Cold plasma treatment of chickpeas: Disinfestation potential, milling and baking quality of the flour

by

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Abstract

Chickpeas (*Cicer arietinum* L.) are an essential legume known for their high protein content, dietary fiber, and micronutrient composition. However, post-harvest losses due to insect infestations, particularly from *Callosobruchus maculatus* (F.), and Cowpea Weevil, pose significant storage and food security challenges. Traditional fumigation techniques rely on chemical treatments, contributing to environmental pollution and raising concerns about pesticide resistance and food safety. This research uses cold plasma technology, an innovative non-thermal pest control method for disinfesting *C. maculatus* different life stages.

This research involved subjecting chickpea samples infested with *C. maculatus* eggs, larvae, pupae, and adults to varying cold plasma exposure conditions (15-25 kV for 5-15 min). The effectiveness of treatment was evaluated based on insect mortality rates and changes in physicochemical properties. Results demonstrated significant mortality rates**,** with 100% larval mortality achieved at 25 kV for 10 min and complete egg eradication at 20 kV for 15 min. Further, cold plasma treatment preserved the physical and nutritional properties of chickpeas, ensuring no adverse effects on bulk density, true density, moisture content, or kernel weight.

Beyond disinfestation, this research also examined the potential of cold plasma-treated chickpea flour in food formulations, particularly in cookie production. Cookies made with treated chickpea flour were assessed for physical properties, texture, and sensory attributes. The findings confirmed that cold plasma treatment maintains the overall quality of the final product. This research showcases cold plasma as a sustainable alternative to chemical fumigation, effectively reducingpost-harvest losses from *C. maculatus*. Its findings advance food safety, grain storage, and non-thermal processing and play a key role in sustainable agriculture and food security.

Table of Contents

List of Figures

List of Tables

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Chapter 1 – Introduction

Chickpea (*Cicer arietinum* L.) is a globally important legume recognized for its high protein content, dietary fiber, and essential micronutrients. Its unique nutritional profile makes chickpea flour an attractive ingredient for functional food products, particularly in developing gluten-free and health-focused baked goods. Additionally, post-harvest losses due to bruchids further limit their shelf life and utilization potential (Bourke et al., 2018; Zhang et al., 2021). Bruchids, commonly known as bean weevils, are significant pests that affect stored legumes, leading to substantial post-harvest losses. These pests, particularly the species *C. maculatus* (F.), cowpea weevil, infest various leguminous crops, including chickpeas, lentils, and beans, resulting in reduced quality and nutritional value of the produce.

Bruchid infestation refers to the attack of stored legumes by bruchid beetles, primarily the species *C. maculatus* , which are commonly known as bean weevils. These pests are notorious for infesting a wide range of leguminous crops, including chickpeas, lentils, and various types of beans(Southgate, 1979). The infestation typically occurs during storage, where adult beetles lay their eggs on the outside of the grain kernel. Once the larvae hatches, they bore into the kernel, feeding on the cotyledons and disrupting the grain structure. Larval feeding not only reduces the nutritional quality of the legumes but also results in significant economic losses due to decreased market value and increased waste. Additionally, bruchid infestations can compromise the safety of stored legumes by promoting mold growth and other microbial contamination(Pitt & Hocking, 2009). Effective management and control strategies are crucial to minimize the impact of bruchid infestations on food security and storage practices.

Traditional thermal and chemical treatments have been used to improve chickpea flour's functional properties and to prevent pest infestations. Still, these methods often degrade their nutritional quality and introduce unwanted sensory changes. In response to these limitations, non-thermal processing technologies, such as cold plasma treatment, have emerged as innovative and sustainable alternatives for enhancing the quality and safety of food materials (Misra et al., 2016; Bayati et al., 2024). Cold plasma technology, which utilizes reactive oxygen and nitrogen species to modify the surface properties of food, has been shown to improve hydration capacity, protein solubility, and microbial safety without compromising the nutritional integrity of grains and flours (Thirumdas et al., 2017; Bourke et al., 2018).

Chickpea flour's growing demand for gluten-free and plant-based diets highlights the need for advanced processing methods to optimize its functional properties for food formulations like cookies. Cookies made with chickpea flour can serve as healthier snack alternatives, offering higher protein and fiber content compared to traditional wheat-based cookies. However, achieving desirable sensory and structural characteristics in these cookies remains a challenge due to the absence of gluten and the chickpea flour's intrinsic properties (Pathan et al., 2022; Shabbir et al., 2024).

Cold plasma treatment presents a promising solution to these challenges by improving the physicochemical and functional properties of flour while also serving as an effective and sustainable approach for controlling bruchid infestations (Hajam et al., 2022; Tiwari et al., 2021; Pathan et al., 2021; Krik Bardleyy et al., 2023). Despite its potential, limited research exists on the specific effects of cold plasma treatment on chickpea flour and its applications in cookie formulations. This study aims to bridge this gap by evaluating the impact of cold plasma on

chickpea flour and its influence on the quality and sensory properties of cookies. The findings will contribute to the development of innovative, sustainable processing techniques that enhance food safety and quality while meeting the demands of health-conscious consumers.

1.1. Problem Statement

Chickpea is a globally significant legume valued for its high nutritional profile and versatility in food applications. However, chickpea production and storage face considerable challenges due to pest infestations, particularly by *C. maculatus* which is one of the most destructive storage pests of legumes. Infestations by *C. maculatus* lead to substantial post-harvest losses, including direct seed consumption, contamination, and reduced seed viability. These losses are further exacerbated by suboptimal storage conditions that promote pest proliferation, such as high humidity and temperature levels (Hollis et al., 2019; Mishra et al., 2020).

The latest forecast for global cereal production in 2024 estimates a total output of approximately 2,841 million MT (Food and Agriculture Organization of the United Nations FAO, 2024). Despite this substantial production, post-harvest losses remain a critical challenge. According to a World Bank study, an estimated 12–16 million MT of food grains are lost annually due to storage pests, significantly impacting global food security and economic stability (Sharon et al., 2014).

The United States, a leading producer of chickpeas, experiences annual post-harvest losses ranging from 20% to 30% due to storage pests, accounting for millions of dollars in economic losses (Dugesar et al., 2021). The infestations result in significant quality deterioration, affecting both domestic consumption and export markets (FAO, 2023; United States Department of Agriculture USDA, 2022). Beyond the economic implications, pest infestations pose food safety concerns, as insect activity often leads to microbial contamination and mycotoxin development

during storage, further compromising the quality of chickpeas (Bourne et al., 2021; Abass et al., 2023).

Traditional pest control methods, including chemical fumigation and synthetic pesticides, have been widely used to mitigate these losses. However, these methods often leave harmful residues, pose health risks to consumers, and contribute to environmental pollution. Additionally, the overuse of chemical treatments has led to pest resistance, rendering these approaches less effective over time (Zettler & Arthur, 2000; Silva et al., 2024).

Considering these challenges, cold plasma technology has emerged as a promising, non-thermal, eco-friendly alternative for addressing pest infestations and ensuring food safety. Cold plasma generates reactive oxygen and nitrogen species capable of inactivating pests and microbes without the need for harmful chemicals. Studies show its effectiveness in various grains, for example, Akasapu et al. (2020) demonstrated improved nutrient retention in rice, Singh et al. (2022) highlighted starch modification in wheat, and Pathan et al. (2021) reported significant pest control in pulses Despite these advancements, limited research focuses on the application of cold plasma for controlling *C. maculatus* infestations in chickpeas, leaving a critical gap in our understanding of its efficacy and scalability.

1.2. Research Objectives

This study seeks to address this gap by evaluating the impact of cold plasma treatment on chickpeas, with a focus on its ability to reduce pest infestations, enhance storage quality, and maintain nutritional integrity. These findings could offer a balanced solution to post-harvest losses and support the broader goal of improving global food security. The objectives are as follows:

1. To evaluate the efficacy of cold plasma treatment in controlling *C. maculatus* infestations in chickpeas across different developmental stages.

2. Formulate cookies using cold plasma-treated chickpea flour at varying proportions and assess their physical properties.

1.3. Research Outline

This thesis is structured into five chapters, each contributing to a comprehensive understanding of the study. Chapter 1: Introduction - Provides an overview of the research problem, the objectives of the study, and the thesis outline. It establishes the relevance of cold plasma treatment in addressing the challenges of chickpea flour utilization. Chapter 2: Nonthermal Techniques for Disinfestation of Bruchids (*C. maculatus* Literature Provides a comprehensive literature review of nonthermal techniques for the disinfestation of bruchids.This chapter identifies knowledge gaps and highlights the novelty of this research. Chapter 3: Discuss the disinfestation efficacy of cold plasma treatment on various life stages of *C.s maculatus* in chickpeas (*Cicer arietinum*). Chapter 4: Quality Characterization of Cookies Made from Cold Plasma-Treated Chickpea (*Cicer arietinum)* Flour is described in Chapter 4. Chapter 5: Lastly, a summary of findings, conclusions, and recommendations for future work based on the two studies presented are reported in Chapter 5.

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Chapter 2 - Nonthermal Techniques for Disinfestation of Bruchids (*C. maculatus*): Literature Review

Abstract

C. maculatus (F.), cowpea weevil, a storage grain pest, is responsible for most of the post-harvest loss. In 2023, US. pulse exports totaled $6,245,368, reflecting a significant decline compared to previous years [USDA FAS]. A significant challenge for pulse crops is posed by storage pests, particularly the bruchid, which leads to economic losses during post-harvest storage. These pests can cause weight loss, reduce germination potential, and impact the overall quality of pulses(Bamaiyi et al., 2006). Bruchids, such as *C. maculatus,* complete most of their life cycle inside the grain and emerge as freshly born adults. Traditionally, fumigation is employed to kill storage pests. However, fumigation techniques result in various health problems like cancer (carcinogens), respiratory issues, and neurological problems, affecting overall human health (Rani et al., 2021; Kaushik, 2021). This triggered the search for alternative non-fumigation methods for the disinfestation of *C. maculatus* . This review investigates the success rates of different nonthermal chemical-free pest management techniques, including gamma irradiation, cold plasma, ozone, and UV-C treatments. The primary goal of this review is to elucidate the potential of non-thermal methods in controlling the infestation in pulses by *C. maculatus,* thereby minimizing the storage loss. Additionally, it focuses on improving pulse storage practices, promoting environmentally friendly solutions, influencing policy and best practices, guiding future research, and educating stakeholders on sustainable pest management approaches.

2.1. Introduction

The interest in plant-based foods is not only précised to vegetarian and vegan consumers; it also extends to non-vegetarians as well (McClements et al., 2021). Pulses play a major role in providing a significant share of protein, minerals, and calories in the diets of people globally. In 2022, the US pulse production reached 1,050,838 MT, with dry peas, lentils, and chickpeas being significant contributors (USDA, 2023). According to the USDA Foreign Agricultural Service (2023), the economic importance of pulses is underscored by the $666 million worth of pulse exports from the US in 2023.

The urgency is for effective pest management strategies to safeguard the quality and quantity of stored pulses. It emphasizes the significant economic losses caused by pest infestations, particularly in regions where pulses are a major source of nutrition and income (Jadhav et al., 2012; Amusa et al., 2013). In the realm of food sources, pulses hold the distinction of being the second most crucial after cereals. Within this category, *Cicer arietinum* (chickpeas), among various legumes, stands out for its superior bioavailability of vitamins, minerals, and protein content. They emerge as a standout contributor, containing a notable 15-25% protein (Kakaei et al., 2024). Chickpeas are also tolerant to natural drought and heat climatic changes.

A fundamental challenge in pulse production arises from post-harvest losses linked to bruchids. These losses, estimated at 30-40% within the initial 6 months of storage, underscore the significant impact of bruchid infestations on pulse crops. If not promptly addressed, the situation has the potential to exacerbate, leading to staggering losses of up to 100% (Govindan et al., 2020). Among bruchids, *C. maculatus* is the most pernicious pest of stored grain pulses. They penetrate the fully grown matured pods and grains in fields and during post-harvest storage (Hajam et al., 2022).

The consequences of infestation by these pests extend beyond mere economic impact, involving weight loss in stored pulses, diminished germination potential, and a decline in overall pulse quality. To reduce the storage pests, different pest management techniques are utilized, like thermal and non-thermal treatments, and using fumigants like methyl bromide, phosphine, methyl formate, etc. Processes such as ionizing radiation, which uses electromagnetic waves to disrupt the DNA and cellular structure of pests, and controlled atmosphere storage, which modifies oxygen and carbon dioxide levels, create unfavorable conditions for insect survival and growth (Hallman, 2011). Despite maintaining sanitary conditions, long-term storage exposes commodities to both biotic and abiotic factors that can cause contamination and damage. Biotic agents such as insects, mites, rodents, birds, and microbes significantly degrade stored grains, resulting in substantial financial losses. Among these, bruchids, particularly from the genus *Callosobruchus,* are considered the most destructive pests of stored pulses, especially in tropical and subtropical regions.

The impact of these pests can be catastrophic, with yield losses often exceeding 50% within a short storage period due to their rapid infestation rates. Initial infestations commonly begin in the field and increase substantially during storage, where seed damage can reach up to 100% within two to three months (Mishra et al., 2018; Ragul et al., 2024; Bhoge et al., 2023). Among the various bruchid species, the cowpea weevil (*C. maculatus*) and the azuki bean weevil (*Callosobruchus chinensis*) are particularly notorious for their destructive potential (Mishra et al., 2018; Ragul et al., 2024). Storage pests lead to substantial yield losses and degrade the market value and quality of economically significant grain legumes. Among these, mung beans (*Vigna radiata*) are particularly affected due to their high nutritional value and widespread consumption, especially in

India and other Asian countries. Recognized as a rich source of dietary protein, mung beans play a crucial role in food security.

However, pest infestations compromise their quality, reducing both nutritional integrity and economic viability (Mishra et al., 2018). Dielectric heating, which uses an electromagnetic field to generate heat within the product, can also effectively kill insects. However, these treatments often alter the physicochemical properties of the grains being treated (Moirangthem et al., 2021). The use of chemicals for fumigation in pulses presents several drawbacks and concerns. It has been associated with adverse effects on seed germination, particularly (Singh et al., 2003). This triggered the exploration of non-thermal disinfestation methods. Paul et al. (2020) reported a status report on disinfestation techniques for major cereals. Mir et al. (2023) explored multiple advanced techniques for managing insect infestations in stored grains. These methods can be categorized into thermal and non-thermal approaches.

Thermal approaches to disinfestation utilize heat to eliminate pests from stored grains. Infrared (IR) heating employs electromagnetic radiation to generate heat and effectively kill insects. Microwave (MW) heating is a rapid heating method that disrupts insect physiology through thermal energy, while radiofrequency (RF) heating penetrates the grain bulk, raising temperatures to eradicate pests. These methods provide effective pest control but may also impact the structural and chemical properties of the grains. Non-thermal approaches, on the other hand, offer alternative solutions that do not rely on significant heating. Cold plasma technology utilizes reactive oxygen and nitrogen species to inactivate pests without altering grain temperature. Gamma radiation employs ionizing radiation to disrupt insect reproduction and development, preventing further infestation. Ultraviolet (UV) radiation targets insect DNA and microbial contaminants, offering an effective pest control strategy without substantial heat exposure.

Additionally, Mohapatra et al. (2015) discussed various strategies for insect pest management in stored pulses, further highlighting the diverse range of available disinfestation techniques.

Overall, pest management in stored grains is a critical challenge that requires effective and safe disinfestation techniques. While thermal approaches such as infrared, microwave, and radiofrequency heating provide efficient pest eradication, they often alter the physicochemical properties of the grains. Non-thermal methods, including cold plasma, gamma radiation, and ultraviolet radiation, offer promising alternatives that mitigate these issues while maintaining grain quality. The shift toward non-thermal techniques is driven by concerns over chemical fumigation and the need for sustainable, residue-free disinfestation methods. By leveraging both thermal and non-thermal technologies, researchers continue to explore innovative strategies that balance effectiveness, grain quality preservation, and economic feasibility in pest control.

In this direction, cold plasma has gained attention as an innovative, non-thermal, eco-friendly alternative to traditional pest control methods. It is an innovative technology due to its ability to inactivate pests without heat while preserving grain quality, making it an eco-friendly solution for future pest control strategies. Annapure et al. (2021) demonstrated the effectiveness of cold plasma in controlling *Callosobruchus chinensis* (L.), adzuki bean weevil, in chickpeas, while Bradley et al. (2022) confirmed the potential of atmospheric cold plasma in combating *C. maculatus* , a common pest in stored chickpeas. Additionally, Pashkov et al. (2023) examined the impact of cold plasma treatment on improving the germination process of chickpeas, suggesting enhanced seed vigor and early plant growth. Li et al. (2023) investigated cold plasma's effects on chickpea protein functionality, revealing improvements in solubility and digestibility, key factors in developing plant-based protein products. Dong et al. (2023) studied the impact of cold plasma

on chickpea starch, demonstrating its potential to improve quality and texture in chickpea-based foods.

Given the limited comprehensive studies on pest control and the quality of chickpea-based products, further research is necessary. Future studies should focus on effectiveness in managing insect infestations, extending shelf life, and improving insect mortality rates, thereby providing a deeper understanding of its potential applications in enhancing food quality and safety. The uniqueness of this review lies in its contribution to the food and grain storage industry, helping stakeholders adopt non-thermal disinfestation techniques for *C. maculatus* management while enhancing shelf life without compromising grain properties.

2.2. Current Challenges in Pest Management

Traditional pest management strategies for bruchids, including chemical treatments, are often deemed economically unsustainable and environmentally hazardous. These approaches can also pose health risks, leading to the exploration of alternative and sustainable methods. Among these, developing host-plant resistance to bruchid infestations has emerged as the most viable long-term solution. However, achieving adequate levels of resistance through conventional breeding has proven challenging due to the genetic complexity of the traits involved. Insights into the molecular mechanisms of resistance could facilitate the identification of resistance genes or quantitative trait loci (QTLs), which can be introgressed into cultivated varieties using marker-assisted backcross breeding techniques (Mishra et al., 2018; Bhoge et al., 2023).

2.3. Life Cycle and Behavior of *C. maculatus*

Understanding the biological and behavioral traits of *C. maculatus* is critical for developing effective management strategies. The pest undergoes complete metamorphosis with four distinct life stages: egg, larva (grub), pupa, and adult. Female weevils lay numerous eggs on legume seeds,

which develop rapidly, enabling populations to grow quickly. Early-stage eggs are small, shiny, oval-shaped, and tightly adhered to the seed surface, making detection difficult. Upon hatching, larvae burrow into the seed's endosperm, progressing through four developmental phases known as instars. Instars 2, 3, and 4 are particularly destructive as larvae consume significant portions of the seed's endosperm, weakening its structure. Larvae pupate within the seed and later emerge as adults, boring small holes through the seed coat, further reducing seed quality and marketability.

The life cycle of *C. maculatus* varies based on environmental conditions and host plant species. Under optimal laboratory conditions at 30°C, the life cycle can be completed within 3-4 weeks when reared on preferred hosts like mung beans (Singh, 2024). However, it may extend to seven weeks when feeding on less favorable legumes (Zahra et al., 2023). Once adults emerge, they mature within 24-36 hours and begin reproduction, laying eggs on new seeds and perpetuating the infestation cycle. This rapid life cycle allows *C. maculatus* to cause extensive internal seed damage, often compromising seeds by the time adults emerge, leading to severe economic losses (Sharma et al., 2007; Hosamani et al., 2018; Omar and Mahmoud, 2020).

A critical aspect of *C. maculatus* biology is the production of the filial (F_1) progeny, which refers to the first generation of offspring produced by an adult population. These F_1 individuals are pivotal in infestation dynamics as they contribute to the rapid escalation of pest populations within a short time frame. Studies have shown that F_1 progeny, under optimal conditions, can emerge in as little as 3-4 weeks, leading to rapid population growth and compounding seed damage (Sharma et al., 2007; Hosamani et al., 2018). F_1 progeny develops entirely within the same seed or batch of seeds where eggs were laid, consuming the endosperm and pupating within the seed, which further weakens the seed structure (Omar & Mahmoud, 2020; Amusa et al., 2013). Proper management strategies aim to disrupt the emergence or reproduction of this F_1 progeny to prevent exponential

growth and mitigate seed damage. Techniques such as cold plasma, gamma irradiation, and controlled storage environments have shown promise in targeting and limiting F_1 progeny development (Anbarasan et al., 2023; Ashraf et al., 2022).

There are many factors that influence the life stages of *C. maculatus* , Temperature is a critical factor, as optimal temperatures ranging from 25–30°C promote faster development and higher reproductive rates (Osman et al., 2015; Verma et al., 2018). Humidity also plays a significant role; high humidity levels can enhance the survival and development of larvae, while low humidity can lead to increased mortality rates (Yan et al., 2016; Umoetok Akpassam et al., 2017). The availability of food, particularly the quality and type of leguminous seeds, directly affects the growth and survival of all life stages. Additionally, the presence of natural enemies, such as predatory insects and parasitic wasps, can impact population dynamics and development. Lastly, genetic variability among populations can result in differences in resilience and adaptability to environmental conditions, influencing their life cycle and reproductive success. Understanding these factors is crucial for developing effective pest management strategies for *C. maculatus* in stored legumes.

2.4. Geographical Distribution and Polymorphism

C. maculatus is a cosmopolitan pest, believed to have originated in Africa before spreading to tropical and subtropical regions globally (Kebe et al., 2017). The species exhibits polymorphism with two distinct adult morphs: a flightless morph and a dispersal morph, each exhibiting unique morphological, physiological, and behavioral traits, which have significant implications for pest management. For example, the flightless morph is primarily sedentary and focuses on reproduction within stored grains, while the dispersal morph is adapted for movement, facilitating the pest's rapid colonization of new storage areas. Understanding these variations is crucial for designing

effective and targeted management strategies. By addressing the unique behaviors and habitat preferences of each morph, integrated pest management approaches can minimize infestation risks and mitigate the global spread of this pest. Consequently, incorporating knowledge of these morphs into pest control measures will enhance efficacy.

2.5. Economic and Nutritional Impact

The damage caused by *C. maculatus* extends beyond storage losses, resulting in wasted resources such as labor, time, land, and funds invested in the cultivation process. Infestations lead to post-harvest losses estimated at 15-30% of stored grain weight for legumes, including mung beans, chickpeas, and lentils (FAO, 2016). This translates to substantial financial losses, with studies estimating an annual loss of $2.5 billion in chickpeas alone in the Indian subcontinent due to pest damage, contamination, and decreased market value (Sutar et al., 2021).

From a nutritional perspective, *C. maculatus* larvae feed on the endosperm of grains, causing a reduction of up to 25% in protein content and up to 20% in crude fat (Saxena & Saxena, 2011). The pests' feeding behavior also increases ash content due to contamination with pest residues and feces, further reducing the grain's overall nutritional quality and safety for consumption. Furthermore, affected grains exhibit poor germination rates, with germination potential declining by as much as 40-50% in severely infested batches (Adedire et al., 2011).

In crops like mung beans, which serve as a vital source of dietary protein in India and other Asian countries, these impacts are particularly devastating. A single infestation cycle of *C. maculatus* can lead to a 20-50% reduction in grain quantity within three to six months of storage, depending on environmental conditions (Shaheen et al., 2020). This directly affects food security and income for small-scale farmers, exacerbating the economic burden on agricultural stakeholders.

2.6. Life Cycle Resistance and Vulnerability

The larval stage of *C. maculatus*, particularly when larvae are inside the seed, is the most resistant phase due to the protection offered by the seed coat. This internal protection makes it challenging for insecticides or environmental conditions to penetrate and affect the pest during storage (Singh et al., 2021; Hamdi et al., 2017). In contrast, the adult stage is the most vulnerable, especially immediately after emergence, as adults are more exposed and can be effectively targeted by insecticides or other control measures before reproduction occurs (Adedire et al., 2011).

Given its rapid life cycle and destructive feeding behavior, controlling *C. maculatus* is essential to prevent significant economic losses. Effective pest management strategies, such as fumigation, insecticides, or airtight storage, are critical in mitigating damage (Sutar et al., 2021). However, *C. maculatus* exhibits resistance to certain control measures, particularly during its larval stages, making early detection and timely intervention vital to minimize infestations (Shaheen et al., 2020).

The growing resistance of pests like *C. maculatus* to chemical insecticides, coupled with increasing concerns about food safety and environmental impact, underscores the urgent need for more eco-friendly and non-invasive pest control methods (El-Aziz et al., 2014; Mahmood et al., 2016). Traditional thermal and chemical methods, while widely used, can lead to quality degradation in food products, chemical residues, and environmental harm (Singh & Jackai, 1995).

2.7. Emergence of Non-thermal Techniques

Non-thermal techniques, as described in **Figure 1** have emerged as innovative solutions for pest management in stored grains, addressing the shortcomings of traditional methods. Unlike conventional approaches, non-thermal methods aim to minimize adverse effects on grain quality while ensuring effective pest control (Beyrer et al., 2020; Jaddu et al., 2024). These techniques,

ranging from ozone treatment to cold plasma, offer eco-friendly alternatives by leveraging physical and chemical properties without exposing grains to extreme temperatures or harmful residues (Saxena & Saxena, 2011).

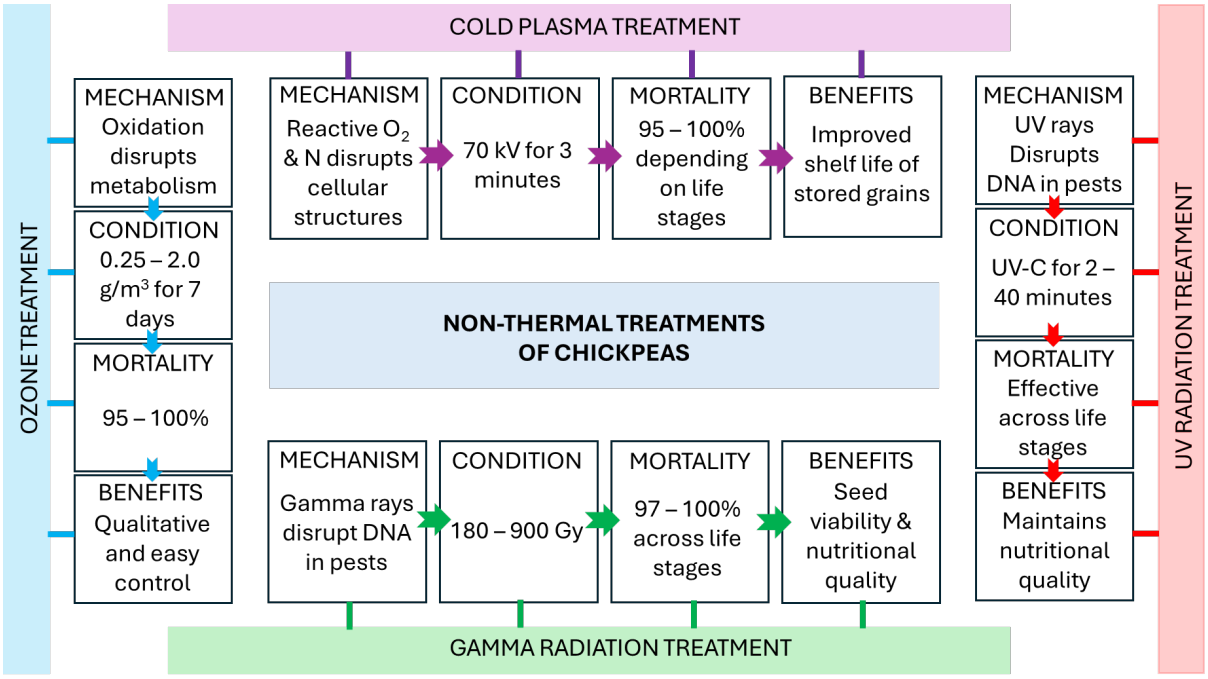

Figure 1: Comparative description of non-thermal treatments for pest control and quality maintenance in stored grains depicting cold plasma treatment, UV radiation treatment, gamma radiation treatment, and ozone treatment involving mechanism, effective condition, pests mortality and benefits.

This review explores the principles, applications, and challenges of various non-thermal methods for the effective disinfestation of *C. maculatus*. Among these methods, cold plasma has shown promise for reducing pest populations without affecting nutritional content, while ozone treatment and UV irradiation have proven effective for surface sterilization but require optimization for broader applications (Grand View Research, 2020). Gamma irradiation remains a powerful method for pest sterilization but faces challenges in public acceptance and regulatory compliance (FAO, 2016). The effectiveness of these non-thermal methods in pest management highlights the need for continued research to address specific challenges associated with each

technique. By tailoring these approaches to target particular life stages of *C. maculatus*, more effective pest control measures can be developed, ultimately enhancing the quality and safety of stored grains.

2.7.1. Ozone-induced disinfestation of *C. maculatus* in grains

Ozone is an allotrope of oxygen produced when atomic oxygen reacts with oxygen molecules (Qasim et al., 2022). It is a strong oxidizing agent and has various applications in the food and grain industry. Ozone has been used in gaseous and aqueous forms to disinfest various food items (Pandiselvam et al., 2019). It acts as a disinfectant by disrupting insect metabolic functions and cellular structure. Ozone is formed when ultraviolet light (UV) reacts with oxygen (O_2) molecules, leading to the formation of ozone (O_3) molecules.

2.7.1.1. Mechanism of action

2.7.1.1.1. Oxidation of Cell Components (Lipid Peroxidation)

Ozone oxidizes lipids and proteins in the cell membrane, increasing permeability and leading to cell lysis. This oxidation disrupts the structural integrity of microbial cells and insect tissues, ultimately leading to death (Rangel et al., 2022).

2.7.1.1.2. Disruption of Enzymes and Proteins

Ozone interacts with amino acids, enzymes, and structural proteins in microbial cells and insect tissues. This leads to the inactivation of key metabolic enzymes, affecting respiration, energy metabolism, and reproductive processes, thereby halting microbial growth and insect development (Kaur et al., 2023).

2.7.1.1.3. DNA and RNA damage

Ozone generates reactive oxygen species (ROS), including hydroxyl radicals (OH•) and singlet oxygen ($^1O_2$), which interact with nucleic acids. These ROS induce DNA fragmentation, mutations, and inhibition of replication, preventing microbial growth and reducing insect reproduction (Tuteja et al., 2001).

2.7.1.1.4. Dehydration and Water Removal

Ozone treatment oxidizes organic matter, leading to moisture loss in microbial cells and insects. This dehydration process is crucial because microbial and insect survival depends on water availability. The moisture loss weakens cell membranes, leading to cell collapse and microbial inactivation (Filippini et al., 2024). Consequently, this method effectively reduces pest populations and enhances the overall safety of stored grains.

2.7.1.1.5. Interference with Insect Respiratory Systems

For stored grain pests, ozone enters through the spiracles (respiratory openings) and oxidizes internal tissues, effectively blocking oxygen uptake. This results in asphyxiation and insect mortality, making it a promising pest control method for stored grains (Michael-Kordatou et al., 2018).

Treatment with ozone benefited microbial safety and improved shelf life. In addition, ozone also had extended applications in storage pest management (Sivaranjani et al., 2020). For instance, Hassan et al. (2021) discussed the effectiveness of ozone in suppressing *C. maculatus* and *Callosobruchus chinensis* in cowpea seeds. Ozone application ranging from 0.25-2.0 g/m^3 resulted in 100% mortality of *C. chinensis* within 7 days after application and immediate death for *C. maculatus.*

A decrease in F_1 progeny is also observed at high ozone exposure of 2.0 g/m^3. Ingegno et.al (2022) studied the effectiveness of ozone exposure in insect mortality in spices. Within the domain of 14400-144000 ppm × min, a 95% mortality rate was reported. Gad et al. (2021) worked on the efficacy of ozone for *C. maculatus* and *Callosobruchus chinensis* on stored cowpea seeds and its impact on seed quality. They reported that a complete adult mortality of *C. maculatus* was possible within 1-2 g/m^3 exposure to ozone. Despite this, significant suppression in progeny production occurred after a severe ozone exposure (2.0 g/m^3). On top of that, ozone treatments did not show any significant changes in cowpea germination. However, a slight decrease in protein, fat, carbohydrates, moisture, and tannins content was observed in ozone-treated cowpea seeds compared to untreated ones (Gad et al., 2021). Interestingly, ozone toxicity was higher at the bottom of the chamber compared to the top. Increasing ozone doses led to decreased water content in the grains, while germination rates remained above 90% (Gad et al., 2021)

Overall, ozone was found to be toxic to *C. maculatus* without affecting grain quality, suggesting its potential use for pest management in stored cowpea grains (Ramos et al. 2023). Nickhil et al. (2021) focused on developing ozone disinfestation systems for treating chickpea grains during storage. They achieved 100% insect mortality and 79% germination after an ozone exposure of 1000 ppm over 5 consecutive days. Pilot-scale trials confirmed these findings and demonstrated improvements in chickpea quality, including protein content, germination ability, surface morphology, milling, and cooking properties, compared to untreated samples. Pandiselvan et al. (2019) also proved 90% mortality of *C. maculatus* adults when exposed for 274.40 min at 500 ppm ozone. Abreu et al. (2022) also proved that 84.40 min of exposure at 3500 (μg L^{-1}) was required to kill 95% of the insect population. Importantly, the pulse quality remained unchanged even after ozone exposure.

On a similar note, Kulwinder et al. (2023) recommended exposure to 1000 ppm ozone for 2 h to attain 104.0%, 90.1%, 86.8%, and 83.5% mortality for eggs, larvae, pupae, and adults of *C. maculatus,* respectively. Simultaneously, a slight decrease in protein, fat, and mineral content in ozone-treated ozone samples was observed compared to the control pulse sample. Based on the results discussed, ozone treatment demonstrated significant potential for effectively disinfesting *C. maculatus* while maintaining pulse quality and preserving germination ability.

2.7.2. Cold plasma-induced disinfestation of *C. maculatus* in grains

Cold plasma (CP) is a non-thermal, innovative technology recently introduced to the food processing industry with great potential as an eco-friendly pest control method. Cold plasma consists of a partially ionized gas made up of electrons, ions, neutral particles, and reactive species (Scholtz et al., 2015). These reactive species, including reactive oxygen and nitrogen species (ROS and RNS), are produced under atmospheric or low-pressure conditions, providing cold plasma with unique antimicrobial and insecticidal properties (Misra et al., 2016).

2.7.2.1. Mechanism of action

2.7.2.1.1. Generation of Reactive Species

Cold plasma is created by applying high-voltage electrical discharges to gas (such as air, oxygen, nitrogen, or argon), producing a partially ionized state of matter. This process generates highly reactive species, including Reactive Oxygen Species (ROS): O_2^- (superoxide anion), OH• (hydroxyl radical), H_2O_2 (hydrogen peroxide), and O_3 (ozone) (Thirumdas et al., 2021). Reactive Nitrogen Species (RNS): NO• (nitric oxide), NO_2• (nitrogen dioxide), and peroxynitrite ($ONOO^-$) (Anbarasan et al., 2023).

2.7.2.1.2. Disruption of Insect Physiology

Cold plasma affects stored-grain pests, including *C. maculatus* , through several mechanisms:

A. Oxidative Stress and Cellular Damage

Oxidative stress plays a critical role in cellular damage and insect mortality during the disinfestation processes. Reactive oxygen species (ROS) and reactive nitrogen species (RNS) actively attack the insect cuticle, causing oxidative degradation of essential biomolecules such as proteins and lipids. This degradation compromises the structural integrity of the cuticle, leading to dehydration and ultimately resulting in insect death (Misnal et al., 2022). In addition to cuticle damage, ROS also disrupts mitochondrial function within insect cells, impairing the energy production pathways necessary for survival. By interfering with mitochondrial activity, ROS leads to severe energy depletion, further weakening the insect's physiological functions and accelerating mortality (Ziuzina et al., 2021). This dual mechanism of oxidative stress, affecting both the external protective layers and internal cellular processes, highlights its effectiveness as a key mode of action in non-thermal disinfestation techniques.

B. DNA and RNA Damage

Plasma-generated UV radiation and reactive oxygen species (ROS) play a significant role in inducing DNA and RNA damage in insects, contributing to effective pest control. These agents cause DNA strand breaks and mutations, which disrupt normal replication processes and impair genetic integrity (Ramanan et al., 2018). As a result, insects experience severe reproductive limitations, with defective egg development and reduced viability. This genetic damage ultimately leads to a decline in the F_1 progeny, significantly lowering pest population growth over successive

generations (Zilli et al., 2022). By targeting the genetic material of pests, plasma-based treatments provide a sustainable and nonchemical approach to long-term insect population management. Additionally, this method minimizes the risk of resistance development compared to conventional chemical pesticides, making it a promising alternative for integrated pest management strategies.

C. Disruption of the Nervous System

Plasma exposure affects the insect nervous system by altering ion channel function in neurons, which interferes with normal neurotransmission. Ion channels are essential for transmitting electrical signals between nerve cells, and any disruption can impair neural communication (Mir et al., 2023). As a result, insects may experience paralysis and a loss of coordination, impacting their ability to move or respond to external stimuli. This neurological disruption can ultimately lead to mortality, making plasma treatment an effective method for insect control (Kirk-Bradley et al., 2023).

Cold plasma effectively eliminates microorganisms and pests on food products while preserving quality. The reactive species generated, such as free radicals and nitrogen atoms, create an oxidative environment that disrupts biological surfaces (Niemira, 2012). Its primary mechanism of action involves damaging the insect's external cuticle, which serves as a protective barrier against environmental stress. Cold plasma breaks down the molecular structure of the insect cuticle (Zhang et al., 2019), induces oxidative stress within insect cells, and damages proteins, lipids, and nucleic acids, leading to insect mortality (Jiang et al., 2017).

Cold plasma has gained attention for its ability to effectively eliminate contaminants in food products (Birania et al., 2022). This nonthermal method does not affect the quality of the product, flavor, and nutrient contents. It is also used for increasing the shelf life of different food

materials (Mir et al., 2016, Khaneghah, 2021). Among these decontamination methods, cold plasma is an emerging technique that has shown efficacy in disinfecting food grains at low pressures (Anbarasan et al.,2023). Plasma generation can be done by a variety of equipment. The basic method followed is arc discharge, where the voltage difference between two electrodes is generated (Becker et al., 2000)

Krik-Bradley et al. (2023) demonstrated the efficiency of atmospheric cold plasma (ACP) treatment for disinfesting *C. maculatus* in cowpea grains (*Vigna unguiculata*). Their study showed that ACP treatment could achieve over 90% mortality for the egg and larval stages of *C. maculatus* with just 3 min of exposure at 70 kV. For complete adult mortality, a 3-minute exposure followed by a 5-day post-treatment retention was required. Mir et al. (2023) highlighted new approaches for controlling insect infestations in stored grains, with cold plasma treatments applied to chickpea seeds showing high resistance against *Callosobruchus* species over four years. Additionally, cold plasma treatment effectively disinfected *C. maculatus* in soybean seeds, achieving the highest mortality at 2.0 kV after 24 min of exposure. The effectiveness varied based on insect life stages and sex, with cold plasma treatment also improving soymilk extraction yields without compromising product quality (Anbarasan et al., 2023).

Cold plasma successfully eliminates insect infestations in various grains without affecting their quality, flavor, or nutrient content while also enhancing their shelf life. As an innovative non-thermal technology, cold plasma holds great promise for adoption in the food industry, with high success rates in disinfestation. Its ability to generate reactive species effectively targets insect pests at different life stages, reducing reliance on chemical fumigants. Additionally, cold plasma treatment can be tailored to specific grains and infestation levels, ensuring minimal impact on

sensory and functional properties. Continued research and optimization of treatment parameters will further improve its efficiency and scalability for commercial applications.

2.7.3. Gamma irradiation induced disinfestation of *C. maculatus* in grains

Gamma irradiation is a non-thermal process that utilizes high-energy gamma rays to control pests, pathogens, and spoilage organisms in food products without raising the temperature. This technique relies on ionizing radiation, which damages the DNA of pests and microorganisms. The radiation disrupts essential cellular processes, ultimately killing these organisms or rendering them unable to reproduce (*Farkas, 2006; Ahmed et al., 2016; Gunes et al., 2018*).

2.7.3.1. Mechanism of Action

2.7.3.1.1. Ionizing radiation and DNA damage

Gamma rays, a form of ionizing radiation, penetrate deep into food materials and pests. The high-energy radiation generates highly reactive species, such as free radicals, that damage the DNA of pests and microorganisms. These reactive species cause DNA strand breaks, disrupt nucleotide bases, and hinder repair mechanisms. The resultant damage impairs the organism's ability to perform critical cellular processes, such as replication and protein synthesis, ultimately leading to cell death or sterility (Gunes et al., 2018; Farkas, 2006).

2.7.3.1.2. Inhibition of Reproduction

Even if pests or microorganisms survive the initial exposure to gamma rays, the induced DNA damage renders them sterile or unable to reproduce. This characteristic is particularly significant for pest control in stored grains, as it prevents infestation from spreading or recurring

(IAEA, 1999). The ability of gamma irradiation to sterilize pests is well-documented and serves as a sustainable alternative to chemical fumigants (Ahmed et al., 2016).

2.7.3.1.3. Biochemical Disruption

In addition to DNA damage, gamma irradiation affects enzymatic systems and cellular membranes. The disruption of these biochemical processes compromises the structural integrity of cell walls and membranes, leading to leakage of cellular contents and metabolic dysfunction. These effects contribute to the ultimate inactivation or death of pests and microorganisms (Diehl, 1995; Farkas, 2006).

Gamma irradiation is generated using specific radioactive isotopes, most commonly cobalt-60 (^60Co) and sometimes cesium-137 (^137Cs). The gamma rays ionize atoms and molecules, disrupting the biological functions of insects, bacteria, and other microorganisms. Importantly, gamma irradiation leaves no residual radiation in the food, ensuring its safety for consumption. Ashraf et al. (2022) investigated the efficacy of gamma radiation against the pulse beetle *C. maculatus (F.)* in cowpeas and observed that different life stages exhibited varying susceptibility. They reported 100% mortality for one-day-old eggs at 16-24 Gy and complete adult mortality at 24 Gy.

These isotopes emit gamma rays as they decay. Gamma rays are highly energetic photons that can penetrate deeply into food products, making this method especially effective for treating larger quantities of food or packaged products while preserving food quality. During the process, food or packaging is placed inside a controlled irradiation chamber where it is exposed to the gamma rays emitted from the radioactive source.

Gamma irradiation not only disinfected stored cowpeas but also preserved their nutritional quality. 100% mortality for eggs showed at 180 Gy, and larval and pupal stages showed 100% mortality at 800 Gy (Supawan et al., 2005). This gamma irradiation treatment resulted in reduced melanization and decreased phenol oxidase activity as the radiation doses increased, demonstrating its high potential in effectively controlling cowpea weevil infestations. Echereobia et al. (2014) also highlighted the efficacy of gamma irradiation for controlling cowpea bruchids.

Gamma irradiation at 800, 900, and 1000 Gy doses achieved 97.7% insect mortality within 96 hours of exposure to irradiation. This irradiation also resulted in low seed perforation and high-water absorption capacity, but there was no significant effect on seed viability. Fawki et al. (2018) reported the lower doses (20-70 Gy) of gamma irradiation-induced sterility on male *C. maculatus* . 70 Gy achieved 100% mortality, while the rest of the lower doses (20, 40, and 50 Gy) showed decreased hatchability, reduced fecundity, and adult emergence across generations. Soje et al. (2021) also proved the efficiency of gamma irradiation in preserving cowpea seeds against *C. maculatus* . Doses ranging from 300 to 900 Gy significantly reduced the weevil population and seed damage compared to non-irradiated seeds. These dosage conditions were also effective in preserving the seeds during storage and increasing their shelf life.

Hastuti et al. (2020) also worked on the gamma Co-60 radiation effect on *C. maculatus* in mung beans. Results showed that a 222.2 Gy dose effectively eradicated weevil eggs, but it did not show any effect on adult weevil development. Gamma radiation doses does not impact the seed quality, maintaining the same water and protein content levels. Gabarty et al. (2022) showed the effect of electron beam irradiation on the developmental stages of *C. maculatus* . Their evaluated results showed no adult emergence from 3-day-old treated eggs upon exposure to 304.8 Gy. Similarly, larvae and pupae exposed to 103.6 Gy did not produce any adults in the subsequent

F_1 generation. A dose of 414.3 Gy was confirmed to be effective as a phytosanitary treatment against *C. maculatus* .

Gamma irradiation is a proven non-thermal method for controlling *C. maculatus* across its developmental stages, with specific doses ensuring 100% mortality and reduced reproduction. Studies highlight its efficacy in preserving seed quality, extending shelf life, and maintaining nutritional value. Doses like 414.3 Gy are confirmed effective for phytosanitary treatment, showcasing its potential for sustainable pest management. Furthermore, gamma irradiation not only targets pests effectively but also helps in preventing the spread of pests and pathogens, thereby enhancing overall food safety in stored products.

2.7.4 UV light-induced disinfestation of *C. maculatus* in grains

UV light can be artificially produced using specialized lamps, particularly mercury vapor lamps or UV-C light-emitting diodes (LEDs). In these lamps, an electrical discharge is passed through mercury vapor, which excites the mercury atoms, causing them to emit UV radiation, specifically in the UV-C range. This UV-C light is commonly used in sterilization processes due to its ability to break down the DNA and cellular structures of pests and microorganisms, making it highly effective for disinfection and pest control.

Short-wave ultraviolet (UV-C) treatment is an effective non-thermal pest management technique, capable of eliminating both inoculated and naturally occurring pests. It serves as an excellent alternative to heat-based methods, particularly for treating heat-sensitive materials (De Souza, 2012). Nobel Singh et al. (2019) was conducted to determine the effect of ultraviolet (UV) radiation on *C. maculatus* . *Callosobruchus maculatus* eggs that were one day old were subjected to different durations of UV-C (254 nm) exposure for 2, 4, 6, and 8 min each. They found that UV-

C treatment significantly reduced the emergence of adult beetles from the irradiated eggs. This indicates a strong inhibitory effect on the early developmental stages of the beetle. After 2, 4, 6, and 8 min of treatment with UV-C, the percentage of reproduction control became 20, 29, 37, and 42%, respectively (Nobel Singh et al., 2019).

In addition, the advancement of *C. maculatus* in the treated groups was slower compared to that in the control groups that were not treated, meaning that UV-C irradiation disrupts normal growth processes. Sedaghat et al. (2011) worked on the effect of ultraviolet (UV) radiation on the hatching rate of *C. maculatus* . The study focused on exposing eggs of different ages, 1, 2, and 3 days old, to UV light at 254 nm for a range of durations: 2, 4, 8, 16, 24, 32, and 40 min, respectively. For one-day-old eggs, there was a decline in hatching percentages from as high as approximately 72% after 2 min, and it dwindled to less than 8% (7.8%) after 40 min (Sedaghat et al.2011). Two-day-old eggs showed a more pronounced fall with their hatching dropping from about 44% at 2 min to around 5.5% at 0 min (Sedaghat et al.2011).

These drops were even more substantial in three-day-old eggs, where their hatchings went down from ~40%, after 2 min 19.43%, to just below 2% (1.86%) after 40 min (Sedaghat et al.2011). Singh et al. (2019) investigated how UV-C irradiation impacts the life cycle parameters of *C. maculatus* . They exposed eggs of different ages to UVC radiation and observed the impacts it had on hatching rates as well as subsequent adult reproduction. They found that longer exposure times significantly decreased the hatching rates, with older eggs showing greater sensitivity to UV-C treatment. Notably, eggs that were two days old and treated with 4 min of UV-C irradiation exhibited the lowest reproduction rates among the resulting adults. Arul et al. (2022) worked on adult beetles exposed to UV light at a wavelength of 254 nm for different durations ranging from 2 to 16 min. The increased sensitivity of older eggs suggests that targeted UV exposure could

enhance disinfestation efforts at critical developmental stages. Further research is needed to optimize UV treatment parameters for different grains and storage conditions to maximize its efficacy.

Results showed a gradual decrease in fecundity, hatchability, population, and adult emergence upon increasing exposure time. Importantly, untreated beetles showed the highest reproductive and fecundity at 180 eggs per 2 females, hatchability at 87.4%, pupation at 81.7%, and adult emergence at 124 individuals (Arul et al., 2022). Conversely, beetles exposed to UV radiation for 16 min showed markedly reduced values: fecundity at 42 eggs per 2 females, hatchability at 34.1%, pupation at 25.4%, and adult emergence at just 3 individuals (Arul et al., 2022). In general, all the described non-thermal techniques are further elaborated in **Table 1**.

Table 1: Non-thermal techniques for the disinfestation of stored pulses and their effectiveness against Callosobruchus species

Grains Exposed	**Effective Conditions**	**Insect Targeted**	**Highest Mortality Achieved (%) & Life Stage**	**Most Resistant Life Stage**	**Other Findings**	**References**
Stored cowpea seeds; Chickpeas	0.25-2.0 g/m³ for 7 days; 2.0 g/m³ immediate mortality	*C. maculatus*	95-100%; All life stages	Adults	Immediate pest control - Maintains germination - Slight decrease in protein and fat content	Hassan et al. (2021) Gad et al. (2021) Ramos et al. (2023)
Stored spices	14400-144000 ppm × min (144 to 5760)	General pest control	95%; All stages	Not specified	High mortality across pest species - Maintains grain quality - Suitable for spices	Ingegno et al. (2022)

Stored chickpea grains	1000 ppm over 5 days	*C. maculatus*	100%; Adults	Eggs	- Improved protein and germination - Better milling properties - Pilot-scale trials confirm effectiveness	Nickhil et al. (2021)
Stored pulses	3500 µg/L for 84.4 min	*C. maculatus*	95%; All stages	Not specified	- Maintains pulse quality - Effective insect control - Retains nutritional content	Abreu et al. (2022)
Stored pulses	1000 ppm for 2 hours	*C. maculatus*	83.5%-104%; Egg to adult stages	Pupae	Effective for all life stages - Minimal protein and mineral loss, - maintains storage quality	Kulwinder et al. (2023)
Grains Exposed	**Effective Conditions**	**Insect Targeted**	**Highest Mortality Achieved (%) & Life Stage**	**Most Resistant Life Stage**	**Other Findings**	**References**
Soybean seeds; Chickpeas	70 kV for 3 min; Atmospheric Cold Plasma for 5 days	*C. maculatus*	90-100%; Early life stages	Pupae	- Improved shelf life - Protein digestibility - Effective against early life stages	Krik-Bradley et al. (2023); Mir et al. (2023)
Soybean seeds	2.0 kV for 24 min	*C. maculatus*	100%; Larvae and adults	Not specified	- Better soymilk yields - Effective for larvae and adults - Retains grain quality	Anbarasan et al. (2023)
Stored pulses	Various power levels	General pest control in pulses	Effective at varied mortality rates	Not specified	- Enhanced germination and protein digestibility; - Potential application for shelf-life extension.	Li et al. (2023); Dong et al. (2023)

Cowpea seeds; Mung beans	180-900 Gy; 20-70 Gy for sterility	*C. maculatus*	97.78%-100%; All stages	Eggs	- Maintains seed viability - Reduces reproduction - Effective across all life stages	Ashraf et al. (2022); Soje et al. (2021)
Cowpea seeds; Chickpeas	414.3 Gy phytosanitary treatment	*C. maculatus*	100%; Eggs	Adults	- Sterility achieved in males - Effective phytosanitary treatment - Preserves nutritional quality	Hastuti et al. (2020); Gabarty et al. (2022)
Stored pulses	300-900 Gy	*C. maculatus*	Significant reduction; Adults	Pupae	- Reduces pest populations - Maintains storage quality - Prolongs shelf life	Fawki et al. (2018); Supawan et al. (2005)
Grains Exposed	**Effective Conditions**	**Insect Targeted**	**Highest Mortality Achieved (%) & Life Stage**	**Most Resistant Life Stage**	**Other Findings**	**References**
cowpea seeds	20-70 Gy	*C. maculatus*	Induces sterility		-Reduced hatchability and fecundity across generations; - Effective for pest management	Fawki et al. (2018)
Stored chickpeas	UV-C light at 254 nm for 2-8 min	*C. maculatus*	Significant inhibition; Eggs	Adults	- Reduces fecundity and reproduction - Maintains nutritional quality - Effective pest control	Arul et al. (2022); Nobel Singh et al. (2019)
Cowpea seeds; Chickpeas	2-40 min at 254 nm	*C. maculatus*	20-42% reduction in reproduction		-Reduces reproductive and developmental stages; Effective	Singh et al. (2019); Sedaghat et al. (2011)

					against eggs and adults;- Maintains nutritional content	
Stored chickpeas	16 min at 254 nm	*C. maculatus*	80–100% at susceptible	larvae and pupae	Marked reduction in fecundity and reproduction; Maintained seed quality	Sedaghat et al. (2011); Arul et al. (2022)
Stored chickpeas	1-3 days old eggs exposed for 240 min at 254 nm	*C. maculatus*	Reduced hatching rates significantly		Older eggs showed greater sensitivity; Effective reduction in reproductive rates	Singh et al. (2019); Sedaghat et al. (2011)

2.8. Conclusions

In an era where sustainable and efficient pest management is critical for ensuring food security, nonthermal techniques have emerged as innovative solutions to address the challenges posed by traditional methods. This review highlights the immense potential of techniques such as ozone treatment, cold plasma, gamma irradiation, and UV radiation in managing storage pests like *C. maculatus* . These methods offer a unique combination of efficacy, environmental friendliness, and the ability to preserve the nutritional and sensory quality of stored grains.

While these advancements are promising, the journey toward widespread adoption is challenging. Industrial scalability, cost-effectiveness, and long-term impacts on pest resistance and grain quality remain key research gaps. Comparative studies to benchmark the effectiveness of these techniques under identical conditions are essential to identify the most practical solutions. Moreover, addressing regulatory and operational barriers, especially for methods like gamma

irradiation and cold plasma, is crucial for their integration into large-scale storage systems. The adoption of non-thermal techniques offers a sustainable and innovative solution for protecting stored grains, ensuring they remain safe, nutritious, and accessible for the future.

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Chapter 3 - Disinfestation Efficacy of Cold Plasma Treatment on Various Life Stages of *Callosobruchus maculatus* in Chickpeas (*Cicer arietinum*)

Abstract

Chickpeas (*Cicer arietinum*), a globally significant legume, are frequently compromised by infestations from *C. maculatus* (F.), cowpea weevil, resulting in notable post-harvest losses. Traditional pest untreated techniques often rely on chemical fumigants that pose ecological risks and promote resistance in pest populations. This study evaluates cold plasma as an innovative, non-thermal pest management method. Using a range of voltage levels (15, 20, and 25 kV) and exposure durations (5, 10, and 15 min), cold plasma treatment was applied across four life stages of *C. maculatus* (egg, larva, pupa, adult) inoculated in chickpea samples. Results revealed significant pest mortality, particularly at higher voltages and prolonged exposure, with 100% larval mortality achieved at 25 kV for 10 min and egg eradication at 20 kV for 15 min. Additionally, cold plasma treatment preserved chickpeas' physical and nutritional properties, with no significant changes in bulk density, true density, kernel weight, or moisture content. These findings underscore cold plasma as a viable, eco-friendly alternative for insect infestations in stored legumes, supporting sustainable agriculture by reducing the reliance on chemical pesticides.

3.1. Introduction

Chickpeas (*Cicer arietinum L.*) are a highly valuable legume crop grown worldwide, prized for their rich plant-based protein content. In addition to their protein benefits, chickpeas are an excellent source of dietary fiber, positioning them among the most nutritious legumes in the pulse family (Jukanti et al., 2012). Furthermore, they provide essential amino acids, vitamins, and minerals, making them an integral component of many diets, particularly for vegetarians and vegans (Iqbal et al., 2006). Their high protein content and low glycemic index make them especially important for addressing food security and reducing malnutrition in developing regions (FAO, 2016).

Chickpeas are commonly sold in various forms, including seeds, flour, or canned products. Globally, chickpeas rank second among legumes in terms of cultivation, following soybeans (FAO, 2021; ICRISAT, n.d.; Foyer et al., 2016). Global chickpea production reached approximately 12 million MT in 2020, with the bulk of production concentrated in countries such as India, Australia, Turkey, and Pakistan (FAOSTAT, 2020).

In the United States (US), chickpea production has also been on the rise due to increasing consumer demand for healthy, protein-rich, plant-based foods. The US produced approximately 300,000 metric tons of chickpeas in 2020, with major production areas located in Montana, North Dakota, Idaho, and Washington (USDA, 2021). The rise in US production has been driven largely by the growing demand for chickpeas in the domestic market, with applications ranging from hummus and other processed chickpea products to their use in soups and salads. Between 2015 and 2020, chickpea acreage in the US nearly tripled, reflecting this surging demand (USDA-ERS, 2020). With the increasing demand for plant-based protein and rising consumption in developing countries, the global chickpea market is projected to surpass USD 6 billion by 2027 (Grand View

Research, 2020). India alone accounted for nearly 70% of global chickpea production in 2020, followed by Turkey and Australia (FAOSTAT, 2020).

Despite increased production, post-harvest losses due to insect infestations, particularly by *C. maculatus* pose a major challenge. These pests can cause significant damage to stored chickpeas, resulting in reduced quality, nutritional loss, and substantial financial impact (Shaheen et al., 2020). Research from FAO (2016) on food waste in pulses emphasizes that chickpeas, as a primary legume crop, contribute to the larger issue of pulse loss in global food systems. Post-harvest losses for legumes are estimated to range from 15-30%, primarily due to inadequate drying, storage, and untreated pest practices (FAO, 2016).

Post-harvest losses, largely due to pests and plant diseases, contribute to roughly 40% of global agricultural losses (Mahmood et al., 2016). During storage, pests like *C. maculatus* can cause both physical and nutritional damage to grains, rendering them unsuitable for consumption. Internal feeders, such as *C. maculatus* larvae, consume the grain's endosperm, resulting in significant weight loss and decreased nutritional quality (Hamdi et al., 2017; Singh et al., 2021). This internal damage can also compromise grain quality and viability (El-Aziz et al., 2014). In addition, external feeders further degrade stored grain by laying eggs on the surface, contaminating it with feces, and leaving behind dead insects (Richard-Molard, 2003; El-Aziz et al., 2014; Sutar et al., 2021). The infestation of *C. maculatus* is one of the biggest threats to stored chickpeas, causing significant post-harvest losses. This pest can cause both physical and nutritional degradation, leading to perforated grains, weight loss, and reduced market value.

Without effective treatment, up to 50% of stored chickpeas can be lost to infestation (Hamdi et al., 2017; Singh et al., 2021). Nutritionally, these infestations decrease total crude fat and carbohydrates while increasing ash and protein content, affecting the quality of the crop

(Saxena & Saxena, 2011). Moreover, *C. maculatus* can lead to secondary infestations, risking total crop loss within a few months (Singh & Jackai, 1995). These findings highlight the urgency of implementing effective pest control strategies to mitigate storage losses and preserve chickpea quality. Without intervention, infestations not only reduce yield but also alter the nutritional composition, potentially impacting both consumer health and market value. The increase in ash and protein content suggests metabolic activity from the pests, leading to the degradation of essential nutrients. Additionally, secondary infestations exacerbate the issue, making long-term storage and trade challenging. Therefore, adopting non-thermal disinfestation methods, such as cold plasma, could offer sustainable solutions to minimize losses and maintain grain integrity.

Traditionally, farmers have used a variety of fumigation methods to treat this pest, including heat and cold treatments and chemical fumigants like phosphine and methyl bromide, as well as natural essential oils. However, these methods often fall short due to issues with residual toxicity and environmental safety (Mahfuz & Khalequzzaman, 2007). Chemical insecticides and fumigants, while common, are becoming increasingly problematic as pests develop resistance, raising concerns about chemical residues, environmental impact, and health risks (Rajendran & Sriranjini, 2008; Holland, 2021).

As *C. maculatus* larvae are internal feeders, it is challenging to target them effectively using fumigants. Thus, there is an urgent need for more ecological and safer untreated methods that ensure food safety while maintaining the nutritional and textural qualities of chickpeas. This challenge has prompted researchers to explore innovative non-thermal treatment methods. Non-thermal processing treats food at ambient room temperature for a very short duration, preserving the food's texture, color, and nutritional properties without causing significant heating (Beyrer et al., 2020; Jaddu et al., 2024). Plasma, first described by Langmuir in 1925, refers to the fourth state

of matter, composed of ionized gases (Darrow et al., 1948). Plasma treatments can be classified into two categories: thermal and non-thermal (cold plasma). Thermal treatments operate at high temperatures and use high voltage (50 kV and 200 kV), whereas cold plasma operates at room temperature and uses moderate voltages (2 kV and 45 kV) (Pipliya et al., 2024; Pankaj, et al., 2014; Misra et al., 2016; Islam et al., 2024).

Cold plasma is an exciting, non-thermal technology that's making waves in the food processing industry, especially as an eco-friendly method for pest untreated. Unlike traditional states of matter like solids, liquids, or high-temperature plasmas, cold plasma is an ionized gas at low temperatures, produced through techniques like dielectric barrier discharges (Bora et al., 2022). It's a unique mix of electrons, ions, neutral particles, and reactive molecules (Scholtz et al., 2015). This ionized gas generates reactive oxygen and nitrogen species (ROS and RNS) that can be created under atmospheric or low-pressure conditions, giving cold plasma special insecticidal properties (Misra et al., 2016). These reactive molecules are effective not only in neutralizing pests but also in reducing microbial contaminants, including harmful bacteria and fungi, which enhances food safety (Science News, 2021). Cold plasma has been shown to effectively eliminate microorganisms and pests on food products while preserving product quality.

Additionally, cold plasma is explored for its potential to replace traditional chemical preservatives, as it allows minimal alternation of food's original flavors and nutritional composition (Bora et al., 2022). By generating reactive species such as free radicals, nitrogen atoms, and NO radicals, cold plasma creates a dynamic oxidative environment that disrupts biological surfaces (Niemira, 2012). Its primary mechanism of action involves damaging the external cuticle of insects, which acts as a protective barrier against environmental stress. The reactive species produced by plasma break down the molecular structure of the insect cuticle

(Zhang et al., 2019). Moreover, cold plasma induces oxidative stress within insect cells, which disrupts cellular functions by damaging proteins, lipids, and nucleic acids, ultimately leading to insect mortality (Jiang et al., 2017). This process not only damages the physical structure of insects but also interrupts their internal biological processes, including metabolism and respiration (Bousserrhine et al., 2018).

Cold plasma technology is gaining attention for its wide-ranging benefits in pest untreated. Unlike traditional methods, it targets pests at various life stages, effectively inhibiting egg hatching, killing larvae, and reducing adult pest activity (Jiang et al., 2017). Notably, cold plasma treatments don't affect the taste, quality, or nutrient content of food and even help extend the shelf life of treated items (Mir et al., 2016; Khaneghah, 2021). A big advantage of cold plasma over chemical pesticides is that it leaves no harmful residues on food, making it a safer and more sustainable choice for protecting crops like chickpeas (Niemira, 2012). Given that chickpeas are often consumed in raw or minimally processed forms, safety is a paramount concern for consumers. Consumer perceptions of cold plasma-treated chickpea kernels are influenced by a variety of factors, including safety, nutritional benefits, and the innovative nature of the technology. As awareness of the health benefits associated with legumes increases, particularly chickpeas, consumers are more inclined to explore new processing methods that enhance food safety and quality. Cold plasma treatment, recognized for its non-thermal and chemical-free properties, aligns well with contemporary consumer preferences for clean and minimally processed foods (Bora et al., 2022; Bayati et al., 2024).

Cold plasma technology is effective in inactivating pathogens, thereby potentially reducing foodborne illness risks (Bora et al., 2022). This technology supports sustainable agriculture by cutting down on chemical pesticide use, reducing harmful residues, and lessening environmental

impact (Misra et al., 2019). Several studies have explored the effectiveness of cold plasma disinfestation for stored grains, demonstrating its potential to control stored product pests.

However, the cold plasma disinfestation of chickpeas across all life stages of *C. maculatus* has not been studied yet. Therefore, this study aims to explore the potential of cold plasma exposure to disinfest chickpeas infested with *C. maculatus* at all life stages. By investigating the effects of varying cold plasma treatment parameters, this research seeks to identify optimal conditions that maximize pest control while preserving the quality of the chickpeas. Ultimately, the findings could provide valuable insights for developing sustainable pest management strategies that benefit both agricultural practices and food safety.

For example, Anbarasan et al. (2023) studied cold plasma disinfestation of *C. maculatus*-infested soybeans, highlighting its impact on insect mortality and soymilk extraction yield. Mir et al. (2023) reviewed novel approaches, including cold plasma, as a safer method for the disinfestation of stored grains compared to chemical fumigation, and Ziuzina et al. (2020) examined the effects of atmospheric cold plasma on wheat grain microbiomes and its antimicrobial efficacy. The study found significant reductions in pest infestations while ensuring grain safety. Although cold plasma has been studied for the disinfestation of grains, its effectiveness against *C. maculatus* across all life stages in chickpeas has not yet been studied. Therefore, the present study aims to explore the potential of cold plasma exposure to disinfest chickpeas infested with *C. maculatus* at all life stages.

3.2. Materials and Methods

3.2.1. Raw Materials

The raw materials used in this study include chickpeas (*Cicer arietinum*), which serve as the primary host grain for rearing the insect species *C. maculatus*, The chickpeas were purchased online from Palouse Brand via Amazon, specifically a 5 lb. bag of garbanzo beans. The initial population of *C. maculatus* used for laboratory rearing was obtained from an established stock culture maintained in an entomology lab. This selection of raw materials is essential for the successful cultivation and study of the cowpea weevil in a controlled environment. The brief methodology, as described in **Figure 2**, explains the steps and procedures considered in the inoculation and incubation of the cultured chickpeas for pest mortality assessment.

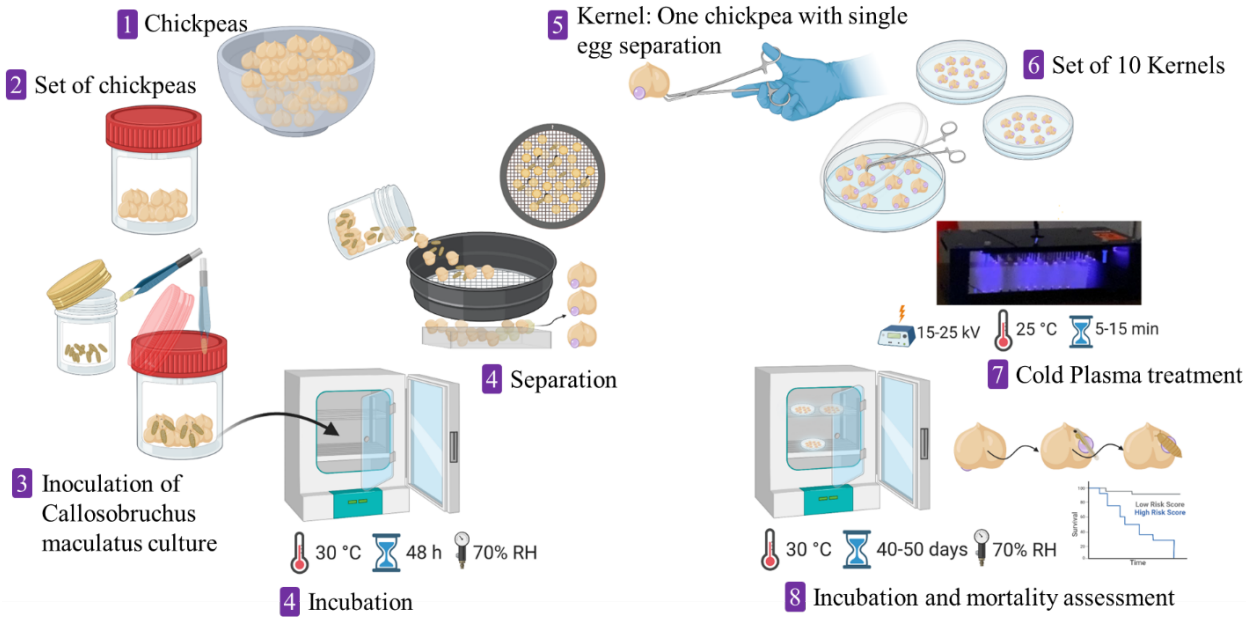


Figure 2: Experimental setup illustrating insect inoculation, incubation at specific conditions, separation of kernels containing a single egg, treatment with cold plasma, followed by incubation and assessment of mortality rates post-incubation.

3.2.2. Equipment

The equipment utilized in this study includes a cold plasma generator, which is essential for the plasma exposure treatment of chickpeas and the cowpea weevil (*C. maculatus*). A

Dielectric Barrier Discharge (DBD) Plasma Generator (Model: LCP-LPS-CP) developed by INGENIUM Technologies, India (2022), was employed for the treatment. The system operates at a frequency of 60 Hz with a power input of 1200 KVA. Additionally, a temperature and humidity control chamber is employed to maintain optimal rearing conditions for *C. maculatus*, ensuring that the insects thrive in a suitable environment. A compound microscope is also used for monitoring the various life stages of *C. maculatus*, allowing for detailed observation and analysis of the insect's development throughout the rearing process. Together, these pieces of equipment facilitate effective research on the impact of cold plasma treatment on both the host grain and the insect. Precise control over these experimental conditions ensures the reproducibility and reliability of the findings, strengthening the study's conclusions.

3.2.3. Rearing of *C. maculatus*

The *C. maculatus* required for the study was obtained from infested Chickpea samples, cultured at the Entomology lab, Kansas State University, KS. The eggs are prepared by inoculating *C. maculatus* cultures in 200 g of chickpea (Kabuli) (seeds with moisture content $11.0 \pm 0.4\%$) samples obtained from Food to Live, New York, USA. After inoculating the adults in different glass jars, covered with wire mesh upon Whatman No. 1 filter paper and kept in incubators (temperature: $30 \pm 2°C$; RH: $74 \pm 4\%$) undisturbed for 24 h. After incubation for 24h, the seeds containing a single egg on each kernel were collected carefully using an aspirator and tongs. Likewise, other life stages—larva, pupa, and adult—were identified by examining the visual characteristics of the seed coat. This involved breaking open the kernel and observing larval features under an electron microscope. All the separated eggs, larvae, and pupa are treated with cold plasma under different voltage (15, 20, and 25 kV) and time (5, 10, and 15 min) combinations.

A full factorial design (3 × 3 × 4: Voltage × Time × Life Stages) was employed with three independent runs to ensure experimental replicability. The control samples consisted of untreated chickpeas infested with various life stages of *C. maculatus* , which were handled identically to the treated samples, without any plasma exposure. These samples were placed in identical glass petri dishes and subjected to the same environmental conditions as the treated groups, i.e., 30 ± 2°C and 74 ± 4% RH.

The different life stages (egg, larvae, pupae, and adult) of *C. maculatus* were identified visually while inspecting under a Compound microscope. This allowed precise differentiation between life stages based on morphological characteristics (El-Aziz, 2014). Eggs were identified by their attachment to the seed surface, while larvae were distinguished by their burrowing behavior and damage to the seed coat. Eggs were identified on day 1, first instar larvae on day 9, second instar larvae on day 16, and pupae on day 23 after insect culture. Pupae were detected inside the seed based on their hard, immobile exoskeleton, while adults were identified by their distinctive color, size, and wing structure (Hamdi et al., 2017).

3.2.4. Optical emission spectroscopy

To analyze the optical emissions from the plasma discharge, a 5-mm collimating lens was used to capture the emitted light within the discharge region above the sample. This captured light was directed into a 600 μm core diameter optical fiber, which transferred the signal to an Ocean Optics High-Resolution spectrometer (HR200191, Ocean Optics Inc., Florida, USA). The collimating lenses were positioned 6 cm from the edge of the electrode plate to ensure optimal light collection. Spectral data were recorded within the wavelength range of 200 to 800 nm, with an integration time of 1 second. To enhance the signal-to-noise ratio, each spectrum was obtained

by averaging ten consecutive recordings. Dark current and background noise were subtracted using Ocean View software (Ocean Optics, FL, USA) to ensure accurate spectral representation.

3.2.5. Cold plasma treatment of the infested chickpeas

The cold plasma used in this study was generated using a pin-to-plate plasma generator (LCP-LPS-CP model, 2022) manufactured by Ingenium Technologies, India. (**Figure 3**). The device is capable of operating at adjustable voltages up to 30 kV. The plasma was generated using air as the ionizing gas, under atmospheric pressure, and directed vertically over the chickpea samples at 3 cm between the electrode edge and the surface of the chickpeas being treated. Voltage and treatment time were adjusted using the device's integrated untreated panel, allowing for a range of treatment times from 5-15 min. This cold plasma was directed vertically over chickpea samples containing inoculated eggs, larvae, pupae, and adult weevils. Each life stage was treated under varying cold plasma conditions, combining three voltage levels (15, 20, and 25 kV) with three treatment durations (5, 10, and 15 min), resulting in nine treatments for each life stage.

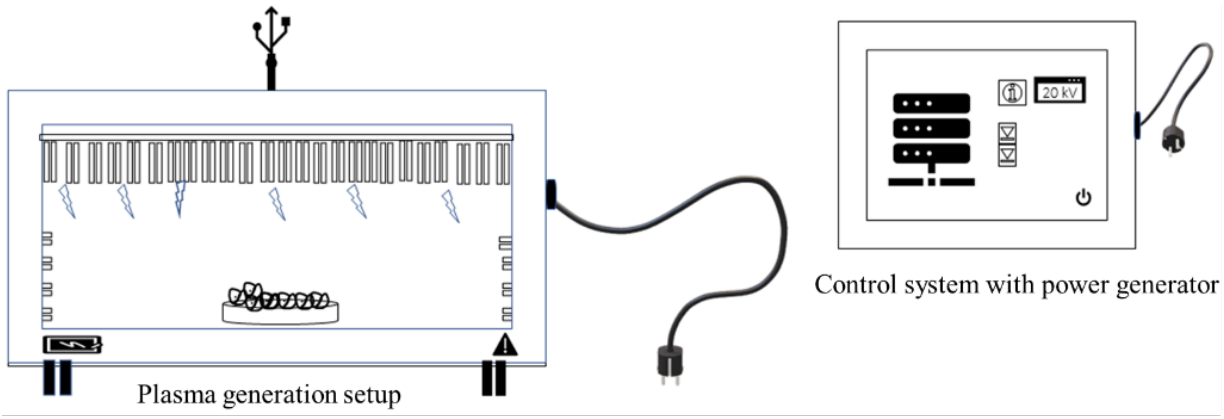


Figure 3: Pin-to-plate cold plasma setup with power generator

A set of 30 seeds with single eggs are placed in a glass petri dish and treated at 15, 20, and 25 kV voltages for 5, 10, 15 min under 25°C environmental conditions. During exposure to 25 kV,

the seeds exhibited noticeable swirling and wagging movements due to the high-voltage plasma discharge effect. Similarly, other different life stages were also treated under the same conditions. Treated eggs, larva, and pupa were placed in plastic vials (30 mL) with wire mesh openings covered to provide aeration and kept in an incubator. The vials were checked at regular intervals and observed for the number of adult beetles that emerged from the chickpeas after treatment. The corrected mortality(Abbott's) of all immature stages was determined by considering those that did not successfully develop into adults.

The corrected mortality(Abbott's) rate measured using equation 1, offers a more reliable metric for evaluating the effectiveness of cold plasma, especially when assessing its potential as a pest control method. By applying this calculation, the study was able to provide a comprehensive analysis of how different life stages of *C. maculatus* eggs, larvae, pupae, and adults were affected by the cold plasma treatment. This approach highlighted the varying susceptibilities of each life stage to the plasma exposure and allowed for a more detailed understanding of how plasma can be used to reduce insect populations effectively over time. The findings contribute to the growing body of knowledge about cold plasma as a nonchemical alternative for pest management, showing its potential for reducing C. maculatus infestations in a variety of contexts. The corrected mortality(Abbott's) rate was adjusted for background mortality in the control group, offering a more precise evaluation of the plasma treatment's impact.

$$Percent\ control\ = \left(\frac{X - Y}{X}\right) 100 \quad (1)$$

X = Percent survival in the control (untreated) group

Y = Percent survival in the treated group

X−Y = Percent reduction in survival due to treatment

Observations were recorded every 24h for 40 days post-treatment. The specific parameters monitored included the number of live and dead insects at each life stage, the development of larvae into pupae or adults, and the overall mortality rate. For eggs, hatching success was tracked, while for larvae and pupae, the focus was on development into adults (Jiang et al., 2017). The data collected provided insights into the efficacy of cold plasma treatment over time, highlighting any delayed effects of the treatment on insect mortality. Mortality percentages were calculated for each stage (egg, larva, pupa, adult) by comparing treated samples to untreated samples. Additionally, corrected mortality(Abbott's)(Abbott's) rates were computed to account for natural mortality in the control group.

3.2.6. Data analysis

The data collected on insect mortality were thoroughly analyzed using various statistical methods to ensure the accuracy and reliability of the results. All the experiments were done in triplicate. SAS software (version 9.4, SAS Institute Inc., Cary, NC) was used for all statistical analyses. Key techniques applied include analysis of variance (ANOVA), means comparison, and error estimation. Post-hoc analysis, using Tukey's Honest Significant Difference (HSD) test, was applied to evaluate specific pairwise differences between voltage levels and treatment times. Statistical significance was set at a p-value of less than 0.05.

3.3. Results and Discussion

3.3.1. Corrected mortality(Abbott's) rates across various *C. maculatus* life stages.

From **Table 2**, at 25 kV, egg mortality was highest at 15 min (85.15±20.95), indicating a strong cumulative effect of exposure at this voltage. However, at 10 min, a sudden drop in mortality (13.3±18.9) suggests a possible adaptive physiological response at this intermediate

exposure time (López-Martínez & Hahn, 2012). Larvae 1 exhibited fluctuating mortality, peaking at 10 min (14.81±5.23) but decreasing at 15 min (11.11±0.00), which might be due to varying tolerance levels among individuals. Larvae 2 mortality remained consistently low, with 0.00±0.00 at 15 min, suggesting higher resistance in later larval stages. Pupae mortality was relatively low across all durations, peaking at 10 min (7.40±5.23), which aligns with previous studies indicating that pupae are the most resistant insect life stage to environmental stressors (Hallman & Thomas, 2015; Neven, 2015).

Table 2: Corrected mortality(Abbott's) rates (mean ± SE) of various life stages of C. maculatus inoculated in chickpeas when exposed to 25-15 kV of cold plasma treatment for 5-15 min time conditions. Dissimilar alphabets in lowercase letters (a, b, c) signify that the mean values for mortality rate are statistically different at $p<0.05$ within treatment times for the same life stage. Dissimilar alphabets in uppercase letters (A, B, C) signify that the mean values for mortality rate are statistically different at $p < 0.05$ between life stages at a fixed treatment time.

Mean (± SE) mortality				
25 kV treatment				
TRMT time	Egg	Larvae 1	Larvae 2	Pupae
5 min	70.3 ± 41.90 a, A	3.70 ± 5.23 b, B	3.70 ± 5.23 a, B	3.70 ± 5.23 a, B
10 min	13.3 ± 18.90 b, B	14.81 ± 5.23 a, B	11.11 ± 0.00 b, A	7.40 ± 5.23 a, B
15 min	85.15 ± 20.95 a, A	11.11 ± 0.00 a, B	0.00 ± 0.00 b, C	3.70 ± 5.23 a, C
20 kV treatment				
5 min	3.70 ± 5.23 b, A	7.40 ± 5.23 a, A	7.40 ± 5.23 a, A	7.40 ± 5.23 a, A
10 min	0.00 ± 0.00 b, C	3.70 ± 5.23 b, B	11.11 ± 0.00 a, A	3.70 ± 5.23 b, B
15 min	11.11 ± 0.00 a, A	7.40 ± 5.23 a, A	3.70 ± 5.23 b, B	3.70 ± 5.23 b, B
15 kV treatment				
5 min	3.70 ± 5.23 a, A	7.40 ± 5.23 b, B	7.40 ± 5.23 a, B	7.40 ± 5.23 b, B
10 min	0.00 ± 0.00 a, A	3.70 ± 5.23 b, B	11.11 ± 0.00 a, A	3.70 ± 5.23 a, B
15 min	11.11 ± 0.00 b, B	7.40 ± 5.23 a, B	3.70 ± 5.23 b, A	3.70 ± 5.23 a, A

Further, at 20 kV, egg mortality was negligible at 5 min (3.70±5.23) and completely absent at 10 min (0.00±0.00), with only a slight increase at 15 min (11.11±0.00). This suggests that 20kV is not as effective for egg mortality unless the exposure duration is increased (Johnson & Neven, 2011). Larvae 1 peaked at 5 min (7.40±5.23) but declined at later durations, possibly due to developmental adaptations reducing susceptibility. Interestingly, Larvae 2 mortality was highest at 10 min (11.11±0.00), indicating that this voltage and duration might be optimal for targeting this stage. Pupae mortality remained low (maximum of 3.70±5.23), reinforcing the notion that pupae are the most resistant stage to electrical treatments (Santos et al., 2020).

Furthermore, at 15 kV, egg mortality was relatively low across all durations, with the highest recorded at 10 min (11.11±9.07), reinforcing that lower voltage treatments are less effective unless exposure time is increased (Neven, 2015). Larvae 1 mortality remained minimal, peaking at 15 min (7.40±5.23), which is lower than what was observed under 25kV and 20kV. Larvae 2 showed the highest mortality at 15 min (14.81±5.23), suggesting that prolonged exposure at even a lower voltage can still be effective in targeting this stage. Pupae mortality was highest at 10 and 15 min (11.11±9.07), which is significantly higher than the 20kV treatment but still lower than what was observed in eggs, confirming that pupae generally require higher voltages or longer durations for effective control (Hallman & Thomas, 2015).

The results indicate that higher voltages (25kV) and longer exposure durations (15 min) are the most effective in increasing mortality, particularly in eggs. Larvae 2 appear to be the most resistant, while pupae are the least affected across all treatments, reinforcing previous studies on insect development and stress tolerance (Neven, 2015; Santos et al., 2020). The non-linear mortality response, particularly in eggs at 25 kV for 10 min, suggests that certain exposure conditions might trigger physiological stress responses rather than direct mortality, a phenomenon

observed in previous studies on electromagnetic stress effects in insects (López-Martínez & Hahn, 2012). These findings are crucial for refining pest management strategies, emphasizing the need to tailor treatment intensity and duration based on the specific developmental stage of the target insect.

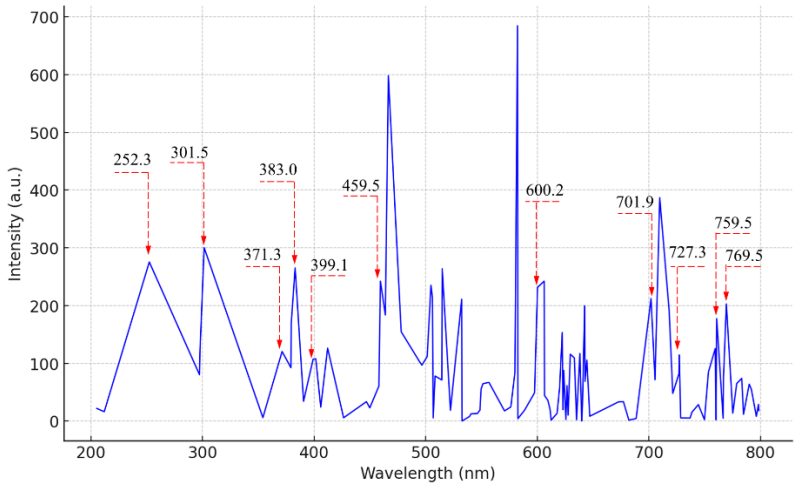
3.3.2. Optical Emission Spectroscopy

From **Figure 4**, the presence of hydroxyl radicals (OH•) was confirmed by emission peaks at 297.7 nm and 301.5 nm, indicating strong hydroxyl activity within the plasma environment. Hydroxyl radicals are highly reactive species known for their role in oxidative reactions, particularly in modifying enzyme structures and breaking down organic compounds. Their strong oxidative potential suggests that cold plasma treatment may influence enzymatic activity by altering protein conformation and functionality. Reactive nitrogen species (RNS) were identified through peaks at 371.3 nm, 379.6 nm, and 383.0 nm, corresponding to N_2 and N_2^+ transitions. These nitrogen-based species contribute to nitration reactions, which can modify enzyme activity and impact metabolic pathways in biological systems

The presence of reactive nitrogen species (RNS) highlights the potential of cold plasma to induce biochemical changes through nitrogen-driven modifications, which could affect microbial viability and protein stability. A recent study by Orlowski et al. (2025) supports this by reporting the generation of reactive oxygen species (ROS) (OH•, HO_2•, O_2^-, O_3, and singlet oxygen $^1O_2$) and RNS (NO, NO_2, and peroxynitrite $ONOO^-$) in cold plasma-treated plant seeds. Research has shown that plasma exposure duration significantly influences ROS and RNS concentrations. As exposure time increases, the concentrations of ROS (O_2^-, O_3, and OH•) and RNS (N_2 transitions, NO, NO_2) also rise, impacting oxidative and nitration reactions. Short-lived species, such as OH• and NO,

reach their peak intensities within the first few seconds of plasma exposure. In contrast, longer-lived species, such as O_3 and NO_2, gradually accumulate over extended treatment durations. These findings are supported by studies from Sardella et al. (2020), Ji et al. (2019), and Liu et al. (2017), which demonstrate the temporal variation of ROS/RNS formation and accumulation during plasma exposure.

Reactive oxygen species (ROS), detected through oxygen molecular bands at 759.5 nm, 769.5 nm, 802.1 nm, and 826.3 nm, indicate the presence of singlet oxygen and atomic oxygen transitions. These oxygen species are known for their role in oxidative modifications of proteins and biomolecules, potentially leading to structural changes in grains and stored products. The interaction of ROS with organic matter suggests that cold plasma treatment may alter functional properties, enhancing food preservation by reducing microbial contamination and improving protein interactions.



Peak (nm)	Possible Electron Shift
252.3	Possible N_2 transition
297.7	Possible OH (Hydroxyl Radical)
301.5	OH ($A^2\Sigma^+ \rightarrow X^2\Pi$) transition
371.3	N_2 ($C^3\Pi u \rightarrow B^3\Pi g$)
379.6	N_2^+ ($B^2\Sigma^+ u \rightarrow X^2\Sigma^+ g$)
383.0	N_2 ($C^3\Pi u \rightarrow B^3\Pi g$)
399.1	N_2 ($C^3\Pi u \rightarrow B^3\Pi g$)
459.5	N_2 second positive system
600.2	Atomic Oxygen (O) transition
701.9	O_2 (Singlet Oxygen)
727.3	Oxygen molecular band
759.5	O_2 ($2s^22p^33p \rightarrow 2s^22p^33s$)
769.5	O_2 ($2s^22p^35P \rightarrow 2s^22p^33S$)
802.1	O_2 transition
826.3	O_2 (possible excited molecular transition)

Figure 4: Optical emission spectra obtained during cold plasma (25 kV/10 min) treatment. ROS, reactive oxygen species; RNS, reactive nitrogen species.

3.4. Comparative Analysis Across Voltages and Life Stages

When comparing voltages, the data shows that higher voltages (20 kV and 25 kV) consistently produced higher mortality rates across all life stages, with shorter exposure times (5 to 10 min) being sufficient to achieve significant mortality. In contrast, at 15 kV, a longer exposure (15 min) was necessary to achieve comparable results, especially for the larvae-2e stage. 100% mortality for larvae-2e was achieved at 25 kV for 10 min, which is lower than 40 kV (Misra et al., 2016) but slightly higher than the 15–20 kV range (Scholtz et al., 2015). At 20 kV, significant mortality was achieved within 5–15 min, which aligns with Scholtz et al. (2015), who reported inactivation occurring within 10 seconds to 30 min, whereas bacteria with spores requiring longer exposure times up to 20–30 min, depending on plasma conditions and target organisms. At 20 kV, significant mortality was achieved within 5–15 min, which is in line with Scholtz et al. (2015) but with shorter exposure times than what they reported. The overall trends indicate that cold plasma treatment is most effective at 20 kV and 25 kV. Compared to 15 min of exposure at lower voltages, a shorter duration of 5–10 min at 20 kV and 25 kV demonstrated greater efficiency in eliminating pests, particularly in their later life stages. The statistical significance across all treatment groups ($P < 0.05$) confirms the efficacy of this non-thermal treatment as a reliable method for post-harvest pests untreated in chickpeas, offering a residue-free alternative to traditional chemical fumigants.

Cold plasma treatment demonstrated significant mortality effects across all life stages of *C. maculatus* in chickpeas at 15, 20, and 25 kV, with increasing efficacy at higher voltages and longer exposure times. At 25 kV, larvae-2e showed the highest sensitivity, achieving 100% mortality at 10 min, while both young larvae and pupae reached over 90% mortality at shorter exposures, underscoring the treatment's efficiency in late-stage pests. At 20 kV, egg mortality

peaked at 100% at 15 min, indicating effective untreated of early life stages, which is essential for preventing pest development. At 15 kV, mortality was high across all stages with significant results even at 5 min, highlighting the treatment's broad-spectrum efficacy. Cold plasma's generation of reactive species (RONS) effectively disrupts cellular structures across stages, making it a promising, non-chemical pest untreated method in stored legumes.

The 20 kV, 15-min cold plasma treatment was identified as the optimal condition for achieving 100% mortality in early life stages, including eggs and young larvae of *C. maculatus* . These stages are particularly destructive, and by achieving complete eradication at this phase, the treatment prevents any progression to later stages like pupae and adults. Consequently, eliminating these initial stages ensures that no further development or reproduction occurs, maximizing the efficacy of untreated pests.

From **Table 3**, the bulk density of both the untreated and cold plasma-treated chickpeas remains identical at 0.76 g/cm³**,** with no noticeable change after treatment. This suggests that cold plasma treatment does not alter the macroscopic structural properties of the chickpeas to a degree that would affect the bulk density. This is likely because bulk density is influenced more by the particle packing and overall structure, which may remain relatively unchanged by cold plasma. The treatment may lead to a more uniform particle distribution, potentially affecting the flour's density, solubility, and water-holding capacity (Pankaj, et al., 2014). The preservation of bulk density indicates that cold plasma treatment does not induce significant structural breakdown or

compaction in chickpeas. This stability is advantageous for food processing applications where maintaining product integrity is crucial.

Table 3: Physical characteristics of the treated chickpeas (20 kV for 15 min) and untreated samples.

Variable	Untreated	Treatment
Bulk density (g/cm^3)	0.76±0.05 A	0.76±0.05 A
True density (g/cm^3)	6.79±0.05 A	6.81±0.06 A
1000 kernel weight (g)	348.3±0.1 A	348.3±.0.1 A
Chickpeas moisture (%)	13.9±0.1 A	13.9±0.0 A

The true density, which measures the density of the solid material excluding the air spaces, shows 6.79 – 6.81 g/cm^3 after cold plasma treatment (**Table 3**). This small change suggests that cold plasma treatment may have caused some minor internal structural modifications at the molecular level within the chickpeas. While these changes do not seem to affect the external structure (as seen from the bulk density), they could indicate slight compaction or alteration in the internal composition. Cold plasma is known for its ability to alter surface properties, and in this case, it may have caused subtle changes that increase the true density without impacting other physical characteristics (Pankaj, et al., 2014; Misra, et al., 2019).

The 1000 kernel weight remains essentially unchanged between the untreated and treated samples, with an almost identical value of around 348.3 g. This indicates that cold plasma treatment does not cause any change in nutritional quality (Bora et al., 2022; Sruthi et al., 2022). Stable kernel weight is an important indicator that plasma treatment does not result in moisture loss or degradation of the chickpeas' structure. Maintaining kernel weight is crucial in industrial

processing, as weight consistency ensures product quality (Misra, et al., 2019). There is no statistically significant difference in moisture content between the untreated (13.9%) and treated (13.9%) chickpea samples. This finding indicates that cold plasma treatment does not have a measurable impact on the moisture content of chickpeas.

By stabilizing moisture levels after cold plasma treatment, chickpeas retain their nutritional quality, physical integrity, and microbial safety, extending their shelf life without introducing chemical preservatives. This finding aligns with previous studies on atmospheric Dielectric Barrier Discharge (DBD) Cold Plasma, which confirm that non-thermal plasma treatments effectively decontaminate grains while preserving their essential properties. It also aligns with studies on the impact of cold plasma treatment on the nutritional and physicochemical characteristics of various legumes (Sani et al., 2024). These studies reported that atmospheric cold plasma treatment at 5 W for 10 min had no significant effect on the total carbohydrate, protein, and fat content of kidney beans, mung beans, and adzuki beans. Additionally, they demonstrated cold plasma's ability to sanitize and enhance microbial safety without altering the moisture content or nutritional properties of foods (Pankaj et al., 2014; Wu et al., 2023; Li et al., 2024). Additionally, cold plasma treatment has been associated with the maintenance or even enhancement of the nutritional profile of treated chickpeas (Patil et al., 2024; Bora et al., 2022).

3.5. Conclusion

This study demonstrates the efficacy of cold plasma treatment as a chemical-free method for controlling *C. maculatus* infestations in chickpeas across various life stages (eggs, larvae, pupae, and adults). Cold plasma treatment at 20 kV and 25 kV for 10 to 15 min significantly increases mortality rates for *C. maculatus*. The larvae and pupae showed the highest susceptibility, with 85.15% mortality achieved at 25 kV for 15 min.

The cold plasma treatment at 20 kV for 15 min did not influence the bulk density, true density, 1000 kernel weight, and moisture content of chickpeas. In light of these results, cold plasma technology presents a viable approach to reducing post-harvest losses and supporting food security while aligning with sustainability goals by reducing the need for harmful chemical pesticides and lowering the environmental footprint of agricultural practices. Further research should focus on optimizing treatment parameters for various grain types and evaluating the long-term impact of cold plasma on food quality during storage to harness its potential in commercial grain storage and pest control.

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Chapter 4 - Quality Characterization of Cookies Made from Cold Plasma-Treated Chickpea (*Cicer arietinum)* Flour

Abstract

Cold plasma treatment is a novel non-thermal technology with significant potential in food processing. This study investigates the impact of cold plasma-treated chickpea flour on the quality of cookies made from it. The objective was to evaluate changes in the physical, chemical, and functional properties of chickpea flour and their influence on cookie characteristics. Chickpea flour was subjected to cold plasma treatment, and cookies were analyzed for dimensions, color, texture, and nutritional properties. The experimental plan involved treating whole chickpea kernels with cold plasma before milling them into flour. The treated flour was then evaluated for its properties in cookie formulations using established methods.

Results demonstrated that cold plasma treatment enhanced cookie characteristics, with an increase in height ranging from 0.4% to 6.5%, particularly in 25 WWF and 100 CPF formulations. Width and weight were minimally affected, with variations below 3.3%. Treated cookies exhibited a darker color, with lightness (L) decreasing by up to 11 units in 100 CF formulations*, while ΔE changes remained below 2 in other formulations due to Maillard reactions. Hardness increased by 15-22% in most formulations, with 100 CF experiencing a much higher increase (~110%), suggesting improved dough structure and better moisture retention. Importantly, protein content remained stable (variation <1%), and moisture content showed no significant difference ($p > 0.05$), ensuring the nutritional integrity of the treated flour was preserved. This study concludes that cold plasma technology enhances the functional and sensory properties of bakery products, particularly in texture and color, without compromising nutritional quality.

4.1. Introduction

Cold plasma treatment is a cutting-edge, non-thermal technology revolutionizing the food processing industry by enhancing the quality and safety of food products. Particularly in chickpea flour-based applications, such as cookies, cold plasma offers a promising alternative to traditional methods. Unlike thermal or chemical treatments, cold plasma deactivates pests without relying on heat or chemical additives, earning it the reputation of being a "clean and green" technology (Pathan et al., 2022; Jaddu et al., 2024; Shabbir et al., 2024; Bayati et al., 2024; Fernandes et al., 2024).

At the core of its effectiveness is the generation of reactive oxygen species (ROS) and reactive nitrogen species (RNS)—molecules such as atomic oxygen (O), singlet oxygen ($^1O_2$), superoxide anion (O_2^-), and nitric oxide (NO•). These reactive species target and disrupt microbial cell membranes, leading to effective microbial inactivation. Additionally, the presence of water enhances the decontamination capabilities by forming hydroxyl radicals (OH•) and hydrogen peroxide (H_2O_2), which further aid in pathogen elimination (Pathan et al., 2022). Therefore, when applying cold plasma technology to chickpeas, it is essential to balance the benefits of microbial decontamination with preserving nutritional integrity (Bayati et al., 2024; Fernandes et al., 2024).

One of the attractive aspects of cold plasma is that its non-thermal nature largely preserves the nutritional integrity of chickpea flour (Yawut et al., 2024; Harikrishna et al., 2023; Birania et al., 2022; Mahnood et al., 2024). Chickpea flour, also known as gram flour or besan, is rich in protein, dietary fiber, and essential micronutrients, making it an excellent ingredient for healthy, gluten-free cookies. It contains approximately 24.7% protein and offers 476.5 kcal per 100 grams, with all eight essential amino acids. These properties make it particularly appealing to health-conscious consumers, vegetarians, and vegans, as it supports satiety and weight management

(Sharma et al., 2024; Silva et al., 2024). Treating whole chickpeas instead of flour ensures a uniform and controlled interaction with reactive oxygen species, minimizing nutrient loss while enhancing hydration and milling properties (Patra et al., 2024; Pathan et al., 2022; Shabbir et al., 2024).

In addition to improving functional properties, cold plasma enhances the interaction between water and the components of chickpea flour. This yields dough with better elasticity and moisture retention, which is crucial for achieving the desired texture in gluten-free baking (Bora et al., 2022). These advantages position cold plasma as a sustainable, forward-thinking approach that aligns with the rising global demand for healthier snack alternatives (Sharma et al., 2024; Silva et al., 2024).

The primary aim of applying cold plasma to chickpea flour is microbial decontamination; its influence on functional properties offers additional advantages in baking applications. Studies by Kaur et al. (2024) and Charoux et al. (2021) revealed that cold plasma-treated legume flours demonstrated improved protein solubility, altered hydration properties, and potentially better emulsification capacity. While these changes could enhance the texture and structure of chickpea-based cookies, they are considered secondary benefits rather than the main objective of plasma application.

Zare et al. (2022) explored the effects of atmospheric pressure cold plasma (ACP) on quinoa flour, finding improvements in starch gelatinization, protein solubility, and water absorption, which are properties relevant to cookie formulations. While quinoa differs from chickpea flour, these findings suggest potential benefits in baking applications. Felisiak et al. (2024) examined chickpea flour in shortbread cookies, assessing its quality and bioactive properties without cold plasma treatment, highlighting the need for research on its impact when

processed differently. Wang et al. (2024) investigated cold plasma-treated chickpea protein isolate (CPI), reporting enhanced solubility, emulsification, and gelation, which could be advantageous for chickpea-based baked goods, though cookies were not directly tested. Together, these studies indicate that cold plasma may enhance chickpea flour's functionality in cookie formulations, yet direct evidence remains limited.

Despite growing research on cold plasma's effects on protein isolates and whole legumes, there is little direct evidence of its impact when chickpea flour is used in cookie formulations. Most studies focus on microbial reduction and functional enhancements, but their relevance to baking applications remains unclear. Additionally, while Ruiz-Zambrano et al. (2024) highlighted the potential benefits of cold plasma in composite flours, research on how cold plasma-treated chickpea flour interacts with wheat or other gluten-free flours in hybrid formulations for baked products is still lacking. Understanding these synergies is crucial for optimizing texture, structure, and overall cookie quality. Therefore, this study aims to assess whether cold plasma treatment alters the functional properties of chickpea flour in ways that improve or hinder cookie quality and to determine if hybrid formulations combining cold plasma-treated chickpea flour with wheat flour maintain structural integrity and enhance cookie texture.

4.2. Materials and Method

4.2.1. Milling

Chickpeas were milled using a laboratory-scale Ross roller mill (Model 915, Ross Machine and Mill Supply, Oklahoma City, OK, USA) following the procedure developed by Pulivarthi et al. (2021). Before milling, the chickpeas underwent cold plasma treatment at 25 kV for 15 min to ensure microbial decontamination while preserving nutritional and functional integrity. This

treatment aligns with the study's objective of evaluating whether cold plasma alters the functional properties of chickpea flour in ways that impact cookie quality and assesses its synergy in hybrid formulations with wheat flour. Chickpeas were tempered overnight to a moisture content of 13%. The roll gaps were adjusted according to the roll gaps sheet, as mentioned in **Table 4**. The "break" process is the initial step in milling grain into flour. The break purpose is to crack open the grain kernel and separate its components. The "reduction" process follows the break system and involves refining the coarse endosperm fragments into fine flour. Chickpeas were milled into flour (< 250 μm) and then sieved to remove larger particles, thereby minimizing contamination and ensuring a uniform particle size. The flour yield is calculated by dividing the weight of flour obtained by the original weight of grains.

Table 4: Roller Milling Parameters and Flour Yield for Chickpea Processing (Corr- Corrugations per inch).

Breaks	Corr./in	Roll gaps (μm)
1st Break	15	0.11
2nd Break	15	0.055
3rd Break	25	0.025
1st Reduction	No Corr.	0.008
2nd Reduction	No Corr	0.003
Flour yield (%)	-	86.6±0.5

4.2.2. Flour Characterization

Particle size and mean geometric diameter analysis of the chickpea flour was performed using the Ro-tap sieve [ANSI/ASAE S319.4 FEB2008 (R2017 ED)] method. 100 g of flour was

passed on to the top sieve 4760 (µm) US sieve size, as mentioned in **Table 5**. Contents were tapped for 10 min and the mass of material on all sieves was determined and recorded.

Table 5: Ro-tap sieve numbers and sizes and mean (±SE) mass of each material retained

US Sieve Number	US Sieve Size (di) (µm)	Mass Retained on the sieve (Mean ± SE) (g)
4	4760	0±0
6	3360	0±0
8	2380	0±0
12	1680	0±0
16	1190	0±0
20	841	0±0
30	595	0±0
40	420	0.6±0
50	297	50.1±0.01
70	210	43.0±0.06
100	149	4.1±1.0
140	105	1.4±0.1
200	74	0±0
270	53	0±0
Pan	-	0±0

Table 6: Impact of cold plasma treatment on the geometric mean diameter (±SE) of chickpea flour.

Flour	Geometric Mean Diameter ±*SE* (µm)	
	Control (Mean ± SE)	Treated (Mean ± SE)
Chickpea flour	291.6 ±1.13	289.7 ±1.34

The Geometric Mean Diameter (GMD) tabulated in **Table 6** is calculated as follows:

Geometric Mean Diameter (GMD)

$$GMD = exp\left(\frac{\sum(w_i \ . \ ln D_i)}{\sum w_i}\right) \quad (2)$$

where w_i is the weight fraction, and D_i is particle size in μm.

4.2.3. Proximate Composition and Starch Damage

Ash, protein, and moisture were determined by following the AACC method 08-01.01, 46-30.01, 44-17.01 (AACC International, 2010, 2016; AACC Approved Methods of Analysis, 1961; AACC Approved Methods of Analysis, 1999), respectively. Flour starch damage was measured using SDmatic TM equipment (Chopin Technologies, Villeneuve la Garenne, France) following the AACC 76–33.01 method (AACC Approved Methods of Analysis, 2007). In short, 1.5 g of citric acid powder, 3 g of potassium iodide powder, and one drop of 0.1*N* sodium thiosulphate solution were mixed with 120 mL of distilled water in the SDmatic glass reaction vessel. Then, 1 g of flour sample was added to the sample holder, and the test was initiated. The SDmatic operates by quantifying the ability of damaged starch to hydrolyze rapidly under controlled conditions and measures the amount of damaged starch in it. All the proximate composition data are reported on a dry basis.

4.2.4. Bulk Density

The bulk density of the chickpea flour was measured using a standard Winchester cup arrangement (Seedburo Equipment Co., Des Plaines, IL, USA). The sample was allowed to fall from a height of 10 cm into an empty cup until it overflowed. A scraper was used to remove the excess sample over the cup, and the bulk density was measured by taking the final sample weight (g) and cup volume using the expression below.

Equation for calculating bulk density

$$Bulk\ Density\ (g/cm^3) = \frac{Weight\ of\ Sample\ (g)}{Volume\ (cm^3)} \quad (3)$$

4.2.5. True Density

The true density of the chickpea flour fractions was measured using a helium gas pycnometer (AccuPync II 1340, Micromeritics, Norcross, Georgia). The helium gas was passed through the chamber to determine the volume occupied by solid particles by filling the pore spaces between and within the grain/flour fractions. The weight and volume occupied by the sample were used to obtain true density. The mean value of true densities was obtained from three replications for each sample. The Hausner's ratio and porosity of the flour samples were determined from the following Equations. These measurements provide essential insights into the flowability and packing characteristics of the flour, which are crucial for processing and formulation in the food industry.

Equations for evaluating flow and packing properties of powders

$$Hausner's\ ratio = \frac{Tap\ density}{Bulk\ density} \quad (4)$$

$$Porosity\ (\%)\ = \left(1 - \frac{Bulk\ density}{True\ density}\right) \times 100 \quad (5)$$

4.2.6. Cookies Formulations

Cookies were made according to the AACC 10-53.01 Macro Wire cut formulation method. The milled Chickpea flour was mixed with shortening and other dry and wet ingredients (**Table 7**) in a Kitchen Aid mixer until they were uniformly mixed. The dough was rolled out using an adjustable rolling pin fitted with 7 mm guide rings to achieve a uniform thickness. A circular dough cutter (8 cm diameter) was used to shape the dough. The prepared dough pieces were then baked in a calibrated convection oven set to 400˚F (204.44˚C) for 11 min, with the baking time precisely monitored using a digital timer

Table 7: Ingredient Composition for Cookie Formulation at 21 ± 1°C (70 ± 2°F)

Ingredients at 21 ± 1˚C	Weight (g)
Sucrose	94.5
Non-fat dry milk	2.3
NaCl	2.8
Sodium bicarbonate	2.3
All-purpose shortening	90.0
High-fructose corn syrup (HFCS), 42%	3.4
Ammonium bicarbonate	1.10
Deionized water; 225g flour + 49.5g	Variable (274.5)

Flour	225.0

4.3. Physical Evaluation of Cookies

4.3.1. Color measurement

Surface color plays a crucial role in the overall appeal of cookies, as it reflects the extent of thermal energy applied during baking and influences consumer perception. The color of the cookies was assessed using units L*a*b*, which is an international standard for color measurements, adopted by the Commission Internationale de l'éclairage (CIE). A point in the CIE color space is defined by these three components of L*a*b* where L* indicates the lightness (black to white), and parameters a* (from green to red) and b* (from blue to yellow) indicate the chromatic coordinates. The color measurement of fresh and processed samples was carried out with a colorimeter (xHP-200 colorimeter, Hanpu Guangdian Science and Technology Ltd., China) in terms of L^*, a^*, and b^* values. To figure out the changes in the surface color of cookies, the total color change (ΔE*) with time was determined using the following equation;

Equation for total color difference (ΔE) in CIE Lab color space

$$\Delta E = \sqrt{(L_2^* - L_1^*)^2 + (a_2^* - a_1^*)^2 + (b_2^* - b_1^*)^2} \quad (6)$$

where L_1*, a_1*, and b_1* were the values of three components of CIE color space for the cookie dough before baking. L_2*, a_2*, and b_2* represented the values for cookies at any time of baking. Before using the colorimeter, calibration was performed using black and white standard plates. To reduce the experimental errors influenced by irregular color on the cookie surface, measurements were carried out at least 6 times per group.

4.3.2. Cookies height, weight, and width measurement

Cookie diameter (cm) was measured by laying six cookies edge to edge with the help of a scale and then rotating them by 90°and re-measuring. The average diameter of the cookies was calculated accordingly. According to the American Association of Cereal Chemists (AACC), cookie height (cm) was determined by stacking six cookies on top of one another, restacking, and re-measuring. The average height of the six cookies was measured. Cookie weight is recorded by taking the weight of each cookie individually. These measurements ensure consistency in cookie dimensions and weight, allowing for accurate comparisons.

4.3.3. Cookie texture

A texture analyzer (TAXT2, Stable Microsystems, USA) equipped with a 50 kg-f load cell was used for cookie texture evaluation. Cookies were evaluated for hardness within 24 h by measuring the peak force during breaking using a TA-42 knife blade with a 45° chisel end and bell lock. The analyzer was set at a 'return to start' cycle, a pre-test, test, and post-test speed of 1.5 mm/s, 2 mm/s, and 10 mm/s, respectively, and a penetration distance of 5 mm. A force/penetration plot was made for every test. The cookie spread ratio was calculated as follows.

Equation for calculating the Spread Ratio (SR)

$$Spread\ Ratio\ (SR) = \frac{Diameter\ of\ the\ Cookie\ (mm)}{Height\ of\ the\ Cookie\ (mm)} \quad (7)$$

4.4. Data Analysis

The results from all treatments were conducted in triplicates. SAS software (version 9.4, SAS Institute Inc., Cary, NC) was used for all statistical analyses. ANOVA was employed to assess

the significant differences between treatments across multiple variables, helping identify any statistically significant effects of different factors on cold plasma-treated chickpea flour. Post-hoc analysis with Tukey's honest significant difference (HSD) test was conducted to determine which specific treatments differed from one another.

4.5. Results and Discussion

From **Table 8**, the control and treated samples have an identical protein content of 24.5%, suggesting that cold plasma treatment does not affect the protein concentration in chickpea flour. mThis is consistent with findings by Misra et al. (2019), who reported that non-thermal plasma treatments typically do not alter protein content because proteins are relatively stable under the conditions used for plasma exposure. Similarly, Dharini et al. (2023) confirmed that cold plasma treatment preserves the primary structure of proteins, ensuring no significant degradation or loss in protein levels. Ash content, representing total mineral content, was slightly higher in the treated samples (2.7%) compared to the control (2.63%).

Table 8: Nutritional and physical properties of chickpea flour after cold plasma treatment. Dissimilar alphabets (a, b) denote that the mean values for particular variables are statistically different at $p<0.05$. Pr-Probability factor.

Variable	Control Mean(±SE)	Treatment Mean(±SE)	F-value	Pr>F	Df 1	Df 2
Protein content (%)	24.5±0.00	24.5±0.00	-	-	1	4
Ash (%)	2.63±0.05	2.7±0.15	4.50	0.1012	1	4
True density (g/cm^3)	4.61±0.01 a	4.62±0.02 a	931.15	< 0.0001	1	4
Damage starch (%)	88.33±5.21	90.93±0.65	1.22	0.3315	1	4
Chickpeas moisture (%)	12.41±12.41	12.44±0.11	0.18	0.6924	1	4

Bulk density (kg/m^3)	0.56±0.05	0.57±0.002	0.09	0.7792	1	4

However, the difference was not statistically significant (P=0.1012). This observation aligns with studies by Thirumdas et al. (2015) and Bourke et al. (2018), which demonstrated that cold plasma treatment does not significantly impact mineral content as it primarily affects surface characteristics rather than altering inherent chemical compositions.

The treated chickpea flour had a slightly higher percentage of damaged starch (90.9%) compared to the control (88.3%), but the difference was not statistically significant (P=0.3315). Dharini et al. (2023) also observed that cold plasma treatment may minimally increase starch damage due to the physical and chemical interactions of reactive species with starch granules. However, as shown by Thirumdas et al. (2017), the increase is typically not substantial unless high-energy plasma conditions are employed, which was not the case here. The moisture content remained unchanged between the control (12.4%) and treated (12.4%) samples, supporting the conclusion that cold plasma treatment does not significantly impact moisture levels. This observation is consistent with the findings of Jaddu et al. (2020), who reported that plasma treatments primarily modify surface properties and have minimal influence on bulk moisture content.

Misra et al. (2015) also highlighted that the non-thermal nature of plasma avoids evaporation or absorption of water, preserving the sample's moisture balance. Further, the protein and ash content in dry chickpeas were 24.5 % and 2.7 %, whereas the moisture content in the chickpea flour was 12.4%. These data are in line with the literature reports. For instance, the protein content in dry chickpeas ranges from 19.9% to 25.5%, while the ash content ranges from 2.80% to 3.42% (Khan et al., 1995; Rincon et al., 1998; Singh et al., 2004). The moisture content

in chickpea flour varies between 7.9% and 20.9%, depending on the variety (Durakova et al., 2005; Debnath et al., 2003; Guo et al., 2008).

Bulk density showed negligible differences between the control (0.56 g/cm³) and treated samples (0.57 g/cm³), indicating no significant effect of the treatment. As noted by Thirumdas et al. (2015) and Jaddu et al. (2020), non-thermal plasma does not cause extensive mechanical or thermal disruption that could affect particle packing or flowability, leading to no substantial changes in bulk density. The particle size distribution from **Figure 5** reveals that both the control and treated samples display a peak in sample retention around sieve numbers 40-50, indicating a dominant particle size within this range. This trend suggests that cold plasma treatment, applied to grains before milling, did not induce significant changes in the particle size distribution of the resulting flour. This consistency is expected since cold plasma treatment primarily modifies the surface of the grains without affecting their internal structural properties, which subsequently determine flour particle size during milling. This observation aligns with the findings of Pankaj et al. (2014), who reported that short-duration plasma treatment impacts surface properties like porosity or microbial decontamination without altering bulk structural characteristics.

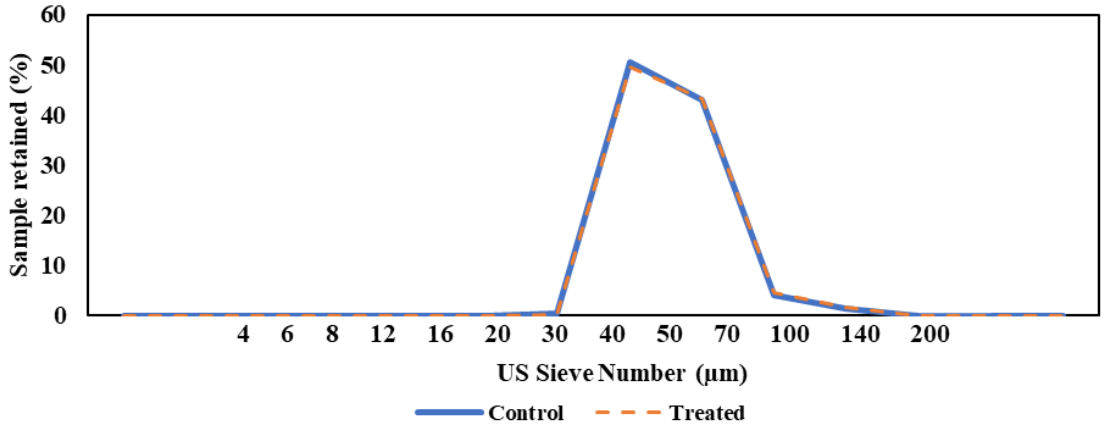

Figure 5: Particle size distribution of cold plasma-treated chickpea flour

The graph further supports the conclusion that cold plasma-treated and untreated samples exhibit comparable functional qualities in terms of particle size distribution, an important factor influencing flour properties such as water absorption and dough handling. The retention of this distribution after treatment highlights the suitability of cold plasma as a non-invasive, non-thermal processing method. Research by Misra et al. (2019) confirms that under moderate power and exposure conditions, cold plasma minimally disrupts the physical integrity of grains while maintaining their core attributes. This ensures the functional consistency of treated flour, particularly in applications such as baking.

The hardness of the control samples (100% chickpea flour) shows a lower value (1.28±0.15 Kg) compared to the treated sample (2.69±0.129 Kg). Chickpea flour tends to have a high protein content and lower gluten than wheat flour, which can result in a crumblier texture in the untreated sample. However, after treatment, the higher hardness may result from changes in the protein network or interactions between starch and proteins during the baking process, leading to a firmer structure (Rout et al., 2024; Chaple et al., 2020; Sarkar et al., 2023). This hardness increase post-treatment may also be attributed to improved water absorption, which is crucial in maintaining the moisture and freshness of cookies (Bora et al., 2022).

At 25% WWF substitution, both control (2.51±0.21 Kg (**Table 9**)) and treated (2.53±0.217 Kg (**Table 10**)) samples maintain relatively high hardness values. This suggests that the introduction of whole wheat flour strengthens the structure of the cookies due to its fiber content and gluten, contributing to a more robust texture. The minimal difference in hardness between the control and treated samples indicates that the treatment may not have significantly altered the internal structure at this substitution level, and the high fiber content in WWF may have helped balance the effects of treatment by maintaining a consistent hardness. The observed increase in

hardness in the treated sample (2.25 ± 0.138 Kg) compared to the control (2.02 ± 0.53 Kg) at 50% WWF substitution could be attributed to the effects of cold plasma on the flour's functional properties rather than directly on the hardness itself.

Table 9: Effect of non-cold plasma treatment on color parameters (L*, a*, b*) and hardness (kg-F) of chickpea flour (CF) and whole wheat flour (WWF) blend cookies. CF- Chickpea Flour; WWF- Whole Wheat Flour.

Proportion	Control			
	*L**Mean(±SE)	*a**Mean(±SE)	*b**Mean(±SE)	Hardness (N)Mean(±SE)
100 CF	37.05±5.26A,a	7.41±0.625B,b	23.01±2.89A,a	1.28±0.15B,b
25 WWF	25.86±1.66A, a	10.35±1.35A,a	16.09±0.55A,c	2.51±0.21A,a
50 WWF	28.52±4.43A,a	8.91±0.71A,a	17.2±2.00A,b	2.02±0.53A,a
75 WWF	25.16±1.2B, b	9.87±1.14A,a	16.09±0.46A,c	1.91±0.15A,b

Table 10: Effect of cold plasma treatment on color parameters (l*, a*, b*) and hardness (kg) of chickpea flour (CF) and whole wheat flour (WWF) blend cookies. CF- Chickpea Flour; WWF- Whole Wheat Flour.

Proportion	Treated			
	*L** Mean(±SE)	*a** Mean(±SE)	*b** Mean(±SE)	Hardness (N)Mean(±SE)
100 CF	25.78±0.39B,a	10.90±0.67A,a	17.4±0.52B, a	2.69±0.12A,a
25 WWF	25.01±1.20A,a	10.09±0.40A,a	16.49±0.63A,b	2.53±0.21A,a
50 WWF	27.19±0.89B,a	8.75±0.70A,a	16.09±0.92B,b	2.25±0.13A,a
75 WWF	27.2±0.31A,a	9.24±0.29AB,a	16.4±0.29A,b	2.26±0.14A,a

Cold plasma treatment primarily modifies the surface chemistry of flour particles, enhancing water absorption, protein solubility, and cross-linking interactions between proteins and starch. These changes indirectly influence the dough and baked product's textural properties, such as hardness (Chaple et al.,2020; Sarkar et al.,2023). The chickpea flour’s impact on hardness

appears to be moderated as the gluten network from WWF supports the dough's structure, even post-treatment. The increase in hardness after treatment is marginal, suggesting that chickpea flour's protein-starch interactions continue to influence texture, but the presence of whole wheat flour limits drastic changes. For the 75% WWF substitution, the control sample's hardness is 1.91±0.15 Kg, with a slightly higher value in the treated sample (2.26±0.146 Kg). At high levels of WWF substitution, the texture of the baked product is primarily governed by the gluten network from WWF, which provides structural integrity and prevents excessive hardening.

Cold plasma treatment is known to enhance protein functionality and starch-protein interactions, which can result in increased dough strength and subsequent firmness in baked goods (Misra et al., 2019; Zhang et al., 2021). The stabilization of hardness in the samples with whole wheat flour (WWF) substitution suggests that the gluten network in WWF provides structural elasticity. This elasticity balances the effects of chickpea flour's high protein and compact structure by introducing flexibility to the matrix. Cold plasma treatment may enhance these effects by increasing water absorption and promoting better integration of the two flour types (Bourke et al., 2018; Thirumdas et al., 2017).

The heat applied during baking contributes to protein denaturation and moisture loss, which are important in the formation of a compact structure in chickpea-based cookies. However, cold plasma treatment further influences these outcomes by modifying protein and starch surface properties before baking. The less pronounced changes in hardness with higher proportions of WWF may indicate that the elastic gluten network minimizes the effects of cold plasma-enhanced protein interactions on textural properties.

The L* (lightness), a* (redness), and b* (yellowness) values indicate color changes that can be linked to the baking process and ingredient interactions. Higher proportions of whole wheat

flour tend to darken the cookies, as reflected by the decrease in L* values. This is expected since whole wheat flour has a darker appearance than chickpea flour. The a* and b* values show minor changes across the samples, with some influence from chickpea flour and whole wheat flour. Color is also influenced by the Maillard reaction during baking, especially in the treated samples, which can lead to browning and caramelization, contributing to changes in hardness and texture.

Equations for calculating the Browning Index (BI), an indicator of browning intensity in food products

$$Browning\ Index\ (BI)\ =\ \frac{100\ (\ X-0.31)}{0.17} \tag{8}$$

$$X\ =\ \frac{(\ a^*\ +\ 1.75L^*)}{(5.645\ L^*\ +\ a^*\ -3.012\ b^*)} \tag{9}$$

Table 11: BI index for control and treated chickpea cookies of different proportions varying from 25-100%.

	Control		Treatment	
Proportion	X	Browning Index (BI)	X	Browning Index (BI)
100 CF	0.490641	106.2596	0.514978	120.5755
25 WWF	0.515498	120.881	0.518741	122.7886
50 WWF	0.498057	110.6216	0.534904	132.2967
75 WWF	0.5211	124.1762	0.527427	127.8979

The browning Index (BI) analysis from **Table 11** reveals that the treated samples exhibit higher browning at 100% Control Flour (CF) and 25% Whole Wheat Flour (WWF), suggesting increased Maillard reactions or enzymatic browning in these compositions. However, at higher WWF levels (50% and 75%), the treatment results in a lower BI, indicating reduced browning. Lightness (L*) decreases significantly in treated 100% CF, making it darker, but at higher WWF

levels, the treated samples appear slightly lighter than the control. Overall, the treatment enhances browning in lower WWF compositions but reduces it in higher WWF samples, likely due to variations in moisture content, sugar composition, or enzyme activity affecting color development.

The control sample has a relatively high L* value (37.05±5.26) compared to the treated sample (25.78±0.39), indicating that the control cookies were significantly lighter than the treated ones. The decrease in lightness after treatment is consistent with browning reactions, particularly the Maillard reaction, which occurs between reducing sugars and proteins in chickpea flour. This reaction typically intensifies during baking, resulting in a darker color. During baking, color changes in the final product occur due to Maillard reactions, caramelization, and pigment degradation, which influence the browning intensity and overall appearance. Factors such as ingredient composition, temperature, baking time, and moisture content play a crucial role in determining the extent of browning. The interaction between reducing sugars and amino acids leads to the formation of brown pigments, contributing to the characteristic baked color and texture

4.5.1. Color Analysis

Cold plasma treatment significantly influenced the color parameters (L*, a*, b*), particularly in 100% chickpea flour (CF) cookies, while its impact diminished with increasing whole wheat flour (WWF) substitution due to the dominance of wheat pigments. Lightness (L*) decreased significantly in 100% CF cookies (37.05 ± 5.26 → 25.78 ± 0.39), suggesting cold plasma-induced oxidation of pigments, enhancing the Maillard reaction and resulting in darker coloration. However, at higher WWF substitutions (25–75%), L* values showed minimal variation, indicating that whole wheat's natural pigments masked the impact of cold plasma. Interestingly, at 75% WWF, treated cookies were slightly lighter than the control, possibly due to

reduced enzymatic browning from plasma treatment. Redness (a*) increased in 100% CF cookies (7.41 ± 0.625 → 10.90 ± 0.67) post-treatment, aligning with enhanced Maillard reactions and caramelization.

However, at higher WWF levels, the effect was negligible, as whole wheat's inherent pigments dominated the color profile. Yellowness (b*) significantly decreased in 100% CF cookies (23.01 ± 2.89 → 17.4 ± 0.52), likely due to cold plasma-induced carotenoid degradation followed by baking-induced caramelization. At higher WWF substitutions (25–75%), b* values remained stable, suggesting WWF's natural pigments buffered any changes from plasma treatment. Cold plasma had a darkening effect on 100% CF cookies, mainly due to pigment oxidation and intensified Maillard reactions. However, at higher WWF levels, the influence diminished, as wheat's natural pigments masked changes in lightness, redness, and yellowness.

4.5.2. Width

sFrom **Table 12** and **Table 13,** across all compositions, the width of both control and treated cookies ranged between 8.4 and 8.6 cm. Control cookies at 25% WWF showed a width of 8.5 cm compared to treated cookies, which had a width of 8.4 cm. At 50% WWF, both control and treated cookies had the same width (8.4 cm), suggesting no significant impact of treatment at this composition level. However, at 100% CPF, the treated cookies had a slightly larger width (8.6 cm) compared to the control cookies (8.5 cm). This could be attributed to the higher water absorption capacity of chickpea flour following cold plasma treatment, which enhances dough hydration and elasticity, allowing for more spread during baking. Singh et al. (1991) reported that chickpea flour absorbs more moisture due to its high protein content, which can influence dough consistency and spreading behavior. Sarkar et al. (2023) also highlighted that cold plasma-treated flours exhibit

enhanced hydration properties, leading to subtle changes in dough flow and spread. The impact of cold plasma is subtle and largely depends on flour composition (Misra et al., 2019; Zhang et al., 2021). Two-way ANOVA revealed no significant effect of flour composition ($F(3,32)=1.04, p=0.39$)($F(3,32) = 1.04, p = 0.39$)($F(3,32)=1.04, p=0.39$), treatment ($F(1,32)=0.63, p=0.44$)($F(1,32) = 0.63, p = 0.44$)($F(1,32)=0.63, p=0.44$), or their interaction ($F(3,32)=0.75, p=0.54$)($F(3,32) = 0.75, p = 0.54$)($F(3,32)=0.75, p=0.54$) on cookie width

Table 12: Mean (±SE) Cookies dimensions for control (non-cold plasma treated) samples. Different upper case letters (A, B, C, D) signify that the corresponding mean values are statistically dissimilar at $p<0.05$ across the column (or chickpea proportion). The mean values for width, height, or weight sharing the same lowercase letter (a or b) in a row are not significantly different within the control and treated samples.

Composition	Control		
	Width(cm) Mean(±SE)	Height (mm) Mean (±SE)	Weight (g) Mean (±SE)
25 WWF	8.5±0.05AB,a	84.7±0.50E,a	37.3±3.4A,a
50 WWF	8.4±0.07ABC,a	86.3±0.88D,a	38.1±1.13A,a
75 WWF	8.4±0.05BC,a	94.7±0.97A,a	37.7±3.00A,a
100 CPF	8.5±0.05AB,a	84.7±0.55E,a	35.8±0.71A,a

Table 13: Cookies dimensions for cold plasma treated samples. Different upper case letters (A, B, C, D) signify that the corresponding mean values are statistically dissimilar at $p<0.05$ across the column (or chickpea proportion). The mean values for width, height, or weight sharing the same lowercase letter (a or b) in a row are not significantly different within the control and treated samples.

Composition	Treated		
	Width (cm) Mean(±SE)	Height (mm) Mean(±SE)	Weight (g) Mean(±SE)
25 WWF	8.4±0.05BC,a	89.4±0.52B,a	36.9±2.00A,a

50 WWF	8.4±0.08BC,a	87.3±0.38CD,a	37.2±0.60A,b
75 WWF	8.4±0.06BC,a	95.1±0.74A,a	36.8±0.35A,b
100 CPF	8.6±0.08A,a	88.1±0.48C,b	36.0±0.86A,a

4.5.3. Height

The height of the cookies exhibited more variations between the control and treated groups across different flour compositions. At 25% WWF substitution, the treated cookies had a height of 89.4 ± 0.52 mm compared to the control's 84.7 ± 0.50 mm, indicating a statistical increase. This can be attributed to the enhanced water absorption capacity and protein solubility induced by cold plasma treatment (Misra et al., 2019; Zhang et al., 2021), which likely led to better gas retention during baking and an increase in dough expansion. As the proportion of WWF increased, the differences in height became consistent. At 50% WWF substitution, the treated cookies (87.3 ± 0.38 mm) maintained a slightly higher than the control (86.3 ± 0.88 mm), but the difference was less pronounced. This stabilization can be attributed to the dominant gluten network from WWF, which regulates dough elasticity and limits excessive expansion, even in the presence of plasma-treated flour.

Interestingly, at 75% WWF, the treated cookies exhibited a height of 95.1 ± 0.74 mm, slightly higher than the control (94.7 ± 0.97 mm), showing that whole wheat flour's structural integrity supports a uniform rise during baking, irrespective of treatment. For 100% CPF, the treated cookies (88.1 ± 0.48 mm) had a significantly higher height than the control (84.7 ± 0.55 mm), reflecting cold plasma's ability to improve hydration and protein functionality, leading to enhanced dough aeration and baking performance.

4.5.4. Weight

The weights of control and treated chickpeas-baked cookies did not show large variability across the different compositions, suggesting that cold plasma treatment had a minimal impact on the overall weight of the cookies. For the 25% WWF cookies, control and treated cookies exhibited similar weights (37.3 g and 36.9 g, respectively), indicating that treatment had no significant effect on weight at this level. However, at 50% WWF and 75% WWF, a slight but reduction in weight was observed in the treated cookies. The treated 50% WWF cookies weighed 37.2 g compared to 38.1 g in the control sample, while the treated 75% WWF cookies weighed 36.8 g versus 37.7 g in the control group. This could be due to water evaporation or less moisture retention in treated samples during baking (Misra et al., 2019; Thirumdas et al., 2017). For 100% CPF, the weight difference between control and treated cookies was negligible (35.8 g vs. 36.0 g), suggesting that chickpea flour's unique properties may have played a role in stabilizing weight regardless of treatment (Zhang et al., 2021). Chickpea flour’s proteins can form dense networks during baking, which may retain moisture more effectively compared to wheat flour (Sarkar et al., 2023; Yadav et al., 2012).

The results suggest that while treatment had minimal impact on cookie width, it is affected the height and, to a lesser extent, the weight of the cookies. The differences in cookie height and weight can be attributed to the flour composition and the specific interactions between the ingredients under the conditions of treatment. Specifically, chickpea flour appeared to respond differently than whole wheat flour, possibly due to its higher protein and fiber content, which could affect dough structure and moisture retention. The observed changes in height, particularly the increased height in treated samples, could be beneficial in terms of product texture and appearance.

The geometric mean diameter (GMD) of chickpea flour showed no statistical differences between the control (291.6 ± 1.13 μm) and treated samples (289.7 ± 1.34 μm). The observed stability in particle size is consistent with studies that emphasize the non-thermal nature of cold plasma, which avoids excessive mechanical or thermal stress that could lead to particle breakdown. Thirumdas et al. (2017) also noted that cold plasma does not significantly affect the bulk physical dimensions of treated grains or flours. From a functional perspective, preserving particle size distribution indicates that cold plasma treatment does not compromise the flour's ability to form cohesive dough. This stability is particularly important for applications in baking, where particle size influences texture, water absorption, and dough handling.

Table 14: Cookies spread ratio. Values within the same row followed by different superscript letters (A and B) are significantly different ($p < 0.05$) based on a statistical significance test (e.g., t-test). Values followed by the same superscript letter (A) indicate no statistically significant difference ($p > 0.05$) between the Control and Treated group.

Composition	Control	Treated
0 % WWF	8.05±**0.09** A	7.80±**0.10** B
25% WWF	8.07±0.06 A	7.53±0.08 B
50% WWF	7.86±**0.13** A	7.72±**0.08** A
75% WWF	7.13±**0.06** A	7.06±**0.09** A
100% WWF	7.51±**0.70** A	7.80±**0.07** A

From **Table 14**, the spread ratio of cookies demonstrates a marked decrease as the proportion of whole wheat flour (WWF) increases from 0% to 75% in both control and treated samples. However, the spread ratio of the treated group rebounds slightly at 100% WWF, surpassing its control counterpart. The progressive decrease in the spread ratio with increasing

WWF could be attributed to the higher fiber content, which reduces dough extensibility and moisture mobility (Kaur et al., 2017; Zouari et al., 2016). The treated group's improvement in the spread ratio at 100% WWF suggests a possible enzymatic or thermal modification of the dough properties (Kumar & Sudha, 2021; Adeyeye, 2016). Zhao et al. (2020) noted that whole-grain substitutions generally reduced spread ratios due to the lower extensibility of whole-grain doughs. The slight improvement at 100% WWF may be linked to specific interactions between treatment and whole-grain properties. Cold plasma does not seem to impact the spread ratio at lower WWF levels (0%–75%). At 100% WWF, plasma treatment appears to enhance spread, possibly due to structural, hydration, or enzymatic modifications.

4.6. Conclusions

This study demonstrates that the application of cold plasma technology to chickpea flour has the potential to enhance its functional properties, making it a promising tool for improving the quality characteristics of cookies. The treatment was found to preserve the structural and functional integrity of the flour, with minimal changes in attributes such as particle size and composition. These findings underscore the suitability of cold plasma for non-thermal food processing, ensuring quality retention while enabling innovative product development. By maintaining the nutritional and functional properties of chickpea flour, cold plasma treatment offers an effective approach. Furthermore, the ability of cold plasma to modify surface properties without altering the core composition of chickpea flour presents opportunities for broader applications in the food industry. Enhanced hydration and protein functionality may lead to improved dough aeration, texture, and overall baking performance, making it particularly beneficial for gluten-free formulations. Additionally, the non-thermal nature of the treatment minimizes potential nutrient degradation, ensuring that the final product retains its nutritional value. Future research can explore optimizing

plasma parameters to maximize these benefits while evaluating long-term storage stability and consumer acceptance of plasma-treated flour-based products.

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Chapter 5 - Summary and Future Work

This study explored the efficacy of the nonthermal treatment of cold plasma (CP) technology as a sustainable method for killing *C. maculatus* (cowpea weevil) pests in chickpeas. This research focused on applying cold plasma treatment across different life stages (eggs, larvae, pupae, adults) of cowpea weevil at various voltage levels (15, 20, and 25 kV) at different exposure times (5, 10, and 15 min). Based on the results of this research, the following conclusions were made,

In this study on insect mortality induced by cold plasma treatment, complete (100%) mortality was observed in the larvae-2l stage of *C. maculatus* following exposure to 25 kV for 10 min. The eggs were identified as the most sensitive life stage, with 100% mortality reached at a lower voltage of 20 kV for 15 min. Conversely, the pupae were the most resistant life stage, requiring 25 kV for 10 min to achieve 96% mortality; however, 100% mortality was recorded in larvae-2e under the same conditions. The optimal treatment conditions were determined by evaluating maximum insect mortality, minimum exposure time, and energy efficiency. Although 25 kV for 10 min proved to be the most effective overall, the treatment of 20 kV for 15 min also resulted in high mortality rates for egg stage (100%), young larvae (90%), larvae-2e, and pupae(93%) indicating its potential as an efficient alternative.

The impact of cold plasma treatment on grain and chickpea flour was assessed in several key areas. Notably, there was no significant impact on the milling yield following the cold plasma treatment, indicating that the process does not adversely affect the quantity of flour produced. The proximate composition of the chickpea flour remained largely unchanged, except a reduction in true density, suggesting that the overall nutritional profile of the flour was preserved. However,

grain surface modifications and oxidation effects were observed, which resulted in noticeable color changes and potential structural alterations in the chickpeas. These modifications may have implications for the functionality of the flour, warranting further analysis to fully understand their effects on properties such as texture, baking quality, and overall usability in food applications.

The effects of cold plasma treatment on cookie quality were evaluated across various formulations. The width of the cookies remained largely unchanged, indicating consistency in size regardless of the flour composition. However, notable changes were observed in the hardness of the cookies, particularly in those made with 100% chickpea flour (CF) samples. Additionally, significant color changes were detected in the 100% CF cookies, characterized by a decrease in lightness (L) and yellowness (b), alongside an increase in redness (a). These color alterations suggest an enhanced Maillard reaction resulting from the cold plasma treatment, which may contribute to flavor development and overall appearance. Despite these changes, the overall quality of the chickpea flour remained stable, successfully preserving key nutritional and functional properties essential for cookie formulation.

Overall, this research highlights the effectiveness of cold plasma treatment as a non-thermal method for managing insect populations and preserving food quality. The findings demonstrate that cold plasma can achieve complete insect mortality across various life stages while maintaining the milling yield and proximate composition of chickpea flour. The observed modifications in grain surface properties and color changes in cookies suggest potential improvements in flavor and shelf life, indicating that cold plasma could be a valuable addition to food processing technologies.

It suggests that cold plasma not only serves as an effective alternative to traditional chemical fumigation methods but also has the potential to enhance the overall quality and safety

of food products. By optimizing treatment parameters and exploring commercial scalability, stakeholders can leverage cold plasma technology to address pressing food safety concerns while promoting environmentally sustainable practices. The ability of cold plasma to reduce microbial contamination and inhibit pest infestations further supports its application in extending shelf life and improving the storage stability of food products, making it a promising approach for the future of food preservation and pest management.

Based on the key findings of this study, the following recommendations are proposed. First, regulatory bodies and stakeholders should consider implementing cold plasma as a viable alternative to chemical fumigation, as it can enhance food safety while minimizing environmental impact. Second, further research is necessary to optimize cold plasma parameters, including voltage levels and exposure times, for a diverse range of grain types and pest species to maximize its effectiveness. Additionally, exploring the feasibility of scaling up cold plasma technology for commercial applications is crucial, with a focus on cost-effectiveness and integration into existing storage infrastructure. Lastly, cold plasma treatment shows promise in extending shelf life and improving storage stability by reducing microbial contamination, inhibiting insect infestation, and modifying surface properties to enhance preservation. Implementing these recommendations could lead to more sustainable and effective pest management and food preservation practices.

Appendix 1: *C. maculatus* mortality %

The mortality % was calculated by directly counting the number of insects that did not survive and develop into the adult stage after treatment. This initial measurement provided a simple indication of the overall impact of the plasma treatment on *C. maculatus* populations. However, % mortality does not account for any natural mortality that may have occurred in the untreated control group, which could skew the assessment of treatment effectiveness. Therefore, % mortality was used primarily as a preliminary indicator, and further analysis with corrected mortality(Abbott's) was conducted, as reported in Chapter 3 to refine the evaluation of the cold plasma treatment's efficacy.

Mortality rates at 25 kV for different treatment durations across various life stages

Egg mortality

From **Table 15** the data show that cold plasma treatment impacts egg mortality at varying exposure times. At egg mortality was 20.0%. A 5-minute treatment raised mortality to 26.7%, though this increase was not statistically significant (P = 0.0589). Mortality sharply increased to 86.7% after 10 min, indicating this as the most effective exposure duration (Krik-Bradley et al., 2023). However, at 15 min, mortality unexpectedly dropped to 13.3% due to noticeable swirling and wagging movements due to the high-voltage plasma discharge effect. The F-value of 3.78 and P-value of 0.0589 suggest a near-significant trend across treatments. The mortality range spanned 13.3% to 86.7%, demonstrating a dose-dependent response, with efficacy peaking at 10 min before declining at 15 min. This anomaly at 15 min may be due to equipment limitations, as kernel "arching and wagging" under high voltage can reduce plasma contact with eggs, impacting treatment consistency. This dose-dependent trend, with optimal exposure times, often maximizes

egg lethality by generating reactive oxygen and nitrogen species (RONS) that disrupt cellular structures. However, studies indicate that extended plasma exposure can prompt stress-adaptive responses or be limited by equipment effects, reducing efficacy at higher exposure times.

Table 15: Mortality rates of various life stages of C. maculatus inoculated in chickpeas when exposed to 25 kV of cold plasma treatment for different time conditions. (Dissimilar alphabets in lower case (a, b, c) signify that the mean values of mortality rate are statistically different at $p<0.05$ for various treatment times while being a certain life stage. Dissimilar alphabets in upper case (A, B, C) signify that the mean values of mortality rate are statistically different at $p<0.05$ for various life stages while being treated for a fixed treatment time).

Treatment (min)	Mean ± SE number of adult mortalities produced [a, b]				*F value*	*P value*	DF
	Egg	Young Larvae	Larvae-2e	Pupa			
5	26.7±26.7 B	86.7±3.3 a, A	93.3±3.3 ab, A	93.3±3.3 a, A	5.56	0.0234	3, 8
10	86.7±13.3	83.3±8.8	100.0±0.0 a	96.7±3.3 a	0.95	0.4602	3, 8
15	13.3±13.3 B	93.3±6.7 a, A	90.0±0.0 b, A	93.3±3.3 a, A	14.36	0.0014	3, 8
F value	3.78	5.31	28.72	9.98	-	-	-
P value	0.0589	0.262	0.0001	0.0044	-	-	-
DF	3, 8	3, 8	3, 8	3, 8	-	-	-

Young larvae mortality

Also, from **Table 15**, cold plasma significantly impacted young larvae mortality, with clear dose-dependent results across treatment times. The untreated group had an adult mortality rate of 30.0 ± 17.3%. A 5-min exposure notably increased mortality to 86.7 ± 3.3% (P = 0.0234), highlighting a significant effect. Both 10-min and 15-min treatments yielded high mortality rates (83.3 ± 8.8% and 93.3 ± 6.7%, respectively), with an F-value of 5.31, confirming significant

differences among groups (P = 0.0234). This trend suggests that cold plasma treatment is effective for young larvae mortality at exposure times of 5 min or longer, achieving the highest mortality at 15 min. Literature supports plasma's high efficacy in insect larvae untreated through the generation of reactive species that compromise cellular functions, demonstrating 95% mortality for young larvae and 85% mortality for pupae after just 2 min of exposure (Krik-Bradley et al., 2023).

Larvae-2e mortality

Larvae-2e showed a high sensitivity to cold plasma across all tested durations. Mortality in the untreated was 56.6 ± 3.3%, while a 5-min exposure significantly increased it to 93.3 ± 3.3% (P < 0.05) (**Table 15**). At 10 min, mortality reached 100.0 ± 0.0%, indicating total lethality. The 15-minute treatment maintained high mortality (90.0 ± 0.0%), suggesting an effective lethal dose achieved in 10 min. The F-value for larvae-2e mortality was 28.72, indicating a very significant treatment effect (P = 0.0001). The high F value and low P value suggest that the cold plasma treatment duration has an impact on the mortality of larvae-2e. This data points to cold plasma's strong impact on larvae-2e, aligning with other studies where plasma exposure disrupts cellular integrity (Lui et al., 2021; Krik-Bradley et al., 2023).

Pupa mortality

Pupa mortality also exhibited significant increases across treatment times, with an untreated mortality rate of 50.0 ± 5.7% (**Table 15**). A 5-minute exposure raised mortality to 93.3 ± 3.3% (P < 0.05), with 10-min and 15-min exposures resulting in mortality rates of 96.7 ± 3.3% and 93.3 ± 3.3%, respectively. The treatment effect remained consistent across exposure times, likely due to the developmental stage of the pupae. At this stage, the insect has already completed

most of its growth inside the kernel, leading to a thinner and more fragile surface layer. This structural change may have made the pupae highly susceptible to cold plasma exposure, resulting in high mortality even at shorter treatment times. As a result, increasing the exposure beyond 5 min did not significantly enhance mortality. The F-value of 9.98 and P-value of 0.0044 underscore a significant effect among treatment groups. The high mortality rates for pupae across treatments suggest that cold plasma is equally effective in targeting insect pupae (Lui et al., 2021). Literature confirms that pupal stages can be susceptible to reactive oxygen and nitrogen species generated by plasma, which damage proteins and nucleic acids (Krik-Bradley et al., 2023).

Mortality rates at 20 kV for different treatment durations across various life stages

Egg mortality

From **Table 16** at 20 kV, egg mortality increased with treatment duration, starting from a baseline of 46.6 ± 8.8% in the untreated group (0 min). A 5-minute treatment led to a substantial rise in mortality to 93.3 ± 3.3% (P = 0.0002), indicating a significant effect. Mortality remained high at 90.0 ± 0.0% with 10 min of exposure and reached 100% at 15 min(Krik-Bradley et al., 2023). The F-value of 24.62 and the low P-value (P = 0.0002) confirm significant differences in egg mortality across treatment times, highlighting that prolonged exposure at 20 kV effectively eradicates egg stages. This trend aligns with studies showing that reactive oxygen and nitrogen species (RONS) produced by plasma disrupt cellular structures, making eggs particularly susceptible (Kutcherov et al., 2020; Krik-Bradley et al., 2023)

Table 16: Table 3: Mortality rates of various life stages of C. maculatus inoculated in chickpeas when exposed to 20 kV of cold plasma treatment for different time conditions. Dissimilar alphabets in lower case(a, b, c) signify that the mean values of mortality rate are statistically different at $p<0.05$ for various treatment time while being a certain life stage. Dissimilar alphabets in upper

case(A, B, C) signify that the mean values of mortality rate are statistically different at $p<0.05$ for various life stages while being treated for a fixed treatment time.

Treatment (min)	Mortality rate (%) for different life stages [a, b]			
	Egg	Young Larvae	Larvae-2e	Pupa
5	93.3±3.3 ab, A	90.0±5.7 a, A	96.6±3.3 a, A	96.6±3.3 a, A
10	90.0±0.0 b, A	86.6±3.3 a, A	86.6±6.6 a, A	86.6±6.6 a, A
15	100±0.0 a, A	90.0±5.7 a, A	93.3±3.3 a, A	93.3±3.3 a, A
F value	24.62	5.74	16.62	14.44
P value	0.0002	0.0215	0.0008	0.0014
DF	3, 8	3, 8	3, 8	3, 8

Young larvae mortality

Young larvae mortality also showed significant increases with plasma exposure at 20 kV (**Table 16**). The untreated mortality was 50.0 ± 5.7%, which increased significantly to 90.0 ± 5.7% after 5 min (P = 0.0215). At 10 min, mortality slightly decreased to 86.6 ± 3.3%, but remained high. A 15-minute exposure maintained this high level at 90.0 ± 5.7%. The F-value of 5.74 and P-value of 0.0215 indicate that treatment time had a statistically significant impact. This result suggests that 20 kV plasma treatment is killing young larvae at shorter exposures that plasma-induced reactive species can disrupt cellular processes in larval stages (Krik-Bradley et al., 2023). The mortality rates across different exposure times highlight the effectiveness of plasma treatment in targeting young larvae. This suggests that plasma-induced reactive species play a crucial role in disrupting cellular integrity, leading to significant insect mortality.

Larvae-2e mortality

Cold plasma treatment at 20 kV significantly impacted larvae-2e. The untreated group had a mortality rate of 26.6 ± 3.3% (**Table 16**). After 5 min of exposure, mortality rose sharply to 96.6 ± 3.3% (P = 0.0008), demonstrating the high effectiveness of plasma at this exposure. Mortality slightly decreased to 86.6 ± 6.6% at 10 min and then increased again to 93.3 ± 3.3% after 15 min. The F-value of 16.62 and P-value of 0.0008 confirm a highly significant difference across treatments, with prolonged exposure yielding high mortality in larvae-2e. Literature on cold plasma treatment supports its lethal effects on more developed larvae due to oxidative stress induced by reactive species, compromising cellular integrity (Lui et al., 2021; Krik-Bradley et al., 2023). These findings suggest that cold plasma exposure effectively disrupts the physiological resilience of larvae-2e, leading to high mortality even at shorter treatment durations.

Pupa mortality

Cold plasma treatment at 20 kV also resulted in high mortality rates in pupae. In the untreated, pupal mortality was 40.0 ± 5.7% (**Table 16**). After 5 min, mortality increased significantly to 96.6 ± 3.3%, with 10-minute and 15-minute exposures yielding mortality rates of 86.6 ± 6.6% and 93.3 ± 3.3%, respectively. The F-value of 14.44 and P-value of 0.0014 show that plasma exposure duration significantly influenced mortality. Krik-Bradley et al.(2023) These results highlight that cold plasma is highly effective against pupal stages at this voltage, which is consistent with studies indicating that pupal stages are susceptible to protein and nucleic acid damage induced by reactive species in cold plasma treatments.

Mortality rates at 15 kV for different treatment durations across various life stages

Egg mortality

From **Table 17** at 15 kV, cold plasma treatment led to a significant increase in egg mortality with extended exposure. The untreated group showed a low mortality rate of 13.3 ± 3.3%. Mortality sharply increased to 86.6 ± 6.6% after 5 min of exposure (P = 0.0012), indicating a significant effect. Mortality remained high at 80.0 ± 5.7% after 10 min and reached 90.0 ± 5.7% at 15 min. The F-value of 15.17 and low P-value (P = 0.0012) confirm statistically significant differences across treatments. These results suggest that even at 15 kV, cold plasma treatment effectively targets egg stages, likely due to the formation of reactive oxygen and nitrogen species (RONS), which compromise egg viability by disrupting cellular structures (Kutcherov et al., 2020; Krik-Bradley et al., 2023). The consistent mortality rates across extended exposures suggest that a threshold plasma effect is reached within the first few min of treatment. This further supports the potential of cold plasma as a non-chemical alternative for controlling insect infestations at the egg stage.

Young larvae mortality

Young larvae mortality also increased significantly with treatment duration at 15 kV. In the untreated group, mortality was 20.0 ± 5.7%, which rose sharply to 86.6 ± 3.3% after 5 min (P = 0.0003) (**Table 17**). The high mortality was sustained at 86.6 ± 6.6% at 10 min and slightly increased to 90.0 ± 5.7% at 15 min. The F-value of 21.58 and very low P-value (P = 0.0003) demonstrate a significant impact of treatment time on young larvae mortality. These results align with literature showing that cold plasma effectively induces mortality in young larvae due to reactive species, which disrupts metabolic processes critical for survival (Krik-Bradley et al., 2023).

Table 17: Mortality rates of various life stages of C. maculatus inoculated in chickpeas when exposed to 15 kV of cold plasma treatment for different time conditions. Dissimilar alphabets in lower case(a, b, c) signify that the mean values of mortality rate are statistically different at $p<0.05$ for various treatment times while being a certain life stage. Dissimilar alphabets in upper case(A, B, C) signify that the mean values of mortality rate are statistically different at $p<0.05$ for various life stages while being treated for a fixed treatment time.

Treatment (min)	Mortality rate (%) for different life stages [a, b]			
	Egg	Young Larvae	Larvae-2e	Pupa
5	86.6±6.6 a, B	86.6±3.3 a, B	90.0±5.7 a, A	83.3±3.3 a, B
10	80.0±5.7 a, AB	86.6±6.6 a, B	90.0±5.7 a, A	86.6± 3.3 a, B
15	90.0±5.7 a, A	90.0±5.7 a, A	76.6±3.3 a, B	80.0±5.7 a, A
F value	15.17	21.58	14.55	12.35
P value	0.0012	0.0003	0.0013	0.0023
DF	3, 8	3, 8	3, 8	3, 8

Larvae-2e Mortality

Larvae-2e showed notable sensitivity to 15 kV cold plasma treatment, with mortality rates increasing significantly with longer exposures. The untreated group mortality was low at 20.0 ± 5.7%, which increased markedly to 90.0 ± 5.7% after 5 min (P = 0.0013) (**Table 17**). This high mortality level was maintained across 10 and 15 min of exposure, with rates of 90.0 ± 5.7% and 76.6 ± 3.3%, respectively. The F-value of 14.55 and P-value of 0.0013 indicate that treatment duration significantly affected larvae-2e mortality. These findings are consistent with other studies demonstrating that plasma-induced reactive species can compromise cellular integrity in maturing insect stages (Lui et al., 2021; Krik-Bradley et al., 2023).

Pupa Mortality

Cold plasma treatment also significantly impacted pupal mortality at 15 kV. The untreated group showed a mortality rate of 23.3 ± 6.6%, which increased significantly to 83.3 ± 3.3% after 5 min of exposure (**Table 17**). Mortality was maintained at a high level, with 86.6 ± 3.3% at 10 min and 80.0 ± 5.7% at 15 min. The F-value of 12.35 and P-value of 0.0023 confirm statistically significant differences in mortality across treatment times. These results suggest that cold plasma treatment at 15 kV effectively targets pupal stages, with literature supporting the susceptibility of pupae to plasma-generated reactive species, which damage proteins and impede development (Krik-Bradley et al., 2023).