

Review

# Potential of Microwave Heating and Plasma for Biosecurity Applications

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**Abstract:** This review explores the use of microwave heating and microwave-generated plasma for biosecurity applications. Microwave heating has been shown to rapidly heat and kill a wide range of pests and pathogens. Examples of microwave thermal disinfestation of soils, grains, hay, and timber are presented and discussed. Microwave energy can also ionize various gasses, including air, to create plasma. Plasmas are described by many characteristics, such as temperature, degree of ionization, and density. In the “after glow” (cold plasma) of a plasma discharge, there are sufficient charged particles and excited atoms to generate elevated UV levels and ionize the surfaces of objects. Examples of cold plasma and plasma-activated water disinfestation of grains and other commodities are also presented and discussed. Brief comments on the scale-up of this technology have also been presented.

**Keywords:** biosecurity; microwave; heating; plasma; pathogens; pests



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## 1. Introduction

Biosecurity is critical to protect ecological and agricultural production systems around the planet. Biosecurity applies various procedures or measures, which are designed to protect the population and environment against the introduction, establishment and spread of harmful biological organisms or substances. The fumigant methyl bromide is an important biosecurity tool for disinfesting internationally traded commodities against quarantine pathogens, pests, and weeds. However, methyl bromide is implicated in the degradation of stratospheric ozone and non-quarantine and pre-shipment uses are being regulated and phased out under the 1992 Montreal Protocol [1]. Quarantine uses of methyl bromide are not currently regulated under the Protocol and are defined as “treatments to prevent the introduction, establishment and/or spread of quarantine pests (including diseases), or to ensure their official control”. Similarly, pre-shipment uses of methyl bromide are currently not regulated and are defined as “non-quarantine applications applied within 21 days prior to export to meet the official requirements of the importing country or existing official requirements of the exporting country” (Decisions VI/11, VII/5 and XI/12 of the Montreal Protocol). Currently, the official worldwide use of methyl bromide for quarantine and pre-shipment purposes as reported by Parties to the Montreal Protocol under Article 7 averages 10,000 tons per year (see link to reference). Therefore, alternative biosecurity technology must be explored.

Among the non-chemical concepts for commodity disinfestation is thermal treatment using solar energy and steam [2]. An advantage of these approaches is there is little risk of resistance developing in pest and pathogen populations. However, these conventional thermal treatments are very time and energy intensive, and this often makes their adoption uneconomical. In contrast, microwave energy is a very fast and efficient method that can be used for directly heating various materials [3]. An advantage of the microwave is that it can heat the commodity and not the container or the environment and this makes the process very efficient. It is even possible to efficiently heat, melt, and process metals using specially

designed industrial microwave ovens [4]. Microwave energy can also be used to ionize various gasses, including air, to create plasma [5–7], which can be used to sterilize surfaces.

Therefore, due to the importance of exploring alternative disinfestation methods, this paper reviews the potential of microwave heating and microwave-generated plasma for biosecurity applications. The paper is divided into two main themes based on these two techniques.

## 2. Thermal Disinfestation

Over the past 50 years, the use of electromagnetic energy, especially in the radio and microwave frequency range, has been proposed as a method of controlling pests in soils, grains, and timber products [8]. Initially, most research was focused on thermal treatment of the commodity [8,9]; however, electromagnetic energy can be used in other ways to disinfect or disinfect materials.

Focusing firstly on the thermal effects of electromagnetic treatment, the fatal impacts of high temperatures on botanical and zoological specimens have been studied in detail for over a century [10]. An empirical relationship between lethal temperature and temperature holding time has been developed by Lepeschkin [11]:

$$T = 79.8 - 12.8 \cdot \log_{10} Z \quad (1)$$

where  $T$  is the lethal temperature ( $^{\circ}\text{C}$ ), and  $Z$  is the lethal temperature holding time, in minutes [10]. Individual relationships for different species of plants and pathogens [11–13] have been developed over time. Ultimately, heat can provide similar lethal effects to chemicals and therefore has been used in soil, timber, hay, and grain sanitation processes [14] for some time.

Various heat sources have been considered for sanitation applications. These include, solarization, solar heating, flaming, steam heating, and electromagnetic heating. The following section highlights how electromagnetic heating, especially using microwave energy, has been used for thermal sanitization.

## 3. Microwave Heat Sanitization

Many studies have considered microwave heating as a method of sanitization. Table 1 provides an overview of some key research. One of the main areas of study has been the deactivation of weed seeds in the soil. Davis, et al. [15] demonstrated the efficacy of microwave energy for weed management. They developed a prototype system, called the “Zapper” [16], which could treat the soil in situ, using a variant on a horn antenna to apply the microwave energy to the soil’s surface. To obtain consistent pre-emergent control of both broadleaved weeds and grasses, it was necessary to apply at least  $183 \text{ J cm}^{-2}$ . Brodie, et al. [17] later confirmed that  $185 \text{ J cm}^{-2}$  of microwave energy, when applied to moist soil, could effectively kill various *Lolium* spp. (ryegrasses) seeds to a depth of 5 cm. Treating seeds in dry soil required over  $550 \text{ J cm}^{-2}$  of microwave energy to kill seeds to a depth of only 2–3 cm [17].

The energy required to control emerged weeds using a horn antenna is quite variable ( $77\text{--}500 \text{ J cm}^{-2}$ ) [15,18], depending in the species and the height of the horn antenna above the ground. Recent experiments using a 15 cm wide slow-wave applicator, connected to a 5-kW microwave source, and being towed at an equivalent speed of approximately  $0.6 \text{ km h}^{-1}$  ( $17 \text{ cm s}^{-1}$ ), has demonstrated that applying  $20 \text{ J cm}^{-2}$  of microwave energy can kill most emerged weeds (unpublished).

### 3.1. Effects on Soil Biota

It has been demonstrated that microwave soil heating has an immediate impact on soil microbial communities [19]. The populations of some species are significantly reduced [20]; however, other species, including nitrifying bacteria and archaea, are relatively unaffected, except at extremely high doses of microwave energy [21–23]. Soil bacterial and fungal community compositions change significantly due to microwave soil treatment and re-

covery of biological diversity takes more than 4–5 weeks [22]. Recent experiments have demonstrated that microwave soil treatments, with the similar intensity necessary to kill weed seeds, significantly reduce the number of soil-borne fungal pathogens, including *Fusarium* spp., *Macrophomina phaseolina*, and *Thielaviopsis basicola* (unpublished).

### 3.2. Implications for Cropping Systems

The combination of removal of weed competition and soil disinfestation provided by microwave soil treatment results in significant crop yield increases. In field experiments, increases in crop yield of between 18% and 84%, compared with the untreated or hand-weeded controls, have been observed [24,25]. Pot experiments have demonstrated that a single microwave soil treatment can provide significant crop yield increases over several seasons, with the longest observations spanning three years, so far [26].

**Table 1.** Summary of microwave-based weed management research.

Microwave Frequency	Power Level	Irradiation Duration	Treatment Scenario	Target Weed Species	Percentage Weed/Seed Destruction	Reference
39 MHz	–	4–37 s	Pre-emergence	Hard Red Winter Wheat <i>Zea mays</i> , <i>Arachis hypogaea</i> , <i>Prosopis juliflora</i> , <i>Cucumis</i> <i>sativus</i> , <i>Brassica</i> spp., <i>Rumex</i> <i>crispus</i> , <i>Echinochloa colonum</i> , <i>Amaranthus</i> sp., <i>Gossypium</i> <i>hirsutum</i> , <i>Glycine max</i> , <i>Sorghum vulgare</i> and <i>Triticum vulgare</i>	50% seed mortality	[27]
2.45 GHz	600 W	60 s	Pre-emergence (dry, 4 h soaked and 46 h germinated seeds)	Johansongrass Moringglory Redroot Pigweed Texas panicum Barnyardgrass Sunflower London rocket Rigseed euphrobia	17% reduction in germination in dry seeds but 100% in case of moist seeds at 10 s of exposure	[15]
2.45 GHz	600 W	8 s	Post-emergence (aquatic weed)	Duckweed ( <i>Wolffia punctat</i> )	50%	[28]
2.45 GHz	2–4 kW	Varying exposure time (not mention properly)	Pre- and post-emergences	London rocket (13 cm deep in soil profile) and Sunflower (2.5 cm seeded depth)	For post-emergence MW treatment 309 J cm <sup>-2</sup> energy was required for 100% control (field conditions) while for pre-emergence MW weed control 73 J cm <sup>-2</sup> gave 85–100% control (Glass house conditions)	[29]
2.45 GHz	45–720 J cm <sup>-2</sup>	No information	Pre-emergence	Clover and Turnip	87% for London rocket and 93% for Sunflower	[30]
2.45 GHz	100–750 W	120–1200 s	Pre-emergence	Black Medic, Barnyard grass, Foxtail purslane, Redroot pigweed, Large crabgrass <i>Brassica napus</i> , <i>Linum</i> <i>usitatissimum</i> , <i>Avena fatua</i>	60–78% reduction in seeds germination	[31]
2.45 GHz	0.1–1.5 kW	Varying exposure time	Pre-emergence of seeds in soil	<i>Zea mays</i>	50%	[32]
2.45 GHz	–	360 s	Pre-emergence	<i>Trifolium</i> and <i>Medicago</i>	85–95%	[33]
9 GHz	10–30 mW cm <sup>-2</sup>	22–24 h	Post emergence	<i>Avena fatua</i>	100% growth inhibitions	[34]
2.45 GHz	1.2 kW	5–45 s	Pre-emergence	Wild Oat and Wheat	85% reduction in germination	[35]
2.45 GHz	500 W	30 s	Pre-emergence	<i>Avena sativa</i> and native weed seeds	60% (based on seed moisture)	[36]
2.45 GHz	1.5 kW	0, 10, 20, and 30	Pre-emergence	<i>Abutilon theophrasti</i> , <i>Panicum</i> <i>miliaceum</i> , Lucerne and Rapeseed	90–100%	[37]
2.45 GHz	–	120 s	Pre-emergence	Rubber vine, Parthenium and Bellyache bush	Reduced weed seeds emergence	[38]
2.45 GHz	900 W	4, 8, 16, 32, 64, 128, and 256 s	Post-emergence	<i>Malva parviflora</i> and <i>Triticum aestivum</i>	Complete dehydrating of plants	[39]
2.45 GHz	800 W	120, 240, 420 and 960 s	Pre-emergence	<i>Lolium perenne</i> and <i>Lolium rigidum</i>	88% (Rubber vine), 67% (Parthenium) and 94% (Bellyache bush) mortality at 960 s irradiation	[40]
2.45 GHz	0.10–1.24 kWh m <sup>-2</sup>	30–300 s	Pre- and post-emergence	Prickly Paddy melon	100% destruction of tested specie at 0.65 kWh m <sup>-2</sup>	[41]
2.45 GHz	700 W	120, 240, 320 and 720 s	Pre-emergence treatment of soil	Ryegrass and Wild Radish	100% seed mortality was achieved at 240 s of MW irradiation	[17]
2.45 GHz	750 W	5, 15, 30 and 60 s	Pre- and post-emergence		100% debilitation of plants	[42]
2.45 GHz	2 kW	5, 10, 15, 30, 60 s	Post-emergence		100% mortality	[18]

While disinfestation of soil has been a key focus of microwave research, other disinfestation and disinfestation studies have been undertaken. Table 2 lists some of the key findings from several examples.

**Table 2.** Table of investigations into thermal control of pests and pathogens using microwave heating.

Author	Title	Main Finding	Reference
Almaiman et al.	Effects of microwave heat treatment on fungal growth, functional properties, total phenolic content, and antioxidant activity of sorghum ( <i>Sorghum bicolor</i> L.) grain	Microwave heating significantly reduced fungal incidence in the sorghum grain. No significant changes were found in the crude protein and digestibility of protein, water holding capacity, and oil holding capacity of sorghum; however, application of higher microwave caused a sharp reduction in the protein solubility, foaming capacity, emulsion capacity, and the emulsion stability. Conversely, a significant increase in total phenolic content and antioxidant activity was observed after microwave heat treatment. Microwave soil treatment significantly reduced fungi and bacteria that could be cultured from the soil. At the highest energy level (15 kJ kg <sup>-1</sup> soil) both fungi and bacteria were eliminated.	[43]
Mahdi et al.	Effect of Microwave Radiation on Bacteria, Fungi and Some Growth Characteristics of Cowpea <i>Vigna unguiculata</i> L.	Cowpea above ground biomass and root mass significantly increased (103% increase above the control) for moderate doses of microwave energy (10 kJ kg <sup>-1</sup> soil), but almost returned to the same level as the untreated control for very high doses of microwave energy (15 kJ kg <sup>-1</sup> soil). Pulse beetle ( <i>Callosobruchus maculatus</i> ) in blackgram ( <i>Vigna mungo</i> (L.) Hepper) kernels, were subjected to radio frequency and microwave heating. The pupa stage of the insect's life cycle was found to be more resilient to heat treatment than eggs, larvae, and adults.	[44]
Tiwari et al.	Dielectric heating-assisted disinfestation of black gram and its effect on protein profile: A comparative study on radio frequency and microwave heating	There were also measurable changes in the amino acid profile of the blackgram. Microwave irradiation was investigated as a controlled soil biocidal treatment which could selectively kill microbial biomass. Under the experimental conditions chosen, irradiation of the soil sample for 90 s gave a kill of microbial biomass equal to that achieved by CHCl <sub>3</sub> fumigation. Extractable mineral N was increased after incubation of irradiated soil, and after 90 s irradiation was only slightly lower than that of fumigated soil.	[45]
Speir et al.	Effects of microwave radiation on the microbial biomass, phosphatase activity and levels of extractable N and P in a low fertility soil under pasture	Microwaves may be an alternate to chemical methods of killing insects in grain as their application do not leave any undesirable residues and thus might be very effective for controlling insect infestation compared to other available methods. Microwave disinfestation can provide a continuous process to allow large quantities of products to pass in a shorter period. Microwave disinfestation is considered safe and competitive alternative method to fumigation as it avoids environmental pollution.	[46]
Yadav et al.	Microwave technology for disinfestation of cereals and pulses: An overview		[47]

Most microwave treatments are focused on the temperature range below 100 °C. This is partly because of the very high latent heat of vaporization for water; however, some studies have shown that microwave heating can achieve much higher temperatures. For example, Falciglia and Vagliasindi [48] achieved temperatures of up to 260 °C, when 180 MJ kg<sup>-1</sup> of microwave heat was applied to soil at 12% moisture content (dry weight basis). These temperatures are high enough to kill most pathogens, including anthrax spores. Microwave

assisted pyrolysis at temperatures above 700 °C have been achieved [49,50]. At these temperatures, organic molecules are decomposed to produce syngas, bio-oil, and biochar. These products can be regarded as sterile.

### 3.3. Microwave Treatment of Animal Fodder

Weed seeds, pests (e.g., fire ants), and pathogens are often transported in hay [51]. Fumigation can manage these problems; however, like with soil disinfestation, it is possible for heat to overcome these problems as well [52]. In accordance with the temperature–time response of all living things, the mortality of insects, seeds, and pathogens increases with temperature and exposure time [53].

Dong, et al. [54] discovered that the organic matter degradability of wheat straw in the rumen of yaks was increased by around 20% after 4 min of treatment in a 750 W, 2.54 GHz, microwave oven. Sadeghi and Shawrang [55] showed that microwave treatment of canola meal increased in vitro dry matter disappearance, including substances that were deemed undegradable in the rumen. Sadeghi and Shawrang [56] also showed that microwave treatment reduced the rumen degradable starch fraction of corn grain and decreased crude protein degradation of the soya bean meal compared with untreated samples. Small scale in vitro pepsin-cellulase digestion experiments [57] demonstrated that microwave treatment: increased dry matter percentage with increasing microwave treatment time; increased in vitro dry matter disappearance with increasing microwave treatment time; but had no significant effect on post-digestion crude protein content. Live animal trials, using microwave-treated lucerne hay showed an 8.1% higher increase in body weight, compared with animals fed with untreated hay [57].

### 3.4. Microwave Treatment of Timber for Pest Control

Non-chemical treatments for infested timber have been reported for some time [58]. The efficacy of excessive heat or cold, electrocution and microwave energy have all been investigated. Heating of the timber using high temperature kilns resulted in 90 to 96% mortality of Western Drywood Termites (*Isoptera: Kalotermitidae*), three days after treatment [58]. It was interesting to note that mortality rose to 98% after 4 weeks [58].

When exposed to radiofrequencies/microwaves the first reaction of insects is an attempt to escape; this is followed by loss of motor coordination, stiffening, immobility and, after a certain time interval, death [59]. Lewis [60] demonstrated that Western Drywood Termites could be controlled by microwave heating, with 100% mortality being achieved at a microwave dose density of 65 J cm<sup>-2</sup>. Massa, Panariello, Pinchera, Schettino, Caprio, Griffo and Migliore [59] investigated the efficacy of microwave treatment of standing live palm trees for the control of Red Palm Weevil (*Rhynchophorus ferrugineus Olivier*). Their objective was to thermally control the weevil without killing the host plant. Differences in susceptibility of the insect were found between development stages within the species. Their results showed that the adult insects are much more sensitive to heat than the larger larvae with 20 min at 50 °C and only 4 min at 80 °C causing adult death. Lethal time for the larvae varies with weight and the most resistant were those weighting between 4 and 6 g, requiring 30 min at 50 °C [59]. Based on their successful experimental work, a semi-commercial prototype has been developed to treat palm trees, in situ, using a ring applicator to apply the microwave energy to the tree.

### 3.5. Scale-Up of Microwave Heating Systems

Scale-up of microwave heating systems has been explored for over 40 years [61]. Many efficient industrial microwave heating plants have been established in several industries, including: the food industry, the rubber processing industry, the timber industry, several waste management industries, and for general product drying [61]; however, scale-up of most microwave technologies still requires much more investigation and refinement. Most research is completed at laboratory scale, where the field densities in small samples are

high. Achieving similar field densities when processing large volumes of material can be challenging.

The two important parameters that limit field density inside the material are: the penetration depth of the microwave fields into the processed material and available power. Penetration depth depends on the dielectric properties of the material and the operating frequency of the microwave system [62–65]. Penetration depth is directly dependent on microwave frequency, so the first step in the scale-up process usually implies the use of lower industrial, scientific, and medical (ISM) frequencies. For example, many microwave heating experiments are performed at 2450 MHz, which is the frequency used in domestic microwave ovens; however, industrial-scale systems usually operate at 915 MHz (or 868 MHz or 922 MHz, depending on how this ISM band is defined in different parts of the world), which is the next lower ISM frequency [66]. Adopting the lower frequency increases the penetration depth of the microwave fields. The trade-off of increasing penetration depth is that the volume of material which is absorbing microwave energy significantly increases and therefore the resulting field density in the larger volume is significantly reduced. The field density can be increased by increasing the available microwave power.

Microwave generators, varying from a few hundred watts up to 100 kW can be used individually [3] or in multiple generator designs [67,68] for microwave heating applications. Although they only seem to have been used for research and military applications, single relativistic cavity magnetrons have been shown to produce in excess of 4 GW [69]. Multiple phase-locked sources output of 3 GW total output; repetitive, near-GW peak powers at pulse repetition rates up to 1 kHz, and long-pulse operation with energy per pulse of about 1 kJ have also been achieved [69].

Installations vary in design, depending on the commodity being processed. Continuous processing can be achieved using tunnel applicators [3]; however, careful electromagnetic design of the in-feed and out-feed ports is needed to prevent radiation leakage into the environment. Microwave cavities [3,70,71] can better prevent microwave field leakage; however, they must be used for batch processing. Therefore, the major challenges of microwave heating scale-up are material handling and achieving similar field densities inside the commodity as was achieved during laboratory-scale experiments.

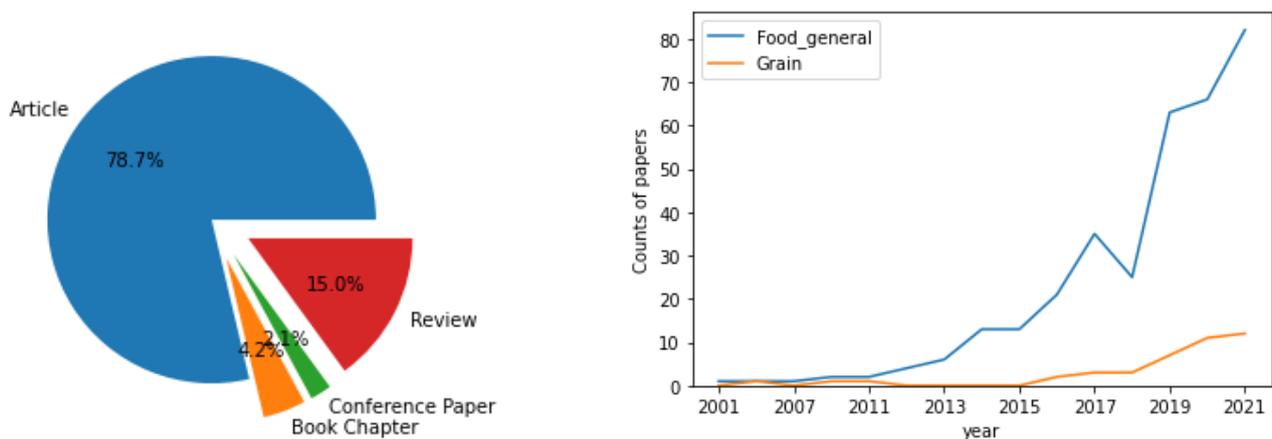
#### **4. Grain Treatment Using Non-Thermal Plasma Technology for Pest and Pathogen Control**

Pre-storage thermal treatment of grains and fresh produce is a non-chemical solution for reducing post-harvest decay. These treatments decrease the reliance on fungicides and protect the environment. However, most thermal treatments affect the quality attributes of the commodity before the completion of disinfestation. Therefore, non-thermal methods of processing are gaining more popularity for pasteurization and sterilization in the food and agricultural industries. Plasma is the fourth state of matter. The excited and ionized atoms react strongly with other materials and release UV photons as the excited electrons decay to their ground states and electrons recombine with ions [72]. Exposing grains to intense plasma would damage the commodity; however, exposure to the plasma afterglow or “cold plasma” provides a good surface sterilization [73]. Moreau, Moisan, Tabrizian, Barbeau, Pelletier, Ricard and Yahia [73] demonstrate *Bacillus subtilis* spores can be killed by oxygen atoms and ultraviolet photons from the cold plasma.

Cold plasma is an emerging technology that appears to be effective against many microorganisms and pests. The attractiveness of plasma in the food industry is its short treatment time and non-thermal conditions, as well as leaving no residues in the food. In non-thermal plasma, the temperature of the electrons, which are responsible for the reactions, reaches 10,000–100,000 K (1–10 eV) while the gas and ion temperatures remain at room temperature [74]. Plasma treatment can not only control pests and pathogens but also has great potential to enhance seed germination rate [75], water uptake, and cooking quality [76]. Therefore, cold plasma can act as one tool to manage all the contaminations

present in grains and eliminate the need for applying different chemicals for pest and pathogen controls.

An overview of publications on cold plasma treatment of different kinds of foods with the aim of pest/pathogen/fungi/bacteria control is represented in Figure 1. The publications are taken out from the Scopus database with Elsevier API (application programming interface). The pie chart is showing that more than 78% of the publications were research papers and just 2.1% of these research works were presented at conferences. The line graph represents the increasing number of publications that accelerated dramatically after 2018. The orange line shows the number of publications related to grains and it is around 10% of the publications from which 10% are review papers.



**Figure 1.** An overview of the published documents on cold plasma on food-related topics, pie chart: proportion of different types of publications; line graph: number of publications for 20 years (blue line: total and orange line: related to grain).

Based on these records of publications and the importance of cold plasma in the grain industry, it seems necessary to obtain an overview of the research on grain treatment using cold plasma, with the special aim of pest and pathogen control, and presenting the gaps in the research work in this area.

#### 4.1. Grain Treatment with Cold Plasma

##### 4.1.1. Disinfestation from Microorganisms and Pathogens

There are many studies on the inhibitory effects of cold plasma on food-related microorganisms and there are recent reviews on the microbial and fungal decontamination of grains using non-thermal plasma [77,78]. For example, Park, et al. [79] used a microwave-driven cold plasma (power of 1 kW) through a quartz tube with argon gas at 100 L/min and atmospheric pressure to control methicillin-resistant *Staphylococcus aureus* and *Escherichia coli*. They showed that cold plasma ruptured the cell wall of the bacteria and reduced their size by releasing the cell contents. Avramidis, et al. [80] also reported that air plasma treatment of mycelia of *Fusarium culmorum* and *Ascochyta pinodella* for 180 and 360 s caused some cracks on their walls and the cytoplasm leaked out, which finally flattened and killed the mycelium. They concluded that when the vegetative cells are not accumulated on each other, their growth could be inhibited within a very short time (60 secs). They utilized compressed air as the plasma gas and in a dielectric barrier discharge (DBD) system, with a power of 560 W and a peak voltage of 20 kV.

Having studied the effect of cold plasma on the natural flora of chickpea, Mitra, et al. [81] indicated that apart from exposure time; size, shape and surface area of the seeds could have a significant impact on the reduction of surface microorganisms by plasma treatment and suggested that the efficiency of the treatment could increase by shaking the samples. They showed that microbial inactivation by this method was better described by the Weibull model with no shoulder and tail than a first-order linear model.

In terms of different microorganisms, it is generally concluded that the elimination of fungi is much more difficult than bacteria. In one type of plasma source and gas such as corona discharge, it takes 2–4 min to inactivate bacteria while the time needed for fungi could increase to 20–30 min [82]. In a study by Zahoranová, et al. [83] naturally occurring bacteria in maize were eliminated after 60 s, while a treatment time of 180 s was required to remove all the naturally occurring fungi. This could stem from a more complex structure of filamentous fungi compared to bacteria, and as it was stated earlier, it was more challenging to reduce fungal pathogens when their vegetative cells were accumulated on each other.

Fungal pathogens could have very different susceptibilities to the treatments depending on their position in or on the seeds and whether they are present in the form of spores, mycelium, or any other form in their lifecycles. This makes it more difficult to draw a firm conclusion about the efficiency of cold plasma for the treatment of naturally infected grains. Fungi can also be more resistant to UV radiation than bacteria due to a layer of melanin present in their cell wall [84]. Therefore, it seems necessary to evaluate the susceptibility of different life stages of a fungus to cold plasma treatment and inoculate the grain with the most resistant life stage of the target fungus.

A summary of plasma treatment of grain for the control of microorganisms is represented in Table 3. Overall, cold plasma has proven to be effective in the disinfestation of important grains from a wide range of bacteria and fungi. However, decontaminations are mostly investigated on surface microorganisms and there is a lack of information on the plasma efficiency on removing the fungal pathogens of grains and seeds which penetrate inside the seeds.

#### 4.1.2. Disinfestation from Pests

Unlike the numerous research completed on the inactivation of microorganisms of grains, there are very few studies on insect control using cold plasma. In a recent study, helium plasma was more effective than argon plasma in the mortality of *Tribolium castaneum* while using radio frequencies (RF) as the source of creating cold plasma [85]. However, the final temperatures of the beetles were not reported as the temperature of helium plasma is higher than argon plasma. The more efficient eradication of the beetles could be the result of higher temperature. Both [85] and [86] applied RF vacuum plasma for insect control; however, neither study considered vacuum alone or inert gases alone (without applying RF) as controls in the work to evaluate if the mortalities are caused by plasma or vacuum/gases.

A summary of insect control using cold plasma is represented in Table 4. In general, this area of cold plasma seems to be very new, and a lot of research is still required to explore the efficacy of cold plasma for insect control and its effect on different life stages of insects. Furthermore, there is a need for exploring different application technologies for this purpose.

#### 4.1.3. Inactivation Mechanism

The microorganism inactivation, which depends on the type of gas and relative humidity, is also related to UV radiation and high energy ions and radicals such as atomic oxygen (O), hydroxyl radical (OH•), ozone (O<sub>3</sub>), hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>) and peroxynitrite [87]. Other reactive species such as NO<sub>x</sub>, excited atoms, and molecules depend on the processing gas. Generally, reactive oxygen species (ROS) and reactive nitrogen species (RNS) have significant roles in the inactivation of microorganisms. Reactive species interact with microbial cells and change their structural and biochemical characteristics, which mostly involves the oxidation of lipid and protein of cells. However, the mechanism could slightly differ among different microorganisms or different plasma apparatus and process parameters.

The responsible phenomenon in a microwave-driven plasma, with argon as the discharge gas, is reported as mostly UV radiation. However, when the humid gas or combination of argon and oxygen is used in the process, some radicals are also responsible for the

disinfestation [88]. Working at low pressure could make UV radiation a dominant reason for the disinfestation [89], while at atmospheric pressure, a large amount of UV is absorbed by the gas and thus UV may not have a significant role in the decontamination [78]. The mechanisms of damage to microorganisms have been reported to be rupturing of their cell walls by charged particles and oxidation by the reactive species or UV [88]. Therefore, the mechanism of inactivation of the microorganisms can be decided by correctly selecting the plasma gas and operating pressure.

Regarding insect mortality with cold plasma treatment, an increase in catalase, glutathione S-transferase, and lipid peroxidase activities in the larvae of *Tribolium castaneum* and *Trogoderma granarium* was observed after treatment with DBD at 15.9 W causing 50% mortality among them. The treatments additionally caused a reduction of glutathione and protein content in the treated larvae [86]. Other reported modes of action involved in insect mortality after plasma treatment include DNA disruption, glycolysis inhibition/metabolic arrest, surface etching, cell disruption [90], and lipid peroxidation of the cellular membrane [91].

Ziuzina, et al. [92] reported a reduction in respiration rate in red beetles (*Tribolium castaneum*) after 5 min exposure to direct DBD plasma. This was accompanied by an increase in the activity of GST (glutathione S-transferase) and the level of lipid peroxidation of insects, but no change in SOD (superoxide dismutase) and CAT (catalase) activities occurred.

Overall, there is plenty of data to confirm the mechanism of action against microorganisms while there is still a lack of information about the interaction of cold plasma with different life stages of insects and the effect on their host after a complete disinfestation.

#### 4.1.4. Effect of Process Parameters

The inactivation of microorganisms depends on many parameters, such as plasma source, type of exposure to cold plasma, input power and voltage, type and speed of the gas, the water content of gas, specimen, the specimens' surface characteristics, microbiological species, and concentration, exposure time, and food shape [93]. Regarding the position of the food, there are three types of exposure to plasma: far from the generation point, which is indirect treatment or exposure to late afterglow; close to the generation point or exposure to early afterglow; and inside the plasma field [89]. The remote or indirect treatment is more flexible, but the reactive species are fewer (secondary chemicals) as the life span of most reactive species finishes before reaching the sample. The second category, which is direct treatment, has a higher concentration of active components; however, this type of exposure can have an adverse effect on the host quality. In the final group, samples are placed between two electrodes and exposed to the highest amount of active and antimicrobial species.

Having compared the direct and indirect exposures, Fridman, et al. [94] showed that direct plasma could be a better surface sterilizer than indirect plasma in a DBD (dielectric barrier discharge), which could be the result of exposure to more effective species. However, Hertwig, et al. [95] indicated that remote microwave-driven plasma treatment was more effective than a plasma jet for the disinfestation of black peppercorns. This difference could be the result of different apparatus of plasma with different process parameters, which makes it difficult to decide whether direct or indirect exposure is more effective.

Lu, et al. [96] compared the effect of different process parameters such as gas mixture, voltage, and type of exposure on the decontamination efficiency of *E. coli* and *Listeria monocytogenes* and observed that indirect exposure in a DBD plasma with a gas containing CO<sub>2</sub> could be more effective than the direct treatment of the microorganisms. They concluded that this effect depends on the type of gas mixture. In their study, they hypothesized that the possible recombination of the species, such as ozone with longer shelf life, could occur and some other reactive species could form, which have more bactericidal impacts. They also mentioned that the gas containing more oxygen and less nitrogen was more efficient in bacterial eradication, due to the production of more effective species such as OH•, O<sup>3</sup>, and O. An interesting finding of their research was that at a very short exposure time, there

was leakage from bacterial cells that was the result of membrane injury, but it had no effect on their DNA, which could lead to their recovery.

Concerning input power and voltage, the effect of process parameters in a fluidized bed cold plasma was investigated by Dasan, et al. [97]. They reported that the eradication efficiency of spores of *Aspergillus flavus* and *Aspergillus parasiticus* on hazelnuts increased by increasing the voltage and frequency and by decreasing the diameter of the reactor. They also concluded that dry air was more efficient than nitrogen as the discharge gas. Zhou, et al. [98] used a dielectric discharge barrier to enhance tomato seed germination and concluded that the effect of plasma at different voltages is different on tomato yield. They indicated that there is an optimum voltage at which the maximum yield could be obtained and the Gaussian distribution curve could be used to describe the relation between voltage and fruit yield. Lu, Patil, Keener, Cullen and Bourke [96] also indicated that the efficiency of eradication of *E. coli* and *L. monocytogenes* was higher at a higher applied voltage in a DBD plasma, both for direct and indirect exposure of the samples. This fact stemmed from more energy and therefore the production of more antimicrobial reactive species. Butscher, et al. [99] investigated the effect of increasing power and percentage of oxygen in argon gas in a fluidized bed plasma for decontamination of wheat seeds. They observed that by increasing power, the plasma would be intensified, and the plasma zone was longer in the tube. However, by considering effective exposure time, increasing power did not have any beneficial effect. They also concluded that increasing the percentage of oxygen in the gas increased the efficiency of spore inactivation. Comparing different gasses in a diffuse coplanar surface barrier discharge (DCSBD) was shown that using oxygen as the working gas was more effective than nitrogen or air in reducing fungi in hazelnuts [100]. It was proven that plasma created from oxygen contained mostly ozone, while air-generated plasma consisted of reactive species made of N and O, and nitrogen-created plasma was mostly UV-A.

Concerning the host surface, Ziuzina, et al. [101] reported that indirect cold plasma inactivation of bacteria from complex surfaces such as strawberries was much more difficult than from the surface of cherry tomato. Having measured the lower concentration of ozone in strawberry packages, they suggested that more pores on the surface could cause ozone to be decomposed, which was the reason for the less effectiveness of the plasma.

Relative humidity (RH) of the plasma gas is another important factor affecting the antimicrobial efficiency of the plasma. The importance of this factor was studied on the inactivation of bacterial spores [102]. Air with more RH was more effective at eliminating the spores and presenting more humidity in the air led to producing more effective particles such as  $\text{OH}^-$  and  $\text{H}_2\text{O}_2$  that sped up the disinfestation process. Additionally, high RH could cause spores swelling which caused more damage to them.

Generally, the effect of all the process parameters should be considered while considering a plasma process for grain treatment. It is beneficial to find the optimized parameters for the best outcomes in the case of cost and efficiency of disinfestation as well as host quality attributes. Plasma dosage definition is one topic that has been recently argued [103]. This is because different plasma sources and process parameters have been applied by researchers which makes it difficult to replicate the research. Therefore, standardization of the plasma dose is now a necessity, which needs to be considered by researchers who use different apparatuses for creating plasma.

#### 4.1.5. Effect of Plasma Treatment on the Host Quality

Considering the host's quality, plasma proved to increase the wettability and decrease the hydrophobicity of the biological materials by etching the surface [104] or by oxidizing and reducing the lipid content on the surface [83]. This effect has been reported to be one of the reasons for increasing seed germination and their seedling growth, which itself is an attractive application of cold plasma but is not in the scope of this review.

Both enzyme inactivation (such as lysozyme and peroxidase) and enzyme activity enhancement (e.g., amylase, protease, and phytase) have been reported in different food

matrices [105]. Therefore, the reduction of antinutritional compounds was one of the investigated topics of cold plasma treatment. It was proven to effectively reduce soybean trypsin inhibitor in soybean milk [106], to enhance phytase and protease activity, and reduce phytic acid and trypsin inhibitor after 12 and 24 h of germination in mung beans [107], and to reduce soybean agglutinin in soybean milk [108]. However, there is not sufficient information on whether plasma treatment of the grain with the aim of pest and pathogen control affects any of the antinutrients.

Amino acids and the secondary structure of protein could be very susceptible to cold plasma; however, this susceptibility opened an opportunity for plant protein modification, which has recently gained attention. It has proven to enhance pea protein gelation at reduced temperature [109] and improve plant protein solubility, hydrophilicity, emulsifying, and foaming properties [110], which are particularly important for grain/legume protein isolation with food applications. Cold plasma proved to increase carbonyl content and decrease free sulfhydryl groups via interaction with reactive species and free radicals. Protein thermal stability could reduce as a result of cold plasma treatment at a lower voltage of up to 20 kV (a reduction in denaturation temperature) and then increase at a higher voltage of 40 and 50 kV due to further aggregation and cross-linking of protein molecules [111].

However, all the effects of cold plasma are dependent on the plasma dosage (time, power, voltage, etc.). Therefore, evaluation of the effect of the optimized parameters of cold plasma on protein secondary structure of grains storage protein and their amino acids should be considered more in-depth depending on the final application of the seeds.

A change in color and other sensory properties after plasma treatment of the seeds is one of the concerns that could occur depending on the type of food, plasma source, treatment time, voltage, power, the distance of food from electrodes, gas type, and gas flow. For example, increasing gas flow in a cold plasma jet reduced  $L^*$ ,  $a^*$ , and  $b^*$  and it was attributed to a larger density of ozone [112]. Lentil seed color shifted toward redness and yellowness with a reduction in lightness as a result of prolonged exposure (30 min and 24 h) to plasma processed air [113]. While color might not be of importance for seeds considered for sowing, this could affect consumer acceptance in those commodities in the market. The mechanism of color change should also be examined in more in detail.

**Table 3.** Treatment of grains using cold plasma with the aim of disinfestation.

Material	Gas	Pressure	Temperature	Source	Pathogens	Treatment Time	Quality Change	Efficiency of Decontamination	Reference
Wheat, bean, chickpea, soybean, barley, oat, rye, lentil, corn	Air or SF6	Vacuum	Room		<i>Aspergillus</i> spp. and <i>Penicillium</i> spp.	5–20 min	No change in germination and nutritive components (like gluten)	3-log reduction after 15 min	[114]
-	Air (3 m/s)	Atmospheric	<45 °C	DBD high-voltage pulse generator (13 kHz)- $p = 560$ W	<i>Ascochyta pinodella</i> , <i>Fusarium culmorum</i>	60, 180, 360 s	-	100% inhibition after 360 s; heating at 55 °C for 720 s did not remove the fungi	[80]
Blue lupine, catgut, honey clover, and soy				5.28 MHz-RF-0.6 W/cm <sup>3</sup>	<i>Fusarium</i> , black spot, Stemphiliosis, Anthracnose	10–15 min	10–20% increase in germination- more than 15 min decrease in G	3–15% decrease in <i>Fusarium</i> , black spot, Stemphiliosis- no effect on Anthracnose	[115]
Wheat, spring barley, soy, field pea	Air	40 pa (vacuum)	Max. 310 °C	RF-5.28 MHz-0.1–0.7 W/cm <sup>3</sup>	Fungi	2, 5, 7, 10, 15, 20 min	An increase of 4–7% in seed germination	6–14% reduction	[116]
Rice and lemon	Air	Atmospheric		Five discharge electrodes and a UV lamp, 7–10 kV	Mold and <i>E. coli</i>	20 min for lemon and 90 min for rice seeds	No significant damage to the host	Complete eradication of the mold, combination of plasma and UV was more effective	[117]
Rice seeds	Air	Atmospheric	<45 °C	DBD- 3 W, 30 kV	Seed-borne <i>Gibberella fujikuroi</i>	120 s	No effect on the seedling emergence and height A strong improvement in the seed germination (89.2%), speed of germination	More than 92%	[118]
Chickpea	Air		Max. 5 °C above ambient T	An electrode based on the surface micro-discharge (SMD)	Natural flora	2–5 min	(7.1 ± 0.1 seeds/day), and seed vigor, after 3 min (optimum 1 min)	1–2 log reduction	[81]

Table 3. Cont.

Material	Gas	Pressure	Temperature	Source	Pathogens	Treatment Time	Quality Change	Efficiency of Decontamination	Reference
Barley and corn	Air	15 Pa		Glow discharge, 100–200 W	Background fungi	20 min	No change of germination and seedling length, an increase in corn fresh weight	25% reduction in the number of fungi in barley seeds and 40% reduction in corn seeds	[119]
Wheat	Argon (15 nL/min) with oxygen (5–10%)	5 mbar	Thermal- max. surface T: 90 °C	RF-13.56 MHz-700–900 W	<i>Bacillus amyloliquefaciens</i> endospores	30 s	No effect on flour and baking quality	2 log reduction-spore inactivation is more with more O <sub>2</sub>	[99]
Wheat	Air	Atmospheric	Low temperature	Cylindrical electrodes, 8 kV	Natural fungi	3–30 s	No significant effect on seed germination and vigor	Significant reduction of all the present fungi after 10 s	[120]
Barley and wheat	1: air-100 SCCM 2: air 8 bars	1: low pressure-100 pa 2: atmospheric		1: pulsed MW 2: gliding arc discharge-50 Hz	Mycotoxins, namely deoxynivalenol (DON), deoxynivalenol-3-glucoside (D3G), and trichothecene mycotoxins (T-2). Endospores of <i>Geobacillus stearothermophilus</i> (as a model)	1–5 min	1: An increase in germination 2: A reduction in germination no nutritive change	1 more effective than 2	[121]
Wheat	Pulsed argon (2.8 nL/min)	Atmospheric		Pulse frequency (5–15 kHz) and pulse voltage (6–10 kV) 5–10 kV;	<i>Aspergillus flavus</i> and <i>Aspergillus parasiticus</i> spores	60 min	No change in gluten	3 log reduction (1 log after 10 min)	[122]
Maize	Air and N <sub>2</sub> (3000 L/h)	Atmospheric	Non-thermal	18–25 kHz (at a maximum power of 655 W)-nozzle with 4 mm		1–5 min	-	5.48 and 5.20 log reduction	[123]
Rice	Air/ argon (2.5 L/min)	Atmospheric	<30 °C	Hybrid cold-discharge plasma with 10 × 10 twin-tip electrodes, 14 kV	Natural fungi	1 min	An enhancement of seed wettability and germination	No fungi growth on the treated seedlings after 14 d while the untreated seedlings were invaded by fungi	[124]
Wheat	Air	Atmospheric	Cold	100 W/cm <sup>3</sup> (400 W)	<i>Fusarium nivale</i> , <i>F. culmorum</i> , <i>Trichothecium roseum</i> , <i>Aspergillus flavus</i> , <i>A. clavatus</i>	10–80 s for seeds and 30–300 s for microbes	20–50 s: an increase in germination rate, dry weight, and vigor of seedlings	Fusarium spp. most sensitive; <i>Fusarium nivale</i> > <i>F. culmorum</i> > <i>Trichothecium roseum</i> > <i>Aspergillus flavus</i> > <i>A. clavatus</i>	[125]
Rice	Humid L/min water)	Atmospheric		Surface discharge electrode, 20 kV, a 10 cm distance between the seeds and the end of the quartz tube	<i>Fusarium fujikuroi</i> , <i>Burkholderia plantarii</i>	10 min (2 min interval shaking)	No adverse effect on the seed germination and seedling growth	A reduction of 18.1% (fungal blight) and 38.6% (bacterial blight) in the disease severity	[126]
Rice culture	Air-130 sL/min-330 K	Atmospheric	Max. 60 °C	4 W/cm <sup>2</sup> , 38 kV	Mycotoxins produced by <i>Fusarium verticillioides</i> , <i>Fusarium avenaceum</i> , <i>Aspergillus nidulans</i> , and <i>Fusarium graminearum</i> (FB1, ErnB, ST, and ZEN) Native microflora, pathogenic bacteria, and fungi: <i>E. coli</i> , <i>Bacillus</i> and <i>Lactobacillus</i> , <i>B. atrophaeus</i> endospores	60 s	-	Removed completely when pure, 2 log reduction after 30 s	[127]
Wheat and barley model media	Air	Atmospheric		DBD, 80 kV	<i>E. coli</i> , <i>Bacillus</i> and <i>Lactobacillus</i> , <i>B. atrophaeus</i> endospores	30 min	-	Maximum of 4.4 log reduction, more reduction on the hydrophobic surface than hydrophilic	[128]
Wheat and barley	Air	Atmospheric		DBD, 80 kV	Native microflora, pathogenic bacteria	5 and 20 min with 2 or 24 h retention time	No change in germination after 5 min, but a reduction after 20 min	Resistance to plasma treatment: <i>E. coli</i> > <i>P. verrucosum</i> (spores) > <i>B. atrophaeus</i> (vegetative cells) > <i>B. atrophaeus</i> (endospores)	[129]
Soybean	O <sub>2</sub> /N <sub>2</sub> (6 nL/min)	Atmospheric		Quasi-stationary DBD, 25 kV, 65 or 85 W	Seed-borne <i>Diaporthe/Phomopsis</i> complex	1, 2, 3 min	29% reduction in catalase activity, 30% increase in glutathione content	49–81% reduction in the number of infected seed	[130]
Pine seeds		Atmospheric		Diffuse coplanar surface barrier discharge (DCSBD)	<i>Fusarium circinatum</i>	5–300 s	Dramatic reduction of germination at 60 s or more	50% reduction after 10 s treatment	
Maize and barley	Ar/N <sub>2</sub> -O <sub>2</sub> (500 and 1000 SCCM)	2–8 mbar	<40 °C	The afterglow of microwave discharge, 25 W	<i>Fusarium graminearum</i> and <i>Fusarium verticillioides</i>	3 min (barley), 4 min (maize)	An improvement in the germination of contaminated seeds	With a mixture of gases, infected seeds reduced to below 10 or 40% and seed germination increased to 80% Complete elimination of natural bacteria and <i>F. culmorum</i> after 60 s and natural filamentous fungi after 180 s; <i>A. flavus</i> reduced to 10% and <i>A. alternata</i> reduced to 30% (from 100%) after 120 s	[131]
Maize	Air	Atmospheric		DCSBD, 20 kV, 400 W	Natural microbiota and <i>A. flavus</i> , <i>A. alternata</i> , <i>F. culmorum</i>	30–300 s	A decrease in water contact angle, removal of the lipid from the seed surface, no effect on seed germination up to 120 s		[83]
Barley	Air	Atmospheric		DBD, 0–34 kV, 3500 Hz, 300 W, in an RH controlled chamber, electrode gap 5 mm, distance from barley 2 mm,	<i>Deoxynivalenol (DON)</i>	6 and 10 min	No significant effect on germination, protein, beta-glucan, and moisture content	48.9% and 54.4% reduction in DON	[132]

Table 3. Cont.

Material	Gas	Pressure	Temperature	Source	Pathogens	Treatment Time	Quality Change	Efficiency of Decontamination	Reference
Wheat	Air	Atmospheric		DBD, 80 kV, 50 Hz, diameter 15 mm, electrode distance 50 mm, sample holder height 20 mm, 2 g samples, direct and indirect exposure	Natural bacterial and fungal pathogens isolated from wheat	0–20 min and 24 h of retention time		20 min significantly reduced all pathogens, <i>B. atrophaeus</i> vegetative cells the highest reduction, <i>B. atrophaeus</i> spores the most resistant	[133]
Brown rice	Air	Atmospheric		Corona discharge plasma jet (CDPJ), 20 kV,	Aerobic bacteria, yeasts and molds	10 min	No effect on germination, increased weight and length of the seedlings, improved phenolic content of seedling, no effect on sensory properties of seedlings	>1.5 log reduction in all contaminants	[134]
canola grain, canola meal, and barley grains	Air	Atmospheric		DBD, 0–30 kV, 70% duty cycle, 10 $\mu$ s pulse width, and 0–1 A current, the gap between sample and high voltage electrode 2 mm	Zearalenone (ZEA)	3 min		91.6, 83.2, and 64.8% reduction for canola grain, canola meal, and barley grains, respectively	[135]

Table 4. Plasma treatment of grains for insect pest control.

Material	Gas	Pressure	Reactor/Plasma Generator	Pest	Treatment Time	Grain Quality Change	Efficiency of Decontamination	Reference
	Air	Atmospheric	Plasma jet, 500 W, samples in plasma maintaining chamber,	<i>Aphis gossypii</i> , <i>Bemisia tabaci</i> , <i>Helicoverpa armigera</i> , <i>Tetranychus kanzawai</i> , and <i>Thrips palmi</i>	3–20 min		LT50 of 6.3 and 9.6 min for <i>B. tabaci</i> and <i>T. palmi</i>	[136]
Rice	Air (70% RH), 10 L/h	Atmospheric	DBD, 200 V, 7.5–20 W, indirect exposure in fumigation chamber	<i>Tribolium castaneum</i>	8, 16, 24 h	No change in cooking properties, texture, hydration, pasting profile, color, and moisture content	100% mortality after 24 h compared to 86.67% mortality with 200 ppm Phosphine	[137]
Wheat (in package)		Atmospheric	DBD, 44–47 kV,	<i>Rhizopertha dominica</i>	4–7 min	Enhancement in milling yield, protein, and fiber content, reduced carbohydrate, increased lightness	88.33% mortality at 47 kV-7 min treatment, after 24 h	[138]
	Argon, helium	150 Kpa	RF (13.56 MHz), 100 W	Red flour beetle <i>Tribolium castaneum</i>	0–90 s		He more efficient than Ar, adult more tolerant than larvae and pupae, 100% mortality with 90 s and He	[85]
Wheat	Air	Atmospheric	DBD, 15.9 W	<i>Tribolium castaneum</i> and <i>Trogoderma granarium</i> (adults and larvae)		Enhancement of seed germination,	Adult more tolerant than larvae,	[86]
Chickpea		0.5 mbar	RF (13.56 MHz), 40, 50 and 60 W	<i>Callosobruchus chinensis</i>	10, 15, 20 min		100% mortality of all life stages over 2 years of storage	[139]
	Air	Atmospheric	DBD, 80 kV, 50 mm electrodes distance, direct and indirect exposure	<i>Tribolium castaneum</i> (egg, young and old larvae, pupae)	0.5–5 min		100% mortality after 24 h with 5 min direct exposure, adults most resistant stage, indirect exposure up to 20 min was less effective	[92]

Conclusively, plasma treatment could be used for modification of protein properties and inactivation of antinutritional factors. However, it should be considered how these modifications and alterations could affect those seeds with the final application of sowing.

#### 4.2. Plasma Apparatus for Seed Treatment

Non-thermal or cold plasma can be created by different sources from high voltage to high frequencies. They include DBD (dielectric barrier discharge), corona discharge, atmospheric pressure plasma jet (APPJ), and microwave discharge. High voltage sources have recently gained more attention in food and agricultural research, probably due to the lower plasma temperature and the feasibility of creating the plasma in atmospheric air, which reduces the costs to a large extent. The different types of plasma apparatuses used for disinfestation and disinfestation of grains are represented in Tables 3 and 4.

In a microwave plasma discharge, commonly working at 2.45 GHz, the plasma applicator is an important part. It defines the efficiency of plasma generation as well as the minimum and maximum powers that can be absorbed by the working gas. It also defines the size and uniformity of the plasma. There are different types of microwave plasma generators with different applications having been reviewed by Lebedev [140], Lebedev [141]. The attractiveness of a microwave plasma is that it can be operated without using any

electrodes and because of its ability to generate very high-density plasma (high density of reactive species). However, a magnetron as a microwave generator has the drawback of a short working life or instability in working frequency. This problem can be overcome by replacing magnetron generators with a solid-state source, which has more flexibility and stability at lower powers.

#### 4.3. Indirect Treatment of the Seeds Using Plasma

##### 4.3.1. Plasma Processed Air (PPA)

Plasma activated water (PAW) and plasma processed air (PPA) have been recently explored as potential tools for seed and fresh produce treatment. The idea of PPA is producing reactive species of ROS and RNS using microwave as the plasma generators and air as the plasma gas. This process was described by Schnabel, et al. [142] for fresh produce decontamination. PPA is also known as remote or indirect plasma processing. It was explained by Schnabel, et al. [143] as an indirect method of treatment for microbial decontamination of *Brassica napus* seeds, which was shown to be more efficient than dielectric barrier discharge (DBD) treatment. They used a microwave source and ignited the plasma for 7 s with a power of 1.2 kW with 20 L/min air as working gas following by different holding times in the bottle in which PPA was captured. Considerations should be toward the efficiency of PPA as some of the reactive species are short-lived or they recombine when they hit a surface. Delivering tubes might need to be very short and made of a special material to increase the efficiency and prevent loss of reactive species.

##### 4.3.2. Plasma Activated Water (PAW)

PAW can be created by treating water with non-thermal plasma. The treated water contains ROS and RNS (and other reactive species depending on the processing gas) and has been proven to have great potential for the disinfection of food and agricultural commodities [144]. Processing of water, using cold plasma, could reduce the pH of the water due to the formation of reactive species from nitrogen and oxygen, and a part of the antimicrobial effect could stem from its acidic condition [145]. However, a high level of nitrite and nitrate also plays a crucial role in the disinfection of fresh produce [146]. PAW was shown to have great potential for the disinfection of fresh produce such as strawberries [147] and baby spinach leaves [148]. It reduced *E. coli* of mung bean seeds by 6 log CFU/mL after 6 h of treatment without any change in the seed germination [149].

In addition to the disinfecting role, PAW has the potential to enhance seed germination and plant growth. Zhang, et al. [150] demonstrated that PAW enhanced lentil crop growth when it was utilized for their irrigation. They additionally showed that activated tap water contains much more nitrate than the activated demineralized water, while they contain the same amount of hydrogen peroxide. Deionized water activated by N<sub>2</sub> (10 L/min)-O<sub>2</sub> (0.4 L/min) microwave plasma contained hydrogen peroxide, nitrite, and nitrate ions as well as NO [151]. However, NO was confirmed to react continuously with the dissolved oxygen content of the water, which reduced its concentration over time.

The initial pH value of the processed water (liquid) can affect the type and quantity of the produced reactive species. Antibiotic norfloxacin degraded more and with lower exposure time to microwave plasma when the pH of the wastewater was 4 compared to the initial pH of 2, 7, and 11 [152].

Therefore, these two new technologies could be compared with thermal or non-thermal (plasma) treatments of grain seeds, with the aim of seed-borne pathogen control. The comparisons still need to be carried out regarding the efficiency of pathogen control, any quality changes in the host, and the fixed and operating costs. The other consideration would be how to scale up using indirect treatments in the grain industry as this involves very large-scale processing equipment.

#### 4.4. Industrialization and Scale-Up

Some of the scale-up attempts were reviewed by Cullen, et al. [153]. They include applying multiple plasma sources to have a uniform and higher density plasma, micro-plasma arrays, surface, and coplanar DBD for continuous or in-package treatment of fresh produce.

Large-scale grain treatment with cold plasma has not been explored widely and most of the research has been focused on lab-scale experiments. One of the attempts for a pilot-scale trial was carried out by Lee, et al. [154] for decontamination of rice germ in combination with pulsed UVC light. Their system had an output voltage and frequency of 1 kV and 30 Hz for producing plasma via four nozzles with the plasma jet dimensions of 5 mm × 18 mm capable of treating 226 kg/h of the product. However, the maximum microbial decontamination was achieved when treating 200 g of the sample (rice germ) for 7 min and the reduction rate was reduced by increasing the sample amount.

Scale-up of cold plasma processing of grains seems to remain a challenge. More work should still be focused on having a uniform plasma for the continuous treatment of grains with direct exposure to cold plasma. Indirect exposure to the plasma-processed air could be an alternative; however, the efficiency of the treatment should be considered as both the short- and long-lived species have significant roles in the disinfection/disinfestation of grains.

### 5. Brief Discussion

Microwave heating is fast, efficient, and finely controllable. Microwave heating is volumetric, negating the reliance on the thermal properties of the treated material to transfer heat from the surface to the core of the material; however, microwave heating is reliant on the dielectric properties of the material.

Plasma, the fourth state of matter, consists of highly excited molecules and free electrons, which can directly react with many organic compounds. The recombination of electrons and ionic compounds or the decay of atoms from excited states to their ground states also releases high-energy photons, often in the UV frequency ranges.

Microwave heating and microwave-generated plasma have both been demonstrated to kill pathogens and pests in other commodities. Many of the studies have been completed at the experimental scale, with some systems being scaled to the pilot scale. Because of the importance of biosecurity, understanding the specific application of microwave heating or plasma for quarantine purposes needs to be properly demonstrated.

Microwave-generated plasma has shown great potential for crop growth enhancement and seed sanitation. However, there is still a lack of data about its effect on many fungal diseases of grain crops as well as a deep investigation of the host quality. PAW and PPA are also two new technologies that have been introduced recently to be considered in fresh produce and grains with a very good potential for sanitization and growth enhancement. These technologies are still in the phase of research and development and by providing enough information on all the aspects of the concerns, such as sensorial effects or long-term effects on the pathogens, they could enter the phase of industrialization.

There have been different conclusions in review papers about future works on the plasma treatment of grains. Based on the literature, the focus of future works could be listed as below:

- Optimizing process parameters and developing a pilot-scale system for rice and grain treatment [155];
- Regulatory review, in-depth understanding of the effect of cold plasma on food functionality, combination with other conventional methods and development of prototypes [105];
- Shelf-life study based on quality parameters, analytical flavor analysis, and sensory analysis [112];
- Combination with other novel technologies such as nanotechnology for food surface decontamination [156];

- Development of large-scale systems and addressing the biofilm issue in the electrodes that could significantly affect laboratory results [77];
- Field studies with plasma treated seeds, unifying optimization of cold plasma parameters to be able to compare them for a specific grain and their pathogens [78];
- Optimization of the process parameters and appropriate equipment design with affordable prices for commercial-scale processing [157];
- Combination with conventional disinfestation methods [90], investigation of the effect on different life stages of insects and pathogens and their impact on treatment efficiency [158];
- Introducing plasma units or plasma dose standardization, mechanisms of action for microbial decontamination, and plasma “vaccination” of crops by activating their immune system [159];
- Scale-up of both microwave heating and microwave-generated plasma for continuous treatment must also be properly understood. This requires ongoing research and development.

## 6. Conclusions and Future Work

Microwave technologies have the potential for the disinfestation of various commodities. Microwave heating is a mature technology and has been applied to commodity processing, particularly in the food industry, for some time. Repurposing these technologies for quarantine purposes should be achievable; however, properly understood, and ongoing development of industrial-scale systems must be achieved.

Plasma, especially microwave-generated plasma, is regarded as a novel technology for commodity processing. The non-thermal treatment of grains with microwave-driven cold plasma is an emerging technology that should be explored further.

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