YEDITEPE UNIVERSITY
GRADUATE SCHOOL OF NATURAL AND APPLIED SCIENCES

A NOVEL SEED COATING FORMULATION FOR THE MANAGEMENT OF SEED-BORNE DISEASES

A Thesis Submitted
by
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In Partial Fulfillment
of the Requirements for the Degree of
Master of Science
in
Biotechnology

Supervisor
Prof. Dr. Fikrettin Şahin

Istanbul - 2025

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ABSTRACT

A NOVEL SEED COATING FORMULATION FOR MANAGEMENT OF SEED-BORNE DISEASES

Almost 90% of the world's food crops are grown from seeds. Seed quality is affected by abiotic and biotic factors. Abiotic factors include environmental conditions, while biotic factors arise from bacteria, fungi, viruses, insects, and nematodes. Seed-borne microorganisms cause serious yield losses (approx. 27-42%) and affect large agricultural areas by transmitting them to seasons/regions via contaminated seeds. Therefore, several physical, biological, and chemical seed treatment methods have been developed and used to control seed-borne diseases up to now. Indeed, seed treatment can become the most effective way to eliminate or reduce seed-borne pathogens. However, none of the existing methods has effectively controlled seed-borne endophytic and epiphytic pathogens.

This study aims to develop a new seed-coating formulation that eliminates all types of plant pathogens (bacterial and fungal pathogens) from seeds without harming germination. In this study, piroctone olamine (PO) based on a novel chemical formulation developed and used for coating wheat, tomato, and corn seeds. Different doses including 0.4, 0.1, 0.08, 0.05, 0.03, and 0.01% of PO-based formulation were tested against CMM, PSS, PST bacteria and two fungal pathogens like *Aspergillus niger* and *Penicillium* sp. *in vitro* conditions. According to antimicrobial activity test results, seed coating formulations containing different concentrations of PO, except 0.01%, were effective against all microorganisms tested. It was also observed that all endophytic and epiphytic bacteria and fungi species were eliminated on/in seeds treated with PO seed coating formulations. It was observed that the application PO seed coating formulations at rates of 0.4% and 0.1% was almost 100% effective in pathogen control. On the other hand, it was determined that 0.1% PO formulation did not have any negative effects on root and shoot development of treated seeds. This is the first study to show that 0.1% PO-based seed coating formulation may have great potential to eradicate seed-borne pathogens of different crops in agriculture. Therefore, further study is required to evaluate the effect of PO based formulations against viral pathogens in different crop seeds/seeds *in vitro* and on yield and quality parameters.

ÖZET

TOHUM İLE TAŞINAN HASTALIKLARIN KONTROLÜ İÇİN YENİ BİR TOHUM KAPLAMA FORMÜLASYONUNUN GELİŞTİRİLMESİ

Dünyadaki tarım ürünlerinin neredeyse %90'ı tohumlardan üretilmektedir. Tohum kalitesi, abiyotik ve biyotik faktörlerden etkilenir. Abiyotik faktörler sıcaklık, nem gibi çevresel koşullardan, biyotik faktörler ise bakteriler, funguslar, virüsler, böcekler ve nematodlardan oluşur. Tohumla taşınan mikroorganizmalar, ciddi verim kayıplarına (yaklaşık %27-42) yol açmakta ve bulaşık tohumlarla uzun süre canlılığını koruyarak ve uzak mesafelere taşınarak büyük tarım alanlarını etkilemektedir. Bu nedenle, tohum kaynaklı hastalıkları kontrol etmek amacıyla fiziksel, biyolojik ve kimyasal metotlar geliştirilmiştir. Tohum kodlama yöntemi, tohumla taşınan patojenleri ortadan kaldırmanın en etkili yolu olabilir. Ancak, mevcut yöntemler, tohumdaki patojenleri etkili bir şekilde kontrol edilmesini sağlayamamıştır. Bu çalışmanın amacı, tohum çimlenmesine zarar vermeden tohumla taşınan fungal ve bakteriyel patojenleri ortadan kaldıran yeni bir tohum kaplama formülasyonu geliştirmektir. Bu çalışmada, buğday, domates ve mısır tohumlarının kaplanmasında piroctone olamine (PO) formülasyonu test edilmiştir. PO içerikli formülasyonun farklı dozları (%0.4, %0.1, %0.08, %0.05, %0.03, %0.01) üç bakteri (CMM, PSS, PST) ve iki mantar patojeni (*Aspergillus niger*, *Penicillium* sp.) üzerine *in vitro* koşullarda test edilmiştir. In vitro antimikrobiyal aktivite testleri (disk difüzyon metodu), PO içeren formülasyonun %0.01 doz dışındaki tüm dozlarının etkili olduğunu göstermiştir. Tohumlar üzerinde yapılan çeşitli testler, PO formülasyonunun tohumla taşınan endofitik ve epifitik fitopatojen bakteri ve fungus türlerini kontrol altına aldığını göstermiştir. %0.4 ve %0.1 oranda PO içeren formülasyonlar tohumdaki bakteri ve funguslar üzerinde %100 etkili olup, %0.1 dozda PO formülasyonu çimlenen tohumların kök ve filiz gelişimini olumsuz etkilememiştir. Bu, %0.1 PO içerikli tohum kaplama formülasyonunun büyük bir potansiyele sahip olduğunu gösteren ilk çalışmadır. PO içerikli formülasyonların tohumla taşınan viral patojenlere karşı etkisi ile verim ve kalite parametrelerinin değerlendirilmesi için daha fazla çalışma gerekmektedir.

ACKNOWLEDGEMENTS

I would like to express my deepest gratitude to Professor Fikrettin Şahin for his support and assistance. Working on my thesis under his supervision was an experience that broadened my mind and provided me with an unlimited source of learning.

I would like to thank Dr. Zeynep İşlek Köklü for opening the door of her laboratory and Özlem Beydilli for her friendship and support.

Finally, I thank my family for their endless love and support, making everything more beautiful.

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PSS	*Pseudomonas syringae* pv. *syringae*
CMM	*Clavibacter michiganensis* subsp. *michiganensis*
PST	*Pseudomonas syringae* pv. *tomato*
XT	*Xanthomonas translucens*
BCA	Biocontrol Agents
FST	Fungicide Seed Treatment
PO	Piroctone Olamine
FDA	Food and Drug Administration
PDA	Potato Dextrose Agar
NA	Nutrient Agar
ISTA	International Seed Testing Association
GR	Germination rate
CMC	Carboxymethylcellulose

1. INTRODUCTION

The food production and quality have been the most important subjects for humanity since the transition of people from a hunting culture to a settled life. Increasing yield and controlling plant diseases have become the most notable issues with the start of agricultural product use. The protection of seed health is accepted inception and the most critical for agricultural production.

1.1. BEGINNING OF AGRICULTURE

Wheat and maize are staple foods for humanity. Around 10,000 BC, agriculture began in the Fertile Crescent region. Fertile Crescent is the core area of domestication of einkorn (*T. monococcum*), diploid wheat. This area, a small region of southeast Turkey and northeast Syria around the Euphrates, might be the cradle of agricultural innovation (Figure 1.1) [1]. Wheat/Maize is almost grown in every part of the planet. Apart from being the basic ingredient bread, wheat grain is also used to make pasta, a variety of pastries, starch, alcohol, malt, dextrose, fuel, feed, and many other products. Domestication of maize, potatoes, beans, cotton, tomatoes, tobacco, and sunflowers dates back to the central region of the Americas, 8,000 years ago [2]. Maize might be planted (sown) at more than 3400 meters, and its morphologic, nucleotide, and structural diversity is very different compared to other plants. Maize spread northward from Mexico to the United States and Canada, and southward from Mexico to the Caribbean coast of South America, the Caribbean, and from the Mexican highlands to the Guatemalan highlands. The first documented history of maize's arrival to Europe dates back to 1493 when Columbus of Spain brought the crop from the Caribbean. In years, maize gained popularity in northern Spain and southwest France in the 17th century [3].

Figure 1.1. Map of the Middle Euphrates region showing the archeologic sites [2]

1.2. SEED PRODUCTION AND TRADE

In recent decades, wheat, rice, and maize have become popular other vegetables such as tomatoes for human consumption. The wild type of tomato cultivated in the Andean region of South America is considered the ancestor of other types of tomatoes. Tomatoes were transported to Europe by warriors in the sixteenth century. For years, these crops have been produced almost worldwide. They are not only used in food but also in industrial products; these plants are economically significant for countries. In terms of food and nutrition, wheat has a very important place in the world. In 2022, 800 million tons of wheat were produced all over the world. By 2050, there will be a 60% rise in the demand for wheat [4]. Approximately, China tops the list of countries with 138 million tons of wheat. It is followed by India, Russia, the US, Australia, France, Canada, Pakistan, Germany and Argentina. Turkey produces 3% of the total global wheat production with 20 million tons. Wheat ranks first in crop production in Turkey, followed by sugar beet, tomato, barley, and corn. Maize was harvested from 203,470,007 ha and produced 1162.4 million tons all over the world, in 2022. The main producers of maize are the USA, China, Brazil, Argentina, Ukraine, India, and Mexico. In Turkey, maize is cultivated in an area of 911,499 ha, with 8,500,000 tonnes of production in 2022. The global tomato production is 186,107,972.48 tons, 13,000,000 tons of which are produced in Turkey. Turkey is the third producer in the world [5].

1.3. MECHANISM OF SEED GERMINATION

Almost 90% of the world's food crops are grown from seed [6]. Seed is the basic unit of crop production. Seed germination is triggered by water imbibition, mobilization of food reserves, protein synthesis, and consequent radicle protrusion [7]. The presence of water affects the enzymatic hydrolysis of proteins, lipids, and carbohydrates, and the transportation of metabolites. When water is reintroduced during imbibition, metabolic activities can restart. Once full metabolic status is reached, component turnover or replacement takes place across several hours. During the water imbibition phase, the oxidative and glycolytic pentose phosphate pathways both restart and the enzymes of the Krebs cycle are activated [8]. Reserve compounds in germinating wheat seeds stimulate the embryo to secrete phytohormones, primarily gibberellic acid (GA), which can diffuse into the aleurone layer and initiate a signaling cascade that produces hydrolytic enzymes such as α-amylases. The endosperm then secretes hydrolytic enzymes required for the hydrolysis of nutritive reserve [9]. Because the endosperm can emit signals to regulate the growth of the embryo, germination is thought to be a response involving bidirectional interactions between the embryo and endosperm [7]. Phytohormones regulate germination mechanisms such as gibberellic acid (GA), indole acetic acid (IAA), cytokine (CKs), abscisic acid (ABA), ethylene (ET), brassinosteroids (BRs), and jasmonates (JAs). ABA regulates the seed dormancy, while GA and ET have a counteracting effect on ABA. The observed modulation of radicle growth and endosperm weakening during germination have been linked to ROS and phytohormone imbalances, which may account for the delayed endosperm rupture observed in crack mutants [10]. ABA biosynthesis genes are signaled by sorting different plant species. Many ABA biosynthesis genes are implicated in the regulation of seed dormancy in different plant species. For example, NCED6 and NCED9 have a key role in dormancy of *Arabidopsis* seeds, while in rice, the expression of OsNCED2 at a low or high level causes dormant or non-dormant seeds. Gibberellic acid (GA) is needed to control ABA activity. Release of GA causes prolongation of radicle and breaking dormancy. GA and ABA regulate other phytohormones, such as auxin, jasmonate (JA), brssinosteroids (BR), and ethylene. While auxin encourages seed germination, jasmonic acid is found in no dormant seeds [11].

1.4. HISTORY OF SEED-BORNE DISEASE AND SEED TREATMENT

Seed germination is affected not only by hormones and environmental conditions but also by seed-borne pathogens, which play a critical role in seed germination. Seed-borne diseases induced by fungi, bacteria, viruses, and nematodes cause important loss of yields. The first seed-borne disease was found by Greek physician Galen around 100 A.D. He related some mysterious objects in wheat to human abortions and hallucinations. After 15 centuries, the pathogens were understood with using a microscope and these microorganisms were defined. In the Middle Era, French botanist Tillet reported that wheat seed surface poisonous substances cause stinking and recommended seed coating with copper sulfate in 1755. In 1807, Prevost discovered that *Tilletia caries* led to stinking. Moreover, he showed that copper sulfate was effective in controlling this disease. *Colletotrichum lindemuthiaum* is known as the first entophytic seed-borne fungus was identified by Frank in 1883. In 1897, formaldehyde was used for smut control in wheat seeds [12]. Alternaria blight causes the most significant yield loss in crops worldwide. The genus *Alternaria* was noticed by Nees in 1817. Later, Saccardo changed the name to *A. brassicae* [13]. In 1882, Millardet's research revealed that grape vines sprayed with a bluish-white solution of lime and copper sulfate to deter thieves retained their leaves throughout the growing season, but vines that weren't sprayed lost them [14] (Table 1.1). Copper, sulfur, and mercury compounds were used as fungicides. However, new chemicals were needed, and development resulted in these chemicals being toxic to humans and animals [15].

Table 1.1. Sequence of the preliminary [14]

Year	Fungicide	Primary use
1637	Brine	Cereal seed treatment
1755	Arsenic	Cereal seed treatment
1760	Copper sulfate	Cereal seed treatment
1824	Sulfur (dust)	Powdery mildew and other pathogens
1833	Lime sulfur	Broad spectrum foliar pathogens
1885	Bordeaux chloride	Broad spectrum foliar pathogens
1891	Mercury chloride	Turf fungicide
1900	$CuOCl_2$	Especially *Phytophthora infestans*
1914	Phenylmercury chloride	Cereal seed treatment
1932	Cu_2O	Seed and broad spectrum foliar diseases
1934	Dithiocarbamates patented	Broad spectrum protectants
1940	Chloranil, Dichlone	Broad spectrum seed treatment

Wheat is important not only for production and nutrition but also for trade, exporting, and importation all over the world. Therefore, seed health is significant for agricultural areas, storage, and trade. Seed-borne pathogens affect wheat production and yield. Farmers and crop growers have specific expectations for the quality of the seeds they plant all over the world. Primarily, they expect coherent their agricultural field. Second, they want the seed to successfully and uniformly produce a crop without weeds and pathogens-free [16]. Producers want store their seeds to used next year. Therefore, seed health and quality are critical factors for them. Seed-borne pathogens not only affect yield but also can survive in the soil for a long time, affecting the production and yield of future years. For example, *Rhizoctonia solani* is known by the name bare patch and leads to very severe damage to wheat, as it inhibits seed germination and makes appear empty areas in fields. In addition, this fungus remains viable in the soil or weeds for more than two years, which affects the yield of the next years (Figure 1.2) [17]. *Fusarium* species can result in yield losses of up to 80% for crops grown in greenhouses as well as fields, which can lead to significant financial losses [18]. *Fusarium* spp. can remain viable in soil for years [19].

Figure 1.2. *R. solani* causes dwarf in wheat [18]

1.5. ORGANISATIONS

In the following years, different plant pathogens were found and treatment chemicals were discovered to protect seeds. To protect plants and seeds, different organizations were established, such as the European Plant Protection Organization (EPPO) and the International Seed Testing Association (ISTA). There shall be established a European and Mediterranean Plant Protection Organization, a recognized regional plant protection

organization , established by the Food and Agriculture Organization of the United Nations (FAO). The aims of the organization are:

- To support the Member Governments' goal of ensuring plant health while safeguarding human and animal health and the environment;
- To pursue and expand, through collaboration among Member Governments, the protection of plants and plant products against pests and the prevention of their international spread, particularly their introduction into threatened areas.
- To establish internationally harmonized plant health and other official plant protection regulations and, if needed, elaborate standards to that effect;
- To submit the Member Governments' collective opinions, as appropriate, to FAO, WTO, other regional plant protection organizations, and any other entities with relevant duties [20].

1.6. SEEDBORNE PATHOGENS

Seeds can be contaminated/infected by fungi, bacteria, viruses and nematodes. Seed-borne pathogens are transmitted in three different ways as follows:

1.6.1. Externally Seed-Borne Pathogens

The pathogen is present on the surface of the seed and attaches to the seed by spores, sclerotia, mycelium, bacteria, nematodes and viral particles. For example, spores of *Alternaria, Fusarium, Helminthnosporium, and Stemphylium*, many smuts, some rust fungi and many other fungal species on the seed can be detected by direct observation under a microscope [21].

1.6.2. Internally (Infected) Seed-Borne Pathogens

The pathogens are carried inside the seed and are located within the tissues or soil. Under a binocular microscope, dry seeds can appear completely healthy and exhibit no outward symptoms of infection. Pathogens, on the other hand, have the ability to colonize the many layers surrounding the seed, including the testa, endosperm, pericarp, and seed coat. Such pathogens can spread by spores, vegetative cells, nematodes, pycnidia, or virus particles. Some examples are *Ustilago nuda* (loose smut of barley), *Ustilago nuda tritici* (loose smut

of wheat), *Colletotrichum lindemuthianum* (bean anthracnose), *Phoma lingam* (black leg of cabbage), *Alternaria zinnia* (alternaria disease of zinnia), *Ascochyta* spp. (ascochyta blight of pea), *Xanthomonas campestris* pv. *malvacearum* (bacterial blight of cotton), *X. campestris* pv. *campestris* (black rot of crucifers), common bean mosaic virus on French beans, and Tobacco ring spot virus on soybeans [22].

1.6.3. Concomitant Contamination

Infected plant debris, fungal sclerotia, nematode cysts, seeds of phanerogamic plant parasites, bacterial ooze, infected soil particles, etc. are examples of concurrent contamination that contains the inoculum mixed with seeds. Although often disregarded, this kind of pathogen dispersal is quite significant. Since concomitant contamination detect hardly, this way disease infection is higher than known. Plant detritus can harbor inocula that cause significant disease outbreaks, such as those brought on by rusts, downy mildew, and bacterial infections. Infected plant debris, fungal sclerotia, nematode cysts, seeds of phanerogamic plant parasites, bacterial ooze, infected soil particles, etc. are examples of concurrent contamination that contains the inoculum mixed with seeds. Although often disregarded, this kind of pathogen dispersal is quite significant. Since concomitant contamination detect hardly, this way disease infection is higher than known.

Fungal contamination likely has an indirect as well as direct impact on seed quality. Direct effects on grain quality could result from the fungus spreading throughout the kernel and producing metabolites that change the composition or metabolism of the grain or make it unfit for human or animal consumption. Reductions in yield attributed to contamination are known as indirect impacts. The establishment of economically significant plant diseases in the field that seriously impact agricultural production is highly due to infected seeds. In addition, poor germination and field establishment result from bad seed quality associated with infected seed, negatively affecting the market value [22].

Field fungi that infect grain seeds are mostly facultative parasite species. Three subgroups can be distinguished among the fungi in this category. This covers specific pathogens attacking germinated or mature seeds and may not show symptoms or damage. The second category consists of extremely pathogenic, non-specialized fungi that infect grain during development. Last but not least, there are the unspecialized saprophyte fungi that infiltrate mature, wet grain. Field fungi commonly colonizing cereal grains include *Fusarium* species,

Alternaria alternata, *Verticillium lecanii*, *Epicoccum purpurascens*, and *Cladosporium cladosporioides*. *Aspergillus flavus* and *Penicillium* species are also present [23]. Fungi that penetrate the seed coat are generally more harmful than those that are only present on the outside. *Trichothecium*, for instance, can decrease Douglas-fir seed batches' germination by twenty percent. Almost 9.4% of global yield losses are attributed especially harmful fungi. Fungi through fatty acid accumulation, decreased germination, mustiness, and eventually grain spoilage impact grain quality. The fact that fungi produce poisons that are harmful to both human and animal health is another reason [24].

Seed-borne disease affects seed quality during the storage period for wheat. Several reports showed that seed-borne diseases lead to a loss of yield from 14 to 100% [25]. *Urocystis tritici* causes a yield decrease of more than 50% [26]. The seed treatment method is the most preferred method to prevent fungal pathogens. Chemical agents are preferred reasons for epiphytic and endophytic pathogen control. *Bipolaris sorokiniana, Alternaria tenuis, Curvularia lunata, Aspergillus niger, Fusarium* spp., and *Aspergillus flavus* are wheat pathogens [27]. *The most* important seed-borne disease of wheat known for centuries is stink rust, caused by *Tilletia tritici*. The fungal pathogen attaches to the seed surface before infecting the wheat seedling as it germinates. This fungus infection result in an unpleasant smell that is similar to that of fish and is harmful to human consumption [21, 26]. The black point of wheat (*Alternaria* spp.) causes the seed's embryo to shrink and turn black. Besides, *Alternaria* blight causes a 36.88% loss per plot obtained in mustard seed yield, and it also dropped 28.22 in 1000 mustard grain weight [28]. On the first two leaves of seedlings, the disease's signs (small lesions) can be seen in the field as early as three weeks after sowing. Several diseases, such as seed rot, early-season seedling mortality, and leaf blights later in crop growth, are mostly transmitted by contaminated seeds. While foliar blights can result in yield losses of up to 20% on average, with the potential to increase several times in extreme circumstances, seed rot and seedling blight reduce plant stand [29]. *Plant* rot is occurred by *Fusarium moniliforme*, a fungus that is typically spread by seeds. *Fusarium* spp. causes color changes primary and secondary roots, also known as root rot, in the primary and secondary roots of corn (Figure 1.3.) [30].

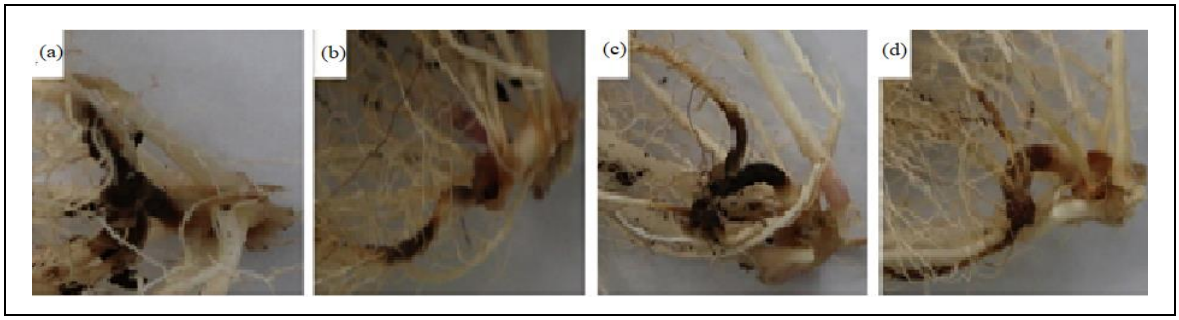

Figure 1.3. *Fusarium* temperature infections root of maize [30]. Four different corn species (a) Hikchal, (b) Mibeak-2ho, (c) Chagong-chal, (d) Oluckdehack-chal

Furthermore, a variety of fungi, mainly *Aspergillus* and *Penicillium* species, are responsible for storage rots. They could be found alone or in conjunction with other fungi. The powdery green or blue-green mold between the kernels, typically at the tip of the ear (spike in wheat), is the most common known indication of *Penicillium*. Fungal expansion usually results in streaking and bleaching of the kernels. One of the most prominent fungi that produce mycotoxin is *Aspergillus*, which contaminates an extensive variety of crops and creates tan, greenish-yellow, or black blooms on and between seeds [31]. *Rhizopus* species that are known as rhizonins produce mycotoxin. Mycotoxins lead to loss of yield and quality crops and are dangerous to humans and animals [32].

The two most commonly reported seed-borne bacterial diseases that reduce wheat production yield globally are *Xanthomonas translucens* and *Pseudomonas syringae* pv. *syringae*. Bacterial leaf blight (BLB) affects wheat seeds and is caused by *P. syringae* pv. *syringae* (PSS). PSS can be present in many sections of wheat seed tissue. BLB disease symptoms in wheat plants were reported to be light tan or white leaf necrosis, resulting from the formation of water-soaked patches. PSS actively encourages frost damage, creating the ideal environment for other diseases.

Xanthomonas translucens (BLS) brings about bacterial leaf streak (BLS). Exudates resembling honey were generated by *X. translucens* in humid conditions, and the disease BLS was typified by translucent stripes. It was concluded that some *PSS* strains and *XT*, as wheat microbial communities, coexist in the same ecological niche (wheat seed) with a synergistic effect. Certain *PSS* strains and *XT*, as wheat microbial communities, could infect together seed [33].

When it comes to tomato seeds, one of the most dangerous diseases is *Didymella lycopersici*. There have been numerous reports of 50–90% seedling losses both before and after emergence [34].

Pseudomonas syringae pv. *tomato* produces dark superficial dots on green fruit, brown to black leaf spots that are occasionally bordered by a chlorotic margin, and sunken specks on ripe fruit encircled by a zone of delayed ripening (Figure 1.4.) [35, 36].

The bacterial spot disease of pepper (*Capsicum annum* L.) and tomato (*Lycopersicum esculentum* Mill.) plants is caused by *Xanthomonas campestris* pv. *vesicatoria* dye. The typical symptom of the disease is necrotic patches on fruit and foliage. Lesions on pepper leaves grow into ~~a~~ blight in particularly favorable conditions. Usually, sepal lesions result in the abscission of flowers or undeveloped fruit. Defoliation and floral abscission result in smaller and fewer fruits. The crop's disease, whether early developed, is affected, and early infection leads to more yield loss [37].

Clavibacter michiganensis subsp. *michiganensis* (CMM): Gram-positive bacterium, *Lycopersicum esculentum* is thought to be the most significant tomato bacterial disease, inflicting significant economic losses around the globe. CMM is a quarantine organism under the European Union Plant Health Legislation. It causes bacterial wilt and canker in tomatoes. It was discovered that there was a strong association between the amount of introduced bacteria to seeds and the disease incidence [38]. Wilt and canker symptoms are typically caused by this vascular pathogen, which enters and multiplies in the conduction tissue through natural holes or lesions. There is an extensive prevalence of both the invasion of tomato seeds by CMM and symptomless disguised infections. Early infection of plants (young seedlings) induces systemic infections that impact fruit quality and yield and usually bring about plant death. On the other hand, foliar infections in older plants often end in chlorosis of the leaves; nonetheless, this may or may not have an impact on both the quantity and quality of the current crop. When CMM invades and multiplies in xylem veins, internal vasculature browns, and vascular tissues gradually deteriorate. This causes wilting in the very beginning stages of infection by obstructing water transfer. While this illness is infrequent and losses from outbreaks can also be minimal, field experiments have shown yield losses of up to 84% in commercial fields in Ontario, Canada; 20–30% in France; and 46% in the USA [39].

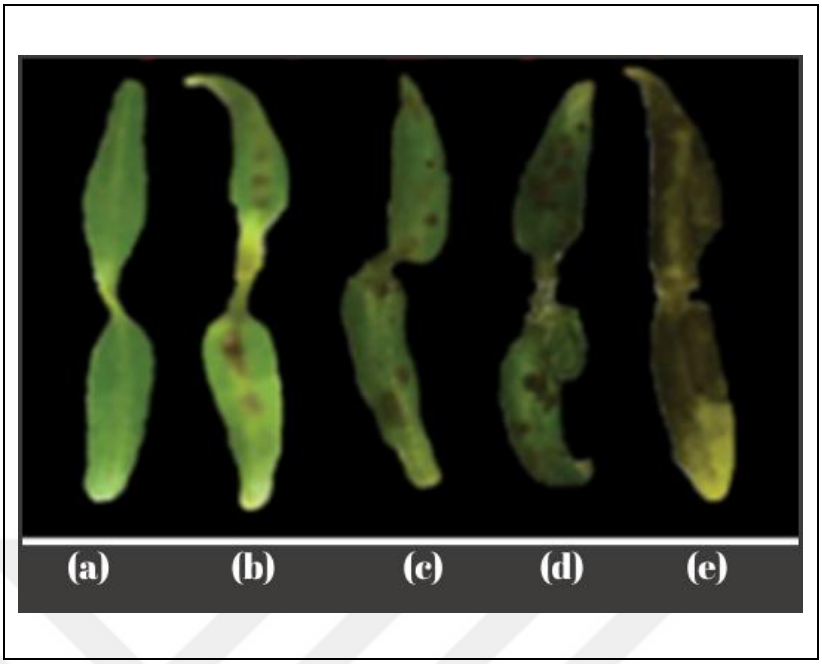

Figure 1.4. Tomato leaves were infected by *Pseudomonas syringae* pv. *tomato*. The disease severity index was used from 0 to 4. (a) healthy plant - 0, (b) Mild - 1 (c) Moderate - 2 (d) Severe – 3 (e) Death - 4 [36].

1.7. SEED TREATMENT METHODS AND IMPORTANT FUNGICIDES FOR SEEDS

Seed treatment protects the process from seed germination to sprouting, which is crucial for the development of strong and healthy plants [40]. Typically, seed treatments such as seed dressing, seed coating, or seed pelleting are applied prior to sowing. The most popular kind of seed treatment, known as "seed dressing" involves applying dry or wet fungicide and insecticide formulations to the seeds. For crops with tiny seeds, like carrots and onions, seed pelleting is used; seed coating is typically done by industries for huge quantities of seeds. To preserve quality throughout seed storage and transportation, seeds can also be treated at the time of harvest.

In general, the potential of fungicide resistance emergence by damping-off pathogens, notably oomycetes, rises when fungicides are administered to aerial plant parts or as a soil drench. Fungicide resistance risk rises when fungicide modes of action are not cycled. Almost all field crops are impacted by the same soilborne viruses that cause damping-off; hence, these crops are frequently treated with the same chemicals. For example, metalaxyl/mefenoxam has been used to suppress oomycetes, but fludioxonil has been used

to combat *Fusarium* sp, leading to damping off. Nonetheless, the possibility of resistance establishment seems to be reduced through seed treatment, though resistance development by soilborne fungi to fungicides recorded in seed treatments has been recorded [41] and 46% in the USA [39].

Seed treatment has some advantages in pathogen control or crop enhancement measures such as

- Provide nourishment for seeds during storage and seeding in soil.
- Diminished first inoculum.
- Minimize environmental consequences. Reduce the risk to the targeted organism, avoid drift, and restrict the area of land exposed to active chemicals while increasing efficacy. Reduce the rate of application per hectare, lowering disease control expenses while providing superior control of seed-borne, soil-borne, and foliar diseases.
- Maximize seed vigor for successful field emergence and establishment.
- Apply the chemical evenly and consistently.
- Combining treatments improves accuracy [42].

Breaking seed dormancy improves emergence and plant stand. High concentrations of fungicides can damage plant metabolism [43]. Most fungicides reduce root nodule growth and the development of mycorrhizal fungi. Research has also shown that Benomyl inhibits root development. Fungicide sprinkling additionally hampers carboxylation performance and reproduction of ribulose 1,5 bisphosphates, which affects CO_2 assimilation [44].

Seed treatments are different from conventional crop protection products; they offer cheaper, reduced application efforts, and save time. Consequently, seed treatment is currently the agricultural chemicals industry with the quickest rate of growth and has a major economic impact on markets, especially in the United States and Europe.

Seed treatment methods can be chemical treatments, physical treatments, and biocontrol agents [45].

1.7.1. Physical Seed Treatment Methods

Agricultural chemicals are less suitable for use because they affect the environment, the soil, and, therefore, the food consumed by humans and animals. In this century, it is very important to investigate the application of sustainable techniques, including physical means. Thanks to new technologies, they are now economically viable.

- Dry or aerated heat: Thermal seed treatment is applied using different methods. Dry heat treatment is a common way of solarization by radiation from the sun. Treatment might be used in warm countries, but industrial agriculture is less applied because of low susceptibility and challenges with large-scale treatment. Moreover, it has no good efficacy for seed-borne fungi. In addition to species, seed lots from the same crop and variety can vary different seed varieties of the same species may respond differently to sanitation practices and may be sensitive. Hot water and hot humid treatments have been used since the late 19th century. This treatment has a high cost and low sensitivity and causes non-germinated seeds [46].
- Hot Water: Hot water treatment has been known for a long time and is more eco-friendly, but seed viability can be affected. Application time and degrees of temperature are significant for the viability of seed and the control of pathogens. Cooler temperatures can fail the control of pathogens, while long-term application can damage seeds. Each seed needs a different temperature and different treatment times so hot water treatment should prefer small and young seeds after being tried on a small sample. Old seeds might be severely affected by this treatment [46].
- Radiation: Several types of electromagnetic radiation can be distinguished such as gamma rays, high-energy electrons, ultrasonic radiation, and UV radiation. Low doses of gamma rays stimulated root growth, while non-injurious levels of gamma irradiation (0.10-0.50 kiloGray) decreased microbial infestation in seeds of all four rice cultivars tested. The seeds treated with 0.10 kiloGray (kGy) had the highest rate of germination of the seed. Although laser light has numerous uses in agriculture, more research is needed to promote its uses as an alternative to manage diseases from the seed stage, particularly those caused by internal fungi [46, 47].

1.7.2. Biocontrol Agents

It is thought that losses ranging from 27% to 42% caused by plant diseases will double if no application is made [48]. Biopesticides or BCA are conspicuous due to being eco-friendly. The first disadvantage of this method is that the results are not consistently reproduced in field trial plots. Second, BCAs are generally pathogen specific such as *Pseudomonas chlororaphis*, strain MA 342 used as bio-pesticide to control fungal pathogen *Ustilago hordei* (*U. hordei*) is a causal agent of smut disease on barley and oat plants. This strain was effective against *U. hordei*, but *U. nuda* was unaffected [49]. *T. virens* is used against *Fusarium* wilt on tomato seeds; the *Serratia plymathica* HRO-C48 strain is effective for *Verticillium* wilt on *Brassica napus* L. [50]. This result demonstrated that BCA had a selective effect on particular microorganisms but no general activity on all bacterial and/or fungal pathogens.

1.7.3. Chemical Treatment

Chemical seed treatment is prevalent and has a wide spectrum of ability for disease control. Developing technologies causes faster and high-accuracy applications [46]. Seed chemicals could be used for pests, nematodes, and fungi. Seed chemicals for fungus cannot control bacteria because of different active needs, which results in more chemical uses.

The active ingredients in seed coating agents, such as plant growth regulators and pesticides, are typically combined with inert ingredients, such as color, suspension concentration, and film-forming agents. It has been demonstrated that seed coating with pesticides encourages plant growth, boosts wheat output, and prevents disease infestation [51]. Some of the chemicals that are commonly used for pathogen control are investigated as new chemicals because of environmental risks. The majority of fungicides for seeds have a low toxicity. The World Health Organization (WHO) (Table 1.2) identified hazard categories. [52]. The most common fungicides used for seeds are toxic; they should not come into contact with human skin, so workers or farmers must wear protective clothing (Table 1.3.).

The research showed that Triadimefon or Triadimenol can disrupt branchial development in rat embryos and cause teratogenic effects if exposed during neurulation. Moreover, triadimenol, which is a metabolite of triadimefon, is toxic more than triadimefon, and both have toxic effects on aquatic organisms [53]. Sensitization rashes were seen in agricultural

workers whose dithiocarbamates were treated. Besides, some agricultural workers did not wear protective clothing, gloves, hats, and boots, so there were observed cases of diffuse erythema and eczematoid epidermatitis of the eyelids and inguinal regions in contact with Zineb or Maneb [54].

Table 1.2. Oral and Dermal toxicity classification. (Class: LD50 for the rat (mg/kg body weight) [a]- the terms "solids" and "liquid" refer to the physical state of the active ingredient being classified.) [52]

Class		LD_{50} for the rat (mg/kg body weight)	
		Oral	**Dermal**
1a	Extremely hazardous	<5	<50
1b	Highly hazardous	5-50	50-200
2	Moderately hazardous	50-2000	200-2000
3	Slightly hazardous	>2000	>2000
U	Unlikely to present acute hazard	5000 or higher	

Seed-borne pathogens do not need a high-level inoculum to cause severe damage, loss of yield, and move to a new area. So protecting seeds and seed health have a critical role in sustainable agriculture.

FST is used to control:

- fungal pathogens that are seed surface-borne, such as those that cause covered bunts of wheat and black points of cereal grains.
- (endophytic) seed-borne fungal pathogens such as the loose-smut fungi of cereals.
- Soil-borne pathogens that attack germinating seeds and seedlings both pre- and post-emergence [41].

Table 1.3. Some FST class and information on hazards

Name of active	Oral acute toxicity (LD50 for rats) (mg/kg)	Dermal toxicity	Inhalation dose	Genotoxicity Mutagenity Carginogenity	NOAEL	Hazardous Categories
Imazalil [55]	227 to 664	>2000 mg/kg	2.43 mg/l	No genotoxic	0-0.05 mg/kg/day	Class II
Triadimenol [56]	1470	>2000 mg/kg (rabbit)	>3.57 mg/l	No genotoxic	3.4 mg/kg/day	Class II
Triadimefon [56]	752	>2000 mg/kg (rabbit)	>2.58 mg/l	No genotoxic	3.4 mg/kg/day	Class II
Triazoles (Difenoconazole) [57]	1453	2010	3.3 mg/l	No genotoxic	1 mg/kg	Class II
Metalaxyl [58]	670	>2000 mg/kg	2.3 mg/l	No genotoxic	19 mg/kg	Class II
Dithiocarbamates (Maneb) [59]	6750	>5000 mg/kg	Not evaluated	No genotoxic	1000 mg/kg	Class II
Piroctone olamine [60]	8100	>2000	4.9 mg/l	No genotoxic	100 mg/kg	Class II

1.8. PIROCTONE OLAMINE

Piroctone olamine (PO) is composed of monoethanolamine and piroctone (Figure 1.5.). The chemical formula of PO is C16H30N2O3, and its molecular weight is 298.42 g/mol. PO is similar to ciclopirox and pyrithione with a substituted group of pyridine. PO is used at the highest dose of 1% for hair cleaning, which was synthesized in 1979 by Schwarzkopf-Henkel (Germany) [61, 62]. PO is efficient against Gram (+) bacteria, Gram (-) bacteria, and fungi. The substance enters the cell and combines with iron (III) ions to block the energy metabolism in mitochondria. Furthermore, it is a novel active collagenase inhibitor [63]. PO has been used as an antidandruff agent in the shampoo and cream rinse formulations, both at 0.2% and 0.3% of doses in humans [63]. Moreover, the active agent has antifungal activity against *Malassezia* spp. and *Candida* spp. While the PO Minimum Inhibitory Concentrations (MICs) for all strains of *Candida* were low, ranging from 0.125 to 0.5 μg/mL, *in vivo* assay the PO efficient doses against *Malassezia* species were between 0.625 and 5 mg/mL [64, 65]. On the other hand, the antibacterial activity of PO toward *Actinomyces viscous* (PTCC 1202) and *Streptococcus mutans* (ATCC 35668) was observed with 64 μg/mL and 53.33 μg/mL doses, respectively [66, 67]. Additionally, researchers have conducted *in vitro* and *in*

vivo studies on antifungal activity, often involving clinical and dermatologic pathogen organisms. PO is approved by the FDA for controlling dandruff, seborrheic dermatitis, and psoriasis and is recommended for use at doses ranging between 0.05% and 1%. Antifungal activity of PO had been assessed previously *in vitro* as well as *in vivo* for clinical and dermatologic pathogen research. The antifungal efficient does was measured at 1.25 to 10 μg/ml for *Malassezia* spp. [67]. So far, the antimicrobial activity of PO has not been investigated against phytopathogens for agricultural applications.

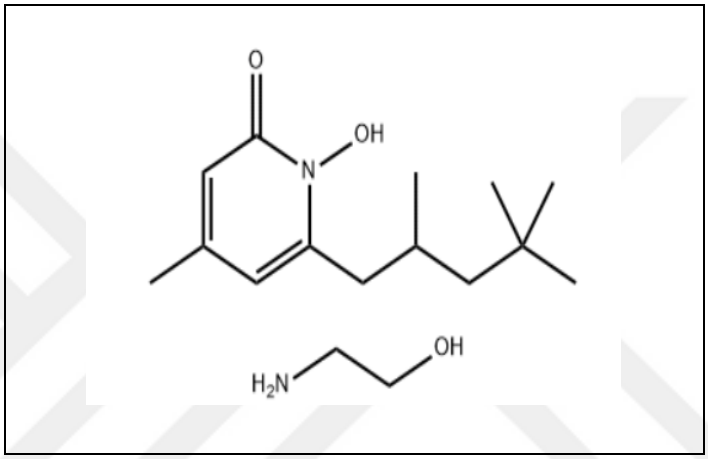

Figure 1.5. Structure of piroctone olamine [61].

The aim of this study is to develop a new seed coating formulation that will eliminate all bacterial and fungal pathogens on/in seeds of different plant species with a single active molecule that does not negatively affect seed germination and development.

2. MATERIAL AND METHOD

2.1. SEED SOURCES

In this study, Yakamoz variety (*Triticum aestivum* L.), 75MAY75 variety (*Zea mays* L.), and Demiröz F1 variety *(Solanum lycopersicum* L.) were used as wheat, corn, and tomato seed sources, respectively.

2.2. MICROORGANISMS

Strains and isolates of important plant pathogenic bacterial and fungal species listed in Table 2.1 were used in this study. *Clavibacter michiganensis* subsp. *michiganensis* and *Pseudomonas syringae* pv. *tomato* strains were provided by Prof. Dr. Yeşim Aysan from Department of Plant Protection, Faculty of Agriculture, Cukurova University, Adana, Turkey. All of the remaining bacterial and fungal strains/isolates used were obtained from Yeditepe University Genetics and Bioengineering Department Culture Collection.

2.3. CHEMICALS AND PREPARATION OF COATING SOLUTION

Piroctone olamine (PO) was obtained from BLD Pharma (CAS No: 68890-66-4) with a purity of 99.00%. Polysorbate 80, used as a solvent, was sourced from Duchefa Biochemie (CAS No: 9005-65-6), while carboxymethylcellulose (CMC) from Sigma-Aldrich (Germany) was employed as a thickening agent. The preparation of the coating solution involved the use of PO in its powdered form. Initially, Tween 80 was added to the PO, and the mixture was stirred at 800 rpm for 10 minutes. Subsequently, distilled sterile water was introduced into the homogeneous solution, followed by the addition of 2% CMC (Table 3.1). Solutions with varying solvent ratios were prepared, and the homogeneous solution with the lowest solvent ratio (1:8) was selected for use as the coating solution.

To prepare coating formulations with varying concentrations of PO, the required amounts of PO were calculated to achieve final concentrations of 0.4%, 0.1%, 0.08%, 0.05%, and 0.03%. For each formulation, the measured amount of PO was first dissolved in Polysorbate 80, which served as the solution, and the mixture was stirred at 800 rpm for 10 minutes to ensure homogeneity. Subsequently, sterile distilled water was gradually added to the homogeneous solution, followed by the incorporation of 2% carboxymethylcellulose (CMC)

as a thickening agent. This procedure was systematically repeated for each concentration, resulting in a series of coating formulations tailored to the specified PO dosages.

Table 2.1. List of the fungi and bacteria species and media used in this study

Species identification	**Media**	**Growth Conditions (time and temperature)**	**Origin**
Pseudomonas syringae pv. *syringae*	NA	24 h at 27 ±1°C	Lab. Collection
Clavibacter michiganensis subsp. *michiganensis*	NA	24 h at 27 ±1°C	Çukurova University, Faculty of Agriculture, Department of Plant Protection Prof. Dr. Yeşim Aysan Laboratory collection [68]
Pseudomonas syringae pv. *tomato*	NA	24 h at 27 ±1°C	Çukurova University, Faculty of Agriculture, Department of Plant Protection Prof. Dr. Yeşim Aysan Laboratory collection [69]
Enterococcus faecium ATCC 6057	TSA	24 h at 37 ±1°C	ATCC, USA
Escherichia coli ATCC 10536	TSA	24 h at 37 ±1°C	ATCC, USA
Staphylococcus aureus ATCC 6538	TSA	24 h at 37 ±1°C	ATCC, USA
Aspergillus niger ATCC 16404	PDA	72 h at 27 ±1°C	ATCC, USA
Penicillium sp.	PDA	72 h at 27 ±1°C	Lab. collection
Aspergillus flavus	PDA	72 h at 27 ±1°C	Lab. collection
Candida krusei	PDA	72 h at 27 ±1°C	Lab. collection

2.4. *IN VITRO* ANTIMICROBIAL ACTIVITY TESTS

100 µL suspensions of freshly grown bacterial (108 CFU mL-1) and fungal (104 spores mL-1) cultures were spread on Nutrient Agar (NA) (Fluka) and Tryptic Soy Agar (TSA) (Fluka) for bacteria and Potato Dextrose Agar (PDA) (Merck) for fungus. The formulation's antimicrobial activity was tested under *in vitro* conditions based on the disc diffusion method. Fungal isolates were grown on Potato Dextrose Agar (PDA), and bacterial strains were grown on Nutrient Agar (NA) and Tryptic Soy Agar (TSA). The plates were incubated at 30°C for 48 hours for bacteria and 96 hours for fungi [70].

Discs (Oxoid, USA) were impregnated with 19µl of the solution and placed on agar plates that has been previously inoculated with phytopathogenic fungi and bacterial species. Nystatin and ofloxacin antibiotic discs were used as positive controls to determine the sensitivity of for fungi and bacteria species, respectively.

According to the disk diffusion method, paper disks saturated with an antimicrobial substance (formulation) are placed on bacteria and/or fungi cultivated on the surface of an agar medium and incubated for 48-72 hours, then the presence or absence of an inhibition zone around the disk in petri dishes is measured. Each test was done in triplicates. Antimicrobial activity was evaluated according to the zone diameter formed. If the inhibition zone diameter was smaller than 10 mm, it was considered negative on the tested microorganism, if the zone was larger than 10 mm, it was regarded as positive effect on the microorganism tested. All experiments were carried out in 90 mm diameter Petri dishes and repeated at least three times [71].

2.5. SEED COATING

Seeds of different plant species (corn, wheat, and tomato) were immersed in the solution for 30 min under shaking at 20 rpm (Stuart, Rotator SB3). Then, the coated seeds were dried on blotting paper (Brand name/code. Maceherey-Nagel/MN 615) for 24 h at room temperature.

2.6. SEED GERMINATION TESTS

2.6.1. The Blotter Paper Method

The Blotter paper method was used to evaluate seed germinations after coating, according to ISTA with some modifications. Ten seeds were placed between two layers of filter papers previously moistened with sterile distilled water into 90 mm diameter, soda-lime glass dishes. Three milliliters of sterilized distilled water was added to each petri dish and incubated at 20-22^{0}C for 14 days. The cycle was organized as 12 hours of darkness and 12 hours of light [72]. If the radicle protrusion is longer than 2 mm, seeds will be accepted as germinated [73]. Number of germinated seeds controlled daily for 10 days.

2.6.2. Effect of PO seed coating application on the microbial population in/on the seeds

PDA were poured into sterile Petri dishes (90 × 17 mm) under aseptic conditions. After the seeds were coated with the formulation containing different doses of PO, 10 seeds with/without seed coatings were placed in each petri and incubated at 28±1^{0}C and 12 hours of darkness and 12 hours of light for two weeks [72]. Microbial growth was observed around the seeds of each plant species daily. The absence of microbial growth around all seeds germinated on the petri plates after the incubation period was accepted as the effective seed sterilization dose compared to the control. Then all of the following studies were carried out with effective dose application.

Untreated seeds were used as the negative control. Seeds treated with 2% NaClO solution for one minute on a shaker and then rinsed three times with sterile distilled water were used as positive control [74].

In addition, 10 seeds of each plant species that were and were not treated with different concentrations of PO formulation were thoroughly crushed in a mortar under sterile conditions, and then the powdered seed samples were transferred to 50 ml of PBS buffer solution and shaken at 28 ± 1 °C and 100 rpm for 30 minutes. Then, 100 µL were taken from each application tube and serial dilutions were made in PBS buffer at least 4 times, with a two-fold dilution each time. Then, 100 µL were taken from each tube and spread in NA and PDA petri dishes. After 72 hours of incubation, the colonies formed in each petri dish were counted [75]. All of the experiments were repeated at least three times..

2.7. EXPERIMENTAL DESIGN

2.7.1. Effect of Piroctone Olamine on Wheat, Corn, and Tomato Seed Germination, Radicle, Sprout Length and Rate of Germination

Ten seeds were placed in each petri dish. All experiments were repeated three times. After 14 days, root and sprout length, and the number of germinated seeds were checked daily for seven days. The germination rate was calculated according to the following formulas [76]:

$$GR = \left(number\ of\ germinated \frac{seed}{number} of\ total\ seeds\right).100$$

2.8. STATISTICAL ANALYSIS

Antimicrobial activity, GR, and seedling measurement were evaluated as general mean with a confidence level of 95%. Results below or above this value were evaluated differently. Root and shoot length comparisons were done with Ordinary One-way ANOVA. The results were compared with multicomparison. The p-value was accepted as α=0.05 and the significant difference was accepted as less than 0.05. Both the nonparametric and parametric analyses were conducted using the GraphPad 9.0. When evaluating the results, the evaluation will be made according to the $p>0.05$ no significant "ns", $p\leq0.05$ significant "*", $p\leq0.01$ very significant "**", $p\leq0.001$ highly significant "***", $p\leq0.0001$ extremely significant "****" values and will be symbolized in the graphics.

3. RESULTS

3.1. SEED COATING SOLUTION

The seed coating formulation in liquid form used in this study was prepared with 2% CMM carrier polymer, Tween-80 (1:8 ratios), and different doses (0.01%-0.40%) PO content (Table 3.1.).

Table 3.1. Concentration of PO in coating solutions.

Concentration of PO (g/L)	Concentration of Tween-80 (ml/L)
4	32.0
1	8.0
0.8	6.4
0.5	4.0
0.3	2.4
0.1	0.8

3.2. *IN VITRO* ANTIMICROBIAL ACTIVITY TESTS

In this study, the antimicrobial activities of the seed coating formulation prepared at different PO concentrations (1%, 0.5%, 0.1%, 0.06%, 0.03%, and 0.01% doses coded as 1, 2, 3, 4, 5, and 6, respectively) were determined under *in vitro* conditions using the disc diffusion test method. The test results are shown in Figure 3.1, 3.2 and summarized in Table 3.2. The results indicated that all seed coating formulations with PO content of 0.08% and above have antifungal and antibacterial activity. The activity increases depending on the dose (Figure 3.1, 3.2 and summarized in Table 3.2). It was observed that bacterial pathogens CMM, PSS, and PST, and fungal pathogens *Penicillium* spp. and *A. niger* were susceptible to PO doses of 0.03% and above. Except for 0.01% used in the seed coating formulation, all PO concentrations were determined to have antifungal activity against *A. flavus* and *C. krusei*. On the other hand, bacterial strains of *E. faecium*, *S. aureus*, and *E. coli* were found to be susceptible to 0.05% and higher PO doses in the seed coating formulation (Table 3.2.) (Figure 3.1., Figure 3.2.). The antimicrobial activity of the PO treatment was effective both on and in the seeds. Seeds (10 pieces each) treated with five different doses of PO were powdered and shaken in the PBS buffer solution for 30 minutes. After 1:10 serial dilution, when 100 µl of each dilution suspension was taken and placed on NA and PDA petri dishes,

no microbial growth was observed in the samples taken from 0.4% and 0.1% PO4-coated seeds of different plant species at the end of the incubation period, and each colony with microbial growth was counted and calculated according to the dilution coefficient in the counted petri dish and listed for each dose in Table 3.3. (Figures 3.3, 3.4, 3.5, 3.6, 3.7, 3.8). On the other hand, bacterial and fungal growth was observed in the control and 0.08% and lower dose PO-seed coating groups after a 3-day incubation period (Table 3.3 and Figure 3.3, 3.7).

Table 3.2. Disc diffusion test results (zone diameter (mm)±std. deviation). Nystatin was used as a positive control for fungal species; ofloxacin was used as a positive control for bacterial species. Their results take place in the control column.

Bacteria and fungi species	**Control**	**Treated doses (%)**					
		0.40	**0.10**	**0.08**	**0.05**	**0.03**	**0.01**
CMM	30±1	21±1	18±0.1	14±1	13±1	12±1	11±1
P. syringae* pv. *tomato	12±1	16±1	14±0.0	14±1	12±1	11±1	8±1
PSS	25±1	14±1	12±0.1	12±1	11±1	10±1	7±0
E. faecium	12±1	12±1	11±0.1	10±0	10±0	00±0	0.±0
S. aureus	29±1	13±1	12±0.0	10±1	9±0	7±1	0.±0
E. coli	38±2	12±0	11±0.1	10±0	8±1	0±0	0.±0
A. flavus	12±1	21±1	17±0.0	12±1	10±1	8±1	0.±0
C. krusei	22±0	19±0	17±0.1	16±1	16±0	12±1	0.±0
***Penicillium* sp.**	15±1	21±1	18±0.1	17±1	16±1	13±1	10±1
A. niger	18±1	20±1	17±0.1	17±1	16±1	16±1	15±1

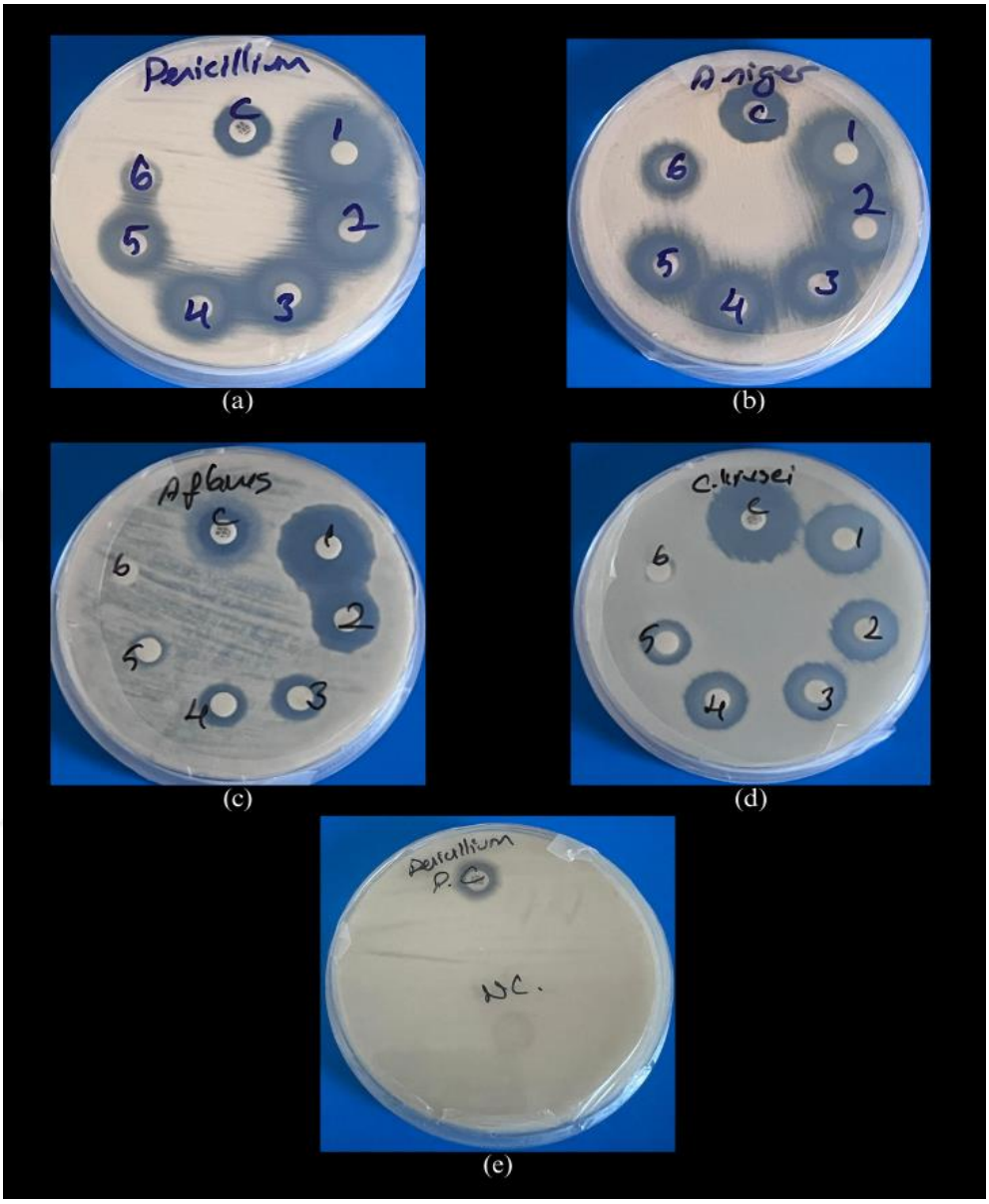

Figure 3.1. Antimicrobial effects of PO *in vitro* conditions for plant pathogenic fungi isolates on agar plates. (C=nystatin) (a) *Penicillium* spp (b) *A. niger* (c) *A. flavus*. (d) *C. krusei (e) Penicillium* spp. compared with 100% Tween-80 (NC= Tween-80, PC= Nystatin)

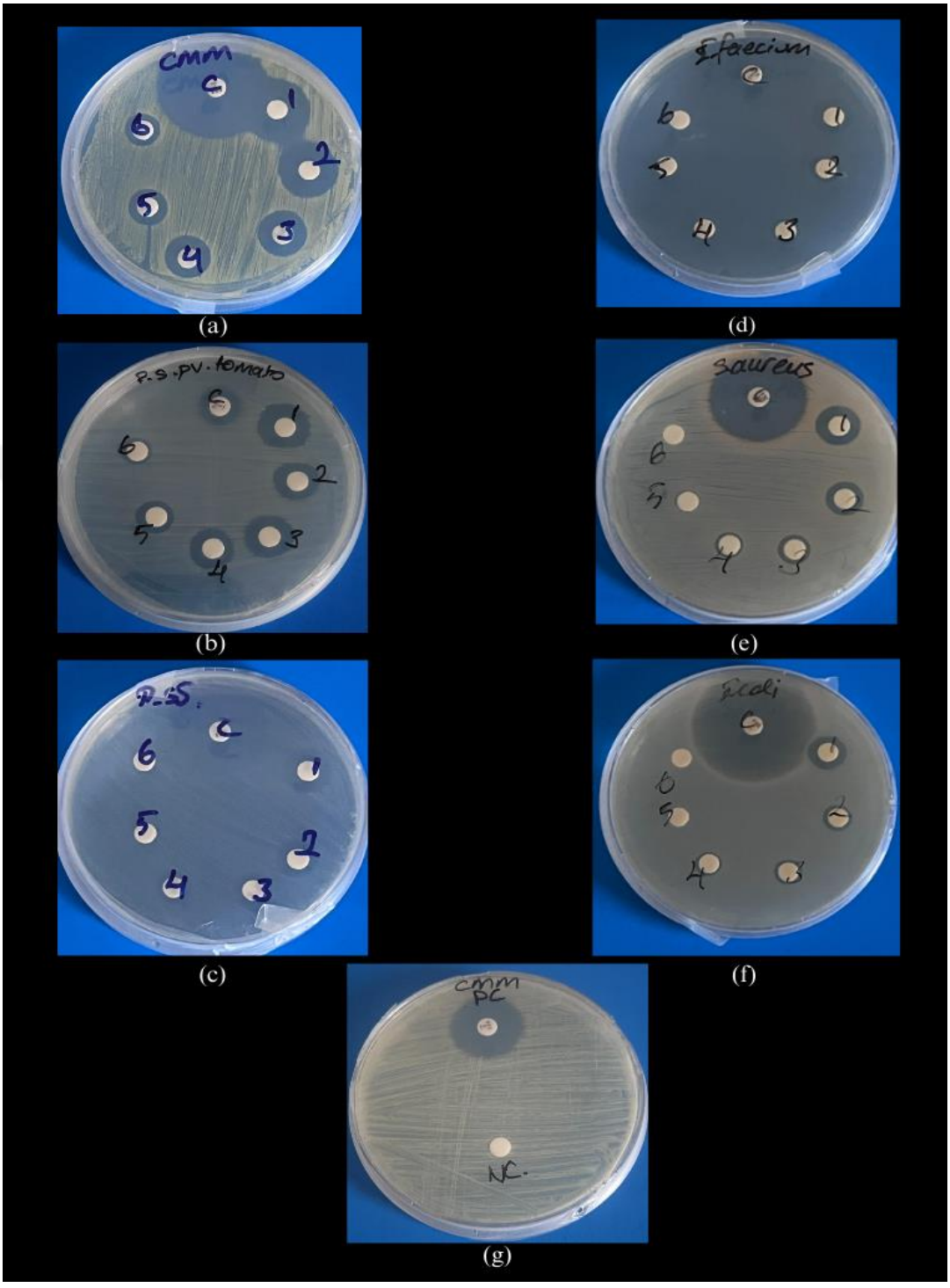

Figure 3.2. Antimicrobial effects of PO *in vitro* conditions for plant pathogenic bacterial strains on agar plates. (C= Ofloxacin) (a) CMM (b) *P. syringae* pv. *tomato* (c) PSS (d) *E.faecium* (e) *S. aureus* (f) *E.coli* (g) CMM compared with 100% Tween-80 (PC= ofloxacin, NC=Tween-80)

Table 3.3. Effect of PO seed coating application on the microbial population in/on the seeds. After 72 hours of incubation, was counted colonies in petri dishes. Colony counts were made in petri dishes containing approximately less than 100 colonies and the results were obtained by calculating the dilution coefficient.

		PO doses in seed coating formulation tested						
Plant Seeds	**Type of Media**	**0.4%**	**0.1%**	**0.08%**	**0.05%**	**0.03%**	**Non-treatment**	**Surface sterilized**
Wheat	**NA**	0.0 ac	0.0 ac	$0.4\pm0.2x10^{1}$ ac	$0,4\pm0.2x10^{1}$ ac	$0.7\pm0.1x10^{1}$ ac	$4.4\pm1.1x10^{5}$ b	$2.1\pm0.6x10^{2}$ c
	PDA	0.0 ac	0.0 ac	0.0 ac	0.0 ac	$0.1\pm0.0x10^{1}$ ac	$2.2\pm0.3x10^{5}$ b	$2.5\pm0.3x10^{3}$ c
Tomato	**NA**	0.0 ac	0.0 ac	0.0 ac	$2\pm0.0x10^{1}$ ac	$2.1\pm0.7x10^{1}$ ac	$2.7\pm0.6x10^{5}$ b	$1.1\pm0.1x10^{3}$ ac
	PDA	0.0 ac	0.0 ac	0.0 ac	0.0 ac	$0.3\pm0.1x10^{1}$ ac	$2.1\pm0.2x10^{4}$ b	$1.4\pm0.4x10^{3}$ c
Corn	**NA**	0.0 ac	0.0 ac	0.0 ac	$1,2\pm0.2x10^{1}$ ac	$1,8\pm0.2x10^{2}$ ac	$2.3\pm0.2x10^{5}$ b	$1.2\pm0.1x10^{4}$ c
	PDA	0.0 ac	0.0 ac	$0.2\pm0.1x10^{1}$ ac	$1.8\pm0.2x10^{1}$ ac	$5.3\pm0.3x10^{1}$ ac	$3.9\pm0.3x10^{5}$ b	$1.3\pm0.2x10^{3}$ c

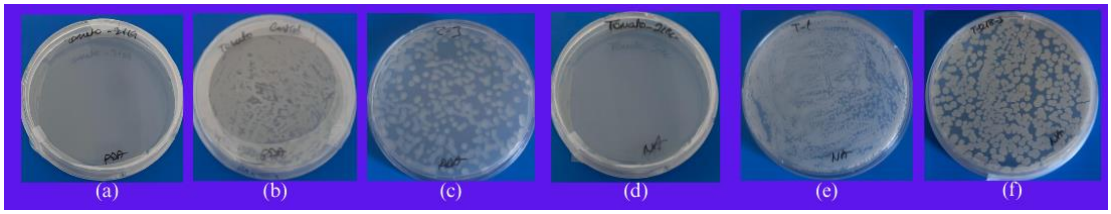

Figure 3.3. Total microbial population of tomato seeds w/wo PO seed coating treatment under *in vitro* conditions (a) Seeds treated with 0.1% PO seed coating formulation in PDA (b) Untreated seeds in PDA (c) Tomato seeds surface-sterilized with 10^{0} diluted in PDA (d) Seeds treated with 0.1% PO seed coating formulation in NA (e) Untreated seeds in NA (f) Surface-sterilized tomato seeds in NA.

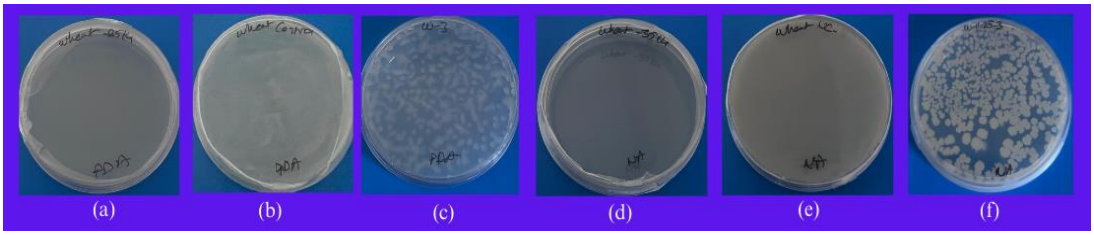

Figure 3.4. Total microbial population of wheat seeds w/wo PO seed coating treatment under *in vitro* conditions (a) Seeds treated with 0.1% PO seed coating formulation in PDA (b) Untreated seeds in PDA (c) Wheat seeds surface-sterilized with 10^0 diluted in PDA (d) Seeds treated with 0.1% PO seed coating formulation in NA (e) Non-coated seeds in NA (f) Surface-sterilized wheat seeds in NA.

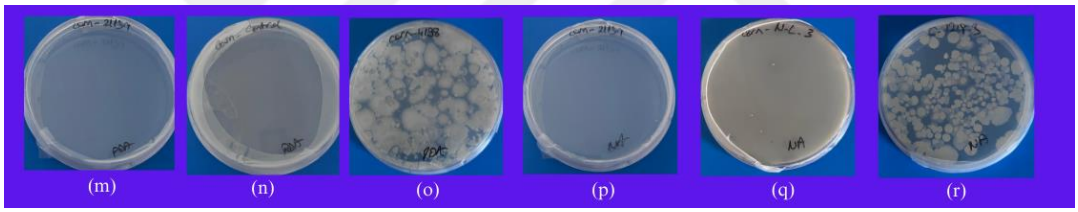

Figure 3.5. Total microbial population of corn seeds w/wo PO seed coating treatment under *in vitro* conditions (a) Seeds treated with 0.1% PO seed coating formulation in PDA (b) Untreated seeds in PDA (c) Corn seeds surface-sterilized with 10^0 in PDA (d) Seeds treated with 0.1% PO seed coating formulation in NA (e) Non-coated seeds in NA (f) Surface-sterilized corn seeds in NA.

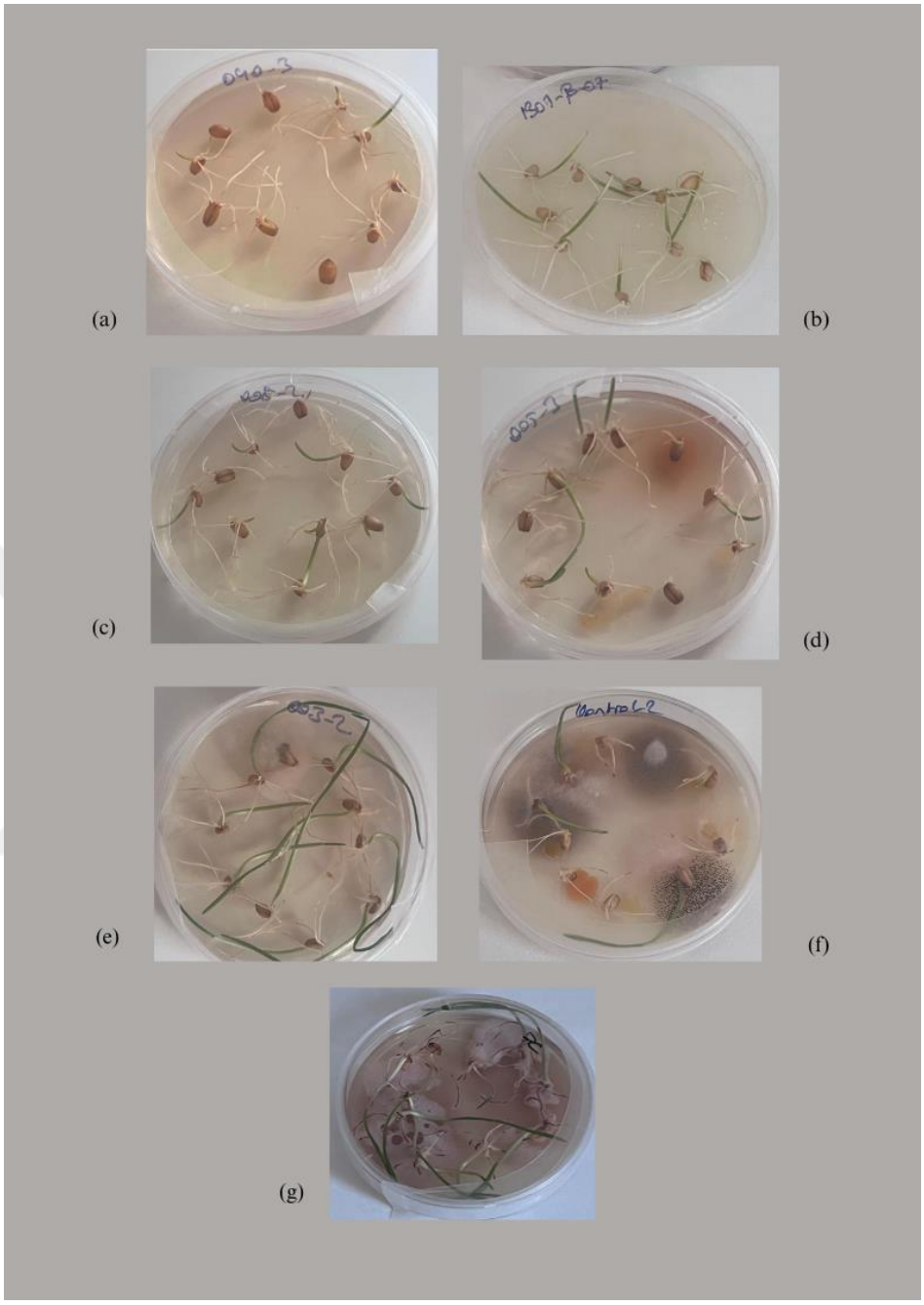

Figure 3.6. Antimicrobial activity and germination effect of PO seed coating formulation on wheat seeds in agar plate. (a) 0.40% dose of PO-coated wheat seeds, (b) 0.10% dose of PO-coated wheat seeds, (c) 0.08% dose of PO-coated wheat seeds, (d) 0.05% dose of PO-coated wheat seeds, (e) 0.03% dose of PO-coated wheat seeds, (f) non-coated seeds, (g) surface sterilized wheat seeds (7^{th} day)

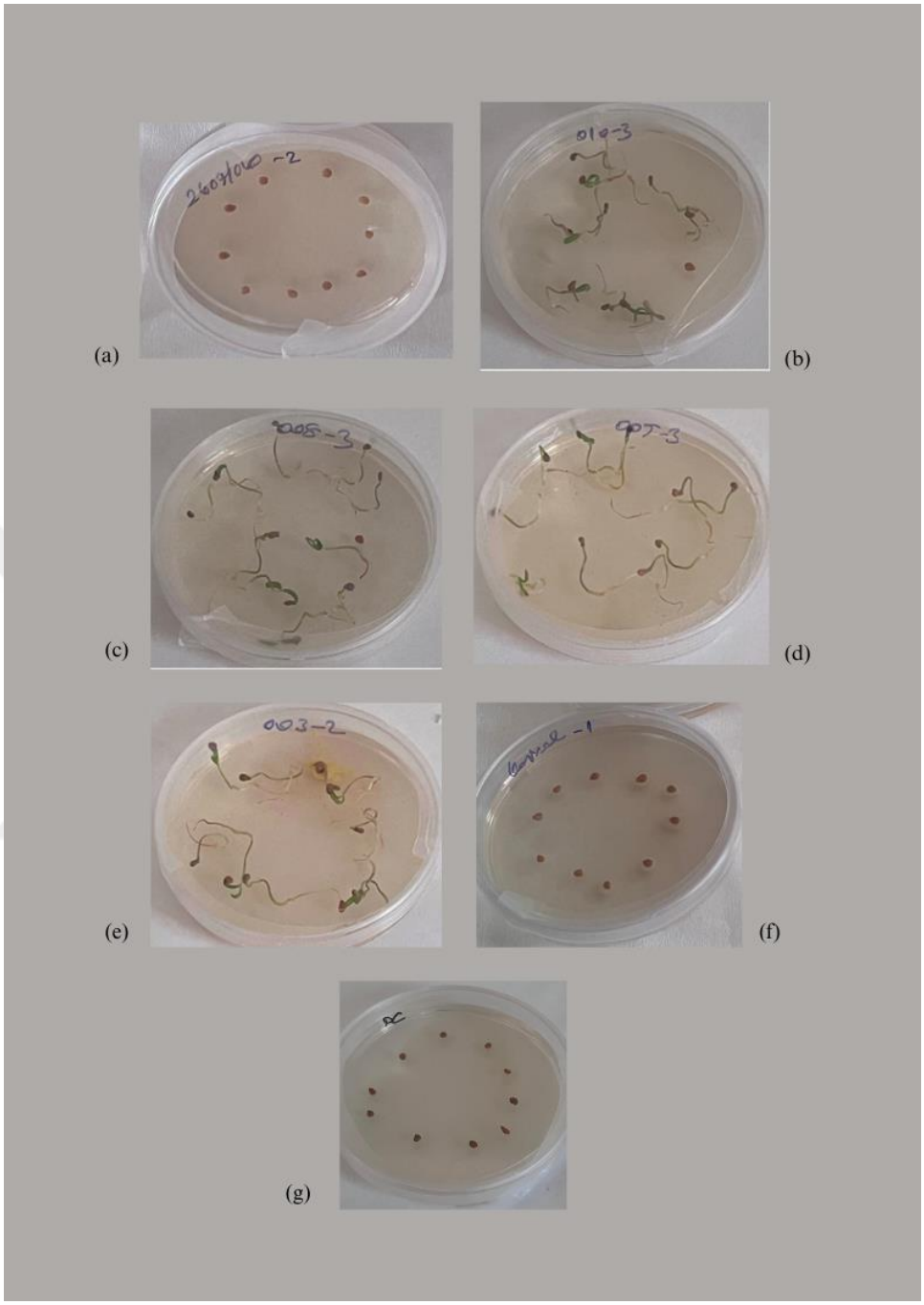

Figure 3.7. Antimicrobial activity and germination effect of PO seed coating formulation on tomato seeds in agar plate. (a) 0.40% dose of PO-coated tomato seeds, (b) 0.10% dose of PO-coated tomato seeds, (c) 0.08% dose of PO-coated tomato seeds, (d) 0.05% dose of PO-coated tomato seeds, (e) 0.03% dose of PO-coated tomato seeds, (f) non-coated tomato seeds, (g) surface sterilized tomato seeds (7th day)

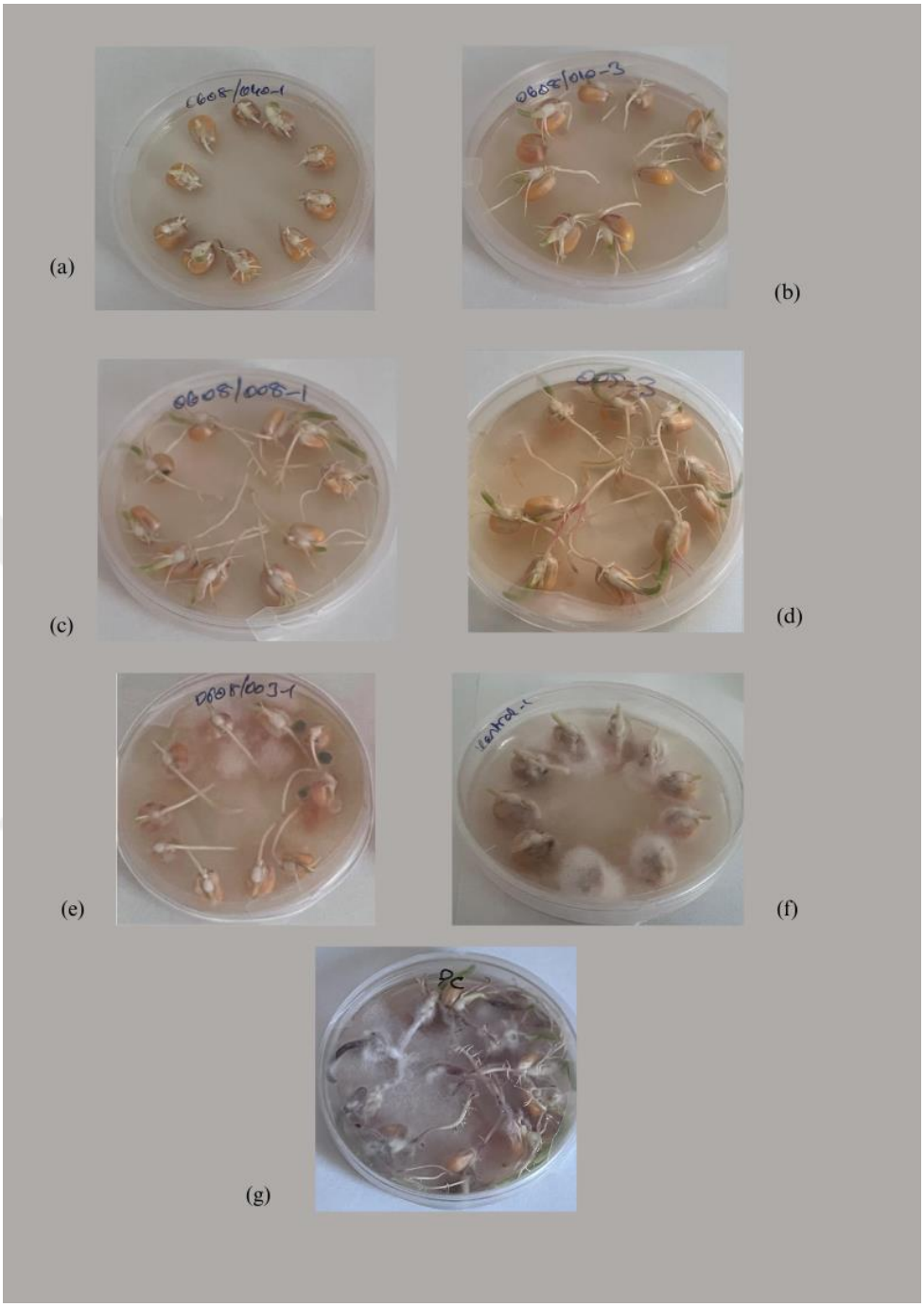

Figure 3.8. Antimicrobial activity and germination effect of PO seed coating formulation on corn seeds in agar plate. (a) 0.40% dose of PO-coated corn seeds, (b) 0.10% dose of PO-coated corn seeds, (c) 0.08% dose of PO-coated corn seeds, (d) 0.05% dose of PO-coated corn seeds, (e) 0.03% dose of PO-coated corn seeds, (f) non-coated corn seeds, (g) surface sterilized corn seeds (7th day)

3.3. SEED GERMINATION TESTS

Seeds of corn, wheat, and tomato plants were treated with seed coating formulation containing different concentrations (0.03%-0.40%) of PO. Seeds with/without seed coating formulation were placed between two layers of filter paper in petri dishes and kept in the incubator for 10 days.

Then the number of germinated seeds in each treatment was determined and summarized in Tables 3.4., 3.5., and 3.6. The lowest germination rate in wheat seeds was obtained from seed application containing 0.4% PO. No significant difference in seed germination rate was observed between all the other PO seed coating formulations tested in wheat and the negative control (Table 3.4.) (Figure 3.9., 3.12.). The effect of PO seed applications on the germination rate of corn seeds was similar to the results in wheat seeds. It was determined that only 0.4% PO seed coating formulation delayed the germination of corn seeds (Table 3.5.) (Figure 3.11, 3.14.). No significant difference was observed between the germination rates of tomato seeds treated with seed coating formulations containing different PO concentrations and the negative control (Table 3.6.) (Figure 3.10, 3.13.). When the seed germination test results were examined, it was determined that there was no significant change in the results at the end of 7 days within the 10-day observation period (Figure 3.15.).

Table 3.4. Effect of PO seed coating application on seed germination rate in wheat. Observations were recorded daily for 10 days. This formula is calculated: (Number of germinated seeds divided by total tested number of seeds) x%

Plant Seed	Doses	Germination rate (%) of wheat seeds observation days										Germination Rate (%)
		1	2	3	4	5	6	7	8	9	10	
wheat	0.4%	0	28.9	48.9	60.0	66.7	68.9	70.0	70.0	70.0	70.0	70±23.4
wheat	0.1%	0	64.4	75.6	82.2	86.7	86.7	88.9	88.9	88.9	88.9	90.0±2.6
wheat	0.08%	0	71.1	88.9	90.0	90.0	92.2	92.2	92.2	92.2	92.2	91.1±1.9
wheat	0.05%	0	72.2	81.1	83.3	85.6	88.9	92.2	92.2	92.2	92.2	92.2±7.7
wheat	0.03%	0	76.7	83.3	88.9	91.1	93.3	93.3	93.3	93.3	93.3	93.3±3.3
wheat	Surface sterilized seeds	0	72.2	80.0	84.4	86.7	91.1	91.1	91.1	91.1	91.1	91.1±3.8
wheat	No-treated seeds	0	94.4	95.6	96.7	97.8	97.8	97.8	97.8	97.8	97.8	97.8±1.9

Table 3.5. Effect of PO seed coating application on seed germination rate in tomato. Observations were recorded daily for 10 days. This formula is calculated: (Number of germinated seeds divided by total tested number of seeds) x%

Plant Seed	Doses	Germination rate (%) of tomato seeds Observation days										Germination Rate (%)
		1	2	3	4	5	6	7	8	9	10	
tomato	0.4%	0	34.4	75.6	93.3	97.8	98.9	98.9	98.9	98.9	98.9	99±1.7
tomato	0.1%	0	30.0	82.2	91.1	93.3	94.4	94.4	94.4	94.4	94.4	94.3±2.3
tomato	0.08%	0	40.0	84.4	96.7	96.7	96.7	96.7	96.7	96.7	96.7	96.7±5.8
tomato	0.05%	0	43.3	85.6	92.2	94.4	94.4	94.4	94.4	94.4	94.4	92±8.5
tomato	0.03%	0	50.0	84.4	93.3	94.4	96.7	96.7	96.7	96.7	96.7	96.7±5.8
tomato	Surface sterilized seeds	0	57.8	83.3	96.7	100.0	100.0	100.0	100.0	100.0	100.0	100±0.00
tomato	No-treated seeds	0	58.9	91.1	98.9	100.0	100.0	100.0	100.0	100.0	100.0	100±0.00

Table 3.6. Effect of PO seed coating application on seed germination rate in corn. Observations were recorded daily for 10 days. This formula is calculated: (Number of germinated seeds divided by total tested number of seeds) x%

Plant Seed	Doses	Germination rate (%) of corn seeds Observation days										Germination Rate (%)
		1	2	3	4	5	6	7	8	9	10	
corn	0.4%	0	0	13.3	53.3	70.0	71.1	74.4	74.4	74.4	74.4	74±13.5
corn	0.1%	0	0	34.4	93.3	95.6	98.9	98.9	98.9	98.9	98.9	98.9±1.9
corn	0.08%	0	0	43.3	86.7	94.4	95.6	97.8	97.8	97.8	97.8	98.9±1.9
corn	0.05%	0	0	45.6	94.4	98.9	100.0	100.0	100.0	100.0	100.0	100±0.0
corn	0.03%	0	0	63.3	94.4	100.0	100.0	100.0	100.0	100.0	100.0	100±0.0
corn	Surface sterilized seeds	0	0	84.4	87.8	88.9	91.1	91.1	91.1	91.1	91.1	91.1±6.9
corn	No-treated seeds	0	0	97.8	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100±0.0

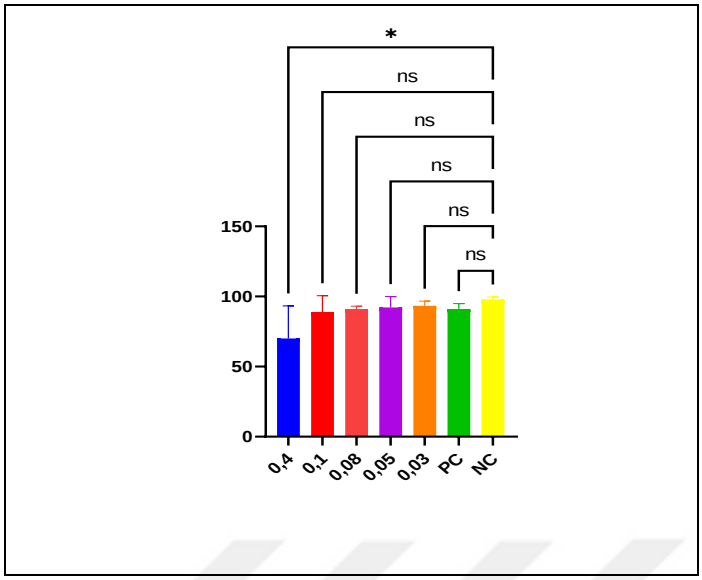

Figure 3.9. Effect of PO seed coating applications on seed germination rate in wheat compared to control (NC= non-coated seeds, PC= surface sterilized seeds) (95% Confidence level, α=0.05, ns= no significance difference >0.05, *= p value 0.0273).

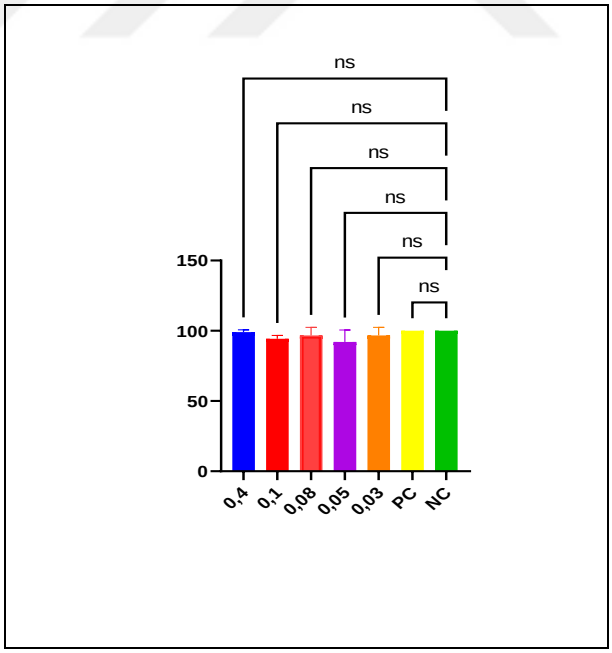

Figure 3.10. Effect of PO seed coating applications on seed germination rate in tomato compared to control (NC= non-coated seeds, PC= surface sterilized seeds) (95% Confidence level, α=0.05, ns= no significance difference >0.05)

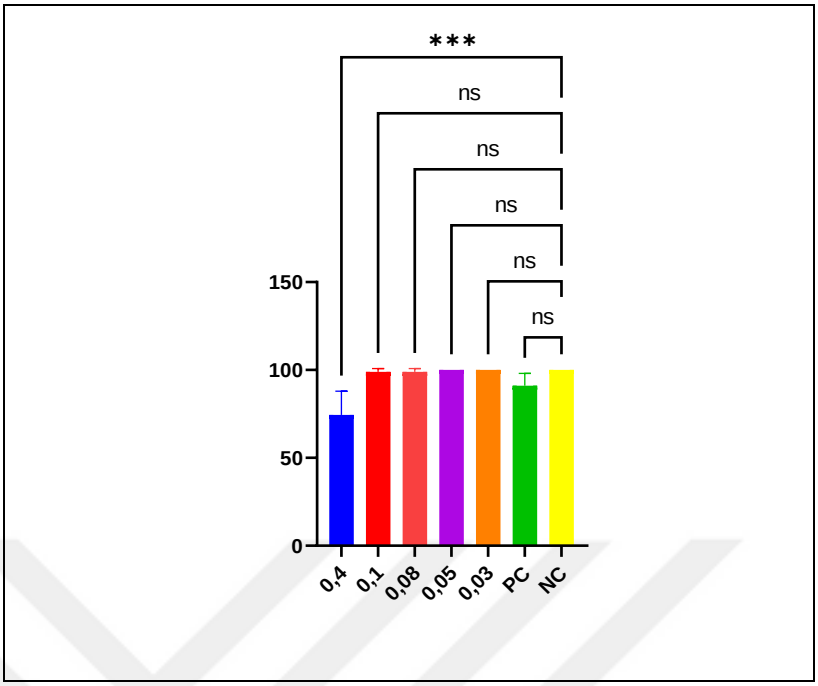

Figure 3.11. Effect of PO seed coating applications on seed germination rate in corn compared to control (NC= non-coated seeds, PC= surface sterilized seeds) (95% Confidence level, α=0.05, ns= no significance difference >0.05, ***=p value 0.0015)

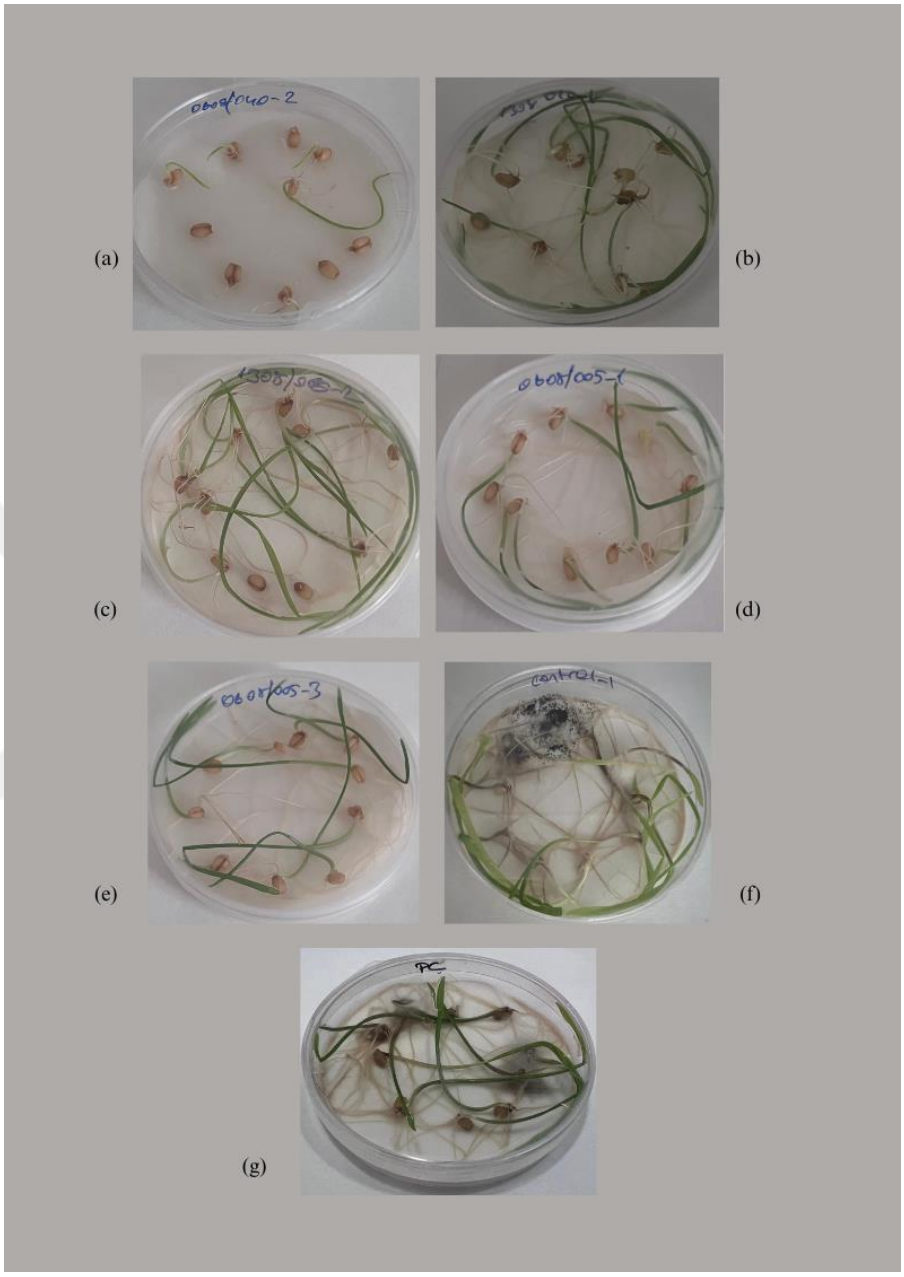

Figure 3.12. Effect of PO seed coating formulation on wheat seed germination on blotter paper. (a) 0.40% dose of PO-coated wheat seeds, (b) 0.10% dose of PO-coated wheat seeds, (c) 0.08% dose of PO-coated wheat seeds, (d) 0.05% dose of PO-coated wheat seeds, (e) 0.03% dose of PO-coated wheat seeds, (f) non-coated wheat seeds, (g) surface sterilized wheat seeds (7th day).

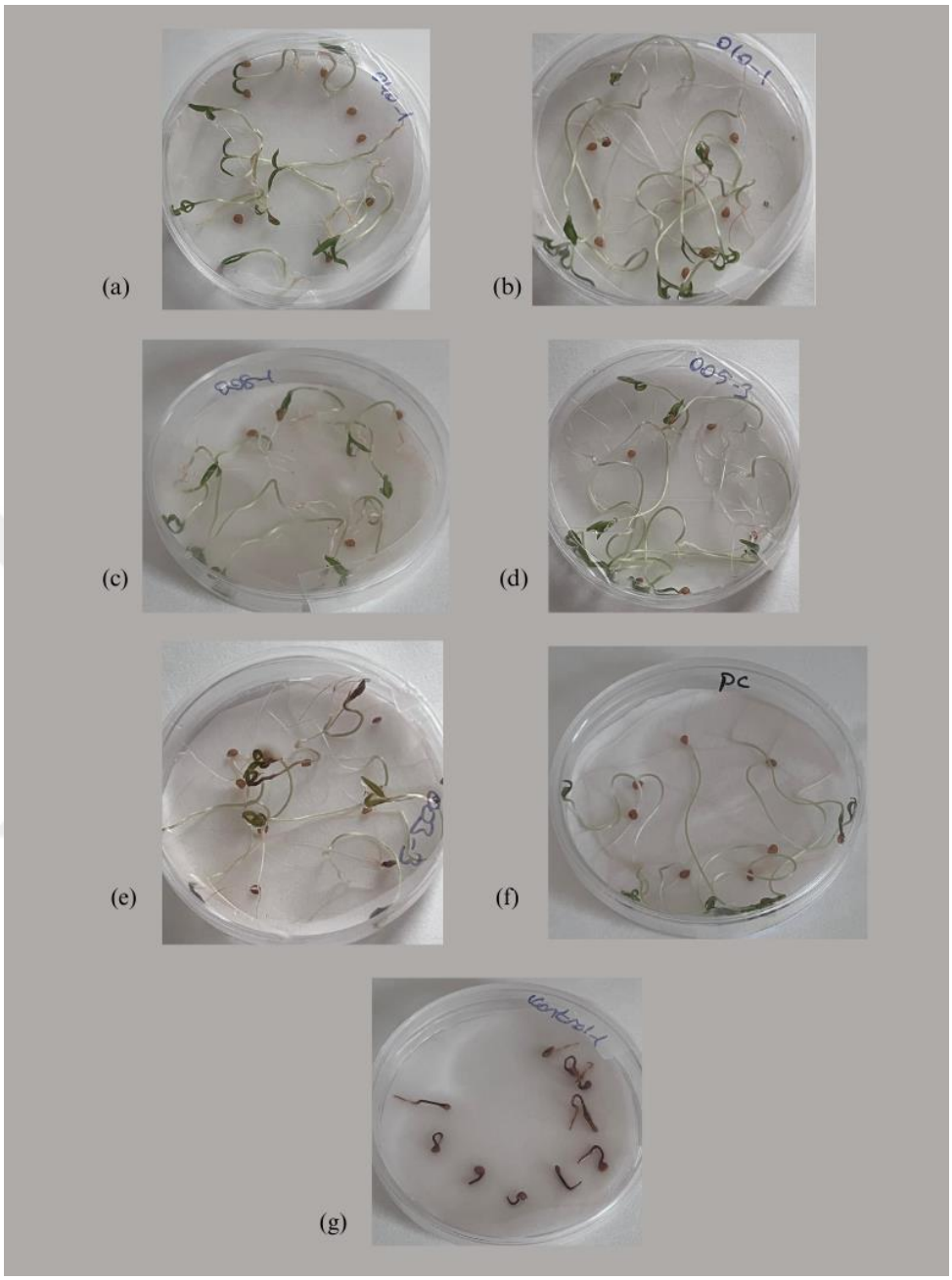

Figure 3.13. Effect of PO seed coating formulation on tomato seed germination on blotter paper. ((a) 0.40% dose of PO-coated tomato seeds, (b) 0.10% dose of PO-coated tomato seeds, (c) 0.08% dose of PO-coated tomato seeds, (d) 0.05% dose of PO-coated tomato seeds, (e) 0.03% dose of PO-coated tomato seeds, (f) non-coated tomato seeds, (g) surface sterilized tomato seeds (7th day).

Figure 3.14. Effect of PO seed coating formulation on corn seed germination on blotter paper. (a) 0.40% dose of PO-coated corn seeds, (b) 0.10% dose of PO-coated corn seeds, (c) 0.08% dose of PO-coated corn seeds, (d) 0.05% dose of PO-coated corn seeds, (e) 0.03% dose of PO-coated corn seeds, (f) non-coated corn seeds, (g) surface sterilized corn seeds (7th day).

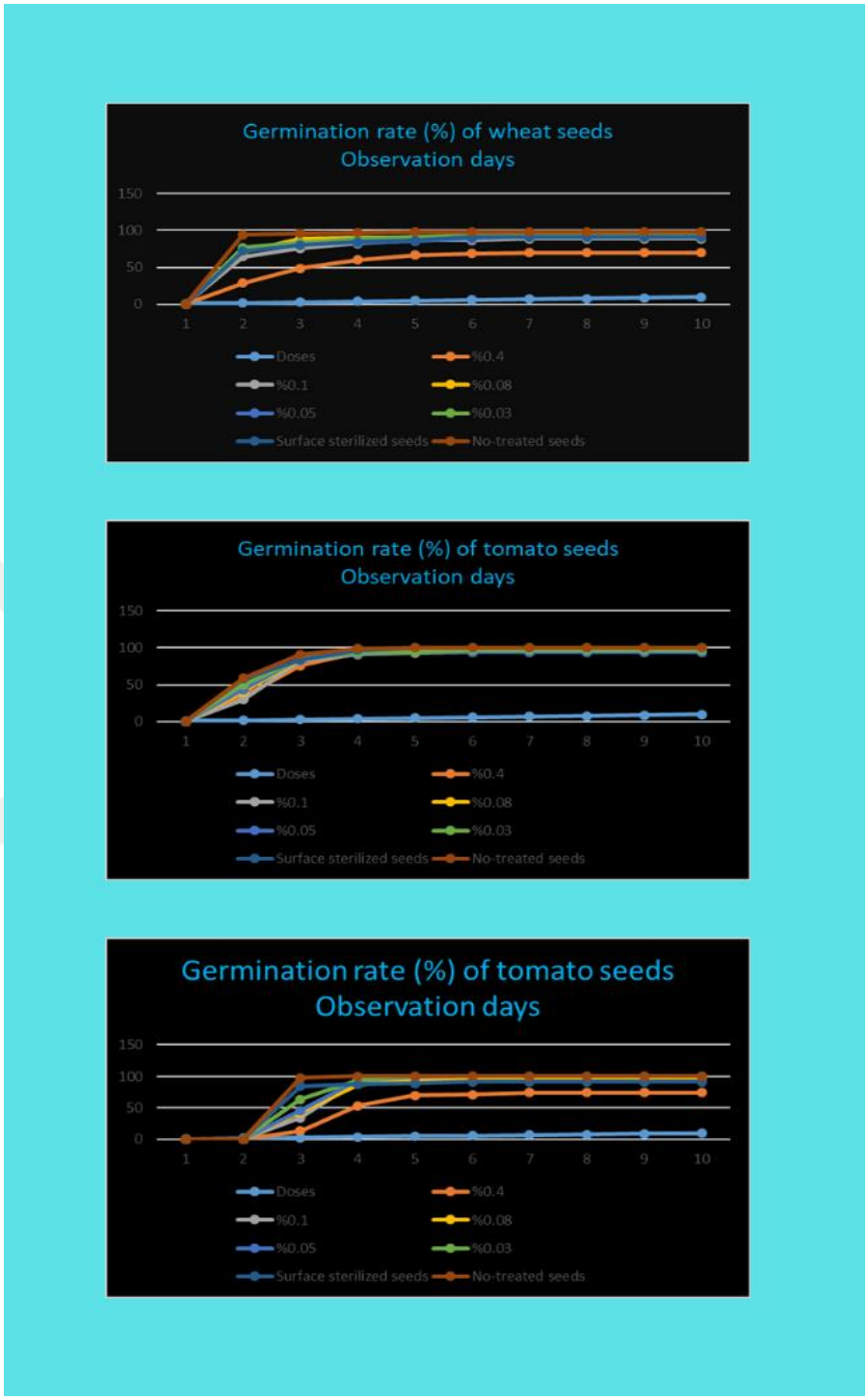

Figure 3.15. Daily germination of seeds (a) wheat, (b) tomato, (c) corn

3.4. ROOT AND SPROUT LENGTHS

3.4.1. Root Lengths

The effects of wheat, tomato, and corn seeds treated with seed coating formulation containing different PO doses on root development are summarized in Table 3.7 in comparison with the negative control application. When the root lengths of germinated seeds in wheat were examined, no statistical difference was observed between the seed coating applications with PO concentrations of 0.08% and below and the negative control, while a significant decrease in root lengths was detected at higher PO concentrations (Table 3.7) (Figures 3.16, 3.22). When the root lengths of tomato seedlings were examined, it was determined that the root lengths of seedlings obtained from seeds treated with 0.1% and/or lower dose PO seed coating formulation were greater than those of the negative control. No statistically significant difference was observed at other doses. (Table 3.7.) (Figure 3.17, Figure 3.23).

In the case of the root lengths of the corn seedlings that were evaluated, it was found that the root lengths of the corn seedlings obtained from treated seeds with the PO seed coating formulation were much longer than those of the control group. The lowest root length was measured in untreated seeds. It was observed that the root lengths of the corn seedlings of treated seeds with a high PO dose (0.4%) seed coating formulation were lower than those of the other application groups. The highest root length values among the other application groups were found in the seeds treated with formulations containing 0.05%, 0.08%, and 0.1% PO, respectively (Table 3.7) (Figure 3.18, Figure 3.24).

3.4.2. Sprout Lengths

The effects on shoot development of seedlings obtained from wheat, tomato and maize seeds treated with seed coating formulation containing different PO doses are summarized in Table 3.7 as comparisons to the negative control. The best shoot lengths in wheat seedlings were observed in seed treatments containing 0.1% and 0.08% PO and were even better than the negative control (Table 3.7.) (Figure 3.19., 3.22).

When the shoot lengths of tomato seedlings were examined, it was determined that seed treatments containing 0.1% and lower PO doses had better shoot lengths than the negative

control. No significant difference was found between the other treatments and the control in terms of shoot length, but high dose PO application (0.4%) had a negative effect on the shoot length of tomato seedlings (Table 3.7.) (Figure 3.20, Figure 3.23).

When the shoot lengths of the seedlings obtained from corn seeds to which PO seed coating was applied were compared and evaluated with the untreated control, it was seen that the tested PO applications affected the shoot lengths positively and that the shoot lengths were better than the shoot lengths of the corn seedlings without the application. It was also determined that the shoot lengths of the seedlings obtained from the seed applications containing 0.08% and 0.1% PO were longer than the other applications and the negative control. (Table 3.7) (Figure 3.21, Figure 3.24).

Table 3.7. Effect of PO seed coating applications on root and shoot development of wheat, tomato and corn seedlings after incubating for 14 days (mean (cm) ±SD). Negative control=non-coated seeds, Positive Control=Surface sterilized seeds. Within each line, means sharing the same letter was not significantly different at the 5% level of significance (α=0.05).

Plant Species	**PO doses in seed coating formulation**					**Negative Control**	**Positive Control**
	0.4%	**0.1%**	**0.08%**	**0.05%**	**0.03%**		
Wheat							
Root Length	1.4±1.3 a	11.0±3.6 b	13.4±3.3 c	12.5±4.8 bcd	10.7±4.7 bd	13.8±4.7 c	14.0±2.0 c
Sprout Length	3.9±2.5 a	10.3±3.5 b	10.5±3.4 b	8.8±4.3 c	8.7±4.1 cd	7.2±2.7 cde	6.6±2.03 e
Tomato							
Root Length	0.9±0.4 a	6.4±2.1 b	4.6±2.5 c	6.2±2.9 b	6.3±2.1 b	4.7±1.3 c	8.8±1.8 d
Sprout Length	2.2±0.8 a	4.3±1.1 b	3.8±1.1 bc	4.8±1.4 d	4.6±1.1 bde	3.8±1.2 c	4.8±0.7 d
Corn							
Root Length	0.8±0.4 a	9.3±4.0 b	10.4±4.3 bc	10.9±3.4 c	9.6±3.9 bc	2.2±1.2 a	11.3±2.5 c
Sprout Length	1.3±0.6 a	2.1±0.7 b	2.4±1.1 b	2.1±0.7 b	1.7±0.8 a	0.7±0.6 c	1.4±0.6 a

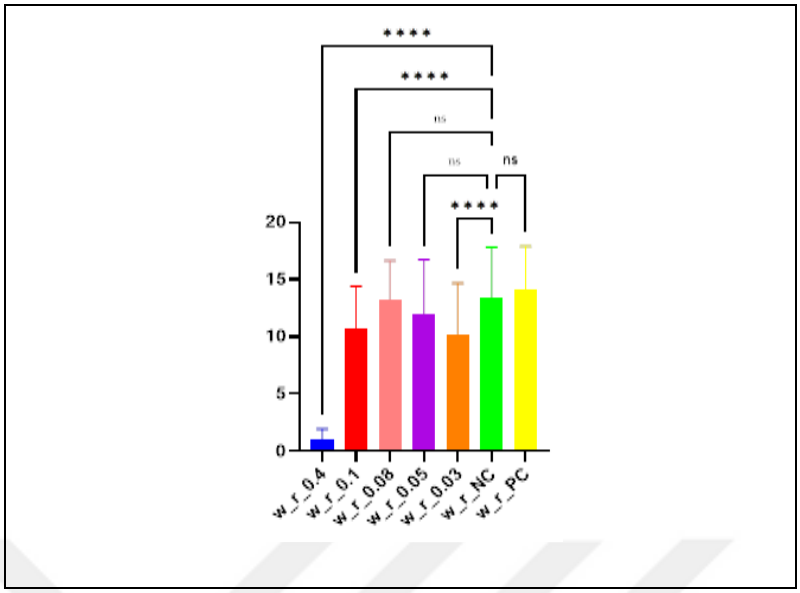

Figure 3.16. Effect of PO seed coating applications on root development of wheat seedlings (mean (cm) ±SD). NC= non-coated seedlings, PC= surface sterilized seedlings (no significant difference >0.05, p<0.0001 =****, Wheat root 0.08xNC p value=0.9997=*, 0.05xNC p value=0.0902=*, NCxPC p value=0.7077)

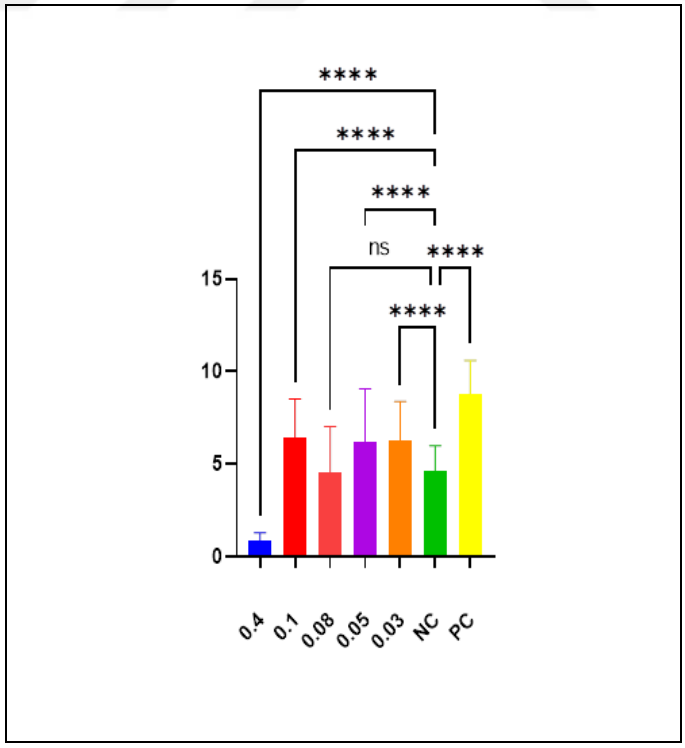

Figure 3.17. Effect of PO seed coating applications on root development of tomato seedlings (mean (cm) ±SD). NC= non-coated seedlings, PC= surface sterilized seedlings (no significant difference >0.05, p<0.0001 =****)

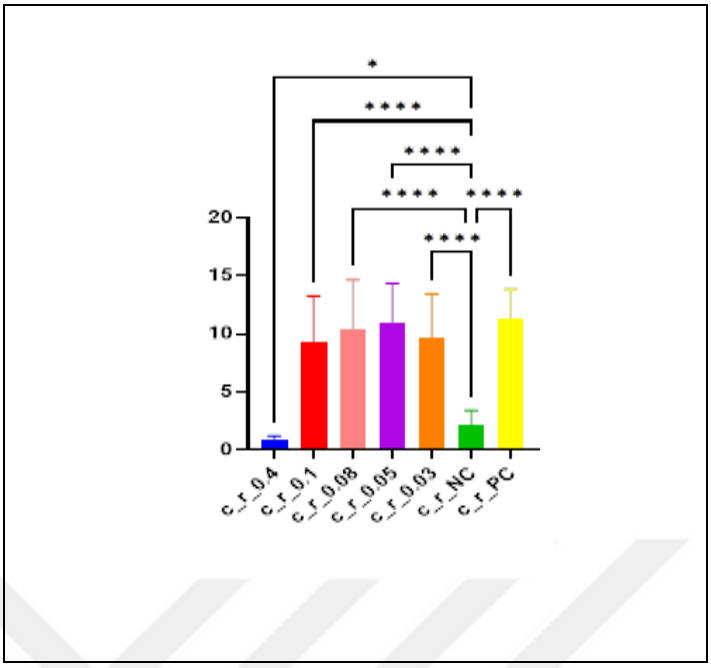

Figure 3.18. Effect of PO seed coating applications on root development of corn seedlings (mean (cm) ±SD). NC= non-coated seedlings, PC= surface sterilized seedlings (no significant difference >0.05, p<0.0001 =****)

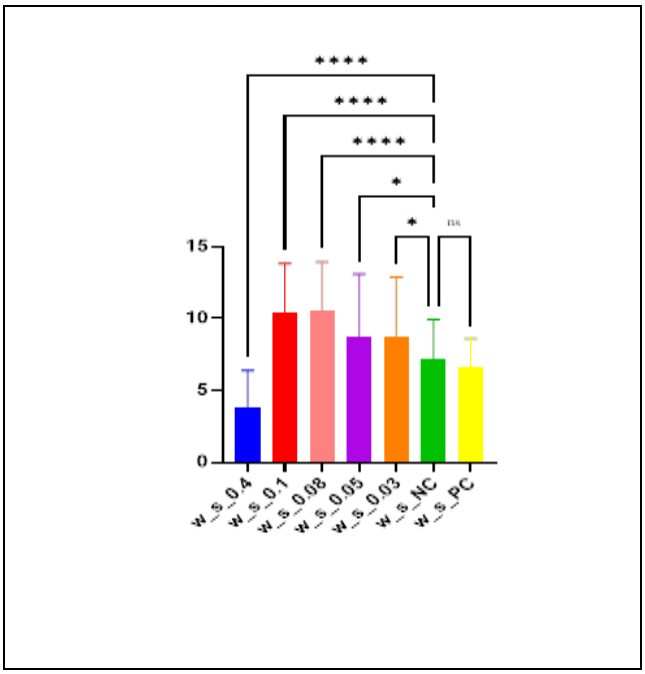

Figure 3.19. Effect of PO seed coating applications on sprout development of wheat seedlings. NC=non-coated seedlings, PC= surface sterilized seedlings. (No significant difference >0.05, p<0.0001 =****, p value=0.0624 for 0.03x NC sprout, NCxPC sprout p value=0.8628)

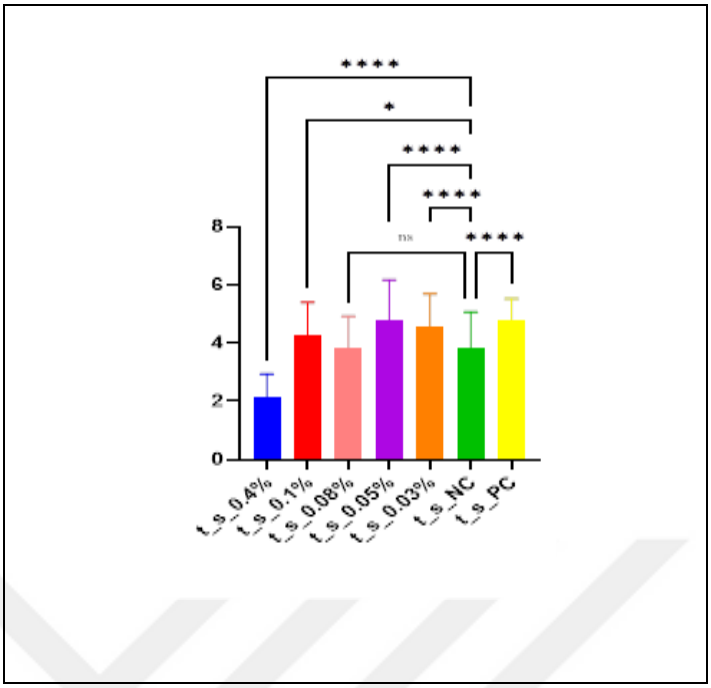

Figure 3.20. Effect of PO seed coating applications on sprout development of tomato seedlings. NC=non-coated seedlings, PC= surface sterilized seedlings. (No significant difference >0.05, p<0.0001 =****, sprout of tomato seedling 0.1xNC p value= 0.1493=*)

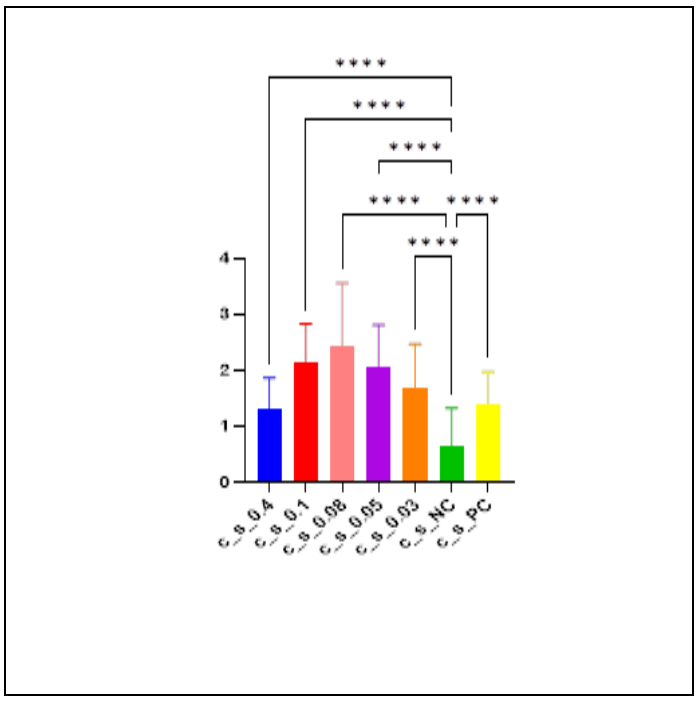

Figure 3.21 Effect of PO seed coating applications on sprout development of corn seedlings. NC=non-coated seedlings, PC= surface sterilized seedlings. (No significant difference >0.05, α=0.05, p<0.0001 =****)

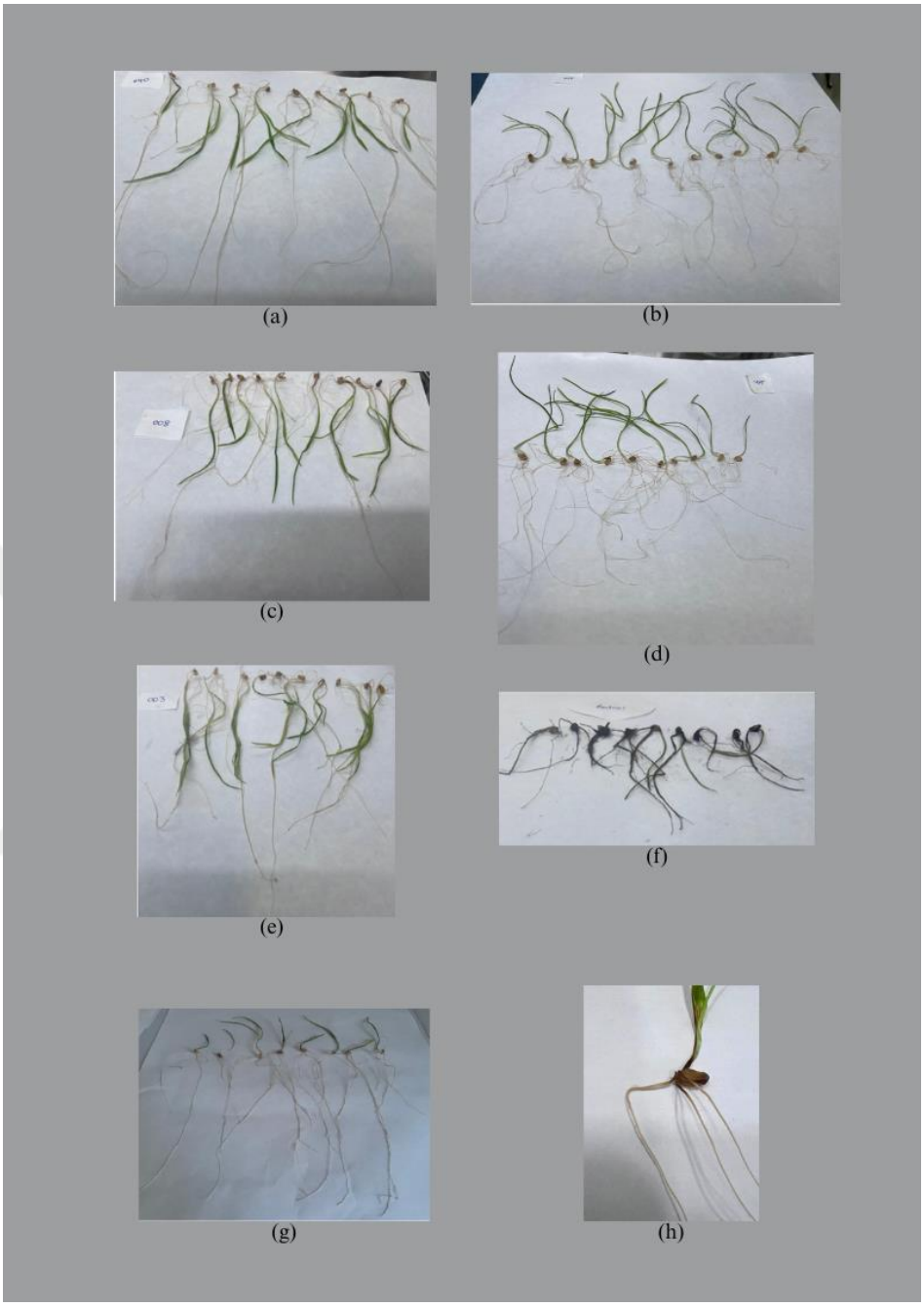

Figure 3.22 Root and shoot lengths of wheat seedlings obtained from seeds treated with seed coating formulations containing different PO concentrations (a) 0.40%, (b) 0.10%, (c) 0.08%, (d) 0.05%, (e) 0.03% , (f) Non-treated seeds, (g) Surface sterilized seedlings (h) Cotyledon of surface sterilized seed showed that cotyledon was affected by endophytic miroorganisms

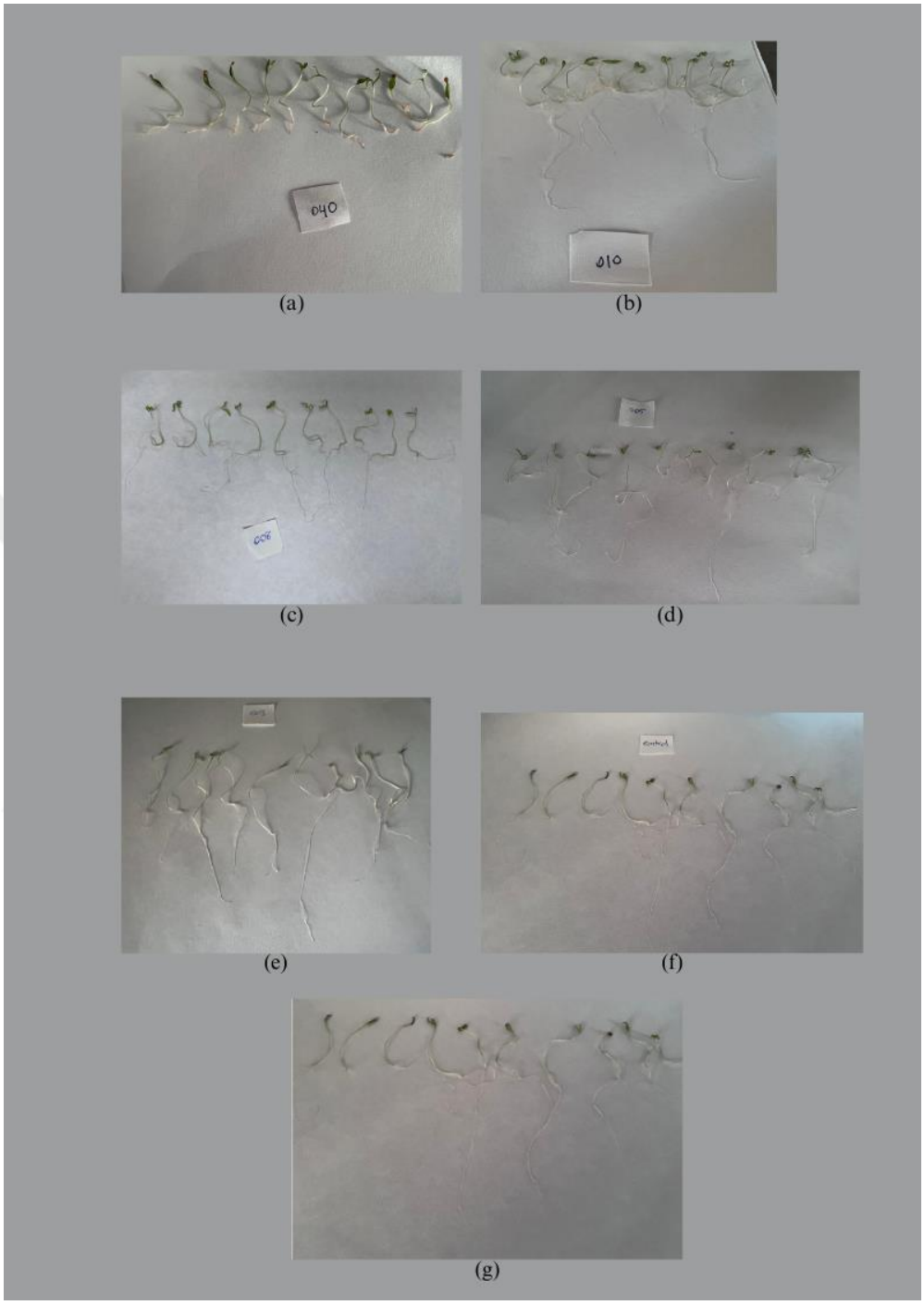

Figure 3.23 Root and shoot lengths of tomato seedlings obtained from seeds treated with seed coating formulations containing different PO concentrations (a) 0.40% (b) 0.10% (c) 0.08% (d) 0.05% (e) 0.03% (f) non-coated seedlings (g) Surface sterilized seedlings

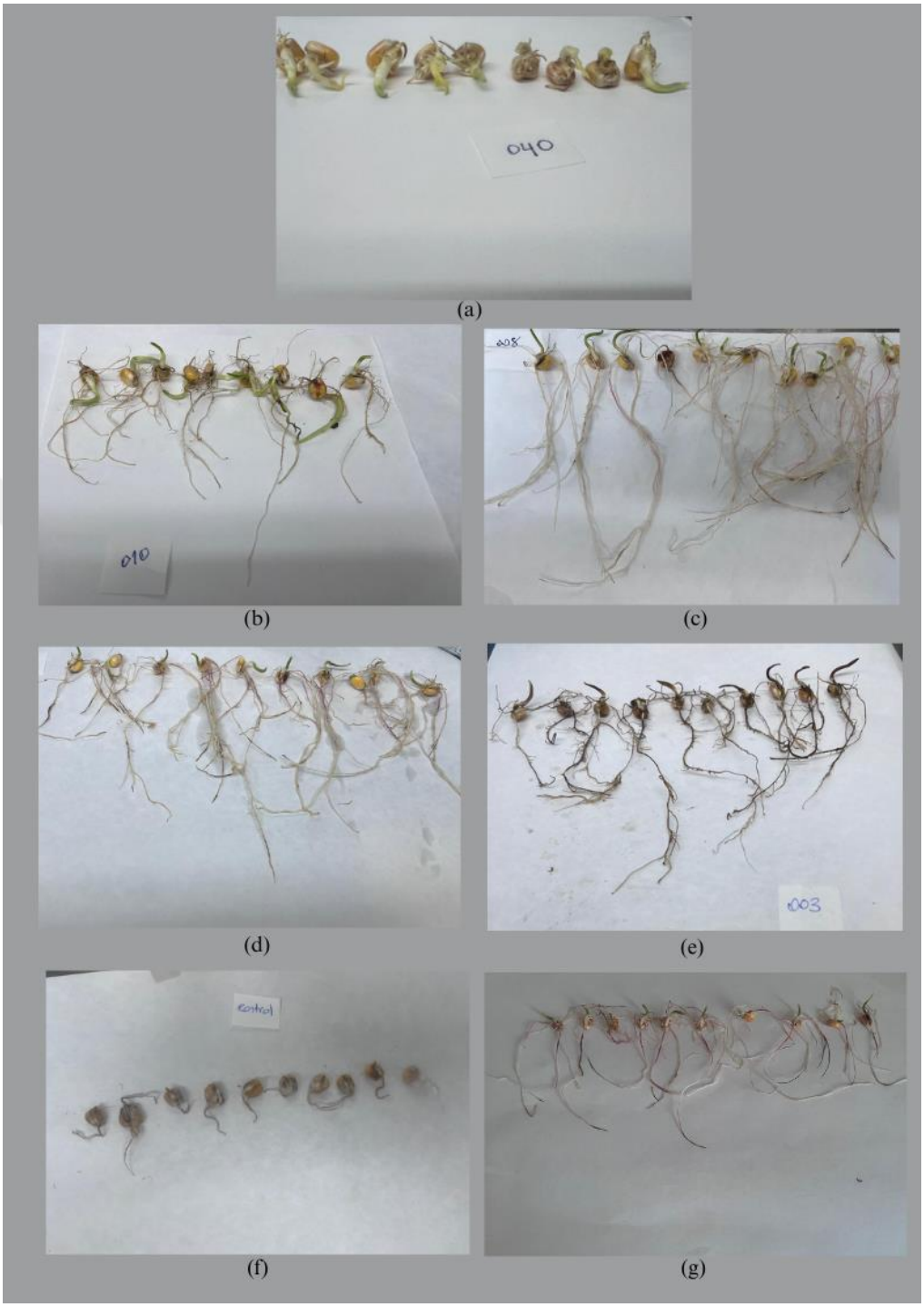

Figure 3.24 Root and shoot lengths of corn seedlings obtained from seeds treated with seed coating formulations containing different PO concentrations ((a) 0.40% (b) 0.10% (c) 0.08% (d) 0.05% (e) 0.03% (f) non-coated seedlings (g) Surface sterilized seedlings

4. DISCUSSION & FUTURE PERSPECTIVES

The findings of this study underscore the transformative potential of a piroctone olamine (PO)-based seed coating formulation in addressing the persistent challenges posed by seed-borne pathogens. These pathogens are a significant barrier to global agricultural productivity, causing yield losses, quality degradation, and increased production costs. By integrating antimicrobial efficacy with seed health preservation, this study provides a sustainable and innovative approach to seed treatment technologies.

Seed-borne diseases remain a critical issue in agriculture and the global seed trade [6]. Pathogens such as fungi, bacteria, and viruses, carried on or inside seeds, are primary sources of inoculum for widespread infections in fields [76]. These infections not only cause poor germination and plant vigor but also lead to economic losses. For instance, *Fusarium* head blight and common bunt in wheat account for annual global losses exceeding $2.7 billion [77]. Moreover, trade restrictions imposed on countries failing to meet phytosanitary standards exacerbate these challenges, limiting market access and reducing revenue streams [25, 26].

Certified pathogen-free seeds, though recommended as a management strategy, are often inaccessible due to high production costs, inadequate regulatory frameworks, and limited availability, especially for smallholder farmers. As such, there is a growing demand for effective seed treatment methods to eliminate pathogens while preserving seed viability and germination potential [78, 79].

Traditional methods, including physical, chemical, and biological treatments, have been employed to manage seed-borne diseases. However, these approaches have inherent limitations. Physical treatments such as hot water, UV radiation, and ozone therapy often compromise seed viability and require significant energy inputs [80]. Similarly, biological treatments, though environmentally friendly, are pathogen-specific and influenced by environmental conditions, resulting in inconsistent efficacy [49, 50]. Chemical treatments, while effective against external pathogens, pose environmental risks and are less effective against internal infections. Additionally, resistance to fungicides and bactericides has been increasingly reported, necessitating novel solutions [41].

The efficacy of physical treatments is often limited by factors such as seed type, pathogen load, and environmental conditions during application. Heat-based methods like dry heat and hot water treatments can lead to thermal damage to seed embryos, reducing germination rates. While UV and ozone therapies offer chemical-free alternatives, their scalability remains a challenge due to high equipment costs and energy consumption [80, 81].

Biological treatments, leveraging beneficial microbes or natural compounds, provide a sustainable alternative to chemical methods. However, their performance is highly variable under field conditions. Factors such as soil type, temperature, and moisture can influence the survival and efficacy of biocontrol agents like *Trichoderma* spp. and *Bacillus subtilis*. Additionally, their narrow-spectrum activity often necessitates the use of multiple agents for comprehensive protection, increasing complexity and cost [49, 50, 82].

Chemical treatments, while widely adopted, face growing scrutiny due to their environmental and health impacts. Commonly used fungicides and bactericides often leave residues that can contaminate soil and water systems. Furthermore, the emergence of resistant pathogen strains underscores the need for new active ingredients with novel modes of action [41, 83].

Piroctone olamine (PO), a compound with proven antimicrobial properties in cosmetic applications, offers a dual advantage of pathogen control and environmental safety. This study demonstrated that PO-based formulations, particularly at concentrations of 0.1% and 0.08%, effectively inhibit the growth of key bacterial and fungal pathogens, including *Clavibacter michiganensis* subsp. *michiganensis* (CMM), *Pseudomonas syringae* pv. *tomato* (PST), and *Aspergillus niger*. The mechanism of action involves iron chelation and the generation of reactive oxygen species, disrupting microbial metabolic pathways [62, 66].

Unlike traditional fungicides, PO operates through a multifaceted mechanism that reduces the likelihood of resistance development. By targeting iron metabolism and inducing oxidative stress, PO disrupts critical cellular processes in pathogens. Its broad-spectrum activity, demonstrated across diverse microbial species, highlights its potential as a versatile seed treatment agent [62, 66, 84].

Importantly, the 0.1% PO concentration emerged as the optimal dose, ensuring robust seed germination and seedling growth while maintaining pathogen control. Unlike higher doses, which exhibited mild growth inhibition, this concentration balanced efficacy and safety,

making it a viable candidate for large-scale agricultural use. The results suggest that PO's efficacy is not solely dose-dependent but also influenced by its formulation components, such as Tween-80 and carboxymethylcellulose [46, 81, 84].

Carboxymethylcellulose (CMC) played a critical role in enhancing the efficacy and stability of PO-based formulations. As a binder and film-forming agent, CMC ensured uniform application and prolonged contact time between the active ingredient and the seed surface. Its biodegradability and biocompatibility further align with sustainable agricultural practices. Studies have shown that CMC improves the adhesion quality of coatings, reduces degradation under field conditions, and facilitates the absorption of antimicrobial compounds into the seed, targeting internal pathogens [46, 84, 85].

The incorporation of CMC into the PO formulation also addressed challenges associated with the physical stability of seed coatings. By enhancing the viscosity and film-forming properties of the formulation, CMC reduced the risk of premature degradation or loss of active ingredients during handling and storage. This ensured consistent application and sustained efficacy across different seed types and environmental conditions [46, 86].

Compared to traditional treatments, PO-based seed coatings provide comprehensive protection without harmful residues. The absence of toxicity to non-target organisms and compatibility with industrial-scale applications highlight its economic feasibility. Additionally, the low environmental footprint of PO aligns with global sustainability goals, addressing concerns related to soil health degradation, water contamination, and biodiversity loss [41, 86].

The scalability of PO formulations further enhances their appeal. The use of simple, readily available components like Tween-80 and CMC reduces production costs and facilitates large-scale implementation. Furthermore, the compatibility of PO with existing seed treatment infrastructure minimizes the need for additional investments, making it accessible to both large-scale and smallholder farmers [85, 86].

In this study, all seed coating formulations with antimicrobial activities determined by disc diffusion method at 0.4%, 0.1%, 0.08%, 0.05% and 0.03% PO doses were applied to wheat, tomato and corn seeds. Then, the effects of seed treatments on seed sterilization and germination rates were observed for 10 days. At the same time, the effects of these treatments on root and shoot lengths were measured for 14 days.

The results showed that all tested PO seed coating formulations had antifungal and antibacterial activity in a dose-dependent manner. *In vitro* antimicrobial activity tests including disc diffusion, and agar plate demonstrated that all seed coating formulations with 0.03% and/or higher PO concentration were effective against all bacterial species CMM, PSS, and PST, *E. faecium, S. aureus*, and *E. coli* , and fungi species *Penicillium* spp., *A. niger, A. flavus* and *C. krusei*. tested in this study. Similarly, the antimicrobial activity and total microbial population data of corn, wheat and tomato seeds treated with PO formulations supported the *in vitro* antimicrobial activity test results.

Seed germination test results showed that there was no significant difference between all PO seed coating formulations and the negative control in wheat, maize and tomato. However, the seed coating formulation containing the highest PO (0.4%) significantly reduced the seed germination rate of corn and wheat seeds.

The effects of PO seed coating formulation on root and shoot development of wheat, tomato and corn seedlings were investigated and the results showed that a significant decrease in root and shoot lengths was detected in seed coating treatments at only 0.4% PO concentrations in all plant species compared to the negative control. It was found that all other treatments had no negative effect on root and shoot lengths, and some treatments even increased root and shoot lengths compared to the untreated control.

The overall evaluation of all data regarding *in vitro* antimicrobial activity tests, seed sterilization, seed germination, root and shoot development of seedlings in this study suggested that seed coating formulation containing 0.08% PO may be the best for seed treatment in order to control all bacterial and fungal seed-borne diseases in different plant species without adversely affecting seed germination rate and seedling development.

The integration of PO-based formulations into seed treatment practices offers significant long-term benefits. By reducing dependency on synthetic fungicides, these formulations minimize environmental impact and enhance soil fertility. Furthermore, their potential compatibility with integrated pest management (IPM) strategies could revolutionize agricultural systems, promoting resilience and sustainability.

Future research should focus on evaluating PO’s efficacy under diverse environmental conditions and its potential against viral pathogens. Long-term field trials and studies on its compatibility with organic farming practices could further establish its role in sustainable

agriculture. Additionally, exploring the synergistic effects of PO with other biocontrol agents or natural compounds could expand its applications and enhance its efficacy [41, 77, 84].

The adoption of PO-based seed coatings offers significant economic and environmental advantages. By reducing yield losses and improving seed health, this technology minimizes the need for additional pest control measures, lowering overall production costs. Its environmentally friendly nature aligns with global sustainability initiatives, addressing critical issues such as soil health degradation, water contamination, and the ecological impact of synthetic agrochemicals [46, 77, 85].

Moreover, the cost-effectiveness of PO formulations, coupled with their scalability, ensures accessibility for diverse agricultural stakeholders. From large-scale commercial operations to smallholder farmers, the versatility and affordability of this technology hold promise for widespread adoption. By enhancing crop yields and reducing dependency on synthetic chemicals, PO-based formulations contribute to food security and agricultural resilience in the face of global challenges such as climate change and population growth [46, 77, 85].

5. CONCLUSION

This study successfully demonstrated the potential of a piroctone olamine (PO)-based seed coating formulation to combat seed-borne diseases caused by bacterial and fungal pathogens in wheat, corn, and tomato seeds. The 0.1% PO concentration proved to be particularly effective, offering complete pathogen control while enhancing seed germination and seedling growth. This dual functionality marks a significant advancement in seed treatment technologies, addressing critical gaps in current methodologies.

The introduction of PO as an agricultural seed coating agent represents a pioneering innovation. This is the first study to document its application in this context, setting a precedent for the development of safer, more sustainable seed treatment solutions. By reducing reliance on conventional chemical treatments and their associated risks, PO-based coatings contribute to improved agricultural productivity and environmental sustainability.

The broader implications of this research extend to addressing global challenges in agriculture, including climate change, soil degradation, and the need for increased food production. The scalability, safety, and efficacy of PO-based seed coatings position them as a transformative tool for modern farming practices. Future advancements in formulation and application methods could further expand their utility, including the control of soil-borne and airborne pathogens.

By establishing a novel approach to pathogen control, this study not only enhances seed health but also sets the foundation for sustainable agricultural innovations. The integration of PO-based coatings into commercial practices has the potential to revolutionize seed treatment methodologies, ensuring higher yields, healthier crops, and a more resilient agricultural sector. To deal with diseases like wilt that damages plant vascular bundles and fungal, bacterial, and viral plant pathogens that cause chlorosis and mosaic symptoms in leaves, various PO formulations can be made. Phytotoxicity tests can be conducted on these formulations, and commercial products can be created using them. Thus, disease-related losses for growers can be avoided while yield and quality are raised.

Further study is required to evaluate the effects of PO-based formulations on yield and quality indicators when employed against viral infections in different agricultural crops and

in vitro conditions. Investigations into PO's impact on seed germination and seedling development are necessary. Their use may be further enhanced by future advancements in formulation and application methods, which could enable them to effectively combat plant suspension culture with antimicrobials and manage soil-borne and airborne infections.

REFERENCES

1. Araus JL, Ferrio JP, Buxó R, Voltas J. The historical perspective of dryland agriculture: lessons learned from 10 000 years of wheat cultivation. *Journal of Experimental Botany*. 2007 Jan 1; 58(2):131-45.

2. Velimirović A, Jovović Z, Pržulj N. From neolithic to late modern period: Brief history of wheat. *Genetika*. 2021; 53(1):407-17.

3. Tenaillon MI, Charcosset A. A European perspective on maize history. Comptes Rendus. *Biologies*. 2011; 334(3):221-8.

4. McMillan VE, Canning G, Moughan J, White RP, Gutteridge RJ, Hammond-Kosack KE. Exploring the resilience of wheat crops grown in short rotations through minimising the build-up of an important soil-borne fungal pathogen. *Scientific Reports*. 2018 Jun 22;8(1):9550.

5. Food and Agriculture Organization of the United Nations FAOSTAT Crops and Livestock Products [cited 2024 10 September] Available from: https://www.fao.org/faostat/en/#data/QCL

6. Abdulsalaam S, Shenge KC. Seed borne pathogens on farmer-saved sorghum (*Sorghum bicolor* L.) seeds. *Journal of stored products and postharvest research*. 2011 Feb;2(2):24-8.

7. Ali AS, Elozeiri AA. Metabolic processes during seed germination. *Advances in seed biology. 2017* Dec 6;2017:141-66.

8. Bewley JD, Black M. *Physiology of Development and Germination.* 2nd ed. New York:Plenum Press; 1994 445 p

9. Lee SJ, Kang JY, Park HJ, Kim MD, Bae MS, Choi HI, Kim SY. DREB2C interacts with ABF2, a bZIP protein regulating abscisic acid-responsive gene expression, and its overexpression affects abscisic acid sensitivity. *Plant physiology*. 2010 Jun 1;153(2):716-27.

10. Oracz K, Karpiński S. Phytohormones signaling pathways and ROS involvement in seed germination. *Frontiers in Plant Science*. 2016 Jun 15;7:864.

11. Skubacz A, Daszkowska-Golec A. Seed dormancy: the complex process regulated by abscisic acid, gibberellins, and other phytohormones that makes seed germination work. *Phytohormones—signaling mechanisms and crosstalk in plant development and stress responses*. 2017 Aug 16:77-100.

12. Srivastava S, Kumar R, Bindal S, Singh VP, Rana M, Singh JP, Sinha A. Ancient, mid-time, and recent history of seed pathology. *Seed-borne diseases of agricultural crops: detection, diagnosis & management*. 2020:81-103.

13. Ghosh T, Biswas MK, Aikat K. A review on seed borne mycoflora associated with different oilseed crops and their management. *Int J Pure Appl Biosci*. 2018;6:1526-38.

14. Morton V, Staub T. A short history of fungicides. *APSnet Features*. 2008 Jun 10;308:1-2

15. Mancini V, Romanazzi G. Seed treatments to control seedborne fungal pathogens of vegetable crops. *Pest management science*. 2014 Jun;70(6):860-8.

16. Gebeyaw M. Review on: Impact of seed-borne pathogens on seed quality. *Am. J. Plant Biol*. 2020 Nov;5:77-81.

17. Aktaş H, Bolat N, Keser M, İnce T. Eskişehir ili hububat ekim alanlarında hububat kök ve kökboğazı çürüklüğü hastalık etmenlerinin saptanması, buğday ve arpada Drechslera sorokiniana (Sacc.) Subram. and Jain'ya karşı genitör çeşit ve hatların belirlenmesi. *Plant Protection Bulletin*. 2000;40(1):71-83.

18. Blanco R, Aveling TA. Seed-borne *Fusarium* pathogens in agricultural crops. *InVII International Symposium on Seed, Transplant and Stand Establishment of Horticultural Crops-SEST2016 1204* 2016 Sep 11 (pp. 161-170).

19. Bilgili A, Güldür ME. GAP Bölgesinde Biberlerde *Fusarium oxysporum* f.sp *vasinfectum* Kök Çürüklüğü Hastalığına Arbüsküler Mikorizal Fungusların Etkinliği. *Harran Tarım ve Gıda Bilimleri Dergisi*. 2018;22(1):88-108.

20. European and Mediterranean Plant Protection Organization [cited 2024 10 September] Available from: https://www.eppo.int/ABOUT_EPPO/eppo_convention

21. Gebeyaw M. Review on: Impact of seed-borne pathogens on seed quality. Am. J. *Plant Biol*. 2020 Nov;5:77-81.

22. Akaranuchat P, Noimanee P, Krittigamas N, von Hoersten D, Vearasilp S. Control of seed-borne fungi by radio frequency heat treatment as alternative seed treatment in barley (*Hordeum vulgare*). *Journal of Plant Diseases and Protection*. 2008, 115(1)

23. Amare A, Dawit A, Mengistu H. Mycoflora, aflatoxins, and resistance of groundnut cultivars from eastern Ethiopia. *Sinet*. 1995;18(1):117-31.

24. Amza J. Seed borne fungi; food spoilage, negative impact and their management: A review. *Seed*. 2018;81:70-9.

25. Hossain I, Azad AK. *Bipolaris sorokiniana,* its reaction and effect on yield of wheat. *Prog. Agric*. 1994;5(2):63-9.

26. Moumni M, Brodal G, Romanazzi G. Recent innovative seed treatment methods in the management of seedborne pathogens. *Food Security*. 2023 Oct;15(5):1365-82.

27. Islam MS, Sarker MN, Ali MA. Effect of seed borne fungi on germinating wheat seed and their treatment with chemicals. *International Journal of Natural and Social Sciences*. 2015;2(1):28-32.

28. Bal RS, Kumar A. Studies on the epidemiology of white rust and *Alternaria* leaf blight and their effect on the yield of Indian mustard. *African Journal of Agricultural Research*. 2014 Jan 9;9(2):302-6.

29. Sharma AB, Singh A, Singh TP. Effect of black point on seed germination parameters in popular wheat cultivars of Northern India. *Indian Phytopathology*. 2021 Mar;74(1):271-5.

30. Tagele SB, Kim SW, Lee HG, Lee YS. Potential of novel sequence type of *Burkholderia cenocepacia* for biological control of root rot of maize (*Zea mays* L.) caused by *Fusarium temperatum*. *International Journal of Molecular Sciences*. 2019 Feb 26;20(5):1005.

31. Gomaa FH. Detection of maize seed-borne fungi and induce resistance against both of *Aspergillus niger* and *Fusarium verticillioides*. *Archives of Phytopathology and Plant Protection*. 2021 Dec 14;54(19-20):2051-66.

32. Goko ML, Murimwa JC, Gasura E, Rugare JT, Ngadze E. Identification and characterisation of seed-borne fungal pathogens associated with maize (*Zea mays* L.). *International Journal of Microbiology*. 2021;2021(1):6702856.

33. Afkhamifar A, Moslemkhani C, Hasanzadeh N, Razmi J. Interactions of seed-borne bacterial pathogens *Xanthomonas translucens* and *Pseudomonas syringae* pv *syringae* on wheat. *Journal of Plant Pathology*. 2023 Aug;105(3):859-67.

34. Kasselaki AM, Malathrakis NE, Goumas DE, Cooper JM, Leifert C. Effect of alternative treatments on seed-borne *Didymella lycopersici* in tomato. *Journal of applied microbiology*. 2008 Jul 1;105(1):36-41.

35. Preston GM. *Pseudomonas syringae* pv. *tomato*: the right pathogen, of the right plant, at the right time. *Molecular plant pathology*. 2000 Sep;1(5):263-75.

36. Morella NM, Zhang X, Koskella B. Tomato seed-associated bacteria confer protection of seedlings against foliar disease caused by *Pseudomonas syringae*. *Phytobiomes Journal*. 2019 Oct 13;3(3):177-90.

37. Stall RE. *Xanthomonas campestris* pv. v*esicatoria*. *Prokaryotes*. 1995 Jan 1;1:167-84.

38. Hadas R, Kritzman G, Klietman F, Gefen T, Manulis S. Comparison of extraction procedures and determination of the detection threshold for *Clavibacter michiganensis* ssp. *michiganensis* in tomato seeds. *Plant Pathology*. 2005 Oct;54(5):643-9.

39. Nandi M, Macdonald J, Liu P, Weselowski B, Yuan ZC. *Clavibacter michiganensis* ssp. *michiganensis*: bacterial canker of tomato, molecular interactions and disease management. *Molecular plant pathology*. 2018 Aug;19(8):2036-50.

40. Madruga FB, Rossetti C, Saraiva CR, da Silveira Pasa M, da Silva Almeida A, Gadotti GI, Villela FA. Seed treatment: importance of products and equipment. *In Colloquium Agrariae*. ISSN: 1809-8215 2023 Mar 23 (Vol. 19, No. 1, pp. 105-115).

41. Lamichhane JR, You MP, Laudinot V, Barbetti MJ, Aubertot JN. Revisiting sustainability of fungicide seed treatments for field crops. *Plant Disease*. 2020 Mar 17;104(3):610-23.

42. Sharma KK, Singh US, Sharma P, Kumar A, Sharma L. Seed treatments for sustainable agriculture-A review. *Journal of Applied and Natural Science*. 2015 Jun 1;7(1):521-39.

43. Ayesha MS, Suryanarayanan TS, Nataraja KN, Prasad SR, Shaanker RU. Seed treatment with systemic fungicides: time for review. *Frontiers in Plant Science*. 2021 Aug 2;12:654512.

44. Dias MC. Phytotoxicity: An overview of the physiological responses of plants exposed to fungicides. *Journal of botany*. 2012;2012(1):135479.

45. Mancini V, Romanazzi G. Seed treatments to control seedborne fungal pathogens of vegetable crops. *Pest management science*. 2014 Jun;70(6):860-8.

46. Sharma KK, Singh US, Sharma P, Kumar A, Sharma L. Seed treatments for sustainable agriculture-A review. *Journal of Applied and Natural Science*. 2015 Jun 1;7(1):521-39.

47. Groot SP, Birnbaum Y, Rop N, Jalink H, Forsberg G, Kromphardt C, Werner S, Koch E. Effect of seed maturity on sensitivity of seeds towards physical sanitation treatments. *Seed Science and Technology*. 2006 Jul 1;34(2):403-13

48. Priyadarsni P, Rout P, Kuldip R, Bhuyan S. *Uses of Biocontrol Agents to Mitigate Seed-Borne Diseases*. Integrated Publications TM New Delhi. 2023:11.

49. Johnsson L, Hökeberg M, Gerhardson B. Performance of the *Pseudomonas chlororaphis* biocontrol agent MA 342 against cereal seed-borne diseases in field experiments. *European Journal of Plant Pathology*. 1998 Sep;104:701-11.

50. Sooda M, Kumarb V, Rawalc R. Seed biopriming a novel method to control seed borne diseases. *Biocontrol agents and secondary metabolites: applications and immunization for plant growth and protection*. Elseivier, Amsterdam. 2020 Nov 13:181-223.

51. Ren XX, Chen C, Ye ZH, Su XY, Xiao JJ, Liao M, Cao HQ. Development and application of seed coating agent for the control of major soil-borne diseases infecting wheat. *Agronomy*. 2019 Jul 26;9(8):413.

52. World Health Organization. (2020). The WHO recommended classification of pesticides by hazard and guidelines to classification 2019 [Cited 2024 10 September] Available from: https://www.who.int/publications/i/item/9789240005662

53. Hou L, Jin X, Liu N, Luo Y, Yan Z, Chen M, Liu Y, Xie H, Giesy JP, Wu F, Xu J. Triadimefon in aquatic environments: occurrence, fate, toxicity, and ecological risk. *Environmental Sciences Europe*. 2022 Dec;34(1):12.

54. Zakharov S, Csomor J, Urbanek P, Pelclova D. Toxic epidermal necrolysis after exposure to dithiocarbamate fungicide mancozeb. *Basic & Clinical Pharmacology & Toxicology*. 2016 Jan;118(1):87-91.

55. FAO Specıfıcatıon And Evaluatıon S For Plant Protectıon Products Imazalil [cited 2024 28 September] Available from: https://openknowledge.fao.org/server/api/core/bitstreams/a251280f-5d9f-4be3-8323-a44746794f4d/content

56. Eligibility R. Triadimefon And Tolerance Reassessment for Triadimenol [cited 2024 28 September] Available from: https://archive.epa.gov/pesticides/reregistration/web/pdf/triadimefon_red.pdf

57. FAO Specıfıcatıon And Evaluatıon S For Plant Protectıon Products Difenoconazole 2005

58. World Health Organization. Toxicological evaluations: sponsored jointly by FAO and WHO with the support of the International Programme on Chemical Safety (IPCS), Joint Meeting of the FAO Panel of Experts on Pesticide Residues in Food and the Environment and the WHO Core Assessment Group, Rome 16-25 September 1996. (No Title).

59. Iris E. Integrated Risk Information System (IRIS) US Environmental Protection Agency. *Chemical Assessment Summary National Center for Environmental Assessment.* IRIS Assessments. Browse A to Z List of Chemicals. IRIS Assessments. Browse A to Z List of Chemicals, in. 2011.

60. Klug P, Kreiling R, Care RD. FDA Docket No. 2004N-0050 Safety and Effectiveness Information for Piroctone Olamine Submitted Pursuant to 21 CFR 5 330.14 (f) and 5 330.10 (a)(2).

61. Tang CF, Pudney PD, Lane ME. Investigation of piroctone olamine delivery to the skin from single, binary and ternary solvent systems. *International Journal of Cosmetic Science*. 2024 Jun;46(3):357-67.

62. Kim Y, Alpmann P, Blaum-Feder S, Kraemer S, Endo T, Lu D, Carson D, Schmidt-Wolf IG. Increased in vivo efficacy of lenalidomide by addition of piroctone olamine. *In vivo*. 2011 Jan 1;25(1):99-103.

63. Futterer EB. Evaluation of efficacy of antidandruff agents. *J Soc Cosmet Chem*. 1981 Nov;32:327-8.

64. Youn HJ, Kim SY, Park M, Jung WH, Lee YW, Choe YB, Ahn KJ. Efficacy and safety of cream containing climbazole/piroctone olamine for facial seborrheic dermatitis: a single-center, open-label split-face clinical study. *Annals of Dermatology*. 2016 Dec 1;28(6):733-9.

65. do Couto FM, do Nascimento SC, Júnior SF, da Silva VK, Leal AF, Neves RP. Antifungal activity of the piroctone olamine in experimental intra-abdominal candidiasis. *SpringerPlus*. 2016 Dec;5:1-4.

66. Ghorbanizadeh S, Karami F, Delfani S, Shakibaie M, Razlansari A, Rezaei F. Antibacterial effects and cellular mechanisms of iron oxide magnetic nanoparticles coated by piroctone olamine against some cariogenic bacteria. *Annals of Medicine and Surgery*. 2022 Sep 1;81.

67. Ghorbanizadeh S, Karami F, Delfani S, Shakibaie M, Razlansari A, Rezaei F. Antibacterial effects and cellular mechanisms of iron oxide magnetic nanoparticles coated by piroctone olamine against some cariogenic bacteria. *Annals of Medicine and Surgery*. 2022 Sep 1;81.

68. Dillon MM, Ruiz-Bedoya T, Bundalovic-Torma C, Guttman KM, Kwak H, Middleton MA, Wang PW, Horuz S, Aysan Y, Guttman DS. Comparative genomic insights into

the epidemiology and virulence of plant pathogenic pseudomonads from Turkey. *Microbial genomics*. 2021 Jul 6;7(7):000585.

69. Sen Y, Aysan Y, Mirik M, Ozdemir D, Meijer-Dekens F, van der Wolf JM, Visser RG, van Heusden S. Genetic characterization of *Clavibacter michiganensis* subsp. *michiganensis* population in Turkey. *Plant disease*. 2018 Feb 17;102(2):300-8.

70. Youn HJ, Kim SY, Park M, Jung WH, Lee YW, Choe YB, Ahn KJ. Efficacy and safety of cream containing climbazole/piroctone olamine for facial seborrheic dermatitis: a single-center, open-label split-face clinical study. *Annals of Dermatology*. 2016 Dec 1;28(6):733-9.

71. Biemer JJ. Antimicrobial susceptibility testing by the Kirby-Bauer disc diffusion method. *Annals of Clinical & Laboratory Science*. 1973 Mar 1;3(2):135-40.

72. Choudhary M, Lakhran L. Detection Methods of Seed Borne Pathogens. *AGRICULTURE & FOOD e-NEWSLETTER*. 2020 Feb:11

73. Maccioni A, Santo A, Falconieri D, Piras A, Manconi M, Maxia A, Bacchetta G. Inhibitory effect of rosemary essential oil, loaded in liposomes, on seed germination of Acacia saligna, an invasive species in Mediterranean ecosystems. *Botany*. 2019;97(5):283-91

74. Sauer DB, Burroughs R. Disinfection of seed surfaces with sodium hypochlorite. *Phytopathology*. 1986 Jul 1;76(7):745-9..

75. Bouri M, Iyigündoğdu Z, Lök P, Şahin F. Efficacy of a newly developed seed coating formulation for the control of seed-bornediseases. *Turkish Journal of Agriculture and Forestry*. 2022;46(3):280-91.

76. Nallathambi P, Umamaheswari C, Lal SK, Manjunatha C, Berliner J. Mechanism of seed transmission and seed infection in major agricultural crops in India. *Seed-borne diseases of agricultural crops: Detection, diagnosis & management*. 2020:749-91.

77. Matny ON. Fusarium head blight and crown rot on wheat & barley: losses and health risks. *Advances in Plants & Agriculture Research* 2015;2(1):00039.

78. Rennie WJ, Cokerell V. *Seedborne diseases*. The epidemiology of plant diseases. 2006:357-72.

79. Gaur A, Kumar A, Kiran R, Kumari P. Importance of seed-borne diseases of agricultural crops: Economic losses and impact on society. *Seed-borne diseases of agricultural crops: detection, diagnosis & management*. 2020:3-23.

80. Kalasariya HS, Patel NB, Yadav A, Perveen K, Yadav VK, Munshi FM, Yadav KK, Alam S, Jung YK, Jeon BH. Characterization of fatty acids, polysaccharides, amino acids, and minerals in marine macroalga chaetomorpha crassa and evaluation of their potentials in skin cosmetics. *Molecules*. 2021 Dec 11;26(24):7515.

81. Davis MG, Piliang MP, Bergfeld WF, Caterino TL, Fisher BK, Sacha JP, Carr GJ, Moulton LT, Whittenbarger DJ, Punyani S, Schwartz JR. Scalp application of the antioxidant piroctone olamine reduces hair shedding in an 8-week randomized, double-blind, placebo-controlled clinical study. *International Journal of Cosmetic Science*. 2021 Nov;43:S26-33.

82. Akbarimoghaddam H, Galavi M, Ghanbari A, Panjehkeh N. Salinity effects on seed germination and seedling growth of bread wheat cultivars. *Trakia journal of Sciences*. 2011 Mar 1;9(1):43-50.

83. Reygagne P, Mandel VD, Delva C, Havlíčková M, Padlewska K, Khalil R, Meuleman V, Miranda GA, Nevskaya M, Michelet JF, Pouradier F. An anti-hair loss treatment in the management of mild androgenetic alopecia: Results from a large, international observational study. *Dermatologic Therapy*. 2021 Nov;34(6):e15134.

84. Master of Bioactive Molecules [cited from 2024 06 May] Available from: https://www.medchemexpress.com/Piroctone_olamine.html

85. Singh J, Sastry ED, Singh V. Effect of salinity on tomato (*Lycopersicon esculentum* Mill.) during seed germination stage. *Physiology and Molecular Biology of Plants*. 2012 Jan;18:45-50.

86. U.S. Food and Drug Administration, Substance added to Food (formerly EAFUS) [cited 29 September 2024]. Available from:

https://www.hfpappexternal.fda.gov/scripts/fdcc/index.cfm?set=FoodSubstances&id=CARBOXYMETHYLCELLULOSESODIUMSALT