesting that cyclamate with lower *CMC* value (0.32 mmol L^{-1}) demonstrated lower phytotoxic activity.

3 Conclusion

We have shown that a novel family of cyclamate derivative-based salts are thermally stable and self-assemble in the aqueous solutions. Most of synthesized compounds proved to be ionic liquids with very good biological function. The very low values obtained for MIC, MBC and MFC show the high potential of cyclamates in disinfection. Moreover, didecydimethylammonium and 1-hexadecylpyridinium cyclamates are very efficient feeding deterrents.

4 Experimental

4.1 General

^{1}H NMR spectra were recorded on the Mercury Gemini 300 spectrometer operating at 300 MHz with TMS as the internal standard. ^{13}C NMR spectra were obtained with the same instrument at 75 MHz. CHN elemental analyses were per-

Table 8 The effective dose (ED_{50}) of tested cyclamates for *Lepidium sativum*

Entry	ED_{50} (ppm)	Equation[a)]	ED_{50} (μM)
11	8.14	$y = 0.8640\ln x + 76.061$	20
12	1014.92	$y = 3.9195\ln x + 22.867$	2100
13	22.09	$y = 5.3732\ln x + 33.369$	40
14	0.97	$y = 3.9628\ln x + 50.096$	2
15	23318.48	$y = 23.859\ln x - 189.95$	73550
16	3.05	$y = 1.1509\ln x + 67.266$	6

a) "*x*" denotes concentration in ppm.

formed at the Adam Mickiewicz University, Poznan (Poland). The water content was determined by using an Aquastar volumetric Karl-Fischer titration with Composite 5 solution as the titrant and anhydrous methanol as a solvent. Melting points were determined by visual observation via hot-plate apparatus.

4.2 Synthetic procedure of cyclamates

(0.01 mol) Sodium cyclamate was dissolved in distilled water and added to aqueous solutions containing 0.01 mol of 1-alkoxymethylpyridinium chlorides or 1-alkylpyridinium chlorides or didecyldimethylammonium chloride or benzalkonium chloride or 3-butyl-1-methylimidazolium chloride or 1-methyl-3-tetradecyloxymethylimidazolim chloride. The mixture was stirred at room temperature for 24 h. The product was isolated by one of the following methods:

Method A: The water was evaporated, and crude product was dried under high vacuum at 50 °C. After that dry acetone was added and the mixture was stirred at room temperature for 24 h. The crystalline sodium chloride was removed by filtration and the acetone was removed by distillation. The obtained product was dried overnight in vacuum at 80 °C.

Method B: The product was extracted from the aqueous solution with chloroform. The chloroform phase was separated and washed with distilled water until chloride ions were no longer detected using $AgNO_3$. Solvent was evaporated and the product was dried under vacuum overnight at 80 °C.

1-Alkoxymethylpyridinium chlorides and 1-methyl-3-tetradecyloxymethylimidazolium chloride were prepared according to the procedure described earlier [21, 22]. 1-Alkylpyridinium chlorides, didecyldimethylammonium chloride, benzalkonium chloride and 3-butyl-1-methylimidazolium chloride are commercial products, were used without further purification.

1-Hexyloxymethylpyridinium cyclamate (**3**): ^{1}H NMR ($CDCl_3$) δ in ppm 0.86 (t, J = 6.7 Hz, 3H), 1.05(m, 3H) 1.25(m, 10H), 1.46(m, 2H), 1.53(m, 1H), 1.68(m, 2H), 2.05(m, 2H), 3.11(m, 1H), 3.91(s, 1H), 6.16(m, 2H), 8.18(t, J = 3.7 Hz, 2H), 8.59(t, J = 4.5 Hz, 1H), 9.30(d, J = 5.5 Hz, 2H); ^{13}C NMR ($CDCl_3$) δ in ppm 13.9, 22.4, 25.0, 25.4, 30.5, 31.6, 32.7, 34.1, 52.9, 71.2, 89.5, 128.1, 143.3, 144.2; Elemental analysis calcd (%) for $C_{18}H_{32}N_2O_4S$ (372.52): C 58.03, H 8.66, N 7.52; found C 58.20, H 8.80, N 7.35.

1-Dodecyloxymethylpyridinium cyclamate (**9**): ^{1}H NMR ($CDCl_3$) δ in ppm 0.86 (t, J = 6.7 Hz, 3H), 1.24(m, 20H), 1.58(m, 2H), 1.61 (m, 2H), 1.71(m, 1H), 1.89(m, 2H), 1.98(m, 2H), 2.11(m, 2H), 3.23(m, 1H), 3.48(m, 2H), 6.35(s, 2H), 8.19 (t, J = 7.1 Hz, 2H), 8.61(t, J = 7.7 Hz, 1H), 9.24(d, J = 5.5 Hz, 2H); ^{13}C NMR ($CDCl_3$) δ in ppm 14.0, 22.5, 24.9, 25.6, 25.7, 29.2, 29.2, 29.3, 29.4, 29.5, 29.5, 29.5, 31.8, 34.0, 52.9, 71.5, 89.5, 128.2, 143.3, 146.7; Elemental analysis calcd (%) for $C_{24}H_{44}N_2O_4S$ (456.68) C 63.12, H 9.71, N 6.13; found C 63.31, H 9.59, N 6.05.

1-Dodecylpyridinium cyclamate (**11**): ^{1}H NMR ($CDCl_3$) δ in ppm 0.89 (t, J = 6.7 Hz, 3H), 1.19(m, 3H), 1.23(m, 18H), 1.59 (m, 2H), 1.71(m, 1H), 1.89(m, 2H), 2.01(m, 2H), 2.11(m, 2H), 3.22 (m, 1H), 3.31(m, 1H), 4.82(t, J = 7.4 Hz, 2H), 8.15 (t, J = 7.3 Hz, 2H), 8.49 (t, J = 7.8 Hz, 1H), 9.31(d, J = 5.7 Hz, 2H); ^{13}C NMR ($CDCl_3$) δ in ppm 14.0, 22.5, 24.9, 25.6, 26.0, 29.2, 29.3, 29.4, 29.5, 31.7, 31.8, 34.3, 52.8, 61.9, 128.3, 144.8, 145.1; Elemental analysis calcd (%) for $C_{23}H_{42}N_2O_3S$ (426.29) C 64.75, H 9.92, N 6.57; found C 65.07, H 10.01, N 6.94.

1-Hexadecylpyridinium cyclamate (**12**): ^{1}H NMR ($CDCl_3$) δ in ppm 0.87 (t, J = 6.6 Hz, 3H), 1.23(m, 3H), 1.25(m, 26H), 1.59(m, 2H), 1.71(m, 1H), 1.89(m, 2H), 1.99(m, 2H), 2.12(m, 2H), 3.24(m, 1H), 3.49(s, 1H), 4.84 (t, J = 7.4 Hz, 2H), 8.12 (t, J = 7.2 Hz, 2H), 8.48 (t, J = 6.6 Hz, 1H), 9.34 (d, J = 5.5 Hz, 2H); ^{13}C NMR ($CDCl_3$) δ in ppm 14.0, 22.6, 25.0, 25.7, 26.0, 29.0, 29.3, 29.4. 29.6, 31.8, 31.9, 34.4, 52.8, 52.8, 128.4, 144.8, 145.4; Elemental analysis calcd (%) for $C_{27}H_{50}N_2O_3S$ (482.76) C 67.17, H 10.44, N 5.80, found C 67.40, H 10.69, N 6.16.

Didecyldimethylammonium cyclamate (**13**): ^{1}H NMR ($CDCl_3$) δ in ppm 0.88 (t, J = 6.7 Hz, 6H), 1.01(m, 3H), 1.26(m, 30H), 1.48 (m, 1H), 1.68(m, 6H), 2.08(m, 2H), 2.90(m, 1H), 3.36(s, 6H), 3.47(m, 4H), 3.98(s, 1H); ^{13}C NMR ($CDCl_3$) δ in ppm 14.0, 22.6, 22.7, 25.0, 25.7, 26.2, 29.1, 29.3, 31.7, 34.4, 51.1, 52.7, 63.4; Elemental analysis calcd (%) for $C_{28}H_{60}N_2O_3S$ (504.853) C 66.61, H 11.98, N 5.55; found C 66.40, H 12.24, N 5.69.

Benzalkonium cyclamate (**14**): ^{1}H NMR ($CDCl_3$) δ in ppm 0.88 (t, J = 6.7 Hz, 3H); 1,24(m, 26H); 1,55(m, 2H);

1,71(m, 2H); 2,13(m, 2H); 2,38(m, 2H); 3,24(m, 6H); 3,43(m, 2H); 4,91(s, 2H); 7,44(m, 3H); 7,65(m, 2H); ^{13}C NMR ($CDCl_3$) δ in ppm 14.1; 22.6; 22.8; 25.0; 25.7; 26.2; 29.2; 29.3; 29.4; 29.5; 29.6; 31.8; 34.4; 49.6; 52.8; 63.3; 67.5; 77.2; 127.5; 129.1; 130.5; 133.2.

1-Butyl-3-methylimidazolium cyclamate (**15**): ^{1}H NMR ($CDCl_3$) δ in ppm 0,95 (t, J = 7.4 Hz, 3H), 1.16(m, 5H), 1.35(m, 2H), 1.55(m, 1H), 1.66(m, 2H), 1.87(m, 2H), 2.06(m, 2H), 3.20(m, 1H), 3.92(s, 1H), 4.07(s, 3H), 4.30 (t, J = 7.3 Hz, 2H), 7.49(s, 1H), 7.64(s, 1H), 9.89(s, 1H); ^{13}C NMR ($CDCl_3$) δ in ppm 13.2, 19.2, 22.9, 24.8, 25.5, 28.7, 31.9, 34.2, 36.2, 49.4, 52.6, 121.8, 123.6, 137.4; Elemental analysis calcd. (%) for $C_{14}H_{27}N_3O_3S$ (317.45): C 52.97, H 8.57, N 13.24; found: C 53.28, H 8.25, N 12.97.

1-Methyl-3-tetradecyloxymethylimidazolium cyclamate (**16**): ^{1}H NMR ($CDCl_3$) δ in ppm 0.88 (t, J = 6.7 Hz, 3H), 1.03(m, 3H), 1.25(m, 26H), 1.55 (m, 1H), 1.71(m, 2H), 2.07(m, 2H), 3.13(m, 1H), 3.56 (t, J = 6.5 Hz, 2H), 3.95(s, 1H), 4.10(s, 3H), 5.71(s, 2H), 7.48(s, 1H), 7.55(s, 1H), 10.18(s, 1H); ^{13}C NMR ($CDCl_3$) δ = 14.0, 22.6, 25.0, 25.6, 25.8, 29.2, 29.3, 29.4, 29.5, 29.6, 31.8, 34.2, 36.6, 52.9, 70.6, 79.2, 120.8, 123.8, 138.1; Elemental analysis CHN calcd. (%) for $C_{25}H_{49}N_3O_4S$ (487.74): C 61.56, H 10.13, N 8.62; found: C 61.69, H 9.84, N 8.86.

4.3 Thermal analysis

Melting points and other thermal transitions of synthesized cyclamates were determined by differential scanning calorimetry (DSC) with a Mettler Toledo Star^e DSC unit (Leicester, UK) cooled with an intracooler. The calorimeter was calibrated for temperature and cell constants using indium (melting point 156.61 °C, ΔH 28.71 J g^{-1}). Data were collected at constant atmospheric pressure using samples between 10 and 40 mg in aluminum sample pans. Experiments were performed heating at the rate of 10 °C min^{-1}. An empty sample pan was used as a reference.

Thermal decomposition temperatures were measured in the dynamic heating regime using a Mettler Toledo Star^e TGA/DSC1 unit (Leicester, UK) under nitrogen. Samples between 2–10 mg were heated from 40–500 °C under constant heating at 10 °C min^{-1}. Decomposition temperatures ($T_{onset5\%}$ and T_{onset}) were determined from onset to 5 mass loss and 50% mass loss respectively under nitrogen.

4.4 Surface activity

Surface tension measurements were carried out by the use of a Drop Shape Analysis System DSA100E (KRÜSS GmbH, Germany, accuracy ±0.01 mN m^{-1}), at 25 °C. Temperature was controlled using a Fisherbrand FBH604 thermostatic bath (Fisher, Germany. accuracy ±0.1 °C). The surface tension was determined using the pendant drop method. The pendant drop method is one of the most widely used techniques to measure the surface tension between gas-liquid and liquid-liquid interfaces. Basically, the method consists of fitting the Young-Laplace equation to the digitized shape of a drop suspended from the end of a capillary tube. Geometry of a drop is analyzed optically using a CCD camera. The method involves first locating the edge of the drop from a digital image which yields a set of points spaced along the drop profile. Next, the Young-Laplace equation for an axisymmetric interface is solved for an initial estimate of the parameters on which it depends. The parameters which govern the theoretical drop profile are adjusted and the equation is solved repeatedly until a best fit to the experimental points is obtained. The best fit is determined by minimizing an objective function which is typically a summation of the squared distances between the experimental points and the theoretical drop profile [23].

4.5 Anti-microbial characteristics

The following microorganisms were used: *Micrococcus luteus* NCTC 7743, *Staphylococcus aureus* NCTC 4163, *Staphylococcus epidermidis* ATCC 49134, *Enterococcus faecium* ATCC 49474, *Moraxella catarrhalis* ATCC 25238, *Escherichia coli* ATCC 25922, *Serratia marcescens* ATCC 8100, *Proteus vulgaris* NCTC 4635, *Pseudomonas aeruginosa* NCTC 6749, *Bacillus subtilis* ATCC 6633, *Candida albicans* ATCC 10231, *Rhodothorula rubra* (Demml 1889, Lodder 1934). Standard strains were supplied by the National Collection of Type Cultures (NCTC) London and American Type Culture Collection (ATCC). *Rhodothorula rubra* was obtained from the University of Medical Sciences, Poznan.

Anti-microbial activity was determined by the tube dilution method and described earlier [22]. Bacteria strains were cultured on a Müller-Hinton broth for 24 h and fungi on Sabouraud agar for 48 h. A suspension of the microorganisms at a concentration of 10^6 cfu mL^{-1} was prepared from each culture. This suspension was then used to inoculate each dilution of the broth medium at a 1:1 ratio. Growth of the microorganisms (or lack thereof) was determined visually after incubation for 24 h at 37 °C (bacteria) or 48 h at 29 °C (fungi).

The lowest concentration at which there was no visible growth (turbidity) was taken as the MIC (minimal inhibitory concentration). Then, an aliquot taken from each tube in a sample loop was cultured in an agar medium with inactivates (0.3% lecithin, 3% polysorbate 80, and 0.1% L-cysteine) and incubated for 48 h at 37 °C (bacteria) or for 5 d at 29 °C (fungi). The lowest concentration of the studied cyclamates supporting no colony formation was defined as the MBC (minimal bactericidal concentration) or MFC (minimal fungicidal concentration).

4.6 Bioassays

The bioassay experiments were conducted with *Tribolium*

confusum Duv. (larvae and adults), *Sitophilus granarius* L. (adults), and *Trogoderma granarium Ev.* (larvae). They came from laboratory colonies reared in a chamber maintained at 26 °C and 60% relative humidity on a wheat grain or whole-wheat meal diet. Choice and no-choice tests for insect-feeding were conducted following a previously described procedure [24]. Wheat wafer discs (1 cm in diameter 1 mm thick) were saturated by dipping either in ethanol only (control) or in a solution of the studied compounds (1%) in ethanol to be tested. After evaporation of the solvent (30 min of air-drying) the wafers were weighed and offered to the insects in plastic boxes as the sole food source for 5 d. The feeding of insects was recorded under three sets of conditions: (1) on two control discs (CC), (2) on a choice between one treated disc (T) and one control disc (C; choice test), and (3) on two treated discs (TT; no-choice test). Each of the three experiments was repeated five times with 3 adults of *Sitophilus granarius*, 20 adults and 10 larvae of *Tribolium confusum*, and 10 larvae of *Trogoderma granarium*. The number of individual insects depended on the intensity of their food consumption. The adults used for the experiments were unsexed. 7–10 d old, and the larvae were 5–30 d old. After 5 d the discs were reweighed and the average weight of eaten food was calculated.

In all variants, three deterrent coefficients were calculated as follows:

(a) the absolute coefficient of deterrency, calculated from no-choice test: $A = (CC - TT)/(CC + TT)\times 100$,

(b) the relative coefficient of deterrency $R = (C - T)/(C + T)$,

(c) the total coefficient of deterrency $T = A + R$

in which *CC* is the average weight of the food consumed in the control, *TT* is the average weight of the food consumed in the no-choice test and *T* and *C* are the average weights of the food consumed in the choice test.

The data were statistically analyzed by means of one-way ANOVA. In the cases where ANOVA results were statistically significant at the 5% probability level, Tukey's test was performed at the LSD values derived are presented.

4.7 Phytotoxicity

The influence of some synthesized cyclamates on growth of garden cress (*Lepidium sativum*) was examined using test tube agar method described by Mathur and Kondsdal [25] with our modifications [26]. The test was carried out at six replications. The logistic model for every relationship between compound and concentration was estimated and ED_{50} values were calculated.

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1 (a) Parvulescu VI, Hardacre C. Catalysis in ionic liquids. *Chem Rev,* 2007, 107: 2615–2665; (b) Chowdhury S, Mohan RS, Scott JL. Reactivity of ionic liquids. *Tetrahedron,* 2007, 63: 2363–2389; (c) Greaves TL, Drummond CJ. Protic ionic liquids: Properties and applications. *Chem Rev,* 2008, 108: 206–237; (d) Martins MAP, Fizzo CP, Moreira DN, Zanatta N, Bonacorso HG. Ionic liquids in heterocyclic synthesis. *Chem Rev,* 2008, 108: 2015–2050; (e) Wasserscheid P, Welton T. *Ionic Liquids in Synthesis*, Wiley-VCH, 2008

2 (a) Plechkova NV, Seddon KR. Applications of ionic liquids in the chemical industry. *Chem Soc Rev,* 2008, 37: 123–150; (b) Kirchner B. (vol. ed) *Ionic Liquids*. Berlin Heidelberg: Springer-Verlag, 2009; (c) Olivier-Bourbigou H, Magna L, Morvan D. Ionic liquids and catalysis: Recent progress from knowledge to applications. *Appl Catal A: General,* 2010, 373: 1–56

3 (a) Gorke J, Srienc F, Kazlauskas R. Toward advanced ionic liquids. Polar, enzyme-friendly solvents for biocatalysis. *Biotechnol Bioprocess E,* 2010, 15: 40–53; (b) Petkovic M, Seddon KR, Rebelo LPN, Pereira CS. Ionic liquids: A pathway to environmental acceptability. *Chem Soc Rev*, 2011, 40: 1383–1403; (c) Pham TPT, Cho Ch-W, Yun Y-S. Environmental fate and toxicity of ionic liquids: A review. *Water Res*, 2010, 44: 352–372; (d) Blesic M, Marques MH, Plechkova NV, Seddon KR, Rebelo LPN, Lopes A. Self-aggregation of ionic liquids: Micelle formation in aqueous solution, *Green Chem*, 2007, 9: 481–490; (e) Tariq M, Freire MG, Saramago B, Coutinho JAP, Canongia Lopes JN, Rebelo LPN. Surface tension of ionic liquids and ionic liquid solutions. *Chem Soc Rev*, 2012, 41: 829–868

4 Simmonds MSJ. Chemoecology: The legacy left by Tony Swain. *J Phytochem,* 1998, 49: 1183–1190

5 Pernak J, Syguda A, Mirska I, Pernak A, Nawrot J, Pradzynska A, Griffin ST, Rogers RD. Choline-derivative-based ionic liquids. *Chem Eur J,* 2007, 13: 6817–6827

6 Cybulski J, Wisniewska A, Kulig-Adamiak A, Lewicka L, Cieniecka-Roslonkiewicz A, Kita K, Fojutowski A, Nawrot J, Materna K, Pernak J. Long-alkyl-chain quaternary ammonium lactate based ionic liquids. *Chem Eur J,* 2008, 14: 9305–9311

7 Carter EB, Culver SL, Fox PA, Goode RD, Ntai I, Tickell MD, Traylor RK, Hoffman NW, Davis Jr JH. Sweet success: Ionic liquids derived from non-nutritive sweeteners. *Chem Commun,* 2004, 630–631

8 Pernak J, Stefaniak F, Węglewski J. Phosphonium acesulfamate based ionic liquids. *Eur J Org Chem*, 2005, 650–652

9 Hough-Troutman WL, Smiglak M, Griffin S, Reichert WM, Mirska I, Jodynis-Liebert J, Adamska T, Nawrot J, Stasiewicz M, Rogers RD, Pernak J. Ionic liquids with dual biological function: Sweet and anti-microbial, hydrophobic quaternary ammonium-based salts. *New J Chem*, 2009, 33: 26–33

10 Burgard A. Antimicrobially active acesulfame complexes, process for their preparation and their use. US Pat. 0023084, 2003

11 Jodynis-Liebert J, Nowicki M, Murias M, Adamska T, Ewertowska M, Kujawska M, Piotrowska H, Konwerska A, Ostalska-Nowicka D, Pernak J. Cytotoxicity, acute and subchronic toxicity of ionic liquid, didecyldimethylammonium saccharinate, in rats. *Regul Toxicol Pharmacol*, 2010, 57: 266–273

12 Jodynis-Liebert J, Nowicki M, Adamska T, Ewertowska M, Kujawska M, Petzke E, Konwerska A, Ostalska-Nowicka D, Pernak J. Acute and subacute (28-Day) toxicity studies of ionic liquid, didecyldimethyl ammonium acesulfamate, in rats. *Drug Chem Toxicol*, 2009, 32: 395–404

13 O'Brien NL. *Alternative Sweeteners*. New York: Marcel Dekker. Inc., 2001

14 Kroger M, Meister K, Kava R. Low-calorie sweeteners and other sugar substitutes: A review of the safety issues. *Compr Rev Food Sci F,* 2006, 5: 35–47

15 (a) Mitchell H. *Sweeteners and Sugar Alternatives in Food Technology*". Blackwell Publishing, Oxford, 2006; (b) Spillane WJ, Ryder CA, Walsh MR, Curran PJ, Concagh DG, Wall SN. Sulfamate sweeteners. *Food Chem,* 1996, 56: 255–261; (c) Spillane WJ, Hanniffy GG. Is the negative charge on $RNHSO_3$-M+ an essential requirement for sulfamate sweetness? *J Agric Food Chem*, 2003, 51:

3056–3059
16 de Carvalho LC, Pinotti Segato M, Spezia Nunes R, Novak C, Gomes Cavalheiro T. Thermoanalytical studies of some sweeteners. *J Therm Anal Calorim*, 2009, 97: 359–365
17 (a) Rosen MJ. *Surfactants and Interfacial Phenomena.* New York: Wiley, 1989
18 Beyer K, Leine D, Blume A. The demicellization of alkyltrimethylammonium bromides in 0.1 M sodium chloride solution studied by isothermal titration calorimetry. *Colloid Surface B,* 2006, 49: 31–39
19 Ley SV. et al. The synthesis of azadirachtin: A potent insect antifeedant. *Chem Eur J,* 2008, 14: 10683–10704
20 Morgan ED. Azadirachtin, a scientific gold mine. *Bioorg Med Chem,* 2009, 17: 4096–4105
21 Pernak J, Czepukowicz A, Poźniak R. New ionic liquids and their antielectrostatic properties. *Ind Eng Chem Res*, 2001, 40: 2379–2383
22 Pernak J, Kalewska J, Ksycińska H, Cybulski J. Synthesis and anti-microbial activities of some pyridinium salts with alkoxymethyl hydrophobic group. *Eur J Med Chem*, 2001, 36: 899–907
23 Thiessen DB, Chione DJ, McCreary CB, Krantz WB. Robust digital image analysis of pendant drop shapes. *J Colloid Interface Sci,* 1996, 177: 658–65
24 Nawrot J, Bloszyk E, Harmatha J, Nowotny L, Drozdz, B. Action of antifeedants of plant origin on beetles infesting stored products. *Acta Entomol Bohemosl*, 1986, 83: 327–335
25 Matur SB, Kongsdal O. *Common Laboratory Seed Health Testing Methods for Detecting Fungi*. International Seed Testing Association, 2003
26 Walkiewicz F, Materna K, Kropacz A, Michalczyk A, Gwiazdowski R, Praczyk T, Pernak J. Multifunctional long-alkyl-chain quaternary ammonium azolate based ionic liquids. *New J Chem*, 2010, 34: 2281–2289