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Calcium disodium ethylenediaminetetraacetate as a safe compound for crop protection with the potential to extend the basic substances group

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Abstract: Excessive use of commercial synthetic fungicides in agriculture is a globally discussed issue. This topic is seen as particularly important in modern plant protection and cultivation systems, where the total fungicide burden of the agroecosystem should be controlled and reduced. Basic substances (BSs) are a relatively novel, legally recognised group of substances which can be applied. The present study tested calcium disodium ethylenediaminetetraacetate (CaNa₂-EDTA), a substance whose properties and environmental safety make it another useful alternative for plant protection in modern farming. The study demonstrated the high antifungal activity of the substance against *Pyrenophora* (anamorph: *Drechslera*) *tritici-repentis* (Died.) Drechsler, (MIC₅₀ 0.195–0.223 mg/mL), safety for plant tissue and especially for non-target organisms, and positive effects on the yield of wheat (*Triticum aestivum* Linnaeus). CaNa₂-EDTA surpassed the effect of chitosan hydrochloride, a registered and utilised substance, and a synthetic conventional fungicide. CaNa₂-EDTA is an excellent candidate for registering within the BS group, with benefits for environmentally sound systems in plant protection.

Keywords: CaNa₂-EDTA; low risk active substances; antifungal effect; non-target organism; yield; organic farming

In conventional agricultural practice, applying synthetic commercial fungicides is one of the basic measures for maximising yield. This is often a crucial and also, sometimes, a controversial input from the grower. Unnecessary and routine applications of synthetic fungicides into the crop create a major problem, even in the current period, because they often do not consider the economic threshold of monitoring the harmfulness and fungal pathogens. Such overuse is disadvantageous for the grower and especially harmful to the environment, non-target organisms and human health (Geiger et al. 2010; Özen & Darcan 2011; Lozowicka 2015). In addition, this approach is responsible for the increase in the occurrence of resistant strains of fungal pathogens in general (Brent & Hollomon 1995; Verveij et al. 2009; Avenot & Michailides 2010). In agriculture,

the increasingly popular new environmentally friendly trends can be seen as a modern tendency. Here, it is possible to apply novel and environmentally safe products to reduce or even eliminate the need for conventional and synthetic fungicides. Plant protection, however, poses the biggest challenge in organic farming, since the use of commercial synthetic fungicides is impossible and strictly prohibited in such systems (Hillocks 2012). For these reasons, the use of natural, botanical pesticides and environmentally safe substances is becoming increasingly attractive (Dubey et al. 2010; Žabka et al. 2014; Marchand 2015). Some examples supporting this trend include the introduction of new, legally recognised groups of substances in the member states of the European Union (EU) referred to as basic substances (BSs) and also low-risk active

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substances (LRASs) as part of the (EC) Regulation No.1107/2009 (Article 23). A positive contribution can be seen in the simplification of the legislation in regards to the BSs registration process, which greatly accelerates the implementation of these substances and environmentally safe products into agricultural practice (EFSA 2017). This significant move particularly exists in the developed countries and regions of the world. BSs form a promising group that offers alternative protection products reflecting the environmental trends in modern agriculture. This study is focused on a promising and not-yet-registered substance, calcium disodium ethylenediaminetetraacetate (CaNa₂-EDTA). The advantage of CaNa₂-EDTA involves a high level of safety for humans and other warm-blooded organisms. This substance is known very well globally; in European countries, it is permitted for use in the food industries as a food additive (E385) and it is widely applied in the cosmetics industry as well and even in human medicine (Ernst 2000; Flora et al. 2008; Jiménez 2014; Van de Sande et al. 2014). Since experiments have revealed that the substance has promising antifungal and ecological characteristics, as well as the potential for the protection and treatment of plants, it could become recognised by legislation and then included in the group of BSs. This study also confirmed, through laboratory methods, its effectiveness against *Pyrenophora tritici-repentis* (PTR), one of the most serious pathogens of wheat which causes leaf tissue chlorosis and necrosis (tan spot) leading to severe yield losses (Moreno & Perelló 2010). More importantly, the registration process. The aim of this study was also to confirm the positive effect of the foliar application of this safe antifungal substance on the final grain yield of winter wheat. The effect on the grain yield was compared with the effect of hydrochloride chitosan, an already registered BS, and with a standard commercial synthetic fungicide possessing both a direct and systemic effect. The present study could support the idea of a further extension of the group of BSs, as well as the effective implementation of CaNa₂-EDTA in plant protection and cultivation.

MATERIAL AND METHODS

Chemicals used in experiments

CaNa₂-EDTA and chitosan hydrochloride were sourced from Sigma-Aldrich (Czech Republic). Both of the substances were of analytical grade. The commercial

insecticide Vaztak[®] (BASF, Czech Republic) was used as the positive control for the acute toxicity test of CaNa₂-EDTA to non-target organisms. Active substance: alpha-cypermethrin 50 g/L. The commercial fungicide Soligor[®] (Bayer, Czech Republic) was used as a positive control for the field experiments.

Active substances: Prothioconazole 53 g/L, Spiroxamine 224 g/L and Tebukonazole 148 g/L. During the experiment, all the substances were kept at 19 °C in a dark and dry area. The application solutions were prepared immediately before the application.

Growth inhibitory effect of CaNa₂-EDTA on PTR and minimum inhibitory concentration

In testing the anti-fungal effect, three strains of PTR were applied; JV8-17001; JV8-17002 and JV8-17004. The strains were sourced from the collection of phytopathogenic fungi maintained at the Crop Research Institute, Prague, Czech Republic. The strains were preserved on slant agar (V8 Agar) at 4 °C (Lamari & Bernier 1989). The sub-culturing, preparation and all the other handling operations were undertaken under sterile conditions.

The antifungal inhibitory effect of CaNa₂-EDTA on the growth of PTR strains was tested using the agar dilution method. CaNa₂-EDTA was properly dissolved in an equal volume of nano-pure water. The dissolved compound was then properly diluted in potato dextrose agar (PDA) at a basic concentration of 2 mg/L. The final concentration of the solvent in the PDA was 0.25% (v/v). The Petri dishes (9.0 cm in diameter) with the amended PDA were aseptically inoculated with assay discs (0.4 cm) cut from the periphery of a 7-day-old culture of the target fungal strains. The control sets were subsequently prepared using an equal volume of water without the tested compound. Incubation was carried out in the dark at 21 °C for seven days. The percent inhibition of the radial growth of the target fungi was calculated according to the following Formula 1 (Abbott 1925):

$$\text{Percent inhibition (\%)} = \frac{D_c - D_T}{D_c} \times 100 \quad (1)$$

where: D_c – the colony diameter of the control sets; D_T – the colony diameter of the treated sets.

The minimum inhibitory concentrations (MIC₅₀) were determined by the method of graded concentration of the compound (from 0.1, 0.25, 0.5, 1.0 and 2.0 mg/mL) in the PDA. Cultivation was carried out in the same way as before (in the dark at 21 °C, for

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7 days). The MIC_{50} was regarded as the concentration of the compound that resulted in a 50% inhibition of the visible growth when compared with control sets (Žabka et al. 2009, 2013, 2014). The MIC_{50} values were then calculated using statistical analysis.

CaNa₂-EDTA phytotoxicity test

The phytotoxicity to the plant tissue was determined on the basis of the measurement of the photosynthetic rate directly on the leaves of the wheat after the foliar application according to the methods of Hola et al. (2010) and Kuklova et al. (2014). The measurement was carried out as per the applicable guidelines and instructions, using an LCA 4 infrared gas analyser (ADC Bio Scientific Ltd, UK) using the irradiance of 650 $\mu\text{mol m}^2/\text{s}$ of the photosynthetically active radiation. The experimental concentrations of the substance were 0.25, 0.5 and 1.0%. The control option was only with water. The negative effect on the photosynthesis was tested 72 and 168 h after the application. At least 10 replications were carried out for each of the concentrations. The values of the photosynthetic rates were measured under controlled lighting and temperature conditions with a controlled environment at 17 ± 1 °C with a humidity of 70–75%, with a 15 : 9 light-to-darkness cycle (illumination: 21 000 lx). The parameters were adjusted to simulate the natural conditions as much as possible. Subsequently, all the data were evaluated statistically.

CaNa₂-EDTA acute toxicity test on non-target organisms

A model soil species *Eisenia fetida* (Savigny, 1826) (earthworm) was selected for testing the acute toxicity to non-target organisms. The tests were carried out according to the standard OECD methodology (1984). An artificial soil substrate was prepared by mixing sphagnum peat (10%), kaolinite clay (20%) and quartz sand (70%). The pH level of the mixture was adjusted to 6.0 using calcium carbonate. The substrate was mixed with an experimental quantity of the test substance, i.e., CaNa₂-EDTA, the proportion being 100, 50 and 25 mg/kg of dry weight. Water was used as the solvent. An equivalent amount of water was used as the negative control. The pesticide Vaztak was used as the positive control, the concentrations being 1000, 500, and 250 $\mu\text{L}/\text{kg}$ (v/v), which is the equivalent of the α -cypermethrin concentrations of 50.0, 25.0, and 12.5 mg/kg of dry weight of the substrate. The test was conducted in 1-L glass containers filled with 650 g of

the substrate and enclosed using a polyethylene lid with gauze to ensure optimal air circulation. 10 adults of *E. fetida* were placed in the containers; the vessels were then placed in an air-conditioned box and thermostatically regulated at 20 ± 1 °C with a humidity of 80–85%. The light/dark regimen was set in the proportion 8 : 16 (illuminations of 600 lx). The incubation of the earthworms was terminated by establishing mortality after 7 and 14 days. Afterwards, the experiment was evaluated statistically.

The design of the field experiment and testing of the positive effect on the yield of winter wheat

For testing the effect of the foliar application of CaNa₂-EDTA on the model plant, i.e., winter wheat, under field conditions, two different sites were selected in the Czech Republic (CR): (1) Kluky (GPS: 49°18'49.86" N, 14°14'51.65" E) – a moderately warm and moderately dry site, soil type: brown earth, soil texture: loamy-sandy soil, elevation: 420–440 m, total annual precipitation: an average of 572 mm. Average annual temperature: 7.2 °C. (2) Nechanice (GPS: 50°14'21.84" N, 15°37'59.16" E) – a warm, rather dry site, soil type: illimerised brown earth, soil texture: clayey-loamy. Elevation: 239 m. Total annual precipitation: an average of 596.9 mm. Average annual temperature: 8.1 °C. The experiment was undertaken in a period of two consecutive years (2016 and 2017). Small plot trials with three replications were completely randomised at each of the sites. The size of each of the plots equalled 25 m². The winter wheat variety of Forhand was used in the trial. A standard, high-pressure pneumatic (0.3 bar) ZEMS trial plot sprayer was used for the application of the substances.

The winter wheat stand received the conventional herbicide and insecticide treatment only. The foliar application of the test substance CaNa₂-EDTA (rate: 750 g/ha) and registered BS chitosan hydrochloride (rate: 100 g/ha) took place at the stage of BBCH 39 (Zadoks et al. 1974). The commercial product Soligor (rate: 0.9 L/ha) was administered at the same stage as the positive control. The substances were applied after being dissolved in water, rate: 250 L/ha. The negative control was treated using the foliar application of an equivalent quantity of water. Harvesting took place at both sites; in both years, at the hard dough stage (BBCH 87). The weather data were recorded daily and are reported as the mean monthly data for both growing seasons (Figure 1).

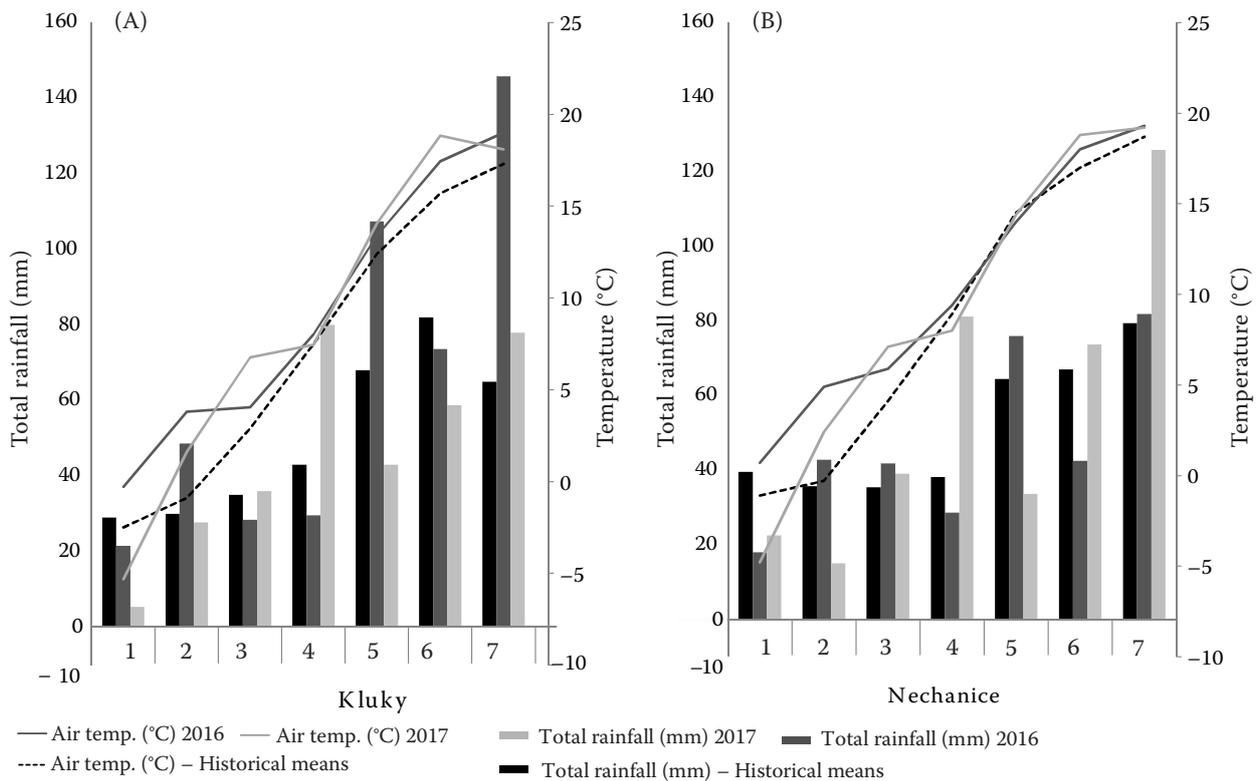


Figure 1. Average monthly air temperature and total rainfall during the 2016–2017 period at (A) Kluky and (B) Nechanice

The grain yield from the experimental plots was weighed precisely. The data were statistically analysed.

Statistical analysis

MIC₅₀ assessment. A Probit Analysis was applied to assess the MIC₅₀ values for each effective compound associated with 95% confidence limits (CI⁹⁵) (Finney 1971). The EPA (Environmental protection Agency) Probit Analysis Program (Version 1.5) was used for the statistical evaluation. The MIC values were statistically calculated and associated with the Chi-square values significant at a $P < 0.05$ level.

Phytotoxicity test, toxicity to non-target organisms and field experiments evaluation. Statistica software (Version 13) was used for the statistical evaluation. The percentages were transformed using arcsine square root ($\arcsin\sqrt{\cdot}$) transformation before

running an ANOVA. The treatment differences were determined by Tukey's test ($P \leq 0.05$).

RESULTS

Inhibitory effect of CaNa₂EDTA on PTR

For CaNa₂-EDTA, the antifungal properties represented by the values of the inhibited growth of the three PTR strains were confirmed with success. The values comprising the % of growth inhibition at the basic experimental concentration of 2 mg/mL and the values of the MIC₅₀ for each strain, including CI⁹⁵ and Chi-square, are shown in Table 1. There is a considerable balance seen in the growth inhibition (83.5–85.8%) within the tested strains at the concentration of 2 mg/mL of the substance. Although the growth inhibition of the pathogen at the basic

Table 1. The inhibitory effect of CaNa₂-EDTA on the target fungi at the concentration 2 mg/mL and MIC₅₀

PTR Strains	Inhibition (%)	S.D.	MIC ₅₀ ^a (mg/mL)	CI ^{95b}	χ^2 ^c
JV8-17001	85.8	± 0.05	0.195	0.138–0.254	4.302
JV8-17002	84.5	± 0.08	0.223	0.162–0.285	4.924
JV8-17004	83.5	± 0.10	0.186	0.124–0.249	2.650

^aThe minimum inhibitory concentration of the compound that resulted in a 50% inhibition; ^b95% confidence intervals; ^c χ^2 -square value, significant at $P < 0.05$

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concentration was not an absolute inhibition, the relatively low MIC₅₀ values of this substance indicate a high efficiency. The MIC₅₀ values were around 0.2 mg in each of the PTR strains.

Safety and non-toxicity of CaNa₂-EDTA toward plant tissue

The safety and non-toxicity of CaNa₂-EDTA toward plant tissue was tested and statistically confirmed by measuring the photosynthetic rate directly on the leaves of the wheat (Table 2). The values for each of the variants after the application of the substance were statistically in accordance with the physiologically normal values of the plants in the control group treated with water only. No statistically significant negative effect was observed after 72 h and even after 168 h of exposure. Statistically significant differences were not observed even at the highest

Table 2. The photosynthetic rate (μmol CO₂ m²/s) after CaNa₂-EDTA tissue exposition

	72 hours	168 hours
Water	4.28 ± 0.98 ^a	3.97 ± 1.14 ^a
EDTA 0.25%	4.20 ± 0.73 ^a	4.32 ± 0.68 ^a
EDTA 0.5%	3.52 ± 0.68 ^a	3.99 ± 1.06 ^a
EDTA 1.0%	3.96 ± 0.35 ^a	3.25 ± 0.66 ^a
<i>df</i>	9	
<i>F</i>	2.826	
<i>P</i>	0.005	

EDTA – Ethylenediaminetetraacetic acid; *df* – total degree of freedom, *F* – *F* value, *P* – the significance level; the means ± SD within a column followed by the same letter do not differ significantly (Tukey's HSD test; *P* < 0.05)

concentration of the applied substance, which was up to five times higher than the real rate of concentration in the treatment of the stands.

Safety and non-toxicity of CaNa₂-EDTA toward non-target organisms

The safety and non-toxicity of CaNa₂-EDTA toward non-target organisms was tested and statistically confirmed using the earthworm *E. fetida*, a useful model soil organism. The experimental data show that there was no statistically significant harmful influence of the test substance on the model organism, in comparison with the negative control (Table 3). Mortality was not observed in any case of the tested experimental concentrations of the test substance, even after 14 days of incubation. On the other hand, a highly significant, up to 100% mortality was found in the case of α-cypermethrin.

Effect CaNa₂-EDTA on the yield of winter wheat under field conditions

The trial, testing the influence of CaNa₂-EDTA in the field situation, demonstrated a positive effect on the overall yield of the winter wheat grain. The yield values provided in Table 4 show a statistically significant increase in the case of the variant treated with CaNa₂-EDTA compared with the negative control, seen on both experimental plots in both years. Comparing the application of CaNa₂-EDTA against chitosan hydrochloride, which is an already registered and used BS, comparable and statistically significantly higher yields were observed for the former at both of the sites in the first year and second year.

The positive control with Soligor showed a statistically more significant increase in the yield in 2016 only. By contrast, in 2017, the increase in yield was

Table 3. The toxicity of CaNa₂-EDTA and α-cypermethrin on the *E. fetida* earthworms

Treatment and conc. (mg/kg)	7 th day* (mortality % ± SD)	14 th day* (mortality % ± SD)
E385 100.0	0.0 ± 0.0 ^a	0.0 ± 0.0 ^b
E385 50.0	0.0 ± 0.0 ^a	0.0 ± 0.0 ^a
E385 25.0	0.0 ± 0.0 ^a	0.0 ± 0.0 ^a
A-CM 50.0	100.0 ± 0.0 ^c	100.0 ± 0.0 ^c
A-CM 25.0	100.0 ± 0.0 ^c	100.0 ± 0.0 ^c
A-CM 12.5	86.0 ± 5.5 ^b	94.0 ± 5.5 ^b
Control	0.0 ± 0.0 ^a	2.5 ± 5.0 ^a
ANOVA	<i>F</i> _{6,21} = 383.05; <i>P</i> < 0.0001	<i>F</i> _{6,21} = 532.32; <i>P</i> < 0.0001

*The average mortality of *E. fetida* (± SD) achieved on the 7th and 14th day after application of CaNa₂-EDTA and α-cypermethrin (A-CM); the means ± SD within a column followed by the same letter do not differ significantly (Tukey's HSD test, *P* < 0.05); % – arcsine square root transformed data; negative control = water

Table 4. The effect of the CaNa₂-EDTA application on the winter wheat yield

Treatment	Nechanice		Kluky	
	yield (t/ha)	increase against control (%)	yield (t/ha)	increase against control (%)
2016				
Chitosan hydrochloride	10.06 ± 0.09 ^b	103.69	9.45 ± 0.26 ^b	105.97
E385 - EDTA	10.23 ± 0.09 ^b	105.44	9.54 ± 0.35 ^b	106.94
Soligor	10.75 ± 0.09 ^c	110.81	10.03 ± 0.10 ^c	112.41
Water	9.70 ± 0.06 ^a	100	8.92 ± 0.28 ^a	100
<i>df</i>	3, 12		3, 12	
<i>F</i>	108.57		28.35	
<i>P</i>	0.0001		0.006	
2017				
Chitosan hydrochloride	9.15 ± 0.30 ^a	103.85	6.73 ± 0.16 ^a	102.7
E385 - EDTA	9.32 ± 0.25 ^b	105.87	6.83 ± 0.05 ^b	104.15
Soligor	9.06 ± 0.08 ^a	102.96	6.86 ± 0.13 ^b	104.6
Water	8.80 ± 0.58 ^a	100	6.56 ± 0.03 ^a	100
<i>df</i>	3, 12		3, 12	
<i>F</i>	15.78		15.48	
<i>P</i>	0.028		0.026	

df – the total degrees of freedom, *F* – the *F*-value, *P* – the significance level; the means ± SD within a column followed by the same letter do not differ significantly (Tukey's HSD test, *P* < 0.05)

statistically comparable when CaNa₂-EDTA was applied; at one of the sites, the yield was even significantly higher than that seen for the commercial synthetic fungicide.

DISCUSSION

In the previous study of Žabka and Pavela (2018), CaNa₂-EDTA also showed a high antifungal effect against the toxigenic and pathogenic species of the *Aspergillus* and *Penicillium* genera, and, particularly, against the *Fusarium* genus. On the basis of the study mentioned earlier, it is possible to conclude that the antifungal effect of this substance in the case of PTR is comparable with the most important antifungal effect on certain *Fusarium* species, which are described as agents of *Fusarium* head blight. This finding adds support to the idea of registering this substance as a BS, expressly for the protection of wheat and other crops. CaNa₂-EDTA is an excellent candidate in terms of both its high efficiency against fungal pathogens – such as PTR – and the breadth of the spectrum of the pathogenic and toxigenic fungi which it can obviously inhibit even in small concentrations. The mechanism of action of

CaNa₂-EDTA on pathogenic fungi is probably in the chelating ability of the compound to bind bivalent ions – particularly Mg²⁺, in the cellular membranes. This increases the permeability and overall energy destabilisation, as also suggested by some studies (Hancock & Wong 1984; Alakomi 2006). According to some of the findings, there is an increase in the effectiveness of commercial fungicides mixed with unmodified ethylenediaminetetraacetic acid (EDTA) due to the increased permeability of the cell membranes (Hachem et al. 2006). Whereas the legally permitted form of CaNa₂-EDTA (E385 in the EU) is also a strong permeabilizer, a similar effect may be achieved even here. For this reason, CaNa₂-EDTA could be regarded not only as the main active substance, but also as a substance supporting the enhanced antifungal effect in order to reduce the consumption of commercial fungicides. As regards to the comparison of the direct antifungal effect, CaNa₂-EDTA shows a significantly higher effect than the one shown by chitosan hydrochloride (Žabka & Pavela 2018), although the direct mechanism of action is based on a similar principle for both substances. Thanks to the chelating ability of chitosan, there is a similar blocking action of the

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major metal ions in the membranes – mainly Ca^{2+} (Goy et al. 2009; Lee et al. 2016). However, other studies instead provide the secondary effect of chitosan on the surface of plants, such as the increased resistance of plants using the elicitation of plant defence mechanisms along with the mechanical barrier of the chitosan layer (Amborabé et al. 2008; El Hadrami et al. 2010).

CaNa_2 -EDTA is a safe substance without a negative effect on green assimilation tissues, expressed as a direct effect on the photosynthetic apparatus.

The applicability of alternative supportive or directly antifungal substances is limited in practice by the frequent phytotoxicity to plant tissue (Kobaisy et al. 2001; Dzoyem et al. 2011). One of the first manifestations of phytotoxicity and damage to plant tissue, hence the photosynthetic processes, is a measurable decrease in the photosynthetic activity. In this experiment, the application of CaNa_2 -EDTA was designed as a rate of 750 g/ha in the form of a 0.3% solution. In the laboratory phytotoxicity tests, this real-life, practically applicable field concentration was evaluated as being safe. Even the experimentally five times higher and, essentially, extreme concentration of 1% proved to be completely safe, without having a negative effect on the photosynthetic apparatus of the tissues. From this perspective, it can be assumed that the possible application of CaNa_2 -EDTA directly on the leaf bears no risk of damage to the plant tissue, thus meeting the possibility for application and plant protection in practice.

For CaNa_2 -EDTA to be registered and included in the group of the BS, the pre-requisite of the absence of a negative effect on non-target organisms must be satisfied. According to the results of this experiment, even this was absolutely fulfilled. CaNa_2 -EDTA showed no toxicity toward the model dummy organism, i.e., *E. fetida* earthworms, even for the highest concentration and exposure of 14 days. *E. fetida* earthworms are among the most useful soil organisms and contribute to soil fertilisation and improving soil structure (Rathore & Nollet 2012; Datta et al. 2016). They also significantly participate in converting nutrients, making them available for the needs of the plants (Jansirani et al. 2012). In addition, it is precisely the organism that is extremely sensitive to soil contamination by synthetic substances. Because of this sensitivity, the damage to *E. fetida* populations occurs very quickly when there is an environmental contamination by commercial pesticides or their residues (Wang et al. 2012; Datta et al. 2016; Vasantha-Srinivasan et al. 2018).

For this reason, research into the group of BSs, as well as its expansion and broader application in the protection of plants, is gaining importance in the current agricultural practice, as is the pre-requisite of compliance with the very strict criteria of safety for humans and homeothermic organisms. This has already been fulfilled through recognition by legislation and the use of this substance in the food and cosmetics industries and, because it is a strong chelator, it is also used in human medicine to treat poisoning by heavy metals (Ernst 2000; Flora et al. 2008). According to the latest research, it is a safe substance with minimal harmful manifestations, which only occur if the dose is very high (Van de Sande et al. 2014).

The foliar application of CaNa_2 -EDTA in the field situation increased the grain yield with statistical evidence. The effect of the increased yield with statistical evidence was apparent not only when compared with the untreated control, but also in comparison with the already registered and known BS chitosan hydrochloride. It is known that, in addition to the direct and secondary antifungal activity, chitosan can affect the metabolism of plants, thus stimulating the yield (Kim et al. 2005; Hadwiger 2013; Malerba & Cerana 2016). In the case of stimulating the yield by applying CaNa_2 -EDTA, the action is not yet clarified in full. In addition to the direct antifungal effect on the fungal pathogens, such as PTR, it can be assumed that certain microelements are made available through the chelating ability and, in particular, their transport and mobility in plant tissues becomes improved (Cieschi et al. 2016). In addition, we can assume that the calcium contained in the molecule of the substance has an effect on the distribution and metabolism of important trace elements such as boron and iron (Gupta & MacLeod 1977). As regards to the unique, however surprising, higher yield when applying CaNa_2 -EDTA in comparison with the application of the conventional synthetic fungicide Soligor, it is important to take into consideration that, according to the meteorological data, both of the experimental years fell among extraordinarily warm and rather dry periods. Therefore, any weaker infestation pressure of the pathogens in the course of the vegetation season should be taken into account. On the other hand, it is clear that under certain conditions the application of this substance only, safe in terms of both the health and environment, could replace the application of commercial fungicides, even without a negative effect on the final yield. Very recently, such circumstances

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may be increasingly frequent and even worldwide, with global climate change observed over the long term. Monitoring the effect of applying other environmentally safe substances – including $\text{CaNa}_2\text{-EDTA}$ – on the occurrence of PTR and other pathogens will be part of our continued work. However, even now, the current experimental facts support the idea of registering $\text{CaNa}_2\text{-EDTA}$ as a legally permitted substance in the EU (i.e., as a BS) to enable alternative methods of plant protection in agriculture. The expansion and broader application of this safe substance with an antifungal potential in the area of plant protection in the field could help reduce the consumption of synthetic fungicides and, in particular, provide a new alternative in organic farming, as well as in other systems where emphasis is placed on environmental protection and the environmentally safe production of agricultural commodities safe for human health.

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