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Chemical stability of chlorine dioxide in the presence of prochloraz manganese

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Abstract: The chemical stability of chlorine dioxide (ClO_2) in the presence of prochloraz manganese (prochloraz-Mn) as biologically active substances used in agrochemical treatments in the cultivation of mushrooms are presented. For model mixtures of the tested components, a relative decrease in ClO_2 content over time was measured in the proportions applied during the mushroom cultivation cycle. Within 20 min after preparing a mixture of 1 500 ppm prochloraz-Mn and 100 ppm ClO_2 in water, the relative decrease in the concentration of ClO_2 was 20%. The obtained results indicate a possibility of simultaneously introducing the working mixture of ClO_2 and prochloraz-Mn salt into the champignon peat casing, assuming its use directly after preparation.

Keywords: champignons; cultivation of mushrooms; peat casing

The prophylaxis of mushroom crop infections includes the use of disinfectant products and plant protection products, the use of which is regulated by the series of independent European legal acts [i.e. Regulation (EC) No 1107/2009; Regulation (EU) No 528/2012]. Biocidal products require authorisation to be issued by a competent authority, usually Ministry of Health or Ministry of Agriculture. The classification of biologically active substance in one of the above-mentioned categories sometimes requires a detailed analysis of the matter to be affected by the applied hygienic procedure. For example, this may be an answer to the question whether the applied agent interacts with or is supposed to affect dead matter, such as mushroom substrate or cover, or living matter, which is the mushroom mycelium or mushroom fruiting bodies. In either case, the main goal is to prevent the development of pathogens without causing a detrimental effect on the growth and quality of the mushrooms. ClO_2, which is popular in the cultivation of edible mushrooms, is a specific type of active ingredient. It can be used for disinfecting the mushroom substrate after its application (Geels *et al.* 1991; Szumigaj-Tarnowska *et al.* 2012). After activation, it disappears very quickly as a gas dissolved in water, by evaporating from the surface or decomposing into neutral chlorides (Gordon 1972). Its biocidal efficacy is achieved over several seconds in surface contact before desorption or neutralisation (Simpson *et al.* 1993; Noszticzius *et al.* 2013). ClO_2 is also approved for the microbiological stabilisation of water in the prevention of secondary infections, carried out by irrigation during the cultivation cycle (Yao *et al.* 2010; Reitz *et al.* 2015; Scarlett 2017; Truchado *et al.* 2018).

ClO_2 is a known non-specific biocide. Due to its strong oxidising properties resulting from its free-radical molecular structure, ClO_2 shows a high bactericidal and virucidal activity which, due to its low harmfulness to higher organisms, results in high interest in its use

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for the prophylaxis of crop plants, including anti-fungal treatments (Motarjemi & Lelieveld 2013).

Studies on the chemical activity of ClO_2 in aqueous solutions, being the subject of many research works, show the possibility of effective and quick reactions with various functional groups by free radical oxidation (Dennis *et al.* 1967; Hull *et al.* 1967, 1969a, b, c; Rosenblatt *et al.* 1967; Davis *et al.*1972; Rav-Acha *et al.* 1985; Choshen *et al.* 1986; Merenyi *et al.* 1990; Hoignè & Tratnyek 1994). The non-specific mechanism of the bactericidal and virucidal actions of ClO_2 can be justified by its course of reactions with numerous molecules of key importance for the organism under attack, at different cell structure levels, in cell membrane, organelles, intracellular processes, or virus (protein coat, genetic material). Due to its strong oxidising properties, in particular in aqueous solutions, ClO_2 exhibits biocidal activity by affecting the balance of cellular processes involving the electron transfer.

Earlier reports demonstrated the ability of ClO_2 to react quickly in aqueous solutions with tyrosine (Napolitano *et al.* 2005), cytosine (Ison *et al.* 2006), or guanosine monophosphate (5'-GMP) (Napolitano *et al.* 2006). An interesting fact sheds light on the mechanism of ClO_2 interaction on living organisms: the results of research show a strong ClO_2 interference in the redox processes determining electrochemical equilibria in the mitochondria and cell membranes, in particular in relation to the $NADH/NAD^+$ redox system responsible for cellular respiration and mediating ATP synthesis (Bakhmutova *et al.* 2008).

Both after the application of the champignon peat casing and during cultivation, plant protection agents, including prochloraz or a complex prochloraz manganese salt (prochloraz-Mn), are used to counteract wet, dry and white rot infections (*Mycogone, Lecanicillium*), dactylium (*Cladobotryum*) or green mould (*Trichoderma aggressivum*) (Gea *et al.* 1996; Grogan *et al.* 2000; Potočnik *et al.* 2008; Carrasco *et al.* 2017).

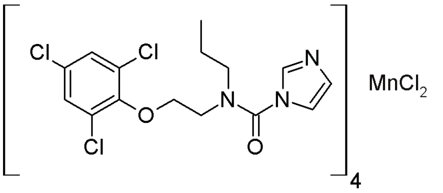

Figure 1. Empirical formula of a complex salt of prochloraz and manganese (II) chloride (dichlorotetrakis [*N*-propyl-*N*-[2-(2,4,6-trichlorophenoxy)ethyl]-1*H*-imidazole-1-carboxamido]-manganese (II), CAS number 75747-77-2, EC No. 278-301-3)

Prochloraz-Mn is a well-known fungicide, widely used in the control and prevention of fungal diseases (Figure 1).

The structural element that determines the biochemical activity of prochloraz is the aromatic imidazole moiety, which has a high ability to produce stable coordination bonds and, therefore, has an affinity to biologically active compounds of living organisms.

In view of the mechanism of biochemical reactions, prochloraz is considered to be a non-competitive inhibitor. Its action is the coordination of the heme iron atom and incorporation as the sixth ligand into the lanosterol 14-demethylase molecule, responsible for the synthesis of ergosterol, which is a component of the fungal cell membrane (Jefcoate 1978; Henry & Sisler 1984; Vinggaard *et al.* 2006).

In cultivation practice there arises a question whether ClO_2 may be administered simultaneously with the prochloraz-Mn solution or if it is necessary to separate the two hygienic procedures and apply these active substances individually, which is more complicated and time consuming in the latter case. The decisive condition for the resolution of the sense of combining both treatments over time is the adequate chemical stability of both components present in the solution and/or suspension.

Considering the possibility that the chemical structure elements of the prochloraz molecule may react with highly reactive ClO_2, the doubt suggested above seems to be justified.

The aim of this paper is to determine the chemical stability of the ClO_2 solution in a working mixture with prochloraz-Mn, as active substances used in the cultivation of mushrooms, in order to optimise the effectiveness of the agrochemical operations performed.

MATERIAL AND METHODS

The ClO_2 solution was prepared by mixing ARMEX 5 commercial product with its MEXACID activator, both from MEXEO, Kędzierzyn-Koźle (Approval No. 2437/05 of the Ministry of Health of the Republic of Poland, and the state registration Certificate No. 3358, issued by Ministry of Agriculture and Food of the Republic of Belarus).

Complex salt of manganese with prochloraz of 97% purity, defined as prochloraz-Mn, was purchased from Hangzhou DayangChem Co., Ltd., Hangzhou, China.

The experiments were carried out in aqueous solutions at the concentrations applied to the peat casing after its

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lining, i.e. 50 and 100 ppm ClO_2, and 1 500 and 750 ppm of prochloraz complex salt, respectively. Changes in the ClO_2 content over time were observed spectrophotometrically by measuring the absorption spectra of UV radiation in the range of 200 to 400 nm. UV spectra were measured using a Hitachi UV 2900 spectrophotometer (Hitachi High-Tech Science Corporation, Tokyo, Japan). The tests were performed at the MEXEO Institute of Technology in Kędzierzyn-Koźle.

RESULTS

Figure 2 represents UV spectra for the pure solution of 200 ppm of ClO_2 in water (Spectrum 1) and saturated prochloraz-Mn solution (Spectrum 2). Figure 3 shows a representative series of the spectra of prochloraz-Mn and ClO_2 solutions, recorded at the intervals of 0, 15, 30, 45, 60, 90, 120, and 180 min from the preparation of the solution. Figure 4 shows the relative decreases in ClO_2 concentration ($\Delta c_{ClO_2}/c_0$) for the four test solutions with different prochloraz and ClO_2 concentrations.

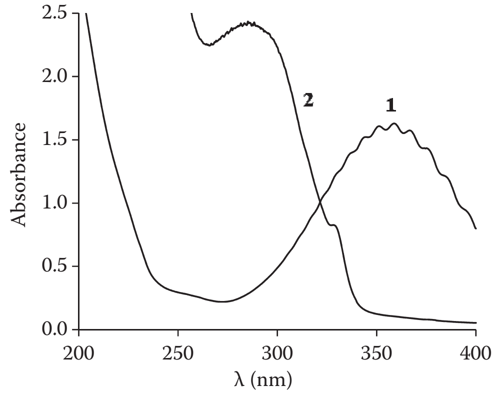


Figure 2. UV absorption spectra of aqueous solutions of ClO_2 200 ppm (1) and saturated solution of prochloraz-Mn salt (2)

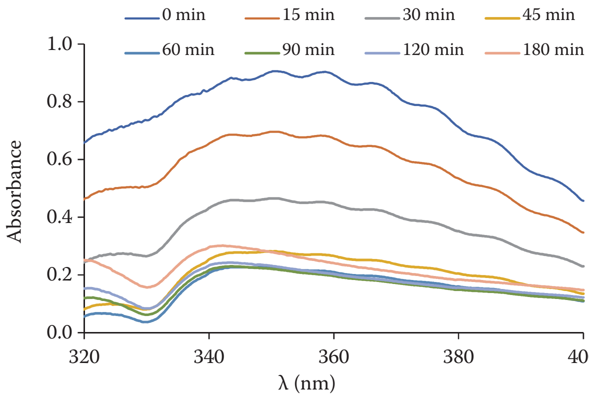


Figure 3. Changes in the UV spectra over time for a solution with 750 ppm prochloraz-Mn and 100 ppm ClO_2

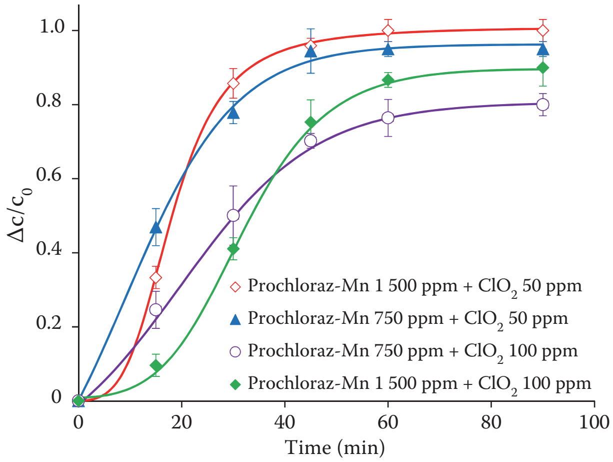


Figure 4. The curves showing the relative decrease in ClO_2 concentration ($\Delta c_{ClO_2}/c_0$) versus prochloraz-Mn and ClO_2 concentrations

DISCUSSION

Premises of possible interactions between ClO_2 and prochloraz. The potential centres of ClO_2 reaction with the prochloraz molecule include the tertiary amino nitrogen atom in the aliphatic chain fragment and the tertiary imino atom of the imidazole moiety. The reactions of ClO_2 with amines were described by Dennis *et al.* (1967), Hull *et al.* (1967, 1969a, b, c), Davis *et al.* (1972), and Merenyi *et al.* (1990).

The mechanism determining the possible route of the prochloraz molecule degradation is presented below.

In the reaction with a tertiary amine, the first step is the electron transfer of the nitrogen atom to the electron deficient active ClO_2 radical to form a cation (1), followed by the proton cleavage to give the azanyl radical according to Equation (2).

The attack of another ClO_2 radical (3) leads to the formation of an active iminium cation which, as a result of hydrolysis in an aqueous medium (4), leads to the

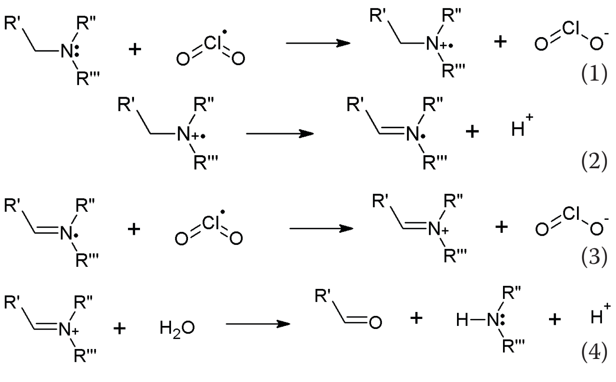
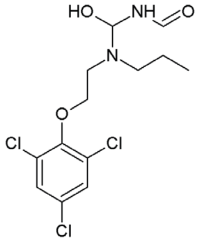


Figure 5. The mechanism of the reaction of ClO_2 with amines

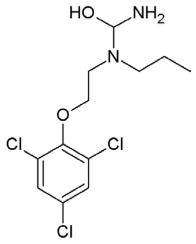
cleavage of the aldehyde molecule and the formation of an amine with the order reduced in (1) (Figure 5).

Based on the above schematic, it is possible to predict the directions of the reaction of prochloraz with ClO_2. The expected preferred reactions may be the ones resulting from the opening of the imidazole ring:

(a) opening of the imidazole ring leading to substituted formamide (I) and next to the diaminomethanol derivative (II);

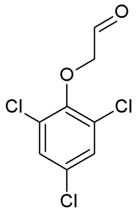


(I)

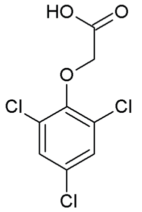


(II)

(b) further oxidation of (I) and/or (II) according to Eqs (1)–(4) with the cleavage of formamide and urea moieties leading to (2,4,6-trichlorophenoxy) acetaldehyde (III) and (2,4,6-trichlorophenoxy) acetic acid (IV).



(III)



(IV)

Compounds (I), (II), (III), and (IV) are known and also belong to a large group of prochloraz metabolites, resulting from its intracellular oxidation (Roberts *et al.* 1999; WHO Geneva 2002).

Experimental result discussion. A significant property of the UV spectrum of ClO_2 (Figure 2) is the absorption peak observed in the range of 270–500 nm with the maximum at $\lambda = 360$ nm. A characteristic feature of this part of the ClO_2 spectrum is either its distinct rotational structure (absorbance oscillations) typical of small molecules with high freedom of rotation. This phenomenon is well known (Vaida & Simon 1995) and it is a hallmark of aqueous ClO_2 solutions enabling both ClO_2 detection and determination of its concentration in aqueous solutions. At a wavelength $\lambda = 360$ nm, there is also a difference in absorbance values between the two components, where the absorbance of the ClO_2 solution reaches the maximum and the absorbance value of the prochloraz-Mn solution is minimal, favourable for the detection and measurement of ClO_2 concentration in the presence of prochloraz.

A comparison of the spectra of a representative series of binary prochloraz and ClO_2 solutions at the ClO_2-specific absorption areas shows a decrease in the absorption of UV radiation in the whole wavelength region, indicating a decrease in ClO_2 concentration over time (Figure 3).

The observed decrease in absorbance is maintained for solutions up to $t = 90$ min followed by a marked increase in absorbance. Moreover, an increase in the absorbance of solutions with a lifespan over 90 min. is accompanied by disappearance of the characteristic for the ClO_2 spectrum rotational structure mentioned earlier, which may indicate further ClO_2 decay, and by appearance of measurable amounts of prochloraz oxidation products, active spectrophotometrically in the studied wavelength range. Accordingly, in further quantitative considerations regarding the drop in ClO_2 concentration, only solutions with a lifespan up to 90 min have been taken into account.

In order to determine the course of a decrease in the ClO_2 content in the test solutions, it was assumed that, at the length corresponding to the absorption maximum ($\lambda = 360$ nm), the absorbance of the ClO_2 solution is directly proportional to the concentration (the solution meets Beer's law), which could be expressed by Eq. (5):

$$A = \varepsilon \times l \times c_{ClO_2} \tag{5}$$

where: A – absorbance; ε – molar absorption coefficient (l/mol/cm); l – thickness of the absorbing layer (cm); c_{ClO_2} – ClO_2 concentration in solution (mol/l)

In view of the above assumption, the relative decrease in ClO_2 concentration (α) over time can be defined by Eq. (6):

$$\alpha = \frac{\Delta c_{ClO_2}}{c_0} = \frac{\Delta A_{360nm}}{A_0} \tag{6}$$

where: $\Delta c_{ClO_2} = c_0 - c$ – decrease of ClO_2 concentration (mol/l); c_0 – initial concentration of ClO_2 (mol/l); ΔA_{360nm} – decrease of the solution absorbance; A_0 – initial value of absorbance

The curves showing the relative decrease of ClO_2 concentration (α) in time for different prochloraz-Mn and ClO_2 concentrations are represented in Figure 4.

The error bars marked in the graphs show the intervals of one standard deviation for each single point depicting the average of 5 measurements.

The values of standard deviations were calculated according to the rule of the propagation of uncertainty (Eq. (7)):

$$s_\alpha^2 = \left(\frac{\delta\alpha}{\delta A}\right) s_A^2 + \left(\frac{\delta\alpha}{\delta A_0}\right)^2 s_{A0}^2 \tag{7}$$

where: s_α – standard deviation of ClO_2 concentration; s_A – standard deviation of absorbance; s_{A_0} – standard deviation of initial value of absorbance

The standard deviation bars describing the variability of parameter α for each of the points indicate that the difference in the position of the two pairs of curves corresponding to ClO_2 concentrations of 50 and 100 ppm is statistically significant.

The course of the two curves corresponding to ClO_2 concentration of 50 ppm for time over 30 min is statistically indistinguishable, which may indicate their convergence to the common value of parameter α (about 0.95).

The difference in the course of the curves that characterise changes in time with ClO_2 concentration of 100 ppm is statistically significant.

The course of the curves shown in Figure 4 reveals that, for the lower ClO_2 concentration, i.e. 50 ppm, the relative decrease in its concentration over time, referred to the initial concentration, is faster. The half-life of ClO_2, corresponding to a 50% decrease in concentration, is about 15 minutes. With ClO_2 concentration of 100 ppm, the relative decrease in ClO_2 concentration per unit of time is smaller, with a half-life of approx. 35 minutes.

It can be shown that the rate of change in the absolute ClO_2 concentration increases with the component concentration product, which is in conformity with the physicochemical (kinetic) background of the observed phenomenon. The above graph also shows that within 1 h after the preparation of the test solution of the active substance mixture at the concentration of 50 ppm almost the total amount of ClO_2 was subjected to degradation whereas at the concentration of 100 ppm ClO_2, the solution still contained approx. 20% of the initial ClO_2 amount (20 ppm). According to the assumed chemical reaction between ClO_2 and prochloraz-Mn, the final content of ClO_2 depended on the initial concentration of that component and was lower at the higher initial concentration of prochlorazMn.

Based on the measurements it can be concluded that it is possible to simultaneously add the working solution of a mixture of ClO_2 and manganese salt of prochloraz to the champignon peat casing, assuming its use immediately after preparation. Within 20 min after the preparation of a mixture of 1 500 ppm prochloraz-Mn and 100 ppm ClO_2, the relative decrease in ClO_2 concentration in the solution of the mixture with prochloraz-Mn is about 20%. During the long-term storage of the prepared working solution, both ClO_2 and prochloraz content will drop significantly.

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