PLANT PROTECTION: INNOVATIONS AND SUSTAINABLE STRATEGIES

Editors
Prof. Dr. Mehmet KAPLAN
Assoc Prof. Dr. Ahmet ÇAT

DUVAR

PLANT PROTECTION: INNOVATIONS AND SUSTAINABLE STRATEGIES

Editors

Prof. Dr. Mehmet KAPLAN

Assoc Prof. Dr. Ahmet ÇAT

Plant Protection: Innovations and Sustainable Strategies
Editors: Prof. Dr. Mehmet KAPLAN, Assoc Prof. Dr. Ahmet ÇAT

Editor in chief: Berkan Balpetek
Cover and Page Design: Duvar Design
Printing : December-2025
Publisher Certificate No: 49837
ISBN: 978-625-8734-48-5

853 Sokak No:13 P.10 Kemeraltı-Konak/İzmir
Tel: 0 232 484 88 68
www.duvaryayinlari.com
duvarkitabevi@gmail.com

TABLE OF CONTENTS

Chapter 1

Potential of Thyme Essential Oils as Biopesticides

Tuğba ÇAKIR[1]*, Hasan MARAL[2]

Abstract

The growing concerns regarding the adverse impacts and environmental risks of chemical pesticides in modern agriculture have accelerated the search for eco-friendly alternatives. This shift has strengthened interest in biological control strategies and created a strong foundation for promoting the use of essential oils at both national and international levels. The widespread adoption of essential oils as biopesticides may represent a significant step toward achieving sustainable agricultural systems and protecting natural ecosystems. As natural products derived from plants, essential oils are effective against insects, fungi, bacteria, and other harmful organisms. Considering Türkiye's agricultural dynamics, climatic conditions, and the prevalence of plant diseases, the potential and limitations of essential oils as biopesticides are particularly important.

This chapter comprehensively discusses the advantages, disadvantages, and success potential of using essential oils as biopesticides, it provides scientific insights into their role and applicability in agriculture. When integrated with alternative practices, essential oils may reduce pesticide resistance, safeguard natural ecosystems, and support sustainable crop production. The study addresses the efficacy, toxicity, storage conditions, and application areas of essential oils, highlighting their promise as natural and environmentally safe alternatives to conventional methods.

1. Introduction

Plant production is vital not only for direct human nutrition but also for supporting livestock production. However, numerous biotic (pathogens, pests and weeds) and abiotic (environmental factors, soil conditions, toxic gases in the atmosphere and improper agricultural practices) factors can cause substantial yield and quality losses. Globally, it is estimated that about 9% of losses in agricultural production result from diseases, 11% from pests, and 15% from

[1] Karamanoğlu Mehmetbey University, Technical Sciences Vocational School, Karaman, Türkiye
*Corresponding author: tyetgin@kmu.edu.tr
[2] Karamanoğlu Mehmetbey University, Technical Sciences Vocational School, Karaman, Türkiye

weeds. When post-harvest losses of 6–12% are also considered, the total loss without pest management may reach 50–70% of potential agricultural yield. To minimize such losses, chemical fertilizers and pesticides are extensively used both worldwide and in Türkiye (Avan and Kotan, 2021).

Pesticides are synthetic organic compounds used to eliminate unwanted organisms in crops. They include all chemicals and preparations used in plant protection in the production of pesticides Although pesticides offer significant benefits, long-term use has been shown to harm ecosystems and human health, leading to regulatory restrictions. The importance of pesticide residues was first recognized in 1948 when organochlorine residues were detected in human tissues. While some pesticides pose no toxicological risk, others have been identified as carcinogenic or neurotoxic. Food products are the main source of pesticide residues. Consequently, the Joint FAO/WHO Codex Committee on Pesticide Residues was established in 1960, setting maximum residue limits in food based on scientific data (Altıkat et al., 2009).

In Türkiye, 936 harmful species affecting cultivated crops have been reported, including 229 pathogens, 415 insect pests, and 292 weed species. Without adequate control measures, pre-harvest yield losses reach around 35%, of which 13% are caused by pests, 12% by pathogens, and 10% by weeds. Despite these losses, chemical pesticides remain widely used. The country's annual pesticide consumption is estimated at 33,000 tons, with insecticides accounting for 47%, herbicides for 24%, fungicides for 6%, and others for 13%. The annual sales volume is approximately 230–250 million USD. Usage is concentrated in polyculture farming areas, particularly the Mediterranean and Aegean regions. For example, Adana, Mersin, and Antalya together account for 40% of Türkiye's pesticide use, while İzmir leads pesticide consumption in the Aegean. Crop-wise, pesticides are used primarily in cotton and cereals (40%), fruits (27%, mainly citrus and grapes), and vegetables (16%) (Kılıç, 2019).

Excessive and uncontrolled use of synthetic pesticides leaves residues in food, soil, water, and air, negatively affecting non-target organisms and human health. According to the World Health Organization (WHO), approximately three million agricultural workers suffer pesticide poisoning annually, with around 18,000 deaths. In developing countries, 25 million workers are at risk each year. Studies also reveal that women exposed to certain pesticides, such as dicofol and endosulfan, during the first eight weeks of pregnancy face an eightfold higher risk of giving birth to autistic children. These findings highlight the urgent need to reduce synthetic pesticide use and promote safer alternatives. Additionally, uncertainties regarding the long-term effects of low-dose exposure and

inadequate monitoring systems for pesticide-related diseases pose further challenges (Kılıç, 2019).

Historically, the earliest pesticides used were of plant origin, such as pyrethrin and rotenone. Except for nicotine, plant-derived pesticides are generally safe for warm-blooded animals. Organochlorine pesticides, such as DDT, introduced in the 1940s, are persistent but not highly toxic to livestock. In contrast, many organophosphates, first developed during World War II as nerve agents and later adapted for pest control, are highly toxic (Yarsan and Çevik, 2007).

Biopesticides, derived from natural sources such as microorganisms (bacteria, fungi, viruses), plants, animals, and minerals, are considered environmentally friendly alternatives to synthetic chemicals (EPA, 2017). They offer lower risks to human health and ecosystems and can be used by commercial producers, ornamental plant growers, and home gardeners alike. Each country regulates the authorization and use of biopesticides according to its laws; in Türkiye, this authority lies with the Ministry of Agriculture and Forestry (Kılıç, 2019).

The modern environmental perspective emphasizes that nature's resources are not limitless, making sustainable agriculture increasingly important. While conventional farming relies heavily on high inputs, good agricultural practices focus on controlled and minimal input use. Given the negative impacts of intensive chemical use in conventional agriculture, alternative strategies are crucial for crop protection (Eryılmaz et al., 2019). Biological control is gaining attention as an alternative to chemical inputs, requiring the development of existing methods and exploration of new approaches (Nohutçu et al., 2021). Among these alternatives, plant essential oils and extracts hold great promise. Currently, around one-third of all plant families contain species that produce essential oils. Notable families include *Asteraceae, Apiaceae, Brassicaceae, Chenopodiaceae, Cupressaceae, Lauraceae, Lamiaceae, Myrtaceae, Rutaceae, Rosaceae, Pinaceae, Poaceae*, and *Zingiberaceae*. Depending on the species, essential oil content ranges from 0.01% to 10%. These oils are widely used in cosmetics, food, and perfumery due to their preservative, fragrance, and disinfectant properties (Nohutçu et al., 2021).

This chapter aims to evaluate essential oils, particularly thyme essential oil, as potential biopesticides, exploring their advantages, challenges, and possible integration into sustainable agricultural systems.

2. Definition and Classification of Pesticides

The Food and Agriculture Organization of the United Nations (FAO) defines pesticides as follows: "Substances intended for preventing, destroying, or controlling any pest, including vectors of human or animal diseases; unwanted

species that interfere with the production, processing, storage, transport, or marketing of food, agricultural commodities, wood and wood products, or animal feed; or pests that may be present on animals. This definition also covers substances used to regulate plant growth, prevent leaf fall, act as desiccants or fruit thinners, prevent premature fruit drop, or protect products from spoilage during pre- and post-harvest storage and transport" (Altıkat et al., 2009).

Pesticides can be classified according to their target organism, chemical structure, or physical state. In broader terms, they may also be categorized into inorganic, synthetic, and biological (biopesticides). A pesticide can be a chemical substance, a biological agent such as a virus or bacterium, an antimicrobial, a disinfectant, or any material that suppresses harmful organisms.

The term pesticide therefore covers a wide array of compounds, including:

- **Algicides** (algae control)
- **Avicides** (bird control)
- **Bactericides** (bacteria control)
- **Fungicides** (fungi control)
- **Herbicides** (weed control)
- **Insecticides** (insect control), with further subgroups such as:
- **Ovicides** (egg control)
- **Larvicides** (larvae control)
- **Adulticides** (adult insect control)
- **Acaricides** (mites and ticks)
- **Molluscicides** (snails and slugs)
- **Nematicides** (nematodes)
- **Rodenticides** (rodents)
- **Virucides** (viruses).

Although pesticides are indispensable in crop protection due to their efficacy, they also pose potential risks to humans and non-target organisms because of their toxicity. Therefore, safe handling and proper application practices are critical to prevent unintended harmful effects (Altıkat et al., 2009).

3. Definition and Classification of Biopesticides

Biopesticides are pest control agents derived from natural sources such as animals, plants, microorganisms, and certain minerals. Unlike synthetic chemical pesticides, they are generally considered safer for humans and the environment. Their use relies on exploiting natural mechanisms of action against pests and pathogens. Based on their origin and active components, biopesticides can be

broadly classified into three main groups: **microbial pesticides**, **plant-incorporated protectants**, and **biochemical pesticides** (Mawcha et al., 2024; Ruiu, 2018; Parveen and Rashtrapal, 2024).

3.1. Microbial Pesticides

These are based on microorganisms such as bacteria, fungi, viruses, and protozoa, which contain bioactive compounds with pesticidal properties. Due to their specificity and minimal impact on non-target organisms, microbial pesticides are considered promising alternatives to synthetic chemicals. They are capable of controlling a wide range of pests. The most widely used microbial pesticide is *Bacillus thuringiensis* (Bt), which is effective against caterpillars on crops such as cabbage and potatoes. Furthermore, diverse microorganisms can be explored to expand the pool of microbial biopesticides, thereby minimizing resistance development and ensuring long-term sustainability (Ayilara et al., 2023).

3.2. Plant-Incorporated Protectants (PIPs)

These are pesticidal substances produced by plants after the insertion of specific genetic material. In other words, PIPs are generated when plants are genetically modified to express pest-resistant traits. For instance, genes encoding pesticidal proteins from *Bacillus thuringiensis* can be incorporated into crop genomes, enabling the plants themselves to produce the bioactive compound. Such plants are then inherently more resistant to insect pests, reducing the need for external chemical inputs (Rani et al., 2022).

3.3. Biochemical Pesticides

Biochemical pesticides are naturally occurring compounds that control pests through non-toxic mechanisms. Unlike conventional pesticides, which typically kill or inactivate pests directly, biochemical pesticides interfere with pest behavior and physiology in alternative ways. These include acting as repellents, disrupting feeding or development, or altering pest morphology and biochemical processes. Their environmentally benign mode of action makes them suitable for integration into sustainable pest management programs (Ody et al., 2025).

4. Chemical Composition of Thyme Essential Oils

Thyme essential oils hold significant importance across various fields, ranging from aromatherapy to traditional medicine. Their chemical composition is highly variable and influenced by several factors, including genetic makeup, environmental conditions, harvest stage, and the method of distillation (Maral and Kırıcı, 2019).

4.1. Monoterpenes

Thymol: A phenolic compound found in thyme, thymol exhibits antimicrobial, antioxidant, and antispasmodic properties. It is particularly known for alleviating respiratory symptoms and supporting immune regulation (Zhao et al., 2023).

Carvacrol: Numerous studies have demonstrated its analgesic, anti-inflammatory, anticancer, antibacterial, and antifungal properties. Carvacrol has also been reported to accelerate wound healing and promote cell proliferation (Yaman et al., 2018).

p-Cymene: A volatile flavor compound that shows higher encapsulation efficiency when combined with β-cyclodextrin compared to other cyclodextrin types (Ak et al., 2023).

γ-Terpinene: Functions as a biosynthetic precursor of both thymol and carvacrol (Maral and Kırıcı, 2023).

4.2. Sesquiterpenes

Although present in smaller amounts in thyme essential oils, sesquiterpenes contribute significantly to aroma and biological activity. Examples include:

Bisabolol: Widely used in cosmetics due to its beneficial effects on skin health (Maurya et al., 2014).

Caryophyllene: Rather than acting directly as an antimicrobial compound, it is known to activate the jasmonic acid signaling pathway in plants, thereby enhancing resistance to pathogens (Gupta and Phulara, 2021).

4.3. Esters and Other Compounds

Bornyl Acetate: Provides aromatic properties and is often used as a food additive, flavoring, and fragrance agent (Anon. 2003).

Geraniol: A monoterpenic alcohol recognized as an important constituent of many essential oils. It is commercially used as a fragrance in cosmetics and household products, while also exhibiting antioxidant and anti-inflammatory activities (Maczka et al., 2020).

Linalool: Contributes to the floral and mildly sweet scent of thyme essential oils. Industrially, it is a common ingredient in perfumes, shampoos, soaps, and detergents (Mitic-Culafic et al., 2009).

5. Effects of Thyme Essential Oils as Biopesticides

The application of thyme essential oils as biopesticides provides an eco-friendly and natural alternative for controlling agricultural pests. Their efficacy has been evaluated against a wide range of targets, including storage pests, weeds,

and mites-areas traditionally dominated by synthetic pesticides (Altundağ and Aslım, 2005; Bayındır Erol and Birgücü, 2020; Şimşek, 2024; Işık and Temur Çınar, 2018; Szczepanik and Zawitoska, 2012; Belgüzer et al., 2016). These studies highlight the potential of thyme essential oils as sustainable substitutes for conventional agrochemicals.

The primary constituents of thyme oils, thymol and carvacrol, exhibit a broad spectrum of biological activities, particularly antioxidant, antimicrobial, antifungal, and insecticidal properties (Ündeğer et al., 2009). Their high efficacy against insects, coupled with low toxicity to humans and environmental safety, make them strong candidates for use in integrated pest management programs (Peng et al., 2025).

The pesticidal activity of thyme essential oils is attributed to several mechanisms:

5.1. Disruption of Cell Membranes

Thymol and carvacrol are lipophilic phenolic monoterpenes that integrate into the lipid bilayers of cell membranes, disrupting their structural integrity. This process leads to increased permeability, leakage of intracellular contents, and ultimately cell lysis. In fungal cells, these compounds cause the loss of membrane-bound enzymes and alter ion homeostasis, which inhibits spore germination and mycelial growth. Similarly, in bacteria and insect cells, membrane disruption interferes with nutrient uptake and energy metabolism, leading to death (Soković et al., 2010).

5.2. Inhibition of Respiration

Another key mechanism is the inhibition of cellular respiration. Thymol and carvacrol can interfere with mitochondrial function by uncoupling oxidative phosphorylation, thereby reducing ATP synthesis. In insects, the vapor-phase activity of thyme essential oils can impair respiratory gas exchange through spiracles, resulting in suffocation. Studies on stored-product insects such as *Sitophilus oryzae* and *Tribolium castaneum* have shown that thyme oil vapors cause significant reductions in oxygen consumption and increase mortality rates (Isman, 2020).

5.3. Neurotoxic Effects

Thyme essential oils also act as neurotoxins against pest species by altering nervous system activity. They can modulate the function of acetylcholinesterase (AChE), an essential enzyme for nerve signal transmission. Inhibition of AChE leads to accumulation of acetylcholine at synaptic junctions, causing continuous

nerve firing, paralysis, and death. This mechanism is particularly relevant for the control of insect pests such as *Aphis gossypii* (cotton aphid) and *Frankliniella occidentalis* (western flower thrips) (Kedia et al., 2015).

6. Application Areas

Thyme essential oils have demonstrated broad-spectrum pesticidal activity and are increasingly recognized as promising alternatives to synthetic chemical pesticides. Their versatility enables use in various agricultural contexts, from pre-harvest disease control to post-harvest protection of stored products.

6.1. Control of Fungal Diseases

Thyme oils are among the most potent antifungal essential oils. Their major constituents, thymol and carvacrol, show strong inhibitory effects on the growth of phytopathogenic fungi such as *Botrytis cinerea*, *Fusarium oxysporum*, *Aspergillus niger*, and *Penicillium expansum*. These effects are linked to their ability to disrupt fungal membrane integrity, suppress ergosterol biosynthesis, and inhibit spore germination. Application of thyme essential oils in vapor or aqueous emulsion form has been shown to effectively reduce fungal incidence on tomato, strawberry, and post-harvest fruit surfaces (Prakash et al., 2011).

6.2. Insect Pest Management

Due to their neurotoxic and repellent properties, thyme essential oils are effective in managing a wide range of insect pests. Laboratory and greenhouse trials have demonstrated significant insecticidal activity against *Aphis gossypii*, Frankliniella occidentalis, *Tetranychus urticae* (two-spotted spider mite), and *Spodoptera littoralis* (cotton leafworm). Furthermore, the volatile nature of these oils allows for their use in fumigation treatments, providing both contact and vapor-phase toxicity. Unlike conventional insecticides, thyme oils leave minimal residues and degrade rapidly in the environment (Pavela, 2015).

6.3. Protection Against Storage Pests

Thyme essential oils also provide effective protection against storage pests that cause severe post-harvest losses. Species such as *Sitophilus granarius* (granary weevil), *Rhyzopertha dominica* (lesser grain borer), and *Tribolium castaneum* (red flour beetle) are highly susceptible to thyme oil vapors. The oils can act as repellents, ovicidal agents, and fumigants, reducing population growth and preventing grain contamination (Kedia et al., 2015). Additionally, their antioxidant and antimicrobial properties help maintain product quality, extending the shelf life of stored cereals and pulses.

7. Mechanisms of Action of Thyme Essential Oils

Essential oils from thyme (*Origanum* spp.) possess a rich chemical profile and a wide range of biological activities, enabling them to act through multiple mechanisms. Understanding these mechanisms is essential for identifying their potential applications across agriculture, medicine, and food industries (Maral, 2022).

7.1. Antimicrobial Mechanisms

The principal active compounds, thymol and carvacrol, are highly effective against bacterial, fungal, and viral infections (Türkmen et al., 2022). Their antimicrobial activity can be explained through several modes of action:

Disruption of cell membranes: Increasing membrane permeability, which causes leakage of intracellular components.

Metabolic interference: Inhibition of microbial energy production pathways, suppressing growth and reproduction.

Nucleic acid inhibition: Blocking the replication and transcription of microbial DNA and RNA.

7.2. Antioxidant Mechanisms

Thyme essential oils exhibit strong antioxidant activity, capable of neutralizing free radicals and reducing oxidative stress:

- Acting as hydrogen donors to stabilize reactive species.
- Preventing lipid peroxidation, thereby protecting cellular membranes from oxidative damage.

7.3. Anti-inflammatory Mechanisms

The anti-inflammatory effects of thyme oils are associated with the regulation of prostaglandin, leukotriene, and cytokine production:

- Suppression of prostaglandin E_2 synthesis, thereby reducing inflammation.
- Inhibition of the NF-κB pathway, blocking the expression of pro-inflammatory genes.

7.4. Herbicidal Mechanisms

Thyme oils also display phytotoxic activity, making them effective natural herbicides:

- Membrane disruption, which compromises cellular homeostasis in weeds.
- Inhibition of respiration and photosynthesis by targeting chloroplasts and mitochondria.

- Allelopathic effects, preventing seed germination and growth of competing plants.

7.5. Cytotoxic and Anticancer Mechanisms

Certain constituents of thyme oils show cytotoxicity against cancer cells:

- Induction of apoptosis through programmed cell death pathways.
- Generation of reactive oxygen species (ROS), which triggers oxidative damage in cancer cells.
- Cell cycle arrest, inhibiting uncontrolled proliferation.

7.6. Neuroprotective Mechanisms

Thyme essential oils by reducing oxidative stress and controlling neuroinflammation:

- Scavenging free radicals in the brain.
- Inhibiting microglial activation, thereby preventing neuroinflammatory damage.

7.7. Antiparasitic Mechanisms

Thyme essential oils can disrupt parasite membranes or interfere with their metabolism. They have shown effectiveness against malaria, intestinal parasites, and protozoan infections.

8. Agricultural Applications of Thyme Essential Oils

Thyme essential oils are versatile natural compounds with considerable potential in agriculture. Owing to their high concentrations of active constituents such as thymol and carvacrol, they can play a key role in plant protection and soil health management.

8.1. Use as Natural Pesticides

Thyme oils possess insecticidal, fungicidal, and nematicidal properties.

Insect control: Thymol and carvacrol affect the nervous system of insect pests, leading to mortality. They may also interfere with insect reproduction, aiding in population management.

Fungal control: The antifungal activity of thyme oil is particularly effective against plant pathogens such as *Fusarium, Alternaria*, and *Botrytis* species.

8.2. Soil Health and Nematode Management

Incorporating thyme essential oils or plant residues into soil can suppress nematode populations. This offers an eco-friendly alternative to synthetic nematicides, contributing to healthier soils and more resilient cropping systems.

8.3. Weed Control (Herbicidal Potential)

Some compounds in thyme essential oils inhibit seed germination and seedling growth, providing a natural option for weed management. Their use can reduce dependence on synthetic herbicides, thus supporting sustainable agriculture.

8.4. Postharvest Protection and Storage

The antimicrobial activity of thyme oil can be harnessed during the storage of harvested crops. By preventing microbial spoilage, it extends the shelf life of perishable products such as fruits and vegetables.

8.5. Contribution to Sustainable Agriculture

Due to their biodegradability, thyme essential oils minimize chemical residues in the environment. Their integration into organic and sustainable farming practices represents a promising strategy to reduce chemical inputs while maintaining crop productivity.

9. Challenges in the Use of Thyme Essential Oils

Although thyme essential oils exhibit strong biological activity and have wide application potential, their practical use in agriculture faces several challenges:

9.1. Lack of Standardization

The chemical profile of thyme oils varies significantly depending on the species (e.g., *Thymus vulgaris*, *Thymus serpyllum*), cultivation conditions (soil, climate), and harvest time. This variability complicates the development of standardized products and makes it difficult to predict efficacy across applications.

9.2. High Concentration and Safety Concerns

Key constituents such as thymol and carvacrol are biologically potent. While beneficial at appropriate doses, they can be toxic at higher concentrations. Overuse or improper application may cause skin irritation, respiratory problems, or allergic reactions.

9.3. Stability Issues

Essential oils are prone to oxidation when exposed to light, heat, or air, which reduces their efficacy and shelf life. Without proper storage such as airtight containers in cool, dark conditions, the oils rapidly degrade.

9.4. Production Costs

Obtaining natural essential oils is expensive, as large amounts of plant material are required for distillation. This increases production costs compared to synthetic alternatives, which can be produced more cheaply but may lack the same biological benefits.

9.5. Efficacy and Application Difficulties

The effectiveness of thyme oil depends on correct dosage and method of application. Under-dosing may render treatments ineffective, while overdosing may lead to toxicity. Achieving the right balance is a challenge for practical use in the field.

9.6. Regulatory Constraints

The use of essential oils in agriculture, food, medicine, and cosmetics is subject to strict regulations, which vary between countries. Obtaining the necessary licenses and approvals for marketing thyme oil products can be time-consuming and costly, particularly for small-scale producers.

9.7. Sustainability and Environmental Concerns

Rising demand for thyme essential oils risks overharvesting natural populations, threatening biodiversity and ecosystem stability. To address this, sustainable cultivation and controlled production practices must be prioritized.

10. Conclusion and Recommendations

Thyme essential oils represent a promising natural alternative to synthetic pesticides due to their broad spectrum of biological activities and environmentally friendly characteristics. Their pesticidal, antifungal, antioxidant, and antimicrobial properties highlight their strong potential in sustainable agriculture. However, challenges such as lack of standardization, safety concerns at high concentrations, stability problems, high production costs, regulatory barriers, and sustainability issues limit their widespread adoption.

Recommendations

To ensure the effective and sustainable use of thyme essential oils as biopesticides, several integrated strategies should be implemented. Standardization efforts must focus on employing advanced analytical techniques to characterize the chemical profiles of thyme oils and establishing specific quality standards tailored to various applications. Education and awareness programs should provide users with training on proper dosage and safe application methods while promoting expert education in medical and aromatherapy contexts. Continued investment in research and development is essential to create more efficient, environmentally friendly distillation technologies that minimize energy consumption and to design innovative delivery systems such as microencapsulation, which can enhance stability and extend shelf life. Regulatory facilitation should aim to harmonize international regulations on essential oils and provide technical support to local producers in meeting licensing and regulatory approval requirements. From a sustainability perspective, controlled cultivation must be encouraged to protect wild thyme populations, alongside promoting certified organic production systems that uphold environmental responsibility. Furthermore, appropriate storage and packaging solutions such as the use of oxidation-resistant materials and maintenance of optimal storage conditions are crucial for preserving product quality and usability. Finally, improving distillation efficiency, offering economic support, and developing cooperative models for small-scale producers can significantly reduce production costs. Collectively, these measures will enable the safer, more effective, and economically viable utilization of thyme essential oils, while contributing to the conservation of natural resources and advancing the overarching goals of sustainable agriculture.

References

Ak, H., Gülşen, İ., & Canbek, İ. (2023). Neuroprotective effects of black cumin seed and seed oil. In Biochemistry, Nutrition, and Therapeutics of Black Cumin Seed (pp. 201–230). Elsevier. https://doi.org/10.1016/B978-0-323-90788-0.00002-0

Altıkat, A., Turan, T., Ekmekyapar Torun, F., & Bingül, Z. (2009). Pesticide use in Türkiye and its environmental impacts. Atatürk Univ. J. of Agricultural Faculty, 40(2), 87–92.

Altundağ, Ş., & Aslım, B. (2005). Antimicrobial effects of thyme on some phytopathogenic bacteria. Orlab Online Microbiology Journal, 3(7), 12–14.

Anon. (2003). Code of Federal Regulations Title 21. Archived October 11.

Avan, M., & Kotan, R. (2021). The use of fungi as microbial fertilizers or biopesticides in agriculture. International Eastern Anatolia Journal of Science, Engineering and Design, 3(1), 167–191.

Ayilara, M. S., Adeleke, B. S., Akinola, S. A., Fayose, C. A., Adeyemi, U. T., Gbadegesin, L. A., Omole, R. K., Johnson, R. M., Uthman, Q. O., & Babalola, O. O. (2023). Biopesticides as a promising alternative to synthetic pesticides: A case for microbial, plant-based, and nano-biopesticides. Frontiers in Microbiology, 14, 1040901.

Bayındır Erol, A., & Birgücü, A. K. (2020). Effects of different plant essential oils on adults of Acanthoscelides obtectus (Say) (Coleoptera: Chrysomelidae: Bruchinae). Turkish Journal of Agricultural Research, 7(2), 143–149.

Belgüzer, S., Yılar, M., Yanar, Y., Kadıoğlu, İ., & Doğar, G. (2016). Antibacterial effects of *Thymus vulgaris* extracts and essential oils on *Clavibacter michiganensis* subsp. *michiganensis*. Turkish Journal of Weed Science, 19(2), 20–27.

EPA. (2017). What are biopesticides? United States Environmental Protection Agency. https://www.epa.gov/ingredients-used-pesticide-products/what-are-biopesticides

Eryılmaz, A., Kılıç, G., & Boz, O. İ. (2019). Evaluation of organic farming and good agricultural practices in Türkiye in terms of economic, social, and environmental sustainability. Yüzüncü Yıl University Journal of Agricultural Sciences, 29(2), 352–361. https://doi.org/10.29133/yyutbd.446002

Gupta, P., & Phulara, S. (2021). Terpenoids: Types and their applications. In Biotechnology of Terpenoid Production from Microbial Cell Factories (pp. 47–78). Elsevier.

Isman, M. B. (2020). Botanical insecticides in the twenty-first century—Fulfilling their promise? Annual Review of Entomology, 65, 233–249.

Işık, D., & Temur Çınar, C. (2018). Investigation of the herbicidal effects of essential oils from some medicinal plants on common lambsquarters (*Chenopodium album* L.). SETSci Conference Proceedings, 3, 744–747.

Kedia, A., Prakash, B., Mishra, P. K., Singh, P., & Dubey, N. K. (2015). Botanicals as eco-friendly biorational alternatives of synthetic pesticides against *Callosobruchus* spp. (Coleoptera: Bruchidae)-a review. Journal of Food Science and Technology, 52(3), 1239–1257. https://doi.org/10.1007/s13197-013-1167-8

Kılıç, E. (2019). Biopesticides as substitutes for chemical pesticides in Erzincan agriculture. Gaziosmanpaşa Journal of Scientific Research, 8(3), 14–28.

Maczka, W., Wińska, K., & Grabarczyk, M. (2020). One hundred faces of geraniol. Molecules, 25(14), 3303. https://doi.org/10.3390/molecules25143303

Maral, H. (2022). Diurnal variations of essential oil content and composition of *Satureja cilicica* P.H. Davis under cultivation. International Journal of Approaches in Agricultural Research, 6(3), 239–245.

Maral, H., & Kırıcı, S. (2019). Changes during the day in essential oil content and composition of zahter (*Thymbra spicata* L.) grown in Ermenek. Turkish Journal of Agriculture – Food Sci. and Technology, 7(2), 13–16. https://doi.org/10.24925/turjaf.v7isp2.13-16.3089

Maral, H., & Kırıcı, S. (2023). Yield and quality properties of endemic *Origanum husnucan-baserii* grown under different ecological conditions. Journal of Tekirdağ Agricultural Faculty, 20(2), 1–11.

Maurya, A., Singh, M., Dubey, V., Srivastava, S., Luqman, S., & Bawankule, D. U. (2014). α-(-)-Bisabolol reduces pro-inflammatory cytokine production and alleviates skin inflammation. Current Pharmaceutical Biotechnology, 15(2), 173–181.

Mawcha, T. D., Kinyanjui, G., Simiyu, W. S., Babalola, O. O., & Ndolo, O. D. (2024). Insecticides in pest control: Impacts, challenges, and strategies.

Mitic-Culafic, D., Zegura, B., Nikolic, B., Vukovic-Gacic, B., Knezevic-Vukcevic, J., & Filipic, M. (2009). Protective effect of linalool, myrcene, and eucalyptol against t-butyl hydroperoxide-induced genotoxicity in bacteria and cultured human cells. Food and Chemical Toxicology, 47(1), 260–266.

Nohutçu, L., Şelem, E., Tunçtürk, R., & Tunçtürk, M. (2021). Use of essential oils against agricultural diseases and pests. Bursa Uludağ Univ. J. of Agricultural Faculty, 35(2), 499–523.

Ody, P. L., dos Santos, M. S. N., Lopes, A. M., Mazutti, A. M., Tres, V. M., & Zabot, L. G. (2025). Biological and biochemical pesticides as sustainable alternatives in organic agriculture: A review. Biocatalysis and Agricultural Biotechnology, 67, 103655.

Parveen, S. S., & Rashtrapal, P. S. (2024). Integrated pest management strategies using endophytic entomopathogenic fungi. Plant Science Today, 568–574.

Pavela, R. (2015). Essential oils for the development of eco-friendly mosquito larvicides: A review. Industrial Crops and Products, 76, 174–187. https://doi.org/10.1016/j.indcrop.2015.06.050

Peng, L., Lu, D., Chen, J., Hu, X., Fang, Z., & Zhao, Y. (2025). Insecticidal toxicity of thymol and carvacrol to brown planthopper: A novel strategy based on disruption of cuticular hydrophobicity. Pesticide Biochemistry and Physiology, 210, 106395.

Prakash, B., Shukla, R., Singh, P., Mishra, P. K., Dubey, N. K., & Kharwar, R. N. (2011). Efficacy of chemically characterized Ocimum gratissimum L. essential oil as an antioxidant and a safe plant-based antimicrobial against fungal and aflatoxin B1 contamination of spices. Food Research International, 44(1), 385-390. https://doi.org/10.1016/j.foodres.2010.10.002

Rani, A. T., Kammar, V., Keerthi, M. C., Rani, V., Majumder, S., Pandey, K. K., & Singh, J. (2022). Biopesticides: An alternative to synthetic insecticides. In Microbial Technology for Sustainable Environment. Springer. https://doi.org/10.1007/978-981-16-3840-4_23

Ruiu, L. (2018). Microbial biopesticides in agroecosystems. Agronomy, 8(12), 235. https://doi.org/10.3390/agronomy8110235

Soković, M. D., Vukojević, J., Marin, P. D., Brkić, D. D., Vajs, V., & Van Griensven, L. J. (2010). Chemical composition of essential oils of thymus and mentha species and their antifungal activities. Molecules, 15(11), 7532–7546.
https://doi.org/10.3390/molecules15117532

Szczepanik, M., & Zawitowska, B. (2012). Insecticidal activities of *Thymus vulgaris* essential oil and its components (thymol and carvacrol) against larvae of lesser mealworm (*Alphitobius diaperinus* Panzer). Allelopathy Journal, 30(1), 129–142.

Şimşek, O. (2024). Effects of some plant essential oils on the biology of storage pest *Tenebrio molitor* (Coleoptera: Tenebrionidae): Their use as

insecticides and environmental impacts. [Master's thesis, Sinop Univ., Graduate School of Natural and Applied Sci.].

Türkmen, M., Kara, M., & Maral, H. (n.d.). Determination of chemical components of essential oil of *Origanum dubium* grown at different altitudes and antifungal activity against Sclerotinia sclerotiorum. Journal of Food Processing and Preservation, 46(6), e15787.

Ündeğer, Ü., Başaran, A., Degen, G. H., & Başaran, N. (2009). Antioxidant activities of major thyme constituents and lack of oxidative DNA damage in V79 hamster lung fibroblast cells at low levels of carvacrol and thymol. Food and Chemical Toxicology, 47(8), 2037–2043.

Yaman, S., Özdemir, Z., Şit, M., Özer, B., & Çatal, O. (2018). Effects of carvacrol from thyme oil on human health. In Proceedings of the 1st International Symposium on Innovative Approaches in Scientific Studies (Vol. 2, pp. 391–392). Antalya, Türkiye.

Yarsan, E., & Çevik, A. (2007). Biopesticides in vector control. Turkish Journal of Hygiene and Experimental Biology, 64(1), 61–70.

Zhao, M., Yang, Y., Nian, Q., Shen, C., Xiao, X., Liao, W., Zheng, Q., Zhang, G., Chen, N., Gong, D., Tang, J., Wen, Y., & Zeng, J. (2023). Phytochemicals and mitochondria: Therapeutic allies against gastric cancer. Phytomedicine, 110, 154608. https://doi.org/10.1016/j.phymed.2022.154608

Chapter 2

Identification of Wheat Fungal Diseases Based on Deep Learning Algorithms

Ahmet ÇAT[1]

Abstract

Wheat (*Triticum* spp.) is one of the most important staple food crops globally, playing a crucial role in ensuring food security. However, wheat production is significantly affected by various biotic and abiotic stresses. Among these, diseases such as rusts, powdery mildew, Septoria leaf blotch, and Fusarium head blight are major threats that severely reduce yield and grain quality worldwide. These diseases not only cause substantial economic losses but also pose challenges for sustainable crop management. In recent years, deep learning techniques have emerged as powerful tools for the early and accurate detection of wheat diseases. Numerous studies have demonstrated the potential of convolutional neural networks (CNNs), hyperspectral imaging, and other advanced machine learning approaches in identifying and classifying wheat diseases with high accuracy. In this study, the significance of major wheat diseases and recent advances in deep learning-based detection and classification approaches are discussed, highlighting their potential applications in precision agriculture and disease management.

Introduction

Wheat (*Triticum aestivum L.*) is a primary staple crop cultivated worldwide, providing approximately 20% of global caloric intake and serving as a major source of carbohydrates and protein for billions of people (FAO, 2022). Global wheat production exceeds 760 million tons annually, with cultivation spread across diverse agro-ecological zones. However, wheat yields are significantly threatened by biotic stresses, particularly fungal diseases, which are among the most devastating causes of crop loss (Savary et al., 2019). Estimates suggest that more than 20% of global wheat production is lost annually due to fungal pathogens (Figueroa et al., 2018). These diseases not only reduce yield but also

[1] Department of Plant Protection, Faculty of Agriculture, Siirt University, Siirt, Türkiye
E-mail: ahmetcat@siirt.edu.tr, Orcid: 0000-0002-5638-0319

impair grain quality through contamination with mycotoxins, leading to severe economic and health implications.

Traditional approaches to fungal disease identification rely on visual field scouting by experts and laboratory-based pathogen isolation. Although reliable, these methods are labor-intensive, time-consuming, and require considerable expertise. In contrast, technological advances in artificial intelligence (AI), particularly deep learning (DL), have transformed plant disease diagnostics. Unlike conventional machine learning methods that rely on handcrafted features, deep learning models automatically extract hierarchical representations from raw images, enabling accurate and scalable identification of complex disease patterns (LeCun et al., 2015). Deep learning methods have achieved significant success in image-based detection of plant diseases. A crucial aspect of this technique is the availability of an appropriate dataset. For this purpose, the PlantVillage dataset, which contains thousands of images of diseased leaves, has been developed. Using this dataset, numerous studies have been conducted by various researchers (Mohanty et al., 2016; Zhang et al., 2018; Saleem et al., 2020). On the other hand, some researchers have performed analyses using their own images of diseased plants collected under different conditions (DeChant et al., 2017; Liu et al., 2018; Singh et al., 2020). In the context of wheat, deep learning algorithms have demonstrated significant promise in recognizing a wide spectrum of fungal diseases under both controlled and field conditions.

In this study, we aimed to evaluate the current state of research on the identification of fungal diseases in wheat using deep learning techniques. It first provides an overview of the major fungal pathogens affecting wheat, followed by an evaluation of the deep learning methods commonly applied in plant disease recognition. In addition, case studies, datasets, existing challenges, and future directions are discussed to provide a comprehensive understanding of how artificial intelligence can be utilized to achieve sustainable wheat production

Major Wheat Fungal Diseases

Wheat is susceptible to more than many fungal pathogens, but a few are particularly significant due to their global distribution and economic impact (Table 1).

Table 1. Information about the major wheat fungal diseases

Disease	Pathogen	Symptoms	Economic Impact	Reference
Powdery mildew	*Blumeria graminis* f.sp. *tritici*	White powdery growth on leaf surfaces	Yield loss up to 30%	Bowen et al. 2019
Septoria leaf blotch	*Zymoseptoria tritici*	Necrotic blotches on leaves, chlorosis	Reduced photosynthesis	Fones & Gurr, 2015
Yellow/Stripe rust	*Puccinia striiformis* f.sp. *tritici*	Yellow-orange pustules on leaves	Yield loss up to 65%	Bever, 1937
Leaf rust	*Puccinia triticina*	Brown lesions on leaves	Global distribution	Kolmer, 1996
Stem rust	*Puccinia graminis* f.sp. *tritici*	Dark red pustules on stems, leaf sheaths	Yield loss up to 55%	Abrahim et al., 2018
Fusarium head blight	*Fusarium graminearum*	Bleached spikelets,	Mycotoxin contamination	

Among of them, the rust diseases, caused by the basidiomycete fungus *Puccinia* species, are historically among the most damaging. Stripe rust also called yellow rust (*Puccinia striiformis f.sp. tritici*) thrives in cool, moist conditions and causes yellow-orange pustules on leaves, leading to severe yield reductions. Leaf rust (*Puccinia triticina*) is more widely distributed and produces brown lesions, while stem rust (*Puccinia graminis f.sp. tritici*) is notorious for causing epidemics that devastate crops, as seen in East Africa with the emergence of the *Ug99* lineage (Singh et al., 2011).

Another devastating pathogen is *Fusarium graminearum*, the primary causal agent of Fusarium head blight. This disease not only reduces yield but also contaminates grains with mycotoxins such as deoxynivalenol (DON), which pose serious risks to food safety (McMullen et al., 2012). Powdery mildew, caused by the obligate biotrophic ascomycete fungal plant pathogen *Blumeria graminis f.sp. tritici*, is characterized by white powdery colonies on leaves and stems (Meyer et al. 2019). Epidemics thrive under cool and humid environmental conditions and exhibit a polycyclic nature, allowing spore populations to increase to exceptionally high levels (Both & Spanu, 2004). The other disease, Septoria leaf blotch (*Zymoseptoria tritici*) produces irregular necrotic lesions that coalesce, reducing photosynthetic capacity (Fones & Gurr, 2015).

The accurate detection of these diseases is challenging because symptoms may overlap or resemble abiotic stresses such as drought or nutrient deficiencies.

For example, early symptoms of stripe rust and leaf rust can be visually similar, and head discoloration caused by Fusarium can be confused with environmental stress. These complexities necessitate the use of advanced computational tools capable of differentiating subtle variations in symptom morphology and color.

Deep Learning Studies in Wheat Fungal Disease

In general, the accurate identification of disease agents is of great importance. However, this often requires specialized pathological expertise, which is not always readily accessible to farmers or agronomists. The development of an automated disease detection system would enable users to easily identify diseases in their crops without the need for expert knowledge, thereby facilitating more efficient and timely disease management. Deep learning has emerged as a powerful tool in computer vision and has been successfully applied to agriculture, particularly in wheat fungal disease identification (Figure 1).

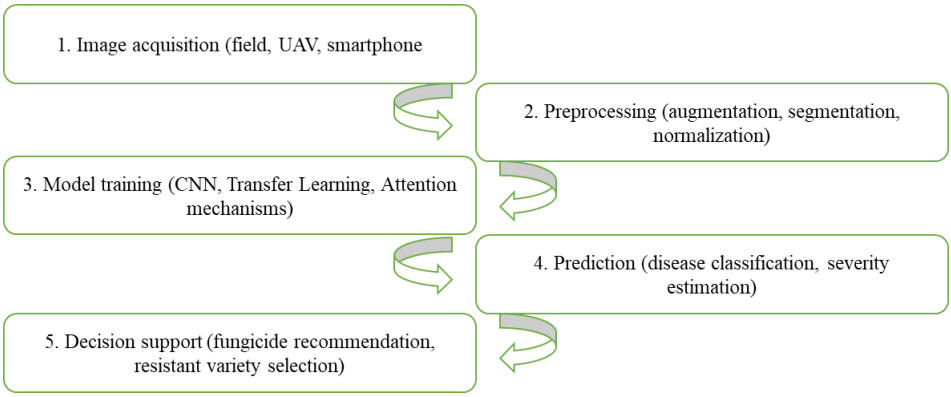

Figure 1. Workflow of wheat fungal disease identification using deep learning

In recent years, advances in machine learning techniques based on neural network algorithms have significantly accelerated the development of technologies for plant disease monitoring using digital RGB images. The distinctive feature of deep learning neural networks compared to other approaches lies in their multilayer architecture, where each layer utilizes the output of the preceding one as input, enabling the extraction of deeper and more meaningful representations of the analyzed object. Convolutional neural networks (CNNs) are the most widely used architectures, consisting of convolutional layers that capture spatial hierarchies, pooling layers that reduce dimensionality, and fully connected layers for classification (Krizhevsky et al., 2012).

In wheat disease detection, CNNs have been applied to leaf, stem, and spike images to classify fungal diseases with remarkable accuracy. Early models such as AlexNet and VGGNet achieved significant improvements over conventional classifiers. Subsequent architectures like ResNet (He et al., 2016), DenseNet (Huang et al., 2017), and InceptionNet (Szegedy et al., 2015) further enhanced feature extraction capacity and reduced overfitting (Table 2).

Table 2. Summary of deep learning models applied in wheat disease detection

Model	Dataset Type	Disease	Accuracy (%)	Reference
AlexNet	PlantVillage images	Leaf rust and stripe rust	90-92	Mohanty et al., 2016
VGG16	Controlled leaf images	Powdery mildew	93	Ferentinos, 2018
ResNet50	Field images	Leaf rust	95-96	Lu et al., 2017
DenseNet121	UAV aerial images	Fusarium head blight	94	Zhang et al., 2019
MobileNetV2	Smartphone-based dataset	Multi-disease	92	Islam et al., 2020
EfficientNet	Multi-location dataset	Stripe rust and Septoria	96-97	Too et al., 2019
EfficientNet-B0	Leaf samples	Multi-disease	94.20	Genaev et al., 2021
CerealConv	Field images	Multi disease	97.05	Long et al., 2023
Ságа	hyperspectral images	Fusarium head blight	89	Wang et al., 2024
MnasNet-SimAM	Wheat images	Multi-disease	91.20	Wen et al., 2024

Transfer learning has become particularly important in agricultural contexts due to the limited size of labeled datasets. By leveraging pretrained models on large-scale datasets such as ImageNet, researchers can fine-tune networks with wheat-specific images, achieving high classification accuracy with reduced computational costs (Ferentinos, 2018). In addition, data augmentation techniques such as rotation, scaling, and color transformation have been used to increase dataset diversity and improve model robustness against varying field conditions.

Recent innovations include the incorporation of attention mechanisms that allow models to focus on relevant image regions associated with disease symptoms. Lightweight networks such as MobileNet and EfficientNet have also facilitated deployment in mobile applications, allowing farmers to identify diseases in real time using smartphones (Too et al., 2019). Furthermore, the

integration of unmanned aerial vehicles (UAVs) and high-resolution imaging has expanded the scope of deep learning applications from single-plant detection to large-scale field monitoring (Kerkech et al., 2020).

Several studies highlight the potential of deep learning for wheat fungal disease identification. For example, Mohanty et al. (2016) demonstrated the effectiveness of CNNs in classifying multiple crop diseases, including wheat rusts, using images from the PlantVillage dataset. Similarly, Lu et al. (2017) applied a transfer learning approach with ResNet for wheat leaf rust detection and achieved over 95% accuracy under controlled conditions.

In another study, Zhang et al. (2019) utilized UAV-based imagery and CNN models to detect Fusarium head blight in wheat fields, demonstrating that deep learning can support large-scale surveillance of disease outbreaks. More recently, Islam et al. (2020) developed a mobile application that integrates CNN-based classification of wheat diseases with user-friendly interfaces, empowering farmers with real-time diagnostic tools. Genaev et al. (2021) reported that the disease-recognition algorithm is based on the convolutional neural network with the EfficientNet architecture. The best accuracy (0.942) was shown by a network with a training strategy based on augmentation and transfer of image styles. In the study conducted by Long et al. (2023), a deep learning-based approach was developed for the early detection of wheat foliar diseases using images captured under real field and glasshouse conditions. The dataset comprised five classes: healthy plants, yellow rust, brown rust, powdery mildew, and septoria leaf blotch. Their proposed model, CerealConv, achieved a high classification accuracy of 97.05%, outperforming experienced plant pathologists by 2% on a subset of test images. Image masking confirmed that the model focused on relevant visual features for disease identification. These findings indicate that the method proposed by Long et al. (2023) demonstrates the strong potential of deep learning networks as reliable and efficient tools for field-level disease detection and classification in wheat. In addition, Wang et al. (2024) reported that their study demonstrated the application of deep learning and hyperspectral imaging for the on-site detection of Fusarium head blight (FHB) in wheat, achieving an accuracy exceeding 89%. They identified significant spectral reflectance differences within the 600-800 nm wavelength range, effectively distinguishing infected wheat ears from healthy ones

Despite promising results, several challenges hinder the widespread application of deep learning in wheat fungal disease detection. A major limitation is the availability of large, diverse, and annotated datasets. Most datasets are collected under controlled conditions with uniform backgrounds, which limits model generalizability to field scenarios where lighting, growth stage, and variety

vary considerably. Another challenge lies in distinguishing between visually similar diseases or differentiating biotic from abiotic stresses, which often leads to misclassification.

Additionally, deep learning models are often criticized for their lack of interpretability. While they provide high accuracy, they do not easily explain their decision-making processes, making them less transparent for plant pathologists. The computational requirements of deep learning, including the need for GPUs and large memory resources, may also limit accessibility in resource-constrained regions.

In summary, the future of deep learning in wheat fungal disease detection lies in integrating multi-modal data and advanced AI techniques. Combining RGB imagery with hyperspectral, thermal, and LiDAR data could improve early detection of fungal infections, even before visible symptoms emerge. Self-supervised and federated learning approaches may address data scarcity and privacy concerns, enabling global collaboration without centralized data sharing (Yang et al., 2021). Vision Transformers (Dosovitskiy et al., 2020), which have revolutionized computer vision, may also enhance wheat disease identification by capturing long-range dependencies in image data. Integration with decision support systems will be crucial for practical adoption. Deep learning models linked to precision agriculture platforms can guide fungicide applications, forecast disease spread under climate change scenarios, and assist breeders in selecting resistant genotypes. Such systems can ultimately transform wheat disease management from reactive to proactive strategies.

Conclusion

Deep learning approaches have been widely applied for the identification of fungal diseases in wheat, offering rapid, accurate, and scalable solutions that surpass traditional diagnostic methods. However, certain challenges persist during implementation such as limited dataset availability, environmental variability, and model interpretability; rapid advancements in artificial intelligence, remote sensing, and computational power are progressively overcoming these obstacles. The integration of deep learning into precision agriculture practices is expected to bring groundbreaking improvements in wheat disease management, contributing significantly to sustainable agricultural production and global food security.

References

Abrahim, A., Wabela, B., Heterat, K. Z., Hailu, E. (2018). Significance of wheat stem rust (*Puccinia graminis* f.sp. *tritici*) in Gurage Zone, Ethiopia. *International Journal of Scientific and Research Publications*, *8*(11), 222-231.

Bever, W. M. (1937). Influence of stripe rust on growth, water economy and yield of wheat and barley. *Journal of Agricultural Research*, *1*, 4-48.

Both, M., & Spanu, P. (2004). *Blumeria graminis* f. sp. *hordei*, an obligate pathogen of barley. Pages 202-218 in: Plant Pathogen Interactions. N. Talbot, ed. Blackwell Publishing, Oxford, U.K

Bowen, K. L., Everts, K. L., & Leath, S. (1991). Reduction in yield of winter wheat in North Carolina due to powdery mildew and leaf rust. *Phytopathology*, *81*, 503-511.

DeChant, C., Wiesner-Hanks, T., Chen, S., Stewart, E.L., Yosinski, J., Gore, M.A. et al. (2017) Automated identification of northern leaf blight-infected maize plants from field imagery using deep learning. *Phytopathology*, *107*, 1426-1432.

Dosovitskiy, A., Beyer, L., Kolesnikov, A., et al. (2020). An image is worth 16x16 words: Transformers for image recognition at scale. *arXiv preprint arXiv:2010.11929*.

FAO. (2022). *FAOSTAT statistical database*. Food and Agriculture Organization of the United Nations.

Ferentinos, K. P. (2018). Deep learning models for plant disease detection and diagnosis. *Computers and Electronics in Agriculture*, *145*, 311-318.

Figueroa, M., Hammond-Kosack, K. E., & Solomon, P. S. (2018). A review of wheat diseases a field perspective. *Molecular Plant Pathology*, *19*(6), 1523-1536.

Fones, H., & Gurr, S. (2015). The impact of Septoria tritici Blotch disease on wheat: An EU perspective. *Fungal Genetics and Biology*, *79*, 3-7.

Genaev, M. A., Skolotneva, E. S., Gultyaeva, E. I., Orlova, E. A., Bechtold, N. P., & Afonnikov, D. A. (2021). Image-based wheat fungi diseases identification by deep learning. *Plants*, *10*(8), 1500.

He, K., Zhang, X., Ren, S., & Sun, J. (2016). Deep residual learning for image recognition. *Proceedings of the IEEE Conference on Computer Vision and Pattern Recognition*, 770-778.

Huang, G., Liu, Z., van der Maaten, L., & Weinberger, K. Q. (2017). Densely connected convolutional networks. *Proceedings of the IEEE Conference on Computer Vision and Pattern Recognition*, 4700-4708.

Islam, M., Dinh, A., Wahid, K., & Bhowmik, P. (2020). Detection of wheat diseases using deep learning. *Plants*, *9*(9), 1255.

Kerkech, M., Hafiane, A., & Canals, R. (2020). Deep learning approach with colorimetric spaces and vegetation indices for vine diseases detection in UAV images. *Computers and Electronics in Agriculture*, *174*, 105446.

Krizhevsky, A., Sutskever, I., & Hinton, G. E. (2012). ImageNet classification with deep convolutional neural networks. *Advances in Neural Information Processing Systems*, *25*, 1097-1105.

Kolmer, J. A. (1996). Genetics of resistance to wheat leaf rust. *Annual review of phytopathology*, *34*(1), 435-455.

LeCun, Y., Bengio, Y., & Hinton, G. (2015). Deep learning. *Nature*, *521*(7553), 436-444.

Liu, B., Zhang, Y., He, D. & Li, Y. (2018) Identification of apple leaf diseases based on deep convolutional neural networks. *Symmetry*, *10*, 11.

Long, M., Hartley, M., Morris, R. J., & Brown, J. K. (2023). Classification of wheat diseases using deep learning networks with field and glasshouse images. *Plant Pathology*, *72*(3), 536-547.

Lu, Y., Yi, S., Zeng, N., Liu, Y., & Zhang, Y. (2017). Identification of rice diseases using deep convolutional neural networks. *Neurocomputing*, *267*, 378-384.

McMullen, M., Bergstrom, G., De Wolf, E., Dill-Macky, R., Hershman, D., Shaner, G., & Van Sanford, D. (2012). A unified effort to fight an enemy of wheat and barley: Fusarium head blight. *Plant Disease*, *96*(12), 1712-1728.

Meyers, E., Arellano, C., & Cowger, C. (2019). Sensitivity of the US *Blumeria graminis* f. sp. *tritici* population to demethylation inhibitor fungicides. *Plant Disease*, *103*(12), 3108-3116.

Mohanty, S. P., Hughes, D. P., & Salathé, M. (2016). Using deep learning for image-based plant disease detection. *Frontiers in Plant Science*, *7*, 1419.

Saleem, M.H., Khanchi, S., Potgieter, J. & Arif, K.M. (2020) Image-based plant disease identification by deep learning meta-architectures. *Plants*, *9*, 1451.

Savary, S., Willocquet, L., Pethybridge, S. J., Esker, P., McRoberts, N., & Nelson, A. (2019). The global burden of pathogens and pests on major food crops. *Nature Ecology & Evolution*, *3*(3), 430-439.

Singh, D., Jain, N., Jain, P., Kayal, P., Kumawat, S. & Batra, N. (2020) PlantDoc: a dataset for visual plant disease detection. *In: Proceedings of CoDS COMAD 2020: 7th ACM IKDD CoDS and 25th COMAD*. New York, USA: Association for Computing Machinery, pp. 249-253.

Singh, R. P., Hodson, D. P., Huerta-Espino, J., et al. (2011). The emergence of Ug99 races of the stem rust fungus is a threat to world wheat production. *Annual Review of Phytopathology*, *49*, 465-481.

Szegedy, C., Liu, W., Jia, Y., et al. (2015). Going deeper with convolutions. *Proceedings of the IEEE Conference on Computer Vision and Pattern Recognition*, 1-9.

Too, E. C., Yujian, L., Njuki, S., & Yingchun, L. (2019). A comparative study of fine-tuning deep learning models for plant disease identification. *Computers and Electronics in Agriculture*, *161*, 272-279.

Wang, X., Polder, G., Focker, M., Liu, C., (2024). Sága, a deep learning spectral analysis tool for fungal detection in grains-a case study to detect fusarium in winter wheat. *Toxins*. *16*(8), 354.

Wen, X., Maimaiti, M., Liu, Q., Yu, F., Gao, H., Li, G., & Chen, J. (2024). MnasNet-SimAM: An ımproved deep learning model for the ıdentification of common wheat diseases in complex real-field environments. *Plants*, *13*(16), 2334

Yang, Q., Liu, Y., Chen, T., & Tong, Y. (2021). Federated machine learning: Concept and applications. *ACM Transactions on Intelligent Systems and Technology*, *10*(2), 1-19.

Zhang, J., Huang, Y., Pu, R., Gonzalez-Moreno, P., Yuan, L., Wu, K., & Huang, W. (2019). Monitoring plant diseases and pests through remote sensing technology: A review. *Computers and Electronics in Agriculture*, *165*, 104943.

Zhang, S., Huang, W. & Zhang, C. (2018) Three-channel convolutional neural networks for vegetable leaf disease recognition. *Cognitive Systems Research*, *53*, 31-41.

Chapter 3

Use of Copper as a Plant Nutrient and Fungicide in Plant Production

Ahmet Şafak MALTAŞ[1]

Introduction

Essential plant nutrients are defined as elements that are necessary for the plant to sustain its life, cannot be replaced by other elements, and are directly required for plant metabolism; in other words, elements without which plant life is not possible (Brown et al. 2022). Plants can continue to develop as long as they receive the plant nutrient elements they need in the required amounts. Plant nutrient elements are generally taken up by plants in the form of anions and cations, but in some cases, they can also be taken up in molecular form (De Bang et al. 2021). In plant production, low or high levels of plant development can lead to yield and quality losses. To reduce yield and quality losses and achieve the highest yields and quality, plant nutrients must be supplied to plants at optimal levels (Barłóg et al. 2022).

To ensure plants produce high yields and high-quality products, fertilizers containing the appropriate amount and ratio of nutrients must be applied to the soil or leaves (Milošević et al. 2022; Noulas et al. 2023). To prevent any nutritional deficiencies in plants, plant nutrient levels should be monitored, and necessary measures should be taken promptly (Grzebisz et al. 2022; Balusamy et al. 2023). Fertilization in plants is important for supplying the nutrients the plant needs and maintaining its nutritional balance. Deficiency or excess of microelements in plants negatively affects plant development (Kumar et al. 2021; Sagwal et al. 2023). In plant production, if the necessary plant nutrition is not provided, even if all other cultural practices are performed without fail, the plant's development will not be at the desired level (Pasternak and Steinmacher 2024). Nutritional deficiencies not only reduce yield but also cause deterioration in the quality of the product obtained and reduce the plant's resistance to stress conditions such as disease, extreme heat and cold, and drought (Abbas et al. 2021; Das and Biswas 2022).

[1] Akdeniz University, Finike Vocational School, Department of Plant and Animal Production, Finike, Turkiye

Nutrient disorders in plants can result from either a deficiency or an excess of any nutrient element (Tewari et al. 2021; Kiani et al. 2022). Plants require at least 16 plant nutrient elements to grow and develop optimally (Figure 1). Even if the 15 plant nutrient elements present in the plant are available at the concentration required by the plant, the excessive deficiency of a single micronutrient element can cause the plant's development to stop completely and lead to a significant drop in yield (Longnecker 2021; Lilay et al. 2021; Monip et al. 2023).

Table 1. Essential plant nutrients required by plants

Essential plant nutrients			
Carbon	Phosphorus	Sulfur	Copper
Hydrogen	Potassium	Iron	Boron
Oxygen	Calcium	Manganese	Molybdenum
Nitrogen	Magnesium	Zinc	Chlorine

Copper (Cu) is a heavy metal with atomic number 29, atomic mass 63.5 g/mol, and density 8.96 g/cm³. Copper is one of the essential micronutrients for plant growth (Kumari et al. 2025). It acts as a structural component of many proteins. It plays a role in cell wall metabolism, photosynthetic electron transport, oxidative stress responses, protein synthesis, hormone signaling, and mitochondrial respiration (Chen et al. 2021; Tang et al. 2025). Due to its ease of reduction and oxidation, copper acts as a cofactor in many enzymes. Some enzymes containing copper as a cofactor play an important role under stress conditions (Tsang et al. 2021).

Copper is a micronutrient element that is essential for plants at optimal levels to support cellular functions, but when present in excessive amounts, it can be toxic and cause adverse effects on the plant's primary production and survival. Deficiency can cause chlorosis or discoloration in young leaves, as well as reduced growth and development (Ghimirey et al. 2024). Plants can live and tolerate conditions of copper deficiency. However, when the concentration of copper in the plant exceeds the optimal level, it becomes toxic to the plant. Copper concentrations higher than the required level negatively affect plant growth and development, reducing root and leaf development and significantly decreasing photosynthesis (Jin et al. 2021; Mir et al. 2021; Guedes et al. 2025). Additionally, high copper concentrations can damage cell development by disrupting the structure of cell components (Jomova et al. 2025; Wang et al. 2025).

The Status of Copper in Soil

Microelements are found in four different forms in the soil: in the composition of primary and secondary minerals, adsorbed on the surfaces of minerals and organic matter, in organic form within the structure of organic and microbial biomass, and in ionic form in the soil solution (Parades-Aguilar et al. 2025).

Copper is present in many soils as Cu+2 compounds. It is found as the Cu+ ion in soils where oxygen is scarce, such as poorly aerated or waterlogged soils. The solubility of copper compounds is high in acidic soils (Zamulina et al. 2022). Solubility decreases as soil pH increases. A large portion of the copper present in the soil is found within the crystal structures of primary and secondary minerals (Redwan et al. 2021). In addition, it is found in the structure of soil organic matter and in the soil solution in ionic form.

The majority of copper present in soil solution is bound to organic matter (Kim et al. 2025). Compared to other cations found in soil structure, the copper element binds very strongly to inorganic ion exchangers. Therefore, despite being in a changeable form, copper ions bound in the soil are not easily taken up by plants (Lui et al. 2023). Copper is quite immobile because it is strongly bound in the soil. Consequently, copper added to the soil in various ways generally accumulates in the upper layers of the soil. Therefore, the movement of copper into the deeper layers of the soil rarely occurs (Yakovets, 2021). However, copper mobility increases when the soil is deeply tilled and the soil pH is between 5.5 and 6.5 (Figure 2). One of the functions of the copper element in the soil is its contribution to symbiotic nitrogen fixation (Kafeel et al. 2023).

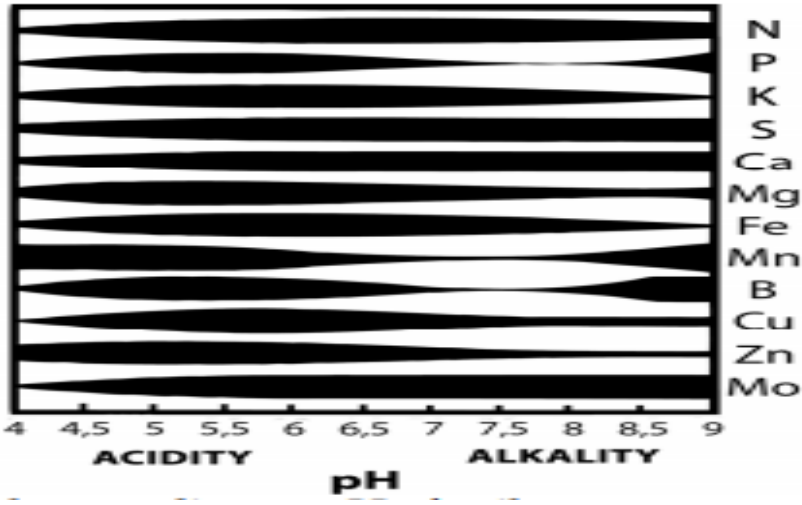

Figure 2. The mobility of copper according to pH of soil

Functions of copper in plants

Copper is a very important element in terms of plant physiology. It plays a role in numerous complex processes such as vitamin, carbohydrate, and protein synthesis, as well as photosynthesis and respiration. Copper is a micronutrient element required by plants for chlorophyll production, respiration, and protein synthesis. Activation in various oxidase enzymes and numerous electron transfers are carried out by copper. It is effective in protein and carbohydrate metabolism. Copper affects the plant's ability to show good resistance to diseases and control plant moisture (Chen et al. 2022; Kaur et al. 2023; Houmani et al. 2024). Copper is absorbed by plants in very low amounts. The Cu content of many plants ranges from 2-20 ppm in dry matter (Mottaleb et al. 2021).

Photosynthesis and respiration can be affected by copper deficiency because Cu enzymes catalyze or activate various steps in these processes. The copper element is essential for the formation of chlorophyll and other thylakoid components. The copper element plays a role in photosynthesis, particularly as a component of ribulose-bisphosphate carboxylase; in respiration, copper deficiency reduces respiration rates (Liu et al. 2024).

Copper uptake is a metabolically controlled active uptake process. Cu and Zn ions have a strong antagonistic effect on each other's uptake. Apart from this, copper uptake is not affected by competition from other cations and is primarily dependent on the amount of available Cu in the soil (Mir et al. 2021). The copper element significantly affects lignin production and the activity of phenol oxidases. Copper deficiency reduces lignin production and the activity of phenol oxidases. In particular, the reduction in lignin production causes leaf and stem tissues to become fragile (Daou et al. 2021; Bahrami-Rad et al. 2024). The presence of sufficient copper affects the synthesis of phenolic compounds that inhibit cell elongation. In the absence of sufficient copper, the amount of auxin hormone in plants decreases. Auxin hormone deficiency causes a decrease in germination (Rolón-Cárdenas et al. 2022).

Copper is not a mobile plant nutrient element within the plant body. It is known that the activation of many enzymes with different functions is carried out by the copper element. Some proteins with important functions in plants also contain copper (Tsang et al. 2021).

The copper element also has an effect on carbohydrate and protein metabolism. In the absence of the copper element, the carbohydrate content of plants decreases (Sun et al. 2023). Copper has a special importance in symbiotic nitrogen fixation. Due to the very high carbohydrate requirement of nitrogen-fixing microorganisms and the effect of copper on carbohydrate content, it is thought that N fixation is affected by copper deficiency (Hu et al. 2023).

Copper Deficiency and Toxicity in Plants

Since copper cannot be easily transferred from older leaves to younger leaves, deficiency symptoms are first seen in younger leaves. Color changes such as a grayish-green color, even whitening, and wilting are observed. Growth weakens. In fruit trees, drying occurs at the tips of the branches. In some cases, larger-than-normal leaves form before tip drying is observed (Magalhães et al. 2023).

Copper deficiency can often be observed in plants grown in soils with high organic matter content. This is because organic matter binds copper very strongly. Copper deficiency causes color changes in young leaves, such as a grayish-green color, chlorosis, and even whitening (Morya et al. 2023).

Copper is an essential plant nutrient for all plants; an average level of 5 ppm in plant leaves is sufficient. However, it can have toxic effects at different concentrations. The toxic effect of copper stems from its displacement of other metal ions, particularly iron, from physiologically important sites. For this reason, copper toxicity often resembles iron deficiency, causing chlorosis and rapid deterioration of root development. Plants are generally very sensitive to copper toxicity. When copper levels in tissues are slightly higher than normal, they cause metabolic disturbances and inhibit plant growth (Mir et al. 2021).

High concentrations of copper inhibit numerous enzymes (Kahlson and Dixon 2022). Copper accumulating in cells leads to the production of free radicals that initiate a peroxidation chain reaction involving membrane lipids and causes inhibition of photosynthetic electron transport. Excess copper can cause oxidative stress in plants by increasing reactive oxygen species. It is an effective inhibitor of plant growth, and its excess often causes symptoms of aging (Gou et al. 2022).

The effect of copper on cell defense

Copper is an important regulator of the plant immune system. It plays a role in the synthesis of phenolic compounds and phytoalexins, making it effective in fighting pathogens. Laccase and peroxidase activities increase, the cell wall hardens, and pathogen penetration becomes more difficult. Therefore, copper deficiency causes plants to become more susceptible to fungal and bacterial infections (Cheng et al. 2022). Copper also affects lignin synthesis, which is present in the structure of the cell wall and influences its strength (Li et al. 2023). Lignin is a phenolic polymer found in the cell walls of plants. It provides mechanical strength to the cell wall (Yadav and Chattopadhyay 2023).

Copper catalyzes the oxidation of phenolic compounds with copper-dependent enzymes such as laccase and polyphenol oxidase. Thus, copper plays

a critical role in lignin synthesis. When there is sufficient lignin in the structure of the cell wall, plant tissues harden, and the stem and veins become durable. Thus, lignin becomes part of the plant's defense system by making it difficult for pathogens to enter the cell. In the absence of lignin, stems become weak, twisted, and prone to lodging. This situation can make plants susceptible to disease (Mydy et al. 2021; Basera et al. 2024).

Copper as a fungicide in crop production

It has been understood that copper promotes plant growth after the use of Bordeaux mixture (a mixture of copper sulfate and lime) for spraying against fungal diseases (Ramchander et al. 2025).

The copper element is likely to react with exudates such as hydroxy and amino acids produced by fungal spores to form soluble toxic copper complexes with “insoluble” copper fungicides. The resulting copper complexes may exhibit direct fungicidal activity. The effect of the copper element on fungi varies depending on the original fungicide and the type of target fungus. Although this is the primary fungicidal effect, it is supported by copper brought into solution by atmospheric factors and host plant exudates. However, these two factors probably play the most important role in phytotoxicity or host plant damage. Finally, cumulative effect is also a contributing factor, but it is part of the effect carried out by spore discharges or exudates (McCallan 1949).

Copper-containing fungicides can be divided into three types. These are a) basic salts, b) normal salts, and c) organic copper compounds. The vast majority of research on copper fungicides has been conducted on Bordeaux mixture. Bordeaux mixture is prepared by mixing a solution of copper sulfate pentahydrate with a suspension of hydrated lime. The proportions of each may vary, but generally the lime is equal to or greater than the copper sulfate by weight (Martin, 1932).

Many companies operating in agriculture offer producers a class of fungicides containing copper, which have antifungal and antibacterial effects. The effectiveness of copper-containing fungicides in high-moisture growing environments stems from their ability to disrupt the protein structures of bacteria and fungi (Burandt et al. 2024). Disruption of protein structure reduces the reproduction of bacteria and fungi and their ability to harm plants. Based on their chemical structure, copper-containing fungicides include: copper sulfate (cupric sulfate; CuSO4), copper acetate (cupric acetate; Cu(OAc)2), cuprous oxide (copper(I) oxide; Cu2O), cupric chloride (copper(II) chloride; CuCl2), copper oxychloride (copper(II) oxychloride; $CuCl_2 \cdot 3Cu(OH)_2$), cuprous chloride (copper(I) chloride; CuCl), cupric nitrate (copper(II) nitrate;

$Cu(NO_3)_2$), copper cyanide (copper(I) cyanide; CuCN), copper soap (solution of copper octanoate), and copper naphthenate.

Conclusion

In today's world, where healthy food production has gained importance, the use of copper elements continues intensively for disease control in plant production. Long-term copper use can cause copper accumulation in agricultural soils and create high levels of residues in fruits and vegetables. Therefore, caution should be exercised in the use of copper in plant production, and applications should be made taking into account the potential risks it may pose to the environment and health after use. Copper is an element involved in many physiological and biochemical processes in higher organisms. Therefore, there is a need for copper consumption; however, care should be taken not to consume high doses of copper in daily consumption. All different chemical forms of the copper element will continue to be one of the most important drugs used in pathogen control in plant production. Therefore, the use of the copper element by producers should be regularly monitored. The dosage and interval of use should be adapted according to the chemical form of the copper used, environmental health, and plant needs.

References

Abbas, S., Javed, M. T., Ali, Q., Azeem, M., & Ali, S. (2021). Nutrient deficiency stress and relation with plant growth and development. In *Engineering tolerance in crop plants against abiotic stress* (pp. 239-262). CRC Press.

Bahrami-Rad, S., & Hajiboland, R. (2024). Differential effects of copper sulfate (CuSO4) and copper tetraamine sulfate complex ([Cu (NH3) 4] SO4) on Cu-enzyme activities, Cu and Fe levels, and lignin deposition in Cu-deficient tobacco (Nicotiana rustica) plants. *Acta Physiologiae Plantarum*, *46*(3), 34.

Balusamy, S. R., Joshi, A. S., Perumalsamy, H., Mijakovic, I., & Singh, P. (2023). Advancing sustainable agriculture: a critical review of smart and eco-friendly nanomaterial applications. *Journal of Nanobiotechnology*, *21*(1), 372.

Barłóg, P., Grzebisz, W., & Łukowiak, R. (2022). Fertilizers and fertilization strategies mitigating soil factors constraining efficiency of nitrogen in plant production. *Plants*, *11*(14), 1855.

Basera, P., Chakraborty, S., & Sharma, N. (2024). Lignocellulosic biomass: insights into enzymatic hydrolysis, influential factors, and economic viability. *Discover Sustainability*, *5*(1), 311.

Brown, P. H., Zhao, F. J., & Dobermann, A. (2022). What is a plant nutrient? Changing definitions to advance science and innovation in plant nutrition. *Plant and Soil*, *476*(1), 11-23.

Burandt, Q. C., Deising, H. B., & von Tiedemann, A. (2024). Further limitations of synthetic fungicide use and expansion of organic agriculture in europe will increase the environmental and health risks of chemical crop protection caused by copper-containing fungicides. *Environmental Toxicology and Chemistry*, *43*(1), 19-30.

Chen, G., Li, J., Han, H., Du, R., & Wang, X. (2022). Physiological and molecular mechanisms of plant responses to copper stress. *International journal of molecular sciences*, *23*(21), 12950.

Chen, G., Li, J., Han, H., Du, R., & Wang, X. (2022). Physiological and molecular mechanisms of plant responses to copper stress. *International journal of molecular sciences*, *23*(21), 12950.

Cheng, F., Peng, G., Lu, Y., Wang, K., Ju, Q., Ju, Y., & Ouyang, M. (2022). Relationship between copper and immunity: the potential role of copper in tumor immunity. *Frontiers in oncology*, *12*, 1019153.

Daou, M., Bisotto, A., Haon, M., Oliveira Correia, L., Cottyn, B., Drula, E., & Faulds, C. B. (2021). A putative lignin copper oxidase from Trichoderma reesei. *Journal of Fungi*, *7*(8), 643.

Das, R., & Biswas, S. (2022). Influence of abiotic stresses on seed production and quality. In *Seed biology updates*. IntechOpen.

De Bang, T. C., Husted, S., Laursen, K. H., Persson, D. P., & Schjoerring, J. K. (2021). The molecular–physiological functions of mineral macronutrients and their consequences for deficiency symptoms in plants. *New Phytologist*, *229*(5), 2446-2469.

Ghimirey, V., Chaurasia, J., & Marahatta, S. (2024). Plant nutrition disorders: insights from clinic analyses and their impact on plant health. *Agriculture Extension in Developing Countries*, *2*(1), 09-17.

Grzebisz, W., Zielewicz, W., & Przygocka-Cyna, K. (2022). Deficiencies of secondary nutrients in crop plants—A real challenge to improve nitrogen management. *Agronomy*, *13*(1), 66.

Guedes, F. R. C. M., Rodrigues, L. S., Lobato, E. M. S. G., Batista, B. L., & Lobato, A. K. D. S. (2025). Exogenous 24-Epibrassinolide Upregulated Photosynthesis in Soybean Plants Under Copper Stress as Evidenced by Electron Transport and Antioxidant Enzymes. *Journal of Soil Science and Plant Nutrition*, 1-16.

Guo, J., Huang, X., Dou, L., Yan, M., Shen, T., Tang, W., & Li, J. (2022). Aging and aging-related diseases: from molecular mechanisms to interventions and treatments. *Signal transduction and targeted therapy*, *7*(1), 391.

Houmani, H., Ben Slimene Debez, I., Turkan, I., Mahmoudi, H., Abdelly, C., Koyro, H. W., & Debez, A. (2024). Revisiting the potential of seed nutri-priming to improve stress resilience and nutritive value of cereals in the context of current global challenges. *Agronomy*, *14*(7), 1415.

Hu, B., Flemetakis, E., Liu, Z., Hänsch, R., & Rennenberg, H. (2023). Significance of nitrogen-fixing actinorhizal symbioses for restoration of depleted, degraded, and contaminated soil. *Trends in Plant Science*, *28*(7), 752-764.

Jin, M. F., You, M. X., Lan, Q. Q., Cai, L. Y., & Lin, M. Z. (2021). Effect of copper on the photosynthesis and growth of Eichhornia crassipes. *Plant Biology*, *23*(5), 777-784.

Jomova, K., Alomar, S. Y., Valko, R., Nepovimova, E., Kuca, K., & Valko, M. (2025). The role of redox-active iron, copper, manganese, and redox-inactive zinc in toxicity, oxidative stress, and human diseases. *EXCLI journal*, *24*, 880.

Kafeel, U., Jahan, U., & Khan, F. A. (2023). Role of mineral nutrients in biological nitrogen fixation. In *Sustainable Plant Nutrition* (pp. 87-106). Academic Press.

Kahlson, M. A., & Dixon, S. J. (2022). Copper-induced cell death. *Science*, *375*(6586), 1231-1232.

Kaur, H., Kaur, H., Kaur, H., & Srivastava, S. (2023). The beneficial roles of trace and ultratrace elements in plants. *Plant Growth Regulation*, *100*(2), 219-236.

Kiani, A. K., Dhuli, K., Donato, K., Aquilanti, B., Velluti, V., Matera, G., & Bertelli, M. (2022). Main nutritional deficiencies. *Journal of preventive medicine and hygiene*, *63*(2 Suppl 3), E93.

Kim, H. B., Kim, J. G., Alessi, D. S., & Baek, K. (2025). Mechanistic Understanding of Copper (Cu) Mobility in Aged Biochar: Influence of Dissolved Organic Matters Release and Oxygen-Containing Groups. *Journal of Hazardous Materials*, 139123.

Kumar, S., Kumar, S., & Mohapatra, T. (2021). Interaction between macro-and micro-nutrients in plants. *Frontiers in Plant Science*, *12*, 665583.

Kumari, S., Rustagi, B., Meena, P., & Warkar, S. G. (2025). Carboxymethyl tamarind kernel gum-based superabsorbent hydrogel for release of copper micronutrient. *International Journal of Biological Macromolecules*, 146010.

Li, Y., Shi, S., Zhang, Y., Zhang, A., Wang, Z., & Yang, Y. (2023). Copper stress-induced phytotoxicity associated with photosynthetic characteristics and lignin metabolism in wheat seedlings. *Ecotoxicology and Environmental Safety*, *254*, 114739.

Lilay, G. H., Thiébaut, N., du Mee, D., Assunção, A. G., Schjoerring, J. K., Husted, S., & Persson, D. P. (2024). Linking the key physiological functions of essential micronutrients to their deficiency symptoms in plants. *New Phytologist*, *242*(3), 881-902.

Liu, A., Xiao, W., Lai, W., Wang, J., Li, X., Yu, H., & Zha, Y. (2024). Potential application of selenium and copper nanoparticles in improving growth, quality, and physiological characteristics of strawberry under drought stress. *Agriculture*, *14*(7), 1172.

Liu, Y., Wang, H., Cui, Y., & Chen, N. (2023). Removal of copper ions from wastewater: a review. *International journal of environmental research and public health*, *20*(5), 3885.

Longnecker, N. (2021). Nutrient deficiencies and vegetative growth. In *Mechanisms of Plant Growth and Improved Productivity Modern Approaches* (pp. 137-172). CRC Press.

Magalhães, D. D. S., Viegas, I. D. J. M., Barata, H. D. S., Costa, M. G., Silva, B. C. D., & Mera, W. Y. W. D. L. (2023). Deficiencies of nitrogen, calcium, and micronutrients are the most limiting factors for growth and yield of smell pepper plants. *Revista Ceres*, *70*(3), 125-135.

Martin, H. (1932). Studıes Upon the Copper Fungıcıdes: The Interactıon of Coppee Sulphate wıth Calcıum Hydroxıde. *Annals of Applied Biology*, *19*(1), 98-120.

McCallan, S. E. A. (1949). The nature of the fungicidal action of copper and sulfur. *The Botanical Review*, *15*(9), 629-643.

Milošević, T., Milošević, N., & Mladenović, J. (2022). The influence of organic, organo-mineral and mineral fertilizers on tree growth, yielding, fruit quality and leaf nutrient composition of apple cv.'Golden Delicious Reinders'. *Scientia Horticulturae*, *297*, 110978.

Mir, A. R., Pichtel, J., & Hayat, S. (2021). Copper: uptake, toxicity and tolerance in plants and management of Cu-contaminated soil. *Biometals*, *34*(4), 737-759.

Monib, A. W., Niazi, P., & Sediqi, S. (2023). Investigating approaches for optimizing agricultural yield: A comprehensive review of the crucial role of micronutrients in enhancing plant growth and maximizing production. *Journal for Research in Applied Sciences and Biotechnology*, *2*(5), 168-180.

Morya, R., Morya, R., Mittoliya, V. K., & Chhirouliya, J. K. (2023). Functions and Deficiency Symptoms of Micro Nutrients. *Dr. Nareshkumar B. Desai*, 35.

Mottaleb, S. A., Hassan, A. Z., El-Bahbohy, R., & Mahmoud, A. W. M. (2021). Are copper nanoparticles toxic to all plants? A case study on onion (Allium cepa L.). *Agronomy*, *11*(5), 1006.

Mydy, L. S., Chigumba, D. N., & Kersten, R. D. (2021). Plant copper metalloenzymes as prospects for new metabolism involving aromatic compounds. *Frontiers in plant science*, *12*, 692108.

Noulas, C., Torabian, S., & Qin, R. (2023). Crop nutrient requirements and advanced fertilizer management strategies. *Agronomy*, *13*(8), 2017.

Parades-Aguilar, J., Agustin-Salazar, S., Cerruti, P., Ambrogi, V., Calderon, K., Gamez-Meza, N., & Medina-Juarez, L. A. (2025). Agro-industrial wastes and their application perspectives in metal decontamination using biocomposites and bacterial biomass: a review. *World Journal of Microbiology and Biotechnology*, *41*(1), 16.

Pasternak, T. P., & Steinmacher, D. (2024). Plant growth regulation in cell and tissue culture in vitro. *Plants*, *13*(2), 327.

Ramchandar, R. V., Kumar, A., Priyanka, E., Bharati, M. R., Bhardwaj, L. K., Khandelia, P., & Narayan, K. P. (2025). A Concurrent Review on Plant-Derived Biopesticides and Synthetic Pesticides: Their Importance in Plant Protection and Impacts on Human Health. *Nature Environment & Pollution Technology*, *24*(3).

Redwan, M., Rammlmair, D., & Berkh, K. (2021). Secondary minerals in a calcareous environment: an example from Um Gheig Pb/Zn mine site, Eastern Desert, Egypt. *Environmental Earth Sciences*, *80*(7), 274.

Rolón-Cárdenas, G. A., Arvizu-Gómez, J. L., Soria-Guerra, R. E., Pacheco-Aguilar, J. R., Alatorre-Cobos, F., & Hernández-Morales, A. (2022). The role of auxins and auxin-producing bacteria in the tolerance and accumulation of cadmium by plants. *Environmental Geochemistry and Health*, *44*(11), 3743-3764.

Sagwal, A., Wadhwa, P., & Kaushal, S. (2023). Essentiality of micronutrients in soil: A review. *International Journal of Plant and Soil Science*, *35*(24), 56-65.

Sun, Z., Shao, Y., Yan, K., Yao, T., Liu, L., Sun, F., & Huang, Y. (2023). The link between trace metal elements and glucose metabolism: evidence from zinc, copper, iron, and manganese-mediated metabolic regulation. *Metabolites*, *13*(10), 1048.

Tang, H., Tang, Q., Zhang, J., Chen, X., Tong, T., Zheng, Q., & Jiang, W. (2025). Molecular Evolution of Cu Transporters and Transcription Factors in Plant Response to Copper Stress. *Plants*, *14*(17), 2710.

Tewari, R. K., Yadav, N., Gupta, R., & Kumar, P. (2021). Oxidative stress under macronutrient deficiency in plants. *Journal of Soil Science and Plant Nutrition*, *21*(1), 832-859.

Tsang, T., Davis, C. I., & Brady, D. C. (2021). Copper biology. *Current Biology*, *31*(9), R421-R427.

Tsang, T., Davis, C. I., & Brady, D. C. (2021). Copper biology. *Current Biology*, *31*(9), R421-R427.

Wang, Y., Yan, Q., Shi, Y., & Long, M. (2025). Copper Toxicity in Animals: A Review: Y. Wang et al. *Biological trace element research*, *203*(5), 2675-2686.

Yadav, S., & Chattopadhyay, D. (2023). Lignin: the building block of defense responses to stress in plants. *Journal of Plant Growth Regulation*, *42*(10), 6652-6666.

Yakovets, L. (2021). Migration of heavy metals in the soil profile. *Norwegian Journal of development of the International Science*, (54-1), 8-12.

Zamulina, I. V., Gorovtsov, A. V., Minkina, T. M., Mandzhieva, S. S., Burachevskaya, M. V., & Bauer, T. V. (2022). Soil organic matter and biological activity under long-term contamination with copper. *Environmental Geochemistry and Health*, *44*(2), 387-398.

Chapter 4

Haploid Production in Vegetable Tissue Culture: Microbial Risks, Limitations and Solutions

Esmanur DEMİREL[1]

1. Introduction

Vegetable breeding is gaining more and more strategic importance every day in the face of the increasing world population and the diverse demands of individuals, climate change, and the need for sustainability in agricultural production. Vegetable breeding studies are now more commonly conducted using classical breeding methods. However, selection cycles are long in developing new varieties with desired traits, and genetic variations can be controlled to a limited extent in the sub-generations (Singh et al. 2023). Therefore, classical breeding techniques need to be supported by modern biotechnological approaches. Double haploid technologies, one of the biotechnological approaches, allow homozygous lines to be obtained in vegetable breeding in a very short time, significantly accelerating the breeding process (Qu et al. 2024). Homozygous lines, which could have been obtained in 6-7 generations through selection cycles, can be obtained in a short time with this technology. Haploid plants can be obtained by extracting and stimulating plant gamete cells at specific stages. Depending on the type of gamete cell used and the method of culturing the gamete cells, techniques such as anther culture, microspore culture, ovule-ovarium culture, and parthenogenesis can be applied (Neumann et al. 2023). The presence of a single gene set in haploid plants results in sterile individuals, which hinders their effective use in breeding. Haploid plants are converted to double haploid by duplicating a single gene set identically using chromosome doubling methods. Thus, homologous chromosome pairs consist of genes with the same traits, and the resulting plants are pure lines (Ren et al. 2017). These lines are a powerful tool for hybrid variety production in vegetable breeding, molecular marker verification, genetic mapping, and QTL analysis. They also increase the frequency of recessive traits in individuals (Srividya et al. 2023).

[1] Department of Plant and Animal Production, Kumluca Vocational School, Akdeniz University, Antalya, Turkiye
Corresponding author: Esmanur DEMİREL
E mail.: esmanurdemirel@akdeniz.edu.tr

There are several factors limiting the effective use of haploids in vegetable breeding. Various limitations, particularly genotype unresponsiveness, lack of appropriate protocol implementation, poor donor plant health, epiphytic and endophytic infection, failure to identify suitable gamete stages, and inadequate culture conditions, negatively impact advances in vegetable breeding (Hale et al. 2022, Cassells 2012). This chapter will systematically review the genetic, physiological, and microbial factors that limit haploid embryo and plant production in vegetable species. Tissue culture and plant protection-based solutions to mitigate these limitations will be discussed within a holistic framework. This will provide a methodological roadmap for phytosanitary safety and high-success haploid production strategies for both researchers and practitioners.

2. Vegetable Haploid Production Techniques

Methods used for haploid production in vegetables are primarily based on culturing gametic tissues or inducing embryo formation through parthenogenetic means (Thakur et al. 2024). The most common of these techniques are androgenesis (anther/microspore culture), gynogenesis (ovule/ovary culture), and parthenogenesis (Figure 1). The aim of the parthenogenesis method is to stimulate the egg cell, using various triggers, as if fertilization had occurred, and initiate embryonic division. These trigger factors include various chemical and hormone applications, temperature shocks, and pollination with pollen sterilized by X and gamma rays (Irradiated Pollen Technique) (Kurtar et al. 2017). This encourages embryo and plant formation from the egg cell with n chromosomes. In cucurbit species (melon, cucumber, watermelon, squash, etc.), irradiated pollen techniques in particular give a highly positive response (Bagheri et al. 2021, Salehian et al. 2023, Kurtar and Seymen 2021, Taşkın et al. 2013).

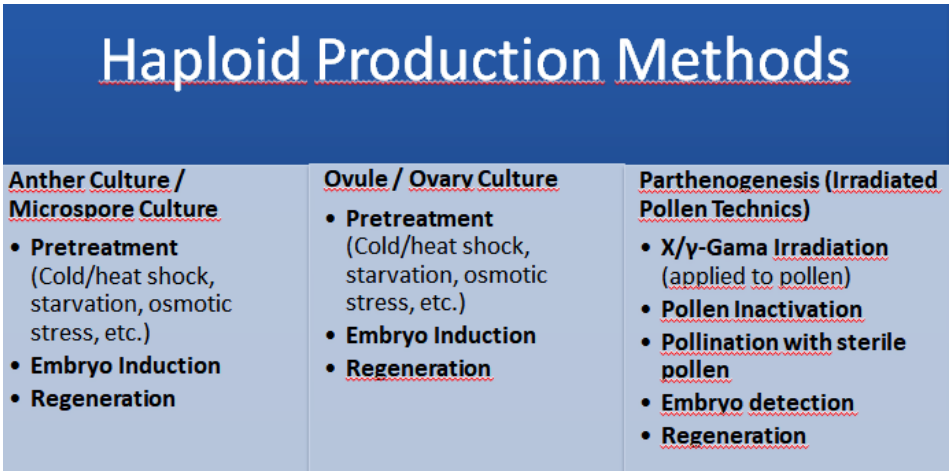

Figure 1. Overview of haploid production methods in vegetable crops.

The gynogenesis technique aims to produce haploid embryos and plants by culturing female gametes in a laboratory environment. This technique is performed either by directly culturing the ovary (ovary culture) or by inducing ovules separated from the ovary to develop into plants (ovule culture). Studies have yielded results, particularly on cucumber, onion, squash, sugar beet, and melon (Demirel and Onus 2021, Deng et al. 2020, Khan et al. 2020, Gürel et al. 2021, Zou et al. 2018, Ermolaev and Domblides 2022). The androgenesis method, on the other hand, involves directly transferring anthers containing microspores capable of developing into embryos to a suitable nutrient medium (anther culture) or culturing microspores isolated from the anthers in liquid nutrient medium (microspore culture). This method has been quite successful in species such as pepper, eggplant, broccoli, cabbage, and carrot. (Supena 2021, Calabuig et al. 2021, Qin et al. 2015, Pilih et al. 2018, Palacios and Sagui-Simarro 2021, Kiszczak et al. 2018)

These three basic methods are critical for haploid production in vegetable breeding; however, their widespread adoption has been limited due to genotype dependence, low regeneration rates, and technical challenges (Dong et al. 2016, Seguí-Simarro 2011). Therefore, correctly identifying these limiting factors and transforming the developed protocols into a sustainable structure will bring success in this field.

3. Limitations in Vegetable Haploid Embryo and Plant Production

According to studies, genotype is reported to be one of the most significant limiting factors in embryo formation in vegetable haploid studies (Hale et al. 2022). An effective protocol used for one species does not yield the same

response across all genotypes of the species. This poses a serious obstacle to the widespread application of haploid technologies, particularly in vegetables such as tomato, pepper, and cucumber (Seguí-Simarro 2011, Dong et al. 2016). Genotype dependence is a factor that limits not only embryo induction rates but also the regeneration capacity of the resulting embryos (Zhao et al. 2022).

Stress has been shown to stimulate the haploid mechanism in gamete cells. Studies have shown that osmotic stress (mannitol/sorbitol), starvation/carbon source modulation (sucrose/maltose levels), and pH optimization stimulate embryo formation (Sohrabi et al. 2021, Islam, S., and Tuteja 2012). Conversely, stress conditions (sudden temperature changes, high osmotic pressure, nutrient contents) can also lead to the accumulation of ethylene and reactive oxygen species (ROS) in cells. ROS and ethylene negatively affect haploid embryo formation. Ethylene, in particular, reduces regeneration and directs cells toward callus formation (Neves et al. 2021, Chandra-kuntal 2022). Some studies have reported that the addition of ethylene inhibitors (AgNO3, activated charcoal, etc.) to nutrient media promotes embryo formation and plant regeneration. It has been reported that ROS enzymes, which cause increased oxidative stress, can be controlled by antioxidants (glutathione, ascorbic acid, etc.) (Ozougwu 2016).

The type and dose of plant growth regulators added to the nutrient medium is also one of the most important determining factors in the haploid formation mechanism. It has been reported that exposure to auxin for a certain period in the induction medium for embryo formation triggers embryo formation (Smit and Weijers 2015). It is then recommended that the regeneration medium be cytokinin-dominated. Among the auxin-group hormones, IAA, IBA, NAA, and 2,4-D are the preferred hormones. The cytokinin-group hormones used in this field are BA, BAP, Kinetin, and Zeatin. Additionally, some complex additives added to the nutrient medium can function as hormones, such as coconut water and casein hydrolysate (Khierallah and Hussein 2013).

Recent studies have also shown that genotype effects are primarily at the genetic level. (Dwivedi et al. 2015). It is thought that the presence of these genes in vivo may also enable the formation of haploid lines. Studies have reported that the presence of the DMP, CENH3, and Baby BOOM genes, in particular, will reduce tissue culture dependency (Shen and Zhao 2023). Furthermore, identifying these genes at the molecular level could allow for gene editing and transfer of genes to desired individuals, potentially increasing haploid formation levels (Zhao et al. 2022). In addition to improving the genetics of the donor plant, the plant's growing conditions and the stress factors it is exposed to have been shown to be important (Thakur et al. 2024). It has been observed that factors that may negatively affect the formation of embryos

from gamete cells, such as temperature, humidity, and nutrient deficiencies, have negative effects on the development of embryos from gamete cells (Demirel 2022).

Research suggests that these differences can be reduced through a number of procedures. Selecting the flower bud at the appropriate gamete stage has been shown to increase embryo formation frequency. For example, for anther/microspore, the gamete should be at the mid- to late single-nucleus stage; It has been reported that the appropriate stage can be associated with bud size/anther color according to genotypes (Sahana et al. 2024, Prem et al. 2012). However, cold/hot (4-10 °C/32-35 °C) pre-heat treatments applied to gamete cells in ovule/ovarium cultures and anther/microspore cultures can reduce the genotype effect (Durna et al. 2025, Diao 2009). Considering the light requirements of the donor plant, it has also been reported that studies on light intensity and wavelength during the cultivation phase or as a pre-treatment after culturing trigger embryo induction (Rivas-Sendra et al. 2020, Han et al. 2025).

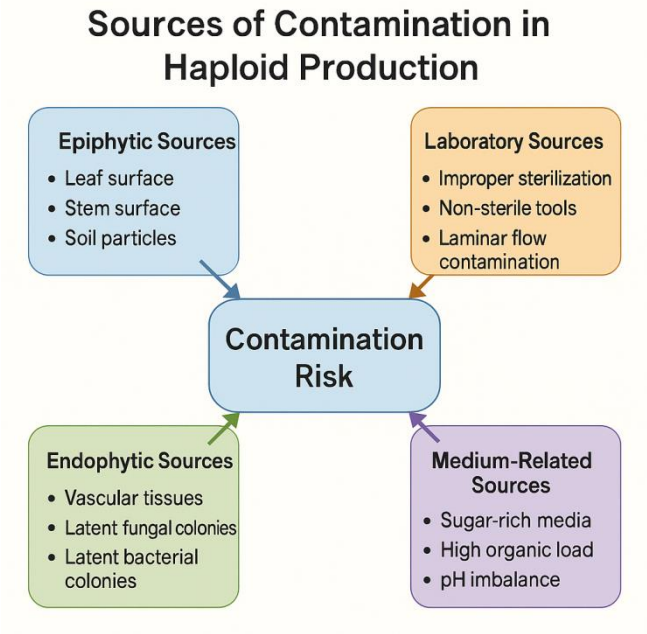

Figure 2. Sources of contamination in haploid production. This schematic summarizes the major contamination pathways encountered during *in vitro* haploid production, including epiphytic, endophytic, laboratory, and medium-related sources, each contributing to increased microbial load and reduced embryogenic success. This schematic representation has been prepared as an illustrative example based on previously published studies (Ali vd. 2024, Yendyo ve Pandey 2018, Yadav vd. 2022)

In addition to factors that can reveal genotype effects, there are also limiting factors that can arise during or after cultivation. One of the most significant problems researchers face is contamination (Figure 2). Plant tissues harbor epiphytic (outer surface) and endophytic (inner surface) microorganisms. These can have either harmless or beneficial effects on plants in nature (Rahayu et al. 2021). However, when ideal growth conditions such as high humidity, sugar, and organic matter are provided *in vitro*, they proliferate rapidly and suppress plant cell proliferation, preventing differentiation and causing contamination. A sterilization protocol is primarily applied to eliminate epiphytic microorganisms. Different methods, chemicals, and doses can be effective depending on the plant species and variety. The most commonly used chemicals are ethanol, sodium hypochlorite, and hydrogen peroxide. In addition to surface sterilization, sterilization of the culture environment and laboratory environment is also crucial. Surface sterilization of all materials used during cultivation should be performed in autoclaves or ovens. Cultivation should be carried out in laminar flow HEPA-filtered cabinets. It is crucial for researchers to pay attention to clothing, clothing, and hand hygiene (Tripathi et al. 2025). Despite careful implementation of all sterilization protocols, contamination problems may still occur due to endophytic microorganisms. These microorganisms may initially remain latent and later emerge (Volk et al. 2022). Therefore, the haploid production process is highly dependent not only on cultural sterilization practices but also on the plant protection program of the donor plant under field/greenhouse conditions. The phytosanitary status of the starting material is one of the most fundamental factors determining whether embryo development will continue. It is crucial to cultivate donor plants in healthy conditions and to manage disease and pest control effectively. In terms of plant protection, the use of fungicides (e.g., propamocarb, mancozeb) and bactericides on donor plants before *in vitro* culture, along with cultivation in sterile media (e.g., peat-perlite mixtures), is effective in reducing endophytic populations (Yadav et al. 2022). Chemical methods are not the only methods used for endophytic control of donor plants. The use of beneficial microorganisms (e.g., PGPR species such as *Bacillus subtilis* and *Pseudomonas fluorescens*) or competitive fungi (*Trichoderma harzianum*) has been shown to suppress the colonization of harmful endophytes (Ali et al. 2024, Yendyo and Pandey 2018) (Table 1). The integrated use of both chemical and biological control methods can be beneficial in achieving successful results in this regard. Under laboratory conditions, antibiotics (cefotaxime, streptomycin, rifampicin, etc.) and antifungals (nystatin, amphotericin B, Benomyl, etc.) can be added to nutrient media, taking into account the harmful effects of endophytic microorganisms

under all circumstances (Asif et al. 2013, Magbalot-Fernandez et al. 2024). Furthermore, pretreatment heat treatments or prolonged storage (cryopreservation) of plant tissues, cells, and embryos at low temperatures (usually liquid nitrogen –196 °C) can reduce endophytic activity (Volk et al. 2022). In addition, methods such as photoperiod (e.g., 16 h light/8 h darkness), low sugar content nutrient medium, and use of activated charcoal in the nutrient medium that will keep plant metabolism in balance are effective in suppressing endophytic microorganisms (Egorova et al. 2021, Armas et al. 2017, Islam and Zobayed 2000).

Light intensity, light quality, and photoperiod of the growth medium in which cultured explants develop can be considered the most important environmental factors that direct morphological, physiological, and biochemical development in vegetable tissue cultures (Liu et al. 2022, Paradiso and Proietti 2022). The maintenance of photosynthetic activity in plant tissues, chloroplast formation, regulation of hormone balance, and the direction of embryogenesis–organogenesis processes are largely dependent on the intensity, quality, and duration of light (Kulus and Woźny 2020). Low light intensity reduces differentiation, while excessive light intensity inhibits embryo development by inducing photooxidative stress in plant tissues (Fan et al. 2022). Therefore, a light intensity between 30–60 μmol m^{-2} s^{-1} is generally preferred in vegetable tissue cultures; this range can trigger both embryogenesis and embryo differentiation. (Hunter and Burritt 2004, Lercari et al. 1999). Another light-dependent factor is reported to be light quality. Light quality is measured in visible light wavelength ranges in tissue culture studies. Studies have shown that red light (approximately 660 nm) promotes shoot formation and cell differentiation, while blue light (450 nm) can limit callus development but increases chlorophyll synthesis and photosynthetic activity (Naznin et al. 2019). Light duration, i.e., photoperiod, is another factor that plays an important role in the physiological response of plant cells. While a 16-hour light/8-hour dark photoperiod is generally used in vegetable tissue cultures, complete darkness conditions during the embryo induction phase have been reported to increase the rate of embryogenesis, while controlled lighting during the regeneration phase supports plantlet development (Demirel and Onus 2021). Classic fluorescent lamps were previously used in growth chambers, but in recent years, LED (Light Emitting Diode) systems have become increasingly popular because they allow for the selection of specific wavelengths, offer higher energy efficiency, lower heat generation, and adaptability to specific plant species spectral selection. Explant viability, root-to-shoot ratio, and chlorophyll levels were found to be higher in tomato, eggplant, and pepper plants cultivated under

LED lighting compared to traditional light sources (Barceló-Muñoz et al. 2021). Furthermore, increased photosynthetic activity under favorable lighting conditions may indirectly reduce the likelihood of microbial contamination by reducing carbon source utilization in the culture medium.

Table 1. Sterilization and donor plant health management practices used to reduce contamination in haploid production.

Stage	Approach	Chemicals / Methods	Purpose	References
Donor Plant Management	Fungicide and bactericide applications; use of beneficial microbes	Propamocarb, mancozeb; *Bacillus subtilis*, *Pseudomonas fluorescens*; *Trichoderma harzianum*	Reduce epiphytic and endophytic microbial load before explant excision	Yadav et.al., 2022; Ali et.al., 2024; Asif 2013, Magbalot-Fernandez et.al. 2024
Surface Sterilization	Chemical sterilization of explants	Ethanol (70%), sodium hypochlorite, hydrogen peroxide,	Eliminate epiphytic microorganisms on plant surfaces	Kurtar and Balkaya 2010, Demirel and Onus 2021
Laboratory Sterility	Sterilization of tools, equipment, and workspace	Autoclave, dry oven, laminar-flow hood, aseptic handling	Prevent laboratory-derived contamination	Tripathi et.al. 2025
Medium Sterility and Additives	Use of antimicrobial agents and medium optimization	Cefotaxime, streptomycin, rifampicin; Nystatin, amphotericin B, benomyl; reduced sugar; activated charcoal	Suppress microbial growth during culture	Asif et.al. 2013, Magbalot-Fernandez et.al. 2024
Environmental Control	Optimization of light, temperature, and photoperiod	16 h light / 8 h dark photoperiod; stable temperature; LED lighting	Maintain explant health and reduce stress-induced contamination	Volk et.al. 2022, Egorova vd. 2021 Islam ve Zobayed 2000

4. Conclusion

Vegetable tissue culture double haploid studies accelerate the genetic breeding process. However, the healthy and successful management of this method depends on controlling several limitations. Chief among these are genotype dependence and donor plant and explant health. Genotype dependence is one of the most important limiting factors affecting embryo induction and regeneration rates. To reduce this dependence, significant advances have been made in determining the appropriate gamete stage, applying stress pretreatments (cold, heat, osmotic), optimizing hormone balance, and identifying and activating haploid induction genes (DMP, CENH3, BBM) at the molecular level.

Furthermore, losses due to contamination remain one of the most significant problems in vegetable tissue culture. Epiphytic and endophytic microorganisms proliferate rapidly *in vitro*, reducing success rates. In this regard, the combination of plant protection activities with biotechnology plays a crucial role. It appears that sterilization protocols applied in *in vitro* culture are insufficient, and the implementation of plant protection activities during donor plant cultivation is of great importance. Cultivating donor plants under appropriate conditions, pre-cultivation with fungicides/bactericides, and the use of sterile substrates and biological control agents (e.g., *Trichoderma harzianum*, *Bacillus subtilis*, *Pseudomonas fluorescens*) contribute to reducing endophyte populations and increasing sterility (Yendyo and Pandey 2018). Under laboratory conditions, antimicrobial additives, activated carbon, low sugar concentrations, and correct light and photoperiod balance are supportive measures that suppress microbial growth.

Consequently, the sustainable implementation of haploid production in plant tissue culture depends not only on comprehensive management at the genotype level, but also on integrated management of plant protection, culture medium composition, and environmental conditions. This integrated approach can produce materials with high genetic purity while also providing healthy growing material free from microbial risks. It is anticipated that this field, where genotype-based breeding and plant protection biotechnology intersect in the future, will offer sustainable solutions for vegetable production in terms of both economics and biosecurity. Therefore, in the design of haploid production protocols, considering the tissue culture phase and plant protection strategies that directly impact donor plant health is critical to the sustainability of the method.

5. References

Ali, M., Ahmed, T., Ibrahim, E., Rizwan, M., Chong, K., & Yong, J. (2024). A review on mechanisms and prospects of endophytic bacteria in biocontrol of plant pathogenic fungi and their plant growth-promoting activities. *Heliyon*, 10. https://doi.org/10.1016/j.heliyon.2024.e31573.

Armas, I., Pogrebnyak, N., & Raskin, I. (2017). A rapid and efficient *in vitro* regeneration system for lettuce (Lactuca sativa L.). *Plant Methods*, 13. https://doi.org/10.1186/s13007-017-0208-0.

Asif, M., Eudes, F., Randhawa, H., Amundsen, E., Yanke, J., & Spaner, D. (2013). Cefotaxime prevents microbial contamination and improves microspore embryogenesis in wheat and triticale. *Plant Cell Reports*, 32, 1637-1646. https://doi.org/10.1007/s00299-013-1476-4.

Barceló-Muñoz, A., Barceló-Muñoz, M., & Gago-Calderón, A. (2021). Effect of LED Lighting on Physical Environment and Microenvironment on *In Vitro* Plant Growth and Morphogenesis: The Need to Standardize Lighting Conditions and Their Description. *Plants*, 11. https://doi.org/10.3390/plants11010060.

Calabuig-Serna, A., Porcel, R., Corral-Martínez, P., & Seguí-Simarro, J. (2021). Anther and Isolated Microspore Culture in Eggplant (Solanum melongena L.).. *Methods in molecular biology*, 2288, 235-250 . https://doi.org/10.1007/978-1-0716-1335-1_14.

Cassells, A. (2012). Pathogen and biological contamination management in plant tissue culture: phytopathogens, vitro pathogens, and vitro pests.. *Methods in molecular biology*, 877, 57-80 . https://doi.org/10.1007/978-1-61779-818-4_6.

Chandra-kuntal, K. (2022). Ethylene and ROS crosstalk in plant developmental processes. Ethylene in plant biology, 125-177.

Demirel E. (2022) The Effect of the Donor Plant's Age And Season Effect on Haploid Embryo And Plant Formation in Cucumber (Cucumis sativus L.) via Gynogenesis, PhD Thesis in Department of Horticulture, Akdeniz University Institute of Science

Demirel, E., & Onus, A. N. (2021). Obtaining haploid embryo and plant by gynogenesis in some cucumber (Cucumis sativus L.) cultivars and types. International Journal of Agricultural and Wildlife Sciences, 7(3), 360-367. https://doi.org/10.24180/ijaws.969741

Deng, Y., Tang, B., Zhou, X., Fu, W., Tao, L., Zhang, L., & Chen, J. (2020). Direct regeneration of haploid or doubled haploid plantlets in cucumber (Cucumis sativus L.) through ovary culture. *Plant Cell, Tissue and Organ*

Culture (PCTOC), 142, 253 - 268. https://doi.org/10.1007/s11240-020-01839-w.

Diao, W., Jia, Y., Song, H., Zhang, X., Lou, Q., & Chen, J. (2009). Efficient embryo induction in cucumber ovary culture and homozygous identification of the regenetants using SSR markers. *Scientia Horticulturae*, 119, 246-251. https://doi.org/10.1016/j.scienta.2008.08.016.

Dong, Y., Zhao, W., Li, X., Liu, X., Gao, N., Huang, J., Wang, W., Xu, X., & Tang, Z. (2016). Androgenesis, gynogenesis, and parthenogenesis haploids in cucurbit species. *Plant Cell Reports*, 35, 1991 - 2019. https://doi.org/10.1007/s00299-016-2018-7.

Durna, P., Geboloğlu, N., Polat, E., & Kurtar, E. (2025). Effect of Genotype, Cold Pre-Treatment, Incubation Conditions and Media on Embryo and Plantlet Formation in Pepper Anther Culture. *Akademik Ziraat Dergisi*. https://doi.org/10.29278/azd.1662151.

Dwivedi, S., Britt, A., Tripathi, L., Sharma, S., Upadhyaya, H., & Ortiz, R. (2015). Haploids: Constraints and opportunities in plant breeding.. *Biotechnology advances*, 33 6 Pt 1, 812-29 . https://doi.org/10.1016/j.biotechadv.2015.07.001.

Egorova, K., Sinyavina, N., Artemyeva, A., Kocherina, N., & Chesnokov, Y. (2021). QTL Analysis of the Content of Some Bioactive Compounds in Brassica rapa L. Grown under Light Culture Conditions. *Horticulturae*. https://doi.org/10.3390/horticulturae7120583.

Ermolaev, A., & Domblides, E. (2022). Optimization of steps in the technology of obtaining doubled haploids of summer squash (Cucurbita pepo L.) in the culture of unpollinated ovules *in vitro*. *Vegetable crops of Russia*. https://doi.org/10.18619/2072-9146-2022-5-5-14.

Fan, C., Manivannan, A., & Wei, H. (2022). Light Quality-Mediated Influence of Morphogenesis in Micropropagated Horticultural Crops: A Comprehensive Overview. BioMed Research International, 2022(1), 4615079.

Gurel, S., Pazuki, A., Aflaki, F., & Gurel, E. (2021). Production of Doubled Haploid Sugar Beet (Beta vulgaris L.) Plants Through Gynogenesis.. *Methods in molecular biology*, 2289, 313-323 . https://doi.org/10.1007/978-1-0716-1331-3_21.

Hale, B., Ferrie, A., Chellamma, S., Samuel, J., & Phillips, G. (2022). Androgenesis-Based Doubled Haploidy: Past, Present, and Future

Perspectives. *Frontiers in Plant Science*, 12. https://doi.org/10.3389/fpls.2021.751230.

Han, J., Li, Y., Zhao, Y., Sun, Y., Li, Y., & Peng, Z. (2025). How does light regulate plant regeneration?. *Frontiers in Plant Science*, 15. https://doi.org/10.3389/fpls.2024.1474431.

Hunter, D. C., & Burritt, D. J. (2004). Light quality influences adventitious shoot production from cotyledon explants of lettuce (Lactuca sativa L.). *In Vitro* Cellular & Developmental Biology-Plant, 40(2), 215-220.

Islam, N., & Zobayed, S. M. (2000). Microbial contamination under photoautotrophic culture system. Transplant production in the 21st century. Kluwer Academic Publishers. Netherland, 284-288.

Islam, S., & Tuteja, N. (2012). Enhancement of androgenesis by abiotic stress and other pretreatments in major crop species.. *Plant science : an international journal of experimental plant biology*, 182, 134-44 . https://doi.org/10.1016/j.plantsci.2011.10.001.

Khan, P., Vijayalakshmi, G., Raja, M., Naik, M., Germanà, M., & Terry, R. (2020). Doubled haploid production in onion (Allium cepa L.): from gynogenesis to chromosome doubling. *Plant Cell, Tissue and Organ Culture (PCTOC)*, 142, 1 - 22. https://doi.org/10.1007/s11240-020-01831-4.

Khierallah, H. S., & Hussein, N. H. (2013). The role of coconut water and casein hydrolysate in somatic embryogenesis of date palm and genetic stability detection using RAPD markers. *Res Biotechnol*, *4*(3), 20-28.

Kiszczak, W., Kowalska, U., Burian, M., & Górecka, K. (2018). Induced androgenesis as a biotechnology method for obtaining DH plants in Daucus carota L.. *The Journal of Horticultural Science and Biotechnology*, 93, 625 - 633. https://doi.org/10.1080/14620316.2018.1431058.

Kulus, D., & Woźny, A. (2020). Influence of light conditions on the morphogenetic and biochemical response of selected ornamental plant species under *in vitro* conditions: A mini-review. BioTechnologia. Journal of Biotechnology Computational Biology and Bionanotechnology, 101(1), 75-83.

Kurtar, E. S., & Balkaya, A. (2010). Production of *in vitro* haploid plants from in situ induced haploid embryos in winter squash (Cucurbita maxima Duchesne ex Lam.) via irradiated pollen. *Plant Cell, Tissue and Organ Culture (PCTOC)*, *102*(3), 267-277.

Kurtar, E. S., Balkaya, A., Göçmen, M., & Karaağaç, O. (2017). Hıyara (Cucumis sativus L.) Anaç Olabilecek Ümitvar Kabak (Cucurbita spp.) Genotiplerinde Işınlanmış Polen Tekniği İle Dihaploidizasyon. Selcuk Journal of Agriculture & Food Sciences/Selcuk Tarim ve Gida Bilimleri Dergisi, 31(1).

Kurtar, E., & Seymen, M. (2021). Induction of Parthenogenesis by Irradiated Pollen in Cucurbita Species.. *Methods in molecular biology*, 2289, 135-145 . https://doi.org/10.1007/978-1-0716-1331-3_9.

Lercari, B., Moscatelli, S., Ghirardi, E., Niceforo, R., & Bertram, L. (1999). Photomorphogenic control of shoot regeneration from etiolated and light-grown hypocotyls of tomato. Plant Science, 140(1), 53-62.

Liu, K., Gao, M., Jiang, H., Ou, S., Li, X., He, R., ... & Liu, H. (2022). Light intensity and photoperiod affect growth and nutritional quality of brassica microgreens. Molecules, 27(3), 883.

Magbalot-Fernandez, A., Dalumpines, A., Ubaub, L., Cobrado, J., Matuginas, J., Zandi, P., & Basu, S. (2024). *In Vıtro* Test Of Dıfferent Antıbıotıcs On Bacterıal And Fungal Contamınants Of Tıssue-Cultured 'Saba' Banana (Musa balbisiana). *International Journal on Agricultural Sciences*. https://doi.org/10.53390/ijas.2024.15107.

Naznin, M. T., Lefsrud, M., Azad, M. O. K., & Park, C. H. (2019). Effect of different combinations of red and blue LED light on growth characteristics and pigment content of *in vitro* tomato plantlets. Agriculture, 9(9), 196.

Neumann, K., Kumar, A., & Imani, J. (2020). Haploid Techniques. *Plant Cell and Tissue Culture – A Tool in Biotechnology*. https://doi.org/10.1007/978-3-030-49098-0_6.

Neves, M., Correia, S., Cavaleiro, C., & Canhoto, J. (2021). Modulation of Organogenesis and Somatic Embryogenesis by Ethylene: An Overview. *Plants*, 10. https://doi.org/10.3390/plants10061208.

Ozougwu, J. C. (2016). The role of reactive oxygen species and antioxidants in oxidative stress. *International Journal of Research*, *1*(8), 1-8.

Palacios, M., & Seguí-Simarro, J. (2021). Anther Culture in Sweet Pepper (Capsicum annuum L.).. *Methods in molecular biology*, 2288, 279-291 . https://doi.org/10.1007/978-1-0716-1335-1_17.

Paradiso, R., & Proietti, S. (2022). Light-quality manipulation to control plant growth and photomorphogenesis in greenhouse horticulture: The state of the art and the opportunities of modern LED systems. Journal of Plant Growth Regulation, 41(2), 742-780.

Pilih, K., Potokar, U., & Bohanec, B. (2018). Improvements of doubled haploid production protocol for white cabbage (Brassica oleracea var. capitata L.). *Folia Horticulturae*, 30, 57 - 66. https://doi.org/10.2478/fhort-2018-0006.

Prem, D., Solís, M., Bárány, I., Rodríguez-Sanz, H., Risueño, M., & Testillano, P. (2012). A new microspore embryogenesis system under low temperature which mimics zygotic embryogenesis initials, expresses auxin and efficiently regenerates doubled-haploid plants in Brassica napus. *BMC Plant Biology*, 12, 127 - 127. https://doi.org/10.1186/1471-2229-12-127.

Qin, Y., Huang, Y., Pulli, S., & Guo, Y. (2015). Comparison of anther and microspore culture in androgenic embryogenesis and regeneration of broccoli (Brassica oleracea L. var. italica P.). *African Journal of Biotechnology*, 14, 2910-2916. https://doi.org/10.5897/AJB12.333.

Qu, Y., Fernie, A., Liu, J., & Yan, J. (2024). Doubled Haploid Technology and Synthetic Apomixis: Recent Advances and Applications in Future Crop Breeding.. *Molecular plant*. https://doi.org/10.1016/j.molp.2024.06.005.

Rahayu, R., Ramadhani, I., Masrukhin, M., Riastiwi, I., Prawestri, A., & Yuliani, Y. (2021). CONFIRMATION OF ENDOPHYTIC MICROBES CAUSING CONTAMINATION IN WATER SPINACH (Ipomoea aquatica Forssk.) TISSUE CULTURE. , 7, 234-249. https://doi.org/10.29122/jbbi.v7i2.4381.

Ren, J., Wu, P., Trampe, B., Tian, X., Lübberstedt, T., & Chen, S. (2017). Novel technologies in doubled haploid line development. *Plant Biotechnology Journal*, 15, 1361 - 1370. https://doi.org/10.1111/pbi.12805.

Rivas-Sendra, A., Corral-Martínez, P., Camacho-Fernández, C., Porcel, R., & Seguí-Simarro, J. (2020). Effects of growth conditions of donor plants and *in vitro* culture environment in the viability and the embryogenic response of microspores of different eggplant genotypes. *Euphytica*, 216. https://doi.org/10.1007/s10681-020-02709-4.

Sahana, K., Srivastava, A., Khar, A., Jain, N., Jain, P., Bharti, H., Harun, M., & Mangal, M. (2024). Anther-derived microspore embryogenesis in pepper hybrids orobelle and Bomby. *Botanical Studies*, 65. https://doi.org/10.1186/s40529-023-00408-6.

Salehian, H., Shahnazi, S., & Nazari, M. (2023). Production of doubled haploid plants in cucumber (Cucumis sativus L.) via parthenogenesis. *In Vitro*

Cellular & Developmental Biology - Plant, 59, 467-474. https://doi.org/10.1007/s11627-023-10368-y.

Seguí-Simarro, J., Corral-Martínez, P., Parra-Vega, V., & González-García, B. (2011). Androgenesis in recalcitrant solanaceous crops. *Plant Cell Reports*, 30, 765-778. https://doi.org/10.1007/s00299-010-0984-8.

Shen, K., Qu, M., & Zhao, P. (2023). The Roads to Haploid Embryogenesis. *Plants*, 12. https://doi.org/10.3390/plants12020243.

Singh, H., Sekhon, B., Kumar, P., Dhall, R., Devi, R., Dhillon, T., Sharma, S., Khar, A., Yadav, R., Tomar, B., Ntanasi, T., Sabatino, L., & Ntatsi, G. (2023). Genetic Mechanisms for Hybrid Breeding in Vegetable Crops. *Plants*, 12.

Smit, M. E., & Weijers, D. (2015). The role of auxin signaling in early embryo pattern formation. *Current opinion in plant biology*, *28*, 99-105.

Sohrabi, S., Abdollahi, M., Mirzaie-Asl, A., Koulaei, H., Aghaeezadeh, M., & Seguí-Simarro, J. (2021). A refined method for ovule culture in sugar beet (Beta vulgaris L.). Plant Cell, Tissue and Organ Culture (PCTOC), 146, 259 - 267. https://doi.org/10.1007/s11240-021-02065-8.

Srividya, A., Sandal, S., & Walia, P. (2023). Doubled Haploids in Crop Improvement: Unraveling Strategies, Advancements and Prospects for Enhanced Genetics. *International Journal of Plant & Soil Science*. https://doi.org/10.9734/ijpss/2023/v35i173215.

Supena, E. (2021). An Efficient Anther Culture on Double-Layered Media to Produce Doubled Haploid Plants of Pepper (Capsicum annuum).. *Methods in molecular biology*, 2288, 267-278 . https://doi.org/10.1007/978-1-0716-1335-1_16.

Taşkın, H., Yücel, N., Baktemur, G., Çömlekçioğlu, S., & Büyükalaca, S. (2013). Effects of different genotypes and gamma ray doses on haploidization with irradiated pollen technique in watermelon (Citrullus lanatus L.). *Canadian Journal of Plant Science*, 93, 1165 - 1168. https://doi.org/10.1139/CJPS2013-059.

Thakur, P., Kumari, N., Kumar, A., Sharma, P., & Chadha, S. (2024). Recent advances in development and utilization of double haploids (DHs) in economically important vegetable crops. *Plant Cell, Tissue and Organ Culture (PCTOC)*, *156*(1), 15.

Volk, G., Bonnart, R., De Oliveira, A., & Henk, A. (2022). Minimizing the deleterious effects of endophytes in plant shoot tip cryopreservation. *Applications in Plant Sciences*, 10. https://doi.org/10.1002/aps3.11489.

Yadav, U., Bano, N., Bag, S., Srivastava, S., & Singh, P. (2022). An Insight into the Endophytic Bacterial Community of Tomato after Spray Application of Propiconazole and Bacillus subtilis Strain NBRI-W9. *Microbiology Spectrum*, 10. https://doi.org/10.1128/spectrum.01186-22.

Yendyo, S., C, R., & Pandey, B. (2018). Evaluation of Trichoderma spp., Pseudomonas fluorescens and Bacillus subtilis for biological control of Ralstonia wilt of tomato. *F1000Research*, 6. https://doi.org/10.12688/f1000research.12448.3.

Zhao, Y., Zheng, W., Li, J., Qi, X., Feng, H., & Zhang, Y. (2022). Effects of genotype and sodium p-nitrophenolate on microspore embryogenesis and plant regeneration in broccoli (Brassica oleracea L. var. italica). *Scientia Horticulturae*. https://doi.org/10.1016/j.scienta.2021.110711.

Zou, T., Su, H., Wu, Q., & Sun, X. (2018). Haploid induction via unfertilized ovary culture in watermelon. *Plant Cell, Tissue and Organ Culture (PCTOC)*, 135, 179-187. https://doi.org/10.1007/s11240-018-1454-1.

Chapter 5

Virus-Free Plant Production Using Meristem Culture in the Pepper (*Capsicum annuum* L.) Plant: Basic Principles, Applications and Current Approaches

Esmanur DEMİREL[1]

1. Introduction

Pepper (*Capsicum* spp.) is a warm-season horticultural crop and ranks among the most widely consumed vegetables worldwide. Owing to its broad agro-ecological adaptability, it is cultivated across numerous countries and contributes substantially to culinary diversity with its extensive varietal range. Global fresh pepper production reaches approximately 38 million tons, with China, Mexico and Türkiye standing as the leading producers (FAO, 2023). Peppers are also valued for their high content of biologically active compounds—such as carotenoids, phenolics, capsaicin and flavonoids—which confer immune-enhancing and anti-inflammatory properties (Carvalho Lemos et al., 2019).

For producers, peppers are a strategic vegetable variety because they offer a wide production period and export opportunities. It serves as an essential raw material for both fresh-market consumption (e.g., pungent types, bell peppers, capia, Charleston) and various processing industries, including paste, dried red pepper flakes and pickled products. In Türkiye, pepper cultivation takes place in both open-field and greenhouse systems during the summer, whereas winter production is limited to off-season cultivation conducted exclusively under greenhouse conditions. This continuous year-round production—particularly in the Mediterranean and Aegean regions—provides a competitive advantage by ensuring price stability and consistent market supply. Despite these advantages, several challenges adversely affect growers' production preferences.

Abiotic and biotic stress factors significantly limit production. Climate change–driven temperature extremes, improper irrigation and fertilization

[1] Department of Plant and Animal Production, Kumluca Vocational School, Akdeniz University, Antalya, Turkiye
Corresponding author: Esmanur DEMİREL
E mail.: esmanurdemirel@akdeniz.edu.tr

practices, soil-borne pathogens and widespread viral diseases represent major constraints in pepper cultivation (Lee et al., 2017; Bouras et al., 2024; Ojinaga et al., 2022; Zhang et al., 2019). These problems complicate sustainable pepper production, and viral diseases, in particular, contribute to poor production. Once infection occurs, growers often have no option but to uproot diseased plants and shift to alternative crops. Therefore, the use of virus-free and physiologically healthy seedlings and seeds constitutes the most critical step in effective viral disease management.

Breeding programs in pepper have increasingly prioritized the development of cultivars with resistance to major viral pathogens (Şavkan, 2024). However, considerable challenges remain, particularly in the mapping and functional characterization of resistance genes, as well as in the efficient integration of these genes into commercial breeding pipelines (Siddique et al., 2022). Developing new varieties using conventional breeding programs has become a significant challenge, particularly in combating viral diseases such as Tomato spotted wilt virus (TSWV), Potato virus Y (PVY), Cucumber mosaic virus (CMV), Tobacco mosaic virus (TMV), and Pepper mild mottle virus (PMMoV), which are the most common in pepper (Şavkan 2024, Ojinaga et al. 2022). These constraints collectively hinder the sustainable production of virus-free pepper crops.

Given these limitations, *in vitro* tissue culture technologies—particularly meristem culture—have emerged as powerful biotechnological alternatives for the elimination of viral pathogens and the production of healthy planting material. Transmission of viral agents within the plant occurs through transmission bundles (Kappagantu et al. 2020). Meristem tissues are the youngest, rapidly dividing tissues within the plant, and vascular bundles are not formed in these tissues (Myśkow et al. 2019). The virus-free nature of meristem tissue has made meristem culture a tool for obtaining rapid, reliable, and genetically sound plant material in situations where conventional breeding is inadequate. Virus-free plants produced by tissue culture are used both as parent material in breeding studies and as a means of establishing primary productive material during the seedling production process (Bhojwani and Dantu 2013). Eliminating viral inoculum sources in peppers produced with this technique is of strategic importance for preventing viral transmission. From this perspective, meristem culture not only ensures the production of healthy seedlings but also enables the creation of clean genotypes that can be used as starting material in breeding programs (Madhuri and Rajam 2012).

Research on meristem culture-based virus-free pepper plant production is quite limited in the literature. This demonstrates the applicability of this method

as an effective virus elimination strategy in pepper and offers significant potential for future research.

2. Meristem Tissue Characteristics and Principles of Culture

The effectiveness of meristem culture as a virus-elimination strategy in pepper is fundamentally attributed to the distinctive biological structure and physiological properties of meristematic tissues. Meristems are specialized tissues composed of undifferentiated cells capable of continuous division in plants (Xue et al. 2020). These tissues are situated within key growth regions—namely the apical, lateral, and intercalary meristems—and function as central sites for the generation of new cells and organs (Wang et al., 2018).

Meristematic cells are small, have thin cell walls, abundant cytoplasm, and a large nucleus. Because they have not yet undergone the differentiation process, the vascular bundles (xylem and phloem) in these regions are not yet fully developed (Chiatante et al. 2021).

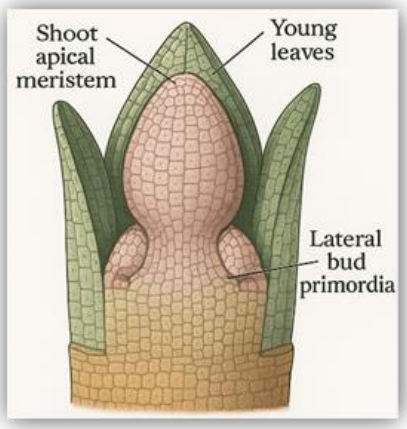

Figure 1

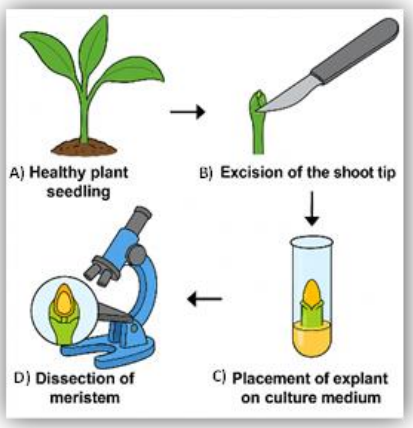

Figure 2

Figure 1. Structure of the shoot apical meristem. Diagram showing the shoot apical meristem with young leaves and lateral bud primordia. Figure 2. Steps of meristem isolation and culture.(A) Healthy seedling selection. (B) Shoot tip excision. (C) Meristem dissection under a microscope. (D) Placement of the isolated meristem onto culture medium.

Meristem tissue culture involves the aseptic excision of meristematic regions—typically the apical or lateral meristems, which constitute the primary growth points of the plant—and their subsequent propagation on a nutrient-enriched culture medium. This technique is widely recognized as an effective approach for generating genetically stable, virus-free, and pathogen-free plant

material (Lakhera et al., 2018; Grout, 1999). Meristem tissues are usually isolated under sterile conditions at 0.1-3 mm in size (Abide et al. 2022, Sasi and Bhat 2018).

For successful culture initiation, the explant is required to include the apical shoot tip accompanied by a few primordial leaf structures (Figure 1). Importantly, the excised tissue should lack differentiated vascular bundles, a characteristic essential for preventing systemic viral carriage (Grout, 1999). The isolated plant tissue is transferred to a nutrient medium containing the appropriate hormone combination. This medium stimulates shoot formation. The regenerated shoots are subsequently subcultured onto a secondary medium designed to induce root formation. Once sufficient rooting and seedling development have been achieved, the plantlets undergo acclimatization—a gradual adaptation process to greenhouse or soil environments (Saeed et al., 1997). This multistep procedure ensures the establishment of vigorous, stable, and virus-free plants suitable for further propagation or field use.

Studies conducted on various vegetable species have consistently demonstrated that the composition of the nutrient medium used in meristem culture is a critical determinant of regeneration success In eggplant, for instance, MS medium supplemented with 2.0 mg L^{-1} BAP was identified as the most suitable medium for meristem culture. Optimal shoot proliferation occurred on the combination of 2.0 mg L^{-1} BAP 1.0 mg L^{-1} NAA, whereas the highest rooting efficiency was achieved on medium containing 1.0 mg L^{-1} IBA. Plants regenerated through this protocol exhibited normal growth following acclimatization, confirming the method's effectiveness for producing virus-free plant material (Sharmin et al., 2008). Similarly, in bitter gourd, the most vigorous shoot development was obtained on MS medium supplemented with 1.0 mg L^{-1} BA + 0.1 mg L^{-1} IBA + 0.3 mg L^{-1} GA_3, while the best rooting response occurred in medium containing 0.5 mg L^{-1} IBA + 0.1 mg L^{-1} NAA (Huda & Sikdar, 2006). In squash (*Cucurbita pepo* L.), Pinky and Walley (1984) reported the highest shoot multiplication using meristem-tip culture on MS medium lacking 1 mg L^{-1} BA and auxin, with rooting achieved within 2–3 weeks on medium containing 8 mg L^{-1} IAA.

In pepper, apical meristem culture has been successfully applied to regenerate complete plants from Capsicum annuum L. cv. Bhivapuri, a cultivar susceptible to viral infection. Meristems approximately 0.8 mm in size produced 5–7 multiple shoots on liquid MS medium enriched with 2 mg L^{-1} BAP. These shoots subsequently rooted on agar-based medium containing 1 mg L^{-1} NAA, ultimately yielding healthy and fully developed plants (Madhuri & Rajam, 2012). In a study using shoot tips containing the apical meristem in

pepper, shoot tip explants from four different cultivars were cultured on MS medium supplemented with 4 mg/L BA + 1 mg/L IAA + 4 mg/L $AgNO_3$, and bud differentiation of over 85% was achieved. This system has been described as an effective *in vitro* propagation method for pepper due to its short regeneration time and high yield independent of genotype (Wen-Xuan 2004). Additionally, cotyledon, shoot-tip and hypocotyl explants obtained from 13-day-old seedlings produced multiple shoot buds within one month when cultured on MS media containing different combinations of NAA and BAP. The highest shoot regeneration rate was observed on medium containing 5 mg L^{-1} BAP, while rooting reached 70% on MS medium supplemented with 0.5 mg L^{-1} IAA (Ebida & Hu, 1993).

3. Dissemination of Plant Viruses Within the Plant System and Common Viral Agents in Pepper

Pepper is among the vegetable crops most severely impacted by viral diseases worldwide, with approximately 68 virus species reported to infect *Capsicum* spp. across different production regions (Kenyon et al., 2014). Among these, Tomato spotted wilt virus (TSWV), Cucumber mosaic virus (CMV), Pepper mild mottle virus (PMMoV), Tobacco mosaic virus (TMV), Potato virus Y (PVY) and Pepper veinal mottle virus (PVMV) are recognized as the most prevalent and economically damaging pathogens in commercial pepper cultivation (Choi, 2023; Ojinaga et al., 2022). Once introduced into plant tissues, these viruses move from infected cells to adjacent healthy cells primarily through plasmodesmata—microscopic intercellular channels that facilitate short-distance viral movement. For systemic infection, viruses subsequently gain access to the plant's vascular system, particularly the phloem, which serves as the principal conduit for long-distance transport within the host (Hipper et al., 2013). Through this pathway, viral particles disseminate efficiently throughout the plant, enabling colonization of new tissues and the establishment of persistent infection.

While the transmission of plant viruses via the phloem leads to the spread of infection throughout the plant system, the underdevelopment of this transport system in meristematic areas prevents the virus from reaching these areas (Bradamante et al. 2021). However, the high metabolic rate, rapid cell division, and the effectiveness of RNA silencing mechanisms in meristem tissues are additional biological factors that reduce virus replication (Schwach et al. 2005). Therefore, keeping meristems virus-free is not only an anatomical defense but also a physiological and molecular defense mechanism. This demonstrates the

biological importance of meristem-based regeneration in species susceptible to systemic viral infections, such as pepper.

Studies on tomato have demonstrated the effectiveness of meristem culture in eliminating multiple viral pathogens. In one investigation, virus-free and high-yielding plants were regenerated by culturing apical meristems measuring 0.3–0.5 mm on MS medium supplemented with GA_3 and IBA. DAS-ELISA analyses confirmed the complete elimination of Tomato mosaic virus (ToMV), Cucumber mosaic virus (CMV) and Tomato leaf curl virus (ToLCV); furthermore, seedlings raised from seeds of these regenerated plants exhibited no symptoms when grown under net-house conditions (Alam et al., 2004). Similarly, in tomato plants infected with Tomato spotted wilt virus (TSWV), culturing meristem tips of 0.2–0.4 mm resulted in the recovery of 100% virus-free plants, a finding validated through IC/RT-PCR. This study clearly demonstrated that TSWV can be effectively eradicated solely through meristem culture without the need for supplemental chemical or thermal treatments (AlKhazindar, 2015). In potato, research conducted on PVY-infected 'Binella' and 'Burren' cultivars showed that the highest percentage of virus-free plants was achieved using meristem tips approximately 100 µm in size, with virus elimination verified by DAS-ELISA. Moreover, integrating meristem culture with thermotherapy (37°C for 40 days), ribavirin chemotherapy (20 mg L^{-1}) and electrotherapy (15 mA for 10 min) increased the PVY eradication rate to as high as 93% (AlMaarri et al., 2012). Virus-free plants have also been successfully obtained in sweet pepper infected with TSWV and TMV. In this case, apical meristems measuring 0.4–0.8 mm were grafted *in vitro* onto healthy rootstock seedlings. The findings demonstrated that *in vitro* grafting, when combined with meristem culture, represents a highly effective strategy for eliminating viral agents in pepper (Katoh et al., 2004).

These studies demonstrate that meristem culture is an effective biotechnological tool for virus elimination in vegetable species. However, higher success rates can be achieved if the effectiveness of meristem culture is increased and supported by complementary methods.

4. Virus Elimination Methods Combined with Meristem Culture

Research on various plant-virus systems reveals that the success of meristem culture depends not only on the tissue source but also on the application conditions. Therefore, meristem culture has been developed as an integrated biotechnological method that provides more effective and sustained virus elimination by combining it with physical and chemical treatments such as thermotherapy, chemotherapy, or cryotherapy (Benke et al. 2023). Applying

these adjunctive techniques before meristem culture significantly increases the virus-free rate of the resulting plants by reducing virus activity (Figure 3).

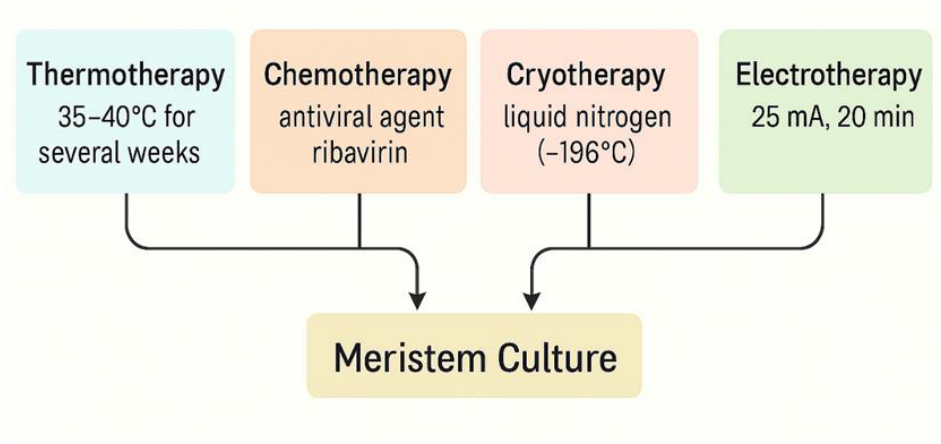

Figure 3. Integrated virus elimination approaches combined with meristem culture. Thermotherapy, chemotherapy, cryotherapy, and electrotherapy can be applied individually or in combination prior to meristem culture to enhance virus eradication efficiency. This schematic representation has been prepared as an illustrative example based on previously published studies (Benke et.al. 2023. Gogile et.al. 2024, Magyar-Tábori et.al. 2021, Nerway et.al. 2020)

4.1. Meristem Culture Combined with Thermotherapy Technique

Thermotherapy is a method that suppresses viral proliferation and maintains meristem tissue viability by heating plant tissues to 35–40°C for specific periods. This practice, when combined with meristem culture, is particularly effective in eliminating phloem-borne viruses (Gogile et al. 2024; Lassois et al. 2012).

A study in potato *(Solanum tuberosum)* examined the effectiveness of meristem culture and 25-day thermotherapy (27–35°C) treatments in virus elimination in potatoes infected with PVY and showed that 35°C significantly increased the virus clearance rate while decreasing meristem viability. Culturing apical meristems measuring 0.2–0.5 mm during the meristem culture phase following the thermoperiod enabled the separation of tissues damaged by heat stress, enabling the regeneration of healthy shoots. At the end of this process, virus clearance success increased up to 43.79% at 35°C, demonstrating a direct, decisive relationship between thermoperiod and meristem regeneration (Ali et al. 2013). Pepino plants (*Solanum muricatum*) were kept under high and continuous thermoperiod conditions with only a 2°C difference (38°C during

the day and 36°C at night) for 40–100 days, and then 0.1–1 mm meristem culture was applied; however, although thermotherapy exceeding 40 days reduced meristem viability, it did not increase ToMV elimination. Consequently, virus clearance success was quite low (4.6%), and meristem regeneration decreased significantly with increasing thermoperiod duration in plants exposed to continuous heat stress due to minimal day-night temperature differences (Szyndel et al. 2008). Similarly, in studies conducted on garlic, it was reported that protocols combined with meristem culture after 30 days of thermotherapy at 37°C significantly increased the clearance, especially in Carlavirus and Potyvirus species; however, elimination was limited due to the high heat resistance of Allexiviruses (Benke et al. 2023).

4.2. Meristem Culture Combined with Chemotherapy Technique

Chemotherapy involves the suppression of viral replication through the incorporation of antiviral compounds—such as ribavirin or acyclovir—into tissue culture media. These nucleoside analogues inhibit viral RNA synthesis, thereby preventing the completion of replication cycles. When used in conjunction with meristem culture, they offer a significant advantage, particularly in eliminating viral agents that exhibit systemic transmission. For example, in sweet potato, the application of 20–30 mg L^{-1} ribavirin increased the elimination rate of Sweet potato feathery mottle virus (SPFMV) to 100%; however, plant viability declined markedly as the concentration increased. Furthermore, the fact that meristem culture alone achieved virus-free survival in all shoots demonstrated the value of chemotherapy as a complementary tool, particularly in cultivars with high viral loads. In another study, Benke et al. (2023) applied ribavirin *in vitro* by supplementing the culture medium with the antiviral compound. When combined with thermotherapy (37°C for 30 days) and meristem culture, this approach resulted in 100% elimination of Carlavirus species (GCLV and SLV) and Potyvirus (OYDV). In the Allexivirus group, ribavirin failed to achieve complete clearance but significantly suppressed replication by reducing viral load by 7.764-fold. However, because ribavirin causes phytotoxicity at high doses, reducing shoot viability, the study emphasized the criticality of dose-toxicity balance for protocol success.

4.3. Meristem Culture Combined with Cryotherapy

The combined use of meristem culture and cryotherapy in virus elimination relies on the high survival capacity of small meristematic cells in particular. When shoot tips are exposed to liquid nitrogen at –196°C, large and highly vacuolated cells undergo lethal injury due to the formation of intracellular ice

crystals, whereas the densely cytoplasmic cells located in the apical dome and young leaf primordia remain viable (Magyar-Tábori et al., 2021). For example, in a study conducted for virus removal in apple rootstocks, thermotherapy, cryotherapy, and a combination of both were applied to plant apical shoot tips. The highest success rate was reported with the combined application of thermotherapy and cryotherapy (Bettoni et al. 2022).

4.4. Meristem Culture Combined with Electrotherapy

Electrotherapy aims to inactivate virus particles by applying electric current to plant tissues. DMV-infected Dahlia stem segments were exposed to 15, 25, and 35 mA current for 10–20 minutes, demonstrating that current intensity and duration were decisive for virus elimination. The highest elimination rate (85%) was achieved by applying 35 mA for 20 minutes, followed by 25 mA for 20 minutes, which produced a 70% clearance rate. These findings indicate that electrotherapy is an effective method with appropriate current-duration combinations, but the risk of phytotoxicity at high current levels should be carefully managed (Nerway et al. 2020). Similarly, a study conducted on apricot cultures reported that the virus elimination rate ranged from 40–60% when electrotherapy was applied at 40 mA. This demonstrated that while effective, the method was less successful than thermotherapy, chemotherapy, and cryotherapy (Khafri et al. 2024).

The integration of supportive techniques such as thermotherapy, chemotherapy and cryotherapy with meristem culture facilitates the removal of viral particles from infected tissues while minimizing the likelihood of reinfection. However, studies in the literature specifically on the application of these combined methods to pepper (*Capsicum annuum* L.) are quite limited. This suggests that the potential of meristem culture in controlling viral diseases in pepper has not yet been fully evaluated.

5. Conclusion

In pepper (*Capsicum annuum* L.) production, viral diseases constitute one of the most critical biotic stress factors, causing substantial yield and quality losses and thereby threatening sustainable cultivation. The challenges faced by conventional breeding programs in developing resistant varieties make the provision of healthy seedling and rootstock material a strategic priority (Kenyon et al. 2014; Şavkan 2024; Ojinaga et al. 2022). In this context, meristem culture stands out as a powerful biotechnological method for eliminating systemically transmitted viruses due to the inadequate colonization of meristem tissues by

vascular bundles, high cell division rates, and the effectiveness of the RNA silencing mechanism (Schwach et al. 2005; Bradamante et al. 2021).

Studies on different vegetable cultivars have shown that, provided that the correct meristem size is selected, the nutrient medium composition is optimized, and the acclimatization phase is carefully planned, a high percentage of virus-free plants can be obtained by meristem culture. These healthy plants can be effectively used as starting material in breeding programs or as rootstock or seed sources in seedling production systems (Madhuri and Rajam 2012; Sharmin et al. 2008; Huda and Sikdar 2006). Moreover, integrating adjunctive treatments—including thermotherapy, chemotherapy, cryotherapy and electrotherapy—with meristem culture significantly enhances viral elimination rates, particularly for PVY, TSWV, ToMV and viruses belonging to the Potyvirus and Carlavirus groups. However, factors such as the temperature regime used, antiviral compound dose, freeze-thaw protocols with liquid nitrogen and electric current density become limiting factors on plant viability and regeneration capacity (Ali et al. 2013; Szyndel et al. 2008; AlMaarri et al. 2012; Benke et al. 2023; Nerway et al. 2020; Khafri et al. 2024).

Despite these advances, the number of studies directly examining integrated meristem-based virus elimination protocols in pepper remains remarkably limited. This indicates that the optimization of meristem culture and supplementary treatments for *Capsicum* species is far from complete. Consequently, there is a clear need for comprehensive experimental studies that systematically compare meristem size, culture medium composition, thermoperiod conditions, antiviral dosages, cryotherapy parameters and electrotherapy intensities across diverse pepper genotypes, supported by robust serological and molecular diagnostics. Such investigations will contribute substantially to securing virus-free and genetically stable propagation material for commercial seedling industries, while also facilitating the availability of clean, uniform starting material for resistance-oriented breeding programs. Collectively, these advances position meristem culture–based virus elimination as a highly promising and strategically important approach for future pepper improvement and production systems.

6. References

Abide, M., Kidanemariam, D., Yimam, T., Worku, Y., Kebede, M., & Abraham, A. (2022). Protocol optimization for elimination of sugarcane bacilliform virus and rapid propagation of virus-free sugarcane using meristem tip culture. Tropical Plant Pathology, 47, 693 - 697. https://doi.org/10.1007/s40858-022-00519-8.

Alam, M. F., Banu, M. L. A., Swaraz, A. M., Parvez, S., Hossain, M., Khalekuzzaman, M., & Ahsan, N. (2004). Production of virus free seeds using meristem culture in tomato plant under tropical conditions. Journal of Plant Biotechnology, 6(4), 221-227.

Ali, M. A., Nasiruddin, K. M., Haque, M. S., & Faisal, S. M. (2013). Virus elimination in potato through meristem culture followed by thermotherapy. *SAARC Journal of Agriculture*, *11*(1), 71-80.

Alkhazindar, M. (2015). Elimination of Tomato Spotted Wilt Virus (genus Tospovirus) from infected tomato plants by meristem tip culture. *Egypt J Bot*, *55*(1), 149-159.

AlMaarri, K., Massa, R., & AlBiski, F. (2012). Evaluation of some therapies and meristem culture to eliminate Potato Y potyvirus from infected potato plants. Plant Biotechnology, 29(3), 237-243.

Benke, A., Krishna, R., Khandagale, K., Gawande, S., Shelke, P., Dukare, S., Dhumal, S., Singh, M., & Mahajan, V. (2023). Efficient Elimination of Viruses from Garlic Using a Combination of Shoot Meristem Culture, Thermotherapy, and Chemical Treatment. Pathogens, 12. https://doi.org/10.3390/pathogens12010129.

Bettoni, J., Fazio, G., Costa, L., Hurtado-Gonzales, O., Rwahnih, M., Nedrow, A., & Volk, G. (2022). Thermotherapy Followed by Shoot Tip Cryotherapy Eradicates Latent Viruses and Apple Hammerhead Viroid from In Vitro Apple Rootstocks. Plants, 11. https://doi.org/10.3390/plants11050582.

Bhojwani, S. S., & Dantu, P. K. (2013). Production of virus-free plants. In Plant Tissue Culture: An Introductory Text (pp. 227-243). India: Springer India.

Bouras, H., Devkota, K., Mamassi, A., Loudari, A., Choukr-Allah, R., & El-Jarroudi, M. (2024). Unveiling the Synergistic Effects of Phosphorus Fertilization and Organic Amendments on Red Pepper Growth, Productivity and Physio-Biochemical Response under Saline Water Irrigation and Climate-Arid Stresses. Plants, 13. https://doi.org/10.3390/plants13091209.

Bradamante, G., Scheid, M., & Incarbone, M. (2021). Under siege: virus control in plant meristems and progeny. The Plant Cell, 33, 2523 - 2537. https://doi.org/10.1093/plcell/koab140.

Carvalho Lemos, V., Reimer, J. J., & Wormit, A. (2019). Color for life: biosynthesis and distribution of phenolic compounds in pepper (Capsicum annuum). *Agriculture*, *9*(4), 81.

Chiatante, D., Montagnoli, A., Trupiano, D., Sferra, G., Bryant, J., Rost, T., & Scippa, G. (2021). Meristematic Connectome: A Cellular Coordinator of Plant Responses to Environmental Signals?. Cells, 10. https://doi.org/10.3390/cells10102544.

Choi, M. (2023). Evaluation of the Weeds around Capsicum annuum (CA) Cultivation Fields as Potential Habitats of CA-Infecting Viruses. The Plant Pathology Journal, 39, 374 - 383. https://doi.org/10.5423/ppj.oa.04.2023.0066.

Ebida, A., & Hu, C. (1993). In vitro morphogenetic responses and plant regeneration from pepper (Capsicum annuum L. cv. Early California Wonder) seedling explants. Plant Cell Reports, 13, 107-110. https://doi.org/10.1007/bf00235301.

Gogile, A., Markos, T., Kebede, M., Kidanemariam, D., & Abraham, A. (2024). Elimination of yam mosaic virus from yam using an optimized combination of meristem culture and thermotherapy. Australasian Plant Pathology. https://doi.org/10.1007/s13313-024-00965-9.

Grout, B. (1999). Meristem-tip culture for propagation and virus elimination.. Methods in molecular biology, 111, 115-25 . https://doi.org/10.1385/1-59259-583-9:115.

Hipper, C., Brault, V., Ziegler-Graff, V., & Revers, F. (2013). Viral and Cellular Factors Involved in Phloem Transport of Plant Viruses. Frontiers in Plant Science, 4. https://doi.org/10.3389/fpls.2013.00154.

Huda, A. K. M. N., & Sikdar, B. (2006). In vitro plant production through apical meristem culture of bitter gourd (Momordica charantia L.). *Plant Tissue Culture and Biotechnology*, *16*(1), 31-36.

Jemal, N., & Feyissa, T. (2020). Production of Virus Free Sweet Potato (Ipomoea batatas (L.) Lam.) through Meristem Culture and Chemotherapy. . https://doi.org/10.21203/rs.3.rs-35805/v1.

Kappagantu, M., Collum, T., Dardick, C., & Culver, J. (2020). Viral Hacks of the Plant Vasculature: The Role of Phloem Alterations in Systemic Virus Infection.. Annual review of virology. https://doi.org/10.1146/annurev-virology-010320-072410.

Katoh, N., Yui, M., Sato, S., Shirai, T., Yuasa, H., & Hagimori, M. (2004). Production of virus-free plants from virus-infected sweet pepper by in vitro grafting. *Scientia horticulturae*, *100*(1-4), 1-6.

Kenyon, L., Kumar, S., Tsai, W.-S. and Hughes, J. d'A. 2014. Virus disease of peppers (Capsicum spp.) and their control. Adv. Virus Res. 90:297-354.

Khafri, A., Zarghami, R., Naderpour, M., Ahmadi, B., & Mirzaei, L. (2024). Assessment of virus eradication methods from infected in vitro-grown apricot cultures. Plant Cell, Tissue and Organ Culture (PCTOC). https://doi.org/10.1007/s11240-023-02621-4.

Lakhera, K., Kumar, A., Rani, A., Dixit, R., & Rana, S. (2018). Plant tissue culture and its application. , 32. https://doi.org/10.5958/2320-3196.2018.00004.6.

Lassois, L., Lepoivre, P., Swennen, R., van den Houwe, I., & Panis, B. (2012). Thermotherapy, chemotherapy, and meristem culture in banana. In Protocols for micropropagation of selected economically-important horticultural plants (pp. 419-433). Totowa, NJ: Humana Press.u

Lee, S., Kim, S., Lee, H., Lee, H., & Lee, J. (2017). Impact of moderate and extreme climate change scenarios on growth, morphological features, photosynthesis, and fruit production of hot pepper. Ecology and Evolution, 8, 197 - 206. https://doi.org/10.1002/ece3.3647.

Madhuri, V., & Rajam, M. (2012). Apical Shoot Meristem Culture in Red Pepper (Capsicum annuum L.). Journal of Plant Biochemistry and Biotechnology, 2, 67-68. https://doi.org/10.1007/bf03262925.

Magyar-Tábori, K., Mendler-Drienyovszki, N., Hanász, A., Zsombik, L., & Dobránszki, J. (2021). Phytotoxicity and Other Adverse Effects on the In Vitro Shoot Cultures Caused by Virus Elimination Treatments: Reasons and Solutions. Plants, 10. https://doi.org/10.3390/plants10040670.

Myśkow, E., Gola, E., & Tulik, M. (2019). Continuity of Procambium and Anomalous Cambium During Formation of Successive Cambia in Celosia argentea. Journal of Plant Growth Regulation, 1-9. https://doi.org/10.1007/s00344-019-09948-3.

Nerway, Z. A. A., Duhoky, M. M. S., & Kassim, N. A. (2020). In vitro elimination of Dahlia mosaic virus by using meristem culture, electrotherapy and chemotherapy. Iraqi Journal of Agricultural Sciences, 51(2).

Ojinaga, M., Guirao, P., & Larregla, S. (2022). A Survey of Main Pepper Crop Viruses in Different Cultivation Systems for the Selection of the Most Appropriate Resistance Genes in Sensitive Local Cultivars in Northern Spain. Plants, 11. https://doi.org/10.3390/plants11060719.

Saeed, N., Zafar, Y., & Malik, K. (1997). A simple procedure of Gossypium meristem shoot tip culture. Plant Cell, Tissue and Organ Culture, 51, 201-207. https://doi.org/10.1023/a:1005958812583.

Sasi, S., & Bhat, A. I. (2018). In vitro elimination of Piper yellow mottle virus from infected black pepper through somatic embryogenesis and meristem-tip culture. *Crop Protection*, *103*, 39-45.

Schwach, F., Vaistij, F. E., Jones, L., & Baulcombe, D. C. (2005). An rna-dependent rna polymerase prevents meristem invasion by potato virus x and is required for the activity but not the production of a systemic silencing signal. Plant Physiology, 138(4), 1842-1852. https://doi.org/10.1104/pp.105.063537

Sharmin, S. A., Kabir, A. H., Mandal, A., Sarker, K. K., & Alam, M. F. (2008). In vitro propagation of eggplant through meristem culture. *Agriculturae Conspectus Scientificus*, *73*(3), 149-155.

Siddique, M., Lee, J., Ahn, J., Kusumawardhani, M., Safitri, R., Harpenas, A., Kwon, J., & Kang, B. (2022). Genotyping-by-sequencing-based QTL mapping reveals novel loci for Pepper yellow leaf curl virus (PepYLCV) resistance in Capsicum annuum. PLoS ONE, 17. https://doi.org/10.1371/journal.pone.0264026.

Szyndel, M. S., Kowalczyk, K., & Pawełczak, A. (2008). Elimination of tomato mosaic virus (tomv) from pepino (Solanum muricatum) plants. *Phytopathologia Polonica*, *49*, 57-63.

Şavkan, A., Başak, H., Uncu, A., & Türkmen, Ö. (2024). Morphological characterization and molecular marker-assisted selection for resistance to Tobacco mosaic virus (TMV) and Tomato spotted wilt virus (TSWV) in S2 population of capia pepper (Capsicum annuum L.). Genetic Resources and Crop Evolution. https://doi.org/10.1007/s10722-024-02013-3.

Wang, B., Smith, S., & Li, J. (2018). Genetic Regulation of Shoot Architecture.. Annual review of plant biology, 69, 437-468 . https://doi.org/10.1146/annurev-arplant-042817-040422.

Wen-Xuan, L. (2004). In Vitro Regeneration from Shoot-Tip Explants of Peppers. Journal of Shanghai University.

Xue, Z., Liu, L., & Zhang, C. (2020). Regulation of Shoot Apical Meristem and Axillary Meristem Development in Plants. International Journal of Molecular Sciences, 21. https://doi.org/10.3390/ijms21082917.

Zhang, L., Wang, D., Hu, Q., Dai, X., Xie, Y., Li, Q., Liu, H., & Guo, J. (2019). Consortium of Plant Growth-Promoting Rhizobacteria Strains Suppresses Sweet Pepper Disease by Altering the Rhizosphere Microbiota. Frontiers in Microbiology, 10. https://doi.org/10.3389/fmicb.2019.01668.

Chapter 6

Mycotoxin Contamination in Cereal-Based Feed Ingredients: Effects on Livestock Health

Onur VURAL [1]

INTRODUCTION

Feed safety is a fundamental element of the animal food chain, and recent food safety incidents have clearly highlighted the importance of feed quality to public health and international trade. Feed is defined as a broad category encompassing all products and additives, whether processed or unprocessed, used in animal nutrition. These products are categorized as forages, grains, compound feeds, and food industry by-products, with feed composition varying significantly depending on animal species and age (FAO, 2024).

Cereals play a central role in the daily diet of animals, and corn and wheat, in particular, constitute the main components of global feed production. In developed countries, the majority of grain harvests are used for feed production. Globally, 56% of corn production is used for feed (Erenstein et al., 2022). In the European Union, most grains are reportedly used for feed production, with approximately 66% directed towards animal feed. Soybeans and their by-products are essential ingredients in modern feed formulations due to their high protein content; approximately 75% of these are used globally as animal feed (Fraanje and Garnett 2020).

Although cereals and plant derived protein sources offer major nutritional advantages in livestock diets, unfavorable climatic and storage conditions create an environment highly conducive to the proliferation of toxigenic fungi. Consequently, the feed chain becomes critical not only from a nutritional standpoint but also regarding chemical and biological hazards. Among these hazards, mycotoxins secondary metabolites produced by fungi are considered major contaminants of concern. These toxic compounds directly impair animal health and compromise the safety of animal-derived food products. Therefore, understanding the occurrence of mycotoxin contamination in animal feed is

[1] Department of Plant and Animal Production, Kumluca Vocational School, A
kdeniz University, Antalya, 07350, Türkiye
Corresponding authors: Onur VURAL, Department of Plant and Animal Production, Kumluca Vocational School, Akdeniz University, Antalya, 07350, Türkiye;
E-mail: onurvural@akdeniz.edu.tr; https://orcid.org/0000-0002-4178-605X

essential for designing effective risk management strategies and protecting livestock health.

1- Mycotoxins

Mycotoxins are secondary metabolites naturally occurring in food and feed, produced by various fungal species either in the field (preharvest) or during storage (postharvest) under favorable environmental conditions. They are most commonly synthesized by fungi belonging to the genera *Aspergillus*, *Penicillium*, *Fusarium*, and *Alternaria* (Table 1).

Table 1: Fungal species of global importance and mycotoxins produced (Kępińska and Biel et al., 2021).

Fungal Species	Mycotoxin(s) Produced
Aspergillus parasiticus	Aflatoxin B_1, B_2; Aflatoxin G_1, G_2
Aspergillus flavus	Aflatoxin B_1, B_2
Fusarium sporotrichioides	T-2 toxin
Fusarium graminearum	Deoxynivalenol (DON); Zearalenone
Fusarium moniliforme	Fumonisin B_1
Penicillium verrucosum	Ochratoxin A
Aspergillus ochraceus	Ochratoxin A

The most studied mycotoxins relevant to food safety include aflatoxins, deoxynivalenol (DON), fumonisins, zearalenone (ZEA), and ochratoxin A (OTA) (Maphaisa et al., 2025). These toxins are widely present in plant-derived materials, particularly cereal grains and their processed fractions. Grain-based feed ingredients constitute a substantial proportion of global livestock diets, and studies from various regions consistently demonstrate the occurrence of multiple mycotoxins in a significant proportion of these feeds. High level exposure in farm animals results in acute mycotoxicosis, which may lead to severe clinical outcomes such as hepatic failure or death. Chronic exposure, even at lower concentrations, can cause immunosuppression, organ damage, reduced productivity, and impaired reproductive performance. Such contamination also facilitates the transfer of mycotoxins into the human food chain through animal products, thereby posing a serious public health concern (Yu and Pedroso, 2023; Akinmoladun et al., 2025).

Global feed analyses further reveal that aflatoxins, fumonisins, DON, ZEA, and OTA frequently co-occur. In poultry feed, fumonisin B_1 and AFB_1 were reported as the most prevalent mycotoxins in Nigeria; ZEA, fumonisins, DON, OTA, and

aflatoxins were commonly detected in South Africa; and high aflatoxin levels were reported in Uganda and Cameroon. Many samples exceeded established regulatory limits, indicating a substantial threat to global feed safety (Kibugu et al., 2024).

Given these findings, the individual evaluation of the most prevalent and toxicologically important mycotoxin groups in animal feed is essential for effective risk management and the safeguarding of animal health. The major mycotoxin groups with critical impacts on livestock production are discussed in detail below.

2- Aflatoxins

Aflatoxins (AFs) are among the most toxic mycotoxins and are primarily produced by *Aspergillus flavus* and A. *parasiticus*. Strains of A. *flavus* synthesize only aflatoxins B1 (AFB1) and B2 (AFB2), whereas A. *parasiticus* produces AFB1, AFB2, AFG1, and AFG2. These toxins occur in many high-fat plant products, including cereals such as corn, rice, barley, oats, and sorghum, as well as peanuts, almonds, walnuts, and cottonseed (Kumar et al., 2017).

AFs are considered the most potent mycotoxin group regarding human toxicity. The International Agency for Research on Cancer (IARC) classifies AFB1, AFB2, AFG1, AFG2, and AFM1 as Group 1 human carcinogens, with AFB1 being the most potent hepatocarcinogenic compound (IARC, 2016). Aflatoxins enter the human food chain through two major routes. The first is direct consumption of contaminated grains, feed, or processed foods. This route is concerning because aflatoxins are heat-stable and resist degradation during conventional processing. The second route is indirect exposure through animal products. Animals consuming contaminated feed metabolize AFB1 into AFM1, which accumulates in tissues, milk, and eggs, facilitating human exposure (Tolosa et al., 2021).

AFB1 is a strong hepatotoxin and also exerts immunosuppressive effects in livestock. Its carcinogenic potential further raises long-term health concerns. Sensitivity to aflatoxins varies among species and is strongly influenced by age and exposure duration. Young calves, lambs, kids, and chicks are particularly vulnerable due to their developing physiological systems (Alshannaq and Yu, 2017). AFB1 disrupts cellular antioxidant defenses and induces oxidative stress. In broilers, AFB1 exposure reduces the activity of major antioxidant enzymes such as glutathione peroxidase (GPx), glutathione reductase, catalase (CAT), and superoxide dismutase (SOD). It also decreases glutathione (GSH) levels and increases malondialdehyde (MDA), indicating lipid peroxidation and cellular damage (Sarker et al., 2023).

Dairy cattle can tolerate low AFB1 levels due to rumen detoxification capacity; however, chronic exposure still reduces milk yield and causes liver damage and immunosuppression. A critical concern is that AFB1 is rapidly metabolized into

AFM1 in the liver and excreted in milk within 12–24 hours, posing a direct public health risk (Malissiova et al., 2024).

Directive 2002/32/EC sets maximum legal concentrations for AFB1 in animal feed within the European Union (EC, 2002). AFB1 is the only mycotoxin with binding legal limits in EU feed legislation. The maximum allowable level is 20 µg/kg in feed materials and 10 µg/kg in complete and complementary feeds, while 5 µg/kg applies to compound feeds for young animals. In contrast, only guidance values exist for OTA, fumonisins, DON, T-2/HT-2, and zearalenone, and no mandatory limits are established (Alshannaq and Yu, 2017).no mandatory maximum limits have been established (Table 2).

Table 2. FDA and EU aflatoxin regulatory guidelines for feeds and feed ingredients (adapted from Tolosa et al., 2021)

Authority	Intended Use / Matrix	Cereals, Cereal By products, Feed or Other Products	MaximumAFB_1 Limits (µg/kg)
FDA	Immature (growing) animals, dairy animals, or other animals with unspecified species	Corn, peanuts, and other feed ingredients	20
	Breeding cattle, swine, and poultry	Corn and peanut products	100
	Finishing (market-weight) swine >45 kg	Corn and peanut products	200
	Finishing beef cattle	Corn and peanut products	300
	Cattle, swine, or poultry regardless of age or production status	Cottonseed meal	300
EU	All feed materials	—	20
	Complete feeds for cattle, sheep, and goats (excluding dairy animals)	—	20
	Complementary feeds for dairy animals	—	5
	Complementary feeds for calves and lambs	—	10
	Complete feeds for pigs and poultry (excluding young animals)	—	20
	Other complementary feeds	—	10
	Complementary feeds for cattle, sheep, and goats (excluding dairy animals, calves, and lambs)	—	20
	Complementary feeds for pigs and poultry (excluding young animals)	—	20
	Other complementary feeds	—	5

3- Ochratoxins

Ochratoxin A (OTA) is a mycotoxin produced by several toxigenic fungal species, most notably *Aspergillus ochraceus*. OTA is a potent nephrotoxin and is extensively evidenced to induce kidney damage. In addition, it exhibits carcinogenic, teratogenic, immunotoxic, and potentially neurotoxic properties. A link between OTA exposure and endemic nephropathy, particularly reported in Balkan regions, has been established. The International Agency for Research on Cancer (IARC) classifies OTA as "probably carcinogenic to humans" (Group 2B) (IARC, 2012).

According to existing regulatory guidelines recommended limits for OTA in animal feed are 250 µg/kg for feed materials, 50 µg/kg for complete and complementary feeds for pigs, and 100 µg/kg for poultry feeds. A global systematic meta-analysis by Sharafi et al. (2023) evaluated OTA concentrations in red meat and edible offal. Their findings show that OTA accumulates at higher levels in metabolically active organs such as the liver and kidney, whereas concentrations in fresh muscle meat are comparatively lower.

OTA exposure also has significant implications for poultry health. Zhai et al. (2021) demonstrated that OTA disrupts the intestinal microbiota by reducing beneficial bacterial populations and promoting the growth of opportunistic pathogens. The toxin further increases intestinal permeability by downregulating tight junction proteins in the epithelial barrier, resulting in compromised immune function. These physiological disturbances contribute to marked production losses, including reduced growth performance, impaired feed conversion efficiency, and increased susceptibility to infectious diseases.

4- Fumonisins

Fumonisins (FBs) are considered an important group of mycotoxins, produced mostly by *Fusarium* species. The primary producers are F. *verticillioides* and F. *proliferatum*. The most important fumonisin compounds are FB1, FB2, FB3, and FB4. Within this group, FB1 has been identified as the most prevalent and toxic compound and is classified as a probable human carcinogen (Group 2B). FBs are reported to not cause direct DNA damage but have toxic effects. The EU has set limit values for FB1 and FB2 at 60,000 µg/kg in corn products and 5,000–50,000 µg/kg in feed (IARC, 2016).

Brown and Herrman (2025) evaluated the validity of the 60 mg/kg guideline level established by the FDA in 2001 for corn and corn by-products for fumonisin contamination in cattle feed based on current scientific data. The analysis revealed that recent studies have not significantly updated the dose-response relationship for fumonisins in cattle. This finding suggests that the current

guideline limits are still appropriate and that there is no new toxicological evidence to warrant changes.

5- Trichothecenes

Trichothecenes (TCs) are a large group of mycotoxins consisting of more than 150 structurally similar compounds produced primarily by *Fusarium* species. While they are divided into four main classes (A–D) based on their chemical properties, the most critical subgroups for animal health are type A (T-2 and HT-2 toxins) and type B trichothecenes (especially deoxynivalenol – DON). Due to their high toxicity, T-2 and HT-2 can cause clinical signs such as reduced feed intake, weight loss, gastrointestinal hemorrhage, oral lesions, reproductive disorders, and, in severe cases, mortality. Deoxynivalenol (DON), the most common member of type B trichothecenes, is one of the most frequently detected mycotoxins globally, despite its relatively low acute toxicity. Known as a " emesis toxin," DON is reported to cause effects such as feed refusal, reduced body weight gain, impaired intestinal barrier integrity, and reduced nutrient absorption. T-2, HT-2, and DON were classified as Group 3 (not classifiable as a human carcinogen) (IARC, 2016).

Gallo et al. (2015) evaluated the effects of feeds containing varying levels of DON on the rumen microbial population and fermentation profile. They noted a decrease in cellulolytic bacteria and changes in the volatile fatty acid profile (especially propionate) in animals fed diets high in DON.

6- Zearalenone

Zearalenone (ZEN) is a mycotoxin with estrogenic activity, synthesized by various *Fusarium* species, primarily *Fusarium graminearum*, but also F. *culmorum*, F. *cerealis*, and F. *equiseti*. This allows it to bind to estrogen receptors and affect the endocrine system. Therefore, ZEN exposure has been associated with hormonal imbalances and various fertility disorders, particularly in animals of reproductive age. ZEN is classified as a Group 3 "unclassifiable carcinogen" for humans (IARC, 2016).

Adverse outcomes such as decreased fertility, increased stillbirth rates, and decreased sperm quality have been reported in farm animals due to ZEN exposure. Exposure during pregnancy is associated with clinical conditions such as decreased embryo viability and low birth weight (Rivera et al., 2024). Ding et al (2021) evaluated changes in the reproductive organs of young female lambs exposed to ZEN. The study showed that ZEN causes uterine inflammation, increased endometrial thickness, and irregular follicular development. Exposure

was associated with imbalances in serum progesterone and estradiol levels. These findings demonstrate that young small ruminants are highly sensitive to ZEN.

According to European Union guidelines, the recommended ZEN levels in feed materials are 2,000 µg/kg for grains and grain products and 3,000 µg/kg for corn products. The maximum permissible levels in complete and complementary feeds are 100 µg/kg for piglets and young sows, 250 µg/kg for other pigs, and 500 µg/kg for calves, dairy cattle, sheep, and goats.

7- Factors Affecting Mycotoxins in Livestock Feeds

The impact of climate change on mycotoxin formation in food and feed has increasingly been recognized as a major public health concern. Rising temperatures, elevated humidity, and the greater frequency of extreme climatic events directly influence the ecology of toxigenic fungi, substantially increasing mycotoxin production (Kępińska and Biel, 2021). Humidity is a critical parameter for the germination of fungal spores and the initiation of mycotoxin biosynthesis, and moisture levels above 9% in stored feed facilitate fungal growth (Nada et al., 2022). The ability of *Aspergillus* and *Penicillium* species to proliferate at moisture levels of 13–18% demonstrates that inadequate drying and poor storage management markedly increase mycotoxin risk.

Temperature is another major environmental factor influencing mycotoxin formation. Although aflatoxin-producing *Aspergillus* species can survive at temperatures as low as 6–8 °C, toxin production peaks between 25–30 °C (Kumar et al., 2017). In contrast, *Fusarium* species thrive in temperate climates, leading to more frequent detection of mycotoxins such as DON, ZEN, and fumonisins, particularly in North America and Europe. Climate change driven increases in temperature are altering the geographic distribution of *Fusarium* mycotoxins and raising contamination risks in several regions (Yu & Pedroso, 2023; Akinmoladun et al., 2025).

The chemical composition of feed ingredients also plays a decisive role in mycotoxin formation. High-oil crops, such as oilseeds, create a favorable substrate for fungal growth and toxin production; fungal metabolism is reported to accelerate more rapidly in carbohydrate-rich grains (Kępińska and Biel, 2021). Multi-mycotoxin surveys indicate the simultaneous occurrence of several mycotoxins in a large proportion of global feed samples. The dominance of Aspergillus-derived aflatoxins in tropical climates and the prevalence of DON, ZEN, and fumonisins in temperate regions reflect a region-specific, climate-dependent risk pattern. Moreover, the detection of AFB1 in Southern Europe as temperatures increase demonstrates a significant shift in the geographical distribution of mycotoxins (Kibugu et al., 2024).

8- Prevention Strategies for Mycotoxin Formation in Feeds

The use of plant-based raw materials such as maize, wheat, oats, soybeans, and their by-products has increased. However, these materials are highly susceptible to mold growth under unfavorable environmental and storage conditions, making them high-risk sources of mycotoxin contamination. Rising feed demand and intensive production systems have led to more frequent mycotoxin occurrence in animal feed, resulting in significant threats to both animal health and the human food chain (Tolosa et al., 2021).

Given current conditions, the main strategies for preventing mycotoxin formation in feeds can be summarized as follows:

1- Controlling temperature and moisture during harvesting, transport, and storage is a key step in preventing mycotoxin formation. High humidity and temperature accelerate fungal growth; therefore, products should be harvested at appropriate moisture levels, storage areas must be well ventilated, and environmental parameters should be continuously monitored.

2- Regular mycotoxin analysis of raw materials is essential for early detection of contamination. In high-risk commodities such as maize and wheat, rapid screening tests combined with LC-MS/MS confirmation methods provide reliable identification of both single and multiple mycotoxins.

3- Limiting fungal growth is also an important strategy for reducing mycotoxin formation. Physical methods such as heat treatment, ozone application, or ultraviolet light, along with the use of antifungal inhibitors, can suppress fungal proliferation and significantly lower toxin production.

4- Mycotoxin binders or degraders serve as additional measures to enhance feed safety. Adsorbents such as bentonite, zeolite, and activated charcoal reduce toxin absorption, while biological degraders convert mycotoxins into less harmful compounds. In cases of multiple contamination, products combining different mechanisms are recommended.

5- Strengthening traceability within the feed supply chain facilitates rapid identification of contamination sources and improves risk management. Digital recording of supplier information, lot numbers, and analytical results ensures transparency and control throughout the process (Liu et al., 2022; Nada et al., 2022).

REFERENCE

Akinmoladun, O. F., Fon, F. N., Nji, Q., Adeniji, O. O., Tangni, E. K., andNjobeh, P. B. (2025). Multiple mycotoxin contamination in livestock feed: Implications for animal health, productivity, and food safety. Toxins, 17(8), 365.

Alshannaq, A., and Yu, J. H. (2017). Occurrence, toxicity, and analysis of major mycotoxins in food. International Journal of Environmental Research and Public Health, 14(6), 632.

Brown, A. A., andHerrman, T. (2025). Evaluating fumonisin contamination in cattle feed: Impact on animal health, the agriculture industry and regulatory considerations. Current Research in Toxicology, 100235.

Ding, R., Qiu, Y., Zhuang, Z., Ruan, D., Wu, J., Zhou, S., ... andYang, J. (2021). Genome-wide association studies reveal the polygenic genetic architecture of litter traits in Duroc pigs. Theriogenology, 173, 269–278.

EC-European Commission. (2002). Directive 2002/32/EC of the European Parliament and of the Council of 7 May 2002 on undesirable substances in animal feed. Official Journal of the European Union, 140, 10–22.

Erenstein, O., Jaleta, M., Sonder, K., Mottaleb, K., andPrasanna, B. M. (2022). Global maize production, consumption and trade: Trends and R&D implications. Food Security, 14(5), 1295–1319.

FAO. (2024). The State of Food Security and Nutrition in the World 2024: Financing to end hunger, food insecurity and malnutrition in all its forms. Rome: FAO. https://doi.org/10.4060/cd1254en

Fraanje, W., and Garnett, T. (2020). Soy: food, feed, and land use change (Foodsource: Building Blocks). Food Climate Research Network, University of Oxford.

Gallo, A., Minuti, A., andTrevisi, E. (2015). Rumen microbial population and fermentation profile are affected by deoxynivalenol in dairy cows. Journal of Animal Science, 93(11), 5303–5313.

IARC. (2012). IARC Monographs on the Evaluation of Carcinogenic Risks to Humans: Chemical Agents and Related Occupations (Vol. 100F). International Agency for Research on Cancer.

IARC. (2016). Agents classified by the IARC Monographs, Volumes 1–116. World Health Organization. https://monographs.iarc.who.int/list-of-classifications

Kępińska-Pacelik, J., and Biel, W. (2021). Mycotoxins—prevention, detection, impact on animal health. Processes, 9(11), 2035.

Kibugu, J., Munga, L., Mburu, D., Maloba, F., Auma, J. E., Grace, D., andLindahl, J. F. (2024). Dietary mycotoxins: An overview on toxicokinetics,

toxicodynamics, toxicity, epidemiology, detection, and their mitigation with special emphasis on aflatoxicosis in humans and animals. Toxins, 16(11), 483.

Kumar, P., Mahato, D. K., Kamle, M., Mohanta, T. K., andKang, S. G. (2017). Aflatoxins: A global concern for food safety, human health, and their management. Frontiers in Microbiology, 7, 235289.

Liu, M., Zhao, L., Gong, G., Zhang, L., Shi, L., Dai, J., ... andSun, L. (2022). Invited review: Improvement strategies for mycotoxin control in feed. Journal of Animal Science and Biotechnology, 13(1), 19.

Malissiova, E., Tsinopoulou, G., Gerovasileiou, E. S., Meleti, E., Soultani, G., Koureas, M., ... andManouras, A. (2024). A 20-year data review on the occurrence of aflatoxin M1 in milk and dairy products in Mediterranean countries—Current situation and exposure risks. Dairy, 5(3), 491–514.

Maphaisa, T. C., Akinmoladun, O. F., Adelusi, O. A., Mwanza, M., Fon, F., Tangni, E., andNjobeh, P. B. (2025). Advances in mycotoxin detection techniques and the crucial role of reference material in ensuring food safety: A review. Food and Chemical Toxicology, 115387.

Nada, S., Nikola, T., Bozidar, U., Ilija, D., andAndreja, R. (2022). Prevention and practical strategies to control mycotoxins in the wheat and maize chain. Food Control, 136, 108855.

Rivera-Chacon, R., Hartinger, T., Castillo-Lopez, E., Lang, C., Penagos-Tabares, F., Mühleder, R., ... and Ricci, S. (2024). Duration of zearalenone exposure has implications on health parameters of lactating cows. Toxins, 16(3), 116.

Sarker, M. T., Wan, X. L., Yang, H. M., and Wang, Z. Y. (2023). Aflatoxin B1 (AFB1) and its toxic effect on the broiler intestine: A review. Veterinary Medicine and Science, 9(4), 1646–1655.

Sharafi, H., Alizadeh, N., Moradi, M., and Mousavi Khaneghah, A. (2023). The prevalence and concentration of ochratoxin A in meat and edible offal: A global systematic review and meta-analysis. Food and Chemical Toxicology. https://doi.org/10.1016/j.fct.2023.113123

Tolosa, J., Rodríguez-Carrasco, Y., Ruiz, M. J., andVila-Donat, P. (2021). Multi-mycotoxin occurrence in feed, metabolism and carry-over to animal-derived food products: A review. Food and Chemical Toxicology, 158, 112661.

Yu, J., and Pedroso, I. R. (2023). Mycotoxins in cereal-based products and their impacts on the health of humans, livestock animals and pets. Toxins, 15(8), 480.

Zhai, S., Zhu, Y., Feng, P., Li, M., Wang, W., Yang, L., andYang, Y. (2021). Ochratoxin A: Its impact on poultry gut health and microbiota—An overview. Poultry Science, 100(5), 101037.

Chapter 7

Weed Seed Dormancy: Mechanisms and Management Strategies

Fırat PALA [1], Hüsrev MENNAN[2]

1. INTRODUCTION

Weeds, the silent yet most persistent competitors of agricultural production, are among the primary biotic stress factors responsible for substantial yield and quality losses. Of all the mechanisms that enable these plants to maintain their presence in fields for decades, the most complex and effective survival strategy is undoubtedly seed dormancy. The success of weeds in agroecosystems depends not only on their rapid growth but also on their extraordinary ability to survive and perpetuate their populations. Among these strategies, dormancy is arguably the most sophisticated and the most difficult to manage.

Dormancy is defined as the failure of a viable seed or vegetative propagule (rhizome, tuber, etc.) to germinate or sprout even when environmental conditions (water, temperature, oxygen) are favorable for germination or emergence. Rather than a passive waiting state, dormancy is a genetically programmed biological "sleep" regulated by hormonal signals, which spreads the risk of species survival over time (Bewley, 1997). This mechanism forms the basis of the "soil seed bank," an enormous underground reservoir consisting of billions of seeds that can remain viable for decades (Holm et al., 1977). The soil seed bank functions as an insurance policy by preventing all individuals of a population from germinating simultaneously and thus protects the species against catastrophic events such as soil tillage, drought, or herbicide applications. For example, seeds of Field Bindweed (Convolvulus arvensis) and Common Mallow (*Malva neglecta*) can remain viable in the soil for more than 50 years, whereas seeds of Common Lambsquarters (*Chenopodium album*) may retain viability for up to 40 years.

This complex biological mechanism has often been a "black box" for researchers, students, and agronomists attempting to conduct controlled studies

[1] Assoc. Prof. Dr., University of Siirt, Faculty of Agriculture, Department of Plant Protection, Siirt, Türkiye
ORCID ID: 0000-0002-4394-8841
[2] Prof. Dr., Ondokuz Mayis University, Faculty of Agriculture, Department of Plant Protection, Samsun, Türkiye
ORCID ID: 0000-0002-1410-8114

in the laboratory. Numerous experiments designed to investigate weed biology, herbicide resistance, or biological control potential have failed at the very first step—seed germination—because the nature of dormancy and ways to break it were not properly understood. Without this knowledge, obtaining homogeneous and healthy plant material is nearly impossible.

The aim of this book chapter is to go beyond describing the theoretical foundations of dormancy and provide clear and reproducible answers to the practical question most relevant for researchers and practitioners: “How can dormancy be broken in practice?” First, the theoretical framework and main types of dormancy will be explained. Then, natural and artificial cues used to break dormancy will be discussed. The core of the chapter consists of step-by-step dormancy-breaking and sowing protocols specifically developed for key weed species important in agriculture. These protocols serve as “recipes” that reveal how to unlock the unique key–lock system of each species. Finally, the protocols are grouped according to similar dormancy types, thereby providing a general framework that fills an important knowledge gap in the field.

The following table presents a comparative overview of the longevity of seeds of common broadleaf and grass weed species in the soil seed bank.

Table 1: Seed Longevity in the Soil Seed Bank by Weed Species

No	Weed Species (Turkish)	Scientific Name	Leaf Type	Seed Longevity
1	Sirken	*Chenopodium album*	Broadleaf	20–40 years or longer
2	Kırmızı köklü tilki kuyruğu	*Amaranthus retroflexus*	Broadleaf	Up to 40 years
3	Tarla Sarmaşığı	*Convolvulus arvensis*	Broadleaf	More than 50 years
4	Yabani Ebegümeci	*Malva neglecta*	Broadleaf	More than 50 years
5	Domuz Pıtrağı	*Xanthium strumarium*	Broadleaf	1–15 years
6	Yabani Hardal	*Sinapis arvensis*	Broadleaf	Up to 50 years
7	Yapışkan Ot	*Galium aparine*	Broadleaf	1–7 years
8	Çoban Çantası	*Capsella bursa-pastoris*	Broadleaf	Up to 35 years
9	Köpek Üzümü	*Solanum nigrum*	Broadleaf	Up to 40 years
10	Kekemik	*Abutilon theophrasti*	Broadleaf	More than 50 years
11	Kısır Yabani Yulaf	*Avena sterilis*	Grass	5–7 years
12	Kanyaş (seeds)	*Sorghum halepense*	Grass	Up to 10 years
13	Köpekdişi Ayrığı (seeds)	*Cynodon dactylon*	Grass	1–5 years

14	Topalak	*Cyperus rotundus*	Grass	Mainly vegetative; seeds survive several years
15	Tilki Kuyruğu	*Alopecurus myosuroides*	Grass	1–7 years
16	Delice	*Lolium temulentum*	Grass	1–5 years
17	Darıcan	*Echinochloa crus-galli*	Grass	7–13 years
18	Yeşil Kirpi Darı	*Setaria viridis*	Grass	Up to 30 years
19	Kuş Yemi	*Phalaris minor*	Grass	3–5 years
20	Püsküllü Çayır	*Bromus tectorum*	Grass	2–5 years

These longevity estimates are based on published literature concerning the persistence of weed seeds in soil seed banks (Pala, 2025).

The data clearly demonstrate that the persistence of weed seeds in the soil seed bank has a decisive influence on management strategies. In species with long-lived seeds (e.g., *Convolvulus arvensis, Malva neglecta, Abutilon theophrasti*), practices such as tillage, crop rotation, mulching, and herbicide applications must be planned within a long-term and integrated framework. In contrast, for grass weeds with short-lived seed banks, intensive monitoring, accurate timing, and cultural practices that enhance crop competitiveness can provide more rapid control. Thus, the table offers practitioners important guidance on how these biological differences should be reflected in weed management plans.

2. TYPES OF DORMANCY AND BREAKING MECHANISMS

Dormancy, which prevents weed seeds or vegetative organs from germinating or sprouting, is not a single mechanism but a complex of different strategies shaped by evolutionary pressures. To manage these strategies effectively, it is essential to understand the source and nature of dormancy. Based on the location of the primary constraint, dormancy can be grouped into three main categories (Lang, 1987).

2.1. Exogenous (External) Dormancy: The Barrier in the Seed Coat

In exogenous dormancy, the embryo is potentially capable of germination, but the seed coat or surrounding fruit tissues (e.g., pericarp) prevent it.

- Physical dormancy: The seed coat (testa) is completely impermeable to water and gases. This condition, often called “hardseededness,” is common in species of the Fabaceae, Malvaceae, and Convolvulaceae families. In nature, such dormancy is gradually broken by slow processes such as freeze–thaw cycles, microbial activity, or soil abrasion (Baskin &

Baskin, 2014). Examples include Wild Vetch (Vicia sativa), Velvetleaf (*Abutilon theophrasti*), and Field Bindweed (*Convolvulus arvensis*).

- Chemical dormancy: The seed coat or fruit tissues contain chemical inhibitors of germination (e.g., phenolic compounds, abscisic acid, coumarins). These inhibitors must be leached by rainfall or degraded by microorganisms before germination can occur. Examples include the bur of Cocklebur (*Xanthium strumarium*) and the seeds of Wild Carrot (*Daucus carota*).

2.2. Endogenous (Internal) Dormancy: The Barrier in the Embryo

In endogenous dormancy, the constraint lies within the embryo itself. Even if the seed coat is permeable to water and gases, the embryo is not yet ready to germinate.

- Physiological dormancy: This is the most common form of dormancy. Although the embryo is morphologically developed, it is metabolically constrained. This "lock" is typically regulated by the balance between the germination-inhibiting hormone abscisic acid (ABA) and the germination-promoting gibberellins (GA) (Baskin & Baskin, 2014). Its release generally requires specific environmental cues such as cold stratification, light, or alternating temperatures. Examples include Sterile Wild Oat (Avena sterilis), Wild Mustard (Sinapis arvensis), and Redroot Pigweed (*Amaranthus retroflexus*).
- Morphological dormancy: When seeds are shed, the embryo is not fully developed and requires time under warm and moist conditions to complete its development before it can germinate.

2.3. Combined Dormancy: A Two-Step Lock

In combined dormancy, both exogenous (e.g., a hard seed coat) and endogenous (e.g., physiological) barriers coexist. To achieve germination, the physical barrier (e.g., by scarification) must first be removed, followed by the release of physiological dormancy (e.g., through cold stratification). Examples include Dog Rose (*Rosa canina*) and Cutleaf Crane's-bill (*Geranium dissectum*).

2.4. Breaking Dormancy: Natural and Artificial Triggers

Breaking dormancy is a delicate "awakening" process in which the seed or vegetative organ senses its environment or receives an external cue to determine the most favorable time to start growth.

Natural triggers include light (especially for small-seeded species), temperature (cold for winter annuals; fluctuating or high temperatures for summer annuals), water (leaching of inhibitors), and naturally occurring soil chemicals such as nitrate.

Artificial triggers used in the laboratory or in agricultural practice mimic or accelerate these natural processes. Common methods include:

- Physical scarification: Abrading hard seed coats with sandpaper, nicking them, or treating with concentrated sulfuric acid (H_2SO_4).
- Cold stratification: Storing seeds of winter annuals under moist, cold conditions (usually around 4°C) to simulate winter.
- Hormones and chemicals: Applying gibberellic acid (GA_3) to release physiological dormancy, potassium nitrate (KNO_3) to stimulate nitrate-sensitive species, and ethylene or ethephon to induce "suicidal germination" in parasitic weeds.

2.5. Dormancy-Breaking Protocols for Important Weed Species

This section presents detailed laboratory and practical protocols for five agriculturally important weed species, each representing a different dormancy type. For each species, two complementary approaches are described:

- Technique 1: Germination in Petri dishes followed by transplanting – ideal for controlled laboratory studies where precise germination data (e.g., percentage, rate) are required.
- Technique 2: Seed soaking and direct sowing – suitable for greenhouse or field trials where large numbers of plants are needed rapidly with less labor.

1. Example of Parasitic Dormancy: Broomrape (*Orobanche* spp.)

The secret of broomrape: a two-step awakening. Broomrape is a holoparasitic plant whose seeds have evolved a highly specialized survival strategy. They do not germinate randomly but only when a suitable host plant is present nearby. This process is controlled in two stages:

1. Preconditioning (readiness): To become capable of perceiving the germination signal, seeds must first be kept for 7–14 days in a moist and warm environment (20–25°C) in darkness (Joel et al., 2013).
2. Chemical signal (germination command): Strigolactones exuded from the roots of the host plant into the soil serve as the "germinate" command for preconditioned seeds. In the absence of this signal, seeds may remain dormant for many years (Yoneyama et al., 2010).

Dormancy-Breaking Protocol for Broomrape (*Orobanche* spp.)

Dormancy type: Parasitic dormancy (requires host signal)

Technique 1: Germination in Petri Dishes and Transplanting

Stage 1: Breaking dormancy in Petri dishes

1. Preconditioning (mandatory): Place surface-sterilized seeds on moist filter paper in Petri dishes and incubate them at 20–25°C in darkness for 7–14 days.
2. Stimulation: Apply a very low concentration (0.1–1.0 μM) solution of a synthetic strigolactone analogue (e.g., GR24) onto the preconditioned seeds.
3. Germination: Return the Petri dishes to darkness at 20–25°C. Germination is typically observed within 3–10 days.

Stage 2: Transplanting (inoculation)

Germinated seedlings are carefully transferred with a fine needle under a stereomicroscope and placed in close contact with the roots of pre-grown host plants. Seedlings that fail to attach to the host root die.

Technique 2: Seed Soaking and Direct Sowing

This method is not suitable for broomrape. Because seeds require an extended preconditioning period followed by exposure to a specific chemical signal (strigolactones), simple soaking cannot satisfy these two sequential requirements.

2. Example of Combined Dormancy: Cocklebur (*Xanthium strumarium*)

The secret of cocklebur: a three-layered lock. The success of Cocklebur is due to a threefold defense mechanism that protects its seeds, known as combined dormancy (Baskin & Baskin, 2014):

1. Mechanical lock: The spiny, hard bur enclosing the seeds physically restricts the movement of water and air to the seeds (Stoller & Wax, 1973).
2. Chemical lock: The bur itself contains water-soluble natural inhibitors that suppress germination (Zimmerman & Weis, 1983).
3. Biological lock: Within each bur, there are two seeds with different dormancy depths (dimorphism). The larger seed tends to germinate earlier, while the smaller one displays deeper physiological dormancy.

Dormancy-Breaking Protocol for Cocklebur (*Xanthium strumarium*)

Dormancy type: Combined (physical/chemical bur + physiological seed)

Technique 1: Germination in Petri Dishes and Transplanting

Stage 1: Breaking dormancy in Petri dishes

1. Remove the bur (mandatory): Crack the burs with pliers and extract the seeds.
2. Washing (mandatory): Wash the seeds under running water for several hours to remove soluble inhibitors.
3. Treatment: Place washed seeds on filter paper moistened with a 250–500 ppm GA_3 solution in Petri dishes.
4. Germination: Incubate Petri dishes in a warm (25–30°C), well-lit environment. Germination is usually observed within 7–14 days.

Stage 2: Transplanting

Transplant germinated seedlings into pots at a depth of 1–2 cm.

Technique 2: Seed Soaking and Direct Sowing

Stage 1: Seed soaking

1. Remove the bur and wash the seeds (mandatory).
2. Soak the seeds in a 250–500 ppm GA_3 solution for 12–24 hours.

Stage 2: Direct sowing

Drain the seeds and sow them into pots at a depth of 1–2 cm. Keep the pots in a warm (25–30°C), well-lit environment. Seedling emergence is expected within 7–14 days.

3. Example of Physiological Dormancy (Cold Requirement): Wild Mustard (*Sinapis arvensis)*

The secret of wild mustard: a biological calendar that waits for winter. The survival strategy of Wild Mustard is based on a sensitive biological calendar capable of perceiving seasonal changes, known as physiological dormancy:

1. Internal lock: In freshly matured seeds, high levels of ABA prevent premature germination in autumn.
2. Environmental trigger: To unlock this internal constraint, the seed must sense that "winter has passed." This signal consists of cold and moist soil conditions, a process referred to as cold stratification, which alters hormonal balance and prepares the seed for germination in spring (Baskin & Baskin, 2014).

Dormancy-Breaking Protocol for Wild Mustard (Sinapis arvensis)

Dormancy type: Physiological dormancy (requires cold stratification)

Technique 1: Germination in Petri Dishes and Transplanting

Stage 1: Breaking dormancy in Petri dishes

1. Cold stratification: Mix seeds with moist sand or perlite and store them in a refrigerator at 4°C for 4–6 weeks.

2. Germination: After stratification, place the seeds in Petri dishes on filter paper moistened with sterile water. Incubate at cool temperatures (15–20°C). Germination is typically observed within 5–10 days.

Alternative: Instead of cold stratification, Petri dishes may be moistened with a 500–1000 ppm GA_3 solution.

Stage 2: Transplanting

Transplant germinated seedlings into pots at a depth of 0.5–1 cm.

Technique 2: Seed Soaking and Direct Sowing

Stage 1: Seed soaking

For freshly harvested seeds, this method is generally less effective, and cold stratification (Technique 1) is preferred. However, as an alternative, seeds can be soaked in a 500–1000 ppm GA_3 solution for 24 hours.

Stage 2: Direct sowing

Drain the seeds and sow them into pots at a depth of 0.5–1 cm. Place the pots in a cool (15–20°C), well-lit environment. Seedling emergence is expected within 7–14 days.

4. Example of Physiological Dormancy (Light/Temperature): Redroot Pigweed (*Amaranthus retroflexus*)

The secret of Redroot Pigweed: an opportunistic germination strategy. The success of this species is based on a sensitive detection system that allows its seeds to germinate only when conditions are ideal:

1. Light signal: Small seeds germinate only when brought close to the soil surface, where they can perceive light via the phytochrome system (Baskin & Baskin, 2014).
2. Temperature fluctuation: The day–night temperature difference in surface soil layers is an additional indicator that the seed is near the soil surface.
3. Nutrient signal (nitrate): High nitrate (NO_3^-) levels in the soil signal fertile conditions (Benech-Arnold et al., 2000).

Dormancy-Breaking Protocol for Redroot Pigweed (*Amaranthus retroflexus*)

Dormancy type: Physiological dormancy (sensitive to light, nitrate, and temperature fluctuations)

Technique 1: Germination in Petri Dishes and Transplanting

Stage 1: Breaking dormancy in Petri dishes

1. Stimulation: Place surface-sterilized seeds on filter paper moistened with 0.2% KNO_3 or a 250 ppm GA_3 solution in Petri dishes.
2. Germination: Incubate the Petri dishes in an illuminated growth chamber with an alternating temperature regime (30°C day / 20°C night).

3. Evaluation: High germination percentages are generally obtained within 3–7 days.

Stage 2: Transplanting

Transplant germinated seedlings into pots at a shallow depth (approximately 0.5 cm).

Technique 2: Seed Soaking and Direct Sowing

Stage 1: Seed soaking

Soak seeds in a 0.2% KNO_3 or 250 ppm GA_3 solution at room temperature for 12 hours.

Stage 2: Direct sowing

Drain the seeds, scatter them on the soil surface in pots, and cover them with a very thin layer of potting mix. Place the pots in a bright, warm environment (25–30°C) with a clear day–night temperature difference. Seedling emergence is expected within 5–10 days.

5. Example of Vegetative Dormancy: Johnsongrass (*Sorghum halepense*)

The secret of Johnsongrass: two distinct survival strategies. The success of Johnsongrass arises from strong dormancy mechanisms in both of its reproductive organs (Radosevich et al., 2007):

1. Seed dormancy (long-term insurance): Seeds possess a water-resistant, hard lemma and palea (physical lock) as well as internal physiological dormancy (biological lock).
2. Rhizome dormancy (rapid spread): The underground rhizomes are controlled by apical dominance. The actively growing rhizome tip suppresses the growth of dormant buds behind it. When rhizomes are fragmented by tillage, this suppression is removed, and each piece can give rise to a new plant.

Dormancy-Breaking Protocol for Johnsongrass (*Sorghum halepense*)

Dormancy type: Physical/physiological (seed); apical dominance (rhizome)

Seed protocol

Technique 1: Germination in Petri Dishes

1. Scarification (mandatory): Lightly abrade the seed coat with sandpaper or immerse seeds in concentrated H_2SO_4 for 5–10 minutes, then rinse thoroughly with water.
2. Treatment: Place seeds on filter paper moistened with a 250–500 ppm GA_3 solution in Petri dishes.
3. Germination: Incubate in a warm cabinet with an alternating temperature regime (30–35°C day / 20–25°C night) until germination.

Technique 2: Seed Soaking and Direct Sowing

1. Soaking: After scarification, soak seeds in a 250–500 ppm GA_3 solution for 12–24 hours. 2. Sowing: Drain the seeds and sow them 1–2 cm deep in pots. Keep the pots in a warm environment (25–30°C) until emergence.

Rhizome protocol (vegetative propagation)

The main means of spread in Johnsongrass is its rhizomes. Cutting rhizomes into 3–5 cm segments, each bearing at least one or two nodes/buds, and planting them directly into pots at a depth of 2–3 cm is the most effective propagation method. This procedure physically breaks apical dominance. A warm environment (25–30°C) is required. Seed soaking or Petri dish techniques are not suitable for rhizomes.

3. CONCLUSION

In weeds, dormancy is not a simple sleep state but a complex, multi-layered evolutionary strategy that ensures the survival of species across generations. The dormancy-breaking protocols detailed in this chapter demonstrate how sensitive, species-specific, and yet predictable these biological mechanisms can be. The inability of hard-seeded Wild Vetch to germinate without scarification, the persistence of Redroot Pigweed seeds in the absence of appropriate light and nitrate signals, or the strict dependence of broomrape seeds on host-derived signals all illustrate that each species possesses its own unique key–lock system.

The practical value of this knowledge is considerable. In the laboratory, these protocols enable researchers to produce homogeneous plant material for controlled and repeatable experiments, thereby increasing the success rate of studies ranging from herbicide resistance to biological control. At the field scale, understanding dormancy biology must be at the core of modern and sustainable weed management. Solving a weed problem in a field is not limited to eliminating the visible plants; it also requires managing the invisible seed bank beneath the soil surface. Consciously manipulating dormancy provides the foundation for proactive control tactics such as the stale seedbed technique, whereas preserving dormancy can enhance the effectiveness of conservation tillage systems.

In conclusion, dormancy should no longer be viewed merely as an obstacle but as a dynamic process that can be strategically managed. The scientifically grounded and practical protocols presented in this chapter offer a roadmap for managing this process, removing bottlenecks in experimental work, and ultimately developing more effective, economical, and environmentally friendly weed management strategies.

REFERENCES

Bangarwa, S. K., & Norsworthy, J. K. (2017). Apical dominance influences purple nutsedge (Cyperus rotundus) tuber sprouting. Weed Science, 65(3), 361–369.

Baskin, C. C., & Baskin, J. M. (2014). Seeds: Ecology, biogeography, and evolution of dormancy and germination (2nd ed.). Academic Press.

Benech-Arnold, R. L., Sánchez, R. A., Forcella, F., Kruk, B. C., & Ghersa, C. M. (2000). Environmental control of dormancy in weed seed banks in soil. Field Crops Research, 67(2), 105–122. https://doi.org/10.1016/S0378-4290(00)00087-7

Bewley, J. D. (1997). Seed germination and dormancy. The Plant Cell, 9(7), 1055–1066.

Holm, L. G., Plucknett, D. L., Pancho, J. V., & Herberger, J. P. (1977). The world's worst weeds: Distribution and biology. University Press of Hawaii.

Joel, D. M., Gressel, J., & Musselman, L. J. (Eds.). (2013). Parasitic Orobanchaceae: Parasitic mechanisms and control strategies. Springer.

Lang, G. A. (1987). Dormancy: A new universal terminology. HortScience, 22(5), 817–820.

Pala, F. (2025). Herboloji (Yabancı Ot Mücadelesi). Akademisyen Publishing.

Radosevich, S. R., Holt, J. S., & Ghersa, C. (2007). Ecology of weeds and invasive plants: Relationship to agriculture and natural resource management (3rd ed.). John Wiley & Sons.

Stoller, E. W., & Wax, L. M. (1973). Periodicity of germination and emergence of some annual weeds. Weed Science, 21(6), 574–580.

Yoneyama, K., Awad, A. A., Xie, X., Yoneyama, K., & Takeuchi, Y. (2010). Strigolactones as germination stimulants for root parasitic plants. Plant and Cell Physiology, 51(7), 1095–1103. https://doi.org/10.1093/pcp/pcq055

Zimmerman, J. K., & Weis, I. M. (1983). Fruit size variation and its effects on germination and seedling growth in Xanthium strumarium. Canadian Journal of Botany, 61(8), 2309–2315.

Chapter 8

Weed Survey Methodology: Data-Driven Approaches From Field to Strategy

Fırat PALA [1], Hüsrev MENNAN[2]

1. INTRODUCTION

Agriculture is the cornerstone of global food security; however, the production process is continuously threatened by numerous biotic and abiotic stress factors. Among these biotic factors, weeds constitute one of the groups with the highest potential to cause significant yield and quality losses. A weed is most simply defined as "a plant growing where it is not desired" (Zimdahl & Basinger, 2024). This definition focuses not on the botanical characteristics of the plant, but on its relationship with human activity and agricultural objectives. For example, barley (Hordeum vulgare) in a wheat (*Triticum aestivum*) field or a daisy (*Matricaria chamomilla*) growing on a well-maintained golf course are considered weeds in this context.

The negative impact of weeds on agricultural production arises from their intense competition with cultivated plants for water, nutrients, light, and space. This competition not only leads to direct yield losses, but also causes indirect damage such as making harvest more difficult, reducing product quality, and serving as hosts for diseases and pests. The primary goal of modern agriculture is to minimize these losses while maintaining both economic profitability and environmental sustainability. Achieving this goal requires moving away from traditional, calendar-based or intuition-driven weed control approaches and adopting the philosophy of data-driven weed management.

The first and most critical step of data-driven management is the accurate diagnosis of the problem. Quantitatively measuring the weed population of a field—i.e., conducting a weed survey—is the foundation of this diagnosis. A survey transforms a subjective observation such as "there are a lot of weeds in the field" into an objective and actionable statement like "there are on average

[1] Assoc. Prof. Dr., University of Siirt, Faculty of Agriculture, Department of Plant Protection, Siirt, Türkiye
ORCID ID: 0000-0002-4394-8841
[2] Prof. Dr., Ondokuz Mayis University, Faculty of Agriculture, Department of Plant Protection, Samsun, Türkiye
ORCID ID: 0000-0002-1410-8114

16 redroot pigweed (*Amaranthus retroflexus*) plants per square meter, and the population covers 90% of the field." This transformation is achieved through systematic data collection.

In a weed survey, three fundamental ecological parameters of weed populations are measured: Density (the number of individuals per unit area), Frequency (the distribution or prevalence of the population across the field), and Cover/Dominance (the competitive strength of the plants and the degree to which they occupy space) (Odum, 1971).

These parameters, particularly density, are essential for applying modern plant protection concepts such as the Economic Threshold of Injury (ETI). ETI is defined as the weed density at which the predicted crop yield loss equals the cost of control (Cousens, 1985). Survey results determine whether the weed density in a field exceeds the ETI, providing a scientific basis for deciding whether herbicide application is economically justified.

2. LITERATURE REVIEW

Weed survey methodology lies at the intersection of plant ecology, population dynamics, and agricultural statistics. The scientific validity of this methodology is grounded in the theories and principles developed within these core disciplines.

Population Ecology and Quantitative Measurement: Weed communities are plant populations that form part of an agroecosystem. Understanding their structure and dynamics requires the quantitative methods established in ecology. Odum (1971) emphasized that characterizing a population requires measurement of fundamental parameters such as density, distribution, and age structure. The metrics used in weed surveys—density, frequency, and cover—are direct reflections of these ecological principles. These metrics reveal not only the presence of a species, but its importance and competitive role within the system.

The foundational works of Mueller-Dombois and Ellenberg (1974) on vegetation ecology and plant sociology established standard methods for sampling and analyzing plant communities. Their methodologies, including the widely used Braun-Blanquet cover scale, have been adapted extensively in weed science.

Sampling Theory: Conducting a complete census of all fields (e.g., all wheat fields in a province) is practically impossible due to constraints in time, labor, and cost. Therefore, scientific studies rely on sampling—a smaller subset of the population that accurately represents the whole. Cochran's (1977) seminal work, 'Sampling Techniques,' forms the basis of modern sampling theory,

establishing how sample size should be determined, how sampling errors can be minimized, and which sampling strategies (simple random, systematic, stratified, etc.) should be used in different situations. In weed surveys, determining the number of fields (sample size) using statistical formulas ensures that results reflect the population with a known level of confidence and error margin.

Economic Threshold Concepts and Management Decisions: The ultimate purpose of weed management is to maintain yield losses at economically acceptable levels. Here, the concept of the Economic Threshold of Injury (ETI) becomes essential. Cousens (1985) developed yield–loss models that quantify the relationship between weed density and crop yield reduction, providing a theoretical foundation for determining when control measures become economically justified.

Thus, a weed survey is not a simple counting exercise—it is a scientifically rigorous methodology rooted in ecological theory and statistical principles, directly informing economic decision-making. Radosevich et al. (2007) highlighted that this integrated approach forms the basis of understanding weed ecology and applying this knowledge for sustainable agriculture and natural resource management.

3. PLANNING THE WEED SURVEY

A successful weed survey begins with meticulous planning. This stage includes foundational steps that determine the purpose, scope, and scientific validity of the research.

3.1 Definition, Purpose, and Importance of a Weed Survey

A weed survey is a systematic and quantitative field investigation carried out to identify, measure, and map weed populations within a specific geographic area (field, district, or region). A survey is far more than simply 'taking a look at the field.' It seeks scientific answers to three fundamental questions:

- What? — Which weed species are present in the field? (e.g., *Chenopodium album*, *Avena sterilis*)
- Where? — Where are these species concentrated within the field? (e.g., Cirsium arvense often clusters at field edges or low-lying moist zones.)
- How much? — What are the density, frequency, and cover of each species?

To answer these questions, surveyors collect data using quadrats—small sampling frames that represent the field as a whole (Radosevich et al., 2007).

Purpose and Importance:

A weed survey is the first step toward shifting from intuition-based weed management to evidence-based Integrated Weed Management (IWM). It enables:

- Accurate Strategy Development: Identifying whether the dominant weeds are broadleaf (dicot) or grass species (monocot) guides the selection of appropriate herbicide groups.
- Economic Decision-Making: Survey-generated density data determine whether the weed population exceeds the Economic Threshold of Injury (ETI). This avoids unnecessary applications and reduces environmental pesticide load.
- Herbicide Resistance Monitoring: Repeated surveys help detect herbicide resistance early. For example, a sudden increase of Alopecurus myosuroides in a field treated for years with the same mode of action suggests resistance.
- Long-Term Ecological Monitoring: Surveys reveal how crop rotation, tillage, or climate change influence weed flora over time, and they help track the spread of invasive species (e.g., *Sorghum halepense*).

Table 1. Key Parameters Used in Determining Sample Size

Parameter	**Description & Common Values**	**Importance**
Population Size (N)	Total number of fields/farmers to be surveyed.	Larger populations require proportionally smaller sample ratios.
Confidence Level	Indicates the reliability of results; typically 95%.	A 95% confidence level means results reflect the population with 95% probability.
Margin of Error (e)	Acceptable deviation from the true population value; typically ±5%.	Lower error requires larger sample size.

3.2 Determining Sample Size

Since surveying all fields within a region is impossible, a statistically valid number of fields must be selected. This number ensures representativeness while maintaining practicality (Cochran, 1977).

Step 1: Initial Sample Size for Very Large Populations

$n_0 = (Z^2 \times p \times (1 - p)) / e^2$

Where Z = 1.96 for 95% confidence, p = 0.5 (maximum variability), e = 0.05.

Thus:

$n_0 = (1.96^2 \times 0.5 \times 0.5) / 0.05^2 \approx 385$

Step 2: Finite Population Correction

If n_0 exceeds 5% of N, apply:

$n = n_0 / (1 + ((n_0 - 1) / N))$

Table 2. Minimum Sample Sizes for Different Population Sizes (p = 0.5)

Population Size (N)	95% Conf., ±5% Error	95% Conf., ±7% Error	95% Conf., ±10% Error
1,000	278	165	88
5,000	357	189	94
10,000	370	192	95
50,000	381	196	96
100,000	383	196	96
>100,000	385	196	97

4. FIELD DATA COLLECTION

Systematic and standardized field data collection is essential to ensure the reliability and scientific value of weed survey results.

4.1 Standard Quadrat Method

The quadrat method is one of the most fundamental and widely used sampling techniques in plant ecology and weed science. Because surveying an entire field is impractical, quadrats—small, representative sample units—are used to objectively gather quantitative field data. The core principle of this method is representativeness.

Survey Preparation and Materials:

- Quadrat (Sampling Frame): Common sizes include 0.25 m² (50 × 50 cm) and 1 m² (1 × 1 m), typically made of foldable metal or plastic.
- Recording Forms or Tablets: Standardized survey forms for data entry.
- Additional Tools: GPS device, camera, sample bags, and weed identification guides.

Methodology: Quadrat Placement Timing, Number, and Pattern

- Timing: Surveys should be conducted when weed identification is most reliable—between the 2–6 leaf stage and the early flowering period (Güncan & Karaca, 2018). This ensures accurate identification before crop competition obscures growth.
- Number of Quadrats: The reliability of a survey is directly related to the number of quadrats placed. Literature and current technical guidelines recommend the following ranges:

Table 3. Recommended Quadrat Numbers for Different Field Sizes

Field Size (da)	Recommended Quadrat Count	Notes
0 – 1 da	3 – 5	Suitable for small garden plots or micro-parcels.
1 – 10 da	10 – 15	Minimum for standard field crops.
10 – 50 da	20 – 30	Ideal for cereal fields in regions like Diyarbakır.
50 – 100 da	30 – 50	Increase number when field uniformity decreases.
> 100 da	50+	Or add 1 extra quadrat per 5–10 da.

Practical Tip: If weed distribution is highly patchy (e.g., slopes, areas with standing water), increasing the number of quadrats reduces sampling error.

Quadrat Placement Pattern:

To avoid bias, quadrats should be placed following a W-shaped or Z-shaped walking path across the field. The surveyor should toss the quadrat backward at predetermined step intervals, preventing intentional selection of weed-infested or clean areas (Pala, 2025).

Quadrat Type and Area Conversion:

In annual crop surveys, 0.25 m² quadrats are most commonly used. Since density is reported in plants per square meter, counts obtained from 0.25 m² quadrats must be multiplied by four.

Density (plants/m²) = (Average individuals per quadrat) × 4

4.2 Recording Auxiliary Data (Metadata)

Numerical data alone—e.g., '10 Chenopodium album plants per m²'—provide only a partial picture. Metadata transform raw data into meaningful context. Metadata provide insight into the underlying conditions that shape weed populations.

1. Recording Phenological Stage: Assessing the 'Magnitude of the Threat' Weed competitiveness and control difficulty vary dramatically by growth stage. For example, five Cirsium arvense plants in the seedling stage represent a far smaller threat than five flowering or bolting individuals.
2. Field Background Information: Understanding 'Why This Pattern Occurred'

Key metadata include:

- Survey details (date, surveyor name)
- Location (province/district, GPS coordinates)
- Agronomic practices (crop type, previous crop, tillage system, herbicide history)

This contextual information enables correct interpretation of ecological patterns. For example, if a field shows extremely low broadleaf weed presence but high Echinochloa crus-galli density, metadata may reveal that the previous year the field was planted with corn and treated heavily with atrazine—a herbicide that controls broadleaf weeds but not grasses.

5. DATA ANALYSIS AND INTERPRETATION

The final stage of a weed survey transforms raw field measurements into meaningful, actionable intelligence that directly informs management decisions.

5.1 Core Ecological Metrics

a) Frequency (%)

Frequency expresses the proportion of quadrats in which a species occurs, indicating its distribution and prevalence within the field.

Formula:

Frequency (%) = (Number of quadrats where species i occurs / Total quadrats) × 100

Example:

If Avena fatua is found in 18 out of 20 quadrats:

Frequency = (18/20) × 100 = 90%

This falls under Class E (Very Frequent), indicating a chronic field-wide issue.

Table 4. Frequency Classification Scale (Adapted from Odum, 1971)

Class	Frequency (%)	Ecological Definition	Agronomic Interpretation
A	1–20	Rare occurrence	Likely new infestation; important for early warning.
B	21–40	Infrequent	Established but not widespread; potential to expand.
C	41–60	Moderate	Significant presence; intervention threshold.
D	61–80	Frequent	Major portion of field affected; key management target.
E	81–100	Very Frequent	Nearly entire field; dominant, chronic weed problem.

b) Density (plants/m²)

Density measures the number of individuals of a species per unit area and is the primary input for economic threshold calculations.

For 0.25 m² quadrats:

Density = (Total individuals / Number of quadrats) × 4

Example:

If 80 *Amaranthus retroflexus* individuals are counted in 20 quadrats:

Density = (80/20) × 4 = 16 plants/m²

Table 5. Density Classification Scale

Level	Density (plants/m²)	Interpretation
Very Low	0–1	No economic impact; scattered individuals.
Low	2–5	Present but low competition risk.
Moderate	6–15	Competition begins; potential yield loss.
High	16–30	Strong competition; economic loss likely.
Very High	>30	Extreme infestation; severe yield loss expected.

c) Cover / Dominance (%)

Cover represents the proportion of ground surface occupied by the foliage of a species. It correlates strongly with competitive ability. The Braun-Blanquet cover-abundance scale is widely used for visual estimation.

Table 6. Braun–Blanquet Cover-Abundance Scale

Scale	Description	Cover Range (%)	Midpoint (%)
+	Rare, negligible cover	<1	0.5
1	Numerous but <5% cover	1–5	3.0
2	5–25% cover	5–25	15.0
3	25–50% cover	25–50	37.5
4	50–75% cover	50–75	62.5
5	Dominant, >75% cover	>75	87.5

d) Recording Phenological Stage

Phenology provides essential context for interpreting the competitive potential and urgency of control. General categories follow BBCH codes.

Table 7. Weed Development Stages and BBCH Codes

Code	Stage Description	BBCH Range
GM	Germination / Emergence	00–09
SD	Seedling (2–4 true leaves)	10–14
RO	Rosette stage (broadleaf weeds)	10–19
ST	Stem elongation / Tillering	20–39
FL	Flowering	60–69
FR	Seed / Fruit formation	70–89

5.2 Advanced Analytical Metrics

a) Importance Value Index (IVI)

IVI provides a holistic measure of a species' ecological dominance by combining three relative metrics:

- Relative Density (RD)
- Relative Frequency (RF)
- Relative Cover (RC)

IVI = RD + RF + RC (maximum = 300)

This index identifies the true dominant weed—i.e., the species that should be prioritized for management.

Table 8. Sample Weed Survey Data (20 Quadrats)

Species	Total Count	Quadrats Present	BB Sum	Density	Frequency (%)
Chenopodium album	80	15	84	16	75
Convolvulus arvensis	30	8	135	6	40
Alopecurus myosuroides	120	18	60	24	90

Table 9. Relative Metrics and IVI Calculation

Species	RD (%)	RF (%)	RC (%)	IVI
Chenopodium album	34.78	36.59	30.11	101.48
Convolvulus arvensis	13.04	19.51	48.39	80.94
Alopecurus myosuroides	52.17	43.90	21.51	117.58

6. CONCLUSION: TOWARD DATA-DRIVEN WEED MANAGEMENT

A weed survey is the most critical step for transforming weed management from intuition-based practices to a scientifically grounded, data-driven decision system. It converts vague impressions such as "there are many weeds" into precise, measurable, and actionable information that can guide effective management strategies.

Core ecological metrics—density, frequency, and cover—provide essential insights into the magnitude, spread, and competitive capacity of each weed species. Density quantifies competition intensity, frequency indicates spatial distribution and persistence, while cover reflects the dominance and shading potential of the species.

Synthetic indices such as the Importance Value Index (IVI) integrate these metrics into a single quantitative value, making it possible to identify the true dominant species in the field. This enables precise prioritization of management efforts and resources. Similarly, similarity indices help evaluate the effectiveness of management over time or across different fields by comparing changes in species composition.

Ultimately, weed surveys provide more than ecological data—they deliver a strategic framework that identifies which weed to control, when to intervene, and which control method is most appropriate. Through this systematic approach, farmers and practitioners can enhance profitability, reduce unnecessary herbicide use, limit environmental impact, and support long-term sustainability of agricultural ecosystems.

REFERENCES

Anonim (2025). Zirai Mücadele Teknik Talimatları. TAGEM Publications.

Braun-Blanquet, J. (1932). Plant sociology: The study of plant communities. McGraw-Hill.

Cochran, W. G. (1977). Sampling Techniques (3rd ed.). John Wiley & Sons.

Cousens, R. (1985). A simple model relating yield loss to weed density. Annals of Applied Biology, 107(2), 239–252.

Güncan, A., & Karaca, M. (2018). Yabancı Ot Mücadelesi. Selçuk University Faculty of Agriculture, Konya.

Mueller-Dombois, D., & Ellenberg, H. (1974). Aims and methods of vegetation ecology. John Wiley & Sons.

Odum, E. P. (1971). Fundamentals of Ecology (3rd ed.). W. B. Saunders Company.

Pala, F. (2025). Herboloji (Yabancı Ot Mücadelesi). Akademisyen Yayınevi.

Radosevich, S. R., Holt, J. S., & Ghersa, C. (2007). Ecology of weeds and invasive plants. John Wiley & Sons.

Zimdahl, R. L., & Basinger, N. T. (2024). Fundamentals of Weed Science (6th ed.). Academic Press.

Chapter 9

Machine Learning Applications in Plant Disease Detection

Utku ŞANVER[1]

ABSTRACT

Machine learning has fundamentally transformed the field of plant disease detection by providing highly accurate, efficient, and scalable solutions that exceed the capabilities of traditional diagnostic methods. This domain employs a diverse range of methodologies, including classical machine learning models, deep learning architectures, time-series forecasting, and hybrid approaches, to tackle the intricate challenges associated with plant pathology. These techniques facilitate early and precise disease identification, support predictive analytics, and enable real-time monitoring, thereby advancing sustainable agricultural practices and reducing crop losses. Despite obstacles such as data scarcity and computational demands, ongoing advancements are continually enhancing model performance and applicability. The integration of machine learning-driven systems presents substantial potential for the advancement of precision agriculture, the assurance of food security, and the promotion of environmentally responsible crop management.

1. INTRODUCTION

Agriculture plays a crucial role in the global economy, serving as a fundamental basis for food security, employment, and economic development. It is vital for addressing the nutritional requirements of the increasing global population and serves as a primary source of income for millions of individuals worldwide. The importance of agriculture transcends mere food production, as it also has significant implications for environmental sustainability and rural development (Bala et al., 2024). The losses attributable to plant protection agents in agriculture are substantial, adversely affecting both crop yields and environmental health. Worldwide, crop losses resulting from pests, diseases, and weeds can reach as high as 42%, with certain regions reporting losses nearing 100% in the absence of effective management strategies. (Canhilal &

[1] Siirt University Faculty of Agriculture Department of Plant Protection SIIRT
utkusanver@siirt.edu.tr., ORCID: 0000-0001-5373-2924

Tiryaki, 2010). Plant pathogens, which encompass fungi, bacteria, viruses, and oomycetes, are responsible for annual yield losses ranging from 20% to 40%. This phenomenon presents a significant challenge to the sustainability of agricultural practices (Ayaz et al., 2023). Correctly diagnosing plant diseases is essential for the development of effective management and control strategies. Accurate identification of the causal agents facilitates targeted interventions, thereby reducing crop losses and safeguarding food security (Donoso & Valenzuela, 2018). Conventional methodologies for diagnosing plant pathogens are associated with several notable limitations that impede effective plant protection. These approaches are often time-intensive and exhibit deficiencies in both sensitivity and specificity, which are essential for precise pathogen identification (Kashyap et al., 2016). Machine learning-based systems provide substantial benefits in the diagnosis and detection of plant diseases, primarily through improved accuracy, efficiency, and sustainability. These systems employ sophisticated algorithms, particularly Convolutional Neural Networks (CNNs), to analyze plant images, facilitating early detection and precise classification of diseases. This capability is essential for enhancing agricultural productivity and minimizing crop losses (Singla et al., 2024). CNNs have exhibited remarkable accuracy rates, achieving as high as 98.7% in disease classification (Singh, 2025). Research indicates that deep learning models consistently surpass traditional methodologies, thereby offering reliable diagnostic capabilities (Joshi & Panse, 2023). Machine learning systems can be integrated into mobile applications, enabling farmers to obtain immediate feedback on plant health (Singh, 2025). This real-time capability facilitates timely interventions, which are crucial for effective disease management (Parida & Stonier, 2024). Early detection diminishes the dependence on chemical pesticides, thereby advancing environmentally sustainable agricultural practices (Rodríguez-Lira et al., 2024). By mitigating crop losses, these systems enhance food security and contribute to economic stability within the agricultural sector (Korkut et al., 2018).

The convergence of computer vision, spectroscopy, remote sensing, and IoT technologies has facilitated the development of machine learning (ML) models capable of identifying disease symptoms, assessing disease severity, and detecting infections prior to the manifestation of visible symptoms. The growing accessibility of extensive image datasets, coupled with advancements in computational power, has positioned ML-driven disease detection as a fundamental aspect of "Agriculture 4.0." (Figure 1).

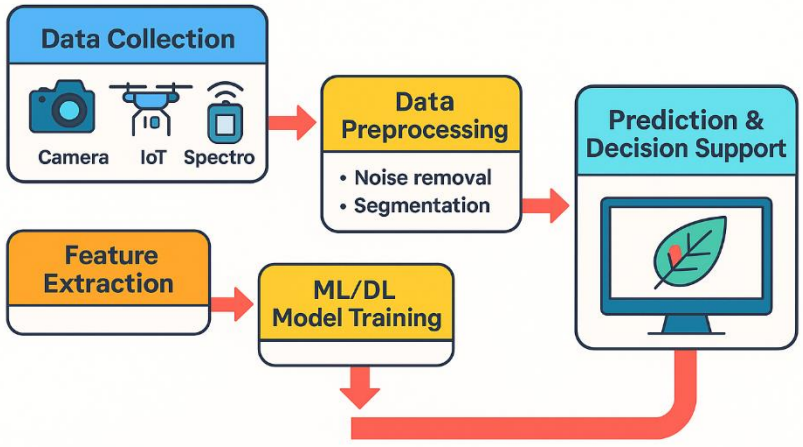

Figure 1. Diagram of the machine learning workflow for plant disease detection

2. MACHINE LEARNING ALGORITHMS USED FOR DISEASE DETECTION

2.1. Traditional Machine Learning Models

Traditional machine learning models have been extensively utilized in the field of plant pathology for the detection and classification of plant diseases. These models, which encompass algorithms such as Support Vector Machines, k-Nearest Neighbors , Random Forest, and Naive Bayes have been employed to analyze features extracted from plant images, including leaf texture and color, to identify disease patterns (Kusumo et al., 2018). While these models have demonstrated potential, they frequently encounter challenges regarding accuracy and computational efficiency when juxtaposed with more advanced deep learning techniques. Nonetheless, they retain value, particularly in contexts where computational resources are constrained or where interpretability is paramount (Gao et al., 2024). The following are key aspects of traditional machine learning models in plant disease detection:

Support Vector Machines (SVM): SVMs are widely regarded for their capacity to manage high-dimensional data and for their performance in scenarios with a clear margin of separation. They have proven effective in situations involving balanced datasets, yielding robust classification results (Ngugi et al., 2024; Waghmare, 2024).

Random Forest (RF): Recognized for its ensemble methodology, RF is particularly adept at addressing imbalanced datasets and is associated with high accuracy in plant disease detection. It is additionally valued for its

interpretability and its capability to manage large datasets without the risk of overfitting (Ngugi et al., 2024; Singh, 2025).

k-Nearest Neighbors (KNN): KNN is a straightforward yet effective algorithm that classifies data based on proximity to the closest training examples within the feature space. It is frequently employed for its ease of implementation and efficacy with smaller datasets (Tanti et al., 2024).

Naive Bayes (NB): While NB is less frequently utilized due to its assumption of feature independence, it can be effective in specific scenarios, although it generally yields lower accuracy when compared to other models (Orchi et al., 2023).

Traditional machine learning models typically exhibit lower accuracy than deep learning models. For example, NB achieved a classification accuracy of only 60.09% in a comparative analysis, whereas deep learning architectures such as InceptionV3 attained accuracies as high as 98.01% (Orchi et al., 2023). Despite their lower accuracy, traditional models are less computationally intensive than their deep learning counterparts, rendering them more suitable for environments with constrained computational resources (Kumar & Singh, 2025). Furthermore, traditional models often offer enhanced interpretability, which is essential for comprehending the decision-making processes involved in plant disease detection (Kumar & Singh, 2025). (Figure 2.)

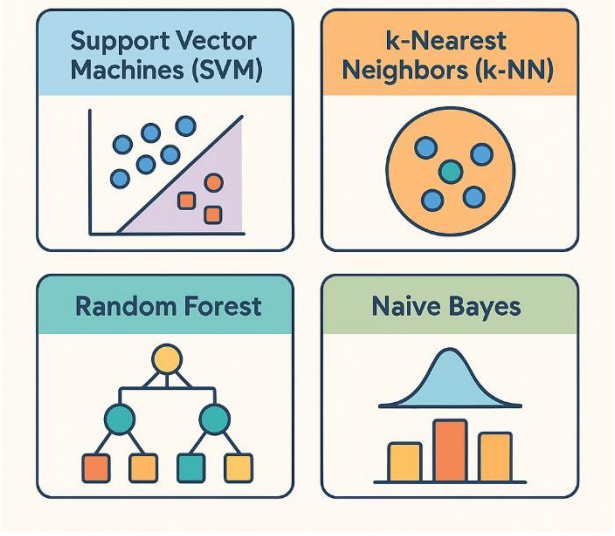

Figure 2. Illustration of traditional machine learning algorithms

2.2. Deep Learning Models

Deep learning models have fundamentally transformed the field of plant disease detection by providing superior accuracy and efficiency in comparison to traditional methodologies. Notably, Convolutional Neural Networks (CNNs) and their various adaptations have been the focus of extensive research due to their capacity to accurately identify diseases from images of plant leaves. This body of research underscores both the strengths and limitations inherent in various deep learning architectures, highlighting their significant potential in the realm of precision agriculture (Marzougui et al., 2020) . The following are critical components of deep learning models in the context of plant disease detection:

Convolutional Neural Networks (CNNs): CNNs are extensively employed in plant disease detection owing to their capability to autonomously extract hierarchical spatial features from images. They have demonstrated remarkable accuracy, with certain studies reporting accuracy rates as high as 98.7% in disease classification (Joshi & Panse, 2023; Singh, 2025).(Figure 3.)

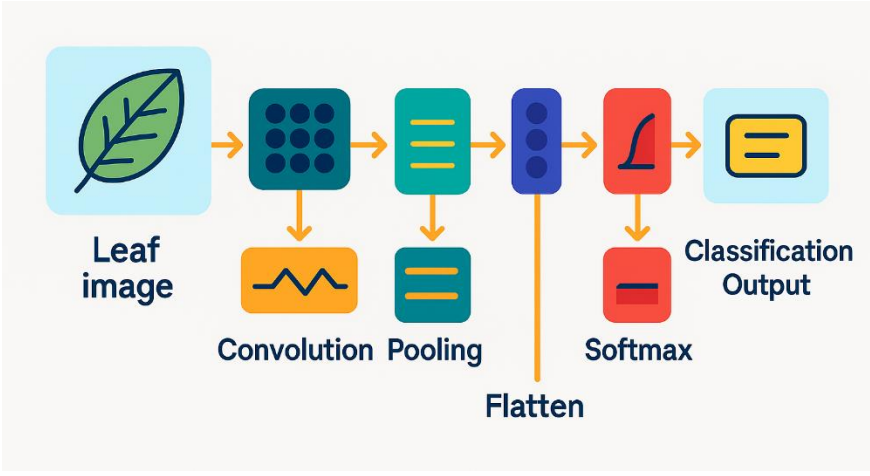

Figure 3. Conceptual workflow of a typical Convolutional Neural Network architecture

DenseNet and VGG16: The densely connected architecture of DenseNet and the profound layer depth of VGG16 are particularly adept at managing intricate patterns associated with diseased leaves, with DenseNet achieving the highest accuracy among the models under investigation (Adiga et al., 2024).

Transfer Learning Models: Models such as MobileNet, VGG19, and ResNet have been assessed for their efficacy when applied to limited training data, a prevalent challenge in agricultural datasets. These models have exhibited

varying levels of success, with MobileNet attaining high accuracy, albeit with slightly lower precision and recall relative to CNNs (Adiga et al., 2024; Khalid & Karan, 2023) (Figure 4.)

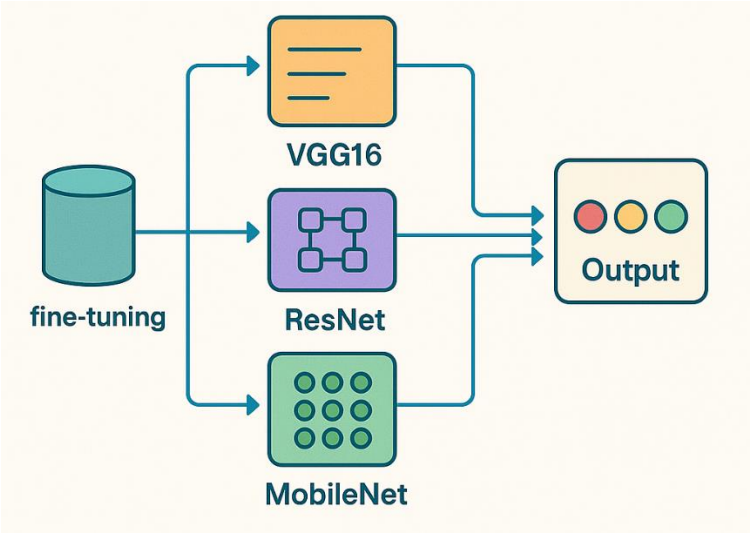

Figure 4. Process schema of Transfer Learning Models

The efficacy of deep learning models frequently diminishes in real-world scenarios due to issues related to data imbalance and a lack of dataset diversity. The availability of high-quality annotated data is essential for the training of effective models (Shivaprasad & Wadhawan, 2023; Zarrouk et al., 2025). Additionally, deep learning models exhibit a propensity for overfitting, particularly when they are trained on limited datasets. The requirement for substantial computational resources further hinders their widespread adoption (Menaka et al., 2025; Shivaprasad & Wadhawan, 2023). The incorporation of eXplainable Artificial Intelligence (XAI) methodologies, such as GradCAM, can facilitate a better understanding of the decision-making processes of deep learning models by providing visual representations of disease indicators (Khalid & Karan, 2023).

2.3. Time-Series Prediction Models

Recurrent Neural Networks (RNN), Long Short-Term Memory (LSTM), and Gated Recurrent Unit (GRU) models have been extensively utilized in the domain of time-series prediction, including the forecasting of plant diseases. These models demonstrate particular efficacy due to their capacity to capture temporal dependencies and patterns inherent in sequential data, a feature that is

vital for accurately predicting the progression of plant diseases (Lalu & Binu Jose, 2021) .

While RNN, LSTM, and GRU models have shown significant promise in plant disease prediction, challenges remain. These include the need for large datasets to train models effectively, the computational resources required, and the scalability of models to different crops and diseases. Future research could focus on developing more efficient models that require less data and computational power, as well as exploring the integration of these models with other machine learning techniques to enhance their predictive capabilities (Metagar & Walikar, 2024; Shinde & Ambhaikar, 2024).

2.4. Hybrid Learning Models

Hybrid learning models in plant disease detection have emerged as a powerful approach to enhancing the accuracy and efficiency of identifying plant diseases. These models integrate various machine learning and deep learning techniques to leverage their respective strengths, thereby offering a robust solution for precision agriculture. The hybrid models not only improve detection accuracy but also provide computational efficiency and scalability, making them suitable for diverse agricultural environments (Nobel et al., 2024)

Hybrid models frequently combine Convolutional Neural Networks (CNN) with other machine learning techniques to enhance feature extraction and classification. For instance, a model that integrates CNN with Random Forest (RF) has demonstrated improved accuracy in classifying tomato diseases by utilizing CNN for feature extraction and RF for robust classification (Chaudhary et al., 2024). Another approach merges CNN with Long Short-Term Memory (LSTM) networks to classify diseases and their severity levels, achieving high accuracy in detecting leaf spot disease in Golden Pothos (Girdher et al., 2023). The combination of CNN with Extreme Gradient Boosting (XGBoost) has proven effective in detecting diseases in vegetable crops, leveraging CNN for pattern extraction and XGBoost for precise categorization (Karim et al., 2025) (Figure 5).

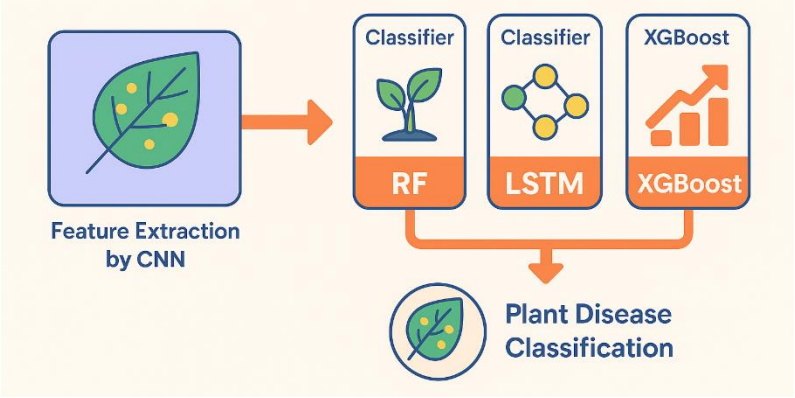

Figure 5. Overview of hybrid learning models for plant disease detection

Hybrid models often employ advanced feature extraction techniques such as Grey Level Co-occurrence Matrix (GLCM) and Local Binary Patterns (LBP) in conjunction with Support Vector Machines (SVM) for classification, providing interpretability and efficiency in resource-limited setups (Kumar & Singh, 2025). The integration of U-Net for segmentation and EfficientNetB7 for feature extraction has yielded state-of-the-art accuracy in potato leaf disease detection, highlighting the significance of precise segmentation in enhancing model performance (Bogireddy & Murari, 2024).

Hybrid models have been applied in various practical scenarios, including online monitoring with drones and diagnostic equipment for farmers, thereby facilitating timely and accurate disease detection (Kumar & Singh, 2025). These models contribute to sustainable agriculture by enabling precise disease identification, reducing the misuse of chemical treatments, and ultimately enhancing crop yields (Sharma et al., 2025). While hybrid learning models offer significant advantages in plant disease detection, it is critical to consider the challenges associated with their implementation. These challenges include the necessity for large annotated datasets, the potential for overfitting due to complex model architectures, and the requirement for domain-specific knowledge to effectively interpret model outputs. Furthermore, the integration of hybrid models into existing agricultural practices may necessitate substantial investment in infrastructure and training for farmers. Nevertheless, the continued development and refinement of hybrid models hold promise for advancing precision agriculture and ensuring food security (Shafik et al., 2025).

3. AREAS OF APPLICATION FOR MACHINE LEARNING IN PLANT DISEASE

Integrating machine learning techniques into existing agricultural practices can significantly improve disease management through several key strategies:

Predictive Analytics for Plant Disease Forecasting: Machine learning models possess the capability to analyze extensive datasets derived from IoT sensors, climatic variables, and historical outbreak records to predict disease occurrences. This predictive capability enables agricultural practitioners to implement preemptive measures to manage potential outbreaks, thereby mitigating crop losses (Delfani et al., 2024). Various machine learning algorithms, including SVM, neural networks, and decision trees, have been employed for plant disease forecasting. Research has demonstrated high levels of accuracy; for example, the application of SVM and feed-forward neural networks to predict potato late blight in Sardinia yielded accuracy rates of 96% and 98%, respectively (Fenu & Malloci, 2019). Additionally, machine learning techniques, such as SVM, have been utilized to develop weather-based disease prediction models. These models incorporate significant weather variables to forecast diseases such as rice blast, thereby providing essential insights to farmers for timely interventions (Kaundal et al., 2006).

Image-Based Plant Disease Detection: The application of computer vision and ML algorithms facilitates the rapid and precise detection of diseases through the analysis of plant imagery. This approach significantly outpaces traditional methods that rely on manual inspections, thereby enabling timely interventions (Dolatabadian et al., 2025). By employing both SVM and DL models, researchers have conducted comparative studies aimed at identifying plant diseases from leaf images. These methodologies are essential to the advancement of precision agriculture, as they allow for the monitoring of disease incidence and severity (Abdu et al., 2020). An automated system utilizing image processing and deep learning has been developed to detect and classify diseases in rice crops. This system employs CNNs and SVMs to accurately identify diseases such as bacterial leaf blight and rice blast through visual analysis (Haridasan et al., 2023). Additionally, a system that integrates artificial intelligence (AI) and wireless sensors for remote monitoring employs machine learning regression techniques to detect and manage cotton leaf diseases. This approach synthesizes soil and environmental data to provide precise disease detection and management recommendations through a mobile application for farmers (Murugamani et al., 2022).

Integration with IoT Devices: The integration of ML with IoT devices enhances the capacity for real-time monitoring and precise management of

agricultural processes. For example, IoT sensors are capable of continuously assessing environmental and soil conditions, while ML models utilize this data for predictive analytics, resource optimization, and disease management (Eze et al., 2025). Through the deployment of IoT sensor networks, this system aggregates environmental and plant-related data to assess the health of grapevines. The data obtained is subjected to analysis via machine learning algorithms to identify and predict grapevine diseases. This configuration enables high-precision management decisions in viticulture, facilitating early detection and prevention strategies (Hnatiuc et al., 2023). Additionally, the RiceTalk project employs non-image IoT devices to detect rice blast by collecting environmental data. This methodology permits real-time monitoring and data analysis through artificial intelligence, thereby providing timely and accurate disease identification without the necessity for labor-intensive manual monitoring (Chen et al., 2020).

Smart Farming Techniques: By leveraging smart farming principles, which incorporate AI, ML, and IoT, farmers can manage resources more efficiently and enhance crop resilience. Smart farming systems are capable of automating critical agricultural functions, including pest and disease control, leading to decreases in resource use and increased yield outcomes (Padhiary & Kumar, 2024).

4. CONCLUSION

Machine learning has fundamentally transformed the field of plant disease detection by providing solutions that are not only highly accurate and efficient but also scalable, thereby surpassing traditional diagnostic methodologies. The incorporation of conventional models, deep learning architectures, time-series forecasting, and hybrid approaches constitutes a robust toolkit for addressing the multifaceted challenges present in plant pathology. These advanced models facilitate early and precise disease identification, enable predictive analytics, and support real-time monitoring through the integration of IoT technologies, thereby enhancing sustainable agricultural practices and reducing crop losses. However, challenges such as data scarcity, computational demands, and complexities associated with implementation persist. Nevertheless, ongoing advancements in model development and explainability continue to enhance their applicability. Ultimately, the adoption of machine learning-driven systems holds significant potential for advancing precision agriculture, ensuring food security, and promoting environmentally sustainable crop management practices.

REFERENCE

Abdu, A. M., Mokji, M. M., & Sheikh, U. U. (2020). Machine learning for plant disease detection: An investigative comparison between support vector machine and deep learning. *IAES International Journal of Artificial Intelligence*, *9*(4), 670–683. https://doi.org/10.11591/ijai.v9.i4.pp670-683

Adiga, A., Gagandeep, N. K., Prabhu, A. A., Pai, H., & Ashok Kumar, R. (2024, December 4). Comparative Analysis on Deep Learning Models for Plant Disease Detection. *International Conference on Emerging Technologies in Computer Science for Interdisciplinary Applications, ICETCS 2024*. https://doi.org/10.1109/ICETCS61022.2024.10543495

Ayaz, M., Li, C.-H., Ali, Q., Zhao, W., Chi, Y.-K., Shafiq, M., Ali, F., Yu, X.-Y., Yu, Q., & Zhao, J.-T. (2023). Bacterial and fungal biocontrol agents for plant disease protection: Journey from lab to field, current status, challenges, and global perspectives. *Molecules*, *28*(18), 6735.

Bala, M., Sharma, R., & Gupta, S. (2024). Integration of hybrid nanomaterials and artificial intelligence for sustainable agriculture. In *Technological Applications of Nano-Hybrid Composites* (pp. 97–118). IGI Global Scientific Publishing.

Bogireddy, S. R., & Murari, H. (2024). A Hybrid Deep Learning Model for Accurate Potato Leaf Disease Detection: Integration of U-Net and EfficientNetB7. *4th International Conference on Power, Energy, Control and Transmission Systems: Harnessing Power and Energy for an Affordable Electrification of India, ICPECTS 2024*. https://doi.org/10.1109/ICPECTS62210.2024.10780105

Canhilal, R., & Tiryaki, O. (2010). Kayseri ve civarında bitki koruma uygulamaları: problemler ve çözüm önerileri. *Erciyes Üniversitesi Fen Bilimleri Enstitüsü Fen Bilimleri Dergisi*, *26*(2), 88–101.

Chaudhary, P., Verma, A., Kukreja, V., & Sharma, R. (2024). Integrating Deep Learning and Ensemble Methods for Robust Tomato Disease Detection: A Hybrid CNN-RF Model Analysis. *2024 11th International Conference on Reliability, Infocom Technologies and Optimization (Trends and Future Directions), ICRITO 2024*. https://doi.org/10.1109/ICRITO61523.2024.10522213

Chen, W. L., Lin, Y. B., Ng, F. L., Liu, C. Y., & Lin, Y. W. (2020). RiceTalk: Rice Blast Detection Using Internet of Things and Artificial Intelligence Technologies. *IEEE Internet of Things Journal*, *7*(2), 1001–1010. https://doi.org/10.1109/JIOT.2019.2947624

Delfani, P., Thuraga, V., Banerjee, B., & Chawade, A. (2024). Integrative approaches in modern agriculture: IoT, ML and AI for disease forecasting

amidst climate change. In *Precision Agriculture* (Vol. 25, Issue 5, pp. 2589–2613). Springer. https://doi.org/10.1007/s11119-024-10164-7

Dolatabadian, A., Neik, T. X., Danilevicz, M. F., Upadhyaya, S. R., Batley, J., & Edwards, D. (2025). Image-based crop disease detection using machine learning. In *Plant Pathology* (Vol. 74, Issue 1, pp. 18–38). John Wiley & Sons, Ltd. https://doi.org/10.1111/ppa.14006

Donoso, A., & Valenzuela, S. (2018). In-field molecular diagnosis of plant pathogens: recent trends and future perspectives. *Plant Pathology*, *67*(7), 1451–1461.

Eze, V. H. U., Eze, E. C., Alaneme, G. U., BUBU, P. E., Nnadi, E. O. E., & Okon, M. Ben. (2025). Integrating IoT sensors and machine learning for sustainable precision agroecology: enhancing crop resilience and resource efficiency through data-driven strategies, challenges, and future prospects. *Discover Agriculture*, *3*(1), 83-. https://doi.org/10.1007/s44279-025-00247-y

Fenu, G., & Malloci, F. M. (2019). An application of machine learning technique in forecasting crop disease. *ACM International Conference Proceeding Series*, 76–82. https://doi.org/10.1145/3372454.3372474

Gao, X., Li, S., Su, X., Li, Y., Huang, L., Tang, W., Zhang, Y., & Dong, M. (2024). Application of Advanced Deep Learning Models for Efficient Apple Defect Detection and Quality Grading in Agricultural Production. *Agriculture*, *14*(7), 1098. https://doi.org/10.3390/agriculture14071098

Girdher, L., Kumar, D., & Kukreja, V. (2023). Detecting and Estimating Severity of Leaf Spot Disease in Golden Pothos using Hybrid Deep Learning Approach. *2023 IEEE 8th International Conference for Convergence in Technology, I2CT 2023*. https://doi.org/10.1109/I2CT57861.2023.10126403

Haridasan, A., Thomas, J., & Raj, E. D. (2023). Deep learning system for paddy plant disease detection and classification. *Environmental Monitoring and Assessment*, *195*(1). https://doi.org/10.1007/s10661-022-10656-x

Hnatiuc, M., Ghita, S., Alpetri, D., Ranca, A., Artem, V., Dina, I., Cosma, M., & Abed Mohammed, M. (2023). Intelligent Grapevine Disease Detection Using IoT Sensor Network. *Bioengineering*, *10*(9). https://doi.org/10.3390/bioengineering10091021

Joshi, G., & Panse, P. (2023). Investigating the Feasibility of Machine Learning Models for Early Detection of Plant Diseases. *International Conference on Self Sustainable Artificial Intelligence Systems, ICSSAS 2023 - Proceedings*, 289–294. https://doi.org/10.1109/ICSSAS57918.2023.10331640

Karim, A., Jumani, T. A., Raza, M. A., Baloch, S. K., Soomro, N. Q., Masud, M., Saeed, M. S., Nahas, M., & Patoli, A. A. (2025). Integration of Deep Learning and Machine Learning Techniques for Advancing the Detection of Plant Diseases. *Engineering Reports*, *7*(6), e70206. https://doi.org/10.1002/eng2.70206

Kashyap, P. L., Rai, P., Sharma, S., Chakdar, H., Kumar, S., Pandiyan, K., & Srivastava, A. K. (2016). Nanotechnology for the Detection and Diagnosis of Plant Pathogens. In *Nanoscience in Food and Agriculture 2* (pp. 253–276). Springer, Cham. https://doi.org/10.1007/978-3-319-39306-3_8

Kaundal, R., Kapoor, A. A., & Raghava, G. P. S. (2006). Machine learning techniques in disease forecasting: A case study on rice blast prediction. *BMC Bioinformatics*, *7*. https://doi.org/10.1186/1471-2105-7-485

Khalid, M. M., & Karan, O. (2023). Deep Learning for Plant Disease Detection. *International Journal of Mathematics, Statistics, and Computer Science*, *2*, 75–84. https://doi.org/10.59543/ijmscs.v2i.8343

Korkut, U. B., Gokturk, O. B., & Yildiz, O. (2018). Detection of plant diseases by machine learning. *26th IEEE Signal Processing and Communications Applications Conference, SIU 2018*, 1–4. https://doi.org/10.1109/SIU.2018.8404692

Kumar, R., & Singh, V. (2025). Advancing Plant Disease Detection: A Comparative Analysis of Deep Learning and Hybrid Machine Learning Models. *Machine Graphics and Vision*, *34*(3), 57–75. https://doi.org/10.22630/MGV.2025.34.3.3

Kusumo, B. S., Heryana, A., Mahendra, O., & Pardede, H. F. (2018). Machine Learning-based for Automatic Detection of Corn-Plant Diseases Using Image Processing. *2018 International Conference on Computer, Control, Informatics and Its Applications: Recent Challenges in Machine Learning for Computing Applications, IC3INA 2018 - Proceeding*, 93–97. https://doi.org/10.1109/IC3INA.2018.8629507

Lalu, J., & Binu Jose, A. (2021). A Survey on Time-Series Data Prediction Models Using Recurrent Neural Networks. In *Second International Conference on Networks and Advances in Computational Technologies* (pp. 285–295). Springer, Cham. https://doi.org/10.1007/978-3-030-49500-8_25

Marzougui, F., Elleuch, M., & Kherallah, M. (2020, November 28). A deep CNN approach for plant disease detection. *Proceedings - 2020 21st International Arab Conference on Information Technology, ACIT 2020*. https://doi.org/10.1109/ACIT50332.2020.9300072

Menaka, K., Hanvitha, D., Nafisa Parvin, S., & Ps, S. S. (2025). Plant Disease Detection Using Deep Learning Model. *2025 International Conference on Sensors and Related Networks, SENNET 2025 - Special Focus on Digital Healthcare (64220)*. https://doi.org/10.1109/SENNET64220.2025.11136045

Metagar, S. M., & Walikar, G. A. (2024). Machine Learning Models for Plant Disease Prediction and Detection: A Review. In *Agricultural Science Digest* (Vol. 44, Issue 4, pp. 591–602). Agricultural Research Communication Centre. https://doi.org/10.18805/ag.D-5893

Murugamani, C., Shitharth, S., Hemalatha, S., Kshirsagar, P. R., Riyazuddin, K., Naveed, Q. N., Islam, S., Mazher Ali, S. P., & Batu, A. (2022). Machine Learning Technique for Precision Agriculture Applications in 5G-Based Internet of Things. *Wireless Communications and Mobile Computing*, *2022*. https://doi.org/10.1155/2022/6534238

Ngugi, H. N., Akinyelu, A. A., & Ezugwu, A. E. (2024). Machine Learning and Deep Learning for Crop Disease Diagnosis: Performance Analysis and Review. In *Agronomy* (Vol. 14, Issue 12, p. 3001). Multidisciplinary Digital Publishing Institute. https://doi.org/10.3390/agronomy14123001

Nobel, S. M. N., Imran, M. A., Bina, N. Z., Kabir, M. M., Safran, M., Alfarhood, S., & Mridha, M. F. (2024). Palm Leaf Health Management: A Hybrid Approach for Automated Disease Detection and Therapy Enhancement. *IEEE Access*, *12*, 9097–9111. https://doi.org/10.1109/ACCESS.2024.3351912

Orchi, H., Sadik, M., Khaldoun, M., & Sabir, E. (2023). Automation of Crop Disease Detection through Conventional Machine Learning and Deep Transfer Learning Approaches. *Agriculture*, *13*(2), 352. https://doi.org/10.3390/agriculture13020352

Padhiary, M., & Kumar, R. (2024). Enhancing Agriculture Through AI Vision and Machine Learning: The Evolution of Smart Farming. In *Advancements in Intelligent Process Automation* (pp. 295–324). IGI Global. https://doi.org/10.4018/979-8-3693-5380-6.ch012

Parida, A. K., & Stonier, A. A. (2024). Plant Disease Detection Using Machine Learning. *2024 4th International Conference on Intelligent Technologies, CONIT 2024*, *3*(4). https://doi.org/10.1109/CONIT61985.2024.10626684

Rodríguez-Lira, D. C., Córdova-Esparza, D. M., Álvarez-Alvarado, J. M., Terven, J., Romero-González, J. A., & Rodríguez-Reséndiz, J. (2024). Trends in Machine and Deep Learning Techniques for Plant Disease Identification: A Systematic Review. In *Agriculture* (Vol. 14, Issue 12, p. 2188). Multidisciplinary Digital Publishing Institute.

https://doi.org/10.3390/agriculture14122188

Shafik, W., Tufail, A., Liyanage De Silva, C., & Awg Haji Mohd Apong, R. A. (2025). A novel hybrid inception-xception convolutional neural network for efficient plant disease classification and detection. *Scientific Reports*, *15*(1), 3936-. https://doi.org/10.1038/s41598-024-82857-y

Sharma, A., -, A. S., -, A. K., -, A. T., & -, satendra K. (2025). A Hybrid Approach for Leaf Disease DetectionUsing Convolutional Neural Networks and VisionTransformers. *IJFMR - International Journal For Multidisciplinary Research*, *7*(2). https://doi.org/10.36948/IJFMR.2025.V07I02.38929

Shinde, N., & Ambhaikar, A. (2024). Comprehensive Survey on Datasets, Models, and Future Directions in Plant Disease Prediction. *International Journal of Image and Graphics*. https://doi.org/10.1142/S0219467826500221

Shivaprasad, K., & Wadhawan, A. (2023). Deep Learning-based Plant Leaf Disease Detection. *Proceedings of the 7th International Conference on Intelligent Computing and Control Systems, ICICCS 2023*, 360–365. https://doi.org/10.1109/ICICCS56967.2023.10142857

Singh, H. (2025). Plant Disease Detection System Using Machine Learning Algorithm. *Indian Scientific Journal Of Research In Engineering And Management*, *9*(5), 1–9.

Singla, A., Nehra, A., Joshi, K., Kumar, A., Tuteja, N., Varshney, R. K., Gill, S. S., & Gill, R. (2024). Exploration of machine learning approaches for automated crop disease detection. *Current Plant Biology*, *40*, 100382.

Tanti, K. S., Gupta, M., Kumar, R., & Obaid, A. J. (2024). A Optimum Review for Plant Leaf Disease Classification using Machine Learning. *Proceedings - 2024 1st International Conference on Technological Innovations and Advance Computing, TIACOMP 2024*, 70–76. https://doi.org/10.1109/TIACOMP64125.2024.00022

Waghmare, U. (2024). Plant Diseases Prediction Using Machine Learning. *International Journal for Research in Applied Science and Engineering Technology*, *12*(10), 1459–1464. https://doi.org/10.22214/ijraset.2024.64917

Zarrouk, Y., Bourhaleb, M., Bouyahrouzi, E. M., Hamdaoui, H., Rahmoune, M., & Hachami, K. (2025). Deep Learning for Plant Disease Detection: Trends and Challenges. *2025 International Conference on Circuit, Systems and Communication, ICCSC 2025*. https://doi.org/10.1109/ICCSC66714.2025.11134855

Chapter 10

Bacteriophage-Mediated Biocontrol of Phytopathogenic Bacteria

Utku ŞANVER[1]

ABSTRACT

Bacteriophage-mediated biocontrol offers a promising and sustainable alternative to conventional chemical and antibiotic treatments for managing phytopathogenic bacteria. This chapter reviews the biology, classification, and life cycles of bacteriophages, emphasizing their lytic mechanisms that enable the targeted destruction of bacterial pathogens. Highlighting their advantages such as high specificity, safety to non-target organisms, self-replication, and biofilm disruption phages emerge as effective agents for controlling major bacterial diseases in crops. Despite challenges related to environmental stability, delivery efficiency, and host specificity, recent advances in formulation and application strategies have enhanced their field efficacy. Empirical evidence demonstrates successful control of key bacterial pathogens through tailored phage therapies, including multi-phage cocktails and integration with agronomic practices. Continued research and optimization are essential to fully realize bacteriophages' potential as integral components of sustainable crop protection systems.

1. INTRODUCTION

Bacterial diseases present a considerable challenge to global agricultural productivity and food security, particularly impacting small-scale farmers (Hoffmann et al., 2025). Approximately 10% of global food production is compromised by plant diseases in developing and emerging economies (Nawaz et al., 2023). Major plant pathogens encompass fungi, viruses, and bacteria. Prominent bacterial genus, including Xanthomonas, Ralstonia, Erwinia, Pseudomonas, and Pectobacterium, are particularly challenging to manage due to their high virulence and adaptability (Nawaz et al., 2023).

Conventional control strategies, especially the use of antibiotics such as streptomycin and tetracycline, as well as copper compounds, are diminishing in

[1] Siirt University Faculty of Agriculture Department of Plant Protection SIIRT
utkusanver@siirt.edu.tr., ORCID: 0000-0001-5373-2924

effectiveness due to the emergence of resistant bacterial strains (Nawaz et al., 2023). Furthermore, the extensive application of these agents raises significant concerns regarding environmental sustainability, human health, and agricultural systems, resulting in issues such as phytotoxicity and diminished microbial diversity in soil (Hoffmann et al., 2025; Nawaz et al., 2023).

In light of these challenges, there is an urgent need for innovative and environmentally sustainable control methodologies (Nawaz et al., 2023). Bacteriophages (phages)—viruses that specifically target and lyse bacteria—have emerged as a promising alternative for disease management in both pre-harvest and post-harvest contexts (Hoffmann et al., 2025; Nawaz et al., 2023). Their natural prevalence in the environment, high specificity, and ability to self-replicate render them ideal biocontrol agents (BCAs) (Hoffmann et al., 2025). This chapter reviews the potential of phages as a sustainable tool, examining their biological characteristics, application strategies, documented success stories, and the critical challenges that must be addressed for widespread implementation.

2. BACTERIOPHAGES: BIOLOGY AND LYTIC MECHANISM

Bacteriophages represent the most abundant biological entities on Earth, characterized by a relatively simple structure that consists of a protein coat encasing genetic material, which may be either DNA or RNA (Dorotkiewicz-Jach et al., 2024; Parija, 2023). These entities undergo various life cycles, including lytic, lysogenic, pseudolysogenic, and chronic cycles, each of which involves distinct interactions with their bacterial hosts (Dorotkiewicz-Jach et al., 2024; Fard, 2016). The lytic cycle culminates in the destruction of the host cell, whereas the lysogenic cycle entails the integration of the phage genome into the host's DNA, thereby permitting its replication alongside that of the host cell (Ansaldi, 2012; Dorotkiewicz-Jach et al., 2024). Bacteriophages play a crucial role in microbial ecology, influencing bacterial populations and driving evolutionary processes through mechanisms such as horizontal gene transfer and lysogenic conversion (Dorotkiewicz-Jach et al., 2024; Fard, 2016). Furthermore, they participate in global geochemical cycles and enhance the genetic diversity of microbial communities (Pieter-Jan & Rob, 2014). While phages can function as both predators and parasites, some researchers advocate for their classification primarily as parasites due to their dependence on host cells for replication (Węgrzyn, 2022).

2.1. Phage Classification

Morphological and Genomic Classification: Bacteriophages are primarily classified based on their morphological characteristics and genomic composition. The predominant category comprises tailed phages, which typically possess double-stranded DNA (dsDNA). These phages are organized into families such as Siphoviridae, Myoviridae, and Podoviridae, all of which fall under the order Caudovirales. Additionally, other phages are classified as cubic, filamentous, or pleomorphic, containing either DNA or RNA (Ackermann, 2011; Moineau & Tremblay, 2017).

ICTV Classification: The International Committee on Taxonomy of Viruses (ICTV) provides a comprehensive hierarchical classification system that is currently undergoing reevaluation to integrate genomic data. This system encompasses two principal orders, Caudovirales and Ligamenvirales, along with various families, subfamilies, genera, and species (Fard, 2016; Pieter-Jan & Rob, 2014).

Genomic Signature Analysis: Recent advancements in phage classification have increasingly involved the analysis of genomic signatures to elucidate evolutionary

relationships and predict host-phage interactions, particularly in distinguishing between lytic and temperate lifestyles (Deschavanne et al., 2010).

2.2. Phage Life Cycle

Lytic Cycle: The lytic cycle involves the infection of a bacterial cell by bacteriophages, which subsequently replicate their genetic material and synthesize new phage particles. This process culminates in the lysis of the host cell, resulting in the release of progeny phages. Characterized by its aggressive nature, the lytic cycle is harnessed for various applications, including biocontrol and phage therapy (Dorotkiewicz-Jach et al., 2024; Kazi & Annapure, 2016).

Lysogenic Cycle: In contrast, the lysogenic cycle entails the integration of phage DNA into the host genome as a prophage, allowing it to replicate alongside the host cell without inducing immediate lysis. This cycle is significant as it may lead to lysogenic conversion, which alters bacterial traits, and facilitates horizontal gene transfer, thereby contributing to bacterial evolution (Dorotkiewicz-Jach et al., 2024; Olszak et al., 2017)

Other Cycles: Additionally, bacteriophages may engage in pseudolysogenic or chronic cycles, during which they persist within the host without integration or lysis. These cycles play a pivotal role in influencing bacterial diversity and adaptive mechanisms (Dorotkiewicz-Jach et al., 2024) (Figure 1).

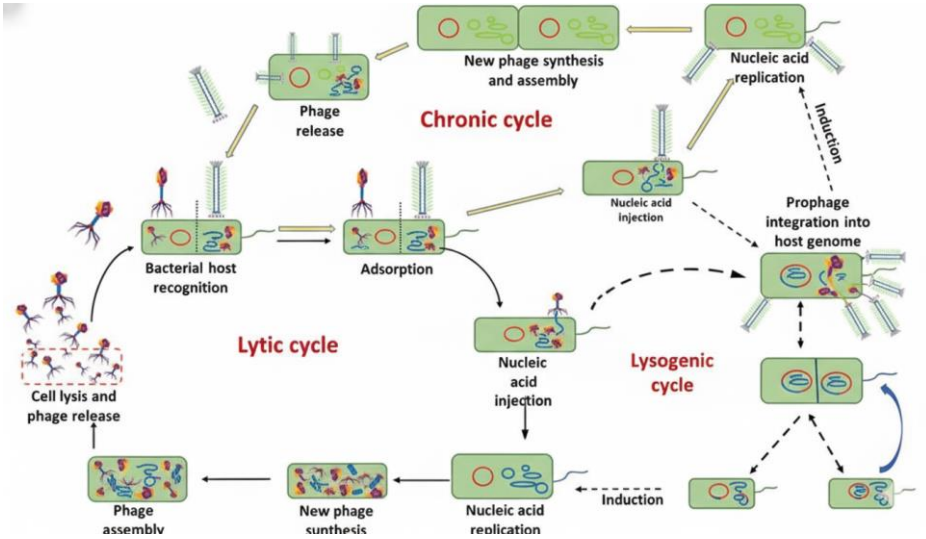

Figure 1. Life cycles of Bacteriophage (Choudhary et al., 2021)

4. ADVANTAGES OF BACTERIOPHAGE BIOCONTROL

Bacteriophages present numerous advantages when compared to conventional chemical treatments, positioning themselves as a viable long-term strategy for pathogen management.

High Specificity and Safety: Bacteriophages are characterized by their high specificity, as they exclusively target designated bacterial pathogens while sparing beneficial microbiota (Hoffmann et al., 2025). This specificity substantially reduces the risk of ecological disturbances in soil and on plant surfaces, thereby aligning with green chemistry principles (Hoffmann et al., 2025). Furthermore, bacteriophages are natural inhabitants of various environments and are non-toxic to eukaryotic cells (including plants, animals, and humans), illustrating an excellent safety profile for both human consumption and environmental application (Hoffmann et al., 2025).

Self-Replication and Overcoming Resistance: In contrast to chemical treatments, lytic bacteriophages possess the capability to self-replicate and exponentially increase in numbers at the site of infection, provided the host bacterium is present. This self-limiting nature allows for a small initial dose to yield sustained antibacterial effects (Sabri et al., 2021).

Moreover, bacteriophages and bacteria engage in continuous co-evolution (Choudhary et al., 2025; Sabri et al., 2021). Bacteriophages exhibit a natural capacity to evolve in response to resistance developed by novel bacterial strains,

an essential consideration in light of the escalating threat posed by antibiotic resistance.

Biofilm Disruption: Phytopathogens frequently persist within biofilms, which confer resistance to metal bactericides and other treatment modalities (Hoffmann et al., 2025; Nawaz et al., 2023). Bacteriophages have developed counter-strategies, including the production of depolymerase enzymes, which dismantle the biofilm matrix, thereby exposing bacterial surface receptors to phages and facilitating successful infection (Hoffmann et al., 2025; Nawaz et al., 2023).

5. CHALLENGES OF BACTERIOPHAGES IN FIELD APPLICATION

The application of bacteriophages in plant protection presents a promising alternative to traditional chemical and antibiotic methods, especially in the face of rising antibiotic resistance. However, several challenges must be addressed to optimize their field application.

Environmental Stability: Bacteriophages exhibit susceptibility to various environmental factors, including ultraviolet (UV) light, extreme temperature conditions, and desiccation, which can considerably diminish their efficacy in agricultural applications. Notably, UV light serves as a significant agent of inactivation for phages on plant surfaces, thereby necessitating the development of protective strategies to mitigate such exposure (Jones et al., 2012; Liu et al., 2024).

Delivery Efficiency: The successful delivery of phages to the site of infection, and their subsequent persistence in adequate quantities, is paramount for therapeutic effectiveness. This task is further complicated by the necessity for high phage populations to be present at critical junctures within the disease cycle (Jones et al., 2012).

Host Specificity and Resistance: Although bacteriophages demonstrate a high degree of specificity toward their bacterial hosts, this characteristic can present challenges, as it necessitates precise matching between phage and pathogen. Furthermore, there exists a potential risk of bacterial populations developing resistance to bacteriophages, akin to the phenomenon of antibiotic resistance (Balogh et al., 2010).

6. APPLICATIONS IN BACTERIOPHAGE THERAPY

The increasing necessity for sustainable agricultural practices has catalyzed research into bacteriophage (phage)-based biocontrol, establishing it as a viable, environmentally friendly alternative to chemical pesticides.

Current literature comprehensively elucidates the efficacy and various application protocols of phages against economically significant phytopathogens, particularly Ralstonia, Xanthomonas, and Pseudomonas species that impact crops such as tomato, potato, and tobacco. Empirical studies have consistently demonstrated the ability of phages to achieve high levels of disease control. For instance, treatments targeting *Ralstonia solanacearum*, the etiological agent of bacterial wilt, have been shown to significantly reduce disease severity through frequent applications of phage cocktails (Wang et al., 2024), with symptom reversal rates reaching as high as 95.6% (Hasanien et al., 2024) and complete control observed in certain experimental trials (Umrao et al., 2021). The efficacy of specific phages, such as PQ43W, has been reported to be superior to that of chemical bactericides in both pot and field settings (Huang et al., 2024).

Similar successes have been documented against Xanthomonas-induced bacterial spot, where phage mixtures have demonstrated to be comparable or superior to conventional copper hydroxide treatments in reducing disease severity (de Sousa et al., 2023). Importantly, advancements in phage formulation technologies, such as the incorporation of nano NAC-ZnS for enhanced UV protection, have effectively addressed issues of phage persistence, thereby improving control under adverse field conditions (Choudhary et al., 2023). In the context of *Pseudomonas syringae* pv. *tomato*, lytic phages have significantly diminished pathogen load and symptoms in tomato foliage (Wu et al., 2024), with one study indicating that phages induce the expression of plant defense genes, thereby suggesting an additional mechanism of action through phage-mediated modulation of plant immunity (Skliros et al., 2023). Moreover, the application of phage cocktails has proven to be more effective than monophage treatments in inhibiting *Pectobacterium caratavorum*, casual agent of soft rot in potato (Parena et al., 2022), highlighting the advantages of multi-phage strategies.

Application protocols are meticulously tailored to the ecological characteristics of the target pathogen. For soilborne pathogens such as *Ralstonia solanacearum*, root drenching or root irrigation are the recommended methods for delivering phages to the rhizosphere (Huang et al., 2024; Umrao et al., 2021). In contrast, foliar spraying is employed for pathogens infecting aerial plant tissues, such as *Xanthomonas spp*. and *Pseudomonas syringae* pv *tomato* on leaves (Wu et al., 2024). Beyond direct phage application, sophisticated strategies include the integration of phage treatment with grafting onto resistant rootstock for comprehensive disease suppression (Nurdika et al., 2023) and the

utilization of stable, broad-range pH and temperature-tolerant phage cocktails to enhance environmental resilience (Hasanien et al., 2024).

Collectively, these findings underscore the versatility of phage therapy, supporting its ongoing development as a fundamental component of future sustainable crop protection.

7. CONCLUSION

Bacteriophage-mediated biocontrol represents a promising and sustainable alternative to conventional chemical and antibiotic treatments for managing phytopathogenic bacteria. Their high specificity, safety profile, self-replicating nature, and ability to disrupt biofilms position phages as effective agents in controlling bacterial plant diseases while minimizing environmental impact. Despite challenges related to environmental stability, delivery efficiency, and host specificity, ongoing advancements in formulation technologies and application strategies are addressing these limitations. Empirical evidence demonstrates significant success in controlling major bacterial pathogens such as Ralstonia, Xanthomonas, and Pseudomonas through tailored phage therapies, including multi-phage cocktails and integration with other agronomic practices. Continued research and optimization are essential to fully harness bacteriophages' potential, paving the way for their widespread adoption as a key component of sustainable crop protection systems.

REFERENCE

Ackermann, H. W. (2011). Bacteriophage taxonomy. *Microbiology Australia*, *32*(2), 90. https://doi.org/10.1071/ma11090

Ansaldi, M. (2012). Cell biology perspectives in phage biology. In *Frontiers in Bioscience - Elite: Vol. 4 E* (Issue 5, pp. 1823–1829). https://doi.org/10.2741/e503

Balogh, B., Jones, J., Iriarte, F., & Momol, M. (2010). Phage Therapy for Plant Disease Control. *Current Pharmaceutical Biotechnology*, *11*(1), 48–57. https://doi.org/10.2174/138920110790725302

Choudhary, M., Bankole, I. A., McDuffee, S. T., Parajuli, A., Poudel, M., Balogh, B., Paret, M. L., & Jones, J. B. (2025). Bacteriophages as Agents for Plant Disease Control: Where Are We After a Century? In *Viruses* (Vol. 17, Issue 8). https://doi.org/10.3390/v17081033

Choudhary, M., Paret, M., Obradović, A., Gašić, K., & Jones, J. B. (2021). Bacteriophages to control plant diseases. In *plantarum.izbis.bg.ac.rs* (pp. 473–506). https://doi.org/10.19103/as.2021.0093.18

Choudhary, M., Pereira, J., Davidson, E. B., Colee, J., Santra, S., Jones, J. B., & Paret, M. L. (2023). Improved Persistence of Bacteriophage Formulation with Nano N-Acetylcysteine–Zinc Sulfide and Tomato Bacterial Spot Disease Control. *Plant Disease*, *107*(12), 3933–3942. https://doi.org/10.1094/PDIS-02-23-0255-RE

de Sousa, D. M., Janssen, L., Rosa, R. B., Belmok, A., Yamada, J. K., Corrêa, R. F. T., de Souza Andrade, M., Inoue-Nagata, A. K., Ribeiro, B. M., & de Carvalho Pontes, N. (2023). Isolation, characterization, and evaluation of putative new bacteriophages for controlling bacterial spot on tomato in Brazil. *Archives of Virology*, *168*(9). https://doi.org/10.1007/s00705-023-05846-y

Deschavanne, P., Dubow, M. S., & Regeard, C. (2010). The use of genomic signature distance between bacteriophages and their hosts displays evolutionary relationships and phage growth cycle determination. *Virology Journal*, *7*(1), 163-. https://doi.org/10.1186/1743-422X-7-163

Dorotkiewicz-Jach, A., Paszkowska, P., Misterkiewicz, A., & Drulis-Kawa, Z. (2024). Bacteriophages: Viruses That Target Bacteria. In *Encyclopedia of Life Sciences* (Vol. 3, pp. 1–12). John Wiley & Sons, Ltd. https://doi.org/10.1002/9780470015902.a0029900

Fard, R. M. N. (2016). A short introduction to bacteriophages. *Trends in Peptide and Protein Sciences*, *1*(1), 7–13.

Hasanien, Y. A., Abdel-Aal, M. H., Younis, N. A., Askora, A., & El Didamony, G. (2024). Bacteriophages as Promising Agents for Biocontrol of Ralstonia

solanacearum Causing Bacterial Wilt Disease. *Egyptian Journal of Botany*, *64*(1), 227–291. https://doi.org/10.21608/ejbo.2023.233873.2474

Hoffmann, A., Sadowska, K., Zenelt, W., & Krawczyk, K. (2025). Bacteriophages as a Sustainable Tool for Plant Disease Management: Benefits and Challenges. *Agronomy*, *15*(11), 2507. https://www.mdpi.com/2073-4395/15/11/2507

Huang, B., Ge, L., Xiang, D., Tan, G., Liu, L., Yang, L., Jing, Y., Liu, Q., Chen, W., Li, Y., He, H., Sun, H., Pan, Q., & Yi, K. (2024). Isolation, characterization, and genomic analysis of a lytic bacteriophage, PQ43W, with the potential of controlling bacterial wilt. *Frontiers in Microbiology*, *15*(9), 686–706. https://doi.org/10.3389/fmicb.2024.1396213

Jones, J. B., Vallad, G. E., Iriarte, F. B., Obradović, A., Wernsing, M. H., Jackson, L. E., Balogh, B., Hong, J. C., & Momol, M. T. (2012). Considerations for using bacteriophages for plant disease control. *Bacteriophage*, *2*(4), e23857. https://doi.org/10.4161/bact.23857

Kazi, M., & Annapure, U. S. (2016). Bacteriophage biocontrol of foodborne pathogens. In *Journal of Food Science and Technology* (Vol. 53, Issue 3, pp. 1355–1362). Springer. https://doi.org/10.1007/s13197-015-1996-8

Liu, S., Quek, S. Y., & Huang, K. (2024). Advanced strategies to overcome the challenges of bacteriophage-based antimicrobial treatments in food and agricultural systems. In *Critical Reviews in Food Science and Nutrition* (Vol. 64, Issue 33, pp. 12574–12598). Taylor and Francis Ltd. https://doi.org/10.1080/10408398.2023.2254837

Moineau, S., & Tremblay, D. M. (2017). Bacteriophage. *Reference Module in Life Sciences*. https://doi.org/10.1016/B978-0-12-809633-8.06122-7

Nawaz, A., Zafar, S., Shahzadi, M., Bukhari, S. M. A. U. S., Khan, N., Shah, A. A., Badshah, M., & Khan, S. (2023). Bacteriophages: an overview of the control strategies against phytopathogens. In *Egyptian Journal of Biological Pest Control* (Vol. 33, Issue 1, p. 108). Springer Science and Business Media Deutschland GmbH. https://doi.org/10.1186/s41938-023-00751-7

Nurdika, A. A. H., Arwiyanto, T., Sulandari, S., Joko, T., & Kandito, A. (2023). Grafting tomato with resistant eggplant and bacteriophages treatment to suppress the development of bacterial wilt disease (Ralstonia pseudosolanacearum). *Archives of Phytopathology and Plant Protection*, *56*(9), 686–706. https://doi.org/10.1080/03235408.2023.2216358

Olszak, T., Latka, A., Roszniowski, B., Valvano, M. A., & Drulis-Kawa, Z. (2017). Phage Life Cycles Behind Bacterial Biodiversity. *Current Medicinal Chemistry*, *24*(36), 3987–4001. https://doi.org/10.2174/0929867324666170413100136

Parena, A. J. S., Silva, B. B. I., Mercado, R. M. L., Sendon, A. A. A., Signabon, F. B., Balidion, J. F., & Encabo, J. R. (2022). Lytic phages display protective effects against soft rot-causing Pectobacterium sp. *Biocontrol Science and Technology*, *32*(11), 1326–1345. https://doi.org/10.1080/09583157.2022.2122403

Parija, S. C. (2023). Bacteriophages. In *Textbook of Microbiology and Immunology* (pp. 733–738). Springer.

Pieter-Jan, C., & Rob, L. (2014). Introduction to Bacteriophage Biology and Diversity. In *Bacteriophages in the Control of Food- and Waterborne Pathogens* (pp. 11–29). ASM Press. https://doi.org/10.1128/9781555816629.ch2

Sabri, M., Benkirane, R., Habbadi, K., Sadik, S., Ou-Zine, M., Diouri, M., & Achbani, E. H. (2021). Phages as a potential biocontrol of phytobacteria. In *Archives of Phytopathology and Plant Protection* (Vol. 54, Issues 17–18, pp. 1277–1291). Taylor and Francis Ltd. https://doi.org/10.1080/03235408.2021.1902033

Skliros, D., Papazoglou, P., Gkizi, D., Paraskevopoulou, E., Katharios, P., Goumas, D. E., Tjamos, S., & Flemetakis, E. (2023). In planta interactions of a novel bacteriophage against Pseudomonas syringae pv. tomato. *Applied Microbiology and Biotechnology*, *107*(11), 3801–3815. https://doi.org/10.1007/s00253-023-12493-5

Umrao, P. D., Kumar, V., & Kaistha, S. D. (2021). Biocontrol potential of bacteriophage ϕsp1 against bacterial wilt-causing Ralstonia solanacearum in Solanaceae crops. *Egyptian Journal of Biological Pest Control*, *31*(1), 1–12. https://doi.org/10.1186/s41938-021-00408-3

Wang, X., Wang, S., Huang, M., He, Y., Guo, S., Yang, K., Wang, N., Sun, T., Yang, H., Yang, T., Xu, Y., Shen, Q., Friman, V. P., & Wei, Z. (2024). Phages enhance both phytopathogen density control and rhizosphere microbiome suppressiveness. *MBio*, *15*(6), e0301623–e0301623. https://doi.org/10.1128/mbio.03016-23

Węgrzyn, G. (2022). Should Bacteriophages Be Classified as Parasites or Predators? In *Polish Journal of Microbiology* (Vol. 71, Issue 1, pp. 3–9). Polish Society of Microbiologists. https://doi.org/10.33073/pjm-2022-005

Wu, Q., An, N., Fang, Z., Li, S., Xiang, L., Liu, Q., Tan, L., & Weng, Q. (2024). Characteristics and whole-genome analysis of a novel Pseudomonas syringae pv. tomato bacteriophage D6 isolated from a karst cave. *Virus Genes*, *60*(3), 295–308. https://doi.org/10.1007/s11262-024-02064-9