

EFFECT OF INORGANIC CHLORIDE SALTS ON WHEAT SEEDS GERMINATION

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Abstract: Examination of effect of five inorganic chlorine salts (NaCl, KCl, CaCl₂, CuCl₂·2H₂O and FeCl₃·6H₂O) on wheat seeds from three different wheat cultivars (“Enola C1”, “Tcarevetz” and “Aleksa”), was conducted in the in vitro conditions. Chloride (Cl) is one of the 17 essential nutrients required for plant growth and development. Chloride plays a major role in plant function, including photosynthesis, enzyme activation, nutrient transport, water movement in cells, stomata activity, accelerated plant development, reduced lodging, and improved disease suppression and tolerance. However some studies reveal the toxic action of chloride salts towards different terrestrial and aquatic plants. The purpose of present study was to investigate if NaCl, KCl, CaCl₂, CuCl₂·2H₂O and FeCl₃·6H₂O have toxic action on germination of wheat seeds. The wheat seeds were placed in the double layer of Whatman filter paper. The papers with seeds were placed in closed plastic bags and 40 ml of the tested solution was poured on the bottom of the bags. The bags were placed in the thermostat at 22 °C for two weeks. Each bag was equal to the one test repetition; each test variant consists of five test repetitions. At the end of the test, percent germination, the length of shoots and roots were measured. Also, the visual observations for phytotoxicity (necrosis, chlorosis and deformations) onto shoots and roots were performed. ANOVA analysis was performed in order to be revealed statistically proved differences between tested variants via R language for statistical computing. The study confirm the toxic action of tested inorganic salts towards wheat and slightly stimulation effect of sodium and potassium chloride at low concentrations on wheat’s growth. Trials confirm the strong phytotoxic action of ferric and especially copper chlorides on wheat seeds and plants. The conducted tests show all tested inorganic chlorine salts can strongly inhibit the germination rates of wheat seeds and growth of shoots and roots. However, some of them as sodium and potassium chloride at very low concentrations – 0.01 – 0.05 % (m/v) can express slight stimulation effect on seeds. Ferric and copper chlorides have strongest inhibition effect on wheat seeds in the conducted research. The fact that ferric chloride can act as ISR promoter in the plants and the results in the given study that the salt in very low concentration does not effects the growth and development of the plants is promising for future researchers in this direction. No differences between tested wheat cultivars were observed towards their response to the salts.

Keywords: chloride salts, wheat, seeds, germination, toxicity

1. INTRODUCTION

Different chlorine inorganic salts can act both as fertilizers, ISR promoters and poisons towards wheat cultivars. The only difference is in the dose. During recent years due to contamination of the environment and the urgent need for new fertilizers and ISR promoters and constantly raised food prices, such kinds of investigations have become an important source of knowledge. In the present study action of NaCl, KCl, CaCl₂, CuCl₂·2H₂O and FeCl₃·6H₂O was examined on wheat seeds from three different cultivars: “Enola C1”, “Tcarevetz” and “Aleksa”. Chloride (Cl) is one of the 17 essential nutrients required for plant growth and development. Chloride plays a major role in plant function, including photosynthesis, enzyme activation, nutrient transport, water movement in cells, stomata activity, accelerated plant development, reduced lodging, and improved disease suppression and tolerance (Grant et al., 2001; Begum et al., 2010) Sourour et al., 2014). Sodium chloride expresses significant reductions in germination rates in high doses for almost all wheat cultivars (Bijanazadeh et al., 2010). Concentration of NaCl (200 mM) can decrease germination by 17.6% compared with control treatment (Dolatabadian et al., 2009).

However, some studies show that under the influence of 0.5 and 1.0% concentration of sodium chloride (NaCl) there is an increase in germination of wheat seeds (respectively, 19.3 and 17.9%), and 1.5% concentration of NaCl leads to a decrease in seed germination by 7.8% than in the control. (Sharipova et al., 2019). Unlike sodium chloride, wheat (*Triticum aestivum* L.) yield increases in the case of fertilization with potassium chloride plus disease suppressions (Fixen et al., 1986; Sweeney et al., 2000). Potassium chloride was established to reduce, by about one-third, the area of the flag and penultimate leaf of the wheat affected by *Septoria tritici* (Mann et al., 2004). Both, potassium chloride and potassium sulfate inorganic salts were able significantly to stimulate the growth of wheat (potassium sulfate was found to be better and economical). Protein content of grains was significantly increased due to K application irrespective of the source and potassium sulfate was found with significantly higher protein content than potassium chloride (Bakhsh et al., 1986). Decreases in crop yield with KCl application occurred in some site-year-cultivar combinations (Grant et al., 2001). Yield responses of wheat (*Triticum aestivum* L.) and barley (*Hordeum vulgare* L.) to additions of KCl fertilizer on high-K soils have been documented. One of the principal

effects of KCl on high-K soils is a reduction in foliar disease (Gaspar et al., 1994). Towards calcium chloride, some researches reveals that seed priming with calcium chloride enhances wheat resistance against wheat aphid *Schizaphis graminum* Rondani (Wang et al., 2021). Also, improving photosynthetic responses during recovery from heat treatments alleviates the negative effect of drought stress (Hairat & Khurana, 2015; Bhardwaj et al., 2018). In the conducted study germination of water-pretreated wheat seeds on 1% NaCl was 8 per cent, while pretreatment with 1% CaCl₂. 2H₂O resulted in 90 per cent germination on 1% NaCl. Calcium chloride alleviates water stress in sunflower plants and chloride effects on salinity-induced oxidative stress, proline metabolism and indole alkaloid accumulation in *Catharanthus roseus* (Jaleel et al., 2007; Ibrahim et al., 2016). The foliar application of calcium chloride and borax influences plant growth, yield, and quality of tomato (Rab & Haq, 2012). Copper chloride was also established to have a negative effect on growth and development of plants and to have cytogenetic effects on *Helianthus annuus* (İşeri et al., 2011; Hussain et al., 2021). Copper chloride can also act as a potential ISR promoter (Rakwal et al., 1996; Liu et al., 2015). The experiments show inhibition effect of copper chloride on growth of Maize (*Zea mays*) (Hussain et al., 2021). The effect of copper chloride on seed germination and seedling growth of *Pisum sativum* and *Phaseolus mungo* has been studied. The growth of radicle and plumule was significantly reduced with respect to increase of CuCl₂.2H₂O concentration. Complete inhibition of germination was observed in the three highest doses (Megha & Heena, 2009). Ferric chloride is a popular reagent for treatment of sewage and industrial wastes, water purification and disinfectant, also reagent in the chemical industry - for phenols detection, Synthesis of dibromotetraalkoxybiphenyls (Soloway & Wilen, 1952; Ching et al., 1994; Boden et al., 2000; Farajnezhad & Gharbani, 2012). In medicine, ferric chloride is used for Arsenic removal, for models of arterial thrombosis (Hering et al., 1996; Grover & Mackman, 2020). However, in agriculture, resistance to rice blast was induced by ferric chloride, di-potassium hydrogen phosphate and salicylic acid which prove the role of the chemical as ISR promoter (Manandhar et al., 1998). Elucidating the effect of microbial inoculum and ferric chloride as additives on the removal of antibiotic resistance genes from chicken manure during aerobic composting was also established (Guo et al., 2020). Ferric chloride was used as peracetic acid pretreatment for effective utilization of sugarcane bagasse (Zhuang et al., 2022). Ferric chloride also reduces the antioxidant activity of the phenolic fraction of virgin olive oil (Keceli & Gordon, 2002).

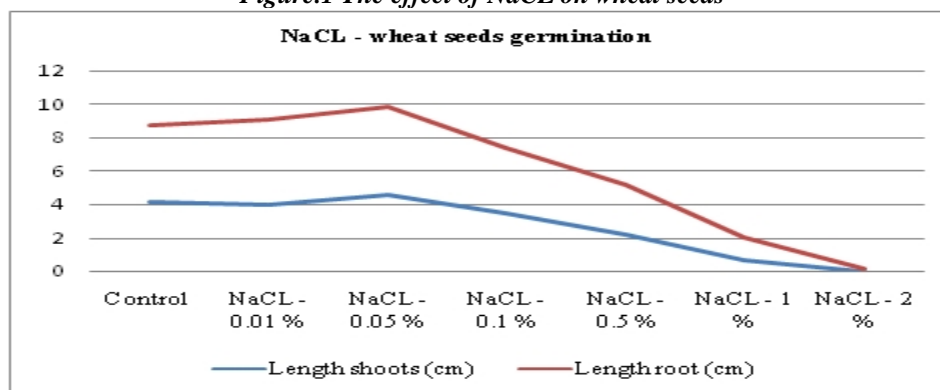
2. MATERIALS AND METHODS

Ten wheat seeds were placed on a double layer of Whatman filter paper. The papers with seeds were placed in closed plastic bags and 40 ml of the tested solution was poured on the bottom of the bags. The bags were placed in the thermostat vertically at 22 °C for two weeks. Each bag was equal to the one test repetition; each test variant consists of five test repetitions. At the end of the test, percent germination, the length of shoots and roots were measured. Also, the visual observations for phytotoxicity (necrosis, chlorosis and deformations) of shoots and roots were performed. ANOVA analysis was performed in order to reveal statistically proved differences between tested variants via R language for statistical computing (R Core Team, 2020)

3. RESULTS

The received results are presented on the figures below:

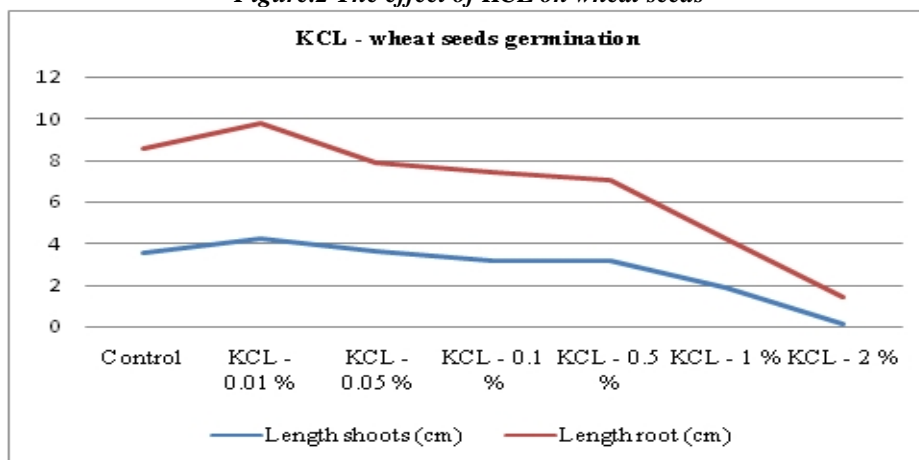
Figure.1 The effect of NaCL on wheat seeds



Source: Microsoft Corporation. (2018). Microsoft Excel. Retrieved from <https://office.microsoft.com/excel>. Own work

The figure illustrates the effect of sodium chloride on germination of wheat seeds. The 0.01 % (m/v) solution had no effect in comparison with the control variant ($p>0.05$). However, at 0.05 % (m/v) concentration was observed slight stimulation effect on shoots and roots from about 10-13 %. Above this concentration, sodium chloride starts expressing the negative effect on wheat seeds. At 1 % (m/v) concentration the suppressing the growth of shoots was approximately 85 %, and the roots – 70 %. Up to 1 %, no change in the percent germination was observed. At 1 %, there was a minus 45 % germination rate in comparison with the control. At 2 % concentration, no one of the tested seeds was able to germinate. There were no statistically significant differences in the action of sodium chloride on seeds according to different wheat cultivars used in the test ($p>0.05$). Hence, sodium chloride, although is high phytotoxic to the wheat seeds at very low concentrations can express stimulation effects.

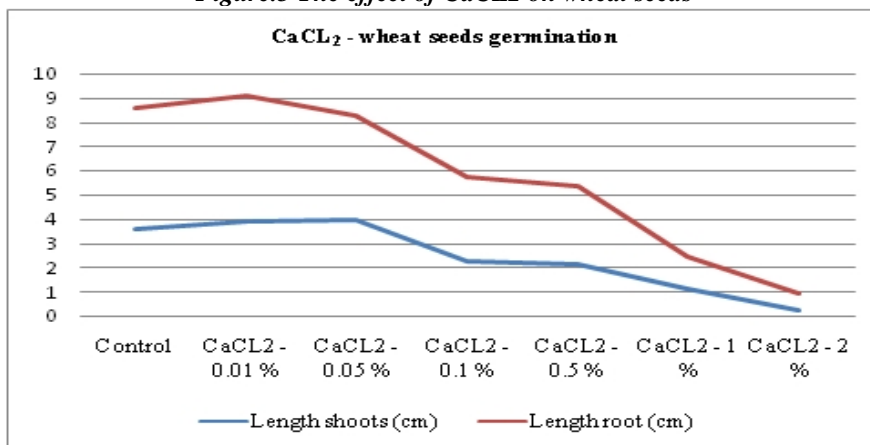
Figure.2 The effect of KCL on wheat seeds



Source: Microsoft Corporation. (2018). Microsoft Excel. Retrieved from <https://office.microsoft.com/excel>. Own work

Although, potassium chloride is a fertilizer, the received results show that only at 0.01 % (m/v) concentration there was a stimulating effect on wheat seeds – approximately 20 % for shoots and 10 % for roots. Up to 0.5 % concentration there were no statistically significant differences from the control variant ($p>0.05$). However unlike sodium chloride where the germination rate at 2 % concentration was 0 %, in the variants tested with potassium chloride it was 30 %. The stimulating effect on wheat seeds from the potassium chloride is achieved in lower concentrations than NaCl although it is a little bit higher.

Figure.3 The effect of CaCL2 on wheat seeds

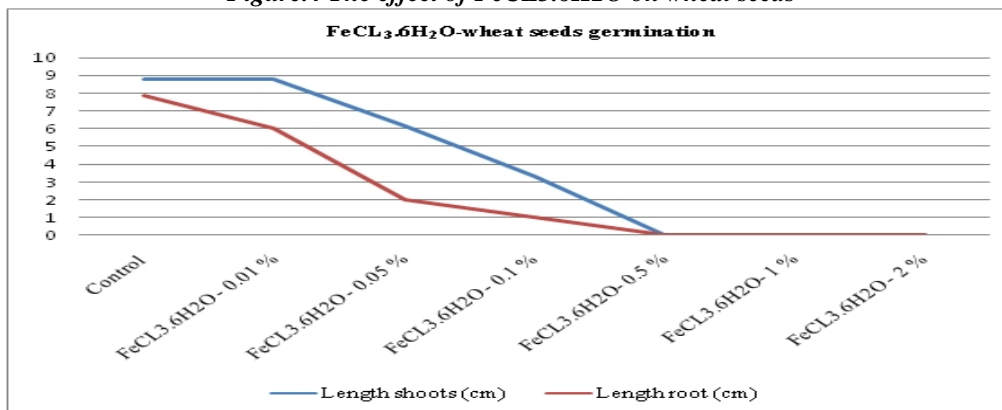


Source: Microsoft Corporation. (2018). Microsoft Excel. Retrieved from <https://office.microsoft.com/excel>. Own work

According to the calcium dichloride, up to the 0.05 % concentration, there were no statistically significant differences with the control i.e. this salt does not express any stimulating effect on the growth of wheat seeds. At 0.1 % concentration, calcium dichloride decreases the growth of shoots and roots by 35 %. The germination rate also decreased by 30 %. At 1 % concentration the decrease in the growth of shoots and roots was 70 % for shoots and above 85 % for roots. The germination rate also decreased by 45 %. At 2 % concentration, the salt inhibits the growth of shoots and roots of wheat seeds almost completely. Just like sodium chloride, there were no statistically significant differences in the conducted test with potassium and calcium chlorides for different wheat cultivars ($p>0.05$).

In the next figure, the effect of $\text{FeCl}_3 \cdot 6\text{H}_2\text{O}$ salt on wheat seeds. Ferric chloride is a popular reagent for treatment of sewage and industrial wastes, water purification and disinfectant:

Figure.4 The effect of $\text{FeCl}_3 \cdot 6\text{H}_2\text{O}$ on wheat seeds

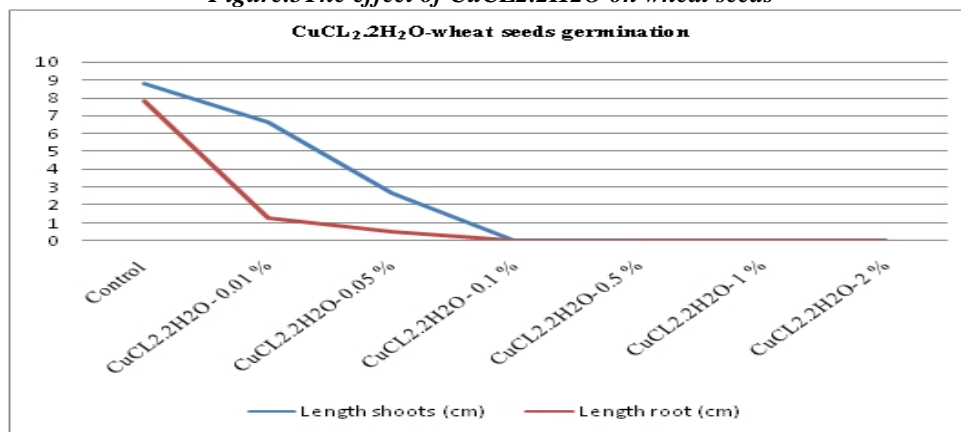


Source: Microsoft Corporation. (2018). Microsoft Excel. Retrieved from <https://office.microsoft.com/excel>. Own work

The results show that at 0.01 % concentration, ferric chloride does not express any effect on wheat seeds ($p>0.05$ towards the control variant). At 0.05 % concentration however, began decreasing effect on growing strongly decreased towards seeds – over 74 % decreasing. Towards shoots the effect was much, slighter in comparison with roots, only 30 % decreasing. At 0.1 % concentration, the inhibition effect on shoots was over 60 %, towards roots – approximately 90 % decreased. An over 0.5 % concentration $\text{FeCl}_3 \cdot 6\text{H}_2\text{O}$ completely blocks the germination of wheat seeds.

In the tests with $\text{CuCl}_2 \cdot 2\text{H}_2\text{O}$, similar results like ferric chloride were observed:

Figure.5 The effect of $\text{CuCl}_2 \cdot 2\text{H}_2\text{O}$ on wheat seeds



Source: Microsoft Corporation. (2018). Microsoft Excel. Retrieved from <https://office.microsoft.com/excel>. Own work

The results show the strong toxic action of the tested chemical on wheat seeds. Even at 0.01 % concentration there was approximately 25 % inhibition of shoots and over 80 % on roots. At 0.05 % concentration, the copper chloride blocks the growth of wheat shoots with 74 % , and roots – with over 93 % . At 0.1 % concentration – full inhibition of the seeds.

4. DISCUSSIONS

The study confirm the toxic action of tested inorganic salts towards wheat (Ayed et al., 2014; Rahim et al., 2019) and slightly stimulation effect of sodium and potassium chloride at low concentrations on wheat's growth. In the trials the strong phytotoxic action of ferric and especially copper chlorides on wheat seeds and plants was verify (El Rasafi et al., 2016; Iannone et al., 2016)

5. CONCLUSIONS

The conducted tests show all tested inorganic chlorine salts can strongly inhibit the germination rates of wheat seeds and growth of shoots and roots. However, some of them like sodium and potassium chloride at very low concentrations – 0.01 – 0.05 % (m/v) can express a slight stimulation effect on seeds. Ferric and copper chlorides have the strongest inhibition effect on wheat seeds in the conducted research. The fact that ferric chloride can act as ISR promoter in plants and the results in the given study that the salt at very low concentration does not affect the growth and development of the plants is promising for future researchers in this direction. No differences between tested wheat cultivars were observed towards their response to the salts.

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