

Harnessing Cold Plasma: An Innovative Strategy for Managing Postharvest Fungal Infections in Plants

ABSTRACT

Researchers in plant pathology in recent times; have faced obstacles in finding chemical-free methods to combat postharvest fungal infections on a large scale. While conventional approaches like heat treatments have been utilized, they often present drawbacks such as altering food quality or causing harm to the environment. An encouraging alternative is a cold plasma, which consists of a blend of gas-derived atoms, excited molecules, and charged particles. Unlike alternative treatments, cold plasma has demonstrated no adverse effects on fresh produce or the environment. This review delves into the potential of cold plasma technology in managing postharvest fungal diseases, offering insights into plasma generation systems and examining both in vivo and in vitro studies. By evaluating the benefits, constraints, and current research gaps, this review seeks to guide for implementation of cold plasma technology in commercial settings.

Keywords: Postharvest diseases, fungal pathogens, Cold plasma technology, Chemical-free control, Commercial scale

1. INTRODUCTION

In 1879, Sir William Crookes identified 'radiant matter' as the predominant form of substance [1]. Later, in 1932, Irving Langmuir introduced the term 'plasma' during a presentation at the International Electrical Congress in Paris [2]. Plasma is characterized as an almost neutral gas that has undergone ionization [3, 4]. It is commonly acknowledged as the fourth phase of matter, succeeding solids, liquids, and gases [5, 6, 7, 8]. Plasma existing at temperatures significantly higher than the surrounding environment, reaching hundreds or even thousands of degrees, is termed thermal plasma. Alternatively, plasma sustained at temperatures spanning from 25 to 450 °C is referred to as non-thermal [3, 9, 10]. Researchers have employed various terms such as atmospheric cold plasma, low-pressure cold plasma, atmospheric pressure plasma, and non-

thermal atmospheric plasma to characterize non-thermal plasma. Nonetheless, in this chapter, it will be designated as cold plasma (CP).

Cold plasma is generated by applying an electric current to a gas or mix of gases, typically including argon, helium, nitrogen, oxygen, or air [8, 11]. It consists of a mix of atoms, energized molecules, charged particles, and reactive oxygen and nitrogen species (RONS) such as superoxide (O_2^-), ozone (O_3), hydroxyl (OH), hydroperoxyl (HO_2), nitric oxide (NO), nitrogen dioxide (NO_2), dinitrogen pentoxide (N_2O_5), and ultraviolet (UV) photons [7, 12, 13, 14]. The ions produced have an extremely short lifespan, ranging from milliseconds to nanoseconds [15]. The composition of cold plasma creates an inhospitable environment for microorganisms such as bacteria and fungi [7, 16, 17], without leaving persistent residues or harmful by-products that could be detrimental to the environment or the treated product [18]. Known for its chemical-free and residue-free decontamination properties [10, 18, 19], cold plasma has been effectively utilized in agriculture and horticulture.

Numerous investigations have highlighted its effectiveness in eliminating bacteria from a variety of fresh produce, including fruits and vegetables [20, 21], nuts [22, 23], seeds [24], spices like black pepper [25, 26], meat [19, 27], dairy items [28], and eggs [29]. Notable postharvest fungi include *Penicillium* spp., *Aspergillus* spp., *Botrytis cinerea*, *Monilinia* spp., and *Colletotrichum* spp., which demonstrate varied host preferences and contribute significantly to economic losses globally. Moreover, some research suggests that CP could offer a potential strategy for controlling fungal pathogens, particularly those causing postharvest ailments [30, 31, 32]. However, there have been no reported cases of CP being applied commercially to treat either bacterial or fungal infections in fresh produce. Additionally, there is a significant lack of available data regarding the use of CP for managing postharvest pathogens on fresh produce. This review aimed to extensively assess the feasibility of using CP as a chemical-free substitute for controlling postharvest diseases caused by fungal plant pathogens. It provides a thorough analysis of CP production methods and explores the range of systems utilized. The review investigates the particular effectiveness of CP in addressing postharvest fungal pathogens. Lastly, it discusses the possibilities and constraints linked with CP, along with providing suggestions for its commercial adoption.

2. COLD PLASMA OVERVIEW

All methods of generating CP involve the use of a gas feed, pressure adjustments during plasma creation - ranging from low pressure in vacuum environments to atmospheric pressure, and the inclusion of a power source. Regardless of the setup, CP is typically administered at room temperature and does not adversely affect the treated material [33].

2.1. Feed Gas

Common gases utilized include air, modified air, or inert gases like argon. The effectiveness of CP varies significantly depending on the gas utilized. For instance, studies have demonstrated that gases such as sulfur hexafluoride and argon are more effective compared to air in controlling fungi such as *Aspergillus parasiticus*. Air is frequently used as the primary feed gas, either independently or combined with other gases like argon or sulfur hexafluoride, in research on postharvest fungi.

2.2. Pressure

Cold plasma can be generated within vacuum chambers under conditions of low pressure and then administered to the surface needing treatment. Alternatively, it can be created under atmospheric pressure. Low-pressure generation provides benefits such as higher energy output and greater accuracy due to the plasma's purity.

2.3. Power supply

CP power can be sourced through various components like transformers, converters (e.g., AC to DC), inverters, or alternative systems like radio frequency power systems, as detailed by Stryczewska et al. [34]. Additionally, CP generation can be facilitated through batteries or solar energy [35, 36]. Despite the specific power supply system used, the resulting CP remains essentially unchanged; however, adjustments in power or voltage levels might affect its antimicrobial efficacy [37, 38].

3. COLD PLASMA APPLICATION

When discussing the treatment of postharvest fungal pathogens, CP generation systems will be classified as direct, indirect, or hybrid to provide clarity and differentiation. In direct plasma

systems, CP is generated between a dielectric surface containing one electrode and the sample, which acts as the second electrode. This setup allows for direct interaction between the sample and the plasma [7, 39]. Direct systems provide high levels of reactive oxygen and nitrogen species (RONS) to the treated surface, resulting in the rapid inactivation or elimination of microorganisms [10, 40]. Indirect plasma systems are generated using closed-loop circuits and an independent electrode arrangement, where plasma is transported to the sample via methods like blown arcs, flames, or jets. Unlike direct systems, the surface under treatment does not come into direct contact with the plasma but rather with the discharge emitted by it. In this configuration, a considerable portion of the short-lived reactive oxygen and nitrogen species (RONS) may not reach the treated surface, resulting in reduced exposure to the antimicrobial components [40]. A notable limitation of this particular CP setup is the generation of increased heat and ultraviolet (UV) radiation at the point of origin [41]. Research conducted by Herceg et al. [8] and Liu et al. [42] provide examples of indirect plasma methodologies employed to combat postharvest fungal pathogens. Their investigations showcased effective spore eradication of *Aspergillus ochraceus*, *Penicillium expansum*, and *P. digitatum* under laboratory conditions. Hybrid plasma setups merge both direct and indirect plasma production techniques. Typically, plasma is directly created near a grounded wiremesh electrode. This electrode acts as a barrier to prevent direct electrical flow through the sample during treatment, ensuring product safety. These systems offer flexibility in their distance from the treated surface (within specific limits), emitting negligible UV radiation and sustaining low temperatures during operation [41]. In the field of postharvest pathology, Khamsen et al. [43] utilized a hybrid plasma method to treat rice seeds, resulting in the complete elimination of seed-borne fungi.

The key difference among the three plasma application methods lies in the amount of reactive oxygen and nitrogen species (RONS) produced on the treated surfaces, as well as in the release of UV radiation and temperature during generation. Indirect plasma systems, particularly CP jets, are predominantly employed in experiments aimed at controlling postharvest fungi and appear to be the preferred choice for CP generation. This preference could stem from its application versatility, allowing for the treatment of various shapes and sizes of the targeted product [41, 44, 45].

3.1. Cold Plasma for Postharvest Disease Control

To date, there has been limited exploration into the use of CP for controlling postharvest fungal diseases. The majority of published research has concentrated on addressing bacterial contamination in plant products, with only two investigations examining its effectiveness against viruses. Despite the nascent stage of research on CP applications for postharvest fungi, both in vivo and in vitro treatments have displayed encouraging antifungal properties against diverse postharvest fungi. These results indicate that CP shows promise in managing postharvest diseases.

3.1.1. In-vivo studies

The predominant focus of research has been on grains treated with in vivo CP systems, which are better suited for such commodities due to their limited surface area. Both direct and indirect CP methods have demonstrated efficacy in managing postharvest fungi on various nuts such as pistachios, hazelnuts, and peanuts. Particularly notable is a documented reduction of 50% in total aflatoxin production by *A. parasiticus* following CP treatment. Moreover, several studies have reported a significant decline in pathogen presence for diverse fungi like *Aspergillus*, *Penicillium*, *Fusarium*, and *Alternaria* across a variety of crops like rice, wheat, maize, barley, lentil, and chickpea. The majority of these experiments utilized indirect plasma. Regarding perishable commodities, investigations involving citrus fruits, berries, and date palm fruits have indicated effective management of postharvest fungi, including species such as *Aspergillus* and *Penicillium* (Table 1).

Table 1 Summary of plasma studies on post harvest fungi in vivo

Host	Pathogen	Time (min)	Impact	Reference
Pistachio	<i>Aspergillus flavus</i>	18	Decreased counts of colony-forming units	[46]
Hazelnut	<i>Aspergillus flavus</i> , <i>Aspergillus parasiticus</i>	5	Decreased counts of colony-forming units	[47]
Hazelnut,	<i>A. parasiticus</i>	20	50% reduction of aflatoxin	[30]

peanut, and pistachio			production	
Rice	<i>Fusarium fujikuroi</i>	10	Notable decrease in bakanae disease incidence among rice seedlings	[48]
Rice	<i>F. fujikuroi</i>	10	Substantial decrease in pathogen germination on rice seeds.	[49]
Rice	<i>Aspergillus oryzae</i>	20	Spores viable but became unculturable	[31]
Maize	<i>Aspergillus flavus</i> , <i>Aspergillus parasiticus</i>	5	Decreased counts of colony-forming units	[50]
Wheat	<i>Alternaria</i> , <i>Aspergillus</i>	0.5	Decreased counts of colony-forming units	[51]
Wheat, barley, lentil, chickpea	<i>Aspergillus parasiticus</i> , <i>Penicillium</i>	20	99% pathogen population reduction	[52]
Satsuma mandarins	<i>Penicillium italicum</i>	10	84% reduction of disease incidence	[38]
Lemon	<i>Penicillium digitatum</i>	20	Complete inactivation of spores	[31]
Grapes, strawberries	<i>Alternaria alternata</i> , <i>Aspergillus niger</i> , <i>Penicillium italicum</i>	7.5	Decreased counts of colony-forming units and mycotoxins	[53]
Blueberry	Native yeast/mould	2	Significant reduction of mould	[54]
Date palm (fruit)	<i>Aspergillus niger</i>	7.5	Complete inactivation of spores	[32]
Mango	<i>Colletotrichum asianum</i>	3	Activities of cell wall degrading enzymes and laccases were reduced and pathogenic genes were down regulated	[55]

Persian lime	<i>Penicillium digitatum</i>	2	Reduced spores to less than 7 CFU/Fruit	[56]
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3.1.2. In-vitro studies

The efficacy of CP against postharvest fungi has been evaluated in laboratory settings, targeting spores and mycelia from various fungal genera. In these studies, particular attention has been given to inhibiting spore germination to assess CP's fungicidal properties. Significant reductions were observed in both spore germination and mycelial growth after a 1-minute CP treatment, resulting in complete inactivation of *Cladosporium fulvum*. Similar to findings from field trials, indirect plasma appears to be the predominant system employed in these experiments (Table 2).

Table 2 Summary of plasma studies on post harvest fungi in vitro

Pathogen	Time (min)	Impact	References
<i>Penicillium digitatum</i>	9	91% inhibition of spore germination	[42]
<i>Aspergillus ochraceus</i> , <i>Penicillium expansum</i>	5	Decreased counts of colony-forming units	[8]
<i>Fusarium oxysporum</i> f. sp. <i>lycopersici</i>	10	Inhibition of spore germination	[57]
<i>Fusarium fujikuroi</i>	10	Inhibition of spore germination	[49]
<i>Cladosporium fulvum</i>	1	Complete pathogen inactivation	[58]
<i>Neurospora crassa</i>	3	Inhibition of spore germination	[59]
<i>Glomerella cingulata</i>	2	100% inhibition of spore germination	[60]
<i>Monilinia laxa</i>	5	Reduction of mycelial growth	[61]
<i>Neurospora crassa</i> , <i>Fusarium</i> <i>graminearum</i> , <i>F. oxysporum</i> f. sp. <i>lycopersici</i>	3	Reduction of mycelial growth	[62]
<i>Botrytis cinerea</i>	20	Inhibitory effects on spore germination and	[63]

3.2. Mode of action

While the specific antimicrobial mechanism of CP remains unclear, it is hypothesized that CP affects pathogens both directly and indirectly upon application to fresh produce. Indirectly, CP may stimulate a defense response in the host. The production of RONS by CP is thought to contribute to both of these proposed mechanisms of action. The impact of CP on pathogens typically involves causing cellular damage, which may include altering the permeability of the cell membrane or wall, leading to the release of cellular contents. Furthermore, CP has the potential to inflict significant damage to intracellular organelles or proteins and disrupt DNA, potentially due to UV radiation generated during plasma species recombination. Microscopy studies suggest that CP primarily affects fungal pathogens such as *Cladosporium*, *Penicillium*, *Aspergillus*, and *Fusarium* by targeting their cell wall or membrane. For instance, investigations have revealed that CP treatment disrupts the cell wall or outer membrane of *C. fulvum* [58] and *P. expansum* [64], leading to the leakage of cytoplasm or distortion of cell organelles. Ye et al. [64] observed various changes in *P. expansum* following CP treatment, including a reduction in protoplasm volume, enlargement of vacuoles, and distortion of the cell membrane. Furthermore, organelles within the cell protoplasm were severely disturbed and completely disrupted. They also observed invagination of the cell membrane, followed by cell lysis and the release of protoplasmic components. Despite these findings, there is a notable gap in understanding the effects of CP on various postharvest fungi, highlighting the necessity for further research to elucidate how CP impacts both mycelia and spores.

RONS (Reactive Oxygen and Nitrogen Species) are considered the main agents responsible for the antimicrobial effects of plasma treatment [65]. The production of RONS, and consequently the efficiency of CP in deactivating pathogens, is significantly influenced by the composition of the feed gas [66]. For example, according to Na et al. [62], the presence of oxygen radicals increased when argon was mixed with air or oxygen, compared to when it was mixed with nitrogen or used alone. A combination of helium and oxygen as the feed gas produced excited oxygen, hydroxyl, and nitrogen species, as observed by Xiong et al. [67], while the use of air as the feed gas only generated active oxygen and nitrogen species alongside hydrogen peroxide, as

reported by Sohbatzadeh et al. [46] and Zhou et al. [68]. Various studies have investigated the impact of RONS on the genetic material of fungi such as *Cladosporium*, *Fusarium*, and *Neurospora* [49, 58, 69]. During CP treatment, RONS cause damage to the DNA and protein molecules within the cells of postharvest fungi. Using scanning electron microscopy, Pannongom et al. [57] illustrated the apoptosis or necrosis of conidia induced by RONS during CP treatment of *Fusarium oxysporum* f. sp. *lycopersici*, which causes tomato wilt. Additionally, RONS play a significant role in enhancing the ability of host plants to defend against microbial infections [70, 71, 72]. Exposing tomato plants to CP treatment resulted in a significant upregulation of genes related to disease resistance, thereby enhancing their ability to resist infection by *F. oxysporum* f. sp. *lycopersici*. Similarly, treating seeds of wheat, lupin, and maize with CP led to a marked reduction in various seedling diseases caused by *Fusarium* spp. and *Ustilago maydis*. In a study by Los et al. [73], they found that ACP effectively fights off various bacteria and fungi commonly found in wheat. They tested individual strains from grains and observed that untreated grains had more diverse and abundant microbial populations compared to those treated with ACP. When directly exposed to plasma for 20 minutes, all pathogens saw a significant reduction in concentration, with *B. atrophaeus* being more susceptible than fungal species. Interestingly, repeated sub-lethal treatments didn't lead to resistance in either *B. atrophaeus* or *A. flavus*. This suggests that ACP treatments can be customized to tackle different microbiological threats, ensuring grain safety and stability. Researchers suggested that administering CP treatment before sowing could enhance plant resistance during the seedling stage. However, further investigation is required to fully understand the mechanism by which CP induces host resistance, particularly in the postharvest context.

3.3. Benefits of CP

Given its antimicrobial properties, CP holds significant potential for managing postharvest pathogens across various commodities. It provides an alternative to fungicides, physical interventions, and biological control methods for both fresh and fresh-cut perishable products. Moreover, it has the potential to extend the shelf life of treated items. Schluter et al. [74] highlighted several key benefits of CP treatment, including (i) effective pathogen control even at low temperatures; (ii) generation of active agents only during treatment; (iii) minimal impact on the internal structure or edible portion of the product; (iv) resource-efficient as it does not require

water or other solvents; and (v) absence of residues after treatment. These advantages hold particular significance in the context of managing postharvest fungal diseases. Cold plasma offers the potential to prolong the shelf life of postharvest produce during transit and sales by preventing or reducing reinfection without compromising product quality. This could result in reduced postharvest losses and associated expenses, increased market worth, and better returns for producers, distributors, and sellers. Furthermore, consumers might be willing to pay more for fresh produce that is free from pesticides or additives, potentially boosting the market value of treated goods. Hence, as a chemical-free approach, CP has the potential to enhance the commercial value of treated products.

3.4. Limitations

Although Cold Plasma (CP) shows considerable promise, the majority of studies have been conducted in controlled environments using pure cultures on artificial media or inoculated seeds/perishables. To progress CP for managing postharvest fungal pathogens in real-world scenarios, it's imperative to validate the findings from these studies under more practical conditions in food storage and distribution facilities. Additionally, discrepancies in experimental setups, such as the type of plasma, feed gas, treatment duration, and distance, present challenges in comparing research results. There's a need to optimize the use of CP for controlling postharvest fungi on a commercial scale. However, recommending a specific CP treatment regimen for commercial application is complicated due to the diverse range of crops and their fungal diseases. The efficacy of CP in fighting pathogens depends on various factors including the type of plasma generation system, the choice of feed gas, and the specific attributes of both the commodity and the pathogen under treatment. Despite ongoing research, there is currently no standardized CP treatment that effectively controls a wide range of postharvest fungi across different types of produce. As a result, the absence of optimization poses a significant obstacle to its adoption in commercial settings.

4. CONCLUSION

Over the past five years, there has been a growing interest in utilizing cold plasma (CP) treatment to combat postharvest fungal diseases, primarily explored through laboratory investigations. Initial findings indicate CP's potential, with fungal control rates reaching up to

100%, suggesting it could serve as a promising postharvest intervention method. However, despite these encouraging results, the optimization of CP treatment remains an area requiring further exploration. Additionally, existing research has predominantly focused on the microbial inactivation aspect of CP treatment, neglecting its potential impacts on other quality parameters. Therefore, there is a need for more comprehensive studies to fill existing knowledge gaps and determine the feasibility of CP as an effective chemical-free strategy for managing postharvest fungal diseases.

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