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Comparison of the effect of additives during gliding arc plasma treatment on the germination of common bunt spores and growth characteristics of wheat

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Abstract: The gliding arc cold plasma treatment of wheat seeds is an alternative to chemical seed dressing, but this treatment is not very effective. This study was focused on testing the addition of salt, water and nitrogenous compounds during plasma treatment to increase the efficiency of the plasma seed treatment. The additives were not supplied in the gaseous state, as usual, but in an aqueous solution by spraying under a plasma nozzle during the treatment. The phytosanitary effect of the plasma treatment was evaluated based on the germination of *Tilletia caries* spores from artificially infected seeds on water agar. The viability of the seeds, growth and yield characteristics of the plasma-treated seeds were evaluated in the laboratory, greenhouse and even in field experiments with the primary goal of reducing spore vitality. The germination of *T. caries* spores was mostly limited to the variant with the addition of water during plasma treatment, where spore germination reached only 32.7% of the control variant while maintaining sufficient growth properties. The experiment demonstrated the suitability of using the addition of various substances during plasma treatment in the form of aqueous solutions and increasing the effectiveness of this treatment.

Keywords: cold plasma; gliding arc; physical seed treatment; *Tilletia* spores; seed emergence; plant development; yield

Due to the intense pressure to reduce the amount of pesticides in agriculture and restrictions on using substances used for seed dressing, breeders and seed producers are faced with significant challenges, and alternative seed treatment methods are being sought. Cold plasma is one of the physical methods tested as a possible substitute for chemical seed treatment. It affects the seed's health and the processes of germination, emergence and plant development. The gliding arc, representative of plasma methods, is simple but still difficult to use for large-scale applications because the treatment takes a relatively long time and can negatively affect seed viability. This study aimed to streamline the treatment process and thus bring it closer to use in mass production. Plasma can act on the treated substrate by temperature, UV radiation, free electrons, excited particles (Fridman et al. 2007), H_2O_2, reactive oxygen particles (ROS) (O_2, O_3, HO) and reactive nitrogen particles (RNS) (NO, NO_2, N_2) (Laroussi & Leipold 2004) and photon flux (Dey et al. 2016). Improvements in treatment efficiency

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can be achieved by increasing the number of reactive particles (ROS and RNS). The most common procedure for increasing the number of ROS and RNS during plasma treatment is to use a carrier gas other than air (Šerá & Šerý 2018). So the usual way is to use gaseous additives. But some studies reported an addition of these beneficial substances in the form of an aqueous solution. This process leads not only to an increase in the content of ROS, RNS and H_2O_2 (Machala et al. 2013). Still, it can also act by increasing the air humidity in the plasma-treated environment, which is also very important for increasing the effectiveness of the treatment (Patil et al. 2014). Kumar et al. (2016) also reported that nitrogenous compounds such as calcium nitrate could positively affect the germination and emergence of wheat as a substance used for halopriming. Also, stimulants such as Terra-Sorb® (Complejo Industrial Bioibérica, Spain), which is based on free L-α amino acids, are used to strengthen crops or on seeds to ensure a more even emergence (Blumenthal & Hilder 1989). Another frequently used compound is sodium chloride, which can increase the efficiency of the plasma treatment process, especially from a phytosanitary point of view. Saline, in combination with plasma, was reported to have strong disinfectant effects (Machala et al. 2013).

Tilletia caries (DC.) Tul. & C. Tul. [syn. *T. tritici* (Bierk.) G. Winter] is a seed-borne pathogen that can cause great damage by degrading grain smelling of trimethylamine. Such grains are unusable for the food industry and very limited use in the feed industry. So far, effective protection against *Tilletia* is only chemical. It is also possible to capture genetic sources of resistance and use them in breeding, which is aided by many studies (Dumalasová & Bartoš 2010; Al-Maaroof et al. 2016). This was the reason why the effect of plasma treatment on the germination of *T. caries* spores from artificially infected seeds was monitored in this study. Spores were used as a measure of the quality of treatment because their destruction or reduction is more difficult than in the case of vegetative stages of fungi or bacteria (Los et al. 2020). It is also very important to compare, together with the effect of the treatment on the phytopathogen, the emergence and growth characteristics because the more the plasma treatment acts on the pathogen, the more vigorously it also affects the seeds (Zahoranová et al. 2016) and it is crucial that seeds are not damaged during treatment.

Plasma treatment directly affects a wide range of the growth characteristics like germination rate and percentage of germination, the energy of emergence and emergence, and the height of young plants and these factors are, therefore, frequent assessment parameters (Iranbakhsh et al. 2017; Meng et al. 2017; Lotfy et al. 2019). Fewer works deal with the evaluation of plants depending on plasma treatment until harvest (Jiafeng et al. 2014; Saberi et al. 2018), and very few results are from such evaluation after treatment with the gliding arc system (Strejckova et al. 2018). Therefore, in this experiment, tillering, heading date, the health status of plants during vegetation, the height of plants (BBCH 87), lodging, weight and quality of harvested grain were evaluated.

MATERIAL AND METHODS

The experiment took place in the laboratory in 2018, field tests in 2018 and 2019. The effect of plasma treatment on the germination of *T. caries* spores from artificially infected seeds, germination characteristics of healthy seeds and plant growth characteristics, including yield and grain quality parameters, were tested. The experiments were performed at Selgen a.s. in the breeding station Stupice, Czech Republic. Seeds of spring wheat cv. Pexeso (Selgen a.s., Czech Republic) was used for the experiment.

Plasma seed treatment. A gliding arc type plasma device GVN-1k-2011 (SurfaceTreat, Czech Republic) at the University of South Bohemia in České Budějovice with a power of 1.3 kW and an airflow of 30 SCFH was used for the treatment. The distance of the nozzle from the seed was 10 cm, and the exposure time was 4 min. Water, $Ca(NO_3)_2$, Terra-Sorb® (Complejo Industrial Bioibérica, Spain) and the mixture of previous were used as added substances. The substances were applied by spraying an aqueous solution every 25 s under a plasma nozzle for 1 mL per 25 g of seeds. In addition to the nitrogen variants, salt variants were tested. NaCl was dissolved in water solutions of the additives mentioned above (S-variants), or the NaCl water solution was applied as the last injection during treatment (SP-variants). The type and concentration of additives are given in Table 1.

Artificial seed infection. Artificial seed infection using spores of *T. caries* (obtained at Crop

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Table 1. Type and concentration of additives used in plasma experiments

Variant	Additives	Concentration (%)
H_2O	water	–
LV	$Ca(NO_3)_2$	0.1
TER	Terra-Sorb	20.0
LVTER	$Ca(NO_3)_2$ + Terra-Sorb	0.1 + 20.0
_S	NaCl added to LV, TER and LVTER	0.9
_SP	NaCl as the last spray	0.9
NO	only plasma treatment without any additives	–
CON	control, without treatment	–

Research Institute, Czech Republic) was performed by mixing 2.5 g of spores with 1 kg of seeds. The infected seeds were stored in a refrigerator at 7 °C. After treatment with cold plasma, seeds were stored for one month. Subsequently, 25 grains were collected in an Erlenmeyer flask and transferred to a sterile box. 3 mL of sterile water with Tween-20 (Sigma, USA) was added to the seeds, and the mixture was mixed thoroughly for 3 min. Then another 3 mL of sterile water was added, stirring the mixture for 0.5 min. In a narrow strip, 20 µL of the suspension was applied to the surface of 2% water agar with antibiotics in a 9 cm Petri dish. After 1 h, the dish was sealed with parafilm and transferred to 7 °C in the dark. The evaluation was performed after ten days of incubation. Germinating spores and all spores in the sample were counted under a stereoscopic microscope (magnified 40×). Spore germination was calculated, and the result was related to the germination of the control sample (infected seeds without treatment).

Laboratory, field and greenhouse experiments. The standard seeds germination test was conducted under laboratory conditions at 20 °C on filter paper. The germination rate of 4 days after the start of the test and the germination percentage of 7 days after the start were assessed. The experiment was arranged in three replicates of 100 grains. The field experiment was set up as a one-row test in five replicates. One hundred grains were sown in each row. Field emergence, young plant height (BBCH 11), tillering, diseases occurring during vegetation, plant height (BBCH 87), thousand-grain weight (TGW) and grain quality were evaluated. A comparative test in perlite was performed in the greenhouse, in which the emergence energy ten days after sowing emergence and the height of young plants 17 days after sowing were evaluated. One hundred grains were sown in triplicate. ANOVA processed data from all experiments.

RESULTS

Seeds artificially infected with *T. caries* spores were exposed to cold plasma with various additives. In the experiments, not only the simple effect of plasma on spore germination and seed viability was observed, but attention was also paid to the effect of the plasma treatment on the emergence, growth and development of plants and, last but not least, on the yield and quality of the harvested grain.

Health status of seeds and germination of *T. caries* spores. The results show that the additives added during the cold plasma treatment and their influence on the germination of *T. caries* spores could be divided into four clusters based on statistical analysis (Figure 1). LVTER and TER variants (see Table 1 for abbreviations) without salt were in the same cluster as the treatment with cold plasma alone (NO) and did not differ statistically significantly. This indicates the same phytosanitary effect of these treatments on the seeds. LV variants (LV alone and LV with added salt) and control variant together with LV_SP variant (see Table 1 for abbreviations) formed two clusters characterised by low efficiency of plasma treat-

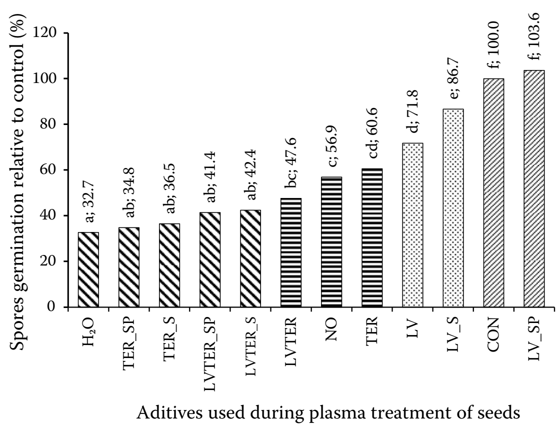

Figure 1. Germination of *Tilletia* spores in dependence on additives used in plasma treatment

[a–f] groups that do not differ based on Tukey's HSD test

Homogeneous subset groups were estimated by ANOVA and multiple range Duncan test (95%)

For treatments explanation see Table 1

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ment. The variants with added water and Terra-Sorb® in combination with salt/calcium nitrate formed a cluster of the most efficient variants, which statistically significantly reduced the germination of *Tilletia* spores compared to the treatment by only plasma. Water addition reduced the germination of *Tilletia* spores on average to 32.7% of the control variant, variants with Terra-Sorb® in combination with salt to 34.8% and 36.5%, respectively, and Terra-Sorb® in combination with salt and calcium nitrate to 41.4% and 42.4%, respectively. However, the addition of only Terra-Sorb® or a combination of Terra-Sorb® and calcium nitrate was not so effective, and a lower reduction of spores germination was recorded (Figure 1). If the results were evaluated with respect to added nitrogen compounds, it could be seen that the addition of salt statistically significantly increased the effect of cold plasma treatment on the germination of *Tilletia* spores in the LVTER and TER variants (Figure 2). The opposite effect was recorded in LV variants, where only calcium nitrate was added during plasma treatment, and the effect of adding salt statistically significantly increased the germination of spores.

Laboratory experiments – germination energy and germination percentage. The laboratory germination test on filter paper showed statistically significant differences in the germination energy of treated seeds (Figure 3). The water variant showed the lowest germination energy, significantly lower than the

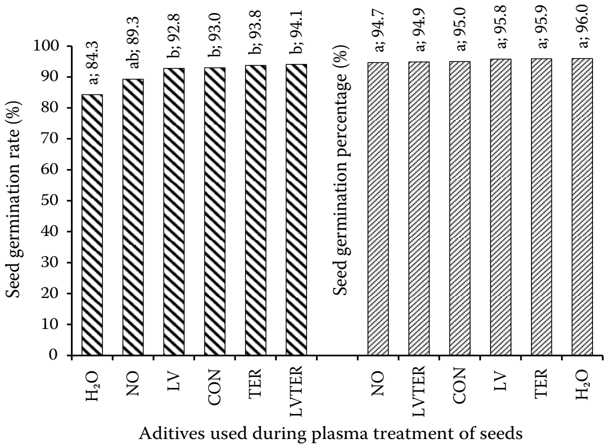

Figure 3. Results from laboratory experiments – assessment of seed germination rate and germination percentage according plasma treatment variants

[a–b] groups that do not differ based on Tukey's HSD test

For treatments explanation see Table 1

nitrogen and control variants. The plasma treatment without any additives differs very slightly from all variants. The differences in germination percentage were equalised, and all variants belonged to the same statistical group. This corresponds to the results of emergence in the perlite and in the field, where there were also no statistically significant differences and the parameters of seed germination, and emergency was not negatively affected by plasma treatment.

Greenhouse experiments. The perlite greenhouse test was designed to evaluate the energy of

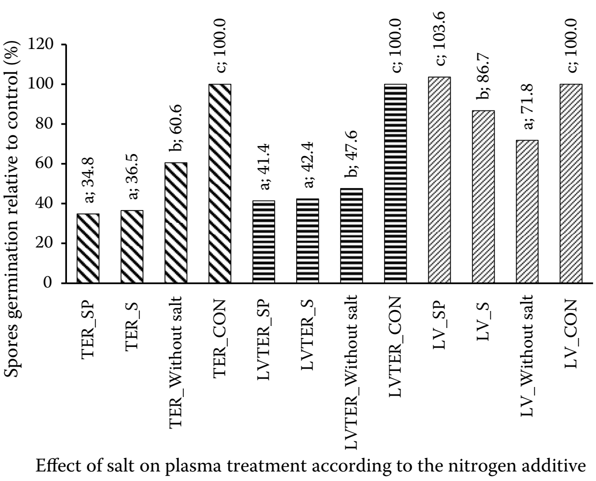

Figure 2. Effect of salt addition to nitrogenous additives on Tilletia spores germination and plasma treatment efficiency

[a–b] groups that do not differ based on Tukey's HSD test

For treatments explanation see Table 1

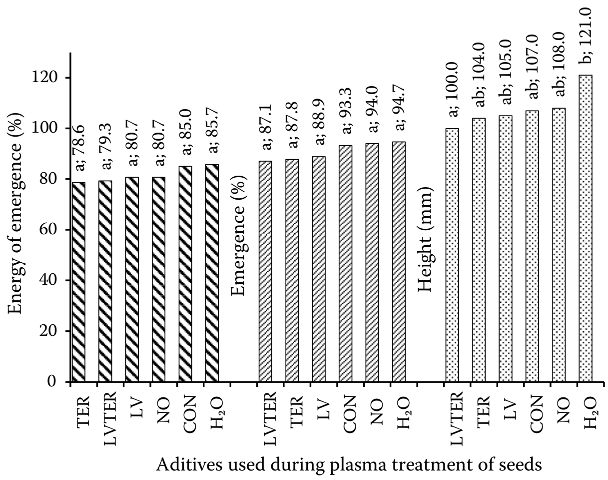

Figure 4. Results from greenhouse experiments – assessment of seed emergence, energy of emergence ant plant height according plasma treatment variants

[a–b] groups that do not differ based on Tukey's HSD test

For treatments explanation see Table 1

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emergence, seed emergence and height of young plants (speed of plant development). The results are presented in Figure 4 and correspond to those achieved in the field experiment. There are no statistically significant differences between the variants in the emergence characteristics; the differences are also minimal in the height of young plants. A statistically significant difference only between the water variant and LVTER variant was recorded, and the plant height was 12.1 cm and 10 cm, respectively. Separate data processing for individual nitrogen variants showed no statistically significant difference between the variants with and without salt (data not shown).

Field experiments. A number of parameters were observed in field experiments; the results are presented for characteristics of field emergence, tillering, plant height, protein content in grains, TGW, and yield in field plots (rows). Figures 5 and 6 show the data converted to percentages related to the control, and values in the range of 97.5–102.5% can be considered identical to the control. No statistically significant differences were recorded between the monitored characteristics in field experiments, except for the protein content, wherein the LV variant was statistically significantly lower protein content, and the water variant was the highest protein content. The plasma-treated variants generally show weaker growth characteristics (except for single plasma) but better results in grain yield and quality (except for LV variants). Separate data processing for individual nitrogen variants showed no statistically significant difference between the variants with and without salt (data not shown).

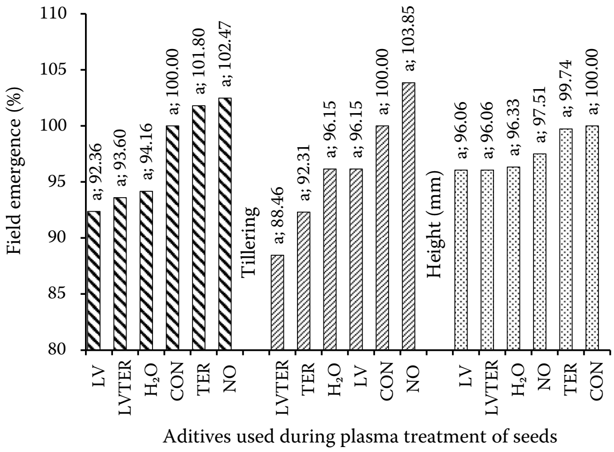

Figure 5. Assessment of plant growth characteristics in field experiments – field emergence, tillering and plant height relative to control variant

[a–b] groups that do not differ based on Tukey's HSD test

For treatments explanation see Table 1

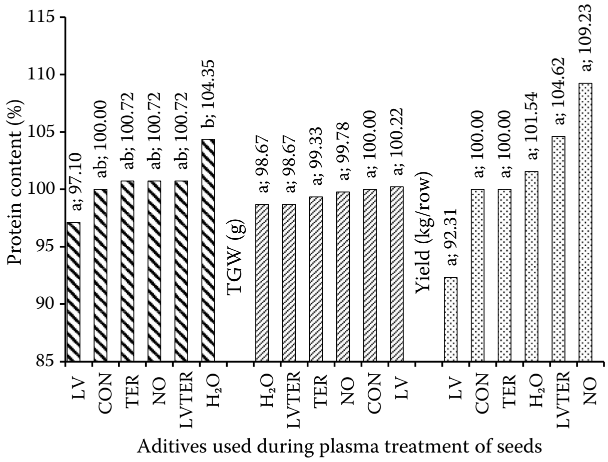

Figure 6. Assessment of quality and yield in field experiments – protein content, thousand-grain weight and yield per one row in field plot relative to control variant

[a–b] groups that do not differ based on Tukey's HSD test

For treatments explanation see Table 1

DISCUSSION

The ever-increasing pressure to reduce the consumption of agrochemicals, including active substances used for seed dressing, makes it necessary to find alternative seed treatment methods. One of these possibilities is the utilisation of the physical methods and the use of cold plasma for seed treatment. In the case of seeds, the issue of plasma application is somewhat more complicated because it is necessary to eliminate pathogens on the surface of seeds but also to preserve the viability of seeds (Zahoranová et al. 2016) and, conversely, it is desirable to support the growth and development of plants and to influence the yield positively. Seed treatment by cold plasma and the positive influence on the elimination of pathogens on the surface of seeds are mentioned by Mitra et al. (2014) and Los et al. (2020). Some studies then focus on the stimulation of seed germination (Lofty et al. 2019), the development of plants (Meng et al. 2017), and some of them also on agricultural characteristics and yield (Saberi et al. 2018; Strejckova et al. 2018). However, most of these studies have the character of laboratory experiments and studies focused on field performance are rare. This was

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also the contribution of this study when the influence of plasma treatment was monitored in both laboratory/greenhouse and field conditions. Cold plasma using gliding arc technology has a number of advantages as well as limitations. These limitations include heat generation, the negative influence of heating on seed germination and lower efficiency. In this experiment, we delivered water, various nitrogen compounds and their combinations with salt to the plasma nozzle space during plasma treatment. Unlike other studies (Laroussi & Leipold 2004; Šerá & Šerý 2018), these additives were delivered in the form of aqueous solutions, as was also reported by Machala et al. (2013) and Patil et al. (2014).

Tilletia sp. are important pathogens of wheat and are transmitted by seed. Therefore, the effect of plasma treatment on spores of *T. caries* was tested. Due to the standard conditions for all treatments, the wheat seed was artificially infected in this model experiment. Artificial seed infection and evaluation of germination of *Tilletia* spores were carried out on the recommendation of Dr. Dumalasová (Crop Research Institute, Czech Republic) and according to protocols of Dumalasová & Bartoš (2008) and Prokinová et al. (2011). The germination of the spores was carried out on an antibiotic medium (ampicillin and streptomycin) at a temperature of 7 °C when the spores germinated more slowly. Still, there is no germination or contamination by other fungi on the spores. Thanks to this, we could simplify the procedure and eliminate the disinfection of the spores. These conditions have been optimised for the evaluation of germination spores of *T. caries*; in the case of testing the effect of plasma on other *Tilletia* species, species-specific spore germination requirements must be taken into account (Váňová et al. 2006). The experiments' results show that the different treatment variants (water, calcium nitrate, Terra-Sorb® and their combinations with salt) differed significantly. The best results of the water variant (addition of only water during plasma treatment) could be explained by the ability of the plasma to decompose water into ROS and H_2O_2. Both of these products show good disinfecting properties. Hydrogen peroxide can react in the presence of nitrites and change to peroxynitrites ($ONOO^-$), which also have a disinfectant effect (Machala et al. 2013). The combination of nitrogen substances with salt was chosen because salt can react under the action of plasma in the presence of oxygen radicals to form the disinfecting agent hypochlorite (Jirásek & Lukeš 2019). But based on these results, it can be assumed that the addition of calcium nitrate solution during plasma treatment inhibits or does not allow the conversion of NaCl to hypochlorite because the difference between the separate LVTER variant and its salt variants is smaller than in TER variants and in LV variants even the addition of salt reduces the efficiency of treatment. Weaker results recorded in LV variants (only calcium nitrate alone or in combination with salt) may indicate less than expected production of RNS or their lower efficiency on spores than ROS. One of the negative effects of RNS on phytopathogens is the change in intracellular pH. But this phenomenon occurs with more difficulty in spores than in vegetative cells (Slonczewski et al. 2009; Hertwig et al. 2015).

However, the suppression of pathogens on the surface of the seeds must not negatively affect the viability of the seeds. Therefore, in addition to the germination of spores and the positive effect of the treatment on health, the parameters of the seed value were monitored. Germination, seed emergence, growth and development of plants were monitored in both laboratory/greenhouse and field experiments. The results of laboratory and greenhouse experiments correspond to positive results published by Kordas et al. (2015). It is also apparent that germination depends on the plasma source. Meng et al. (2017) tested a DBD plasma source with different gases. Compared to the results of this study based on the gliding arc source, the DBD plasma variants with air and nitrogen had significantly better germination than the control variant. However, the great statistical proximity of plasma with air and nitrogen was confirmed as well in the results of this study. The improving effects of wheat growth characteristics in the initial stages were also found in DBD plasma-treated seeds with added nitrogen by Iranbakhsh et al. (2017). There was also an improvement in germination rate, germination percentage and height of young wheat plants in experiments with nitrogen plasma jet (Lotfy et al. 2019). In the presented study positive effect of added nitrogenous substances during the treatment on the germination process was verified. In the laboratory paper germination test, where germination was not affected by the intake of nitrogenous substances from the environment, all nitrogenous variants expressed better germination, similar to results reported by Meng

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et al. (2017) and Lotfy et al. (2019). There was also a slight statistical difference in the germination rate. In emergence tests in the greenhouse and field conditions, the effect of added nitrogenous substances disappeared, probably due to the influence of the environment, as reported in cereals by Strejckova et al. (2018). The results differ from the conclusions of work with planar plasma sources (Mitra et al. 2014; Zahoranová et al. 2016), where demonstrably improved germination and growth characteristics in the initial stages were reported.

The most significant part of the results relates to field experiments. Treatment with cold plasma variants used in this study does not adversely affect seed germination, further plant development or yield and grain quality. In the field experiments, no statistically significant differences were observed between the treatment variants, except for the protein content in water and calcium nitrate variants, as described in the result chapter. Also, Saberi et al. (2018) reported statistically significant improving effects of RF plasma on wheat on vegetation parameters, including protein content but also yield and TGW. As in this experiment, a slight but statistically insignificant yield increase was also reported in the study of Jiafeng et al. (2014) in wheat and Strejckova et al. (2018) in barley using a gliding arc plasma source.

CONCLUSION

The method of adding some chemical substances to the process of gliding arc plasma treatment by spraying in the form of aqueous solutions was verified in this study. The method is suitable for delivering substances, increasing air humidity and decreasing temperature in the area of plasma treatment. The supplied separate nitrogenous substances did not significantly statistically affect either the hygienic aspect or the growth properties except for the germination rate on paper in laboratory tests. In contrast, the addition of water showed a demonstrable effect on the effectiveness of the plasma treatment. It significantly reduced the germination of *T. caries* spores while maintaining the good growth properties of wheat. The importance of adding NaCl to nitrogenous solutions and adding aqueous salt solution at the end of the treatment was also verified. Both of these possibilities of combining salt with Terra-Sorb® and with a mixture of $Ca(NO_3)_2$ plus Terra-Sorb® resulted in a significant reduction in the germination of *Tilletia* spores. For these reasons, the addition of NaCl to the variants with Terra-Sorb®, Ca $(NO_3)_2$ plus Terra-Sorb® and the addition of water appear to be the most effective treatments and can significantly improve the plasma treatment of seeds. Further research will focus on testing cold plasma's effect on other *Tilletia* species. Particular attention will be paid to the scale-up of this treatment technology, optimisation of the financial cost of the treatment and the use of plasma technology in seed processing as an alternative to the chemical treatment of seeds.

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