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Preliminary assessment of the effectiveness of non-fungicide methods of maize seed treatment

Wstępna ocena skuteczności nefungicydowych metod zaprawiania
nasion kukurydzy

Abstract. Many plant diseases are transmitted through seeds. Thus, seed dressing is the first and most important protective measure. It promotes germination, increases seed vigour, improves rooting, and effectively controls pathogens. Due to the reduction of chemical plant protection products on the market, new products are being sought. Therefore, the aim of the present study was to preliminarily assess non-fungicidal methods that significantly reduce seed contamination before sprouting and do not affect germination rates and initial maize growth. The following non-fungicidal seed surface-sterilisation methods were tested: hypochlorous acid, sodium and calcium hypochlorite, peracetic acid and non-ionic nanosilver for 5, 10, 20 or 30 minutes of soaking. Dish and pot experiments were carried out. Among the tested treatments, hypochlorous acid and calcium hypochlorite were the most effective, resulting in the least seed contamination and the highest maize germination. These treatments also significantly enhanced plant height, root elongation and its fresh weight. However, the remaining treatment methods using sodium hypochlorite, peracetic acid and nanosilver were ineffective. Additionally, a pot experiment was carried out to evaluate the effect of non-fungicide seed treatments. The positive effect of hypochlorous acid and calcium hypochlorite on germination capacity, plant growth and weight, as well as its physiological condition, was also confirmed.

Keywords: calcium hypochlorite, hypochlorous acid, seed dressing, germination, natural plant protection products

INTRODUCTION

In contemporary agriculture, farmers face increasing challenges due to climate change, biodiversity loss, and environmental degradation [Gai and Wang 2024]. At the same time, there has been a noticeable rise in plant diseases caused by bacterial, fungal,

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and viral pathogens. According to Nazarov et al. [2020], in favourable conditions, diseases can affect up to 70–80% of the total plant population, and crop yields can decline by as much as 80–98%. Thus, the threat from crop diseases continues to grow, posing a risk to food security and agricultural productivity. Chemical control has long been the mainstay of plant disease management. However, one significant problem in recent years has been the progressive reduction of chemical plant protection products on the market, largely due to regulatory and environmental concerns. Therefore, there is a need to develop more environmentally friendly, safe, and sustainable integrated control strategies and technologies for plant diseases.

Pathogens affect plants at various stages of agricultural production, ranging from seed germination to growth, harvest, and storage. The basic condition for achieving high yields with good quality parameters is the use of high-quality seed material as well as its proper dressing. Seed dressing is a common agricultural practice that improves germination rates, protects against pathogens, and enhances crop productivity. These treatments are of great importance in the development of vigorous and healthy plants from the initial stages of growth [Madruga et al. 2023].

Many plant diseases are transmitted through seeds that are colonised by various bacteria and fungi, and some may be detrimental to the germination and subsequent growth of seedlings [Hong et al. 2023, Surovy et al. 2023]. The result of this damage is a reduction and deterioration in the quality of the obtained crops. Thus, seed treatment is essential and beneficial for all crops, aiming to eliminate and control pathogens that attack seeds, seedlings and plants [Madruga et al. 2023]. It is particularly crucial for maize seeds, which can be susceptible to a range of fungal pathogens, including those belonging to the *Fusarium* genus. Thus, maize seed treatment is the first and most important protective measure during the early stages of its growth. This treatment can significantly increase germination velocity, resulting in improved seedling establishment, particularly under varying environmental conditions [Rossini et al. 2024]. Furthermore, seed dressing inhibits post-emergent diseases, contributing to better plant health [Yang et al. 2022, Kardava et al. 2023].

Due to the withdrawal of chemical dressings from the market, it is necessary to explore alternative methods of dressing seed material before sowing. Maize is an important agricultural crop, ranking third globally in both harvested area and total production [Tobiasz-Salach et al. 2023]. It can be used for fodder, food, and industrial and energy purposes, as well as in the phytoremediation process. Non-fungicide maize seed treatment methods offer viable alternatives to chemical products, particularly in light of growing concerns about pesticide use and its impact on health and the environment. These methods must focus on promoting germination, increasing seed vigour and effectively controlling pathogens. Therefore, the aim of the present study was to preliminarily assess non-fungicidal methods that can significantly reduce seed contamination before sprouting and do not adversely affect germination rates and the appropriate growth of maize.

MATERIAL AND METHODS

The maize seeds came from the 2024 harvest. The following seed dressing methods were used: soaking in 0.05% hypochlorous acid (produced by Bio ActiW), 10% sodium hypochlorite or 10% calcium hypochlorite (Chempur), 1% peracetic acid (Pol-Aura) and

0.01% non-ionic colloidal nanosilver (Uni-Farma). All seeds were soaked in these solutions for 5, 10, 20 or 30 minutes and then washed in sterile distilled water to remove the solution.

After the surface sterilisation, the maize seeds were placed in a 90 mm Petri dish with Potato Dextrose Agar (PDA) medium. The experiment included five replicates for each treatment and time point, with each replicate containing five seeds. The control consisted of undressed seeds. All plates were incubated at 28°C for 4 days. Then, the Petri dishes with seeds were placed in the phytotron at 20°C/15°C under a 14-hour/10-hour (day/night) photoperiod. Germination energy was assessed after 4 days, and germination capacity after 10 days. The percentage of seed infection was calculated at 4 and 14 days after the start of the experiment. Additionally, shoot and root length, as well as fresh root mass, were measured after one month of growth.

At the same time, the experiment was conducted in a greenhouse from June to July 2025. The maize seeds were sown in plastic pots filled with a mixture of Jiffy and QTS substrates (1 : 1, volume basis). Five surface-sterilised seeds were planted in each pot. Three replications for each treatment and sterilisation time point were performed. The plants were grown in a greenhouse at 25°C (± 5) and 80–90% relative humidity for 4 weeks. The watering was performed based on water demand. Then, the shoot length and diameter, average root length and above-ground and below-ground mass of plants were determined. The index of leaf greenness (relative chlorophyll content) expressed in SPAD units was recorded using a portable Chlorophyll Meter SPAD-502 Plus.

Statistical analysis was performed using Statistica v.13.1. An ANOVA and the LSD mean separation test were used at a significance level of 0.05.

RESULTS AND DISCUSSION

Seed dressing is the first and most important protective measure during the early stages of plant growth [Kowalska and Łukaszyk 2022]. It can significantly increase crop yield by improving germination, stimulating root development, enhancing nutrient uptake and protecting plants against diseases [Madruga et al. 2023]. However, the number of registered chemical seed dressings in Poland has been decreasing annually [Kowalska and Łukaszyk 2022]. Additionally, chemical dressings are not allowed in organic farming. Therefore, new, environmentally friendly and non-invasive methods of eliminating pathogens from seed coats are being sought.

Different degree of effectiveness of the treatments used in reducing the level of surface contamination of the maize seed was found. After 14 days, seed infection ranged from 3 to 100%. The most effective was peracetic acid, but it significantly reduced seed germination, even after 5 minutes of soaking (tab. 1). Furthermore, after treatment with peracetic acid for more than 20 min, the maize seeds lost their ability to germinate (fig. 1). Similarly, Wilson [1976] found that the germination of wheat and soybean seeds was reduced after treatment with peracetic acid. Effective disinfectants must significantly reduce seed contamination without significantly reducing the germination percentage. Thus, peracetic acid cannot be recommended for corn dressing. Nevertheless, peracetic acid is gaining increasing attention as a powerful disinfectant, particularly within the agricultural and food processing sectors [Hopkins et al. 2003, Lee et al. 2016, Şehirli et al. 2020]. Its broad-spectrum antimicrobial activity allows it to inactivate various microorganisms, particularly bacteria and fungi, effectively.

Table 1. The effect of different seed treatments on germination and growth of maize plants using the agar method

Time of treatment (min)	Seed infection (%)		Germination energy (%)	Germination capacity (%)	Shoot length (cm)	Root length (cm)	Root mass (g)
	after 4 days	after 14 days					
control							
0	80 ^C	100 ^C	52 ^{BC}	60 ^B	0.9 ^A	1.9 ^{AB}	0.020 ^{AB}
hypochlorous acid							
5	4 ^a	12 ^a	76 ^a	96 ^a	5.7 ^a	18.1 ^{ab}	0.352 ^a
10	0 ^a	8 ^a	72 ^a	92 ^a	10.3 ^b	21.6 ^b	0.536 ^a
20	0 ^a	0 ^a	64 ^a	76 ^a	6.9 ^{ab}	16.3 ^{ab}	0.450 ^a
30	0 ^a	0 ^a	64 ^a	84 ^a	8.2 ^{ab}	14.8 ^a	0.391 ^a
\bar{x}	1 ^A	5 ^A	69 ^C	87 ^C	7.8 ^B	17.7 ^D	0.432 ^D
sodium hypochlorite							
5	0 ^a	8 ^a	40 ^a	64 ^a	0.8 ^a	4.3 ^a	0.105 ^a
10	4 ^a	4 ^a	40 ^a	60 ^a	1.0 ^a	4.6 ^a	0.122 ^a
20	0 ^a	8 ^a	44 ^a	68 ^a	1.1 ^a	5.4 ^a	0.168 ^a
30	0 ^a	4 ^a	36 ^a	48 ^a	0.9 ^a	2.6 ^a	0.056 ^a
\bar{x}	1 ^A	6 ^A	40 ^B	60 ^B	1.0 ^A	4.2 ^B	0.116 ^B
calcium hypochlorite							
5	16 ^a	24 ^a	72 ^{ab}	92 ^b	7.2 ^a	4.8 ^a	0.107 ^a
10	12 ^a	16 ^a	56 ^a	84 ^{ab}	8.9 ^{ab}	9.2 ^{ab}	0.261 ^{ab}
20	12 ^a	12 ^a	56 ^a	80 ^a	10.0 ^{ab}	13.2 ^{bc}	0.360 ^b
30	0 ^a	4 ^a	80 ^b	84 ^{ab}	12.1 ^b	14.6 ^c	0.307 ^b
\bar{x}	10 ^B	14 ^B	66 ^C	85 ^C	9.6 ^C	10.5 ^C	0.259 ^C
peracetic acid							
5	0 ^a	0 ^a	0 ^a	16 ^a	1.2 ^b	7.4 ^c	0.154 ^b
10	4 ^a	8 ^a	0 ^a	48 ^a	0.9 ^b	3.2 ^b	0.083 ^{ab}
20	0 ^a	4 ^a	0 ^a	0 ^a	0.2 ^a	0.0 ^a	0.000 ^a
30	0 ^a	0 ^a	0 ^a	0 ^a	0.0 ^a	0.0 ^a	0.000 ^a
\bar{x}	1 ^A	3 ^A	0 ^A	16 ^A	0.6 ^A	2.8 ^{AB}	0.062 ^{AB}
non-ionic nanosilver							
5	88 ^a	100 ^a	40 ^a	40 ^a	0.7 ^a	1.2 ^a	0.010 ^a
10	96 ^a	100 ^a	44 ^a	44 ^a	0.8 ^a	1.5 ^a	0.014 ^a
20	100 ^a	100 ^a	56 ^a	56 ^a	1.0 ^a	1.0 ^a	0.008 ^a
30	100 ^a	100 ^a	60 ^a	64 ^a	1.0 ^a	2.1 ^a	0.017 ^a
\bar{x}	96 ^D	100 ^C	50 ^B	51 ^B	0.9 ^A	1.4 ^A	0.012 ^A

Different letters within the columns indicate significant differences ($p < 0.05$) according to the LSD test, over-case letters indicate significant differences among treatment time periods ($p < 0.05$) according to the LSD test



Fig. 1. Comparison of maize growth and development depending on the seed treatment:
A) hypochlorous acid, B) sodium hypochlorite, C) calcium hypochlorite, D) peracetic acid,
E) non-ionic nanosilver

One promising method of seed treatment is the use of hypochlorous acid. Several studies have demonstrated that this treatment reduces microbiological contamination on seed surfaces [Goo and Koo 2020, Gilbert et al. 2023, Saikumar et al. 2023]. In this study, this effect was confirmed, as on average, 95% of the maize seeds were not infected. Furthermore, seed infection was completely eliminated by 20- and 30-minute treatments with hypochlorous acid. This capacity for microbial reduction is crucial, as surface contamination can adversely affect seed germination and subsequent plant health. At the same time, seed treatment with hypochlorous acid showed a 27% improvement in germination capacity compared to the control. It suggests that this treatment can be a beneficial method for enhancing seed germination. However, hypochlorous acid must be prepared fresh, as it is unstable and loses its antimicrobial effectiveness within a few days [Gilbert et al. 2023].

The calcium hypochlorite prevented the growth of microbial contaminants present on the maize seed surface to a lesser extent. After 2 weeks, 14% of the maize seeds were infected using the agar method. However, the effectiveness of this treatment increased with the length of soaking time, from 24% to 4% (tab. 1). It can be assumed that the longer the treatment time, the better the effect of seed sterilisation and the greater the reduction in pathogens. Additionally, the use of calcium hypochlorite significantly increased maize germination capacity compared to the control (tab. 1). After 10 days, 85% of the seeds had germinated, a substantially higher rate than the untreated seeds. Similarly, Dempsey and Walker [1973] found that calcium hypochlorite was an effective surface disinfectant of pepper seed and did not reduce germination. Calcium hypochlorite has also been previously tested for the disinfection of alfalfa seeds and sprouts against *Escherichia coli* and *Salmonella enterica* [Gandhi and Matthews 2003, Ding et al. 2013, Kim et al. 2025].

Sodium hypochlorite is a substance commonly used for explant surface decontamination in *in vitro* plant cultures [Yildiz and Ekiz 2014, Hesami et al. 2017, Bošnjak Mihovilović et al. 2024]. In the present study, the sodium hypochlorite significantly eliminated maize seed infection, but the germination rate was similar to that of untreated seeds. This result is consistent with Dempsey and Walker [1973]. They reported that sodium hypochlorite was more effective in sterilising pepper seeds than calcium hypochlorite, but its germination was reduced. On the other hand, Gilbert et al. [2023] obtained different results. They reported that sodium hypochlorite was an effective treatment for reducing fungal growth on seeds, without harming germination rates. However, they used a lower solution concentration – 0.6% instead of 10%. Similarly, the use of nanosilver showed a comparable germination to the control. Additionally, it was found that there is a relationship between the duration of silver treatment and the germination capacity of seeds. Longer exposure to silver was associated with a higher rate of maize seed germination, from 40% to 64%. However, under the silver treatment, all seeds were contaminated by fungal pathogens within 14 days. Nevertheless, the positive impacts of silver nanoparticles have been noted in agriculture [Khan et al. 2023, Rahman et al. 2023, de Almeida Junior et al. 2024, Al Salama et al. 2025].

The impact of non-fungicide seed dressing solutions on the initial growth and development of maize plants was also determined. Among the tested treatments, hypochlorous

acid and calcium hypochlorite were the most effective, with the lowest rate of contamination and the highest germination rates. Additionally, surface sterilisation treatment does not appear to impact the growth of shoots and roots. Maize plants treated with hypochlorous acid and calcium hypochlorite exhibited significantly higher growth compared to the control (tab. 1, fig. 1). These treatments significantly increased plant height, root elongation and its fresh weight. Furthermore, longer exposure to calcium hypochlorite was observed to stimulate shoot growth and root elongation. All other treatments proved to be inhibitors of plant growth. The treatment of maize seeds with nanosilver, peracetic acid and sodium hypochlorite resulted in statistically similar growth of seedlings compared to the untreated control. It can be assumed that microbiological infections at the seed germination stage can inhibit rooting and further plant growth. Moreover, longer exposure to peracetic acid significantly reduced shoot length and root development.

At the same time, a pot experiment was established. After 10 days, the untreated maize seeds germinated at a level of 60%. Similarly, the application of sodium hypochlorite resulted in the same germination rate as the control. In contrast, peracetic acid completely inhibited maize germination, even after 5 min of soaking. Particularly, the use of hypochlorous acid and calcium hypochlorite increased the maize germination capacity (tab. 2). The results of the pot experiment were consistent with those obtained using the agar plate method.

Maize plants treated with calcium hypochlorite exhibited improved growth compared to untreated plants. The plants were on average over 80 cm in height and almost 1 cm in diameter, with roots extending over 20 cm. This seed treatment method was found to be the most effective treatment, resulting in improved shoot and root elongation, particularly during the 20- and 30-minute treatments (tab. 2). Hypochlorous acid treatment was also effective for initial maize growth and development. These plants reached a length of almost 80 cm and a shoot base diameter of nearly 0.8 cm. Faster seed germination is crucial for successful corn growth, as it allows for earlier emergence and reduces the time plants are vulnerable to diseases, pests, and environmental stress. Thus, this leads to better overall growth and potentially higher yields.

In turn, sodium hypochlorite, commonly used for sterilisation, limited the development of maize plants during the studied exposure periods. Similarly, silver nanoparticles, while used for their antimicrobial properties, also inhibited the early stages of maize development. A slower seed germination rate was also observed in silver treatments. However, the use of silver treatment has been shown to have a significant impact on the physiological state of plants. The highest SPAD leaf greenness index was found for silver-treated maize plants. Peracetic acid completely inhibited seed germination, regardless of the treatment duration. Similarly, Vines et al. [2003] found that peracetic acid has phytotoxic effects.

Plant mass production is also a crucial factor in determining plant yield [do Moraes Gatti et al. 2023]. In this study, the fresh shoot mass ranged from 13 to 20 g, while the fresh root mass ranged from 4 to 10 g per plant after a month. The use of calcium hypochlorite proved to be the most effective strategy for increasing the underground and above-ground mass of maize (tab. 2). Longer exposure to calcium hypochlorite was observed to stimulate shoot growth and root elongation. Similarly, the use of hypochlorous acid contributed to increased shoot and root mass.

Table 2. Effect of different seed treatments on maize seed germination and growth in pot experiment

Time of treatment (min)	Germination capacity (%)	Shoot length (cm)	Shoot diameter (mm)	Average root length (cm)	Fresh shoot mass (g)	Fresh root mass (g)	SPAD index
control							
0	60 ^{BC}	74.2 ^{BC}	7.3 ^{BC}	18.0 ^{BC}	13.9 ^{BC}	4.3 ^B	34.4 ^{BC}
hypochlorous acid							
5	93 ^a	79.8 ^a	8.3 ^a	20.0 ^a	17.2 ^a	6.7 ^a	34.0 ^a
10	87 ^a	80.0 ^a	5.8 ^a	19.8 ^a	19.2 ^a	9.2 ^a	34.7 ^a
20	80 ^a	77.6 ^a	9.4 ^a	21.9 ^a	13.6 ^a	7.8 ^a	33.6 ^a
30	80 ^a	75.3 ^a	8.0 ^a	19.0 ^a	17.7 ^a	7.5 ^a	33.1 ^a
\bar{x}	85 ^D	78.2 ^C	7.7 ^B	20.0 ^{CD}	17.2 ^{BC}	7.8 ^C	33.9 ^B
sodium hypochlorite							
5	53 ^a	76.8 ^b	7.7 ^a	17.3 ^a	14.2 ^a	3.7 ^a	33.7 ^b
10	53 ^a	70.3 ^{ab}	7.6 ^a	18.2 ^a	17.6 ^a	5.6 ^a	31.7 ^{ab}
20	73 ^a	57.9 ^a	5.7 ^a	14.9 ^a	9.7 ^a	3.5 ^a	29.3 ^a
30	60 ^a	63.5 ^{ab}	5.9 ^a	16.7 ^a	10.1 ^a	2.7 ^a	33.6 ^b
\bar{x}	60 ^{BC}	67.4 ^B	6.8 ^B	16.8 ^B	13.1 ^B	4.0 ^B	32.0 ^B
calcium hypochlorite							
5	100 ^b	77.2 ^a	9.1 ^a	18.2 ^a	16.6 ^a	7.6 ^a	35.9 ^a
10	73 ^a	80.8 ^a	9.4 ^a	21.0 ^a	19.1 ^a	9.2 ^a	35.0 ^a
20	50 ^a	84.5 ^a	10.7 ^a	24.6 ^a	20.5 ^a	13.8 ^b	37.9 ^a
30	67 ^a	88.2 ^a	10.2 ^a	22.7 ^a	22.0 ^a	10.9 ^{ab}	37.2 ^a
\bar{x}	72 ^{CD}	83.0 ^C	9.8 ^C	21.7 ^D	19.8 ^C	10.3 ^D	36.4 ^C
peracetic acid							
5	0 ^a	0.0 ^a	0.0 ^a	0.0 ^a	0.0 ^a	0.0 ^a	0.0 ^a
10	0 ^a	0.0 ^a	0.0 ^a	0.0 ^a	0.0 ^a	0.0 ^a	0.0 ^a
20	0 ^a	0.0 ^a	0.0 ^a	0.0 ^a	0.0 ^a	0.0 ^a	0.0 ^a
30	0 ^a	0.0 ^a	0.0 ^a	0.0 ^a	0.0 ^a	0.0 ^a	0.0 ^a
\bar{x}	0 ^A	0.0 ^A	0.0 ^A	0.0 ^A	0.0 ^A	0.0 ^A	0.0 ^A
non-ionic nanosilver							
5	40 ^a	81.8 ^b	8.9 ^a	18.4 ^a	15.9 ^{ab}	8.6 ^b	41.3 ^a
10	40 ^a	86.0 ^b	7.3 ^a	18.1 ^a	21.3 ^b	6.4 ^{ab}	39.1 ^a
20	60 ^{ab}	73.9 ^{ab}	7.4 ^a	17.0 ^a	10.3 ^{ab}	3.8 ^a	37.3 ^a
30	80 ^b	59.3 ^a	5.0 ^a	15.0 ^a	8.6 ^a	3.2 ^a	33.4 ^a
\bar{x}	53 ^B	76.1 ^{BC}	7.5 ^B	17.3 ^B	13.7 ^B	5.8 ^{BC}	38.3 ^C

Different letters within the columns indicate significant differences ($p < 0.05$) according to the LSD test, lower-case letters indicate significant differences among treatment time periods ($p < 0.05$) according to the LSD test

CONCLUSIONS

Seed dressing is the most effective method for protecting plants from diseases during the early growth stages. However, one significant problem in recent years has been the progressive reduction of chemical plant protection products on the market. Preliminary studies on non-fungicide seed treatments are promising in maize protection. The use of calcium hypochlorite and hypochlorous acid significantly reduced the seed-borne pathogens, which is beneficial during the period of seed germination. These treatments also enhanced the germination capacity and improved maize plant growth and root development. It was observed that longer exposure to calcium hypochlorite stimulated shoot growth and root elongation. Thus, the use of calcium hypochlorite and hypochlorous acid is a promising method for seed treatment. These treatments can be used as a supplement to chemical plant protection products. However, the research was conducted under *in vitro* and greenhouse conditions and further studies are needed. It is also necessary to extend the research to include field experiments. It should be noted, however, that products used for sterilisation may disturb the microbiological balance under natural conditions.

In contrast, peracetic acid, which significantly reduces microbial growth and significantly reduces seed germination, will not be an effective method for maize seed surface sterilisation. Exposure to peracetic acid significantly reduced the shoot length and root development of maize plants. The remaining seed treatment methods using sodium hypochlorite and nanosilver were also ineffective. Moreover, the silver nanoparticles cannot eliminate all pathogens from the seed coat. Silver nanoparticle treatments were included solely as an experimental option, tested under controlled *in vitro* and greenhouse conditions, and not as a recommended method for field-ready use. Nanoparticle-based treatments are currently not considered standard, environmentally friendly practices in agriculture.

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