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Review

Can Cold Plasma Be Used for Boosting Plant Growth and Plant Protection in Sustainable Plant Production?

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Abstract: Sustainable agriculture with low inputs of chemicals and fertilizers has been recently attracting more attention from producers and researchers in the EU. The main reason for such attention is The European Green Deal—the EU's latest growth strategy concerning environmental degradation and climate change. One of its main components is the Farm to Fork strategy, which especially features the reduction in pesticide and mineral fertilizer application and also supports the development of organic farming. At the same time, food demand is rising. These ambitious challenges require extensive research, development and innovation. Therefore, new non-chemical techniques for improving plant growth and resistance to biotic and abiotic stresses must be explored for their potential in this field. One of the most promising is the use of non-thermal plasma for such purposes. As this physical factor is a complex mixture of ions, atoms, electrons, radicals and molecules, its effect on plants and pathogens is also complex. This review presents different aspects of the effect of non-thermal plasma on seed germination, development of seedlings, plants and pathogens. The literature was explored to provide evidence for the possible use of non-thermal plasma for boosting plant growth and plant protection.

Keywords: cold plasma; plant growth stimulation; plant protection; disinfection

1. Introduction

In recent decades, there has been a rising environmental awareness amongst Europeans, which demonstrates our care for the natural environment. Its devastation, loss of biodiversity and climate change have been a concern of the European Community for years. These issues, especially climate change, are considered to be an existential threat to Europe and the world. In order to overcome these challenges, the European Green Deal was presented in December 2019 [1]. It is the most recent growth strategy and action plan, which will transform the EU into a modern, resource-efficient and competitive economy, ensuring no net emissions of greenhouse gases by 2050. Moreover, it will turn the EU into the first climate-neutral continent (with a reduction in emissions by at least 55% by 2030, relative to 1990). The European Green Deal is an action plan aimed at turning the listed challenges into new opportunities for environment and climate-friendly, sustainable development. As nature is the most important ally in our efforts to reverse climate change and ensure healthier and sustainable EU food systems, two cornerstone strategies of the European Green Deal were launched in May 2020: Farm to Fork and Biodiversity [1]. They constitute elements for the sustainable development of agricultural and rural areas in the EU according to the common agricultural policy. These strategies are essential as half of the global GDP (Gross Domestic Product), EUR 40 trillion, depends on nature. Both strategies are intended to prevent biodiversity loss, as the global population of wild species decreased by 60% over the last 40 years and 1 million species are at risk of extinction, to reduce pollution levels, and to improve food quality. In 2017, over 950,000 deaths were attributed to unhealthy diets (one out of five) [2,3]. Therefore, the following, ambitious goals were set out by the EU within the Farm to Fork strategy: a reduction in the use of chemical plant protection products and dangerous pesticides by 50% by 2030, a reduction in nutrient loss by 50% while preserving soil fertility, a reduction in mineral fertilizer use by a minimum of 20% by 2030, as well as supporting the development of organic farming to achieve 25% of total farmland in the EU [2]. Despite the serious challenge that they pose, they are also an opportunity for dynamic and sustainable development as well as increasing competitiveness for the EU on the global market. However, a literal reduction in agricultural means of production or a simple increase in organic production would inevitably lead to failure. In order to fulfil the set goals, it is important to consider other background facts:

(a) According to the Food and Agriculture Organization of the United Nations (FAO), the human population will increase to nearly 10 billion by 2050, which makes food security one of the greatest challenges and a concern for the EU (food production must increase to about 70%) [4]. However, this issue cannot be solved entirely by the EU policies and actions relating to the reduction in food waste (the commission will propose legally binding targets to reduce food waste across the EU by 2023 by halving per capita food waste at retail and consumer levels by 2030) [2].

(b) On the other hand, the creation of a healthy food environment, which makes a healthy and sustainable choice the easy choice, as well as food labeling to empower consumers to choose healthy and sustainable diets, will raise consumers' demand for healthy food free of chemicals in favor of plants and environmentally friendly means of plant production and protection (the commission will propose mandatory front-of-pack nutrition labeling and develop a sustainable food labeling framework that covers nutritional, climate, environmental and social aspects of food products) [2].

(c) In general, plant pathogens are responsible for economic losses that usually amount to 20–30% on average, e.g., wheat 21.5%, rice 30.0% and maize 22.5% %, but sometimes reaching above 40% (e.g., maize 41.1%) if not correctly controlled [5] with their numbers increasing over the last 100 years [6]. Moreover, globalization is conducive to the faster spreading of pathogens (e.g., the rising issue of quarantine disease occurrence). The number of resistant pathogens is continuously increasing [7] and, at the same time, the number of available pesticides is decreasing. These facts, together with climate warming and the occurrence of more favorable conditions for pathogens, will result in a higher rate of disease incidence and this may in turn lead to producers using more chemicals, the outcome being a higher percentage of pesticide residues found in food products [8].

(d) The climate crisis has become increasingly more evident in recent years, with frequent droughts and irregular rainfall distribution, and the last decade being witnessed as the warmest decade ever [9]. This global climate change makes plant production and disease control more difficult and complicated. The elevation in CO_2 concentration and temperature can lead to the emergence of new diseases due to possible changes in plant susceptibility to infection by specific pathogens [10].

Considering the above mentioned facts, the demand for safer non-chemical-based, effective and sustainable systems for intensive agricultural production is continuously and quickly rising. Therefore, innovative and modern solutions that can pose an alternative to those currently used can guarantee the success of the European Green Deal strategies and meet consumers' expectations. As a result, the search for new non-chemical techniques in accelerating seed germination, improving plant growth and development as well as resistance against abiotic and biotic stress factors has recently reached high importance. Promising results for the use of physical methods such as non-thermal plasma [11], electromagnetic fields [12] and ultrasounds [13,14] for improvement of seed germination and seedlings growth have been more widely reported in the literature.

This paper presents different aspects in which cold plasma affects seeds and plants, as well as its possible applications in non-chemical plant protection and boosting. The literature was reviewed to provide evidence for how cold plasma might be used to achieve the goals of the European Green Deal strategy in accordance with the principles of sustainable agriculture.

2. Plasma Basics and Its Potential in Agriculture

Plasma is the fundamental state of matter in the Universe. Interstellar matter, stars and other elements are in the plasma state of matter. It is estimated that over 99% of the entire Universe mass is in this state [15,16]. Natural plasma is relatively rare on Earth. The gaseous layer of the atmosphere absorbs a large part of the ionizing radiation. However, our planet has an outer plasma layer called the ionosphere. The main, natural source of plasma formation in terrestrial conditions is lightning or the aurora light (polar lights). In general, plasma is referred to as a fourth state of matter. It is an ionized gas characterized by unique properties. Plasma is comprised of excited atomic, molecular, ionic and radical species coexisting with numerous species, such as electrons, free radicals, positive and negative ions, gas atoms and molecules in the fundamental or excited state, e.g., reactive oxygen species (ROS) and reactive nitrogen species (RNS) together with electromagnetic radiation (UV photons and visible light) [16]. It is considered a "quasi-neutral" medium with a zero net electrical charge. However, it is electrically conductive as it contains free charge carriers and exhibits properties distinguishing plasma from neutral gasses and liquids [16,17].

Plasma is usually created by the application of energy to a neutral gas, which in turn generates ions. The process can be supplied with thermal energy, electromagnetic radiation, or electric current [18]. Depending on the conditions of generation, plasma can be divided into equilibrium (thermal) and non-equilibrium (low temperature/cold) plasma. The latter can be further divided into quasi-equilibrium plasma (temperature around 100–150 °C) and non-equilibrium plasma (temperature less than 60 °C). The second group is especially interesting for biologists due to the lack of thermal damages of living material. The cold, non-thermal plasma is usually generated by an electric discharge with direct (DC) or alternating current (AC). However, it can also be produced with a radio frequency discharge (100 kHz–100 MHz) or a microwave discharge (above 100 MHz) [16,19]. The most frequently used types of DC discharges are a corona discharge (the current range is 10^{-7}–10^{-5} A), a glow discharge (current range 10^{-5}–1 A) and an arc discharge (current above 1 A) [20]. The best known and the most common type of AC discharge is a dielectric barrier discharge (DBD). The most characteristic feature of this discharge is two electrodes insulated by a dielectric barrier [21]. The greatest advantage of DBD is the possibility to operate in ambient air and in normal atmospheric pressure. The most common cold plasma generators include a dielectric barrier discharge, a plasma jet, a plasma torch, microplasma arrays, a gliding arc and high voltage nanosecond pulsed plasma [22–27]; the first two are the most frequently used devices. Figure 1 shows an example of DBD generators used by the authors in their research on cold plasma and ozone produced in the process of plasma generation and their effectiveness in decontamination of seeds and loose organic materials (e.g., grounded dry spices) [28]. Depending on the characteristics of the treated material, the construction of the discharge chamber and the electrodes may differ, as shown in Figure 2 [28,29].

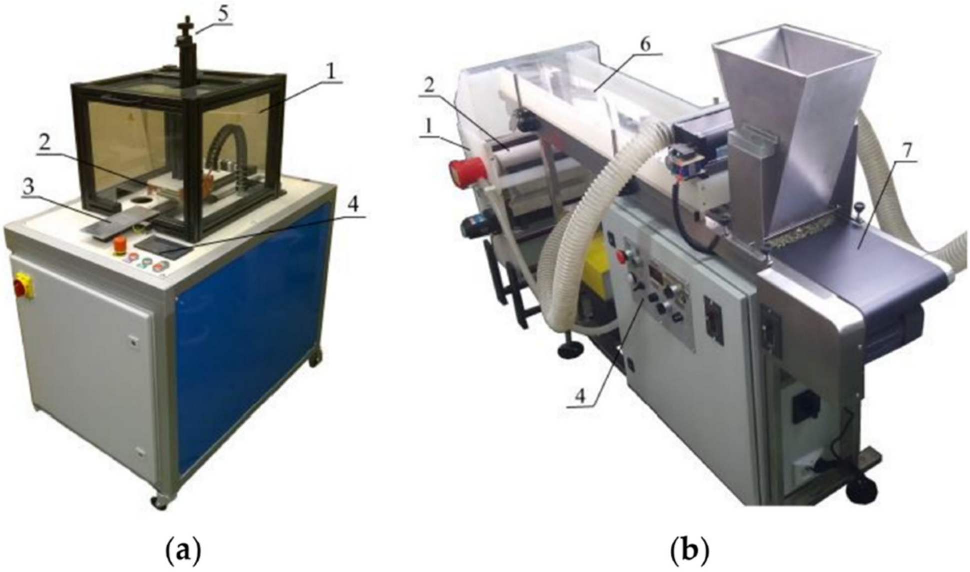


Figure 1. View of devices for decontamination of loose organic materials using DBD discharges tested by the authors: (**a**) device with a sliding electrode; (**b**) device with rotating electrodes; 1—discharge chamber, 2—electrodes assembly, 3—transport trolley, 4—operator panel, 5—electrodes gap adjustment knob, 6—ozone chamber and 7—belt conveyor (adapted from Mućko et al. [28]).

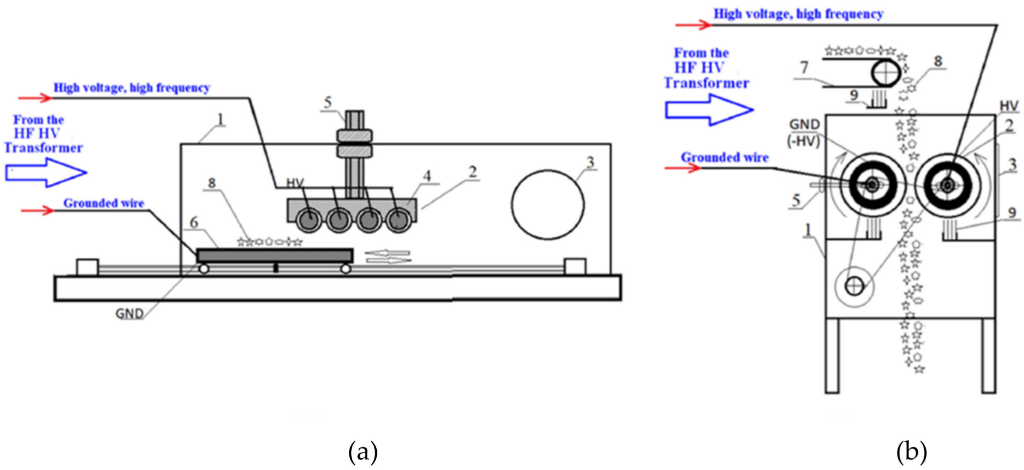


Figure 2. Construction of the devices for decontamination of loose organic material using DBD discharges: (**a**) discharge chamber with a sliding electrode; (**b**) discharge chamber with rotating electrodes; 1—discharge chamber, 2—electrodes assembly, 3—suction hole, 4—insulating support, 5—electrodes gap adjustment knob, 6—transport trolley, 7—belt conveyor, 8—processed material and 9—sweeper conveyor (adapted from Mućko et al. [28], Mućko et al. [29]).

The construction of plasma generators is closely related to their intended purpose. In our first prototype, the discharge chamber used a sliding electrode and was designated for the treatment of smaller sample particles to allow us to carry out laboratory scale research. The second prototype was equipped with rotating electrodes, a reverse transport of ozone and a belt conveyor, which was built for testing larger amounts of plant material in a production scale for that of small to medium sized companies [28,29]. Crucial for consideration in the final result of cold plasma treatments and its characteristics is the type and pressure of the gas being used. Numerous gases can be used for such purposes. Due to ease of use and low cost, the ambient air usually acts as the carrier gas for applications in agriculture [30]. There are also other gases used, such as argon, helium, nitrogen, oxygen and mixtures with oxygen [31]. Depending on these factors, cold plasma properties, such as volume, electron density, gas temperature, production rate of hydrogen peroxide and energy yield, can vary widely. The results of Wang et al. [32] showed that helium plasma had a lower electron density and gas temperature, but a larger volume compared to argon plasma in a nanosecond pulsed plasma discharge generated in a water-film plasma reactor. They also proved that it had production rates and energy yields of hydrogen peroxide slightly higher and more diffusive compared to argon plasma. However, helium plasma had a much lower electron density. The composition and properties of the cold plasma generated are specific to its intended purpose. Lou et al. [33] found that the antimicrobial efficiency for a cold atmospheric plasma jet (CAPJ) against *Escherichia coli* was highly dependent on the application voltage, CAPJ sample distance and treatment time, as well as the flow rate of argon gas. The most influential parameter was treatment time. Optimal bactericidal activity was achieved with an application voltage of 8.5 kV, CAPJ-sample distance of 10 mm, a treatment time of 300 s and an argon flow rate of 500 sccm (standard cubic centimeter per min). There was also an observed positive correlation intensity of hydroxyl radicals to CAPJ antibacterial activity. This would suggest that optimized experimental conditions can ensure high antimicrobial effectiveness of cold plasma.

Cold plasma has wide range of uses, including surface modification of plastics [34,35], ozone generation [36,37], toxic and harmful gas and sewage decomposition [38–40], as well as sterilization of soil and wounds [41–43]. Plasma complexity with its wide range of chemically active species also have a pronounced antimicrobial effect and can be used for surface decontamination [29,44–46], and for reducing the detrimental effect of microorganisms in the food processing industry [47,48] and agriculture [19,49,50]. Cold plasma technology is widely used in food preservation due to its ability to rapidly decontaminate microorganisms in ambient temperatures and normal atmospheric pressure conditions. Moreover, this technology ensures effectiveness without causing perceivable changes in food quality followed by reasonable costs when using ambient air as the working gas. The attractiveness of cold plasma technology is also due to a wide range of target microorganisms, including fungi (yeasts and molds), which have attracted special attention from the food industry as they are a large group of food spoilage microorganisms [51], viruses [52], prions [53], Gram positive and Gram negative bacteria [54,55] and biofilm forming bacteria [56]. There is also evidence that plasma has assisted the degradation of mycotoxins produced by some fungi [57–60].

Cold plasma technology also possesses many advantages desirable in agriculture, such as low operating temperatures, relatively short processing time as well as safeness to seeds, crops, humans and especially to the environment. Those highly reactive species that constitute cold plasma affect seed germination, plant growth, the quality of agricultural produce and cause changes in the density of reactive oxygen and nitrogen species, pH, oxidation–reduction potential and electrical conductivity. Recently, the contribution of cold plasma technologies in agriculture has included the decontamination of seeds or crops intended for sowing or storage, the boosting of seed germination and growth, the reduction in pathogen contamination, the production of nitrogen-based fertilizers, soil remediation, the disinfection of processing surfaces and tools, the removal of ethylene from air to lower the aging rate [61,62] and the activation of plant growth-promoting bacteria [63]. As well as using cold plasma directly, plasma can be applied indirectly through the use of plasma-activated water (PAW) [64–67]. In general, water treatment by exposure to a plasma discharge, such as cold plasma jet, increases its beneficial properties for plants. PAW can significantly affect seed germination, seedling growth [11,68–71] and it can also increase crop yields when used for irrigation during the vegetation period [19,72,73]. The main mechanism is the activation of the synthesis of plant hormones such as auxin and cytokinin, which induce physiochemical changes resulting in the boosting of seed germination and plant growth. Cold atmospheric plasma-assisted nitrogen fixation is also considered a significant source of nitrogen for plants due to the presence of nitric oxide, nitrite, nitrate, dinitrogen trioxide, dinitrogen pentoxide and ammonia [74–76]. These forms of nitrogen are mobile and soluble in soil, which makes them easy for plant uptake [77,78]. Therefore, they can be used as fertilizers [79]. The undeniable benefits for the use of cold plasma in nitrogen fixation are low-energy consumption (high-energy demand of present artificial nitrogen fixation) and low cost, sustainable production (atmospheric conditions) [80,81], non-emission of greenhouse gases [82,83] and lastly, a relatively easy setup for on-site mass production [84]. However, nitrogen-fixed water is acidic (pH 2–3) due to dissolved nitrogen oxides and hydrogen peroxide, which can be harmful for some crops [69,85,86]. An alternative method for neutralization is the immersing of Mg, Al or Zn in water, as proven by Lamichhane et al. [67]. Authors found that the addition of metal ions is very effective in the neutralization of PAW. They observed a high increase in corn germination and plant growth after the treatment with metal ion-enriched PAW. Plants contained more chlorophyll (especially in Mg–PAW) and proteins. Such neutralized, metal ion-enriched and nitrogen-fixed PAW can be used directly for crop irrigation and therefore exhibits a great potential in large-scale sustainable agriculture for both soil-based and hydroponic production [87]. Moreover, some limitations for the present production of nitrogen fertilizers (environmental, establishment, production and transportation [88–90]) can be addressed. However, there is still a need to provide other macro and micronutrients essential for plant growth.

3. Effect of Cold Plasma on Seed Fungal Pathogens

It is scientific knowledge that pathogen-infected seeds are the primary source of plant disease in many types of food crops. A huge number of fungal pathogens, such as *Alternaria*, *Aspergillus*, *Bipolaris*, *Botrytis*, *Colletotrichum*, *Fusarium* or the *Sclerotinia* genera, are transmitted when the seeds are sown and emerge. The pathogens limit the germination and the development of both seedlings and plants, such as soybean [91], cereals [92], oilseed rape [93], vegetables [94–96] and herbs [97], thus negatively affecting the yield and food safety [98,99].

Traditionally, in order to control seed-borne pathogens, fungicides have been widely used [44,100]. As shown in numerous studies, the use of cold plasma is an innovative, efficient, and eco-friendly method for reducing pathogens without the use of pesticides, which are harmful to both the environment and consumer [94,101,102].

Plant pathogens occur mainly in the seed coat [103]. Low-temperature plasma, in addition to causing changes in the external structure of seeds, prevents the possibility of pathogen development [45]. Cold plasma has a direct impact on pathogens, which makes it a useful tool for sterilization of the seed surface. The application of cold plasma meets the criteria for the advanced seed treatment method, for ease of application, effectiveness against a wide range of pathogens and non-toxicity to humans and animals [102,104]. It has been found that a mixture of free radicals, ions, electrons, stable reaction products, reactive oxygen species and molecules, or ultraviolet (UV) radiation which are generated by cold plasma, induce fungicidal effects. Different reactive agents play a role, either independently or in synergistically, in the inactivation of microorganisms. The mechanism of sterilization has not yet been fully elucidated. In general, plasma disrupts the cell exterior and causes cell permeability. The device model and operating parameters, such as gas composition, flow rate, temperature, humidity, voltage, and frequency, also affect the efficacy of cold plasma [73,105–107].

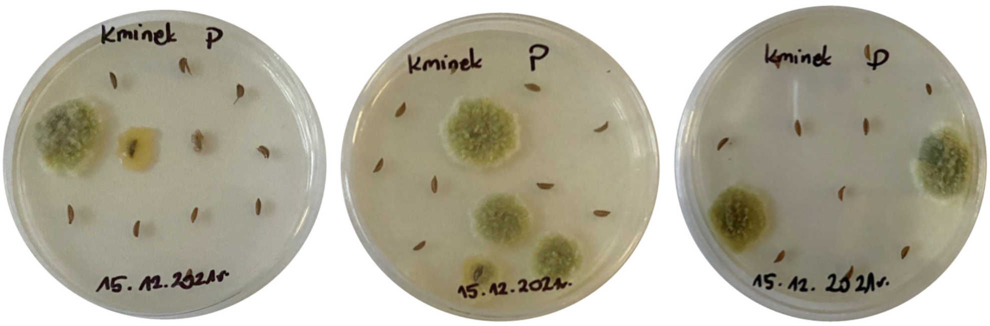
Hydroxyl radicals (OH) have a direct effect on the cell membranes of microorganisms through the ability to readily destroy membrane proteins [107]. Moreover, the contact of biological material with reactive oxygen species (ROS) and reactive nitrogen species (RNS) can lead to serious biological cell damage (oxidative stress). As a result of the oxidation of lipids and membrane proteins, the proper function of the cell membrane is impaired and, eventually, destroyed [108,109].

According to studies conducted on microorganisms, including filamentous fungi, the effect of UV radiation on the inactivation is relatively minor [110]. ROS are much more effective, as demonstrated in the case of inactivation of *Penicillium digitatum* spores [111,112].

Among the reactive oxygen species, ozone is a strong oxidant, second only to the hydroxyl radical [113].

A preliminary study carried out by the authors of this manuscript also proved a high inhibition effect of dielectric barrier discharge plasma (DBD) on growth of some pathogenic fungi contaminated caraway seeds (*Carum carvi* L.). They were settled by the fungi from *Alternaria*, *Aspergillus*, *Colletotrichum*, *Fusarium*, *Penicillium* genera and some bacteria. Cold plasma (DBD) treatment of seeds with 400 W power and 15 s of exposure time was very effective. The weakest result was noted for *Aspergillus ochraceus* (Figure 3).

(**a**)



(**b**)

Figure 3. Control (untreated) (**a**) and treatment (**b**) by dielectric barrier discharge plasma (DBD) seeds of caraway (*Carum carvi* L.) cultivated on PDA medium. Clear growth inhibition of some pathogenic fungi and bacteria after treatment.

Fusarium spp., commonly occurring on seeds, is considered to be one of the most sensitive fungi to cold plasma, for example, the seed treatment of common buckwheat conducted in a low-pressure radiofrequency (RF) system with the use of oxygen, which resulted in a reduction in fungal growth, including *Fusarium* species [114]. Plasma treatment using the diffuse coplanar surface barrier discharge (DCSBD) at atmospheric pressure in ambient air also strongly affected fungi on maize seeds. The fungi of genus *Alternaria* proved to be the most resistant to cold plasma. The reduction of 3.22 log (CFU/g) in *A. alternata* was observed after a 300 s plasma treatment with an input power of 400 W, while for *F. culmorum*, just 60 s was sufficient for its a total elimination [30]. Filamentous fungi inhabiting wheat grains also showed a varied response to cold plasma. The efficiency in the treatment of seeds artificially inoculated with fungal isolates decreased in the following order: *F. nivale*, *F. culmorum*, *Trichothecium roseum*, *Aspergillus flavus* and *Aspergillus clavatus* [115]. High antimicrobial activity of non-thermal plasma generated by an air dielectric barrier discharge (DBD) was also noted for *Gibberella fujikuroi* (*Fusarium fujikuroi*) pathogenic to rice, where treatments at atmospheric pressure effectively reduced the general number of colonies on seed surface [102]. A negligible effect of extended plasma treatment on the number of *F. culmorum* on wheat grains was noted by Kordas et al. [116]. In addition, Hoppanová et. al. [101] observed the possibility of decontamination in wheat and barley seed surfaces from this pathogen, which proves plasma to be an effective tool in the successful treatment of cereal seeds against disease. Treatment in the range of 120–300 s significantly inhibited the growth of *F. culmorum* on seed surface.

Cold plasma has also been shown to be effective in decontamination and enhancement of pepper (*Capsicum annuum* L.) seed quality. Inhibition of mycelium growth and spore germination of *Colletotrichum gloeosporioides*, one of the most destructive plant pathogens [117], was observed [94] in this study where the emulated plasma-like condition was generated by exposing the solution of H_2O_2 with UV-C irradiation to obtain EPAS-H_2O_2. Results indicated that EPAS-5% H_2O_2 treatment completely inhibited the growth of mycelium and spore germination. Fungal mycelium was more resistant to the treatments than spores.

Cold plasma (DCSBD at atmospheric pressure in ambient air) has been shown to successfully reduce *Didymella licopersici* spores from pepper seeds. The elimination of *Cladosporium cucumerinum* and *Didymella bryoniae* spores from cucumber seed was found to require a slightly longer plasma treatment. In the same experiment, no effect of plasma treatment on the presence of cucumber mosaic virus (CMV), zucchini yellow mosaic virus (ZYMV) and watermelon mosaic virus (WMV) on cucumber was found [118]. Nishioka et al. [119] observed the effect of inactivation of *Rhizoctonia solani* on brassicaceous seeds, where 10-min-long treatment with cold atmospheric plasma at low pressure reduced the fungal survival rate from 100% and 83%, respectively, to less than 2%. Plasma also exhibited antifungal activity on ginseng seeds. The number of fungal colonies from genera *Coniochaeta*, *Pyrenochaeta* and *Fusarium* was significantly lower for seeds treated with argon-generated plasma (argon used as feed gas for the DBD plasma generator) than for the untreated controls Lee at al. [120].

Numerous studies show the suppression of *Aspergillus* spp. growth by cold plasma. A reduction in *A. flavus* was noted on maize seeds [30]. Successful elimination of *A. niger* as well as *A. westerdijikiae*, *A. steynii* and *A. versicolor* was observed on coffee treated by cold plasma with helium at 1.5 L min^{-1} flow, which reduced the content of ochratoxin A in roasted coffee [121]. However, in the case of the dark pigmented species of *Aspergillus* spp., melanins, that proved to be involved in their resistance to environmental stress, can limit the cold plasma effectiveness. Such effect was observed for *Aspergillus fumigatus* where melanin increased conidia resistance to ROS [122,123]. It may be suspected that the presence of melanins resulted in a weaker plasma effect on *A. flavus* compared to *Fusarium* species [115]. Melanins are also present in the primary cell walls and the septa of multicellular conidia of *Alternaria* spp. [124], which may explain its previously mentioned resistance to cold plasma action.

The research results mainly show the effectiveness of cold plasma on pathogens inhabiting the seed surface. However, this eco-friendly technology may show efficiency in controlling seed-borne pathogenic fungi developing within the seeds, an example of which is the reduction in species from the *Diaporthe*/*Phomopsis* complex, which occurred on soybean seeds [125]. In these studies, the effectiveness was demonstrated by reactive oxygen and nitrogen species (RONS), which is consistent with the considerations of Ambrico et al. [126], who questioned the effectiveness of UV radiation in such cases, which when used directly against genetic material, destroyed its DNA [127]. The sterilization effect through the use of UV radiation cannot be achieved for microbial cells or spores that are stacked or covered with plant tissues, due to the minimal depth in penetration of UV photons Ambrico et al. [126].

The antifungal impact of cold plasma application against seed-transmitted fungi is documented in many research papers. However, the fungicidal effect of plasma is usually weaker than in the case of bacteria. This is mainly caused by differences in the structure of prokaryotic and eukaryotic cells [126]. Due to the composition of chitin, α and β-linked glucans, glycoproteins, it is relatively difficult to force and destroy fungal cells by external factors. Melanins protect the fungi from damaging agents, such as UV, ionizing, extreme temperatures, the action of hydrolytic enzymes, antifungal substances and free oxygen radicals [128–130].

4. Effect of Cold Plasma on Bacteria Contaminated Seed

The most commonly found bacterial plant pathogens contain representatives of the genera that can be transmitted with seeds, such as *Pseudomonas*, *Xanthomonas*, *Dickeya* and *Ralstonia solanacearum* species [131]. Although plant pathogenic bacteria cause a smaller number of diseases and less economic damage than that of fungi, they have a negative impact on the condition of many plants and, consequently, on the quality and quantity of the crop. For this reason, the cold plasma application on bacteria has been relatively less studied [50]. Plasma is often more effective against bacteria. However, Lee et al. [120] found that plasma treatment disinfects the surface of the ginseng seeds from fungi to a greater extent compared to bacteria. They observed a weaker bactericidal effect, for example, in the case of genus *Pseudomonas*, which exhibited survival rates of 93.8% and 90.4% having used a plasma treatment with argon and Ar/O_2 mixture as feed gases, respectively, while non-pathogenic *Azospirillum* was drastically inhibited.

However, the studies show that, as for fungi, successful elimination of bacteria can be achieved with the use of generated RONS, which probably led to the damage and dysfunction of DNA, proteins and ribosomes, as observed with mung bean seeds artificially inoculated with *Dickeya* and *Pectobacterium* spp. [132]. There are groups of bacteria transmitted with seeds that are of significant importance in agriculture that show sensitivity to plasma. An example is *Xanthomonas campestris* (Gram-negative), which causes black rot, the most important disease in vegetable brassica crops worldwide [133]. Regarding bacterial pathogens, Nishioka et al. [134] eradicated them from cruciferous seeds by cold plasma working under an Argon atmosphere. After plasma treatment of cabbage seeds contaminated artificially by *Xanthomonas campestris* pv. *campestris*, a simultaneous elimination of molds and rot was observed in the seedlings [135].

Irradiation of infected rice seeds with atmospheric-pressure plasma has shown a suppressive effect on *Burkholderia plantarii*, a causal agent of bacterial seedling blight [136]. A beneficial effect observed in the use of cold plasma seed treatment was an increase in young plant resistance to tomato bacterial wilt caused by *Ralstonia solanacearum*. Observation of some parameters suggests that plasma influences the regulation of plant growth, the concentration of H_2O_2 and the activity of enzymes in tomato plants, which results in improved resistance to this pathogen [137].

Both Gram-positive and Gram-negative bacteria are sensitive to plasma treatment, as exemplified by its effect on *Staphylococcus aureus* (G +) and *Escherichia coli* (G −). Plasma directly affects the bacteria that cause a decrease in membrane integrity [138]. However, the outer membrane of Gram-negative bacteria is usually more sensitive to the physical effects of plasma-generated species [139].

The presence of non-plant pathogenic bacteria is problematic in the case of seeds grown for their sprouts, which should be free from microorganisms harmful to humans. Raw sprouts can carry a risk of foodborne illness if they are contaminated [140]. Seeds infected with microorganisms are the primary cause of most contaminations in sprouts, although pathogens could possibly be introduced at various stages of production [141,142].

Salmonella strains, including *S. enterica*, *E. coli* and *Bacillus cereus*, can be found on the surfaces of plant seeds intended for sprouting purposes [143,144]. Exposure of broccoli seeds to corona discharge plasma jet has shown a species-specific variation of susceptibility among bacteria. Aerobic bacteria and *E. coli* were shown to reduce more strongly than *B. cereus*, *Salmonella* spp. as well as molds and yeasts [144]. It was noted that by applying a corona discharge plasma jet for 3 min, these three groups of bacteria were reduced in the range of 1.3–2.1 log CFU/g [145].

The research on the reduction in native microorganisms or artificially applied *E. coli* on sprout seeds of onion, radish, cress and alfalfa has shown more effective inactivation of *E. coli* compared to the native microbiota composed of Gram-negative and Gram-positive bacteria [146].

A positive effect of the reduction in *E. coli* on mung bean sprouting seeds has also been observed after the application of plasma-activated water (PAW) [147].

5. Potential of Cold Plasma for Boosting of Seed Germination and Plant Growth

In the previous chapters, we demonstrated the use of cold plasma for seed decontamination from microorganisms. However, cold plasma is also a useful tool for the enhancement of seed germination and improvement of their viability. In addition to modifying the seed surface morphology, the penetration of active plasma species through the pores affects their physiological response and results in the modification of some metabolic, biosynthetic and signaling pathways [88,115,148–150]. The application of this non-toxic and low-energy consumption method, favoring of long-time storage and supporting seed development, presents cold plasma as a promising tool for the improvement of plant survival [151].

There are a number of factors controlling seed germination and dormancy, including plant hormones, temperature, water uptake and availability of food stores, as well as morphological and physiological alterations [152]. The reactive forms of oxygen and nitrogen (RONS) and nitric oxide produced during plasma discharges eliminate seed dormancy and increase germination probability and biomass. These compounds penetrate into the seeds and may modify the water uptake rate, increasing the wettability, which in turn increases the germination rate [87,153]. As Švubová et al. [154] found for soybean seeds, water intake and thus seed weight increased linearly with increasing plasma dose. Changes in wetting properties of seeds are caused by oxidation of their surface that leads to faster germination and better yield. This was found for wheat and oats [88] and lentils, beans and wheat [155]. According to Park et al. [156], growth enhancement after seed germination observed for barley may be initiated by breakage of the seed coat and stimulation of the growth cycle. Analysis showed that cold plasma induced significant changes on the seed surface [157]. However, morphological changes, such as significant degradation or damage on the seed surface, were not observed. Zahoranová et al. [30], who based their research on maize, concluded that the active plasma species caused a decrease in the lipid group and an increase in polar groups containing oxygen and nitrogen on the seed surface.

Studies suggest the effect of seed treatment with cold plasma on the presence and synthesis of plant hormones in seeds and seedlings as they regulate seed dormancy, germination and plants' vegetative growth [156–161]. Cold plasma treatment acts as an oxidative boost leading to disturbance of the redox equilibrium in plants. RONS can crosstalk with plant hormones, metabolic reactions and signaling cascades that alter physiological and biochemical processes in plants [162]. The main role is played by two antagonistic hormones: abscisic acid (ABA), an inhibitor of germination, and gibberellins (GAs), stimulators of germination. They activate the signaling cascades and enzymes, e.g., amylases that decompose seed reserves and initiate germination [49]. However, the key factor is not only the concentration of a specific hormone, but mainly their ratio, an integrated relationship. Other hormones, such as auxin IAA (indole-3-acetic acid) (negative regulator of germination), ethylene, citokinins, brasinosteroids and strigolactones (stimulate germination by various modes), salicylic acid (SA) and jasmonate (stress hormones) (stimulate or inhibit germination depending on the conditions) affect germination by modulating the effects of ABA/GA balance [163]. Mildažienė et al. [159] reported a noticeable change in the auxin/cytokinin ratio and production of other hormones in sunflower seeds treated with cold plasma and a radio-frequency electromagnetic field. However, there was no direct relationship between changes in the amount of phytohormone and germination kinetics. Ji et al. [23] showed a higher concentration of gibberellin acid (GA3) in wheat seeds after cold plasma treatment, as well as an increased activity of hydrolytic enzymes. Stolárik et al. [157] noted changes in auxin and cytokinins in peas after plasma treatment. They found an increase in indole-3-acetic acid (IAA), (oxidizing IAA) oxIAA and zeatin and a correlation with increased growth parameters. Unclear data suggest a high complexity of phytohormones and their functions involving mutual regulation or functional crosstalk [164]. Therefore, an integrated analysis of plant hormones under cold plasma influence would require further research to provide a better understanding of the exact mechanisms involved.

The increased concentration of plant hormones after low-temperature plasma treatment may also affect the expression of certain genes or modify key gene products [165]. Wang et al. [166] reported that increased auxin levels may result in increased lateral root growth in tobacco under drought stress. Guo et al. [160] noted increased abscisic acid (ABA) content in wheat seedlings treated with cold plasma prior to imbibition. ABA is considered the major signaling factor in regulating the water status of plants and in the process of inducing genes involved in dehydration resistance.

Numerous studies have shown that plasma-treated seeds germinate faster than untreated ones. The result of the improved seed germination is the boosting of seedling and plant growth promotion, and increased yields for numerous crops [148,157,158,167]. Such effect was observed for tomatoes, where plasma enhanced the germination rate. Seedling length and weight were significantly higher than those of untreated seeds [168]. Green onion seeds also responded with an increase in both germination potential and germination rate to cold plasma treatment [169]. Enhancement of seedling vigor (shoot and root lengths) has been observed in the case of maize [30]. Guo et al. [170] found that germination potential, germination index and vigor index significantly increased along with shoot length, root length, dry weight, and fresh weight. Jiang et al. [161] observed that, after seed treatment, wheat plants were significantly taller with longer roots and contained more chlorophyll and nitrogen. In the case of peanuts, apart from the growth of germination potential, germination rate and the growth of dry matter of seedlings, leaf area and thickness, nitrogen concentration in leaves, chlorophyll content, plant height, stem diameter as well as dry root mass of plants in the final stages of vegetation were all found to be significantly increased by cold plasma treatment in another study [171]. Treatment of brown rice seeds have been found to increase the germination percentage, seedling length, and water uptake [172]. De Groot et al. [173] reported an increase in water absorption by seeds after air plasma treatment that improved germination activity, which remained for several months.

Cold plasma may have a positive effect under abiotic stresses. In drought conditions, seed germination improvement and better seedling growth of wheat have been observed [160]. In the case of alfalfa [174] and oilseed rape [175], a significant increase in seedling root and shoot lengths, seedling height and weight as well as lateral root number have been noted.

Problems with seed germination are often caused by microbiological contamination of their surface [176,177]. Therefore, in addition to its direct effect on seeds, cold plasma may also contribute to the boosting of the germination rate indirectly through their sterilization.

6. Cold Plasma Technology for Sustainable Plant Production

The European Green Deal has outlined the need for non-chemical, alternative and environmentally friendly production methods. This has driven scientists in cooperation with agriculture supplying companies and producers to look for new alternative tools for plant production and protection. This process is exceptionally difficult to achieve the goals as the above mentioned background situation imposes serious limitations on agricultural producers. Under such conditions, EU agriculture needs to develop the existing system as well as implement new cutting-edge solutions supporting sustainable agricultural production in line with the formulated requirements as soon as possible [178,179]. The above referenced literature and research findings conducted by the authors prove a significant potential for cold atmospheric plasma in this area. This is also evident in numerous scientific articles on the subject in the last decade, which show a positive effect of cold atmospheric plasma on plant growth and development. Cold plasma-enhanced antioxidant activity, the production of growth hormones and metabolites ensure earlier seed germination, higher germination percentage, improved growth of shoots and roots, and as a result, a yield increase. However, the positive effect of cold plasma treatment on the physicochemical and physiological parameters (e.g., protein and sugar content, seeds wettability plant growth and harvest time) depends on the plant species and cultivars, the type of treated seeds and the environmental conditions. Moreover, the positive direction of the germination and plant growth processes depend to a high extent on plasma gas composition, applied power and the time of seed treatment [180]. The power of plasma generator and the time of exposure are the most important and studied plasma parameters, crucial for the achievement of a desirable effect. These parameters may vary significantly (5 s–30 min) depending on the type and construction of the plasma generator and the plant species. In most of the conducted experiments, shorter exposure time significantly improved the seed and plant growth parameters, while longer treatments often had an inhibitory effect on seed germination percentage. Along with longer exposure time and an increase in plasma power, higher antimicrobial efficiency can be achieved. However, a lower percentage of germination may be also observed [87,157,180–184]. Following the above mentioned observations, it should be stated that in most research cases, a specially dedicated device for cold plasma generation must be constructed. Special attention must be also paid to health and safety measures when operating the cold plasma devices.

The evidence from the literature suggests that cold plasma can provide a reliable method to meet the requirements associated with global climate change in agriculture. Despite the achieved progress in cold plasma research, the available results indicate its use for seed treatment and plant growth boosting mainly based on laboratory experiments. Few studies have been performed in field conditions. Therefore, more detailed research is still required for commercialization purposes [58]. In order to support such needs, dedicated financial resources are allocated at the EU level (e.g., Horizon Europe). This need was perfectly realized by Stella Kyriakides, the EU's Commissioner for Health and Food Safety—"We know that investment will be necessary to encourage innovation and create sustainable food systems. We have made EUR 10 billion available for research and innovation, to support and accelerate this transition, to develop biological pesticides, natural resources as well as the use of digital and nature-based solutions for food production" [178].

7. Conclusions

In order to meet the above listed expectations and to achieve the goals quickly and effectively, it needs to be emphasized that combined efforts of scientists from different, interdisciplinary areas of expertise, producers and agriculture supplying companies must cooperate in order to promptly transfer the new innovative solutions into real practice. The described achievements of cold plasma technology, especially from the last decade, allow us to look optimistically forward to the next decade, which, in view of the aims of the European Green Deal, will bring an important transformation for EU's agriculture. Having outlined the above, we need to raise the question as to whether the European Green Deal can provide an opportunity for non-thermal atmospheric plasma use in agriculture. The worldwide research conducted so far, especially in the past decade, indicates that it should do so.

In conclusion, cold plasma technology has considerable potential for seed treatment, boosting their germination rate and providing support in later stages of seedling and plant development, especially during difficult growth periods. It proves itself as an innovative, efficient and eco-friendly plant protection method. Cold plasma use will reduce the application of fertilizers (in its use to strengthen diseased plants, for their recovery and plasma-assisted atmospheric nitrogen fixation due to available extra resources in PAW) and pesticides (due to antimicrobial activity, e.g., used for seed disinfection) in plant production, providing a more sustainable management, which fits within the scope of the European Green Deal. It is expected that cold plasma will find numerous applications for the agricultural sector in years to come.

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