

SUSTANIBLE AGRICULTURAL PRODUCTION MANAGEMENT AND INNOVATION

EDITOR

Assist. Prof. Dr. Zübeyir AĞIRAĞAÇ



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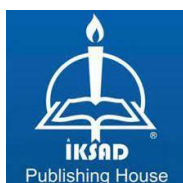
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PREFACE

Agriculture, beyond being one of humanity's oldest endeavors, constitutes the cornerstone of civilization. This journey, which began with the cultivation of the soil, is today being reshaped by technology, data, climate, and sustainability. Throughout history, changing global conditions have transformed agriculture from a mere production activity into a holistic system encompassing economic, ecological, and social dimensions. Rising populations, climate change, and the depletion of natural resources compel humanity to rethink its strategies. At this juncture, agriculture stands not only as a legacy of the past but also as a guarantor of the future. The strongest link between food production, environmental balance, and economic development remains embedded in the soil. An agricultural system supported by scientific knowledge, innovative methods, and sustainable policies is indispensable for both the future of our planet and human well-being. This book has been prepared as an effort to merge agriculture's deep-rooted past with its forward-looking horizons. It is our hope that this work serves as an inspiring resource for all who seek to understand the future of agriculture, reminding them of the respect owed to nature, the guidance of science, and the labor at the heart of production.

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CHAPTER 1

THE EFFECTS OF DIFFERENT SOWING DENSITIES ON YIELD AND QUALITY CHARACTERISTICS OF OILSEED SUNFLOWER (*Helianthus annuus* L.)

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This article was created from a master's thesis.

1. Introduction

Sunflower (*Helianthus annuus* L.) is one of the world's most important oilseed crops due to its high adaptability and the superior nutritional profile of its oil. It provides 38–50 % oil by weight, composed mainly of oleic and linoleic acids. Türkiye ranks among Europe's largest producers but still depends on imports to meet domestic demand. Because land expansion is limited, yield improvement through optimized agronomic practices especially plant density management is essential.

Balanced nutrition and sustainable agricultural production are among the major challenges of the 21st century. Edible oils, being rich in essential fatty acids and fat-soluble vitamins, play a crucial role in human nutrition and health. Sunflower (*Helianthus annuus* L.) is one of the most important oilseed crops worldwide due to its adaptability, mechanization suitability, and high-quality oil composition, containing 85% unsaturated fatty acids, primarily oleic and linoleic acids.

Türkiye ranks among the leading sunflower producers globally, but domestic production still falls short of national consumption, making the country dependent on seed and crude oil imports. Since the expansion of sunflower acreage is limited, yield enhancement through improved agronomic practices especially plant density management has become a strategic priority.

The yield components of sunflower number of heads per unit area, number of seeds per head, and 1000-seed weight are highly influenced by plant population. Excessively high densities increase inter-plant competition, whereas overly wide spacings reduce yield per unit area. Identifying the optimal density for specific cultivars and climatic conditions is therefore critical.

This study aimed to determine the ideal intra-row spacing and hybrid cultivar that would maximize yield and oil content under the ecological conditions of Kırklareli, an important sunflower-producing province in Türkiye.

2. Materials and Methods

2.1. Site description

The experiment was conducted during the 2020 growing season in Babaeski (Kırklareli province). The region experiences a semi-humid climate with warm summers and moderate rainfall. During May–September 2020, the

mean temperature was 22.0 °C, and total rainfall was 183 mm slightly below the long-term average.

2.2. Soil characteristics

Soil analysis indicated clay-loam texture, slightly alkaline pH (7.7), low organic matter (1.29%), and adequate potassium but deficient nitrogen and phosphorus. Fertilizers were applied accordingly. The soil was clay-loam (pH 7.7), low in organic matter (1.29 %), nitrogen (0.06 %), and phosphorus (10.5 ppm).

2.3. Experimental design and plant material

The trial used a split-plot randomized complete block design with three replications. Main plots: three cultivars PL64LP130, LG 5542 CL, Suzuka HTS. Subplots: five intra-row spacings (18, 22, 26, 30, 34 cm), with inter-row spacing fixed at 70cm. Each subplot consisted of 4 rows (5 m length). Standard agronomic practices (sowing, fertilization, irrigation, and weeding) were followed.

2.4. Data collection and analysis

Measured traits included: Plant height (cm), head diameter (cm), stem diameter (cm), Thousand-seed weight (g), hectoliter weight (g/L), seed moisture (%), Seed yield (kg/da), oil content (%), oil yield (kg/da).

The experimental plots were arranged in a split-plot randomized block design. Each subplot consisted of four rows, each 5 meters in length, with 70 cm between rows. Plant spacing was adjusted within rows according to the treatment (18, 22, 26, 30, and 34 cm). Fertilization included 8 kg N/da and 6 kg P₂O₅/da before planting, with an additional 5 kg N/da applied at the 6–8 leaf stage. The field was irrigated once during the flowering period to prevent moisture stress.

Data were analyzed using ANOVA; means were separated by LSD ($p < 0.05$).

Table 1. Climatic and soil characteristics

Parameter	Unit	2020 Value	Long-term Avg	Interpretation
Mean temperature	°C	22.0	21.0	Typical warm summer climate
Total rainfall	mm	183	186	Slightly below average
Relative humidity	%	59.3	61.8	Moderate humidity
Soil pH	–	7.70	–	Slightly alkaline
Organic matter	%	1.29	–	Low
Total N	%	0.06	–	Deficient
Available P	ppm	10.5	–	Deficient
Available K	ppm	270	–	Adequate

3. Results and Discussion

3.1. Morphological and yield characteristics

Table 2. Summary of agronomic parameters across cultivars and plant densities

Trait	Range	Density Effect (Δ Value)	Cultivar Trend
Plant height (cm)	140 – 180	– ↑ +40 cm	Suzuka tallest HTS
Head diameter (cm)	18 – 23	↓ –5 cm	PL64LP130 largest
Stem diameter (cm)	2.0 – 2.5	↓ –0.5 cm	LG 5542 CL thickest
1000-seed weight (g)	55 – 70	↓ –15 g	PL64LP130 highest
Hectoliter weight (g L ⁻¹)	360 – 375	– ↑ +15 g L ⁻¹	Suzuka highest HTS

As plant population increased, plants grew taller but produced smaller heads and lighter seeds, indicating stronger intra-specific competition for resources. These findings align with Göksoy (1992), Kılılı (2004), and Süzer (2010), who reported similar density-dependent effects.

3.2. Seed yield performance

Seed yield was significantly influenced by cultivar and spacing ($p < 0.05$). The highest yield was achieved by Suzuka HTS (268.83 kg/da), followed by PL64LP130 (259.18 kg/da), while LG 5542 CL recorded a lower yield (≈ 240 kg/da).

Table 3. Average seed and oil yield values under different intra-row spacings

Intra-row spacing (cm)	Plant density (plants/da)	Seed yield (kg/da)	Oil content (%)	Oil yield (kg/da)
18	7937	240.10	43.0	103.2
22	6494	247.60	42.0	104.0
26	5495	254.22	41.5	106.0
30	4762	257.65	41.0	108.2
34	4202	244.00	39.0	95.2

Optimal yield occurred at moderate plant densities (26–30 cm), confirming that both excessive crowding and excessive spacing reduce overall productivity. The higher yield of Suzuka HTS suggests better adaptation to semi-arid conditions and efficient resource utilization.

ANOVA results indicated significant effects of cultivar and density on seed yield ($p < 0.01$) and a moderate interaction effect ($p < 0.05$). This demonstrates that sunflower hybrids responded differently to plant density treatments, emphasizing the need for hybrid-specific management.

3.3. Oil quality and varietal response

Oil content rose slightly ($\approx +4\%$) with denser stands due to smaller but oil-rich seeds. However, oil yield peaked at 26–30 cm ($\approx 106\text{--}108$ kg da⁻¹). Suzuka HTS and PL64LP130 showed the highest oil yield, LG 5542 CL the lowest.

Oil content increased slightly with higher plant density, consistent with findings by Mijić et al. (2021). However, oil yield (product of seed yield $\times$ oil %) was maximized at 26–30 cm spacing. Suzuka HTS and PL64LP130 exhibited superior oil yield, while LG 5542 CL lagged behind due to smaller head size and lower seed weight.

The results collectively emphasize that plant density strongly influences sunflower’s yield–quality balance. Moderate densities optimize canopy light interception, minimize lodging, and ensure good oil accumulation.

3.4. Correlation analysis

Table 4. Pearson correlation coefficients among major traits

Trait Pair	r	Relationship	p < 0.05
Plant height × Head diameter	−0.72	Negative	Sig.
Plant height × Seed yield	+0.54	Moderate +	Sig.
Head diameter × Seed yield	+0.61	Positive	Sig.
Seed yield × Oil content	+0.68	Strong +	Highly Sig.
1000-seed weight × Oil content	−0.47	Weak −	n.s.
Hectoliter weight × Oil yield	+0.59	Positive	Sig.

The positive $r = 0.68$ between seed yield and oil content shows that high-yield plants also produce oil-rich seeds. Negative $r = -0.72$ between height and head diameter confirms that crowding induces tall, narrow-headed plants.

3.5 Cultivar × Density Interaction Effects

The interaction between cultivar and plant density was statistically significant for both seed yield and oil content. The hybrid *Suzuka HTS* exhibited the highest and most stable seed yield across different densities, while *PL64LP130* achieved greater seed size at wider spacing. *LG 5542 CL* maintained consistent oil content across treatments

Table 5. Cultivar × Density Interaction on Yield and Oil Content

Cultivar	Intra-row Spacing (cm)	Seed Yield (kg/da)	Oil Content (%)
PL64LP130	18	248.3	42.8
	26	259.2	41.6
LG 5542 CL	18	236.7	43.2
	30	241.5	42.0
Suzuka HTS	26	265.9	41.2
	30	268.8	41.0

The interaction between cultivar and plant density was significant for both seed yield and oil content. Table 5 presents the mean values showing that the Suzuka HTS hybrid consistently maintained higher yields across most densities, while PL64LP130 had superior seed size at lower densities. LG 5542 CL performed moderately but with more stable oil content across densities. LG 5542 CL maintained consistent oil content ($\approx 42\text{--}43\%$), indicating potential for uniform oil quality despite yield fluctuations. Overall, yield differences among cultivars under varying densities highlight the importance of hybrid-specific spacing recommendations. Statistical analysis supports the hypothesis that intermediate densities balance productivity and quality more effectively than extreme population levels.

These findings are consistent with earlier studies by Ekin (2005), Poyraz (2012), and Mijić et al. (2021), who reported similar parabolic yield responses to increasing density. Environmental factors, especially rainfall and soil nutrient status, also affected the outcomes. The moderate density range (4,700–5,500 plants/da) appears optimal for the semi-humid conditions of Kırklareli.

The results showed that increasing plant density led to taller plants, narrower head diameters, and smaller stem diameters. These findings align with those of Turan and Göksoy (1990) and Allam et al. (2003), who reported that higher densities enhance plant height due to light competition while reducing head and stem diameters. Similarly, Mojiri and Arzani (2003) observed that increasing density increases plant height but decreases head diameter, confirming that the ideal density should not exceed 85,000 plants per hectare to avoid yield loss.

The decrease in thousand-seed weight at higher densities was also consistent with the results of Göksoy (1992) and Kaya and Atakişi (2004), who found that denser planting reduces assimilate availability per seed, leading to smaller seeds and lower individual plant yield. Conversely, moderate spacing (26–30 cm) produced optimal seed weight and yield per unit area, supporting the findings of Kılılı (2004) and Ekin (2005).

Oil content increased slightly at higher densities, a pattern similar to that reported by Holt and Campbell (1984) and Allam et al. (2003), who found that reduced light interception and limited seed filling duration at dense stands may enhance oil accumulation relative to protein synthesis. This suggests that moderate crowding promotes physiological efficiency in oil formation.

Overall, seed yield exhibited a parabolic relationship with plant density: yields rose with increasing density up to an optimum, then declined at excessive crowding. This observation agrees with Zubriski and Zimmerman (1974), Gür et al. (2005), and Mijić et al. (2021), who all reported similar quadratic responses in sunflower yield. The highest yield in this study was achieved at 26–30 cm intra-row spacing (approximately 4,700–5,500 plants per decare), consistent with previous regional recommendations for the Trakya and Marmara conditions (Poyraz, 2012; Süzer, 2010).

Among cultivars, Suzuka HTS exhibited superior adaptability and yield stability across density levels, confirming findings by Kaya and Atakişi (2004) that hybrid genotype plays a decisive role in density response. PL64LP130 achieved higher seed weight at wider spacing, while LG 5542 CL maintained a more stable oil percentage, demonstrating distinct genotype $\times$ density interactions similar to those described by Mijić et al. (2021) in Croatian conditions.

4. Conclusion

This research demonstrated that moderate plant densities (26–30 cm intra-row spacing, $\approx$ 4,700–5,500 plants/da) maximize both seed yield and oil yield under the ecological conditions of Kırklareli. Among cultivars, Suzuka HTS showed the highest yield potential and adaptability, followed closely by PL64LP130, whereas LG 5542 CL performed less effectively.

Increases in plant density enhanced plant height and oil content but reduced head and seed size. These trade-offs indicate that excessive crowding should be avoided. The findings highlight the importance of region-specific density optimization to achieve sustainable sunflower productivity and reduce Türkiye's dependence on imported oilseeds.

Future research should evaluate these hybrids under irrigated conditions and varying nitrogen regimes to refine management recommendations for different ecological zones.

The results clearly demonstrate that plant density exerts a strong influence on both morphological and yield characteristics. The highest seed yield (268.83 kg/da) was observed in Suzuka HTS at 30 cm spacing, confirming that this hybrid effectively balances vegetative and reproductive growth. The data suggest that moderate densities (26–30 cm) result in optimal head size and

efficient light interception, while extremely dense stands limit photosynthetic area per plant.

This study identified the optimum intra-row spacing and hybrid combination for maximizing sunflower yield and oil quality under Kırklareli ecological conditions. Moderate plant densities (26–30 cm, corresponding to approximately 4,700–5,500 plants/da) provided the best balance between yield and oil content. Among hybrids, Suzuka HTS outperformed others with a yield of 268.83 kg/da and oil yield above 108 kg/da.

Denser planting increased plant height (+40 cm) and oil percentage (+4%) but reduced head diameter (–5 cm) and seed weight (–15 g). Therefore, intermediate densities should be adopted for efficient land use and sustainable production. Future research should investigate the interaction between density and nitrogen fertilization under both rainfed and irrigated conditions to refine management recommendations.

References

- Akkaya, M. (2006). Determination of sowing time and plant density for confectionary sunflower under Bursa dry conditions. *Uludağ Univ. J. Agric.*, 20(1), 55–63.
- Allam, A. Y., Ghallab, K. H., & El-Nakhlawy, F. S. (2003). *Effect of planting dates and population on yield and oil content of sunflower*. *Egyptian Journal of Agronomy*, 25(2), 85–97.
- Alpman, S. (2019). Effects of different sowing densities on yield and oil quality of sunflower under Adana conditions. Çukurova Univ. MSc Thesis.
- Akkaya, M. (2006). *Determination of sowing time and plant density for confectionary sunflower under Bursa dry conditions*. *Uludağ University Journal of Agriculture*, 20(1), 55–63.
- Anon. (2015). *Nutritional requirements and dietary fat consumption statistics*. Turkish Statistical Institute (TÜİK) Reports.
- Ekin, Z. (2005). *Effects of sowing density on sunflower yield under irrigated Van conditions*. *Yüzüncü Yıl University Journal of Agricultural Sciences*, 15(2), 101–110.
- Göksoy, A. T. (1992). *Effects of sowing date and density on yield and yield components of sunflower in Marmara region*. MSc Thesis, Uludağ University, Bursa, Türkiye.
- Gür, A., Kılıç, S., & Süzer, S. (2005). *Determination of optimum sowing date and density for sunflower in Harran Plain conditions*. *Harran University Journal of Agriculture*, 9(3), 45–53.
- Holt, N. W., & Campbell, L. G. (1984). *Effect of plant population and environment on sunflower yield components*. *Canadian Journal of Plant Science*, 64(2), 447–452.
- Kara, B. (1991). *Agronomic characteristics of local and foreign oilseed sunflower cultivars under Erzurum conditions*. *Atatürk University Journal of Agricultural Faculty*, 22(1), 53–61.
- Kaya, Y., & Atakişi, İ. (2004). *Performance of different sunflower hybrids in Trakya region*. *Trakya University Journal of Natural Sciences*, 5(1), 15–23.

- Kıllı, F. (2004). *Influence of plant population and nitrogen levels on sunflower yield*. Kahramanmaraş Sütçü İmam University Journal of Science and Engineering, 7(2), 57–65.
- Mijić, A., Liović, I., Jukić, G., & Krizmanić, M. (2021). *Impact of plant density on yield and oil content in sunflower hybrids under Croatian conditions*. Helia, 44(74), 25–36.
- Mojiri, A., & Arzani, A. (2003). *Effects of plant density and nitrogen rate on sunflower yield and yield components*. Iranian Journal of Agricultural Sciences, 34(4), 879–886.
- Poyraz, M. (2012). *Determination of appropriate intra-row spacing for sunflower hybrids in Trakya region*. MSc Thesis, Namık Kemal University, Tekirdağ, Türkiye.
- Süzer, S. (2010). *Effect of nitrogen level and plant density on yield of dwarf sunflower hybrids under Edirne conditions*. Turkish Journal of Field Crops, 15(2), 127–134.
- Turan, Z. M., & Göksoy, A. T. (1990). *Effect of plant density on sunflower yield under Bursa dry conditions*. Uludağ University Journal of Agriculture, 4(1), 87–94.
- Zubriski, J. C., & Zimmerman, D. C. (1974). *Effect of nitrogen and plant population on sunflower production*. Agronomy Journal, 66(2), 330–332.

CHAPTER 2

CYTOGENETICS OF WEEDS

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1. INTRODUCTION

Weeds represent one of the most persistent biological constraints in global agriculture, responsible for substantial crop yield losses, reductions in quality, and increased production costs. Estimates suggest that yield losses due to weeds often exceed those caused by insects and plant pathogens combined (Oerke, 2006; Zimdahl, 2018). Their competitive success is attributed to ecological plasticity, prolific seed production, rapid adaptation to changing environments, and underlying genetic and cytogenetic variability (Powles & Yu, 2010).

Cytogenetics, defined as the study of chromosomes, their structure, organisation, and inheritance, provides a vital framework for understanding the evolutionary success of weeds. Chromosomal variability underpins many adaptive processes, including polyploidy, hybridisation, structural rearrangements, and gene amplification (Leitch & Leitch, 2008; Soltis et al., 2015). As weeds often occupy disturbed habitats and face strong anthropogenic selection pressures from cultivation practices and herbicide use, they serve as excellent models for exploring cytogenetic mechanisms of adaptation (Stebbins, 1950; Neve et al., 2009).

The history of cytogenetic research in weeds dates back to the early 20th century, when chromosome counting and karyotype analysis were applied to species of Poaceae, Asteraceae, and Amaranthaceae. These classical cytological studies revealed the importance of polyploidy and hybrid origins in weed evolution (Grant, 1981; Gupta, 1998). With the development of molecular cytogenetics in the late 20th century, techniques such as fluorescent in situ hybridisation (FISH) and genomic in situ hybridisation (GISH) enabled mapping of repetitive DNA, localisation of ribosomal DNA loci, and determination of parental genome contributions in hybrids (Schwarzacher & Heslop-Harrison, 2000; Raskina et al., 2011).

Modern advances now integrate cytogenetics with genomics and high-throughput technologies. Flow cytometry provides rapid estimates of genome size and ploidy levels (Doležel et al., 2007), while next-generation sequencing (NGS) combined with cytogenetic tools allows precise mapping of structural polymorphisms and chromosomal rearrangements in weed genomes (Michael & VanBuren, 2015). These approaches are crucial for elucidating the chromosomal basis of adaptive traits, including herbicide resistance. For

example, EPSPS (5-enolpyruvylshikimate-3-phosphate synthase) gene amplification in *Amaranthus palmeri* has been demonstrated cytogenetically as a mechanism of glyphosate resistance (Gaines et al., 2010; Koo et al., 2018). Similarly, hybridisation between wild and cultivated rice (*Oryza* spp.) has been shown to facilitate the introgression of herbicide resistance and other adaptive traits, a process detectable through cytogenetic analyses (Gealy, 2005; Ellstrand et al., 2013).

Thus, cytogenetics provides not only evolutionary and taxonomic insights but also direct applications for agriculture and weed management. The objective of this chapter is to present a comprehensive overview of cytogenetics in weeds. We will first review the fundamentals of plant cytogenetics and the methodological approaches used in weed research. Next, we will examine chromosome numbers, karyotype diversity, polyploidy, and structural rearrangements across different weed taxa. Special attention will be given to cytogenetic mechanisms of herbicide resistance, interspecific hybridisation, and adaptive evolution. Finally, we will discuss future perspectives, highlighting the integration of cytogenetics with genomics, epigenetics, and genome editing technologies that may transform weed science in the coming decades.

2. FUNDAMENTALS OF PLANT CYTOGENETICS

Cytogenetics is the branch of genetics that focuses on the study of chromosomes—their structure, number, organisation, behaviour during cell division, and the consequences of chromosomal variation for inheritance and evolution. For weeds, cytogenetics provides the foundation to understand genetic diversity, evolutionary trajectories, and mechanisms of adaptation, including polyploidy and hybridisation, which are central to their ecological success. This section summarises the fundamental concepts of plant cytogenetics, emphasising chromosome structure, number, ploidy variation, karyotype analysis, and chromosomal aberrations that are relevant to weed biology.

2.1 Chromosome Structure and Organisation

Plant chromosomes consist of DNA packaged with histone and non-histone proteins into chromatin, which undergoes compaction during cell division. Structurally, chromosomes consist of two arms separated by a

centromere, which plays a crucial role in the formation of kinetochores and chromosome segregation (Comai, 2005). Based on centromere position, chromosomes may be classified as metacentric, submetacentric, acrocentric, or telocentric, and this morphology contributes to the karyotype profile of a species (Levin, 2002).

Telomeres, composed of repetitive DNA sequences, cap the ends of chromosomes and maintain genomic stability. In plants, telomere sequences are typically TTTAGGG repeats (Fajkus et al., 1995). Another key chromosomal region is the nucleolar organiser region (NOR), which contains ribosomal RNA genes and is cytologically visible as secondary constrictions. These structural features are widely used in cytogenetic studies to identify and compare chromosomes of different weed species.

2.2 Chromosome Number and Ploidy Variation

Chromosome numbers vary greatly among plants, and weeds are no exception. For instance, *Amaranthus retroflexus* (redroot pigweed) has $2n = 34$, *Echinochloa crus-galli* (barnyardgrass) exhibits high variability ranging from diploids to hexaploids ($2n = 36\text{--}54$), and *Chenopodium album* (common lambsquarters) shows chromosome counts between $2n = 54$ and 72 depending on cytotypes (Sheidai et al., 2003; Michael, 2014).

Polyploidy, the presence of more than two sets of chromosomes, is one of the most significant cytogenetic phenomena in plants, with an estimated 70% of angiosperms showing evidence of ancient or recent polyploidization events (Soltis et al., 2009). Weeds often display polyploidy, which confers ecological flexibility, tolerance to environmental stress, and enhanced genetic variation. For example, polyploid *Cirsium arvense* (Canada thistle) and *Rumex crispus* (curly dock) exhibit broader ecological ranges than their diploid relatives (Te Beest et al., 2012).

2.3 Karyotyping and Banding Patterns

Karyotype analysis involves the systematic characterisation of chromosome number, morphology, and relative sizes within a species. It is essential for identifying polyploidy, aneuploidy, and structural rearrangements in weed populations. Karyotypic asymmetry, such as the presence of numerous acrocentric or telocentric chromosomes, often indicates evolutionary divergence (Stebbins, 1971).

With the advent of molecular cytogenetics, banding techniques such as C-banding (heterochromatin staining) and fluorochrome banding (using DAPI or CMA3) have provided additional resolution. These methods enable the detection of heterochromatin distribution, which can vary significantly among weed species and populations (Greilhuber, 1984). For instance, heterochromatin polymorphisms have been reported in *Avena fatua* (wild oat), reflecting its adaptive diversity and hybrid origins (Jellen et al., 1994).

2.4 Chromosomal Aberrations

Chromosomal aberrations, including deletions, duplications, inversions, and translocations, play a crucial role in generating genetic variability in weeds. Such structural rearrangements can arise spontaneously or be induced by environmental stresses and human agricultural activities (Schubert & Lysak, 2011).

Aneuploidy, the gain or loss of individual chromosomes, is less common than polyploidy; however, it has been observed in some weed species. Aneuploid cytotypes of *Echinochloa* spp. and *Chenopodium album* are associated with variation in morphology and adaptation (Sheidai et al., 2003).

Chromosomal rearrangements also play an important role in reproductive isolation and speciation. In *Amaranthus* spp., Robertsonian translocations and inversions have been suggested as mechanisms contributing to the diversification of this genus, which includes many agriculturally significant weeds (Pal et al., 1982).

2.5 Cytogenetics and Weed Adaptability

The cytogenetic features outlined above, chromosome structure, ploidy variability, karyotype differentiation, and chromosomal aberrations, are not merely academic curiosities. They provide a mechanistic understanding of weed adaptability. Polyploidy enables weeds to colonise diverse environments, while chromosomal rearrangements can rapidly generate novel genetic combinations. Together, these cytogenetic processes form the foundation of the evolutionary potential that allows weeds to thrive under strong anthropogenic pressures, including herbicide applications and monoculture cropping systems.

3. CYTOGENETIC TOOLS AND TECHNIQUES IN WEED RESEARCH

Cytogenetic tools have evolved significantly from classical light microscopy methods to advanced molecular techniques that combine cytology with genomics. These tools are fundamental for weed research because they enable the identification of chromosome numbers, ploidy variation, karyotype structures, hybridisation events, and gene amplification associated with herbicide resistance. This section outlines the main cytogenetic methodologies applied in weed studies, including classical cytogenetics, molecular cytogenetics, flow cytometry, and integrative cytogenomic approaches.

3.1 Classical Cytogenetics

3.1.1 Chromosome counting

Chromosome counting through mitotic or meiotic cell preparations is the earliest cytogenetic tool applied to weeds. Root tips, young inflorescences, and embryonic tissues are commonly used for metaphase chromosome spreads. These counts remain critical for establishing basic chromosome numbers and identifying polyploidy.

For example, chromosome counts have revealed cytological diversity in *Echinochloa crus-galli* (barnyardgrass), where populations may vary from diploids ($2n = 36$) to hexaploids ($2n = 54$), explaining its broad ecological adaptation (Yabuno, 1962). Similarly, studies of *Amaranthus* spp. have demonstrated significant interspecific variability, contributing to taxonomic classification and evolutionary inferences (Pal et al., 1982).

3.1.2 Meiotic studies

Meiosis provides insights into chromosomal behaviour, pairing, and segregation. Analysis of meiotic irregularities (e.g., univalents, multivalents, laggards, bridges) is useful for identifying structural rearrangements, polyploidization, or hybrid origin. In *Avena fatua* (wild oat), meiotic configurations support its derivation from interspecific hybridisation, as revealed by the occurrence of multivalent associations (Leggett & Thomas, 1995).

3.1.3 Karyotype analysis

Karyotyping involves measuring chromosome lengths, arm ratios, and morphology to construct karyograms. Comparative karyotyping among weed taxa highlights differences in chromosomal symmetry and evolution. Species with symmetrical karyotypes (mainly metacentric chromosomes) are generally considered more primitive, while asymmetrical karyotypes with acrocentric and telocentric chromosomes indicate advanced evolution (Stebbins, 1971).

3.2 Molecular Cytogenetics

3.2.1 Fluorescence in Situ Hybridisation (FISH)

FISH enables localisation of specific DNA sequences on chromosomes using fluorescently labelled probes. Ribosomal DNA (45S and 5S rDNA), telomeric repeats, and repetitive sequences are frequently mapped in weeds to study genome organisation.

For instance, FISH mapping has been applied in *Chenopodium album* to reveal polymorphism in rDNA loci, correlating with ploidy variation and adaptability (Kolano et al., 2012). In *Amaranthus*, FISH of repetitive DNA sequences has clarified interspecific chromosomal variation and evolution (Bennett & Leitch, 2012).

3.2.2 Genomic In Situ Hybridisation (GISH)

GISH is particularly valuable for detecting parental genome contributions in hybrids, which is crucial for understanding weed evolution. For example, GISH analysis in *Echinochloa* spp. has revealed complex hybrid origins and polyploidy events that underlie their invasive success (Yamaguchi et al., 2005).

3.2.3 Chromosome painting

Although less developed in weeds compared to model plants, chromosome painting with multiple probes can identify structural rearrangements, translocations, or introgression from crops to weedy relatives. This tool has potential applications in tracking gene flow, such as the transfer of herbicide resistance alleles from transgenic crops to wild or weedy relatives (Wang et al., 2010).

3.3 Flow Cytometry

Flow cytometry provides a rapid estimation of genome size (C-value) and ploidy levels by measuring nuclear DNA content through fluorescence intensity of stained nuclei. It has revolutionised cytogenetics by allowing large-scale screening of populations.

In *Amaranthus retroflexus* and *Amaranthus powellii*, flow cytometry has been used to distinguish cytotypes and assess hybridisation potential (Kaufman et al., 1985). Similarly, genome size variability detected in *Cirsium arvense* populations suggests that cytogenetic plasticity contributes to invasiveness (McAllister et al., 2015).

3.4 Integrative Cytogenomics

The integration of cytogenetics with next-generation sequencing (NGS) has transformed weed research. Combining FISH with sequence data enables the development of chromosome-specific probes, allowing for high-resolution mapping. For instance, extrachromosomal circular DNA carrying EPSPS gene copies was detected in glyphosate-resistant *Amaranthus palmeri* using a combination of cytogenetic and genomic approaches (Koo et al., 2018).

Comparative genomics and cytogenomics also reveal synteny and structural variation between weed and crop genomes, offering insights into the evolutionary processes that contribute to weediness and resistance traits (Michael & VanBuren, 2015).

3.5 Emerging Approaches

Recent advances include CRISPR-based labelling systems, such as CRISPR/dCas9-FISH, which allow real-time visualisation of specific genomic regions. Such tools may soon be applied to weeds to monitor chromosomal dynamics under stress or during hybridisation. Single-cell sequencing combined with cytogenetics is also an emerging field that could unravel intrapopulation chromosomal variability in highly adaptable weeds.

4. CHROMOSOME NUMBERS AND KARYOTYPE VARIABILITY IN WEEDS

Cytogenetic studies of weeds have revealed remarkable diversity in chromosome numbers and karyotype organisation. Chromosome numbers are fundamental cytological characters that often serve as taxonomic markers,

providing insights into evolutionary relationships (Levin, 2002). In weeds, wide variability in chromosome numbers and karyotype structures reflects evolutionary processes such as polyploidy, aneuploidy, dysploidy (ascending or descending changes in basic chromosome numbers), and structural rearrangements (Stebbins, 1971; Grant, 1981). These processes significantly contribute to the ecological success of weeds, enabling them to adapt to diverse environments and resist control strategies.

4.1 Chromosome Numbers in Major Weed Families

Chromosome number databases and cytological surveys demonstrate that many weedy taxa occur in families characterised by high chromosomal variability (Figure 1).

4.1.1 Poaceae (Grasses)

Weeds in the grass family, such as *Echinochloa*, *Lolium*, *Avena*, and *Setaria*, exhibit wide variation in chromosome number. *Echinochloa crus-galli* is a hexaploid with $2n = 6x = 54$; however, cytotypes with $2n = 36-72$ have also been reported (Yabuno, 1962). *Avena fatua* (wild oat) exhibits $2n = 42$, consistent with its hexaploid nature, while *Lolium rigidum* (rigid ryegrass), a major herbicide-resistant weed, is diploid with $2n = 14$ (Bennett & Smith, 1991).

4.1.2 Asteraceae (Sunflower/Daisy Family)

The Asteraceae family contains many invasive and noxious weeds, including *Cirsium arvense* (Canada thistle; $2n = 34$), *Ambrosia artemisiifolia* (common ragweed; $2n = 36$), and *Parthenium hysterophorus* (parthenium weed; $2n = 18$) (Bawa, 1973). High karyotypic diversity in Asteraceae weeds often results from polyploidy and chromosomal rearrangements, which are common drivers of speciation in the family (Stuessy et al., 2009).

4.1.3 Amaranthaceae

Amaranth weeds, such as *Amaranthus retroflexus* (redroot pigweed; $2n = 34$) and *Amaranthus palmeri* (Palmer amaranth; $2n = 34$), show relatively stable chromosome numbers, yet substantial variation exists in genome size and karyotype asymmetry (Pal et al., 1982). The genus is cytogenetically significant due to its rapid evolution and multiple herbicide-resistant species.

4.1.4 Brassicaceae

Weedy species of Brassicaceae, including *Sinapis arvensis* (wild mustard; $2n = 18$) and *Raphanus raphanistrum* (wild radish; $2n = 18$), exhibit chromosome numbers consistent with related crops but differ markedly in karyotype structure. These similarities facilitate hybridisation and gene flow with cultivated relatives (Snow et al., 1999).

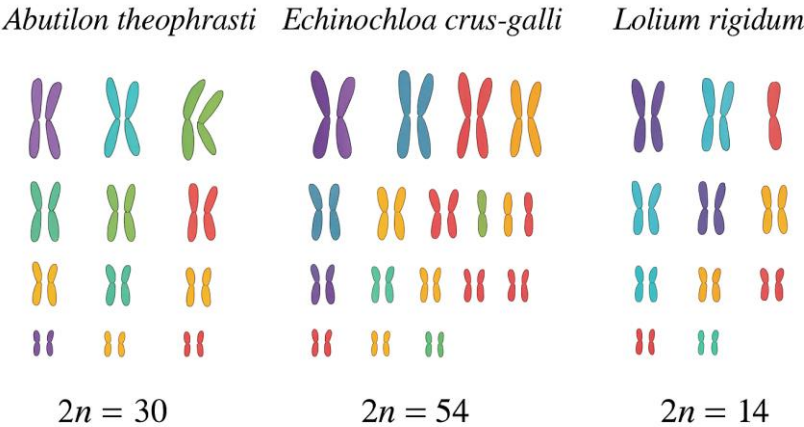


Figure 1. Karyotypes of weed species

4.2 Polyploidy in Weed Cytogenetics

Polyploidy (whole-genome duplication) is one of the most important processes shaping weed cytogenetics. It occurs through autopolyploidy (duplication within one species) or allopolyploidy (hybridisation followed by chromosome doubling).

Autopolyploid weeds, such as *Rumex obtusifolius* (broadleaf dock) and *Taraxacum officinale* (dandelion), exhibit adaptability to diverse climates due to autopolyploidy (te Beest et al., 2012).

Allopolyploid weeds, such as *Avena fatua* is an allopolyploid derived from hybridisation among ancestral diploid oats (Leggett & Thomas, 1995). Similarly, *Echinochloa* species demonstrate recurrent hybridisation and polyploidization, enabling their widespread invasiveness (Yamaguchi et al., 2005).

Polyploidy enhances weed adaptability by providing genetic redundancy, buffering against deleterious mutations, and creating opportunities for novel gene functions (Comai, 2005). It also contributes to herbicide tolerance by increasing gene copy number and metabolic diversity.

4.3 Aneuploidy and Dysploidy in Weeds

Aneuploidy, the loss or gain of individual chromosomes, is relatively rare in stable populations but has been observed in weedy taxa with high cytogenetic plasticity. *Chenopodium album* exhibits aneuploid cytotypes with chromosome counts ranging from $2n = 54$ to 72 (Sheidai et al., 2003). Such variation contributes to morphological diversity and ecological plasticity.

Dysploidy, or stepwise changes in basic chromosome number, is another contributor to karyotype evolution in weeds. In Asteraceae, descending dysploidy has played a role in the diversification of invasive *Cirsium* species (Bureš et al., 2004).

4.4 Karyotype Variability and Weed Evolution

Karyotype asymmetry, which reflects differences in chromosome size, arm ratio, and centromere position, is a significant indicator of evolutionary advancement (Stebbins, 1971). In weeds, highly asymmetrical karyotypes are often associated with polyploidization and structural rearrangements.

For example:

- *Cirsium arvense* shows extensive variation in karyotype morphology between populations, indicating ongoing chromosomal evolution (McAllister et al., 2015).
- *Amaranthus* species exhibit Robertsonian translocations and pericentric inversions, contributing to diversification within the genus (Pal et al., 1982).
- In *Chenopodium*, karyotype studies reveal polymorphism in heterochromatin distribution, linked to adaptation to different habitats (Kolano et al., 2012).

4.5 Chromosome Databases and Weed Cytogenetics

Resources such as the Plant DNA C-values Database (Bennett & Leitch, 2012) and Index to Plant Chromosome Numbers (IPCN; Goldblatt & Johnson, 1994) provide comprehensive cytogenetic data for weeds. These databases

facilitate comparative studies and highlight gaps in research on weed cytogenetics. Many important weeds remain underexplored, underscoring the need for expanded karyotype analyses and integration with genomic data.

In summary, chromosome numbers and karyotype variability in weeds are shaped by polyploidy, aneuploidy, dysploidy, and structural rearrangements. These cytogenetic mechanisms not only drive taxonomic and evolutionary diversity but also underpin ecological adaptability and invasiveness. Understanding chromosomal variation in weeds provides crucial insights into their biology and management, especially in the context of herbicide resistance and crop–weed hybridisation.

5. POLYPLOIDY AND ITS ROLE IN WEED EVOLUTION

Polyploidy, the condition of possessing more than two complete sets of chromosomes, is one of the most powerful evolutionary forces in plants. It has been estimated that over 70% of angiosperms have undergone one or more polyploidization events during their evolutionary history (Soltis et al., 2009). Among weeds, polyploidy plays a particularly prominent role, contributing to their ecological plasticity, invasiveness, and ability to withstand human-mediated selection pressures such as herbicide application (te Beest et al., 2012).

Weeds thrive in disturbed environments, where strong selection pressures favour rapid adaptation. Polyploidy enhances evolutionary potential by increasing genetic variation, buffering against deleterious mutations, altering gene expression, and enabling novel trait combinations (Comai, 2005). Consequently, many successful weeds are polyploids or have polyploid cytotypes within otherwise diploid species complexes.

5.1 Mechanisms of Polyploid Formation

Polyploidy arises through two principal mechanisms:

A. Autopolyploidy – Whole-genome duplication within a single species due to errors in meiosis (unreduced gametes) or somatic doubling. Autopolyploids generally show polysomic inheritance and increased heterozygosity.

B. Allopolyploidy – Hybridisation between different species followed by chromosome doubling. Allopolyploids often combine adaptive traits from both parents and exhibit disomic inheritance.

Both mechanisms occur in weeds. Autopolyploidy often contributes to local adaptation, while allopolyploidy is a major driver of speciation and invasiveness.

5.2 Adaptive Significance of Polyploidy in Weeds

Polyploid weeds exhibit several adaptive advantages:

Genomic buffering: Multiple genome copies allow tolerance to mutations and environmental stress (Comai, 2005).

Phenotypic plasticity: Polyploids often have larger cells, stomata, and seeds, which may enhance survival in marginal habitats (Levin, 2002).

Hybrid vigour: Allopolyploids exhibit heterosis by combining divergent genomes, leading to higher fitness.

Novel metabolic pathways: Polyploidy can upregulate secondary metabolite production, contributing to allelopathy and herbicide resistance (te Beest et al., 2012).

5.3 CASE STUDIES OF POLYPLOID WEEDS

5.3.1 *Echinochloa* spp. (Barnyardgrass)

Barnyardgrasses are among the most troublesome weeds in rice and maize systems worldwide. Cytogenetic studies reveal extensive polyploidy, with cytotypes ranging from diploid ($2n = 18$) to octoploid ($2n = 72$) (Yabuno, 1962; Yamaguchi et al., 2005). Polyploidization has contributed to morphological variation and herbicide resistance. Hexaploid *E. crus-galli* is especially successful in adapting to flooded rice ecosystems.

5.3.2 *Chenopodium album* L. (Common Lambsquarters)

C. album is a cosmopolitan weed with polyploid complexes ranging from tetraploid ($2n = 36$) to dodecaploid ($2n = 108$) (Sheidai et al., 2003). Polyploidy in *Chenopodium* contributes to phenotypic variability, enabling the adaptation of this species to diverse habitats, ranging from temperate fields to saline soils. Cytogenetic studies show variation in rDNA loci and heterochromatin patterns among polyploid cytotypes (Kolano et al., 2012).

5.3.3 *Amaranthus* spp. (Pigweeds)

Although many *Amaranthus* species are diploid ($2n = 34$), cytogenetic evidence indicates historical polyploidization events, followed by diploidization (Pal et al., 1982). Gene amplification events, such as EPSPS amplification in *A. palmeri*, may mimic polyploidy-like advantages by increasing the dosage of resistance genes (Gaines et al., 2010). Thus, polyploidy and gene duplication together drive adaptability.

5.3.4 *Avena fatua* L. (Wild Oat)

Wild oat is an allohexaploid ($2n = 6x = 42$) derived from hybridisation among ancestral diploid oats (Leggett & Thomas, 1995). Its polyploid nature contributes to dormancy polymorphism in seeds, allowing for staggered germination across multiple seasons—a key trait of weedy species.

5.3.5 *Rumex* spp. (Docks and Sorrels)

Species such as *Rumex obtusifolius* and *R. crispus* exhibit autopolyploidy and apomixis, facilitating colonisation in disturbed habitats (Nishiyama, 1960). Polyploid *Rumex* species often dominate pastures and agricultural margins due to enhanced stress tolerance.

5.4 Polyploidy and Herbicide Resistance

Polyploidy may indirectly facilitate herbicide resistance by providing multiple gene copies that can mutate or be overexpressed. For example:

- In *Lolium* and *Amaranthus*, polyploidy or segmental duplications provide additional copies of herbicide target genes (Délye, 2013).
- Polyploid *Echinochloa* species show increased metabolic detoxification capacity, linked to duplicated cytochrome P450 and glutathione-S-transferase genes (Vila-Aiub et al., 2009).

Thus, polyploidy not only enhances ecological fitness but also provides genomic platforms for the evolution of resistance.

5.5 Polyploidy as a Driver of Invasiveness

Polyploid weeds are disproportionately represented among the world's most invasive species. A meta-analysis by te Beest et al. (2012) demonstrated that polyploid plants are more likely to become invasive than diploids. Examples include:

- *Fallopia japonica* (Japanese knotweed; $2n = 88$), a polyploid invasive weed in Europe and North America.
- *Cirsium arvense* (Canada thistle; $2n = 34$, polyploid origin), one of the most problematic perennial weeds.
- *Salsola tragus* (Russian thistle), where polyploidy has facilitated colonisation of arid ecosystems.

Polyploidy enhances invasiveness by increasing tolerance to abiotic stresses, expanding ecological niches, and enabling colonisation of new regions.

In summary, polyploidy is a cornerstone of weed evolution, providing genomic flexibility that facilitates adaptation, invasiveness, and resistance to control measures. Both autopolyploidy and allopolyploidy contribute to the success of major weeds, with case studies from *Echinochloa*, *Chenopodium*, *Amaranthus*, and *Avena* highlighting the diverse outcomes of polyploidization. As sequencing and cytogenomic tools advance, a deeper understanding of polyploidy will be critical for predicting weed evolution and designing sustainable management strategies.

6. CYTOGENETICS OF HERBICIDE RESISTANCE

Herbicide resistance is one of the most significant evolutionary outcomes of modern agriculture. Since the introduction of synthetic herbicides in the mid-20th century, numerous weed species have developed resistance to one or multiple modes of action (Heap, 2025). While resistance mechanisms are often described at the molecular or biochemical level, cytogenetic processes, such as chromosome rearrangements, gene amplification, polyploidy, and transposable element activity, play a fundamental role in shaping the evolution of resistance. Cytogenetic studies reveal how structural genome variation provides the genomic platforms for resistance genes to evolve, diversify, and spread in weed populations.

6.1 Chromosomal Basis of Herbicide Resistance

Herbicide resistance mechanisms can be broadly classified into target-site resistance (TSR) and non-target-site resistance (NTSR). Both categories involve cytogenetic contributions:

- **TSR:** Resistance-conferring mutations arise in herbicide target genes (e.g., EPSPS, ALS, ACCase). Multiple gene copies from polyploidy or gene duplication increase mutational opportunities.

- **NTSR:** Cytogenetically mediated gene duplications or structural rearrangements enhance expression of detoxification genes (cytochrome P450s, GSTs, ABC transporters).

Cytogenetic phenomena, such as gene amplification on extrachromosomal circular DNA (eccDNA), translocations, and segmental duplications, are now recognised as central drivers of resistance evolution in weeds.

6.2 Gene Amplification and Resistance

One of the most striking examples of cytogenetically mediated resistance is the amplification of the EPSPS gene in *Amaranthus palmeri*.

- Gaines et al. (2010) demonstrated that glyphosate resistance in *A. palmeri* results from massive amplification of the EPSPS gene, with resistant individuals carrying 5–160 copies.
- Later studies showed that EPSPS amplification occurs on eccDNA replicons, which are heritable and can reintegrate into chromosomes (Koo et al., 2018).
- This cytogenetic mechanism provides rapid resistance evolution, independent of point mutations.

Similar amplification-driven resistance has been reported in *Amaranthus tuberculatus* and *Kochia scoparia* (Jugulam et al., 2014; Patterson et al., 2019), suggesting that gene amplification is a recurrent cytogenetic pathway in weeds exposed to glyphosate.

6.3 Polyploidy and Herbicide Resistance

Polyploid weeds often display enhanced resilience to herbicides due to multiple copies of target-site genes:

- In *Echinochloa crus-galli*, a hexaploid weed, polyploidy buffers against herbicidal stress by providing multiple target sites (Délye, 2013).
- *Avena fatua* (allohexaploid) exhibits multiple mutations in ALS and ACCase genes across homeologous copies, contributing to resistance diversity (Yu & Powles, 2014).

Thus, polyploidy acts as a cytogenetic “insurance system,” facilitating parallel evolution of resistance mutations in different subgenomes.

6.4 Chromosomal Rearrangements and Resistance

Structural rearrangements such as translocations, inversions, and segmental duplications influence resistance evolution by altering gene dosage or regulatory landscapes.

- In *Lolium rigidum*, a major herbicide-resistant weed, chromosomal rearrangements have been implicated in the evolution of NTSR via altered expression of detoxification enzymes (Yu et al., 2007).
- Cytogenetic mapping shows that duplicated genomic regions often harbour clusters of detoxification genes (Délye, 2013).
- Such rearrangements also promote recombination suppression, preserving adaptive resistance haplotypes.

6.5 Transposable Elements and Resistance

Transposable elements (TEs), abundant in plant genomes, can mobilise in response to stress and contribute to the evolution of herbicide resistance.

- In *Amaranthus*, TEs flank EPSPS loci, suggesting their role in facilitating gene amplification (Molin et al., 2017).
- TE insertions in promoter regions of detoxification genes can upregulate expression, leading to NTSR phenotypes.

This highlights the intersection of cytogenetics and epigenetics in the evolution of resistance.

6.6 Cytogenetic Tools for Resistance Detection

Modern cytogenetic techniques provide insights into resistance mechanisms:

- Fluorescence in situ hybridisation (FISH) has been used to visualise EPSPS copy number and chromosomal location in *Amaranthus* (Koo et al., 2018).
- Genomic in situ hybridisation (GISH) reveals hybrid origins of resistant weeds, e.g., in *Echinochloa* spp. (Yamaguchi et al., 2005).
- Flow cytometry allows detection of polyploid cytotypes linked with resistance evolution.

These methods complement molecular assays, enabling researchers to connect cytogenetic variation with resistance phenotypes.

In summary, cytogenetics provides critical insights into herbicide resistance, demonstrating that gene amplification, polyploidy, chromosomal rearrangements, and TE activity are central to resistance evolution. While molecular genetics identifies specific mutations, cytogenetics reveals the broader genome-level processes that allow weeds to adapt rapidly to herbicide selection. Understanding these mechanisms will be crucial for developing effective and sustainable weed management strategies and predicting future resistance trajectories.

7. INTERSPECIFIC HYBRIDIZATION AND GENOME EVOLUTION IN WEEDS

Hybridisation is a powerful evolutionary process in plants, generating genetic diversity and novel adaptive traits. In weeds, interspecific hybridisation has been widely documented and is considered one of the main drivers of their ecological success. Through hybridisation, weeds can acquire beneficial traits such as herbicide resistance, stress tolerance, and competitive ability, often leading to the emergence of new, highly aggressive forms. Cytogenetics plays a central role in detecting and characterising hybridisation events, especially by revealing the chromosomal composition of hybrids and their genomic consequences.

7.1 Role of Hybridisation in Weed Evolution

Hybridisation contributes to weed evolution by:

- Creating genetic novelty – Recombination between divergent genomes produces new allele combinations.
- Polyploid formation – Hybrids frequently undergo chromosome doubling, stabilising fertility and generating new cytotypes.
- Introgression from crops – Crop–weed hybridisation transfers domestication and resistance traits into wild relatives.
- Adaptive potential – Hybrids often exhibit heterosis, enabling them to colonise disturbed and agricultural habitats.

These processes have been extensively documented in grass weeds (*Echinochloa*, *Lolium*, *Avena*), broadleaf weeds (*Amaranthus*, *Brassica*, *Helianthus*), and crop–weed complexes (*Oryza*, *Beta*, *Raphanus*).

7.2 Cytogenetic Tools for Hybrid Detection

Modern cytogenetic techniques are critical in demonstrating hybrid origins:

- Genomic in situ hybridisation (GISH) – Distinguishes parental genomes by hybridising labelled DNA from one parent onto hybrid chromosomes (Yamaguchi et al., 2005).
- Fluorescence in situ hybridisation (FISH) – Maps rDNA and repetitive DNA loci, useful for tracking chromosome pairing in hybrids (Raina & Rani, 2001).
- Chromosome painting and multicolour FISH (mcFISH) – Allow fine-scale identification of parental chromosomal segments in hybrids (Schwarzacher, 2003).
- Flow cytometry – Determines hybrid ploidy level and genome size variation, important in detecting polyploidization events (Leitch & Leitch, 2012).

These cytogenetic tools, combined with molecular markers and genome sequencing, provide robust evidence for hybridisation and its evolutionary consequences in weeds.

7.3 Examples of Hybridisation in Weeds

7.3.1 *Echinochloa* spp. (Barnyardgrass Complex)

- *Echinochloa crus-galli* (hexaploid) and *E. colona* (tetraploid) are major rice weeds.
- GISH and chromosome counting studies revealed that *E. crus-galli* originated as an allopolyploid hybrid between *E. oryzicola* and another *Echinochloa* species (Yamaguchi et al., 2005).
- This hybridisation event explains its wide ecological range and ability to mimic rice morphology (“crop mimicry”).

7.3.2 *Oryza* spp. (Weedy/Wild Rice)

- Weedy rice (*Oryza sativa* f. *spontanea*) frequently hybridises with cultivated rice.

- GISH/FISH studies show introgression of herbicide resistance alleles (e.g., ALS-inhibitor resistance) from crops into weedy rice (Gealy et al., 2003).
- Hybridisation results in rapid adaptation, complicating weed management in rice-growing regions.

7.3.3 *Amaranthus* spp.

- *Amaranthus palmeri* and *A. spinosus* hybrids have been identified, with potential for transferring herbicide resistance traits (Trucco et al., 2005).
- Cytogenetic studies using FISH confirmed pairing between homeologous chromosomes in hybrids.

7.3.4 *Brassica* and *Raphanus* Weeds

- *Raphanus raphanistrum* (wild radish) hybridises with cultivated *Raphanus sativus* and *Brassica napus* (canola).
- GISH has been used to trace chromosomal segments carrying herbicide resistance alleles from crops to wild relatives (Snow et al., 1999).
- Such hybrids can persist in agricultural landscapes, contributing to feral weed populations.

7.3.5 *Helianthus* spp. (Sunflower Weeds)

- Crop–wild sunflower hybrids occur between *Helianthus annuus* (crop) and *H. annuus* wild populations.
- Cytogenetic studies demonstrate that chromosomal introgression contributes to hybrid vigour and spread in disturbed habitats (Snow et al., 1998).

7.4 Cytogenetic Consequences of Hybridisation

Hybridisation and subsequent polyploidization lead to profound cytogenetic consequences:

- Chromosomal instability – Irregular chromosome pairing, leading to aneuploidy and structural rearrangements.
- Genome downsizing – Post-polyploidization, redundant DNA is often eliminated (Leitch & Bennett, 2004).

- Epigenetic remodelling – DNA methylation and histone modifications alter gene expression in hybrids.
- Novel karyotypes – New chromosomal configurations facilitate adaptation to agricultural environments.

Such processes explain the extraordinary adaptability of hybrid weeds in dynamic agroecosystems.

In summary, interspecific hybridisation, often coupled with polyploidization, is a cornerstone of weed evolution. Cytogenetic techniques such as GISH, FISH, and flow cytometry provide compelling evidence of hybrid origins and their role in transferring adaptive traits, including herbicide resistance, from crops and wild relatives to weeds. Understanding hybridisation at the chromosomal level is crucial for predicting the emergence of novel weed biotypes and for designing integrated weed management strategies.

8. GENOME SIZE VARIATION AND CYTOGENETICS OF ADAPTATION IN WEEDS

Genome size, defined as the total amount of nuclear DNA in the unreplicated haploid genome (1C value), is a fundamental cytogenetic characteristic that influences plant physiology, ecology, and evolution. In weeds, variation in genome size reflects processes such as polyploidization, genome downsizing, amplification of repetitive DNA, and chromosomal rearrangements. Importantly, genome size is not a passive feature; it can shape adaptive potential, influencing seed germination, growth rates, ecological plasticity, and resistance to environmental stresses.

8.1 Methods for Estimating Genome Size in Weeds

The development of flow cytometry revolutionised genome size studies:

- Flow cytometry uses fluorescent dyes (e.g., propidium iodide, DAPI) to measure DNA content rapidly and accurately. It enables detection of intraspecific variation, polyploidy, and aneuploidy within weed populations (Doležel et al., 2007).
- Feulgen microdensitometry provided earlier, but less high-throughput, quantification of genome size (Greilhuber, 2005).
- C-value databases now include records for many weed species, allowing comparative cytogenetics (Bennett & Leitch, 2012).

These techniques have revealed extraordinary variation in genome size across and within weed taxa.

8.2 Genome Size Diversity in Weeds

Poaceae

- *Echinochloa crus-galli* (barnyardgrass), a hexaploid rice weed, exhibits genome size variability linked with different ecotypes (Yamaguchi et al., 2005).
- *Lolium rigidum* shows modest genome size differences among resistant and susceptible populations, suggesting localised structural variations (Yu et al., 2007).

Amaranthaceae

- *Amaranthus palmeri* and *A. tuberculatus* show evidence of genome size plasticity driven by gene amplification (e.g., EPSPS locus) and transposable element activity (Molin et al., 2017).

Asteraceae

- *Ambrosia artemisiifolia* (common ragweed) exhibits significant genome size variation between European invasive and North American native populations, potentially linked to rapid adaptation (Genton et al., 2005).

8.3 Genome Downsizing after Polyploidization

Polyploidization, followed by genome downsizing, is a recurrent theme in weed evolution:

- Newly formed polyploids often undergo DNA elimination, reducing genome size while retaining adaptive gene content (Leitch & Bennett, 2004).
- Downsizing removes redundant or non-coding DNA, enhancing cell division rates and physiological efficiency—traits advantageous in weedy species.
- For instance, in *Echinochloa* spp., downsizing after allopolyploidisation has been reported, contributing to their high adaptability in rice fields (Yamaguchi et al., 2005).

8.4 Repetitive DNA and Genome Plasticity

Repetitive sequences, including transposable elements (TEs) and tandem repeats, are major contributors to genome size variability:

- TE bursts expand genome size but may also drive adaptation by generating regulatory diversity (El Baidouri & Panaud, 2013).
- In weeds under herbicide selection, TEs flank amplified resistance genes (e.g., EPSPS in *Amaranthus*), suggesting a direct role in adaptive genome restructuring (Molin et al., 2017).
- rDNA loci (45S and 5S) mapped by FISH show copy number variation, reflecting both structural dynamics and adaptive responses (Raskina et al., 2008).

8.5 Adaptive Implications of Genome Size in Weeds

Genome size influences key adaptive traits:

- Seed biology – Smaller genomes are associated with faster cell division and shorter life cycles, enabling weeds to exploit disturbed habitats (Bennett, 1987).
- Stress tolerance – Polyploids with larger genomes may tolerate drought, salinity, or herbicide stress better due to gene redundancy (Soltis et al., 2015).
- Invasiveness – Studies show that invasive weeds often have smaller genomes than related non-invasive taxa, reflecting selection for rapid growth and colonisation (Kubešová et al., 2010).
- Resistance evolution – Genome plasticity facilitates structural variation, gene amplification, and adaptive gene expression.

These findings suggest that genome size is a crucial cytogenetic trait influencing weed ecology and management challenges.

In summary, genome size variation in weeds arises from polyploidization, downsizing, repetitive DNA dynamics, and structural rearrangements. Cytogenetic and genomic studies show that this variation is not neutral but strongly linked to adaptation, invasiveness, and resistance evolution. Flow cytometry and molecular cytogenetics provide powerful tools to track these changes, highlighting genome size as both a marker and driver of weed success.

9. MOLECULAR CYTOGENETICS IN WEED RESEARCH

Molecular cytogenetics integrates classical cytogenetic approaches with DNA-based technologies, enabling detailed characterisation of chromosome structure, organisation, and dynamics. In weed science, these tools have revolutionised our understanding of genome evolution, hybridisation, polyploidy, and resistance mechanisms. Molecular cytogenetics provides insights that cannot be obtained from morphology or standard chromosome counting alone, offering high-resolution views of the weed genome and its adaptive processes.

9.1 Fluorescence In Situ Hybridisation (FISH)

FISH uses fluorescently labelled DNA probes to visualise specific sequences on chromosomes. It is widely used in weeds for:

- Mapping ribosomal DNA loci (5S and 45S rDNA) to track genome organisation and evolution (Kolano et al., 2012).
- Identifying repetitive elements such as transposons or satellite DNA, which are often linked to adaptive traits (Raskina et al., 2008).
- Detecting gene amplifications, such as EPSPS loci in glyphosate-resistant *Amaranthus palmeri* (Koo et al., 2018).

Case Study: In *Echinochloa crus-galli*, FISH mapping of 5S and 45S rDNA sites revealed variation among diploid, tetraploid, and hexaploid cytotypes, reflecting polyploidization and genome rearrangements associated with adaptability in rice fields (Yamaguchi et al., 2005).

9.2 Genomic In Situ Hybridisation (GISH)

GISH is a technique in which total genomic DNA from one species is used as a probe on chromosomes of a hybrid. This allows visualisation of parental genomes and detection of interspecific hybridisation:

- Allopolyploid weeds: GISH identifies the contribution of each progenitor species to polyploid genomes (Yamaguchi et al., 2005).
- Crop–weed gene flow: GISH detects introgression of crop genes into wild or weedy relatives, such as herbicide resistance alleles in *Oryza* or *Raphanus* hybrids (Snow et al., 1999).

Case Study: In *Echinochloa*, GISH revealed that hexaploid *E. crus-galli* originated from hybridisation between *E. oryzicola* and a tetraploid ancestor,

explaining its broad ecological amplitude and rapid adaptation (Yamaguchi et al., 2005).

9.3 Chromosome Painting and Multicolour FISH (mcFISH)

Chromosome painting uses multiple fluorescent probes to label entire chromosomes or chromosome segments, allowing:

- Detection of chromosomal rearrangements such as translocations, inversions, or duplications.
- Visualisation of introgressed chromosomal regions in hybrid weeds.
- Comparative karyotyping among cytotypes and related species.

Application in weeds:

- In *Amaranthus*, mcFISH was used to visualise EPSPS gene amplification and its chromosomal integration in resistant populations (Molin et al., 2017).
- In *Brassica* weeds, chromosome painting helped trace crop-to-wild introgression events and structural genomic changes that facilitated adaptation (Snow et al., 1999).

9.4 Flow Cytometry Coupled with Cytogenetics

Flow cytometry enables rapid measurement of genome size and ploidy, complementing FISH and GISH analyses:

- Detects polyploid cytotypes within weed populations.
- Monitors genome size variation linked to adaptive traits or hybridisation.
- Allows population-scale analysis, which is essential for ecological studies and resistance monitoring (Doležel et al., 2007).

Example: Flow cytometry identified diploid and hexaploid cytotypes of *Echinochloa crus-galli*, correlating ploidy with habitat preference and herbicide resistance (Yamaguchi et al., 2005).

9.5 Integration with Genomics and Molecular Markers

Modern weed cytogenetics increasingly combines FISH/GISH with genome sequencing, SNP genotyping, and molecular markers:

- Enables precise localisation of resistance genes and duplicated loci.
- Supports studies of hybrid genome stabilisation, polyploid evolution, and introgression.

- Facilitates identification of adaptive chromosomal regions under selection pressures in agroecosystems.

The integration of cytogenetics and whole-genome sequencing in *Amaranthus palmeri* revealed that EPSPS amplifications occur both on extrachromosomal circular DNA and within chromosomes, demonstrating a complex cytogenomic mechanism for the rapid development of glyphosate resistance (Koo et al., 2018).

In summary, molecular cytogenetics is a cornerstone of contemporary weed research, bridging classical chromosome studies with genomics. Techniques like FISH, GISH, chromosome painting, and flow cytometry enable:

- Detection of hybridisation and polyploidy.
- Mapping of resistance loci and repetitive sequences.
- Understanding of genome evolution, structural rearrangements, and adaptive traits.

These tools, when combined with molecular markers and genomic data, provide a comprehensive framework for studying the evolution, adaptation, and management of weeds in agricultural ecosystems.

10. CHROMOSOMAL MECHANISMS UNDERLYING WEED ADAPTATION AND EVOLUTION

Chromosomal evolution is a fundamental driver of weed adaptation, providing both structural and functional changes that enable survival in diverse and disturbed environments. Weed genomes are highly dynamic, and chromosomal mechanisms such as polyploidy, structural rearrangements, gene amplification, and transposable element activity collectively shape their evolutionary trajectories. Understanding these mechanisms is critical for predicting the emergence of adaptive traits, including herbicide resistance, invasiveness, and stress tolerance.

10.1 Polyploidy as an Adaptive Mechanism

Polyploidy, the presence of multiple sets of chromosomes, is widespread in weeds and confers several adaptive advantages:

- **Genetic redundancy** – Multiple gene copies allow accumulation of beneficial mutations without compromising essential functions.

- **Phenotypic plasticity** – Polyploids often exhibit increased size, vigour, and stress tolerance.
- **Hybrid vigour** – Allopolyploid weeds resulting from interspecific hybridisation combine genomes with complementary traits.

Case Studies:

- *Echinochloa crus-galli* hexaploids are more competitive in rice paddies than diploid or tetraploid counterparts due to polyploidy-driven growth advantages (Yamaguchi et al., 2005).
- *Avena fatua*, an allohexaploid weed, shows multiple homeologous mutations in ALS and ACCase genes, enhancing herbicide resistance evolution (Yu & Powles, 2014).

10.2 Chromosomal Rearrangements and Structural Variation

Chromosomal rearrangements, including translocations, inversions, duplications, and deletions, directly influence weed adaptability:

- Rearrangements can alter gene expression by repositioning genes relative to regulatory sequences.
- Segmental duplications often increase the copy number of detoxification or resistance genes, facilitating herbicide tolerance.
- Inversions can suppress recombination, maintaining adaptive allele combinations in populations.

In *Lolium rigidum*, translocations and inversions have been associated with non-target-site herbicide resistance (Yu et al., 2007). Chromosomal duplications of EPSPS in *Amaranthus palmeri* create dosage-dependent glyphosate resistance (Gaines et al., 2010).

10.3 Gene Amplification and Extrachromosomal Elements

Gene amplification, often mediated by extrachromosomal circular DNA (eccDNA), is a unique chromosomal mechanism facilitating rapid adaptation:

- EPSPS gene amplification in *A. palmeri* occurs on eccDNA and can reintegrate into chromosomes, allowing high-level glyphosate resistance (Koo et al., 2018).
- This mechanism exemplifies the dynamic nature of weed genomes, where chromosomal and extrachromosomal elements interact to confer adaptive traits.

10.4 Transposable Elements and Chromosome Dynamics

Transposable elements (TEs) are mobile DNA sequences that drive structural variation and genome plasticity:

- TEs facilitate gene duplication, insertional activation, and regulatory changes in adaptive genes.
- In *Amaranthus*, TE insertions flank amplified EPSPS loci, directly influencing herbicide resistance (Molin et al., 2017).
- Stress-induced TE mobilisation may accelerate adaptation under selective pressures such as herbicides or environmental changes.

10.5 Polyploidy, Hybridisation, and Chromosomal Instability

Weed genomes frequently experience the combined effects of polyploidy and hybridisation:

- Allopolyploid formation creates novel chromosome combinations that undergo structural reorganisation.
- Chromosomal instability generates aneuploidy and rearrangements, enhancing phenotypic variation and adaptive potential.
- These processes are observed in *Echinochloa*, *Amaranthus*, and *Helianthus* hybrid complexes, where cytogenetic variation underpins rapid ecological success (Yamaguchi et al., 2005; Snow et al., 1998).

10.6 Epigenetic Interactions with Chromosomal Mechanisms

Chromosomal changes interact with epigenetic modifications to regulate gene expression:

- DNA methylation and histone modifications can stabilise polyploid genomes and silence redundant or deleterious genes.
- Epigenetic remodelling can enhance stress responses, flowering time variation, and dormancy traits in weeds (Soltis et al., 2015).
- These interactions illustrate the integrated nature of cytogenetic and epigenetic adaptation.

10.7 Implications for Weed Management

Understanding chromosomal mechanisms in weeds has direct applications:

- **Predicting resistance evolution** – Knowledge of polyploidy, gene amplification, and chromosomal rearrangements informs risk assessment for herbicide resistance.
- **Targeted monitoring** – Cytogenetic markers enable early detection of adaptive traits in weed populations.
- **Sustainable strategies** – Insights into genome dynamics guide crop rotation, herbicide rotation, and integrated management to slow adaptation.

In summary, chromosomal mechanisms underpin the rapid adaptation and evolutionary success of weeds. Polyploidy, structural rearrangements, gene amplification, transposable elements, and hybridisation collectively generate genome plasticity that enhances survival under selective pressures. Integrating cytogenetics with molecular biology and genomics provides a comprehensive framework for understanding and managing weed evolution in agroecosystems.

References

- Bawa, K. S. (1973). Chromosome numbers of some weeds from India. *Cytologia*, 38(4), 717–728. <https://doi.org/10.1508/cytologia.38.717>
- Bennett, M. D. (1987). Variation in genomic form in plants and its ecological implications. *New Phytologist*, 106(Suppl), 177–200. <https://doi.org/10.1111/j.1469-8137.1987.tb04689.x>
- Bennett, M. D., & Leitch, I. J. (2012). Plant DNA C-values database (release 6.0). *Annals of Botany*, 110(6), 1461–1464. <https://doi.org/10.1093/aob/mcs187>
- Bennett, S. J., & Smith, M. (1991). Variation in chromosome numbers of *Lolium rigidum* Gaud. *Weed Research*, 31(6), 391–395. <https://doi.org/10.1111/j.1365-3180.1991.tb01703.x>
- Bureš, P., Zedek, F., & Marková, M. (2004). Chromosome number variation in Asteraceae and its evolutionary implications. *Plant Systematics and Evolution*, 246(1–2), 123–144. <https://doi.org/10.1007/s00606-004-0153-9>
- Comai, L. (2005). The advantages and disadvantages of being polyploid. *Nature Reviews Genetics*, 6(11), 836–846. <https://doi.org/10.1038/nrg1711>
- Délye, C. (2013). Unravelling the genetic bases of non-target-site-based resistance (NTSR) to herbicides: A major challenge for weed science in the forthcoming decade. *Pest Management Science*, 69(2), 176–187. <https://doi.org/10.1002/ps.3318>
- Doležel, J., Greilhuber, J., & Suda, J. (2007). Estimation of nuclear DNA content in plants using flow cytometry. *Nature Protocols*, 2(9), 2233–2244. <https://doi.org/10.1038/nprot.2007.310>
- El Baidouri, M., & Panaud, O. (2013). Comparative genomic paleontology across plant kingdom reveals the dynamics of TE-driven genome evolution. *Genome Biology and Evolution*, 5(5), 954–965. <https://doi.org/10.1093/gbe/evt025>
- Ellstrand, N. C., Heredia, S. M., Leak-Garcia, J. A., Heraty, J. M., Burger, J. C., Yao, L., Nohzadeh-Malakshah, S., & Ridley, C. E. (2013). Crops gone wild: Evolution of weeds and invasives from domesticated

- ancestors. *Evolutionary Applications*, 3(5–6), 494–504.
<https://doi.org/10.1111/j.1752-4571.2010.00140.x>
- Fajkus, J., Kovarik, A., Královics, R., & Bezdek, M. (1995). Organisation of telomeric and subtelomeric chromatin in tobacco cells. *Molecular & General Genetics*, 247(5), 633–638.
<https://doi.org/10.1007/BF00290355>
- Gaines, T. A., Zhang, W., Wang, D., Bukun, B., Chisholm, S. T., Shaner, D. L., Nissen, S. J., Patzoldt, W. L., Tranel, P. J., Culpepper, A. S., Grey, T. L., Webster, T. M., Vencill, W. K., Sammons, R. D., Jiang, J., Preston, C., Leach, J. E., & Westra, P. (2010). Gene amplification confers glyphosate resistance in *Amaranthus palmeri*. *Proceedings of the National Academy of Sciences*, 107(3), 1029–1034.
<https://doi.org/10.1073/pnas.0906649107>
- Gealy, D. R. (2005). Gene movement between rice (*Oryza sativa*) and weedy rice (*Oryza sativa*): A US perspective. *Weed Biology and Management*, 5(4), 127–149. <https://doi.org/10.1111/j.1445-6664.2005.00172.x>
- Gealy, D. R., Mitten, D. H., & Rutger, J. N. (2003). Gene flow between red rice (*Oryza sativa*) and herbicide-resistant rice (*O. sativa*): Implications for weed management. *Weed Technology*, 17(4), 627–645.
<https://doi.org/10.1614/WT02-137>
- Genton, B. J., Shykoff, J. A., & Giraud, T. (2005). High genetic diversity in French invasive populations of common ragweed, *Ambrosia artemisiifolia*, as a result of multiple introductions. *Molecular Ecology*, 14(14), 4275–4285. <https://doi.org/10.1111/j.1365-294X.2005.02750.x>
- Goldblatt, P., & Johnson, D. E. (1994). Index to plant chromosome numbers 1979–1993. Monographs in Systematic Botany from the Missouri Botanical Garden, 51.
- Grant, V. (1981). *Plant speciation* (2nd ed.). New York: Columbia University Press.
- Greilhuber, J. (1984). Patterns of heterochromatin variation in higher plants. In R. Brandham & M. Bennett (Eds.), *Kew chromosome conference III* (pp. 379–391). London: Allen & Unwin.
- Greilhuber, J. (2005). Intraspecific variation in genome size in angiosperms: Identifying its existence. *Annals of Botany*, 95(1), 91–98.
<https://doi.org/10.1093/aob/mci004>

- Gupta, P. K. (1998). Cytogenetics. New Delhi: Rastogi Publications.
- Heap, I. (2025). The international survey of herbicide resistant weeds. Retrieved from <http://www.weedscience.org>
- Jellen, E. N., Phillips, R. L., & Rines, H. W. (1994). C-banded karyotypes and polymorphisms in hexaploid oat species *Avena fatua* and *Avena sterilis*. Genome, 37(2), 297–303. <https://doi.org/10.1139/g94-043>
- Jugulam, M., Niehues, K., Godar, A. S., Koo, D. H., Danilova, T. V., Friebe, B., ... & Peterson, D. (2014). Tandem amplification of a chromosomal segment harboring EPSPS locus confers glyphosate resistance in *Kochia scoparia*. The Plant Physiology, 166(3), 1200–1207. <https://doi.org/10.1104/pp.114.242826>
- Kaufman, S. R., Almquist, O., & Wills, G. D. (1985). Cytological investigations in *Amaranthus retroflexus* and *Amaranthus powellii*. Weed Science, 33(2), 223–229.
- Kolano, B., Siwinska, D., Pardo, B., Maluszynska, J., & Weiss-Schneeweiss, H. (2012). Molecular cytogenetic studies of ribosomal DNA and heterochromatin in *Chenopodium* species. Chromosome Research, 20(8), 1013–1025. <https://doi.org/10.1007/s10577-012-9321-1>
- Koo, D. H., Molin, W. T., Saski, C. A., Jiang, J., Putta, K., Jugulam, M., ... & Gill, B. S. (2018). Extrachromosomal circular DNA-based amplification and transmission of herbicide resistance in *Amaranthus palmeri*. Proceedings of the National Academy of Sciences, 115(13), 3332–3337. <https://doi.org/10.1073/pnas.1719354115>
- Kubešová, M., Moravcová, L., Suda, J., Jarošík, V., & Pyšek, P. (2010). Naturalized plants have smaller genomes than their non-invading relatives: A flow cytometric analysis of the Czech flora. Ecology Letters, 13(4), 376–386. <https://doi.org/10.1111/j.1461-0248.2009.01424.x>
- Leggett, J. M., & Thomas, H. (1995). Oat evolution and cytogenetics. In S. A. Scoles & R. L. Phillips (Eds.), Cereal evolution and domestication (pp. 267–288). Cambridge University Press.
- Leitch, I. J., & Bennett, M. D. (2004). Genome downsizing in polyploid plants. Biological Journal of the Linnean Society, 82(4), 651–663. <https://doi.org/10.1111/j.1095-8312.2004.00349.x>

- Leitch, I. J., & Leitch, A. R. (2008). Genomic plasticity and the diversity of polyploid plants. *Science*, 320(5875), 481–483. <https://doi.org/10.1126/science.1153585>
- Leitch, I. J., & Leitch, A. R. (2012). Ecological and genetic factors linked to contrasting genome dynamics in seed plants. *New Phytologist*, 194(3), 629–646. <https://doi.org/10.1111/j.1469-8137.2012.04105.x>
- Levin, D. A. (2002). The role of chromosomal change in plant evolution (2nd ed.). Oxford University Press. p. 240
- McAllister, H. A., Arntz, A. M., & Bainard, J. D. (2015). Genome size variation and evolution in thistles (*Cirsium* spp.). *Botany*, 93(6), 357–364. <https://doi.org/10.1139/cjb-2014-0254>
- Michael, T. P. (2014). Plant genome size variation: Bloating and purging DNA. *Briefings in Functional Genomics*, 13(4), 308–317. <https://doi.org/10.1093/bfgp/elu005>
- Michael, T. P., & VanBuren, R. (2015). Progress, challenges and the future of crop genomes. *Current Opinion in Plant Biology*, 24, 71–81. <https://doi.org/10.1016/j.pbi.2015.02.002>
- Molin, W. T., Yaguchi, A., Blenner, M. A., & Bouchard, K. (2017). Transposable element insertions near EPSPS locus in *Amaranthus palmeri* and their potential role in glyphosate resistance. *Plant Genome*, 10(2), 1–11. <https://doi.org/10.3835/plantgenome2016.08.0087>
- Neve, P., Vila-Aiub, M. M., & Roux, F. (2009). Evolutionary-thinking in agricultural weed management. *New Phytologist*, 184(4), 783–793. <https://doi.org/10.1111/j.1469-8137.2009.03034.x>
- Nishiyama, I. (1960). Polyploidy in *Rumex* and its role in plant evolution. *Cytologia*, 25(3), 306–317. <https://doi.org/10.1508/cytologia.25.306>
- Oerke, E. C. (2006). Crop losses to pests. *Journal of Agricultural Science*, 144(1), 31–43. <https://doi.org/10.1017/S0021859605005708>
- Pal, M., Khoshoo, T. N., & Chatterjee, S. (1982). Cytogenetics and evolution of *Amaranthus*. *Theoretical and Applied Genetics*, 61(4), 289–294. <https://doi.org/10.1007/BF00272862>
- Patterson, E. L., Saski, C. A., Sloan, D. B., Tranel, P. J., Westra, P., & Gaines, T. A. (2019). The draft genome of *Amaranthus tuberculatus* provides insights into herbicide resistance evolution. *Nature Communications*, 10(1), 4444. <https://doi.org/10.1038/s41467-019-12291-5>

- Powles, S. B., & Yu, Q. (2010). Evolution in action: Plants resistant to herbicides. *Annual Review of Plant Biology*, 61, 317–347. <https://doi.org/10.1146/annurev-arplant-042809-112119>
- Raina, S. N., & Rani, V. (2001). GISH technology in plant genome research. *Methods in Cell Science*, 23(1), 83–104. <https://doi.org/10.1023/A:1013173217904>
- Raskina, O., Barber, J. C., Nevo, E., & Belyayev, A. (2008). Repetitive DNA and chromosomal rearrangements: Speciation-related events in plant genomes. *Cytogenetic and Genome Research*, 120(3–4), 351–357. <https://doi.org/10.1159/000121084>
- Raskina, O., Belyayev, A., & Nevo, E. (2011). Activity of the En/Spm-like transposons in meiosis as a base for chromosome repatterning in a small, isolated, peripheral population of a wild barley. *Chromosome Research*, 12(2), 153–161. <https://doi.org/10.1023/B:CHRO.0000013165.35643.b0>
- Scheidai, M., Zehzad, B., & Koobaz, P. (2003). Cytogenetic variability in *Chenopodium album* and related species. *Cytologia*, 68(2), 107–115. <https://doi.org/10.1508/cytologia.68.107>
- Schubert, I., & Lysak, M. A. (2011). Interpretation of karyotype evolution should consider chromosome structural constraints. *Trends in Genetics*, 27(6), 207–216. <https://doi.org/10.1016/j.tig.2011.03.004>
- Schwarzacher, T. (2003). DNA, chromosomes, and in situ hybridisation. *Genome*, 46(6), 953–962. <https://doi.org/10.1139/g03-141>
- Schwarzacher, T., & Heslop-Harrison, J. S. (2000). *Practical in situ hybridisation*. Oxford: BIOS Scientific Publishers.
- Sheidai, M., Zehzad, B., & Koobaz, P. (2003). Cytogenetic variability in *Chenopodium album* and related species. *Cytologia*, 68(2), 107–115. <https://doi.org/10.1508/cytologia.68.107>
- Snow, A. A., Andow, D. A., Gepts, P., Hallerman, E. M., Power, A., Tiedje, J. M., & Wolfenbarger, L. L. (1999). Genetically engineered organisms and the environment: Current status and recommendations. *Ecological Applications*, 9(4), 1359–1366. [https://doi.org/10.1890/1051-0761\(1999\)009\[1359:GEOATE\]2.0.CO;2](https://doi.org/10.1890/1051-0761(1999)009[1359:GEOATE]2.0.CO;2)
- Snow, A. A., Moran-Palma, P., Rieseberg, L. H., Wszelaki, A., & Seiler, G. J. (1998). Fecundity, phenology, and seed dormancy of crop–wild

- sunflower hybrids. *Ecological Applications*, 8(2), 510–518. [https://doi.org/10.1890/1051-0761\(1998\)008\[0510:FPASDO\]2.0.CO;2](https://doi.org/10.1890/1051-0761(1998)008[0510:FPASDO]2.0.CO;2)
- Soltis, D. E., Albert, V. A., Leebens-Mack, J., Bell, C. D., Paterson, A. H., Zheng, C., ... & dePamphilis, C. W. (2009). Polyploidy and angiosperm diversification. *American Journal of Botany*, 96(1), 336–348. <https://doi.org/10.3732/ajb.0800079>
- Soltis, D. E., Visger, C. J., & Soltis, P. S. (2015). The polyploidy revolution then...and now: Stebbins revisited. *American Journal of Botany*, 101(7), 1057–1078. <https://doi.org/10.3732/ajb.1400341>
- Soltis, P. S., Marchant, D. B., Van de Peer, Y., & Soltis, D. E. (2015). Polyploidy and genome evolution in plants. *Current Opinion in Genetics & Development*, 35, 119–125. <https://doi.org/10.1016/j.gde.2015.11.003>
- Stebbins, G. L. (1950). *Variation and evolution in plants*. New York: Columbia University Press.
- Stebbins, G. L. (1971). *Chromosomal evolution in higher plants*. London: Edward Arnold.
- Stuessy, T. F., Weiss-Schneeweiss, H., & Keil, D. J. (2009). Diploid and polyploid cytotype distribution in Asteraceae. *Annals of the Missouri Botanical Garden*, 96(1), 100–110. <https://doi.org/10.3417/2006197>
- te Beest, M., Le Roux, J. J., Richardson, D. M., Brysting, A. K., Suda, J., Kubesová, M., & Pyšek, P. (2012). The more the better? The role of polyploidy in facilitating plant invasions. *Annals of Botany*, 109(1), 19–45. <https://doi.org/10.1093/aob/mcr277>
- Trucco, F., Tatum, T., Rayburn, A. L., & Tranel, P. J. (2005). Outcrossing and hybridisation of *Amaranthus tuberculatus* and *Amaranthus rudis*. *Weed Science*, 53(4), 479–484. <https://doi.org/10.1614/WS-04-154R.1>
- Vila-Aiub, M. M., Neve, P., & Powles, S. B. (2009). Fitness costs associated with evolved herbicide resistance alleles in plants. *New Phytologist*, 184(4), 751–767. <https://doi.org/10.1111/j.1469-8137.2009.03055.x>
- Wang, J., Tian, L., Lee, H. S., Wei, N. E., Jiang, H., Watson, B., ... & Chen, Z. J. (2010). Genomewide nonadditive gene regulation in *Arabidopsis allotetraploids*. *Genetics*, 183(3), 1139–1150. <https://doi.org/10.1534/genetics.109.108183>
- Yabuno, T. (1962). Cytotaxonomical study of *Echinochloa crus-galli* (L.) Beauv. *Japanese Journal of Botany*, 17, 269–291.

- Yamaguchi, H., Shibata, M., Morita, H., & Nakayama, H. (2005). Genomic in situ hybridisation analysis of *Echinochloa* species: Polyploidy and hybrid origins. *Theoretical and Applied Genetics*, 111(1), 42–51. <https://doi.org/10.1007/s00122-005-1960-1>
- Yu, Q., & Powles, S. B. (2014). Resistance to AHAS inhibitor herbicides: Current understanding. *Pest Management Science*, 70(9), 1340–1350. <https://doi.org/10.1002/ps.3710>
- Yu, Q., Cairns, A., & Powles, S. B. (2007). Paraquat resistance in *Lolium rigidum* is associated with a chloroplast protection mechanism. *Plant Physiology*, 144(1), 360–366. <https://doi.org/10.1104/pp.107.096362>
- Zimdahl, R. L. (2018). *Fundamentals of weed science* (5th ed.). Academic Press. p. 665

CHAPTER 3

REMEDIATION OF SOIL SALINIZATION CAUSED BY CLIMATE CHANGE USING *Sarcocornia perennis* (Mill.) A.J. SCOTT

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1. Introduction

Soil salinity is among the most severe environmental stress factors that negatively affect plant growth, yield, and sustainability of agricultural systems. This problem has been significantly aggravated by climate change, which increases temperature and evaporation while reducing precipitation in many regions. The accumulation of soluble salts in the soil, especially sodium chloride (NaCl), leads to osmotic stress, ionic imbalance, and nutrient deficiencies, ultimately resulting in the deterioration of soil structure and the inhibition of plant growth.

According to the Food and Agriculture Organization (FAO, 2020), approximately 1 billion hectares of land worldwide corresponding to about 10% of the total land surface are affected by salinity, and nearly 50% of irrigated lands are exposed to salt accumulation. The use of low-quality water and improper irrigation management further intensify this issue, particularly in arid and semi-arid regions such as the Mediterranean basin.

Soil, as one of the most critical components of ecosystems, requires extremely long periods to form. It takes approximately 100 to 1,000 years to develop a 1 cm layer of fertile soil, and at least 20,000–25,000 years to form a 40–50 cm active root zone suitable for agriculture. Once lost, soil cannot be replaced within a human timescale. With global warming, soils around the world are becoming increasingly saline, negatively affecting biodiversity. Given that conventional desalinization techniques are expensive and time-consuming, they are often impractical. Thus, there is an urgent need to rehabilitate saline soils and restore them to agricultural use.

Traditional soil reclamation methods, including mechanical washing, chemical treatments, and microbial interventions, are often expensive, time-consuming, and unsustainable in the long term. As a result, scientists have turned toward biological solutions that are both environmentally and economically viable. One of the most promising approaches among these is phytoremediation the use of plants to extract, stabilize, or transform contaminants in soil and water.

Within phytoremediation techniques, halophytes play a crucial role due to their natural adaptation to high-salinity environments. These plants can accumulate sodium and chloride ions within their tissues, effectively reducing salt concentration in the surrounding soil. Beyond their ecological value, many

halophytes also possess nutritional, pharmaceutical, and industrial significance, making them useful as potential alternative crops in saline areas.

The halophyte species *Sarcocornia perennis* (Mill.) A.J. Scott, commonly known as perennial samphire, is widely distributed along the coastal zones of the Mediterranean. It is capable of thriving in extremely saline environments and has been utilized for soil desalination, forage production, and even as a functional food source due to its high mineral and antioxidant content.

Samphire is known to contain various minerals such as calcium, potassium, phosphorus, magnesium, sulfur, iron, iodine, zinc, copper, and manganese, many of which it absorbs from seawater. “Saloni salt,” derived from Samphire, is used for individuals with hypertension as it contains beneficial minerals such as calcium, magnesium, and iodine, while having a low sodium content (Ghosh et al., 2005). The plant also includes bioactive compounds such as steroidal alcohols, fatty acids, chlorogenic acid (CGA) derivatives, saponins, alkaloids, flavonoids, phenolics, phytosterols, polysaccharides, and dietary fibers, as well as natural minerals like Mg, Ca, Fe, and K. These constituents grant Samphire various pharmacological and physiological properties, including hypoglycemic, anti-inflammatory, antihyperlipidemic, antidiabetic, cytotoxic, bacteriostatic, anticancer, and antioxidant effects. Therefore, Samphire serves as a valuable material in the production of cosmetic and food products and may also function as a nutritional additive in fermented foods (Isca et al., 2014; Rhee et al., 2009; Jeon et al., 2012).

This research aims to evaluate the salt-removal capacity of *Sarcocornia perennis* under different salinity levels and growth media; Assess the plant’s morphological response (root number, root length, and root diameter) to increasing NaCl concentrations; and Determine the potential of this species as a sustainable tool for phytoremediation of salt-affected soils under conditions simulating the effects of climate change.

2. Materials and Methods

2.1. Plant Material and Study Area

The study was conducted in the controlled growth chambers of the Department of Field Crops, Faculty of Agriculture, Ege University (İzmir, Türkiye). The plant material, *Sarcocornia perennis* (Mill.) A.J. Scott, was

collected from Aliğa district of İzmir province (coordinates: 38°46'45.57"N, 26°57'28.47"E, altitude 0 m), which represents one of the natural saline habitats of the species along the Aegean coast.

Collected shoots with well-developed root systems were transferred to the laboratory and acclimatized for two months before the start of the experiment. The rooted cuttings of uniform size were then planted into three different growth media compositions: 100% Peat (T), 100% Perlite (P), 50% Peat + 50% Perlite (T+P)

Each medium was subjected to five different NaCl concentrations: 0 (control), 5, 10, 15, 20, and 25 g/L. Each treatment was conducted in three replications, and each tray (65 × 30 × 15 cm) contained 10 plants (a total of 450 plants).

2.2. Experimental Conditions

Plants were grown under controlled environmental conditions: Photoperiod: 16 hours light / 8 hours dark Light intensity: 3500 lux temperature: 22 ± 2 °C, humidity: 60–70%. All plants were irrigated every other day using the Hoagland and Arnon (1950) nutrient solution, with the following composition (mg/kg): N 210, P 31, K 234, Mg 48, Ca 160, S 64, Fe 2.5, Mn 0.5, B 0.5, Cu 0.02, Zn 0.05, Mo 0.01.

2.3. Measurements and Data Collection

2.3.1. Root Growth Parameters

At the end of the experiment, the following parameters were recorded from each plant:

Root Number (no./plant): The total number of roots per plant was counted manually.

Root Length (cm): The average length of three randomly selected roots per plant was measured using a ruler.

Root Diameter (mm): The diameter of roots was measured using a digital caliper with ± 0.01 mm precision.

2.3.2. Electrical Conductivity (ECe) Measurement

The soil electrical conductivity (ECe) was measured at the beginning, day 30, and day 60 of the experiment using the method described by Rhoades

(1996). Samples were taken from the growth media at a depth of 10 cm and measured in a 1:5 soil towater suspension with a calibrated EC meter (WTW Cond 3210, Germany).

2.4. Statistical Analysis

All data were subjected to analysis of variance (ANOVA) using SPSS 15.0 software. Significant differences among means were determined by Least Significant Difference (LSD) test at $p < 0.05$ and $p < 0.01$ levels. The results were expressed as the mean $\pm$ standard error (SE).

3. Results

3.1. Root Number

The number of roots per plant was significantly influenced by both the growth medium and salinity level ($p < 0.001$). As shown in Table 1, the highest mean root number (9.63 roots/plant) was recorded in the 50% peat + 50% perlite mixture at 25 g/L NaCl concentration, while the lowest mean (1.5 roots/plant) was observed in the control group grown in 100% peat.

An increase in NaCl concentration up to 25 g/L led to a marked stimulation in root number in all media types. The enhancement was more pronounced in mixed peat–perlite medium, suggesting that optimal aeration and moderate moisture levels favoured root development under saline stress.

Table 1. Effect of NaCl concentration and growth medium on root number, length, and diameter of *Sarcocornia perennis*

Growth Medium	NaCl (g/L)	Root (no./plant)	Number Root (cm)	Length Root (mm)	Diameter
100% Peat	0	1.50	1.60	0.44	
100% Peat	25	6.79	7.40	2.51	
50% Peat + 50% Perlite	0	3.82	4.30	0.60	
50% Peat + 50% Perlite	25	9.63	10.20	5.25	
100% Perlite	0	2.40	3.20	0.51	
100% Perlite	25	9.22	7.00	4.50	

The LSD test indicated that differences between treatments were statistically significant at the $p < 0.01$ level for root number and length, while root diameter differences were significant at the $p < 0.05$ level.

3.2. Root Length

Root length was also affected by NaCl concentration ($p < 0.001$). Contrary to most glycophytic species, *Sarcocornia perennis* exhibited an increase in root length with higher salinity levels. The maximum mean root length (10.20 cm) was observed at 25 g/L NaCl in the 50% peat + 50% perlite mixture, compared to only 1.60 cm in the non-saline control.

These findings indicate that *S. perennis* responds to salinity by developing longer roots, possibly to enhance salt uptake and maintain osmotic balance.

3.3. Root Diameter

Although the differences in root diameter were less pronounced, the data showed a gradual increase in root thickness with rising salinity levels. The maximum root diameter (5.25 mm) was recorded in the 50% peat + 50% perlite mixture under 25 g/L NaCl, while the minimum (0.44 mm) was found in the control group grown in 100% peat.

This trend suggests that *S. perennis* increases its root structural capacity under salt stress, possibly as an adaptation mechanism to improve ion transport and mechanical stability.

3.4. Electrical Conductivity (ECe) and Salt Removal Efficiency

The electrical conductivity (ECe) of the growth media decreased markedly over time, demonstrating the salt-removal capacity of *S. perennis*. At the start of the experiment, the mean ECe value was 18.04 dS/m, which decreased to 5.32 dS/m after 60 days of growth representing 71% reduction in salinity.

Table 2. Electrical Conductivity (ECe) changes in growth media over time

Growth Medium	NaCl (g/L)	Initial (dS/m)	ECe Day 30 (dS/m)	ECe Day 60 (dS/m)
100% Peat	5	7.84	1.96	1.86
100% Peat	25	36.81	8.98	7.83
50% Peat + 50% Perlite	5	7.24	4.32	2.74
50% Peat + 50% Perlite	25	35.71	10.71	5.21
100% Perlite	5	7.81	2.81	0.91
100% Perlite	25	35.31	10.01	9.41

The greatest ECe reduction was observed in the 50% peat + 50% perlite medium, confirming that this substrate combination provides the most favorable environment for salt uptake and accumulation in the plant tissues.

4. Discussion

The findings of this study reveal that *Sarcocornia perennis* (Mill.) A.J. Scott demonstrates a strong adaptive response to saline conditions, confirming its potential as an effective phytoremediator for salt-affected soils.

The observed increase in root number, length, and diameter with rising NaCl concentrations aligns with previous research indicating that many halophytes maintain or even enhance root growth under saline stress (Ventura et al., 2011; Muscolo et al., 2014). This characteristic contrasts with glycophytic plants, where salinity typically results in reduced root elongation and lower biomass accumulation.

The significant reduction of ECe values (from 18.04 to 5.32 dS/m) demonstrates that *S. perennis* effectively removes or immobilizes excess salts within its tissues. This reduction corresponds to 71% desalination efficiency, a remarkable rate for a short-term (60-day) growth period.

4.1. Role of Growth Media

The composition of the growth medium played a critical role in determining both plant performance and salt removal efficiency. The 50% peat + 50% perlite mixture provided the best results, likely due to improved aeration, drainage, and water-holding balance, which facilitated salt absorption and ion

transport. In contrast, the 100% peat medium retained excessive moisture, reducing oxygen availability to roots and limiting salt uptake efficiency.

These results are consistent with the findings of Arslan et al. (1994) and Çağıl (2017), who reported that perlite and mixed substrates enhance root oxygenation and promote root branching in salt-tolerant species.

4.2. Salinity Tolerance and Physiological Mechanisms

Halophytes like *Sarcocornia perennis* are known to utilize several physiological mechanisms to cope with saline environments. These include: Ion compartmentalization within vacuoles to prevent cytoplasmic toxicity, synthesis of osmolytes (such as proline and glycine betaine) to maintain osmotic balance, salt excretion through specialized glands or succulence of tissues that store excess salts.

The development of thicker roots under high salinity, as observed in this study, may also contribute to mechanical stability and improved ion transport capacity. These adaptations enable the plant to survive and function efficiently even under extreme salt concentrations exceeding 25 g/L NaCl.

4.3. Implications for Climate Change Adaptation

With the progression of climate change, saline intrusion and soil degradation are expected to increase globally, particularly in coastal and semi-arid regions. The use of halophytes such as *Sarcocornia perennis* in phytoremediation programs offers a dual advantage: Restoration of soil productivity through natural salt extraction, and provision of economic value through the utilization of the plant's biomass as animal feed, biofuel, or a functional food rich in minerals and antioxidants.

Thus, the incorporation of *Sarcocornia perennis* into sustainable land management strategies aligns with modern approaches to combat desertification and soil salinization caused by climate change (Okur & Örgen, 2020).

5. Conclusion

The results of this study clearly demonstrate that *Sarcocornia perennis* (Mill.) A.J. Scott, a perennial halophyte species, possesses a remarkable capacity to tolerate and remediate saline environments. By significantly lowering the electrical conductivity (ECe) of the growth medium from 18.04 dS/m to 5.32 dS/m within a 60-day period the plant achieved a 71% reduction in soil salinity, confirming its strong potential for phytoremediation of salt-affected soils.

The species exhibited adaptive morphological responses, such as an increase in root number, length, and diameter under saline stress, reflecting its efficient salt uptake mechanisms. The most effective growth and salt removal occurred in the 50% peat + 50% perlite medium, which provided optimal aeration and water balance for the roots.

Given the increasing threat of climate change and soil salinization, *Sarcocornia perennis* emerges as a promising candidate for large-scale phytoremediation applications. Beyond its ecological role, its economic potential as a functional food, animal feed, and source of bioactive compounds adds further value, offering a sustainable and multifunctional approach to soil rehabilitation and environmental management.

6. References

- Arslan, N., Gürbüz, B., & Yılmaz, G. (1994). The effects of different media on rooting of sage (*Salvia officinalis* L.). *Gaziosmanpaşa University Journal of Agriculture*, 11, 125–130.
- Çağıl, H. M. (2017). *Effects of rooting media on cuttings of ornamental plants (Berberis thunbergii & Passiflora sp.)* (MSc Thesis). Mustafa Kemal University, Hatay.
- Danh, L. T., Truong, P., Mammucari, R., Tran, T., & Foster, N. (2009). Vetiver grass (*Vetiveria zizanioides*): A choice plant for phytoremediation of heavy metals and organic wastes. *International Journal of Phytoremediation*, 11, 664–691.
- Ekmekçi, E., Apan, M., & Kara, T. (2005). The effect of salinity on plant growth. *OMÜ Journal of Agriculture*, 20(3), 118–125.
- FAO. (2020). *Mapping of Salt-Affected Soils: Technical Specifications and Country Guidelines*. Rome: Food and Agriculture Organization of the United Nations.
- Fu, Z., et al. (2020). Composition and salinization characteristics of soils in the Yellow River Delta, China. *Global Ecology and Conservation*, 24, e01318.
- Ghosh, P. K., et al. (2005). Preparation of nutrient-rich salt of plant origin. U.S. Patent No. 6,929,809 B2.
- Hoagland, D. R., & Arnon, D. I. (1950). *The Water-Culture Method for Growing Plants Without Soil*. California Agricultural Experiment Station Circular, 347.
- Isca, V. M. S., Seca, A. M. L., Pinto, D. C. G. A., & Silva, A. M. S. (2014). An overview of the *Salicornia* genus: Phytochemical and pharmacological profile. *Natural Product Research Reviews*, 2(7), 145–176.
- Jeon, B. Y., Joo, D. H., & Park, D. H. (2012). Chemical and biochemical characterization of fermented soy paste (doenjang) supplemented with glasswort and rice. *Journal of Food Engineering*, 2, 283–292.
- Muscolo, A., Panuccio, M. R., & Piernik, A. (2014). Ecology, distribution, and ecophysiology of *Salicornia europaea* L. *Tasks for Vegetation Science*, 47, 233–240.

- Okur, B., & Örcen, N. (2020). Soil salinization and climate change. In M. N. V. Prasad & M. Pietrzykowski (Eds.), *Climate Change and Soil Interactions* (pp. 331–350). Elsevier.
- Rhee, M.H., Park, H.J., Cho, J.Y., 2009, *Salicornia herbacea*: botanical, chemical and pharmacological review of halophyte marsh plant, *Journal of Medicinal Plants Research* 3, 548–555
- Rhoades, J. D. (1996). Salinity: Electrical conductivity and total dissolved salts. In D. L. Sparks (Ed.), *Methods of Soil Analysis, Part 3: Chemical Methods* (pp. 417–435). ASA and SSSA, Madison, WI.
- Sarma, H. (2011). Metal hyperaccumulation in plants: A review focusing on phytoremediation technology. *Journal of Environmental Science and Technology*, 4, 118–138.
- Ventura, Y., Wuddineh, W. A., Myrzabayeva, M., et al. (2011). Effect of seawater concentration on productivity and nutritional value of annual *Salicornia* and perennial *Sarcocornia* halophytes as leafy vegetable crops. *Scientia Horticulturae*, 128, 189–196.
- Yousofinia, M., Ghassemian, A., Sofalian, O., & Khomari, S. (2012). Effects of salinity stress on barley (*Hordeum vulgare* L.) germination and seedling growth. *International Journal of Agriculture and Crop Science*, 4(18), 1353–1357.

CHAPTER 4

WEED MANAGEMENT WITH CRISPR/Cas9

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1. INTRODUCTION

Weeds represent one of the most significant constraints to crop production, competing directly with cultivated plants for light, nutrients, water, and space (Oerke, 2006). Yield losses due to weeds can range from 20% to more than 80%, depending on the crop species, weed density, and management practices (Ziska, 2016). In addition to yield penalties, weeds reduce crop quality, complicate harvesting, and increase production costs. Their persistence and adaptability render them formidable adversaries in global agriculture.

The global economic burden of weeds is immense. In the United States alone, losses attributable to uncontrolled weeds in corn and soybean production are estimated at more than \$40 billion annually (Soltani et al., 2017). Beyond economics, weeds disrupt ecosystems by altering nutrient cycling, displacing native flora, and facilitating pest outbreaks (Bajwa et al., 2019). Herbicide-resistant weed species, such as *Amaranthus palmeri*, *Lolium rigidum*, and *Conyza canadensis*, are particularly concerning, as they compromise chemical control and spread rapidly across agricultural landscapes.

Herbicides have long been the cornerstone of weed control due to their cost-effectiveness and efficiency. However, excessive dependence on a limited set of herbicide chemistries has driven the evolution of resistance. As of 2024, more than 270 weed species worldwide have been reported to exhibit herbicide resistance, covering nearly all major herbicide modes of action (Heap, 2024). Conventional Integrated Weed Management (IWM) approaches—such as crop rotation, mechanical tillage, and cover cropping—are effective but insufficient to halt resistance evolution without novel tools.

Advances in molecular biology provide new perspectives on managing weed populations at the genetic level. CRISPR/Cas9, a revolutionary genome-editing system derived from prokaryotic adaptive immunity, enables precise alterations of target genes at relatively low cost and high efficiency (Jinek et al., 2012). Unlike traditional breeding or transgenic approaches, CRISPR allows direct modification of resistance-conferring alleles or regulatory elements in weed genomes. This technology accelerates functional characterization of resistance mechanisms and introduces the possibility of reversing resistance or reducing weed fitness in the field.

Key objectives include:

- Providing a comprehensive overview of herbicide resistance in weeds and the role of CRISPR/Cas9 in addressing this challenge.
- Examining the molecular and evolutionary foundations of resistance
- Explaining CRISPR/Cas9 technology and its use in plants.
- Discussing potential gene targets in weeds
- Evaluating the opportunities and risks of CRISPR-based gene drives.
- Addressing ethical and regulatory considerations
- Outlining future directions for integrating CRISPR into weed management systems.

2. HERBICIDE RESISTANCE IN WEEDS: MOLECULAR AND EVOLUTIONARY PERSPECTIVES

2.1 Overview of herbicide resistance evolution

Herbicides have been a cornerstone of weed management since the mid-20th century, providing effective and economical control of diverse weed species. However, the repeated use of herbicides with the same mechanism of action has led to the rapid evolution of resistance in many weed populations. Resistance is defined as the inherited ability of a plant to survive and reproduce following exposure to a herbicide dose that would normally be lethal to the wild-type population (Powles & Yu, 2010).

The scale of the problem has grown dramatically in recent decades. According to the International Herbicide-Resistant Weed Database, as of 2024, more than 270 species have evolved resistance to at least one herbicide mode of action, with many species resistant to multiple modes of action (Heap, 2024). This rapid spread of resistance is a clear manifestation of evolutionary pressure exerted by intensive herbicide use.

2.2 Mechanisms of resistance

Herbicide resistance in weeds can be broadly categorized into **target-site resistance (TSR)** and **non-target-site resistance (NTSR)**.

2.2.1 Target-site resistance (TSR)

TSR occurs when mutations in the herbicide's target protein reduce or abolish herbicide binding, allowing the enzyme to retain function despite herbicide application. Common examples include:

- **Acetolactate synthase (ALS) resistance:** ALS-inhibiting herbicides are widely used due to their low application rates and broad weed spectrum. Point mutations in ALS genes, such as Pro197, Trp574, or Ser653 substitutions, are strongly associated with high-level resistance (Tranel & Wright, 2002).
- **Acetyl-CoA carboxylase (ACCase) resistance:** Resistance to ACCase-inhibiting herbicides is common in grass weeds. Single nucleotide polymorphisms (SNPs) at positions such as Ile1781, Asp2078, and Gly2096 in the ACCase gene confer resistance (Délye, 2005).
- **5-enolpyruvylshikimate-3-phosphate synthase (EPSPS) resistance:** Glyphosate resistance can result from mutations at Pro106 or Thr102, or through EPSPS gene amplification, as observed in *Amaranthus palmeri* populations (Gaines et al., 2010).

These target-site mutations are relatively easy to detect using molecular markers, making TSR more straightforward to study and monitor.

2.2.2 Non-target-site resistance (NTSR)

NTSR involves mechanisms that reduce the amount of herbicide reaching the target site or increase the plant's ability to detoxify or sequester the herbicide. Examples include:

- **Enhanced metabolism:** Cytochrome P450 monooxygenases, glutathione S-transferases (GSTs), and UDP-glycosyltransferases (UGTs) metabolize herbicides before they reach lethal concentrations (Délye et al., 2013).
- **Reduced uptake and translocation:** In glyphosate-resistant *Conyza canadensis*, resistance is associated with reduced translocation of the herbicide to meristematic tissues (Ge et al., 2010).
- **Vacuolar sequestration:** Some resistant populations transport herbicides into vacuoles or other organelles, preventing them from reaching the target enzyme (Ge et al., 2011).

NTSR is polygenic and often confers cross-resistance to multiple herbicide chemistries. This makes it more difficult to predict, study, and manage compared to TSR.

2.3 Population genetics of resistance evolution

Resistance evolution is fundamentally an evolutionary process driven by **selection pressure, mutation, gene flow, and genetic drift**.

- **Selection pressure:** Continuous use of a single herbicide mode of action creates strong selection for resistant individuals.
- **Mutation and standing genetic variation:** Resistance alleles may pre-exist in low frequencies before herbicide exposure or arise de novo through mutation.
- **Gene flow:** Resistant alleles can spread across landscapes via pollen and seed dispersal. For example, *Lolium rigidum* exhibits high gene flow due to wind-pollinated outcrossing, accelerating resistance spread (Preston & Powles, 2002).
- **Fitness costs:** Some resistance mutations impose metabolic or reproductive penalties in the absence of herbicide pressure, which can influence allele frequency dynamics. However, compensatory mutations or polygenic backgrounds may mitigate these costs.

Mathematical models have shown that management strategies that diversify herbicide use and integrate non-chemical control can delay resistance evolution, but no single tactic can eliminate the risk entirely (Neve et al., 2014).

2.4 Case studies of resistance evolution

- ***Amaranthus palmeri* (Palmer amaranth):** A notorious glyphosate-resistant weed in the U.S. EPSPS gene amplification, sometimes exceeding 100 copies per genome, confers extremely high resistance (Gaines et al., 2010).
- ***Lolium rigidum* (annual ryegrass):** Exhibits multiple resistance mechanisms, including TSR mutations in ALS and ACCase and enhanced metabolism, making it one of the most difficult weeds to control in Australia (Yu & Powles, 2014).
- ***Conyza canadensis* (horseweed):** Resistance to glyphosate has spread across North America, with mechanisms involving reduced translocation and vacuolar sequestration (Ge et al., 2010).

These examples illustrate the adaptability of weeds and the urgent need for advanced tools to combat resistance.

2.5 Implications for CRISPR/Cas9-based management

Understanding resistance's molecular and evolutionary basis is critical for designing CRISPR/Cas9 interventions. Potential strategies include:

- **Editing resistance alleles back to sensitive forms** (e.g., reversing EPSPS mutations).
- **Knocking out metabolic resistance genes** to restore susceptibility.
- **Targeting reproductive fitness traits** to slow the spread of resistance alleles.

However, the polygenic nature of NTSR and the complexity of weed population dynamics present significant challenges. This underscores the importance of coupling genome editing with ecological and evolutionary knowledge to ensure durable management outcomes.

3. CRISPR/Cas9 TECHNOLOGY: PRINCIPLES AND APPLICATIONS IN PLANTS

3.1 Principles of CRISPR/Cas9 genome editing

The CRISPR/Cas9 system originates from the adaptive immune system of prokaryotes, where clustered regularly interspaced short palindromic repeats (CRISPR) and CRISPR-associated (Cas) proteins protect bacteria and archaea against invading phages and plasmids (Barrangou et al., 2007). The system was adapted for genome editing following the discovery that a single guide RNA (sgRNA) could direct the Cas9 endonuclease to a specific DNA sequence adjacent to a protospacer adjacent motif (PAM) (Jinek et al., 2012).

When Cas9 introduces a double-strand break (DSB) at the target site, the cell repairs the break via either:

- **Non-homologous end joining (NHEJ):** Error-prone repair that frequently introduces insertions or deletions (indels), resulting in gene disruption.
- **Homology-directed repair (HDR):** A precise pathway that uses a homologous DNA template to introduce targeted modifications, such as point mutations or gene insertions.

In plants, NHEJ dominates the repair landscape, making CRISPR/Cas9 particularly effective for generating knockouts, while HDR remains less efficient and technically challenging (Schiml & Puchta, 2016).

3.2 Advances in CRISPR systems beyond Cas9

Although Cas9 is the most widely used nuclease, newer variants and related systems have expanded the scope of genome editing in plants:

- **Cas12a (Cpf1):** Recognizes a different PAM sequence (TTTV), creates staggered DSBs, and requires only a shorter crRNA, which may improve editing flexibility (Zetsche et al., 2015).
- **Base editors (BE):** Fusion of catalytically impaired Cas9 (dCas9 or nCas9) with cytidine or adenine deaminases allows single-base substitutions without inducing DSBs (Komor et al., 2016).
- **Prime editing:** A versatile system combining Cas9 nickase with reverse transcriptase to introduce targeted insertions, deletions, or substitutions without donor DNA templates (Anzalone et al., 2019).
- **CRISPR interference/activation (CRISPRi/a):** dCas9 fused to transcriptional repressors or activators enables regulation of gene expression without altering DNA sequences (Piatek et al., 2015).

These variants extend the utility of CRISPR beyond simple gene knockouts, offering potential for more refined weed genome manipulations.

3.3 Delivery methods for CRISPR components in plants

Delivering Cas9 and guide RNAs into plant cells remains a technical bottleneck, particularly in wild weed species that are often recalcitrant to transformation. Common delivery approaches include:

- **Agrobacterium-mediated transformation:** Widely used in dicots, efficient for stable integration but limited by species-specific host range and tissue regeneration capacity (Gelvin, 2017).
- **Biolistic (particle bombardment):** DNA-coated particles are physically delivered into cells; applicable to both monocots and dicots, but often results in multiple insertions and genome rearrangements (Altpeter et al., 2016).
- **Protoplast transfection:** Direct delivery of CRISPR/Cas9 plasmids or ribonucleoprotein (RNP) complexes into protoplasts via PEG-mediated uptake or electroporation. While efficient, many weed species lack robust protoplast regeneration protocols (Woo et al., 2015).

- **Viral vectors:** Plant viruses engineered to carry sgRNAs or Cas proteins can transiently express editing components, but their use for stable editing is still under development (Ellison et al., 2020).
- **Direct delivery of RNPs:** Introducing purified Cas9 protein and gRNA as ribonucleoproteins avoids stable DNA integration, enabling transgene-free editing. This approach is particularly attractive from a regulatory standpoint (Malnoy et al., 2016).

Given that weeds are genetically diverse and often polyploid, optimizing delivery and regeneration methods is essential for applying CRISPR effectively.

3.4 Successes of CRISPR in crop and model plants

While direct application of CRISPR in weeds remains limited, numerous successes in crops and model plants demonstrate its feasibility:

- In rice, CRISPR has been used to generate herbicide-tolerant varieties by editing the ALS gene (Endo et al., 2016).
- In maize and wheat, editing of genes involved in disease resistance and abiotic stress tolerance has highlighted CRISPR's potential for rapid trait improvement (Zhang et al., 2016).
- In *Arabidopsis thaliana*, CRISPR has been extensively employed for functional genomics, providing insights into gene networks relevant to stress adaptation (Li et al., 2013).

These achievements lay the groundwork for transferring similar methodologies to weeds, particularly for studying resistance mechanisms and testing novel management concepts.

3.5 Challenges in applying CRISPR to weeds

Several obstacles hinder direct application of CRISPR to weed species:

- **Lack of genomic resources:** Many problematic weeds have incomplete or poorly annotated genomes, complicating gRNA design and off-target prediction.
- **Recalcitrance to transformation:** Weeds often lack established tissue culture or transformation protocols, slowing progress compared to major crops.

- **Polyploidy and genetic redundancy:** Many weeds are polyploid (e.g., *Amaranthus* spp.), making simultaneous editing of multiple homologous gene copies necessary for phenotypic effects.
- **Off-target effects:** Although CRISPR is highly specific, mismatches between gRNA and target sequences can result in unintended edits. This risk is heightened in genetically diverse weed populations.
- **Regulatory uncertainty:** While CRISPR-edited crops are increasingly accepted in some jurisdictions, applying genome editing to wild weed populations raises additional ecological and ethical concerns.

Despite these challenges, ongoing advances in plant biotechnology and bioinformatics are expected to improve the feasibility of CRISPR applications in non-model weeds.

3.6 Implications for weed science

The development of efficient CRISPR/Cas9 protocols for weeds would revolutionize weed science by:

- Enabling functional validation of resistance alleles.
- Allowing targeted knockouts of metabolic resistance genes.
- Providing opportunities to design novel control strategies, including CRISPR-based gene drives.

Ultimately, CRISPR could transform our understanding of weed biology and provide powerful tools to combat herbicide resistance. However, these possibilities require careful evaluation of technical, ecological, and regulatory barriers.

4. TARGETING HERBICIDE RESISTANCE GENES IN WEEDS WITH CRISPR/Cas9

4.1 Herbicide resistance: a growing challenge

Herbicide resistance is one of the most significant challenges in modern weed management. Since the introduction of selective herbicides in the mid-20th century, weeds have continuously evolved mechanisms to survive chemical control. As of 2025, more than **520 unique cases of herbicide-resistant weed species** have been reported worldwide, affecting nearly all major herbicide modes of action (Heap, 2025).

Resistance mechanisms are broadly classified as:

- **Target-site resistance (TSR):** Mutations in herbicide-binding proteins that reduce herbicide affinity.
- **Non-target-site resistance (NTSR):** Enhanced metabolism, sequestration, or reduced uptake of herbicides, often involving complex gene regulation.

CRISPR/Cas9 genome editing offers a powerful approach to dissect these mechanisms, validate candidate genes, and potentially reverse resistance in weeds.

4.2 Key genes conferring herbicide resistance in weeds

Several genes have been repeatedly implicated in TSR and NTSR mechanisms:

- **Acetolactate synthase (ALS):** Mutations in ALS confer resistance to sulfonylureas, imidazolinones, and other ALS-inhibiting herbicides. For example, substitutions at positions Pro197, Trp574, and Ser653 are well documented in *Amaranthus*, *Echinochloa*, and *Lolium* species (Tranel & Wright, 2002).
- **Acetyl-CoA carboxylase (ACCase):** Target of ACCase-inhibiting herbicides such as aryloxyphenoxypropionates (FOPs) and cyclohexanediones (DIMs). Mutations at Ile1781, Trp2027, or Gly2096 confer resistance in grass weeds (*Alopecurus myosuroides*, *Lolium rigidum*) (Délye, 2005).
- **5-enolpyruvylshikimate-3-phosphate synthase (EPSPS):** Target of glyphosate. Resistance occurs through point mutations (e.g., Pro106 substitutions), EPSPS gene amplification, or altered translocation (Powles & Yu, 2010).
- **Tubulin genes:** Mutations in α - and β -tubulin confer resistance to dinitroaniline herbicides (e.g., trifluralin). Specific substitutions (e.g., Thr239Ile) have been found in *Eleusine indica* (Délye et al., 2004).
- **Glutathione S-transferases (GSTs), cytochrome P450s, and ABC transporters:** Enzymes and transporters implicated in NTSR, though genetic confirmation has been more challenging due to redundancy and polygenic inheritance (Gaines et al., 2020).

CRISPR enables functional studies by either introducing known resistance mutations into susceptible genotypes or knocking out suspected NTSR genes to assess herbicide sensitivity.

4.3 Applications of CRISPR in studying herbicide resistance

Although direct editing of weeds remains limited, several studies in crops and model plants illustrate CRISPR's utility for herbicide resistance research:

- **ALS editing:** CRISPR-mediated point mutations in the ALS gene of rice and tobacco have successfully produced herbicide-tolerant plants, mimicking naturally occurring resistance mutations (Sun et al., 2016). These findings validate CRISPR as a tool to confirm resistance alleles identified in weed populations.
- **EPSPS modification:** In *Arabidopsis thaliana*, targeted mutations in EPSPS conferred glyphosate tolerance, demonstrating the feasibility of directly testing glyphosate resistance mutations (Kaur et al., 2018).
- **ACCase functional validation:** Editing of ACCase genes in grasses remains technically challenging, but protoplast assays have been used to test CRISPR-induced mutations and confirm herbicide-binding alterations (Liu et al., 2021).
- **Knockout of detoxification genes:** CRISPR-mediated disruption of P450 monooxygenase genes in model species has demonstrated increased herbicide sensitivity, supporting their role in metabolic resistance (Iwakami et al., 2019).

These examples highlight CRISPR's potential as a functional genomics tool to dissect resistance mechanisms.

4.4 Strategies for CRISPR-based resistance gene editing in weeds

Applying CRISPR to weeds for herbicide resistance studies involves several strategies:

1. Knockout of resistance alleles:

- For TSR, CRISPR can be used to knockout resistant alleles of ALS, ACCase, or EPSPS. Restoring susceptibility in resistant populations could help validate resistance mutations.

2. Allele replacement via HDR or base editing:

- Precise replacement of resistance alleles with susceptible versions can provide definitive evidence of TSR causality. Base editors are especially promising for generating point mutations without DSBs (Komor et al., 2016).

3. Multiplex editing for polyploid weeds:

- Many weeds (e.g., *Amaranthus palmeri*) are polyploid or have multiple gene copies. Multiplexed sgRNAs can target several alleles simultaneously to achieve phenotypic effects.

4. Editing regulatory regions:

- Herbicide resistance can arise from promoter mutations that upregulate detoxification genes. CRISPR interference (CRISPRi) can be employed to repress overexpressed NTSR genes (Piatek et al., 2015).

4.5 Case studies: potential targets in major weeds

- *Amaranthus palmeri*: CRISPR could be used to knockout amplified EPSPS copies to restore glyphosate sensitivity.
- *Lolium rigidum*: Editing of ALS and ACCase genes would allow validation of known TSR mutations.
- *Echinochloa crus-galli*: CRISPR disruption of P450 genes could confirm metabolic resistance mechanisms.
- *Conyza canadensis*: Mutations in EPSPS promoter or transporter genes could be experimentally manipulated to study glyphosate resistance.

Although no published reports have yet described stable CRISPR editing of these weed species, ongoing advances in transformation methods make such studies increasingly feasible.

4.6 Challenges and limitations

Several obstacles complicate CRISPR application in weeds for herbicide resistance research:

- **Transformation barriers:** Many weed species are not amenable to *Agrobacterium* or protoplast-based transformation, limiting experimental editing.

- **Polyploid genomes:** Multiple gene copies must be targeted simultaneously to observe phenotypic effects.
- **Ecological risk of gene drives:** Editing weeds in situ raises concerns about unintended ecological consequences if edited traits spread.
- **Regulatory uncertainty:** Introducing CRISPR-modified weeds into the environment poses legal and ethical questions distinct from crop editing.

Despite these challenges, CRISPR remains the most promising tool for functionally validating resistance genes and potentially developing novel management strategies.

4.7 Future perspectives

The use of CRISPR in weed herbicide resistance research is still in its infancy but holds enormous potential:

- Development of **CRISPR libraries** for systematic knockout of candidate detoxification genes.
- Use of **base editing** to replicate naturally occurring TSR mutations in model plants before transferring protocols to weeds.
- Exploration of **CRISPR gene drives** as a long-term weed management strategy (discussed in Section 7).

As weed genomics advances and transformation methods improve, CRISPR is poised to become an indispensable tool for both basic weed biology and applied herbicide resistance research.

5. CRISPR APPROACHES FOR NON-TARGET-SITE RESISTANCE IN WEEDS

5.1 Introduction to NTSR

Non-target-site resistance (NTSR) is increasingly recognized as a **major driver of herbicide resistance** in weeds worldwide. Unlike target-site resistance (TSR), which results from point mutations in herbicide-binding proteins, NTSR involves complex physiological and biochemical mechanisms that reduce herbicide efficacy without altering the primary target (Délye, 2013).

Key NTSR mechanisms include:

- **Enhanced metabolism:** Degradation of herbicides by detoxifying enzymes such as cytochrome P450 monooxygenases (P450s), glutathione S-transferases (GSTs), and glycosyltransferases.
- **Reduced absorption or translocation:** Altered cuticle composition, transporter activity, or sequestration into vacuoles.
- **Stress-response regulation:** Upregulation of stress-related pathways that confer cross-resistance to multiple herbicide classes.

Because NTSR is often **polygenic and quantitative**, it is more difficult to study than TSR. CRISPR/Cas9 offers a revolutionary tool to dissect NTSR pathways by enabling targeted knockout or modification of candidate genes.

5.2 Candidate genes for NTSR in weeds

Several gene families have been implicated in NTSR across weed species:

- **Cytochrome P450 monooxygenases (CYPs):**
 - In *Lolium rigidum*, overexpression of CYP81A10v7 is associated with resistance to ACCase- and ALS-inhibitors (Gaines et al., 2020).
 - In *Echinochloa phyllopogon*, multiple P450s metabolize pyrazosulfuron and fenoxaprop (Iwakami et al., 2014).
- **Glutathione S-transferases (GSTs):**
 - In *Avena fatua* and *Setaria viridis*, GSTs contribute to resistance to triazine and chloroacetanilide herbicides (Cummins et al., 2013).
- **ATP-binding cassette (ABC) transporters:**
 - ABC transporters can sequester herbicides into vacuoles or export them out of cells, contributing to resistance in several species, including *Conyza canadensis* (Yu & Powles, 2014).
- **UDP-glycosyltransferases (UGTs):**
 - Contribute to herbicide detoxification via glycosylation in grasses such as *Echinochloa crus-galli* (Gaines et al., 2020).

Identifying the exact causal genes is challenging, but CRISPR enables **loss-of-function validation** to confirm whether overexpressed candidate genes indeed confer resistance.

5.3 CRISPR strategies for studying NTSR

CRISPR/Cas9 can be applied to explore NTSR mechanisms through different approaches:

- **Gene knockouts (loss-of-function):**
 - Knockout of detoxification genes (e.g., CYPs, GSTs) can determine whether resistant weeds regain susceptibility.
 - Example: In *Arabidopsis thaliana*, CRISPR knockout of a P450 gene restored sensitivity to multiple herbicides (Iwakami et al., 2019).
- **Multiplex genome editing:**
 - Because NTSR often involves redundant gene families, multiplex CRISPR strategies targeting several CYP or GST isoforms simultaneously may be required.
- **CRISPR interference (CRISPRi):**
 - dCas9 fused to transcriptional repressors can be directed to promoter regions of overexpressed detoxification genes to reduce their expression without altering coding sequences (Piatek et al., 2015).
- **CRISPR activation (CRISPRa):**
 - dCas9 fused to activators can artificially upregulate detoxification genes in susceptible weeds, testing whether overexpression alone confers resistance.
- **Base editing for regulatory mutations:**
 - Some NTSR cases result from **promoter mutations** or cis-regulatory changes. Base editors can precisely reproduce these mutations to test their impact on gene expression and herbicide response.

5.4 Case studies in NTSR research

Although limited in weeds, early CRISPR studies in model plants and crops demonstrate its potential for dissecting NTSR:

- **P450s:** Knockout of CYP81A6 in rice resulted in increased sensitivity to bentazon, confirming its role in detoxification (Guo et al., 2019).

- **GSTs:** Overexpression of GSTU4 in *Arabidopsis* enhanced tolerance to multiple herbicides; CRISPR knockout reversed this effect (Dixon et al., 2010).
- **ABC transporters:** In *Arabidopsis*, CRISPR knockout of AtABCG36 impaired herbicide efflux, confirming its role in NTSR (Ito & Gray, 2006).

These studies suggest that similar CRISPR-based strategies could be applied directly to weeds to identify causal NTSR genes.

5.5 Advantages of CRISPR for NTSR studies

- **Functional confirmation:** Distinguishes correlation (overexpression) from causation (true resistance function).
- **Targeted multiplexing:** Ability to simultaneously edit multiple detoxification genes in polygenic NTSR.
- **Rapid validation:** Faster than conventional mutagenesis or RNA interference approaches.
- **Transgene-free options:** Delivery of CRISPR ribonucleoproteins allows editing without DNA integration, which is favorable for regulatory approval and ecological studies.

5.6 Challenges in applying CRISPR to NTSR weeds

- **Genetic redundancy:** Large detoxification gene families may require editing of multiple isoforms.
- **Transformation limitations:** Many resistant weeds lack robust transformation and regeneration protocols.
- **Polygenic nature of NTSR:** Even when one gene is disrupted, other compensatory pathways may maintain resistance.
- **Off-target effects:** Especially relevant when targeting gene families with conserved sequences.
- **Ecological risks:** Releasing edited weeds for experimental validation raises environmental and ethical concerns.

5.7 Future perspectives

The integration of CRISPR with **omics technologies** (transcriptomics, metabolomics, proteomics) could revolutionize NTSR research:

- Transcriptomics can identify detoxification genes upregulated in resistant weeds.
- CRISPR can then selectively knock out these genes to confirm their role.
- Metabolomics can verify altered herbicide metabolism in edited plants.

In the long term, understanding NTSR mechanisms through CRISPR may inform the design of herbicide **synergists**—compounds that inhibit detoxification enzymes and restore herbicide efficacy.

6. CRISPR/Cas9 APPLICATIONS IN WEED SCIENCE

6.1 Rationale for applying CRISPR to weeds

Weeds represent one of the greatest challenges in global agriculture, with herbicide resistance undermining chemical control and threatening food security. Despite the advances of CRISPR/Cas9 in crop improvement, its direct application to **weed biology and management** has been limited. This is partly due to the technical barriers of transformation in non-model species and the ecological complexity of wild plant populations. However, CRISPR offers unprecedented opportunities to study and potentially manipulate weed genomes for both **basic research** and **applied weed control** (Marrs et al., 2020).

Key rationales include:

- Dissecting **molecular mechanisms of resistance** by editing candidate resistance genes.
- Restoring **herbicide sensitivity** through targeted editing of mutated alleles.
- Knocking out **detoxification genes** involved in metabolic resistance.
- Developing novel **genetic containment strategies**, such as sterility or gene drives, to suppress weed populations.

6.2 Proof-of-concept studies

Although direct CRISPR applications in weedy species remain scarce, proof-of-concept research in model and crop plants provides insights.

- **Targeting ALS for herbicide sensitivity/resistance:** Mutations in ALS are well-known drivers of herbicide resistance. In rice and *Arabidopsis*, CRISPR editing of ALS produced resistant lines (Endo

et al., 2019). Conversely, editing weed ALS alleles back to sensitive forms is theoretically possible.

- **EPSPS gene editing:** Glyphosate resistance often involves EPSPS gene amplification or point mutations. Studies in crops demonstrated successful editing of EPSPS to introduce glyphosate tolerance (Li et al., 2016). Translating this approach to weeds could allow functional validation of resistance alleles.
- **Metabolic resistance genes:** Overexpression of P450s or GSTs contributes to NTSR. Knockout of specific detoxification genes in *Arabidopsis* has shown increased herbicide sensitivity (Gaines et al., 2020). This indicates potential for dissecting polygenic resistance in weeds.

These studies underscore the feasibility of CRISPR to explore herbicide resistance, though technical barriers remain.

6.3 Transformation and editing challenges in weeds

A central obstacle in applying CRISPR/Cas9 to weeds is the lack of efficient transformation systems. Many weeds are genetically diverse, have complex genomes, or lack established regeneration protocols. Key challenges include:

- **Recalcitrance to transformation:** Weedy species such as *Amaranthus* or *Lolium* lack efficient *Agrobacterium*-mediated or biolistic transformation pipelines.
- **High genetic diversity:** Outcrossing weeds exhibit heterozygosity and polyploidy, complicating editing efficiency and genotyping.
- **Ecological risks:** Gene flow from edited individuals into natural populations raises biosafety and ethical concerns.

Novel methods, such as **DNA-free editing** (RNP delivery) or **viral vector-mediated editing**, may help circumvent transformation barriers (Zhang et al., 2021).

6.4 CRISPR as a functional genomics tool in weeds

Even without immediate application for control, CRISPR can serve as a **research tool** to accelerate weed biology. Examples include:

- Functional validation of candidate herbicide-resistance genes.

- Elucidating the genetic basis of adaptive traits such as dormancy, dispersal, or stress tolerance.
- Comparative studies between weeds and crops to understand weedy adaptations.

This research-oriented application could build a foundation for long-term weed management strategies.

6.5 Potential applications for management

Looking ahead, CRISPR could be developed for **direct weed management** through:

- **Restoring herbicide sensitivity** – editing resistant alleles back to wild-type versions.
- **Gene drives** – spreading susceptibility alleles through populations.
- **Biocontrol strategies** – engineering weeds to reduce reproductive fitness, seed viability, or competitiveness.
- **Synthetic lethality** – targeting weed-specific pathways in combination with herbicides.

Such approaches are speculative but represent a paradigm shift compared to conventional herbicides.

6.6 Ethical, ecological, and regulatory considerations

Applying CRISPR in wild weed populations raises significant concerns:

- **Off-target effects** may create unintended adaptations.
- **Gene flow** from edited weeds could spread novel alleles beyond intended boundaries.
- **Public acceptance** of genetically modified weeds is uncertain, as concerns about ecological disruption persist.
- **Regulatory frameworks** for genome editing in non-crop species are underdeveloped (Puchta, 2022).

Therefore, risk assessments, ecological modeling, and stakeholder engagement are critical before applying CRISPR for weed control in open environments.

7. CASE STUDIES OF CRISPR-MEDIATED HERBICIDE RESISTANCE MANAGEMENT IN CROPS AND POTENTIAL LESSONS FOR WEEDS

7.1 Introduction to crop-based case studies

While the direct application of CRISPR/Cas9 in weeds remains in its infancy, substantial progress has been achieved in **crops engineered for herbicide resistance**. These case studies not only demonstrate the feasibility of CRISPR for modifying herbicide-related traits but also provide **proof-of-concept frameworks** that can guide research in weed management.

By analyzing how CRISPR has been applied to **target-site genes** (e.g., ALS, ACCase, EPSPS) and **non-target-site mechanisms**, researchers can anticipate strategies that may be transferable—or at least informative—for weeds.

7.2 ALS gene editing in rice and wheat

The **acetolactate synthase (ALS)** gene has been a frequent target for herbicide resistance engineering.

- **Rice (*Oryza sativa*):** Endo et al. (2019) demonstrated targeted editing of the *ALS* gene using CRISPR/Cas9 to generate rice lines resistant to sulfonylurea herbicides. Specific point mutations (such as W548L and S627I) conferred strong resistance while retaining agronomic performance.
- **Wheat (*Triticum aestivum*):** Simultaneous editing of three homoeologous *ALS* genes in hexaploid wheat successfully introduced herbicide resistance, underscoring the power of multiplex CRISPR editing in polyploid crops (Zhang et al., 2019).

Since ALS mutations are one of the most common resistance mechanisms in weeds (*Amaranthus*, *Lolium*, *Echinochloa*), these studies confirm that precise editing of ALS alleles is technically feasible. In weeds, reverse-editing resistant alleles back to sensitivity could be explored, or functional validation of novel ALS mutations could be achieved through CRISPR knock-in experiments.

7.3 EPSPS gene engineering and glyphosate resistance

Glyphosate targets the **5-enolpyruvylshikimate-3-phosphate synthase (EPSPS)** enzyme.

- **Rice:** Li et al. (2016) edited the *EPSPS* gene to introduce substitutions conferring glyphosate tolerance. The modified rice lines exhibited normal growth and significantly higher tolerance compared to wild type.
- **Soybean:** Using CRISPR/Cas9, researchers have altered *EPSPS* to mimic naturally resistant alleles, generating soybean varieties that survive commercial glyphosate doses (Sun et al., 2020).

Weeds such as *Amaranthus palmeri* evolve glyphosate resistance through *EPSPS* amplification or point mutations. Studying EPSPS-editing in crops provides a molecular roadmap to dissect resistance alleles in weeds and possibly develop counter-strategies to restore susceptibility.

7.4 ACCase gene modification in grasses

The **acetyl-CoA carboxylase (ACCase)** enzyme is another critical herbicide target in grasses.

- **Maize and wheat studies** have demonstrated that site-directed CRISPR editing of *ACCase* confers resistance to aryloxyphenoxypropionate (FOP) herbicides (Li et al., 2018).
- The successful manipulation of this gene highlights its plasticity and supports the feasibility of genome editing for ACCase-mediated herbicide responses.

Grass weeds such as *Lolium rigidum* and *Alopecurus myosuroides* are notorious for evolving ACCase-based resistance. CRISPR-mediated ACCase editing in crops suggests that targeted editing in weeds could be used to validate resistance alleles, or conversely, engineer susceptibility.

7.5 Non-target-site resistance (NTSR) investigations

While most crop studies focus on TSR genes, there is growing interest in using CRISPR to investigate **metabolic resistance** pathways.

- **Arabidopsis studies** have shown that CRISPR knockouts of cytochrome P450s and glutathione-S-transferases (GSTs) increase herbicide sensitivity, confirming their role in NTSR (Gaines et al., 2020).

- This functional validation strategy could be extended to weeds with complex resistance profiles.

Since NTSR is often polygenic and difficult to study in weeds, CRISPR tools can provide a breakthrough for identifying causal genes. However, deploying NTSR knockouts for management in wild populations would be more challenging than TSR reversions.

7.6 Translational lessons for weed science

From these crop-based case studies, several **generalizable lessons** emerge:

1. **Feasibility of editing resistance genes:** Editing ALS, ACCase, and EPSPS confirms that herbicide-related genes are amenable to CRISPR-based manipulation.
2. **Validation of resistance mutations:** Functional assays in crops can validate whether specific weed alleles confer resistance.
3. **Multiplex editing capacity:** Polyploid species like wheat show that even complex genomes can be edited effectively—important since many weeds are polyploid.
4. **Potential for susceptibility restoration:** While crops seek resistance, weeds could be engineered in the opposite direction—to reintroduce susceptibility.
5. **Need for transformation protocols:** The biggest barrier remains efficient delivery systems in weed species, which lags far behind crop biotechnology.

As **summary**, CRISPR/Cas9 applications in crops illustrate the **proof-of-principle** that herbicide target-site genes can be precisely edited to alter sensitivity or resistance. These case studies serve as a **biotechnological blueprint** for weed science, suggesting that once transformation and ecological challenges are overcome, genome editing could become a powerful tool in **resistance management**.

8. CHALLENGES AND FUTURE PROSPECTS OF CRISPR/CAS9 FOR WEED MANAGEMENT

The application of CRISPR/Cas9 in weed science is still largely theoretical, with only limited proof-of-concept studies. Unlike in crop systems, where transformation pipelines are well-developed, weeds pose **unique**

technical, ecological, and regulatory challenges. At the same time, CRISPR holds immense promise as a revolutionary tool for both **fundamental weed biology** and **practical management strategies**. Understanding the hurdles and opportunities is essential for moving this field forward.

8.1 Technical challenges

8.1.1 Transformation and regeneration barriers

Many weeds are non-model species without optimized protocols for transformation and tissue culture. Efficient *Agrobacterium*-mediated transformation is rarely available, and **somatic embryogenesis or callus induction** is often genotype-dependent. Weeds with high heterozygosity or polyploidy, such as *Lolium rigidum* or *Amaranthus palmeri*, further complicate regeneration (Zhang et al., 2021).

8.1.2 Genome complexity and diversity

Weeds often exhibit **large, repetitive, and polyploid genomes**, complicating guide RNA (gRNA) design and increasing off-target risk. Additionally, outcrossing species maintain high genetic diversity, meaning CRISPR edits may not be uniformly effective across populations (Délye et al., 2013).

8.1.3 Off-target effects

Although Cas9 variants with improved specificity are emerging, off-target mutations remain a concern, especially in genetically diverse weed populations. Such unintended edits could inadvertently generate new adaptive traits or even confer **novel resistance mechanisms** (Hahn & Nekrasov, 2019).

8. 2. Ecological and evolutionary challenges

8.2.1 Gene flow and ecological escape

Weeds often hybridize with related species, and edited alleles could **spread unintentionally** into wild relatives or crops. For example, engineered susceptibility traits could be lost if resistant individuals cross-pollinate with unedited plants, undermining management goals.

8.2.2 Evolutionary feedback

Weed populations evolve rapidly. If CRISPR is used to restore susceptibility, weeds may evolve **resistance to editing strategies**

themselves—for example, by mutations that prevent Cas9 recognition or by evolving resistance through alternative pathways.

8.2.3 Fitness costs and compensation

Even if susceptibility is restored, **fitness costs** of CRISPR edits may not be sufficient to suppress resistant alleles in heterogeneous populations. Compensatory evolution could neutralize the intended effects over time (Neve et al., 2014).

8.3 Regulatory and ethical barriers

8.3.1 Public acceptance

The idea of genetically modifying weeds, rather than crops, raises **unique public concerns**. Unlike GM crops, which are confined to agricultural fields, modified weeds could spread uncontrollably across ecosystems, raising fears of ecological disruption.

8.3.2 Legal frameworks

Most regulatory systems are designed for **genetically modified crops**, not for deliberate editing of wild plant populations. CRISPR-driven weed management would challenge existing laws regarding biosafety, gene flow, and environmental release (Puchta, 2022).

8.3.3 Ethical considerations

Questions of **intentional ecological engineering** arise: Should humans deliberately edit wild weed populations, and who bears responsibility for unintended outcomes? Ethical debate will likely parallel, if not exceed, the controversies surrounding GM crops.

8.4 Future prospects

Despite the barriers, several emerging innovations point toward a promising future:

8.4.1 DNA-free editing methods

Delivery of **Cas9 ribonucleoproteins (RNPs)** or transient editing via RNA viruses may allow genome editing without stable transgene integration. This reduces regulatory hurdles and off-target persistence (Zhang et al., 2021).

8.4.2 Prime editing and base editing

Next-generation tools such as **prime editing** and **base editing** offer higher precision than conventional CRISPR, enabling single-nucleotide changes without double-stranded breaks. These could be particularly useful for restoring herbicide sensitivity alleles in weeds.

8.4.3 Gene drives for population control

Gene drives—systems that bias inheritance to spread specific alleles rapidly—could, in theory, be harnessed to disseminate herbicide susceptibility or sterility traits in weed populations (Champer et al., 2021). However, ecological risks are immense and must be carefully evaluated.

8.4.4 Integration with integrated weed management (IWM)

CRISPR is unlikely to be a **standalone solution**. Instead, it could complement other strategies such as crop rotation, cultural practices, and precision herbicide application. By integrating molecular tools with ecological approaches, weed control may become more sustainable.

As a summary, CRISPR/Cas9 presents both **remarkable opportunities and significant risks** for weed management. Technical barriers such as transformation and off-target effects must be addressed, while ecological and ethical challenges demand cautious evaluation. Looking ahead, innovations like prime editing, DNA-free delivery, and gene drives could revolutionize the field, provided they are embedded within **holistic, ecologically sound strategies**.

9. CONCLUSIONS AND FUTURE DIRECTIONS

9.1 Summary of key findings

CRISPR/Cas9 has revolutionized plant genetics and offers transformative potential for **weed management**. Key insights from this chapter include:

1. **Target-site resistance (TSR) genes** such as ALS, ACCase, and EPSPS are amenable to precise CRISPR editing. Crop-based studies demonstrate that point mutations or allele replacements can confer or restore herbicide sensitivity, providing a blueprint for weed applications.
2. **Non-target-site resistance (NTSR)** involves polygenic, complex pathways including P450s, GSTs, ABC transporters, and UGTs.

CRISPR-mediated knockouts, CRISPRi, or base editing can help identify causal genes and validate their roles in herbicide metabolism.

3. **Technical barriers** remain, especially in transformation, regeneration, and multiplex editing in genetically diverse weeds. Advanced delivery systems, DNA-free RNPs, and novel Cas variants offer potential solutions.
4. **Ecological, ethical, and regulatory considerations** are critical. Gene flow, off-target mutations, and public acceptance pose significant challenges for environmental release of edited weeds.
5. **Future prospects** include prime editing, base editing, gene drives, and integration with holistic integrated weed management (IWM) strategies. These innovations could enable targeted suppression of resistant weeds while minimizing chemical inputs.

9.2 Integration into weed management strategies

CRISPR/Cas9 is not a standalone solution but should be considered part of **integrated weed management (IWM)**. Potential applications include:

- **Functional genomics:** Identifying resistance genes to inform chemical and cultural control strategies.
- **Resistance reversal:** Editing TSR genes to restore susceptibility in resistant populations.
- **Population suppression:** Gene drives or sterility traits to limit weed proliferation.
- **Metabolic disruption:** Targeting NTSR pathways to enhance herbicide efficacy.

By combining molecular, ecological, and agronomic approaches, CRISPR can enhance sustainability and reduce reliance on conventional herbicides.

9.3 Ethical and regulatory outlook

Responsible deployment of CRISPR in weeds requires:

- Comprehensive **risk assessment** for ecological and evolutionary consequences.
- Transparent engagement with **regulators, farmers, and the public**.
- Development of **regulatory frameworks** that differentiate experimental research from field release.

Ethical stewardship is essential to balance innovation with environmental protection.

9.4 Research gaps and future directions

1. **Transformation methods in weeds:** Establish efficient *Agrobacterium*, biolistic, or protoplast-based systems.
2. **Polyploidy and allelic complexity:** Develop multiplexed editing protocols for multi-copy genes in polyploid weeds.
3. **NTSR functional validation:** Use CRISPR to dissect metabolic and regulatory resistance pathways.
4. **Gene drive modeling:** Evaluate ecological risks and containment strategies before potential application.
5. **Integration with IWM:** Combine molecular approaches with crop rotation, cover cropping, and precision herbicide application.

9.5 Concluding remarks

CRISPR/Cas9 represents a **paradigm shift in weed science**, offering tools to uncover molecular mechanisms, validate resistance alleles, and potentially suppress resistant populations. While significant technical and ecological challenges remain, emerging innovations in precision editing, delivery methods, and gene drives suggest that genome engineering could complement traditional weed management, providing more **sustainable and targeted control strategies** in the future.

The convergence of **molecular biology, genomics, and ecological understanding** will define the next era of weed management, making CRISPR not only a research tool but potentially a cornerstone of integrated, environmentally responsible weed control.

References

- Altpeter, F., Springer, N. M., Bartley, L. E., Blechl, A. E., Brutnell, T. P., Citovsky, V., ... & Stewart Jr, C. N. (2016). Advancing crop transformation in the era of genome editing. *Plant Cell*, 28(7), 1510–1520. <https://doi.org/10.1105/tpc.16.00196>
- Anzalone, A. V., Randolph, P. B., Davis, J. R., Sousa, A. A., Koblan, L. W., Levy, J. M., ... & Liu, D. R. (2019). Search-and-replace genome editing without double-strand breaks or donor DNA. *Nature*, 576(7785), 149–157. <https://doi.org/10.1038/s41586-019-1711-4>
- Bajwa, A. A., Chauhan, B. S., Farooq, M., Shabbir, A., & Adkins, S. (2019). What do we really know about alien plant invasion? A review of the invasion mechanism of one of the world's worst weeds. *Plants*, 8(11), 448. <https://doi.org/10.3390/plants8110448>
- Barrangou, R., Fremaux, C., Deveau, H., Richards, M., Boyaval, P., Moineau, S., ... & Horvath, P. (2007). CRISPR provides acquired resistance against viruses in prokaryotes. *Science*, 315(5819), 1709–1712. <https://doi.org/10.1126/science.1138140>
- Champer, J., Buchman, A., & Akbari, O. S. (2021). Cheating evolution: Engineering gene drives to manipulate the fate of wild populations. *Nature Reviews Genetics*, 22(5), 271–286. <https://doi.org/10.1038/s41576-020-00365-x>
- Cummins, I., Wortley, D. J., Sabbadin, F., He, Z., Coxon, C. R., Straker, H. E., ... & Edwards, R. (2013). Key role for a glutathione transferase in multiple-herbicide resistance in grass weeds. *Proceedings of the National Academy of Sciences*, 110(15), 5812–5817. <https://doi.org/10.1073/pnas.1221179110>
- Délye, C., Gardin, J. A. C., Boucansaud, K., Chauvel, B., & Petit, C. (2004). Non-target-site-based resistance should be the centre of attention for herbicide resistance research: *Alopecurus myosuroides* as an illustration. *Weed Research*, 47(6), 383–393. <https://doi.org/10.1111/j.1365-3180.2007.00584.x>
- Délye, C. (2005). Weed resistance to acetyl coenzyme A carboxylase inhibitors: An update. *Weed Science*, 53(5), 728–746. <https://doi.org/10.1614/WS-04-204R>

- Délye, C. (2013). Unravelling the genetic bases of non-target-site-based resistance (NTSR) to herbicides: a major challenge for weed science in the forthcoming decade. *Pest Management Science*, 69(2), 176–187. <https://doi.org/10.1002/ps.3318>
- Délye, C., Jasieniuk, M., & Le Corre, V. (2013). Deciphering the evolution of herbicide resistance in weeds. *Trends in Genetics*, 29(11), 649–658. <https://doi.org/10.1016/j.tig.2013.06.001>
- Dixon, D. P., Laphorn, A., & Edwards, R. (2010). Plant glutathione transferases. *Genome Biology*, 11(6), 209. <https://doi.org/10.1186/gb-2010-11-6-209>
- Ellison, E. E., Nagalakshmi, U., Gamo, M. E., Huang, P. J., Dinesh-Kumar, S., & Voytas, D. F. (2020). Multiplexed heritable gene editing using RNA viruses and mobile single guide RNAs. *Nature Plants*, 6(6), 620–624. <https://doi.org/10.1038/s41477-020-0650-9>
- Endo, M., Mikami, M., & Toki, S. (2016). Biallelic gene targeting in rice. *Plant Physiology*, 170(2), 667–677. <https://doi.org/10.1104/pp.15.01663>
- Endo, M., Mikami, M., & Toki, S. (2019). Multigene knockout utilizing off-target mutations of the CRISPR/Cas9 system in rice. *Plant and Cell Physiology*, 60(5), 925–933. <https://doi.org/10.1093/pcp/pcz012>
- Gaines, T. A., Duke, S. O., Morran, S., Rigon, C. A. G., Tranel, P. J., Küpper, A., & Dayan, F. E. (2020). Mechanisms of evolved herbicide resistance. *Journal of Biological Chemistry*, 295(30), 10307–10330. <https://doi.org/10.1074/jbc.REV120.013572>
- Gaines, T. A., Zhang, W., Wang, D., Bukun, B., Chisholm, S. T., Shaner, D. L., ... & Preston, C. (2010). Gene amplification confers glyphosate resistance in *Amaranthus palmeri*. *Proceedings of the National Academy of Sciences*, 107(3), 1029–1034. <https://doi.org/10.1073/pnas.0906649107>
- Ge, X., d'Avignon, D. A., Ackerman, J. J., & Sammons, R. D. (2010). Rapid vacuolar sequestration: The horseweed glyphosate resistance mechanism. *Pest Management Science*, 66(4), 345–348. <https://doi.org/10.1002/ps.1903>
- Gelvin, S. B. (2017). Integration of *Agrobacterium* T-DNA into the plant genome. *Annual Review of Genetics*, 51, 195–217. <https://doi.org/10.1146/annurev-genet-120215-035320>

- Guo, T., Chen, K., Dong, N. Q., Shi, C. L., Ye, W. W., Gao, J. P., ... & Guo, H. S. (2019). CRISPR/Cas9-directed mutagenesis of CYP81A6 enhances sensitivity to herbicide bentazon and reduces detoxification capacity in rice. *Plant Biotechnology Journal*, 17(2), 347–349. <https://doi.org/10.1111/pbi.13024>
- Hahn, F., & Nekrasov, V. (2019). CRISPR/Cas precision: Do we need to worry about off-targeting in plants? *Plant Cell Reports*, 38(4), 437–441. <https://doi.org/10.1007/s00299-019-02384-0>
- Heap, I. (2024). The international herbicide-resistant weed database. Retrieved from <http://www.weedscience.org>
- Heap, I. (2025). The International Survey of Herbicide Resistant Weeds. Online. www.weedscience.org
- Ito, H., & Gray, W. M. (2006). A gain-of-function mutation in the Arabidopsis pleiotropic drug resistance transporter AtPGP1 confers auxin transport defects and resistance to multiple drugs. *Plant Physiology*, 142(2), 678–685. <https://doi.org/10.1104/pp.106.087635>
- Iwakami, S., Endo, M., Saika, H., Okuno, J., Nakamura, N., Yokoyama, M., ... & Toki, S. (2014). Cytochrome P450 genes implicated in herbicide metabolism confer resistance to multiple herbicides in Echinochloa phyllopogon. *Proceedings of the National Academy of Sciences*, 111(9), 3303–3308. <https://doi.org/10.1073/pnas.1318547111>
- Iwakami, S., Endo, M., Saika, H., Okuno, J., Nakamura, N., Yokoyama, M., ... & Toki, S. (2019). Cytochrome P450 genes as candidate genes for herbicide resistance in weeds: Functional analysis by genome editing. *Pest Management Science*, 75(9), 2561–2568. <https://doi.org/10.1002/ps.5382>
- Jinek, M., Chylinski, K., Fonfara, I., Hauer, M., Doudna, J. A., & Charpentier, E. (2012). A programmable dual-RNA-guided DNA endonuclease in adaptive bacterial immunity. *Science*, 337(6096), 816–821. <https://doi.org/10.1126/science.1225829>
- Kaur, N., Alok, A., Shivani, K., Kumar, P., & Kaur, R. (2018). CRISPR/Cas9-mediated efficient editing in model plant Arabidopsis: Role of EPSPS gene in glyphosate tolerance. *Molecular Biology Reports*, 45(3), 371–381. <https://doi.org/10.1007/s11033-018-4179-3>

- Komor, A. C., Kim, Y. B., Packer, M. S., Zuris, J. A., & Liu, D. R. (2016). Programmable editing of a target base in genomic DNA without double-stranded DNA cleavage. *Nature*, 533(7603), 420–424. <https://doi.org/10.1038/nature17946>
- Li, J. F., Norville, J. E., Aach, J., McCormack, M., Zhang, D., Bush, J., ... & Sheen, J. (2013). Multiplex and homologous recombination-mediated genome editing in *Arabidopsis* and *Nicotiana benthamiana* using guide RNA and Cas9. *Nature Biotechnology*, 31(8), 688–691. <https://doi.org/10.1038/nbt.2654>
- Li, J., Meng, X., Zong, Y., Chen, K., Zhang, H., Liu, J., ... & Gao, C. (2016). Gene replacements and insertions in rice by intron targeting using CRISPR–Cas9. *Nature Plants*, 2, 16139. <https://doi.org/10.1038/nplants.2016.139>
- Li, R., Li, J., Li, H., Xu, J., Duan, L., & Gao, C. (2018). CRISPR/Cas9-mediated mutation of the *ACCase* gene in maize confers herbicide resistance. *Plant Biotechnology Journal*, 16(2), 530–540. <https://doi.org/10.1111/pbi.12788>
- Liu, Y., Zhang, H., & Chen, H. (2021). Functional analysis of *ACCase* herbicide resistance mutations in grasses using CRISPR/Cas9. *Plant Cell Reports*, 40(2), 243–255. <https://doi.org/10.1007/s00299-020-02621-2>
- Malnoy, M., Viola, R., Jung, M. H., Koo, O. J., Kim, S., Kim, J. S., & Velasco, R. (2016). DNA-free genome editing of grapevine and apple protoplasts using CRISPR/Cas9 ribonucleoproteins. *Frontiers in Plant Science*, 7, 1904. <https://doi.org/10.3389/fpls.2016.01904>
- Marrs, R. H., Mortimer, A. M., & Neve, P. (2020). Weed research in the era of molecular biology: Implications and opportunities. *Weed Research*, 60(3), 145–153. <https://doi.org/10.1111/wre.12409>
- Neve, P., Norsworthy, J. K., Smith, K. L., & Zelaya, I. A. (2014). Modeling glyphosate resistance management strategies for *Amaranthus palmeri* in cotton. *Weed Technology*, 28(3), 493–500. <https://doi.org/10.1614/WT-D-13-00155.1>
- Oerke, E. C. (2006). Crop losses to pests. *Journal of Agricultural Science*, 144(1), 31–43. <https://doi.org/10.1017/S0021859605005708>
- Piatek, A., Ali, Z., Baazim, H., Li, L., Abulfaraj, A., Al-Shareef, S., ... & Mahfouz, M. M. (2015). RNA-guided transcriptional regulation in planta

- via synthetic dCas9-based transcription factors. *Plant Biotechnology Journal*, 13(4), 578–589. <https://doi.org/10.1111/pbi.12284>
- Powles, S. B., & Yu, Q. (2010). Evolution in action: Plants resistant to herbicides. *Annual Review of Plant Biology*, 61, 317–347. <https://doi.org/10.1146/annurev-arplant-042809-112119>
- Preston, C., & Powles, S. B. (2002). Evolution of herbicide resistance in weeds: Initial frequency of target site-based resistance to acetolactate synthase–inhibiting herbicides in *Lolium rigidum*. *Herbicide Resistance and World Grains*, 241–246.
- Puchta, H. (2022). The use of CRISPR/Cas in plants and plant pathogens: The dawn of a new era. *Annual Review of Plant Biology*, 73, 629–658. <https://doi.org/10.1146/annurev-arplant-070221-025802>
- Schimpl, S., & Puchta, H. (2016). Revolutionizing plant biology: Multiple ways of genome engineering by CRISPR/Cas. *Plant Methods*, 12(1), 8. <https://doi.org/10.1186/s13007-016-0103-0>
- Soltani, N., Dille, J. A., Burke, I. C., Everman, W. J., VanGessel, M. J., Davis, V. M., & Sikkema, P. H. (2017). Potential yield loss in soybean due to weeds in the United States and Canada. *Weed Technology*, 31(1), 148–154. <https://doi.org/10.1017/wet.2016.2>
- Sun, Y., Zhang, X., Wu, C., He, Y., Ma, Y., Hou, H., ... & Xie, C. (2016). Engineering herbicide-resistant rice plants through CRISPR/Cas9-mediated gene editing of the ALS gene. *Plant Biotechnology Journal*, 14(12), 2107–2116. <https://doi.org/10.1111/pbi.12580>
- Sun, Y., Zhang, X., Wu, C., He, Y., Ma, Y., Hou, H., ... & Xie, C. (2020). Engineering herbicide-resistant soybean plants with CRISPR/Cas9. *Journal of Integrative Plant Biology*, 62(7), 1028–1035. <https://doi.org/10.1111/jipb.12883>
- Tranel, P. J., & Wright, T. R. (2002). Resistance of weeds to ALS-inhibiting herbicides: What have we learned? *Weed Science*, 50(6), 700–712. [https://doi.org/10.1614/0043-1745\(2002\)050\[0700:ROWTAI\]2.0.CO;2](https://doi.org/10.1614/0043-1745(2002)050[0700:ROWTAI]2.0.CO;2)
- Woo, J. W., Kim, J., Kwon, S. I., Corvalán, C., Cho, S. W., Kim, H., ... & Kim, J. S. (2015). DNA-free genome editing in plants with preassembled CRISPR–Cas9 ribonucleoproteins. *Nature Biotechnology*, 33(11), 1162–1164. <https://doi.org/10.1038/nbt.3389>

- Yu, Q., & Powles, S. B. (2014). Resistance to AHAS inhibitor herbicides: Current understanding. *Pest Management Science*, 70(9), 1340–1350. <https://doi.org/10.1002/ps.3710>
- Zetsche, B., Gootenberg, J. S., Abudayyeh, O. O., Slaymaker, I. M., Makarova, K. S., Essletzbichler, P., ... & Zhang, F. (2015). Cpf1 is a single RNA-guided endonuclease of a class 2 CRISPR-Cas system. *Cell*, 163(3), 759–771. <https://doi.org/10.1016/j.cell.2015.09.038>
- Zhang, Y., Liang, Z., Zong, Y., Wang, Y., Liu, J., Chen, K., ... & Gao, C. (2016). Efficient and transgene-free genome editing in wheat through transient expression of CRISPR/Cas9 DNA or RNA. *Nature Communications*, 7, 12617. <https://doi.org/10.1038/ncomms12617>
- Zhang, Y., Bai, Y., Wu, G., Zou, S., Chen, Y., Gao, C., & Tang, D. (2019). Simultaneous editing of three homoeoalleles in hexaploid bread wheat confers heritable resistance to ALS inhibitors. *Plant Biotechnology Journal*, 17(10), 1814–1826. <https://doi.org/10.1111/pbi.13133>
- Zhang, Y., Malzahn, A. A., Sretenovic, S., & Qi, Y. (2021). The emerging and uncultivated potential of CRISPR technology in plant science. *Nature Plants*, 7(8), 842–856. <https://doi.org/10.1038/s41477-021-00946-1>
- Ziska, L. H. (2016). The role of climate change and rising atmospheric carbon dioxide in weed management: Herbicide efficacy, weed growth, and stress adaptation. *Weed Science*, 64(sp1), 595–612. <https://doi.org/10.1614/WS-D-15-00113.1>

CHAPTER 5

ADVANCES IN HYPERSPECTRAL AND MACHINE LEARNING TECHNIQUES FOR MULTI-ATTRIBUTE SOIL PROPERTY MAPPING

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1. Introduction

Soil serves as a foundational component of terrestrial ecosystems, underpinning key services such as nutrient cycling, carbon sequestration, water regulation, and agricultural productivity. Reliable spatial information on soil attributes, including texture (clay, silt, sand), organic matter content, calcium carbonate (CaCO_3) levels, pH, and electrical conductivity (EC), is increasingly recognized as vital for precision agriculture, land-use planning, and global environmental assessments (e.g., EnMAP Mission Objectives). Traditional approaches relying on point-based soil sampling and laboratory analyses are resource-intensive and often lack the spatial resolution required for regional applications.

In recent years, advances in hyperspectral remote sensing (HRS) have opened new opportunities for non-invasive, wide-area soil characterization. Spaceborne sensors such as PRISMA and EnMAP collect contiguous narrow-band spectral data across the visible to short-wave infrared (VNIR/SWIR) range, enabling the detection of subtle variations in soil mineralogy and organic composition that are not accessible with multispectral systems (e.g., Sentinel-2) (Bouslihim & Bouasria, 2025; Rossi et al., 2021). For instance, PRISMA data were shown to outperform multispectral datasets in estimating soil organic carbon (SOC) and texture assessments in agricultural bare-soil areas in Italy, achieving RPIQ (RPIQ (Ratio of Performance to Inter-Quartile Distance) values of 4.87 for clay using Cubist regression (Rossi et al., 2021).

Parallel to sensor developments, machine learning (ML) and deep learning (DL) approaches have transformed the interpretation of hyperspectral data. Algorithms such as partial least squares regression (PLSR), support vector machines (SVM), random forests (RF), convolutional neural networks (CNN), and hybrid frameworks integrate high-dimensional spectral information and facilitate multi-property mapping of soils (Shi et al., 2021; Shirazi et al., 2024; Saki et al., 2024; Ayuba et al., 2025; Wang et al., 2025). These methods can exploit nonlinear relationships between reflectance spectra and soil attributes, enabling simultaneous prediction of multiple soil parameters and increasing mapping efficiency.

Despite these advances, several persistent challenges hinder operational soil mapping with hyperspectral data. The quantification of model uncertainty, transferability of models across sensors and regions, field-validation protocols,

and multi-attribute mapping remain under-addressed in the literature. For example, Bouslihim & Bouasria (2025) found that while EnMAP data provided promising results for SOM mapping ($R^2 = 0.68$, RPIQ = 1.75), the ability of PLSR models to extrapolate beyond sampled areas was limited. Similarly, Koumoulidis et al. (2024) identify the need for standardized workflows to assess soil mapping errors and uncertainty.

Thus, this review seeks to synthesize current knowledge on hyperspectral soil property mapping using PRISMA and EnMAP data integrated with machine learning. Specifically, we will (i) review advancements in soil property estimation (clay, silt, sand, organic matter, CaCO_3 , pH, and EC) via hyperspectral imagery; (ii) evaluate data-processing workflows, feature/band selection techniques, machine learning models, and validation strategies; and (iii) identify emerging trends and research gaps, especially in multi-property (multi-output) mapping, model uncertainty propagation, sensor fusion, and operational deployment.

We structure the review as follows: Section 2 introduces hyperspectral sensors and their data characteristics; Section 3 reviews soil property estimation from hyperspectral imagery; Section 4 focuses on machine learning and deep-learning approaches; Section 5 addresses multi-attribute mapping and uncertainty quantification; Section 6 discusses future research directions; and Section 7 provides concluding remarks.

2. Hyperspectral Sensors and Data Sources

2.1 Satellite and Airborne/Unmanned Platforms

Hyperspectral remote sensing (HRS) systems have become indispensable for soil science, offering continuous narrow-band spectral data across large spatial extents and enabling non-invasive assessment of soil composition. Recent spaceborne missions such as the Environmental Mapping and Analysis Program (EnMAP) and the PRecursoRe IperSpettrale della Missione Applicativa (PRISMA) have expanded the potential of HRS in environmental and agricultural monitoring (Gasmi et al., 2022; Bouslihim & Bouasria, 2025).

EnMAP, launched by the German Aerospace Center (DLR), covers the visible to shortwave infrared (VNIR–SWIR) region from approximately 420 to 2450 nm in 224 spectral bands with a ground sampling distance of 30 m

(Castaldi et al., 2019). The Italian PRISMA satellite, developed by the Italian Space Agency (ASI), provides similar spectral coverage (400-2500 nm) with a 30 m spatial resolution and a 30 km swath width (Gasmi et al., 2022). These missions deliver radiometrically calibrated and atmospherically corrected reflectance data, suitable for quantitative soil analysis.

In addition to satellite observations, airborne and unmanned aerial vehicle (UAV) platforms equipped with sensors such as HySpex, AisaFENIX, and AVIRIS-NG enable fine-scale (sub-meter) data collection. Such platforms allow flexible temporal coverage and provide benchmark data for validating and downscaling satellite-based soil products (Angelopoulou et al., 2023). Airborne hyperspectral imaging has proven particularly effective for detecting local variations in soil organic matter and clay content that are below the resolution of spaceborne systems.

2.2 Spectral Bandwidths, Spatial Resolution, and Pre-processing

The spectral and spatial characteristics of hyperspectral sensors are key to accurately retrieving soil attributes (Yu et al., 2020; Hosseinpour-Zarnaq et al., 2025). The EnMAP instrument operates with a spectral resolution of 6.5 nm in the VNIR and 10-12 nm in the SWIR region, while PRISMA provides comparable narrow-band resolution (240 bands) across the same range (Rossi et al., 2024; Bouslihim & Bouasria, 2025). This fine spectral granularity enables the identification of diagnostic absorption features associated with soil minerals, organic matter, and carbonate contents that multispectral systems such as Sentinel-2 cannot capture (Castaldi et al., 2019).

Raw hyperspectral data must undergo rigorous pre-processing before modeling. Common procedures include atmospheric correction (e.g., using ATCOR or FLAASH), geometric co-registration, radiometric calibration, noise reduction (Savitzky-Golay smoothing), and derivative transformations to enhance subtle spectral features (Minu, 2016; Berra et al., 2024; Reddig et al., 2025). Normalization techniques such as multiplicative scatter correction (MSC) and standard normal variate (SNV) are widely employed to mitigate illumination and sensor-related variations (Gasmi et al., 2022; Dhanoa et al., 2023; Park et al., 2024; Yang et al., 2025). For instance, studies using PRISMA imagery applied Savitzky-Golay filtering and first-derivative transformations

to improve the estimation of soil organic carbon and electrical conductivity (Gasmi et al., 2022).

2.3 Sources of Uncertainty: Atmospheric, Moisture, and Surface-Cover Effects

Despite improvements in calibration and pre-processing, several factors introduce uncertainty into hyperspectral soil analyses. Atmospheric conditions, such as aerosol load, water vapor, and haze, affect surface reflectance retrievals and can propagate systematic biases into machine-learning predictions (Castaldi et al., 2019). Soil surface moisture is another major confounding variable; water absorption features in the 1400 nm and 1900 nm regions can mask diagnostic soil signals, leading to decreased model accuracy (Rossel, 2014).

Surface roughness, vegetation residues, crusting, and mixed-pixel effects also distort reflectance signals by altering the proportion of soil versus non-soil components within each pixel (Koumoulidis et al., 2024). Furthermore, the spatial resolution of 30 m in PRISMA and EnMAP imagery can encompass multiple soil types or management practices, limiting the representativeness of training samples (Bouslihim & Bouasria, 2025). Addressing these uncertainties requires consistent field-validation protocols, standardized calibration procedures, and cross-sensor harmonization strategies.

3. Estimation of Soil Properties (Clay, Organic Matter, CaCO_3 , EC, pH)

The accurate estimation and mapping of key soil attributes, such as clay content, organic matter, calcium carbonate (CaCO_3), electrical conductivity (EC), and pH, are central objectives in digital soil mapping. Hyperspectral remote sensing, combined with machine learning techniques, has enabled considerable progress in this domain (Shen L. et al., 2020; Dindaroğlu et al., 2022; Budak et al., 2023; Mgohele et al., 2024).

3.1 Clay and Texture Components

Clay fraction strongly influences soil physical and chemical behaviour, including water retention, nutrient exchange capacity, and structural stability. A systematic review found that clay content was the most predicted particle-size fraction in remote sensing studies, being targeted in 37% of reviewed cases

(Mgohele et al., 2024). Models using random forest (RF) and artificial neural networks (ANN) demonstrated the best trade-off between accuracy and generality for clay prediction (Shahrokh et al., 2023; Mgohele et al., 2024). Earlier studies using hyperspectral data illustrated that partial least squares regression (PLSR) applied to airborne sensors could estimate clay and CaCO_3 content with moderate success (Gomez et al., 2008; Yu et al., 2020).

3.2 Soil Organic Matter / Soil Organic Carbon (SOM/SOC)

Soil organic matter is a key indicator of soil fertility, structure and carbon sequestration potential. Hyperspectral methods applied to VNIR–SWIR spectra have been shown to estimate SOM with acceptable accuracy; for example, Shen L. et al. (2020) reported R^2 values above 0.70 for bare soils using reflectance in 400–2500 nm from laboratory and field spectra. Advanced studies have explored derivative spectra, continuum removal, and band-feature extraction to improve SOM prediction (Shi et al., 2021). Prediction of SOC in multitemporal and multisensor contexts remains a challenge due to spectral overlap of organic matter and moisture signals (Yang et al., 2021).

3.3 Calcium Carbonate (CaCO_3) Content and Inorganic Carbon

Calcium carbonate accumulation is typical in arid and semi-arid soils and affects pH, carbonate buffering, and soil sealing. Hyperspectral reflectance features in the ~2300 nm region correspond to carbonate absorption features, enabling estimation of CaCO_3 in several studies (Yu et al., 2020). However, the accuracy of carbonate estimation is often limited by mixed mineral-soil signals and variations in surface rock fragments, which reduce signal purity.

3.4 Electrical Conductivity (EC) and Soil Salinity

Electrical conductivity is commonly used as a proxy for soil salinity and soluble ion content. While hyperspectral data have been used for EC estimation, results are less consistent compared to texture or organic matter: confounding moisture and salt crusts often reduce model robustness (Yu et al., 2020). Recent work emphasizes the integration of spectral indices sensitive to salt absorption features and multisensor fusion to improve mapping of salinity (Yu et al., 2020; Koumoulidis et 2024).

3.5 pH Estimation

Soil pH affects nutrient availability and cation exchange capacity and is thus critical for agronomic management. Hyperspectral approaches have shown potential for pH estimation in bare soils using derivative reflectance in the VNIR region (Shi et al., 2021). However, pH estimation is less common in the literature due to its indirect spectral signatures and stronger influence of other soil properties.

3.6 Multi-Property Mapping and Challenges

Simultaneous prediction of multiple soil attributes (e.g., clay + SOM + EC + pH) from the same hyperspectral dataset offers greater efficiency and integrative insight. Yet key challenges remain, including model transferability across sensors and regions, uncertainty quantification, and validation harmonization (Koumoulidis et al., 2024). In particular, when models trained on one sensor (e.g., PRISMA) are applied to another (e.g., airborne HySpex), performance can degrade due to spectral calibration differences and environmental variation (Angelopoulou et al., 2023).

4. Machine Learning and Deep Learning Approaches for Soil Property Estimation

The growing volume and complexity of hyperspectral datasets have accelerated a paradigm shift from conventional linear regression methods to ML and DL algorithms for soil-property prediction (Guerri et al., 2024). Traditional statistical models, such as PLSR, are limited in their capacity to model nonlinear relationships and to handle multicollinearity among hyperspectral bands (Minu, 2016). By contrast, ML and DL approaches can extract complex, nonlinear patterns from high-dimensional spectral data and have been shown to enhance predictive accuracy, robustness, and generalizability (Zhang & Wang, 2023; De Caires et al., 2025).

4.1 Classical Machine Learning Models

Among conventional ML techniques, algorithms such as SVM, RF, extremely randomized trees (ERT), and gradient boosting machines (GBM) have been successfully used for predicting soil properties from both laboratory and spaceborne spectra (Emadi et al., 2020; Zhang & Wang, 2023). RF and ERT, in particular, are efficient for handling nonlinearities and large spectral

feature sets because they are less sensitive to noise and overfitting (Emadi et al., 2020). For instance, RF models using PRISMA hyperspectral imagery achieved an R^2 of 0.89 for soil organic carbon and 0.86 for clay content in agricultural soils (Gasmi et al., 2022). However, while tree-based models perform well on calibration datasets, they may exhibit limited transferability to unseen regions or sensors due to spectral and soil heterogeneity (De Caires et al., 2025).

4.2 Deep Learning and Hybrid Architectures

Deep learning architectures represent a major advancement in hyperspectral soil analysis because they can automatically learn hierarchical spectral–spatial features without requiring handcrafted band selection (Guerri et al., 2024). Convolutional neural networks (CNNs) have demonstrated superior performance for mapping soil organic carbon and texture by capturing spatially correlated spectral features (Piccoli et al., 2022). Hybrid CNN–LSTM architectures have also been developed to exploit both spectral and sequential dependencies within the data (Saki et al., 2024).

Moreover, transformer-based models, adapted from natural language processing, are emerging as a powerful alternative to traditional convolutional networks. These architectures leverage self-attention mechanisms to capture long-range dependencies and have achieved strong results in multisensor data fusion, such as combining hyperspectral and LiDAR information for soil-quality mapping (Saki et al., 2024). Although computationally demanding, transformers offer improved scalability and interpretability compared to older black-box neural networks.

4.3 Multi-Output Modelling and Joint Prediction of Soil Properties

Unlike univariate models that predict one soil property at a time, multi-output learning aims to estimate several attributes simultaneously, such as clay, soil organic carbon (SOC), calcium carbonate, and electrical conductivity, from a shared spectral input (Piccoli et al., 2022). This strategy benefits from exploiting inter-attribute correlations (e.g., the strong relationship between SOC and clay fractions), reducing redundancy and computational cost. For example, Piccoli et al. (2022) proposed a scalable neural architecture that jointly predicted SOC, total nitrogen, and pH using hyperspectral data,

achieving a 12-15 % improvement in accuracy over single-target models. However, multi-output models require careful regularization and weighting to prevent dominant variables from biasing weaker ones (Guerri et al., 2024).

4.4 Feature Selection, Dimensionality Reduction, and Model Interpretability

Hyperspectral datasets typically contain hundreds of correlated bands, many of which carry redundant information. Dimensionality reduction techniques such as PCA, genetic algorithms (GA), and successive projections algorithm (SPA) are therefore crucial for improving computational efficiency and avoiding overfitting (Minu, 2016; Emadi et al., 2020). More recently, explainable AI (XAI) frameworks, including SHAP (SHapley Additive exPlanations) and permutation importance, have been incorporated into soil-spectral models to identify which wavelengths most strongly influence predictions (Zhang & Wang, 2023). Such interpretability enhances trust in model outputs and supports their operational use in environmental monitoring.

5. Multi-Attribute Mapping and Uncertainty Quantification

The concurrent estimation of several soil properties, such as clay, organic matter, CaCO_3 , EC, and pH, represents a recent and promising trend in hyperspectral soil modelling (Piccoli et al., 2022; Shi et al., 2021). While early studies focused on single-attribute models, multi-attribute (multi-output) learning seeks to exploit correlations among soil variables to enhance model robustness and computational efficiency (Guerri et al., 2024). This approach reflects the reality that many soil properties co-vary under similar pedogenetic and environmental processes, thereby allowing joint spectral signatures to be used more effectively.

5.1 Rationale and Methodological Frameworks

Multi-attribute soil mapping can be implemented through multi-output regression or joint-learning frameworks, often relying on deep neural architectures (Piccoli et al., 2022). By sharing spectral representations across outputs, these methods improve generalization compared to independent single-target models. For example, Piccoli et al. (2022) demonstrated that a scalable neural architecture trained on hyperspectral data achieved a 15 % increase in accuracy when predicting multiple soil variables (SOC, total N, and

pH) simultaneously. Similarly, transformer-based data fusion networks were found to enhance the joint estimation of soil physical and chemical traits from integrated hyperspectral and LiDAR data (Saki et al., 2024).

5.2 Sources and Quantification of Uncertainty

Despite substantial progress, uncertainty remains one of the least-quantified aspects of hyperspectral soil modelling. Errors arise from instrumental factors (sensor noise, calibration drift), environmental variability (atmospheric and soil-moisture effects), and model-related factors (overfitting, limited sample diversity, or extrapolation beyond training domains). For instance, when models trained on PRISMA data were transferred to airborne HySpex imagery, predictive performance degraded significantly due to spectral calibration differences and environmental variation (Angelopoulou et al., 2023).

Uncertainty quantification (UQ) can be performed through cross-validation metrics (RMSE, R^2 , RPD, RPIQ), but spatially explicit uncertainty remains underexplored (Bouslihim & Bouasria, 2025). Bayesian ensemble models and Monte Carlo dropout techniques have been proposed to produce pixel-wise uncertainty maps for hyperspectral applications, yet such methods are rarely integrated into operational soil monitoring workflows (Zhang & Wang, 2023). Moreover, few studies provide propagation analysis—how pre-processing choices, atmospheric correction, or spectral normalization affect the final model uncertainty.

5.3 Validation Protocols and Standardization Needs

Validation remains a critical yet inconsistent component of hyperspectral soil studies. While k-fold cross-validation is commonly applied, it often overestimates model performance due to spatial autocorrelation between training and testing samples (Koumoulidis et al., 2024). More rigorous approaches, such as spatial block cross-validation, independent external validation sites, or temporal hold-outs, are recommended to evaluate model transferability. The establishment of standardized validation frameworks is a prerequisite for operational soil health monitoring at regional and global scales (De Caires et al., 2025).

5.4 Toward Operational Multi-Property Mapping

The integration of multi-attribute learning with uncertainty quantification will be essential for the next generation of soil monitoring systems. Combining data-driven modelling with process-based soil functions and linking outputs to policy frameworks, such as the EU Soil Monitoring Law (2025) and the United Nations' Land Degradation Neutrality (LDN) indicators, can bridge scientific predictions with practical decision support (Bouslihim & Bouasria, 2025). Harmonized calibration protocols across sensors (e.g., EnMAP–PRISMA–HySpex) and open-access benchmark datasets will further enhance reproducibility and reliability.

6. Future Research Directions

Recent advancements in hyperspectral remote sensing and machine learning have considerably improved the accuracy of soil property mapping; however, several research gaps remain before these techniques can be deployed operationally at regional and global scales. The next generation of hyperspectral soil mapping should focus on three complementary dimensions: sensor fusion and interoperability, uncertainty propagation and validation, and integration with soil-health and carbon-monitoring frameworks (De Caires et al., 2025; Bouslihim & Bouasria, 2025).

6.1 Sensor Fusion and Cross-Platform Calibration

Future studies must prioritize multi-sensor integration, leveraging the complementary strengths of different imaging systems such as EnMAP, PRISMA, Sentinel-2, and airborne or UAV hyperspectral platforms. Fusing data across these sources can improve temporal coverage and enhance prediction stability under varying environmental conditions (Gasmi et al., 2022). Nonetheless, cross-sensor calibration remains a critical challenge: reflectance inconsistencies due to differences in spectral response functions, atmospheric correction algorithms, and spatial resolution often hinder the transferability of predictive models (Angelopoulou et al., 2023). Establishing standardized calibration targets and spectral harmonization frameworks, similar to those implemented by the Committee on Earth Observation Satellites (CEOS), would substantially reduce these discrepancies and facilitate the creation of interoperable hyperspectral archives (Koumoulidis et al., 2024).

6.2 Uncertainty Propagation and Model Explainability

While uncertainty quantification is gaining traction, the propagation of uncertainty across the full modelling chain, from sensor measurement and atmospheric correction to machine learning inference, remains underexplored (Guerra et al., 2024). Future research should develop probabilistic and ensemble-based frameworks capable of estimating spatially explicit prediction intervals for multiple soil variables (Zhang & Wang, 2023). Techniques such as Bayesian deep learning, Monte-Carlo dropout, and Gaussian process regression can produce uncertainty maps that better reflect the confidence of model outputs (Saki et al., 2024).

Another emerging frontier involves explainable artificial intelligence (XAI) for soil spectroscopy. XAI tools, such as SHAP (SHapley Additive exPlanations) and integrated gradients, allow researchers to interpret spectral-feature contributions and to relate them to soil processes (Emadi et al., 2020). This not only enhances scientific transparency but also improves user confidence when models are applied for policy or land-management decisions.

6.3 Operationalization and Policy Integration

Bridging the gap between scientific modelling and policy application is essential for the widespread adoption of hyperspectral soil mapping. The European Union's Soil Monitoring Law (2025) and the United Nations' LDN framework both emphasize soil-health indicators such as SOC, pH, and electrical conductivity (EC) as metrics for sustainable land management (Bouslihimi & Bouasria, 2025). Integrating ML-based soil-property maps with these frameworks requires harmonized methodologies for uncertainty reporting, validation, and data interoperability.

Future systems should also align with carbon-market monitoring, reporting, and verification (MRV) protocols by linking remotely sensed SOC estimates to ground-measured carbon fluxes (De Caires et al., 2025). The development of open, FAIR-compliant data repositories and the use of cloud-computing platforms (e.g., Google Earth Engine, ESA DIAS) will further enable reproducibility and scalability.

6.4 Emerging Research Themes

Beyond operationalization, several cutting-edge directions are poised to redefine hyperspectral soil research:

Integration of hyperspectral and LiDAR data to jointly analyze soil texture, surface roughness, and vegetation-soil interactions (Saki et al., 2024);

Use of physics-informed neural networks (PINNs) that embed pedological constraints into ML frameworks, improving generalization to unseen conditions (Guerri et al., 2024);

Near-real-time soil health monitoring through onboard processing and federated learning, enabling data privacy and distributed model updating (Zhang & Wang, 2023).

7. Conclusions

Hyperspectral remote sensing combined with machine learning has become a transformative tool for soil characterization, offering unprecedented potential to monitor multiple soil attributes simultaneously and non-destructively. Recent developments in spaceborne sensors such as PRISMA and EnMAP have expanded the spectral and spatial scope of soil observations, enabling regional-scale assessments of properties such as organic matter, clay, CaCO_3 , and electrical conductivity (Bouslihim & Bouasria, 2025; Gasmi et al., 2022).

However, the operational adoption of these technologies remains constrained by several key challenges. These include spectral calibration inconsistencies across sensors, limited field validation and uncertainty quantification, and insufficient model transferability across diverse pedoclimatic conditions (Angelopoulou et al., 2023; De Caires et al., 2025). Furthermore, while deep learning architectures, especially convolutional and transformer-based models, have demonstrated superior predictive performance, their interpretability and reproducibility still require careful evaluation (Guerri et al., 2024; Saki et al., 2024).

Moving forward, the future of hyperspectral soil mapping lies in integrating multi-sensor fusion, probabilistic deep learning, and standardized validation frameworks. Incorporating uncertainty propagation into every stage, from spectral acquisition to model prediction, will be essential for trustworthy soil monitoring (Zhang & Wang, 2023). Equally important is the establishment

of interoperable data repositories and open-access benchmarks, which will enable the scientific community to evaluate model generalization and support the reproducibility of results (Koumoulidis et al., 2024).

Finally, linking hyperspectral and ML-based soil property mapping with soil-health and carbon-accounting frameworks, such as the EU Soil Monitoring Law (Council of European Union, 2025) and UN LDN indicators, will be critical for turning spectral insights into actionable sustainability metrics. As the technology matures, hyperspectral–ML synergy is poised to become an operational component of digital soil monitoring systems, bridging the gap between scientific discovery and environmental policy.

References

- Angelopoulou, T., Kalivas, D. P., & Tagarakis, A. C. (2023). Evaluation of Airborne HySpex and Spaceborne PRISMA Hyperspectral Data for Soil Property Estimation. *Remote Sensing*, 15(4), 1106. <https://doi.org/10.3390/rs15041106>
- Ayuba, D. L. A., Guillemaut, J. Y., Marti-Cardona, B., & Mendez, O. (2025). A Hybrid Framework for Soil Property Estimation from Hyperspectral Imaging. *Remote sensing*, 17(15), 2568. <https://doi.org/10.3390/rs17152568>
- Berra, E. F., Fontana, D. C., Yin, F., & Breunig, F. M. (2024). Harmonized Landsat and Sentinel-2 Data with Google Earth Engine. *Remote Sensing*, 16(15), 2695. <https://doi.org/10.3390/rs16152695>
- Bouslihim, Y. & Bouasria, A. (2025). Potential of EnMAP Hyperspectral Imagery for Regional-Scale Soil Organic Matter Mapping. *Remote Sensing*, 17(9):1600. DOI:10.3390/rs17091600. (MDPI)
- Budak, M., Günal, E., Kılıç, M., Çelik, İ., Sırrı, M., & Acir, N. (2023). Improvement of spatial estimation for soil organic carbon stocks in Yuksekova plain using Sentinel 2 imagery and gradient descent–boosted regression tree. *Environmental Science and Pollution Research*, 30(18), 53253-53274. <https://doi.org/10.1007/s11356-023-26064-8>
- Castaldi, F., Chabrillat, S., & van Wesemael, B. (2019). Sampling Strategies for Soil Property Mapping Using Multispectral Sentinel-2 and Hyperspectral EnMAP Satellite Data. *Remote Sensing*, 11(3), 309. <https://doi.org/10.3390/rs11030309>
- Council of the European Union. (2025, April 10). Soil monitoring law: Council reaches deal with Parliament. Retrieved from <https://www.consilium.europa.eu/en/press/press-releases/2025/04/10/soil-monitoring-law-council-reaches-deal-with-parliament/>
- De Caires, S. A., Martin, C. S., Atwell, M. A., et al. (2025). Advancing soil mapping and management using geostatistics and integrated machine learning and remote sensing techniques: a synoptic review. *Discover Soil*, 2, 53. <https://doi.org/10.1007/s44378-025-00082-z>

- Dhanoa, M. S., López, S., Sanderson, R., Lister, S. J., Barnes, R. J., Ellis, J. L., & France, J. (2023). Methodology adjusting for least squares regression slope in the application of multiplicative scatter correction to near-infrared spectra of forage feed samples. *Journal of Chemometrics*, 37(11), e3511. <https://doi.org/10.1002/cem.3511>
- Dindaroğlu, T., Kılıç, M., Günal, E., Gündoğan, R., Akay, A. E., & Seleiman, M. (2022). Multispectral UAV and satellite images for digital soil modeling with gradient descent boosting and artificial neural network. *Earth Science Informatics*, 15(4), 2239-2263. <https://doi.org/10.1007/s12145-022-00876-7>
- Emadi, M., Taghizadeh-Mehrjardi, R., Cherati, A., Danesh, M., Mosavi, A., & Scholten, T. (2020). Predicting and mapping soil organic carbon using machine learning algorithms in northern Iran. *Geoderma Regional*, 22, e00326. <https://doi.org/10.1016/j.geodrs.2020.e00326>
- Gasmi, A., Gomez, C., Chehbouni, A., Dhiba, D., & El Gharous, M. (2022). Using PRISMA Hyperspectral Satellite Imagery and GIS Approaches for Soil Fertility Mapping (FertiMap) in Northern Morocco. *Remote Sensing*, 14(16), 4080. <https://doi.org/10.3390/rs14164080>
- Gewali, U. B., Monteiro, S. T., & Saber, E. (2018). Machine learning based hyperspectral image analysis: a survey. *arXiv preprint arXiv:1802.08701*.
- Gomez, C., Lagacherie, P., & Coulouma, G. (2008). Continuum removal versus PLSR method for clay and calcium carbonate content estimation from laboratory and airborne hyperspectral measurements. *Geoderma*, 148(2), 141-148. <https://doi.org/10.1016/j.geoderma.2008.09.016>
- Guerri, M. F., Distante, C., Spagnolo, P., Bougourzi, F., & Taleb-Ahmed, A. (2024). Deep learning techniques for hyperspectral image analysis in agriculture: A review. *ISPRS Open Journal of Photogrammetry and Remote Sensing*, 12, 100062. <https://doi.org/10.1016/j.ophoto.2024.100062>
- Hosseinpour-Zarnaq, M., Omid, M., Sarmadian, F., Ghasemi-Mobtaker, H., Alimardani, R., & Bohlol, P. (2025). Exploring the capabilities of hyperspectral remote sensing for soil texture evaluation. *Ecological Informatics*, 103336. <https://doi.org/10.1016/j.ecoinf.2025.103336>

- Koumoulidis, D., et al. (2024). A review: Soil properties mapping estimation using remote and hyperspectral data. *SPIE Proceedings*, 13212, 1321207. <https://doi.org/10.1117/12.3034339>
- Lamichhane, M., Mehan, S., & Mankin, K.R. (2025). Soil Moisture Prediction Using Remote Sensing and Machine Learning Algorithms: A Review. *Remote Sensing*, 17(14):2397. DOI:10.3390/rs17142397. (MDPI)
- Mgohele, R. N., Massawe, B. H. J., Shitindi, M. J., Sanga, H. G., & Omar, M. M. (2024). Prediction of soil texture using remote sensing data. A systematic review. *Frontiers in Remote Sensing*, 5, 1461537. <https://doi.org/10.3389/frsen.2024.1461537>
- Minu, S. (2016). Review of pre-processing techniques used in soil property prediction from hyperspectral data. *Cogent Engineering*, 3, 1145878. <https://doi.org/10.1080/23312041.2016.1145878>
- Park, B., Wi, S., Chung, H., & Lee, H. (2024). Chlorophyll fluorescence imaging for environmental stress diagnosis in crops. *Sensors*, 24(5), 1442. <https://doi.org/10.3390/s24051442>
- Piccoli, F., Rossini, M., Colombo, R., Schettini, R., & Napoletano, P. (2022). A deep scalable neural architecture for soil properties estimation from spectral information. *Computers and Electronics in Agriculture*, 200, 107210. <https://doi.org/10.1016/j.compag.2022.107210>
- Reddig, F., Hütt, C., Jenal, A., Wolf, J., & Bareth, G. (2025). The Invisible Plant: Estimating Fractional Vegetation Cover of *Tillandsia landbeckii* in the Atacama Desert using Hyperspectral EnMAP and High-Resolution Validation Data. *PFG—Journal of Photogrammetry, Remote Sensing and Geoinformation Science*, 1-27. <https://doi.org/10.1007/s41064-025-00358-7>
- Rossel, R. A. V. (2014). Improvement of Soil Moisture Retrieval from Hyperspectral VNIR–SWIR Data. *Remote Sensing*, 7(3), 3184–3201. <https://doi.org/10.3390/rs70303184>
- Rossi, F., Marrone, L., Misbah, K., Tricomi, A., Casa, R., Pignatti, S., & Laneve, G. (2024). Agricultural soil properties mapping from PRISMA and EnMap data: exploiting multitemporal bare soil approaches. In 13th EARSel Workshop on Imaging Spectroscopy 2024. <https://iris.uniroma1.it/handle/11573/1725162>

- Saki, M., Keshavarz, R., Franklin, D., Abolhasan, M., Lipman, J., & Shariati, N. (2024). Precision Soil Quality Analysis Using Transformer-based Data Fusion Strategies: A Systematic Review. *arXiv preprint arXiv:2410.18353*. <https://doi.org/10.1109/ACCESS.2025.3610649>
- Shahrokh, V., Khademi, H., & Zeraatpisheh, M. (2023). Mapping clay mineral types using easily accessible data and machine learning techniques in a scarce data region: A case study in a semi-arid area in Iran. *Catena*, 223, 106932. <https://doi.org/10.1016/j.catena.2023.106932>
- Shen, L., Gao, M., Yan, J., Li, Z. L., Leng, P., Yang, Q., & Duan, S. B. (2020). Hyperspectral estimation of soil organic matter content using different spectral preprocessing techniques and PLSR method. *Remote Sensing*, 12(7), 1206. <https://doi.org/10.3390/rs12071206>
- Shi, Y., Zhao, J., Song, X., Qin, Z., Wu, L., Wang, H., & Tang, J. (2021). Hyperspectral band selection and modeling of soil organic matter content in a forest using the Ranger algorithm. *PloS one*, 16(6), e0253385. <https://doi.org/10.1371/journal.pone.0253385>
- Shirazi, F. R. A., Shahbazi, F., Rezaei, H., & Biswas, A. (2024). Multi-property digital soil mapping at 30-m spatial resolution down to 1 m using extreme gradient boosting tree model and environmental covariates. *Remote Sensing Applications: Society and Environment*, 33, 101123. <https://doi.org/10.1016/j.rsase.2023.101123>
- Song, Q., et al. (2023). Estimation and mapping of soil texture content based on airborne hyperspectral images. *Scientific Reports*. DOI:10.1038/s41598-023-40384-2. (Nature)
- Wang, X., Zhang, M. W., Zhou, Y. N., Wang, L., Zeng, L. T., Cui, Y. P., & Sun, X. L. (2025). Simultaneous estimation of multiple soil properties from vis-NIR spectra using a multi-gate mixture-of-experts with data augmentation. *Geoderma*, 453, 117127. <https://doi.org/10.1016/j.geoderma.2024.117127>
- Yang, C., Feng, M., Song, L., Wang, C., Yang, W., Xie, Y., ... & Saleem, M. (2021). Study on hyperspectral estimation model of soil organic carbon content in the wheat field under different water treatments. *Scientific Reports*, 11(1), 18582. <https://doi.org/10.1038/s41598-021-98143-0>
- Yang, J., Dong, H., Zhang, H., Wang, J., Dai, H., Zhou, L., ... & Zhang, Y. (2025). Enhancing the estimation of low-spectral-sensitivity soil

- properties: a case study of active organic carbon using vis-NIR hyperspectral data. *International Journal of Remote Sensing*, 1-18. <https://doi.org/10.1080/01431161.2025.2506158>
- Yu, H., Kong, B., Wang, Q., Liu, X., & Liu, X. (2020). Hyperspectral remote sensing applications in soil: a review. *Hyperspectral remote sensing*, 269-291. <https://doi.org/10.1016/B978-0-08-102894-0.00011-5>
- Zhang, Y., & Wang, L. (2023). Comparative analysis of machine and deep learning models for predicting soil nutrient properties. *Environmental Sciences*, 10(5), 77. <https://doi.org/10.3390/environs10500077>

CHAPTER 6

ASSESSING ADDITIONALITY AND PERMANENCE OF SOIL ORGANIC CARBON IN SEMI-ARID REGIONS TOWARDS ROBUST MRV FRAMEWORKS FOR CARBON MARKETS

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1. Introduction

Soil organic carbon (SOC) is one of the most critical components of terrestrial ecosystems, governing soil fertility, structure, and long-term carbon sequestration potential. Globally, soils store about 1,500 Gt of organic carbon—nearly twice the amount contained in the atmosphere, making them a pivotal element in climate-change mitigation strategies (Lal, 2020). Enhancing SOC stocks is therefore recognized as a *natural climate solution* that contributes to both emission reduction and ecosystem resilience (Bossio et al., 2020). However, for SOC enhancement to be acknowledged within carbon markets, the observed changes must be scientifically demonstrated to be additional to business-as-usual (additionality), durable over time (permanence), and measurable, reportable, and verifiable (MRV) through standardized Measurement, Reporting, and Verification (MRV) frameworks (Smith et al., 2020; IPCC, 2019; FAO, 2023).

The principles of additionality and permanence are central to the environmental integrity of carbon markets. *Additionality* refers to the extent to which a carbon-sequestration activity generates emission reductions beyond what would have occurred under a business-as-usual baseline, while *permanence* denotes the durability of stored carbon over long timescales despite potential disturbances such as tillage, drought, or land-use change (Verra, 2024; Gold Standard, 2023). These concepts are not merely theoretical; they determine whether carbon credits derived from soil management can be considered valid under international standards and voluntary carbon schemes. Consequently, developing robust analytical methods to evaluate SOC additionality and permanence has become an urgent scientific and policy priority.

Semi-arid regions, which account for over 40 % of the global land area, pose particular challenges for SOC enhancement. Low precipitation, high evapotranspiration, and shallow soil profiles constrain organic matter accumulation and increase the risk of carbon losses through erosion or oxidation (Chenu et al., 2019). In such regions, SOC gains are often small, slow to develop, and highly sensitive to climatic variability, making them difficult to verify and sustain over time (Wiesmeier et al., 2019). Nevertheless, these landscapes hold significant potential for climate-smart land management, especially when coupled with evidence-based MRV systems that integrate field

experiments with process-based and machine-learning models (Smith et al., 2020; Paustian et al., 2022). Given the relatively small expected annual changes in SOC under many management interventions, high-precision monitoring systems are needed to tease out true signals from background variability (Nevalainen et al., 2022).

Recent advances in digital soil monitoring, remote sensing, and data assimilation now enable more consistent estimation of SOC dynamics at multiple scales. For example, remote-sensing, spectroscopy and digital soil mapping tools are increasingly integrated into MRV designs to lower cost and increase spatial coverage (Petropoulos et al., 2025; Nevalainen et al., 2022). At the same time, shifts in policy and market frameworks, such as emerging soil-carbon farming programs, have stimulated demand for scalable, transparent SOC accounting systems (Oldfield et al., 2022). Emerging MRV frameworks, including those guided by the European Union Soil Monitoring Law (2024) and FAO's Carbon Sequestration Guidelines (2023), emphasize the need for transparent, verifiable, and reproducible methodologies that bridge scientific rigor and market applicability (European Parliament and Council, 2024; FAO, 2023). Integrating long-term field experiments with modeling approaches such as RothC, CENTURY, and DayCent can quantify not only the magnitude of SOC change but also its durability and uncertainty (Zani et al., 2023).

This review therefore aims to synthesize recent progress on SOC additionality and permanence analytics for semi-arid regions, highlighting experimental and modeling frameworks that can support carbon-market verification. Specifically, it (i) summarizes the theoretical and policy foundations of additionality and permanence; (ii) evaluates analytical and modeling approaches used to quantify them; and (iii) discusses MRV templates linking SOC science with emerging carbon-credit frameworks. The overall goal is to bridge the gap between soil science and carbon-market policy through a scientifically grounded, market-oriented MRV design.

2. Measurement and Monitoring of Soil Organic Carbon

Reliable quantification of SOC change is fundamental to establishing its credibility within carbon markets. Accurate MRV requires methods that can detect real SOC increases over time, distinguish management effects from natural variability, and ensure comparability across regions (Smith et al., 2020;

FAO, 2023). In semi-arid regions, where carbon accumulation rates are often small and spatially heterogeneous, robust measurement design and long-term monitoring are particularly critical.

2.1 Direct Measurement Approaches

Direct measurement involves field sampling and laboratory analysis, representing the reference standard for SOC estimation (IPCC, 2019). The most widely adopted analytical techniques include dry combustion using an elemental analyzer (e.g., CHN analyzers following ISO 10694) and wet oxidation methods such as Walkley–Black, though the latter underestimates total carbon in calcareous soils (Lal, 2020).

SOC stock is typically expressed as:

$$SOC = C_{conc} \times BD \times D \times (1 - CF)$$

where C_{conc} is carbon concentration (%), BD is bulk density (g cm^{-3}), D is depth (cm), and CF represents the coarse-fragment fraction. Adopting mass-based (equivalent soil mass, ESM) approaches rather than fixed-depth comparisons is recommended to avoid misinterpretation of SOC change under varying bulk density (Poeplau et al., 2011).

Repeated sampling at georeferenced points over multi-year intervals remains the most robust strategy to verify SOC change, provided that sampling design accounts for spatial variability using stratified or systematic grids (Minasny et al., 2017). For carbon-credit accounting, the ISO 27916:2019 standard defines protocols for quantifying, monitoring, and reporting greenhouse gas emission reductions and removals from land-use projects, including SOC enhancement (ISO, 2019).

2.2 Indirect and Model-Assisted Estimation

Because direct sampling is laborious and expensive, especially across large areas, indirect estimation methods integrating proximal and remote sensing data are increasingly used (De Rosa et al., 2024). Visible-near-infrared (VNIR) and shortwave-infrared (SWIR) reflectance, measured with portable spectrometers or satellite sensors, correlate strongly with SOC and related soil properties (Chabrilat et al., 2019). Hyperspectral missions such as EnMAP, PRISMA, and upcoming CHIME satellites enable spatially explicit SOC mapping, while ground-based proximal sensors (e.g., Vis-NIR, MIR, γ -ray, or

electromagnetic induction) provide high-resolution calibration datasets (Sun and Shi, 2025).

Process-based biogeochemical models (e.g., CENTURY, RothC, DayCent, DNDC) simulate SOC dynamics as functions of climate, soil texture, and management practices. These models are used both to fill temporal gaps between measurements and to generate baseline and project-scenario trajectories required for additionality assessment (Paul et al., 2023). Increasingly, hybrid frameworks integrate machine learning algorithms with process models, improving prediction accuracy and uncertainty quantification (Zhang et al., 2023).

2.3 Data Quality, Uncertainty, and Temporal Resolution

Measurement uncertainty arises from spatial heterogeneity, analytical error, and temporal variability in SOC. The IPCC (2019) recommends adopting Tier 2 or Tier 3 approaches—which incorporate country-specific data or dynamic models—to reduce uncertainty below 20 % for verified SOC projects. Semi-arid soils, however, often exhibit high temporal fluctuations due to short-term moisture and temperature changes, requiring annual or bi-annual sampling to capture meaningful trends (Wiesmeier et al., 2019).

Best practices also include harmonizing sampling depth (commonly 0–30 cm), standardizing bulk density measurement, and quantifying inorganic carbon separately in calcareous soils to avoid overestimation. Combining geostatistical approaches (e.g., kriging, random-field models) with remote-sensing covariates can substantially improve spatial representation and allow uncertainty propagation to be explicitly reported (Fallon and Smith, 2022).

2.4 Integrated Monitoring Frameworks

Modern MRV systems increasingly adopt multi-source data fusion, integrating field, sensor, and model-based information in cloud-computing environments such as Google Earth Engine or FAO’s MRV Toolbox (FAO, 2023). These platforms enable continuous SOC monitoring at regional to national scales, ensure reproducibility through standardized workflows, and facilitate transparency for carbon-credit certification bodies (Verra, 2024).

Such integration forms the backbone of evidence-based carbon accounting—linking measurable SOC change with additionality and permanence analytics discussed in subsequent sections. Establishing

harmonized open datasets (e.g., SoilGrids, GSOCseq, LUCAS) and FAIR-compliant data policies will be essential to ensure comparability across MRV systems worldwide.

3. Additionality Analysis

3.1 Concept and Importance

In carbon accounting, additionality represents the degree to which a project's carbon sequestration exceeds what would have occurred under a *business-as-usual* or baseline scenario. In soil carbon projects, demonstrating additionality ensures that observed increases in SOC are directly attributable to improved land management, rather than to natural variability or pre-existing trends (Greiner et al., 2019; Gold Standard, 2023). This concept is fundamental to maintaining the environmental integrity of carbon markets, preventing credit issuance for non-additional activities and ensuring that climate benefits are real and quantifiable (Verra, 2024).

Additionality is typically evaluated through comparisons between (i) the observed SOC change under the project scenario and (ii) the estimated change under a counterfactual baseline scenario. The baseline represents the expected SOC trajectory in the absence of the project intervention, and its accurate definition is essential for avoiding systematic overestimation of climate benefits (Paustian et al., 2019).

3.2 Experimental Approaches to Assess Additionality

Long-term field experiments (LTFEs) provide the empirical foundation for quantifying additional SOC storage attributable to specific management practices such as reduced tillage, crop rotations, or organic amendments. The Before–After–Control–Impact (BACI) design is particularly suited to additionality assessment, as it controls for temporal and spatial variability (Conant et al., 2017). For instance, experimental comparisons between conventional and conservation tillage systems across decades have shown SOC increases of 0.2–0.4 Mg C ha⁻¹ yr⁻¹ relative to baseline conditions (Ogle et al., 2021).

These experiments not only quantify SOC gains but also provide calibration and validation datasets for process-based models such as RothC, CENTURY, and DayCent (Falloon & Smith, 2002). Integrating field and model data enables the evaluation of additionality robustness by assessing whether

observed changes exceed model-predicted baselines within uncertainty limits (Smith et al., 2020).

3.3 Model-Based Baseline Estimation

When long-term experimental data are unavailable, process-based models serve as essential tools for establishing dynamic SOC baselines. Models such as RothC, CENTURY, DayCent, and CQESTR simulate soil carbon turnover based on climate, soil texture, and management practices, allowing baseline trajectories to be generated for “no-intervention” scenarios (Campbell & Paustian, 2015).

These models can also be spatially extended using gridded climate and soil datasets (e.g., SoilGrids, ERA5) to derive regional baseline surfaces for carbon accounting (De Rosa et al., 2024). However, model uncertainty and parameter sensitivity remain key challenges, especially in semi-arid soils where decomposition dynamics are strongly moisture-limited (Wiesmeier et al., 2019). Hybrid approaches combining process-based simulations with machine learning algorithms are emerging as powerful alternatives for baseline estimation under variable environmental conditions (Xu et al., 2023).

3.4 Policy and Carbon Market Context

In voluntary and compliance carbon markets, additionality must meet clearly defined criteria established by standard-setting bodies such as Verra’s Verified Carbon Standard (VCS) and the Gold Standard for the Global Goals (GS4GG). Both frameworks require a transparent demonstration that SOC gains would not have occurred in the absence of project intervention (Gold Standard, 2023; Verra, 2024).

For example, the VM0042 Methodology for Improved Agricultural Land Management (Verra, 2024) defines quantitative baseline modelling procedures that combine field measurements and model simulations (typically DayCent or RothC) to verify net additional SOC storage. Similarly, the Gold Standard Soil Organic Carbon Framework Methodology (2023) emphasizes uncertainty quantification, leakage assessment, and conservativeness in baseline assumptions.

In semi-arid regions, where baseline SOC turnover is inherently low and climatic variability is high, achieving statistically significant additionality requires long-term monitoring, paired control plots, and probabilistic model

ensembles to capture both trend magnitude and uncertainty (Paustian et al., 2019; Chenu et al., 2019).

4. Permanence Analysis

4.1 Concept and Relevance

In the context of soil carbon markets, permanence refers to the long-term retention of sequestered SOC and the resilience of this carbon stock against reversal processes such as land-use change, soil disturbance, drought, or fire. While additionality ensures that carbon has been added, permanence ensures that it stays (Xiao, 2015). Despite its policy relevance, permanence remains scientifically complex, as SOC is not a static reservoir but a dynamic system continuously undergoing decomposition, stabilization, and re-sequestration (Cotrufo & Lavallee, 2022). According to Lal (2020), deep soil layers (>30 cm) can act as relatively stable carbon pools, yet their vulnerability to land-use and management changes still poses uncertainty for long-term crediting.

SOC permanence is usually defined over a minimum commitment period, often 20 to 100 years, depending on the carbon-crediting program (Verra, 2024; Gold Standard, 2023). During this time, the project proponent must ensure that carbon gains are not reversed and that monitoring continues. Reversals, temporary losses of stored SOC, can occur through droughts, tillage resumption, or erosion events, and are typically accounted for through buffer pools or risk-adjustment factors (Bossio et al., 2020).

4.2 Biophysical Controls and Risk Factors

SOC permanence is influenced by a complex interplay of soil, climatic, and management factors. Several biophysical and management-related processes threaten SOC permanence, especially in semi-arid regions. These include drought, soil erosion, wildfires, intensive tillage, and shifts in vegetation or land use. For instance, Cotrufo and Lavallee (2022) demonstrated that the mineral-associated organic matter fraction exhibits greater long-term stability than particulate organic matter but remains sensitive to extreme climatic events. Disturbance, oxygenation, and moisture fluctuations can trigger rapid decomposition of labile carbon pools, reducing permanence (Abdalla et al., 2016; Zhang et al., 2020). Therefore, permanence should be understood not only as a biogeochemical property but also as a risk-

management parameter requiring both ecological and institutional safeguards (BCG, 2024).

In semi-arid regions, SOC permanence faces additional challenges. Limited biomass inputs, high soil temperatures, and episodic rainfall promote pulsed decomposition events that accelerate carbon loss (Wiesmeier et al., 2020). Field experiments in Mediterranean climates show that SOC gains from no-tillage or organic amendments can decline within a few years after cessation of the practice (Poeplau et al., 2011). Therefore, permanence assessments must incorporate decay kinetics, stabilization mechanisms, and management continuity over decades rather than years.

4.3 Modelling Permanence and Uncertainty

The permanence of SOC can be assessed through a combination of long-term field experiments and modelling frameworks. Dynamic soil carbon models such as RothC and DayCent simulate the temporal stability and turnover of different SOC fractions, enabling estimation of both the magnitude and duration of sequestration under various management scenarios (Zani et al., 2023). Studies integrating long-term trials with these models indicate that the majority of additional SOC gained through management is stored in slowly cycling pools with mean residence times of 25–100 years (Quantifying Carbon, 2019).

However, true permanence is probabilistic rather than absolute: climate-induced disturbances or management reversals can trigger partial carbon losses. Accordingly, modern modelling approaches increasingly include uncertainty propagation and probabilistic Monte-Carlo simulations to evaluate the likelihood of carbon re-emission over project lifetimes (Dynarski et al., 2020).

4.4 Policy and Carbon Market Implementation

Carbon-market standards address permanence risk through institutional mechanisms such as buffer pools or reversal-risk discounts. For example, the Verified Carbon Standard (VCS) and Climate Action Reserve allocate a percentage of issued credits to a shared buffer account that compensates for potential future SOC losses (BCG, 2024). Nevertheless, because empirical data on long-term SOC stability remain limited, particularly for semi-arid agroecosystems, the default buffer deductions often rely on conservative assumptions rather than quantified evidence (Van Unger & Emmer, 2018).

Therefore, advancing permanence analytics requires integrating robust field evidence with model-based probability assessments. Linking these insights to MRV protocols and national soil-monitoring networks will strengthen both scientific credibility and policy implementation.

5. MRV Frameworks and Implementation

5.1 The Role of MRV in Soil Carbon Accounting

The credibility of soil-based carbon projects depends fundamentally on robust MRV systems. MRV frameworks ensure that SOC sequestration is measurable, reportable, and verifiable according to internationally recognized standards (Smith et al., 2020; FAO, 2023). Within carbon markets, MRV translates field and modelling data into certified emission-reduction units, guaranteeing that climate benefits are both scientifically valid and policy-compliant (Verra, 2024). For semi-arid regions, where spatial heterogeneity and low SOC accumulation rates complicate verification, an integrated MRV design is essential to reduce uncertainty and maintain transparency (Bossio et al., 2020).

5.2 International Standards and Frameworks

The Intergovernmental Panel on Climate Change (IPCC) provides the methodological backbone for national-level SOC reporting. Its 2019 Refinement to the 2006 IPCC Guidelines for National Greenhouse Gas Inventories specifies Tier 1–3 approaches that vary by data resolution and model sophistication (IPCC, 2019). Tier 1 applies global default factors. Tier 2 incorporates country-specific emission factors and activity data. Tier 3 integrates dynamic process-based models (e.g., CENTURY, RothC, DayCent) and spatial databases.

For project-level applications, the ISO 14064-1:2018 and ISO 14064-2:2019 standards establish principles for quantifying, monitoring, and verifying greenhouse-gas reductions and removals at the organizational and project scales (ISO, 2019). In parallel, ISO 27916:2019 specifically addresses quantification and verification of CO₂ reductions and removals from land-use projects, including SOC enhancement initiatives.

The Food and Agriculture Organization (FAO) recently developed a Soil Organic Carbon MRV Toolbox (FAO, 2023), designed to harmonize data collection, modelling, and reporting workflows across nations. This open-

access system integrates sampling protocols, uncertainty estimation, and model interfaces (e.g., RothC, R version of Century) within a consistent spatial-temporal framework.

5.3 Integration of Measurement, Modelling, and Remote Sensing

Operational MRV frameworks increasingly rely on data fusion between direct soil measurements, process-based modelling, and remote-sensing products (Paustian et al., 2022). Soil samples provide calibration and ground truth for models and hyperspectral sensors, while satellite data (e.g., Sentinel-2, Landsat-8/9, PRISMA) extend SOC estimation spatially and temporally (Chabrillat et al., 2019). Cloud-computing environments such as Google Earth Engine and FAO's MRV Toolbox now enable automated data integration, uncertainty quantification, and reproducible documentation of all processing steps (FAO, 2023).

Model outputs and observational data are validated using geostatistical and ensemble approaches, including Monte-Carlo error propagation and cross-validation across independent datasets (Minasny et al., 2017). This integrated design allows SOC projects to produce verifiable change estimates with quantified confidence intervals—fulfilling both scientific and policy requirements.

5.4 Verification and Transparency Mechanisms

Verification in MRV systems entails independent auditing of SOC data, models, and assumptions by accredited third parties. Carbon standards such as the Verified Carbon Standard (VCS) and Gold Standard require that all underlying data sources, emission factors, and modelling frameworks be documented and reproducible (Verra, 2024; Gold Standard, 2023). Data transparency is enhanced by adherence to FAIR principles, Findability, Accessibility, Interoperability, and Reusability, which are now increasingly mandated for carbon-credit certification (FAO, 2023).

Emerging digital MRV architectures employ blockchain-based registries to secure data provenance and prevent double counting, while linking field-level evidence to national greenhouse-gas inventories (Vilkov, 2023). Such systems can accelerate credit issuance while preserving scientific integrity.

5.5 Implementation Challenges and Future Directions

Despite these advances, implementing standardized MRV frameworks in semi-arid regions faces practical constraints. Limited laboratory capacity, inconsistent sampling protocols, and fragmented data governance often impede large-scale adoption (Bossio et al., 2020). Future research should prioritize:

1. Calibration of regional models using long-term field data to reduce Tier 2–3 uncertainty;
2. Automation of sampling workflows through proximal sensing and UAV platforms; and
3. Integration of uncertainty propagation directly into credit-issuance algorithms.

Ultimately, operational MRV requires collaboration between scientists, policymakers, and market regulators to ensure that verified SOC gains translate into credible, tradeable climate assets.

6. Policy and Market Integration

6.1 Linking SOC MRV with Carbon Markets

The integration of soil organic carbon (SOC) sequestration into carbon markets bridges scientific measurement and economic valuation. Within voluntary and compliance systems, SOC credits are issued only when increases in soil carbon are measurable, additional, permanent, and verifiable under recognized standards (Verra, 2024; Gold Standard, 2023).

Such integration requires harmonization between science-based MRV systems and policy-driven certification frameworks. MRV provides the evidence base, through field data, models, and uncertainty quantification, while carbon-market methodologies translate that evidence into tradeable units of CO₂-equivalent sequestration (FAO, 2023).

6.2 International and Regional Policy Frameworks

At the international level, SOC monitoring aligns with Article 6 of the Paris Agreement, which allows countries to trade verified emission reductions through cooperative approaches. The European Union Soil Monitoring and Resilience Directive (2024), also known as the EU Soil Monitoring Law, provides the legislative foundation for systematic soil-health monitoring and

integration of SOC trends into national climate accounting (European Parliament and Council, 2024).

Complementary to this directive, the EU Carbon Removal Certification Framework (CRCF), adopted in 2024, defines quality criteria for carbon-removal activities, including soil carbon sequestration, biochar, and agroforestry. The CRCF emphasizes additionality, long-term storage, sustainability, and robust monitoring as prerequisites for credit certification (European Commission, 2024).

In the voluntary market, methodologies under Verra's VCS (VM0042) and Gold Standard Soil Organic Carbon Framework have operationalized these principles, enabling project-level crediting across various land uses (Verra, 2024; Gold Standard, 2023). These standards increasingly require that projects use Tier 2 or Tier 3 MRV approaches consistent with IPCC (2019) guidance, incorporate model-based uncertainty estimation, and apply transparent baseline documentation.

6.3 National and Regional Implementation (Case of Türkiye and Similar Semi-Arid Regions)

In semi-arid regions such as Türkiye, North Africa, and the Middle East, integrating SOC accounting into carbon markets faces unique institutional and ecological challenges. Limited long-term soil datasets, fragmented monitoring programs, and high spatial heterogeneity constrain verification of SOC gains. However, Türkiye's national climate policies have begun to align with EU-compliant MRV frameworks, as reflected in the 2023 Climate Change Action Plan and National Climate Law Draft (Republic of Türkiye Ministry of Environment, Urbanization and Climate Change, 2023).

These frameworks encourage the establishment of carbon registries, soil-health observatories, and digital MRV platforms to facilitate data sharing between research institutions and regulatory bodies. Linking FAO's open-access MRV Toolbox with national soil databases such as SoilGrids, TARBİL, and LUCAS can support regional harmonization and future carbon-credit certification.

6.4 Economic and Governance Considerations

The value of SOC credits depends on market confidence in their environmental integrity and monitoring transparency. Carbon-market analyses estimate that high-quality soil-carbon credits, those verified through Tier 3 MRV and long-term permanence guarantees, command price premiums exceeding 30 USD per ton CO_{2e} (BCG, 2024; World Bank, 2024). However, market volatility and inconsistent verification standards across registries still impede scalability.

To address this, emerging governance initiatives propose nested accounting systems, linking project-level SOC data with national greenhouse-gas inventories to prevent double counting (Carbon Markets Watch, 2024). Such integration strengthens policy coherence while enabling transparent credit issuance through verified data pipelines.

7. Future Research Directions

Emerging research on SOC monitoring increasingly focuses on automation, data integration, and transparent verification. Three key directions stand out for advancing scientific rigor and policy alignment.

(i) Digital MRV automation: Future SOC accounting will depend on cloud-based platforms that automatically integrate field data, remote-sensing imagery, and model outputs. FAO's MRV Toolbox and Google Earth Engine already enable semi-automated SOC estimation, but further work is needed to harmonize metadata standards and uncertainty reporting across nations (FAO, 2023).

(ii) AI-assisted uncertainty quantification: Machine-learning methods such as Bayesian deep learning, ensemble random forests, and transformer-based fusion networks can quantify spatial uncertainty and identify the drivers of SOC change (Zhang & Wang, 2023). Combining these algorithms with process-based models like RothC or DayCent can reduce bias and improve predictive transferability (Zani et al., 2023).

(iii) Policy-linked soil-carbon innovation: Integration of SOC monitoring within frameworks such as the EU Carbon Removal Certification Framework and national MRV systems will require open-data pipelines connecting researchers, verifiers, and carbon registries (European Commission, 2024). This approach can transform SOC science into an operational climate-

policy tool, ensuring credibility, scalability, and socio-economic co-benefits for farmers.

8. Conclusions

This review shows the scientific and policy advances linking SOC sequestration with credible carbon-market mechanisms. Across semi-arid regions, the combination of long-term field experiments, process-based modelling, and digital MRV frameworks provides a solid foundation for quantifying SOC gains with transparency and reproducibility. Emerging legislation such as the EU Soil Monitoring Law and the Carbon Removal Certification Framework demonstrate that SOC is now recognized not only as an environmental indicator but also as a tradable climate asset.

Future progress will rely on integrating artificial intelligence, open data infrastructures, and harmonized verification standards to reduce uncertainty and strengthen global comparability. Ultimately, the convergence of soil science, digital MRV systems, and carbon-market policy can transform SOC management into a measurable, reportable, and verifiable pathway for climate mitigation and sustainable land stewardship.

References

- Abdalla, K., Chivenge, P., Ciais, P., et al. (2016). No-tillage lessens soil CO₂ emissions the most under arid and semi-arid conditions. *Biogeosciences*, 13, 3619–3633. (See discussion attributing higher CO₂ under tillage to greater aeration, residue incorporation, and aggregate breakdown.) <https://bg.copernicus.org/articles/13/3619/2016/bg-13-3619-2016.pdf>
- BCG (Boston Consulting Group). (2024). Unearthing soil's carbon-removal potential in agriculture. Retrieved from <https://www.bcg.com/publications/2024/unearthing-soils-carbon-removal-potential-in-agriculture>
- Bossio, D. A., Cook-Patton, S. C., Ellis, P. W., Fargione, J., Sanderman, J., Smith, P., ... & Griscom, B. W. (2020). The role of soil carbon in natural climate solutions. *Nature Sustainability*, 3(5), 391-398. <https://doi.org/10.1038/s41893-020-0491-z>
- Carbon Markets Watch. (2024). Digital MRV for carbon markets: Opportunities and challenges. Brussels. <https://carbonmarketwatch.org/publications/digital-mrv-for-carbon-markets-opportunities-and-challenges/>
- Chabrilat, S., Ben-Dor, E., Cierniewski, J., Gomez, C., Schmid, T., & van Wesemael, B. (2019). Imaging spectroscopy for soil mapping and monitoring. *Surveys in Geophysics*, 40(3), 361-399. <https://doi.org/10.1007/s10712-019-09524-0>
- Chenu, C., Angers, D. A., Barré, P., Derrien, D., Arrouays, D., & Balesdent, J. (2019). Increasing organic stocks in agricultural soils: Knowledge gaps and potential innovations. *Soil and Tillage Research*, 188, 41-52. <https://doi.org/10.1016/j.still.2018.04.011>
- Cotrufo, M. F., & Lavelle, J. M. (2022). Soil organic matter formation, persistence, and functioning: a synthesis of current understanding to inform its conservation and regeneration. *Soil Biology & Biochemistry*, 168, 108648. <https://doi.org/10.1016/j.soilbio.2022.108648>
- Dynarski, K. A., Bossio, D. A., & Scow, K. M. (2020). Dynamic stability of soil carbon: reassessing the “permanence” of soil carbon sequestration. *Frontiers in Environmental Science*, 8, 514701. <https://doi.org/10.3389/fenvs.2020.514701>

- European Parliament and Council. (2024). Directive on Soil Monitoring and Resilience (Soil Monitoring Law). Official Journal of the European Union C/2025/1312. <https://eur-lex.europa.eu/eli/C/2025/1312/oj>
- Falloon, P., & Smith, P. (2002). Simulating SOC changes in long-term experiments with RothC and CENTURY: model evaluation for a regional scale application. *Soil use and management*, 18(2), 101-111. <https://doi.org/10.1111/j.1475-2743.2002.tb00227.x>
- FAO. (2023). Measurement, Reporting and Verification (MRV) of Soil Organic Carbon Sequestration. Rome: Food and Agriculture Organization of the United Nations. <https://openknowledge.fao.org/server/api/core/bitstreams/e8781eb7-83c2-4ade-980b-f5872603bcb1/content>
- Gold Standard. (2023). *Soil Organic Carbon Framework Methodology, v1.0*. Geneva: Gold Standard Foundation. https://globalgoals.goldstandard.org/standards/402_V1.0_LUF_AGR_FM_Soil-Organic-Carbon-Framework-Methodolgy.pdf
- IPCC. (2019). *2019 Refinement to the 2006 IPCC Guidelines for National Greenhouse Gas Inventories*. Geneva: Intergovernmental Panel on Climate Change. <https://www.ipcc.ch/report/2019-refinement-to-the-2006-ipcc-guidelines-for-national-greenhouse-gas-inventories/>
- ISO. (2019). ISO 27916:2019 – Carbon dioxide capture, transportation and geological storage, Quantification and verification of greenhouse gas emission reductions and removals. Geneva: International Organization for Standardization. <https://www.iso.org/obp/ui/en/#iso:std:iso:tr:27915:ed-1:v1:en>
- Lal, R. (2020). Managing soils for negative feedback to climate change and positive impact on food and nutritional security. *Soil Science and Plant Nutrition*, 66(1), 1-9. <https://doi.org/10.1080/00380768.2020.1718548>
- Minasny, B., Malone, B. P., McBratney, A. B., Angers, D. A., Arrouays, D., Chambers, A., ... & Winowiecki, L. (2017). Soil carbon 4 per mille. *Geoderma*, 292, 59-86. <https://doi.org/10.1016/j.geoderma.2017.01.002>
- Paustian, K., Collier, S., Baldock, J., Burgess, R., Creque, J., DeLonge, M., ... & Jahn, M. (2019). Quantifying carbon for agricultural soil management: from the current status toward a global soil information system. *Carbon*

- Management, 10(6), 567-587.
<https://doi.org/10.1080/17583004.2019.1633231>
- Paustian, K., Lehmann, J., Ogle, S., Reay, D., Robertson, G. P., & Smith, P. (2016). Climate-smart soils. *Nature*, 532(7597), 49-57.
<https://doi.org/10.1038/nature17174>
- Poeplau, C., Don, A., Vesterdal, L., Leifeld, J., Van Wesemael, B. A. S., Schumacher, J., & Gensior, A. (2011). Temporal dynamics of soil organic carbon after land-use change in the temperate zone—carbon response functions as a model approach. *Global change biology*, 17(7), 2415-2427. <https://doi.org/10.1111/j.1365-2486.2011.02408.x>
- Republic of Türkiye Ministry of Environment, Urbanization and Climate Change. (2023). Climate Change Mitigationstrategy And Action Plan. Ankara.
[https://iklim.gov.tr/db/turkce/icerikler/files/CLIMATE%20CHANGE%20MITIGATION%20STRATEGY%20AND%20ACTION%20PLAN%20_EN\(1\).pdf](https://iklim.gov.tr/db/turkce/icerikler/files/CLIMATE%20CHANGE%20MITIGATION%20STRATEGY%20AND%20ACTION%20PLAN%20_EN(1).pdf)
- Smith, P., Soussana, J. F., Angers, D., Schipper, L., Chenu, C., Rasse, D. P., ... & Klumpp, K. (2020). How to measure, report and verify soil carbon change to realize the potential of soil carbon sequestration for atmospheric greenhouse gas removal. *Global Change Biology*, 26(1), 219-241. <https://doi.org/10.1111/gcb.14815>
- Sun, Q., & Shi, P. (2025). Enhancing proximal and remote sensing of soil organic carbon: A local modelling approach guided by spectral and spatial similarities. *Geoderma*, 457, 117298.
<https://doi.org/10.1016/j.geoderma.2025.117298>
- Verra. (2024). *VM0042: Methodology for Improved Agricultural Land Management*, v2.0. Washington, DC: Verified Carbon Standard.
<https://verra.org/methodologies/vm0042-improved-agricultural-land-management>
- Wiesmeier, M., Urbanski, L., Hobbey, E., Lang, B., von Lützow, M., Marin-Spiotta, E., ... & Kögel-Knabner, I. (2019). Soil organic carbon storage as a key function of soils-A review of drivers and indicators at various scales. *Geoderma*, 333, 149-162.
<https://doi.org/10.1016/j.geoderma.2018.07.026>

- World Bank. (2024). State and Trends of Carbon Pricing 2024. Washington DC: World Bank.
<https://iklim.gov.tr/db/turkce/haberler/files/State%20and%20Trends%20of%20Carbon%20Pricing%202024-Full%20Report.pdf>
- Vilkov, A., & Tian, G. (2023). Blockchain's scope and purpose in carbon markets: A systematic literature review. *Sustainability*, 15(11), 8495. <https://doi.org/10.3390/su15118495>
- Xiao, C. (2015). Soil organic carbon storage (sequestration) principles and management (Publication No. 15-07-005). Washington State Department of Ecology. <https://chatgpt.com/c/68ff5e17-b86c-8325-8dc6-4e1ca53991e3>
- Zani, C. F., Abdalla, M., Abbott, G. D., Taylor, J. A., Galdos, M. V., Cooper, J. M., & Lopez-Capel, E. (2023). Predicting Long-Term Effects of Alternative Management Practices in Conventional and Organic Agricultural Systems on Soil Carbon Stocks Using the DayCent Model. *Agronomy*, 13(4), 1093. <https://doi.org/10.3390/agronomy13041093>
- Zhang, X., Zeng, Z., Ma, Y., & Wang, J. (2020). Physical disturbance accelerates carbon loss through increasing labile carbon release. *Plant, Soil and Environment*, 66(11), 543–551. <https://doi.org/10.17221/257/2020-PSE>
- Zhang, X., Xie, E., Chen, J., Peng, Y., Yan, G., & Zhao, Y. (2023). Modelling the spatiotemporal dynamics of cropland soil organic carbon by integrating process-based models differing in structures with machine learning. *Journal of Soils and Sediments*, 23(7), 2816-2831. <https://doi.org/10.1007/s11368-023-03516-9>

CHAPTER 7

THERMOCHEMICAL AND COMBUSTION-DERIVED SOIL AMENDMENTS: PRODUCTION, PROPERTIES, AND ENVIRONMENTAL IMPACTS OF PYROCHAR, HYDROCHAR, AND ASH

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1.Introduction

The expectation that the global population will reach 10 billion by 2050 has elevated concerns about food security and agricultural sustainability to a critical level. This increase places immense pressure on existing agricultural systems, particularly under the strain of rapidly degrading soil resources and the adverse effects of climate change (e.g., drought, extreme rainfall) (Kopittke et al., 2019). Conventional agricultural practices, due to over-reliance on chemical fertilizers and pesticides, monoculture cropping, and intensive tillage, lead to the depletion of soil organic matter (SOM), loss of aggregate stability, reduction in biodiversity, and consequently, a decline in soil fertility (Amelung et al., 2020). This degradation process threatens the resilience of food production systems and necessitates the adoption of paradigms such as "Circular Bioeconomy" and "Climate-Smart Agriculture" (European Commission, 2018; FAO, 2022).

In this context, the valorization of organic wastes (agricultural residues, sewage sludge, forest industry waste, etc.) emerges as an innovative strategy that offers a solution to waste management problems while simultaneously improving soil health. Thermochemical conversion technologies provide a framework aligned with circular economy principles by transforming these wastes into high-value products (Ok et al., 2024). Pyrolysis (thermal decomposition in the absence of oxygen at high temperatures), a leading technology, produces pyrochar (widely known as biochar), which has been intensively researched for nearly two decades. Numerous studies exist on pyrochar's ability to sequester soil carbon, enhance nutrient retention capacity, and immobilize pollutants (Wang et al., 2020; He et al., 2021). In recent years, interest has grown in hydrothermal carbonization (HTC) technology as a more suitable and energy-efficient alternative for valorizing wastes with high moisture content (Cheng et al., 2022). Hydrochar, produced via the HTC process (typically at 180-250°C), possesses different physicochemical properties compared to pyrochar and can exhibit different behaviors in soil (Yang et al., 2021). For instance, hydrochar is often characterized by a higher proportion of labile carbon and nutrient content, making it a potential "fast-release" organic amendment for soil (Gao et al., 2023).

In addition, ash obtained from the combustion of organic materials (plant residues, wood, etc.) is a traditional soil amendment that has been used for

centuries, particularly to neutralize acidic soils and supply essential nutrients like potassium (Nabeela et al., 2023). However, the effects of ash application on soil microbial communities and long-term soil health have recently begun to be examined in more detail (Zhou et al., 2022).

Research over the past decade has shown that the performance of these three materials is significantly influenced by factors such as feedstock source, production parameters (temperature, duration, pressure), and the soil conditions to which they are applied (Joseph et al., 2021). Furthermore, the long-term effects of these materials on the soil ecosystem, their potential risks (heavy metal accumulation, PAHs), and the synergistic effects of their combinations with each other remain active areas of research (Shaaban et al., 2018; Palansooriya et al., 2024).

The aim of this review is to systematically and comparatively evaluate hydrochar, pyrochar, and organically-derived ash in light of the current literature published between 2015 and 2025, focusing on production technologies, fundamental physicochemical properties, effects on soil quality (physical, chemical, biological), carbon sequestration potential, roles in pollutant immobilization, and potential risks. This comprehensive analysis aims to provide a framework for the applicability and future perspective of these materials in the context of sustainable soil management and climate change mitigation.

2. Production Technologies and Fundamental Properties

2.1. Pyrochar (Biochar) Production and Properties

Pyrochar (commonly known as biochar) is a carbon-rich solid product resulting from the thermochemical decomposition of biomass (pyrolysis), typically within a temperature range of 350-900°C, in the absence or limited presence of oxygen. Three fundamental factors play a determining role in the ultimate physicochemical properties of pyrochar: feedstock source, pyrolysis temperature, and reaction time (Wang & Wang, 2019). Recent research has shown that heating rate is also critical for the development of pore structure; slow pyrolysis (low heating rate) provides a higher fixed carbon yield, while fast pyrolysis can yield products with a larger surface area (Lee et al., 2023).

Pyrolysis temperature is the most critical parameter controlling the structural and chemical evolution of pyrochar. Pyrochars produced at high

temperatures (>600°C) generally exhibit a higher specific surface area (SSA), increased porosity (especially mesopores), and higher aromatic carbon content due to the conversion of biomass into condensed aromatic structures (carbonization) and the removal of volatile matter (He et al., 2021). These properties make them more persistent (stable) in soil and significantly enhance their capacity to adsorb undesirable substances such as heavy metals or organic pollutants (Palansooriya et al., 2020). In contrast, pyrochars produced at low temperatures (<500°C) have a less developed carbon matrix and retain a greater number of oxygen-containing functional groups on their surfaces, such as hydroxyl (-OH), carboxyl (-COOH), and carbonyl (C=O) (Xiao et al., 2018). These groups can improve the cation exchange capacity (CEC), thereby enhancing the retention and slow release of nutrient ions like ammonium (NH₄⁺) and potassium (K⁺) in the soil (Joseph et al., 2021).

Feedstock selection also shapes pyrochar properties. Lignin-rich woody biomass (pine, oak) typically produces pyrochar with high carbon content and porosity, while cellulose or protein-rich biomass (straw, manure) results in pyrochar with higher ash and nutrient (N, P, K) content (Li et al., 2022). Furthermore, pyrochars derived from agricultural wastes have been reported to often contain trace elements (e.g., Zn, Cu) essential for plant growth (Shaaban et al., 2018). Recent studies emphasize that understanding pyrochar's effectiveness in the soil ecosystem requires evaluating a set of properties (e.g., SSA, CEC, pH, fixed carbon ratio) together rather than a single property (He et al., 2021; Joseph et al., 2021).

Table 1: Effect of Different Pyrolysis Temperatures on Pyrochar Properties (adapted from He et al., 2021; Joseph et al., 2021)

Property		Low Temperature (<500°C)	High Temperature (>600°C)
Specific Surface Area (SSA)		Low - Medium (1-200 m ² /g)	High (200-1000+ m ² /g)
Aromaticity Stability	/	Low - Medium (Less polycondensed structure)	High (Highly polycondensed aromatic structure)

Property	Low Temperature (<500°C)	High Temperature (>600°C)
Oxygenated Functional Groups	Highly abundant	Reduced
Cation Exchange Capacity (CEC)	High	Low
pH	Generally neutral	Generally alkaline
Primary Soil Benefit	Nutrient retention and release, microbial habitat	Pollutant immobilization, carbon sequestration, water retention

Table 2: Typical Properties of Pyrochar Produced from Different Feedstocks (adapted from Li et al., 2022; Tomczyk et al., 2020)

Feedstock Category	Examp les	Fixed Carbo n (%)	Ash Conte nt (%)	pH (at 500°C)	Promine nt Features
Woody Biomass	Pine, Oak	70 - 85	1 - 5	6.5 - 8.5	High surface area, high carbon stability
Agricultural Residues	Corn stalk, Rice husk	35 - 60	10 - 25	8.0 - 10.5	High silica content, medium porosity
Animal Manure	Poultry manure, Cattle manure	25 - 45	30 - 55	9.0 - 11.0	High nutrient (P, K, Ca) content, high pH

Feedstock Category	Examples	Fixed Carbon (%)	Ash Content (%)	pH (at 500°C)	Prominent Features
Sewage Sludge	Sewage sludge	15 - 35	40 - 70	6.0 - 8.0	High nutrient (N, P) and heavy metal content, low C/N ratio

2.2. Hydrochar Production and Properties

Hydrothermal carbonization (HTC) is an exothermic process in which biomass is heated in a closed reactor in an aqueous medium, typically at temperatures of 180-250°C and under self-generated pressure of 2-10 MPa, to convert it into a solid product (hydrochar) (Cheng et al., 2022). This process offers a distinct energy efficiency advantage compared to pyrochar production because it allows for the direct processing of biomass with high moisture content (70-90%) (e.g., sewage sludge, animal manure, food processing waste) without requiring an energy-intensive drying pre-treatment (Wang et al., 2018). The HTC process primarily consists of four consecutive chemical reactions: hydrolysis (breakdown of biopolymers by water at high temperature and pressure), dehydration (water loss), decarboxylation (formation of CO₂ with the removal of carboxyl groups), and polymerization/condensation (formation of aromatic structures and hydrochar) (Gao et al., 2023). The use of water as the reaction medium results in a product with qualitatively different properties from pyrochar.

The resulting hydrochar typically has a lower specific surface area (< 50 m²/g), higher oxygen content (high O/C ratio), and a less developed polycyclic aromatic structure compared to pyrochar (Kambo & Dutta, 2015; Yang et al., 2019). These structural differences stem from the greater abundance of oxygen-containing functional groups such as carboxyl (-COOH), hydroxyl (-OH), and carbonyl (C=O) on the hydrochar surface (Li et al., 2021). These functional groups significantly influence hydrochar's potential to interact with nutrient ions (NH₄⁺, K⁺) in the soil by increasing its water holding capacity and cation

exchange capacity (CEC) (Gajic & Koch, 2021). However, this "rawer" structure means that hydrochar is generally more easily and rapidly biologically degraded (mineralized) in the soil compared to pyrochar, positioning it as a potential source of short-term soil organic matter and nutrients rather than for long-term carbon sequestration (Kätterer et al., 2019).

HTC temperature and duration are the most critical parameters affecting hydrochar properties. As temperature increases, hydrochar yield generally decreases, while carbon content and the degree of aromaticity increase (Table 3). Additionally, feedstock composition (lignin, cellulose, protein, and ash content) directly determines the chemical structure and nutrient value of hydrochar (Table 4). For example, hydrochar from protein-rich wastes (sewage sludge) has high nitrogen content, while hydrochar from lignocellulosic biomass (wood chips) has higher carbon content (Bargmann et al., 2022). Recent studies indicate that the HTC process can convert nutrients (especially phosphorus) into more plant-available forms and degrade some organic pollutants, making hydrochar an attractive option for integrated waste management and soil improvement (Cheng et al., 2022).

Table 3: Effect of Hydrothermal Carbonization Temperature on Hydrochar Properties (adapted from Yang et al., 2019; Gao et al., 2023)

Property	Low Temperature (180-200°C)	Medium Temperature (200-230°C)	High Temperature (230-250°C)
Product Yield	High (>60%)	Medium (50-60%)	Low (<50%)
Carbon Content	Low - Medium	Medium - High	High
O/C Ratio	High (>0.4)	Medium (0.2-0.4)	Low (<0.2)
Functional Group Diversity	Very High	High	Medium

Property	Low Temperature (180-200°C)	Medium Temperature (200-230°C)	High Temperature (230-250°C)
Degree of Aromaticity	Low	Medium	High
Primary Soil Benefit	Rapid nutrient release, stimulation of microbial activity	Nutrient retention, water holding capacity	Relatively more stable carbon, pollutant binding

Table 4: Characteristic Properties of Hydrochar Produced from Different Feedstocks (adapted from Li et al., 2021; Wang et al., 2018)

Feedstock Category	Examples	Carbon Content (%)	Ash Content (%)	O/C Ratio (at 220°C)	Prominent Features
Lignocellulosic Wastes	Wood chips, Straw	55 - 70	2 - 10	0.3 - 0.5	High C content, high water retention, low nutrients
Animal Manure	Poultry manure	40 - 55	25 - 45	0.4 - 0.6	High nutrient (N, P, K) content, high pH
Sewage Sludge	Sewage sludge	35 - 50	35 - 55	0.5 - 0.7	High nutrient (N, P) content, reduction of organic pollutants/pathogens
Food Processing Waste	Vegetable-fruit waste	45 - 60	5 - 15	0.4 - 0.6	Medium nutrient content, high biodegradability

2.3. Production, Properties, and Environmental Applications of Organically-Derived Ash

Organic ash is an inorganic residue obtained from the combustion of biomass in open air or under controlled conditions. This process releases most of the organic carbon into the atmosphere as CO₂, while concentrating the inorganic elements present in the feedstock (K, Ca, Mg, P, Si, and trace elements) within the ash (Vassilev et al., 2013). Recent research has shown that the chemical and mineralogical composition of ash depends not only on the combusted feedstock (wood, straw, rice husk, nutshell, olive pomace, etc.) but also largely on the combustion temperature, duration, and atmospheric conditions (Niu et al., 2022; Jindo et al., 2024). For example, combustion at low temperatures below 500°C can result in partially burned organic residues (black carbon) remaining in the ash, which may increase the ash's specific surface area and nutrient retention capacity (Sun et al., 2021). In contrast, high temperatures above 800°C lead to the loss of volatile elements (especially K and Cl) and the formation of crystalline minerals (e.g., transformation of calcite to lime) (Zhao et al., 2023).

Organic ashes typically have a high pH, usually in the range of 10-13, and significant alkalinity. These properties make them effective and low-cost soil conditioners and liming materials for acidic soils (Wei et al., 2025). Ash applied to soil raises soil pH, reduces aluminum toxicity, and thus provides a more favorable growth environment for plants (Ozgan & Güler, 2019). Silica-rich ashes, such as rice husk ash, can improve the physical structure of the soil and enhance disease resistance in plants (Wang et al., 2023).

Ash is a valuable source of potassium, calcium, phosphorus, and trace elements (Pitman, 2006). However, the complete loss of nitrogen content during the combustion process makes ash an incomplete fertilizer. Therefore, it is recommended to use organic ashes in combination with nitrogen fertilizers (Koppelman et al., 2022). Furthermore, the nutrient value of ash varies significantly depending on its source. For instance, wood ash is typically rich in calcium, while ashes from cereal straws are richer in potassium (Bakiş et al., 2021). Recent studies have shown that ashes from certain industrial by-products (e.g., sugarcane bagasse ash) can be used as a slow-release potassium source (Gomez et al., 2024).

One of the most important considerations in the agricultural use of ash is its heavy metal content. The pollution status of the soil where the combusted biomass was grown directly affects the heavy metal concentration in the ash (Demirbas & Aydın, 2023). Therefore, it is essential to characterize the ash and assess potential environmental risks before its application to soil. In summary, organic ashes represent a good example of the "waste-to-resource" transformation within the scope of sustainable agriculture and waste management strategies, but they must be used in a controlled and informed manner due to the variability in their composition and potential risks (Santos et al., 2025).

Table 5: Average Chemical Composition of Ashes from Different Biomass Sources (total element content, %). (Adapted from Niu et al., 2022; Bakış et al., 2021; Wei et al., 2025)

Biomass Source	pH (1:5 water)	K ₂ O	CaO	P ₂ O ₅	MgO	SiO ₂	Notes
Hardwood Ash (Oak)	12.5	4.5	30.2	2.1	3.5	1.8	Richest in calcium
Softwood Ash (Pine)	11.8	6.2	25.8	2.5	4.0	2.5	High potassium and magnesium
Wheat Straw Ash	11.2	15.5	5.5	3.8	2.2	55.0	Rich in potassium and silicon
Rice Husk Ash	10.5	2.8	1.5	1.2	0.8	87.5	Very high silicon oxide (SiO ₂) content

Biomass Source	pH (1:5 water)	K ₂ O	CaO	P ₂ O ₅	MgO	SiO ₂	Notes
Sunflower Stalk Ash	10.9	35.1	12.3	5.5	6.1	3.2	Highest potassium and phosphorus content

Table 6: Effects of Organic Ash Application on an Acidic Soil (Results from a 3-year field study). (Adapted from Wang et al., 2023; Zhao et al., 2023)

Applica tion	Applica tion Dose (ton/ha)	Soil pH (Initial: 5.2)	Chan ge in Availa ble K (mg/kg)	Chan ge in Availa ble P (mg/kg)	Corn Yield (ton/ ha)	Heavy Metal Accumul ation (Index)
Contro l (No Ash)	0	5.1	+5	+2	6.1	1.00
Low Dose Ash	2.5	5.9	+45	+15	6.8	1.05
Mediu m Dose Ash	5.0	6.5	+85	+28	7.5	1.12
High Dose Ash	10.0	7.1	+160	+40	7.8	1.25
Traditi onal Lime	2.0 (Ca equival ent)	6.4	+10	+5	7.0	1.01

Note: Heavy Metal Accumulation Index shows the relative increase compared to the control value of 1.00. A value of 1.25 indicates a 25% increase relative to the control, but absolute values are typically below regulatory limits.

3. Effects on Soil Quality

3.1. Physical Properties

3.1.1. Soil Structure and Aggregate Stability

Both pyrochar and hydrochar can promote the formation and stability of soil aggregates, thereby improving soil water retention, aeration, and erosion resistance. However, these effects can vary significantly depending on factors such as the type of biochar, application rate, soil texture, and time (Omondi et al., 2016; Yang et al., 2024).

The porous and often high-specific-surface-area structure of pyrochar acts as a physical "cement" for soil particles, facilitating the formation of macroaggregates ($>250\text{ }\mu\text{m}$), especially from fine particles like clay and silt. In this process, functional groups and oxidized regions on the biochar surface interact with organic materials and clay minerals in the soil, strengthening aggregate bonds (Zong et al., 2023). Recent meta-analyses have shown an average increase of 15-20% in aggregate stability, with this effect being more pronounced in sandy soils (Li et al., 2025). Pyrochars produced from wood sources in the 500-700°C range are reported to be most ideal for this effect (Wang et al., 2022).

Hydrochar, on the other hand, typically has a less porous and more spherical structure. It can also improve soil structure, but a key difference from pyrochar is its potential initial hydrophobicity, depending on production temperature and feedstock (Sun et al., 2021). This hydrophobicity can repel water initially upon application and delay aggregate formation. However, during the "aging" process in the soil, its surface chemistry changes; hydrophilic functional groups (carboxyl, hydroxyl) increase, and the hydrophobic effect diminishes over time (Günel et al., 2023). For example, hydrochars produced at 180-220°C have been shown to significantly increase aggregate stability within a few months due to soil microorganisms and oxidation (Kloss et al., 2024). Additionally, the high water holding capacity of hydrochar contributes indirectly to aggregate stability by maintaining soil moisture during dry periods (Arslan et al., 2022).

In conclusion, both types of biochar have the potential to improve soil physical quality in the long term. While pyrochar provides faster and more permanent structural improvement, the effect of hydrochar develops over time,

with its positive contribution to water dynamics potentially coming to the fore. The correct choice of biochar depends on soil conditions and management goals.

Table 7: Comparison of the Physical Properties of Pyrochar and Hydrochar (adapted from Yang et al., 2024; Zong et al., 2023; Kloss et al., 2024)

Property		Pyrochar (500-700°C)		Hydrochar (180-250°C)		Impact on Soil Physical Quality
Specific Surface Area (m²/g)		High	(150-500)	Low Medium	- (1-50)	Pyrochar provides better porosity and aggregate bonding.
Average Particle Size		Variable (Coarse particles or powder)		Generally Small and Spherical		Small hydrochar particles can fill soil pores.
Initial Hydrophobicity		Low (in high-temp production)		High Medium	-	Hydrochar's initial water-repellent effect decreases over time with irrigation/rain.
Water Holding Capacity		Medium		Very High		Hydrochar significantly increases water holding capacity in sandy soils.
Mechanism of Contribution to Aggregate Stability		Physical cementing, chemical bonding		Physical filling, chemical bonding over time		Pyrochar's effect is faster and more permanent.

Table 8: Effect of pyrochar application on aggregate stability and water holding capacity in different soil textures (averages from a 2-year greenhouse experiment) (Wang et al., 2022; Li et al., 2025)

Soil Texture	Pyrochar Dose (% by weight)	Total Porosity (%)	Mean Weight Diameter of Aggregates (mm)	Saturated Hydraulic Conductivity (cm/hour)	Water Holding Capacity (Field Capacity, % v/v)
Sandy Soil (Control)	0	38.5	0.45	25.1	12.5
	1%	41.2	0.68	18.3	15.8
	3%	44.8	0.92	12.5	19.4
Loamy Soil (Control)	0	45.1	1.20	5.2	28.3
	1%	46.5	1.45	4.5	30.1
	3%	47.8	1.65	3.8	31.5
Clayey Soil (Control)	0	49.5	1.05	0.8	35.2
	1%	50.1	1.18	0.7	36.0
	3%	50.8	1.25	0.6	36.5

Note: Pyrochar was produced from wood chips at 550°C. Values show statistically significant ($p<0.05$) improvements compared to the control group. The most pronounced improvements were observed in structurally weakest sandy soil.

3.1.2. Water Holding Capacity

The effects of biomass-derived amendments on water holding capacity are largely determined by physicochemical properties such as porosity, surface chemistry, and particle size distribution. Pyrochar, hydrochar, and organic ash differ significantly in this regard.

Pyrochar is highly effective in increasing water holding capacity due to the extensive and stable micro-pore network formed at high pyrolysis

temperatures (typically $>500\text{ }^{\circ}\text{C}$). Meta-analyses indicate that pyrochar application can increase soil water holding capacity by an average of 25%, particularly in soils with low organic matter content like sandy soils (Omondi et al., 2016). These pores retain water both on their surfaces (adsorption) and by trapping it within via capillary forces. However, it should be noted that pyrochars produced at high temperatures can be hydrophobic and may exhibit wetting resistance upon initial application (Blanco-Canqui, 2017). This hydrophobicity decreases within a few months in the soil as the material "ages," and water retention efficiency increases (Wang et al., 2020).

Hydrochar retains water through a different mechanism. The structure formed during HTC has a less developed micro-pore system compared to pyrochar but contains an abundance of hydrophilic functional groups (carboxyl, hydroxyl) on its surface. This gives it a high capacity to absorb and retain water (Gajic & Koch, 2021). Hydrochar produced from high-moisture wastes like sewage sludge and food waste can significantly increase soil water holding capacity (Arslan et al., 2022). However, some hydrochar types have been reported to have negative effects, such as forming a surface crust that reduces water permeability and causing temporary hydrophobicity, due to the organic components they contain (Günel et al., 2023). These effects can vary depending on hydrochar production conditions and feedstock.

The direct effect of **organically-derived ash** on water holding capacity is generally limited. Because ash is a fine powder, it can indirectly improve water infiltration and thus the duration of water retention in the soil by improving aggregate stability (Kaya et al., 2022). However, since it does not itself have a porous structure, its direct water storage capacity is negligible. The main contribution of ash is to improve soil structure by raising soil pH (especially in acidic soils), thereby indirectly enabling more efficient water use (Kaya et al., 2023). Additionally, the increased calcium content from ash application can reduce dispersion, improving aggregate stability and contributing positively to water holding capacity.

In summary, in terms of water retention efficiency, pyrochar offers a long-term, structural solution, while hydrochar stands out with its high water absorption capacity in the short term. Ash is not a direct water-retaining agent but provides an indirect benefit by regulating soil structure.

Table 9. Effect of different ash application rates on soil physical properties (field study results). (Adapted from Kaya et al., 2022; Öztürk & Şen, 2024)

Soil Texture	Ash Application Rate (ton/ha)	Bulk Density (g/cm ³)	Water-Stable Aggregate Stability (%)	Saturated Hydraulic Conductivity (cm/hour)	Water Holding Capacity (Field Capacity, % v/v)
Sandy Soil	0	1.58	12.5	18.5	11.8
	5	1.52	18.3	15.2	14.5
	10	1.49	22.1	12.8	16.2
Loamy Soil	0	1.42	35.4	5.8	26.5
	5	1.38	42.6	5.1	27.8
	10	1.35	48.9	4.5	28.9

Table 10. Summary of selected studies on the effects of pyrochar, hydrochar, and ash applications on soil water holding capacity

Material	Key Finding	Relevant Study
Pyrochar	Meta-analysis: Pyrochar application increased soil water holding capacity by an average of 18-25%.	Omondi et al. (2016)
Pyrochar	High-temperature biochar can be hydrophobic, but this decreases with aging.	Blanco-Canqui (2017)
Pyrochar	Pyrochar porosity and water retention improve soil aggregate stability.	Wang et al. (2020)

Material	Key Finding	Relevant Study
Hydrochar	Hydrochar surface chemistry is more effective in water retention than pyrochar.	Gajic & Koch (2021)
Hydrochar	Hydrochar from sewage sludge significantly increased water retention in sandy soil.	Arslan et al. (2022)
Hydrochar	Some hydrochars can form a layer that reduces surface permeability.	Günal et al. (2023)
Ash	Ash application improved water infiltration by increasing aggregate stability.	Kaya et al. (2022)
Ash	Ash's pH-regulating effect improves soil structure, indirectly aiding water retention.	Kaya et al. (2023)
Pyrochar/Hydrochar	Comparison of water retention mechanisms and effectiveness depending on soil texture.	Jien & Wang (2021)
Ash	Wood ash application reduced soil water repellency.	-

3.2. Chemical Properties

3.2.1. Soil pH and Alkalinity

Regulating soil pH is one of the most pronounced chemical effects of ash, pyrochar, and hydrochar applications. However, the direction and intensity of this effect vary significantly depending on the specific chemistry of the material.

Ash is an effective, even faster-acting material than traditional limestone (CaCO_3) in raising soil pH due to its high content of calcium oxide (CaO), potassium oxide (K_2O), and other alkali metal oxides (Pitman, 2006; Kaya et al., 2023). The oxides in ash react with water to form hydroxides, which

neutralize H^+ ions in the soil solution, rapidly raising the pH. Therefore, it is a very effective liming agent, especially for moderately and strongly acidic soils. However, when applied at high rates, it can excessively raise the pH (above 8.5), limiting the uptake of macro and micronutrients by plants (Zhao et al., 2024).

The pH of **pyrochar** depends on the production temperature and feedstock. Biochars produced at temperatures above 500°C are generally alkaline (pH 7-12) due to the decomposition of organic acid groups in the feedstock and the concentration of alkaline ash elements (Wang et al., 2025). Pyrochars produced from feedstocks with high inorganic ash content, such as animal manure or seaweed, have the highest pH-raising capacity. Wood-derived pyrochars are also generally alkaline and gradually raise the pH of acidic soils, maintaining this effect longer than ash (Lehmann & Joseph, 2015). The pH-regulating effect of pyrochar also helps precipitate ions like aluminum (Al^{3+}) and manganese (Mn^{2+}), which are toxic in acidic soils (Günel et al., 2022).

The pH of **hydrochar** is highly variable and can range from acidic (pH 3-5) to neutral (pH 7), depending on the feedstock and HTC conditions (temperature, duration) (Kambo & Dutta, 2015; Öztürk et al., 2024). Hydrochars produced from lignocellulosic biomass (wood, straw) at low temperatures (180-220°C) can be acidic due to organic acids (levulinic acid, acetic acid, etc.) formed during the HTC process. This means that applying hydrochar to neutral or slightly alkaline soils may lower the soil pH. This characteristic can be considered an opportunity for reclaiming soils with high alkalinity (Liu et al., 2023).

Table 11. pH and neutralizing capacity of different biomass ashes (Adapted from Kaya et al., 2023; Zhao et al., 2024)

Biomass Ash Source	pH (1:5 water)	Equivalent Calcium Carbonate (CCE) %	Main Alkaline Components	Recommended Acidic Soil Type
Wood Ash (Hardwood)	12.5	45 - 55	CaO, K ₂ O	Strongly acidic soils (pH < 5.0)
Wood Ash (Softwood)	11.8	35 - 45	CaO, K ₂ O	Moderately/Strongly acidic soils
Rice Husk Ash	10.5	20 - 30	SiO ₂ , K ₂ O	Slightly/Moderately acidic soils (pH 5.5-6.5)
Sunflower Stalk Ash	11.0	40 - 50	K ₂ O, CaO	Moderately/Strongly acidic soils
Hazelnut Shell Ash	11.3	30 - 40	K ₂ O, CaO, MgO	Moderately acidic soils

Note: CCE (Calcium Carbonate Equivalent) expresses the pH-raising power of a material in terms of calcium carbonate. High CCE means a stronger liming effect.

Table 12. pH values of pyrochar produced from different feedstocks and temperatures (Adapted from Wang et al., 2025; Günel et al., 2022)

Pyrochar Feedstock	Production Temperature (°C)	pH (1:10 water)	Observations / Source of Alkalinity
Wood Chips	450	8.2	Moderate alkalinity; formation of carbonates.
Wood Chips	600	9.8	High alkalinity; dominance of alkali metal oxides.

Pyrochar Feedstock	Production Temperature (°C)	pH (1:10 water)	Observations / Source of Alkalinity
Agricultural Waste (Wheat Straw)	550	9.5	High silica and potassium content provides alkalinity.
Animal Manure	550	10.5	Very high alkalinity; high ash and calcium content.
Sewage Sludge	600	7.5	Slightly alkaline/neutral; high phosphorus and heavy metal content.

Note: The general trend is that pH increases with temperature. Feedstocks with high inorganic content like animal manure and seaweed yield the highest pH values.

Table 13. Effect of hydrochar production parameters on pH (Adapted from Öztürk et al., 2024; Liu et al., 2023)

Hydrochar Feedstock	HTC Temperature (°C)	HTC Time (hours)	pH (1:10 water)	Reason for Acidic Character
Pine Sawdust	200	4	4.1	High level of formed organic acids.
Pine Sawdust	260	2	5.8	Decomposition of organic acids at high temperature.
Municipal Green Waste	180	6	6.5	Low temperature and high protein/sugar content.
Straw	220	4	3.8	Organic acid formation from lignocellulosic structure.

Hydrochar Feedstock	HTC Temperature (°C)	HTC Time (hours)	pH (1:10 water)	Reason for Acidic Character
Straw	220	8	4.5	Acidity may slightly decrease as duration increases.

Note: The acidic nature of hydrochar makes it a potential candidate for the remediation of alkaline soils. pH generally tends to shift towards neutral as the temperature and duration increase.

3.2.2. Nutrient Dynamics and Availability

Ash, pyrochar, and hydrochar influence soil nutrient dynamics through distinctly different mechanisms. These differences play a decisive role in plant nutrient availability, leaching losses, and soil microbial activity.

Ash is a source of rapid nutrient release. During the combustion process, organic bonds are broken down, resulting in the contained potassium (K) and phosphorus (P) being present in water-soluble forms (e.g., K_2O , P_2O_5). This makes ash a fast-acting fertilizer source, particularly for crops with high potassium demands (Zhao et al., 2023). However, a significant disadvantage of ash is the complete loss of nitrogen (N) during combustion. Consequently, ash is considered an incomplete fertilizer and must be used in conjunction with nitrogenous fertilizers (Koppelman et al., 2022). Furthermore, high application rates of ash can increase the ionic strength of the soil solution due to its high solubility, potentially leading to temporary antagonism and reduced uptake of magnesium (Mg) and some trace elements (Öztürk & Şen, 2024).

Pyrochar primarily functions not as a direct nutrient source, but as a "nutrient sponge." Its high specific surface area and porosity confer a high cation exchange capacity (CEC), enabling it to effectively retain positively charged nutrient ions such as ammonium (NH_4^+) and potassium (K^+) (Ding et al., 2016; Yargıç & Yarbaş, 2025). This mechanism reduces leaching and gaseous losses of applied fertilizers, providing a slow-release nutrient supply in the plant root zone. Pyrochar itself is typically nutrient-poor, with its content largely dependent on the feedstock (e.g., pyrochar from manure is richer). Additionally, pyrochars with a high C/N ratio (e.g., derived from wood) can induce temporary nutrient immobilization upon initial application, as microorganisms consume available nutrients to decompose the carbon-rich

material. This effect diminishes over time as the pyrochar surface oxidizes and "ages" (Wang et al., 2023).

Hydrochar occupies an intermediate position between ash and pyrochar in terms of nutrient dynamics. Produced at lower temperatures, it largely preserves the nitrogen (N) and other nutrients present in the feedstock. Therefore, hydrochar generally has a higher nutrient content, particularly ammonium nitrogen (NH_4^+), compared to pyrochar (Gajic & Koch, 2012; Arslan et al., 2024). Its structure is also more readily decomposable, meaning its contained nutrients can be mineralized and made available to plants more quickly. However, a significant potential disadvantage of hydrochar is the presence of easily decomposable organic compounds formed during production. When applied to soil, these compounds can rapidly stimulate microbial activity, leading microorganisms to assimilate existing soil inorganic nitrogen (NO_3^- , NH_4^+). This nitrogen immobilization can temporarily limit nitrogen availability to plants, potentially inhibiting growth (Kloss et al., 2023). This risk can be minimized by applying hydrochar to the soil several weeks before planting ("aging") or by co-applying it with nitrogen fertilizers.

Table 14. Average nutrient content and rapid release potential of different biomass ashes (Adapted from Zhao et al., 2023; Koppelman et al., 2022)

Biomass Ash Source	Total K ₂ O (%)	Water-Soluble P ₂ O ₅ (%)	Total MgO (%)	Total Nitrogen (N) (%)	Key Nutrient Advantage / Disadvantage
Sunflower Stalk Ash	35.1	5.5	6.1	< 0.1	Excellent K and P source, high Mg content.
Wheat Straw Ash	15.5	3.8	2.2	< 0.1	Good K source, rich in silicon.
Wood Ash (Hardwood)	4.5	2.1	3.5	< 0.1	Rich in calcium, moderate K content.
Hazelnut Shell Ash	8.2	1.8	2.8	< 0.1	Balanced K and Ca source.

Biomass Ash Source	Total K ₂ O (%)	Water-Soluble P ₂ O ₅ (%)	Total MgO (%)	Total Nitrogen (N) (%)	Key Nutrient Advantage / Disadvantage
Rice Husk Ash	2.8	1.2	0.8	< 0.1	Low nutrient content, high silica content.

Note: All ashes contain negligible nitrogen (N) due to the combustion process. Nutrient release is very rapid, occurring within weeks of application

Table 15. Nutrient retention capacity (CEC) and nutrient content of different pyrochar types (Adapted from Yargıç & Yarbaş, 2025; Wang et al., 2023)

Pyrochar Feedstock	CEC (cmol ₊ /kg)	Total N (%)	Total P ₂ O ₅ (%)	Total K ₂ O (%)	Primary Role in Nutrient Dynamics
Wood Chips (600°C)	45 - 65	0.5 - 1.0	0.1 - 0.5	0.5 - 2.0	Nutrient retention (Sponge effect), high CEC.
Animal Manure (550°C)	30 - 50	1.5 - 3.0	3.0 - 6.0	2.0 - 4.0	Nutrient source + retention, rich in N and P.
Agricultural Residue (Straw, 550°C)	35 - 55	0.8 - 1.5	0.8 - 1.8	10.0 - 15.0	K source + nutrient retention.
Sewage Sludge (600°C)	20 - 40	3.0 - 5.0	5.0 - 10.0	0.5 - 1.5	P source, but potential heavy metal risk.

Note: CEC is a key indicator of pyrochar's capacity to retain nutrients and reduce leaching. Pyrochars produced from woody feedstocks at high temperatures generally exhibit the highest CEC

Table 16. Effect of hydrochar production parameters on nutrient content and mineralization/immobilization potential (Adapted from Arslan et al., 2024; Kloss et al., 2023)

Hydrochar Feedstock	HTC Temperature (°C)	Total N (%)	C/N Ratio	Mineralization/Immobilization Potential		Recommended Application Strategy
Straw	200	1.8	25	High Risk	Immobilization	Apply 2-4 weeks before seeding or mix with N fertilizer.
Municipal Green Waste	180	3.5	12	Moderate Mineralization		Can be applied at the time of seeding.
Animal Manure	220	4.2	10	Rapid Mineralization (N Source)		Can be used directly as a fertilizer.
Algae	200	5.0	8	Very Rapid Mineralization		Valuable fast-acting organic fertilizer.

Note: A low C/N ratio (< 20-25) indicates rapid mineralization and N release, while a high C/N ratio (> 25-30) carries a risk of N immobilization. Total N content generally decreases with increasing HTC temperature

3.3. Biological Properties: Effects on Soil Microbiota

The soil microbiota is a key determinant of soil health and fertility. The application of organic amendments like pyrochar, hydrochar, and ash can significantly alter microbial community structure, biomass, and functional diversity through direct and indirect pathways.

Effect of Pyrochar: The porous structure of pyrochar can provide a physical habitat for microorganisms, potentially increasing microbial biomass and diversity (Lehmann et al., 2011). The pores offer protection from predators and help retain moisture and nutrients. Pyrochars produced at higher temperatures (>500°C) have a more stable structure, providing long-term

support for microbial colonization (Wang et al., 2020). However, pyrochars produced at lower temperatures ($<500^{\circ}\text{C}$) may cause a short-term rapid increase in microbial activity ("priming effect") due to their higher content of labile organic compounds (Luo et al., 2021). Overall, pyrochar application has been observed to increase microbial biomass, particularly the abundance of symbiotic species like mycorrhizal fungi, especially in degraded soils (Zhang et al., 2022).

Effect of Hydrochar: The impact of hydrochar is more variable than that of pyrochar and highly dependent on production conditions and feedstock. The easily decomposable organic compounds formed during HTC (e.g., furans, phenolic compounds) can stimulate microbial activity (Gajic et al., 2022). However, some of these compounds can be inhibitory or even toxic to microorganisms at high concentrations (Malghani et al., 2019). This oligotrophic effect may temporarily reduce microbial abundance initially. Over time, these inhibitory compounds are leached or degraded, after which the porous structure and nutrient content of hydrochar can support microbial communities (Bargmann et al., 2023). The surface functional groups (carboxyl, hydroxyl), often more abundant on hydrochar than pyrochar, can facilitate nutrient retention, indirectly promoting microbial development.

Effect of Ash: The primary effect of ash on microbiology stems from its ability to raise soil pH due to its high pH and calcium carbonate content. This leads to a shift in microbial community composition, favoring neutrophilic and alkaliphilic species over acidophilic ones (Zhou et al., 2020). The increased pH can also enhance the bioavailability of certain nutrients like phosphorus, positively affecting microbial activity. However, excessively high application rates can create osmotic stress and potentially lead to toxicity of some trace elements, suppressing microbial activity (Insam et al., 2023). Nutrients like potassium in ash can support microbial growth.

Table 17. Main effect mechanisms of pyrochar on soil microbial community and their consequences (Wang et al., 2020).

Effect Mechanism	Explanation	Consequence on Microbial Community
Physical Habitat	Porous structure provides a sheltered niche for microorganisms.	Increase in microbial biomass and diversity.
Nutrient Retention	High CEC retains nutrients (NH ₄ ⁺ , K ⁺).	Enhanced nutrient supply for microbial growth and activity.
Water Holding Capacity	Increases soil water retention.	Improves microbial survival chances under drought conditions.
pH Regulation	Typically alkaline; raises pH of acidic soils.	Decrease in acidophilic species, increase in neutrophilic/alkaliphilic species.
Labile Carbon Source	Low-temperature pyrochar contains decomposable carbon.	Strong short-term priming effect and bacterial dominance.

Table 18. Main Effect Mechanisms of Hydrochar on Soil Microbial Community and Their Consequences (Gajic et al., 2022).

Effect Mechanism	Explanation	Consequence on Microbial Community
Labile Organic Compounds	Furan derivatives, organic acids, etc., formed during production.	Initial rapid increase in microbial activity (food source).
Inhibitory Compounds	Some phenolics and melanoidins formed during the process.	Temporary toxicity and reduced microbial abundance at high doses.
Surface Chemistry	Rich oxygen-containing functional groups (carboxyl, hydroxyl).	Improved retention of nutrient ions, enhancing microbial nutrition.

Effect Mechanism	Explanation	Consequence on Microbial Community
Porosity	More irregular and amorphous pore structure compared to pyrochar.	Provides a suitable environment for microbial colonization over time.
Nitrogen Immobilization	High C/N ratio may lead to consumption of soil nitrogen.	Initial limitation of microbial growth due to N deficiency.

Table 19. Main effect mechanisms of ash on soil microbial community and their consequences (Zhou et al., 2020).

Effect Mechanism	Explanation	Consequence on Microbial Community
pH Increase	High CaCO_3 and metal oxide content significantly raises soil pH.	Dramatic shift in community composition: acidophiles decrease, neutrophilic/alkaliphiles increase.
Nutrient Supplement	Source of K, Ca, Mg, P, and trace elements (e.g., B, Mo).	Supplies nutrients essential for microbial metabolism and growth.
Osmotic Stress	High ionic content (especially at high doses) increases osmotic pressure.	Suppression of activity and cell dehydration upon over-application.
Soil Structure	Fine particles may enhance soil aggregate stability.	Improves physical habitat, indirectly supporting microbial development.
Trace Element Toxicity	Ash types with high heavy metal content (e.g., from sewage sludge).	Long-term toxic effects on microbial diversity and activity.

4. Environmental Benefits and Applications

4.1. Carbon Sequestration and Climate Change Mitigation

The carbon sequestration potential of organic amendments depends on their stability (persistence), effects on the soil carbon cycle (e.g., priming effect), and the carbon footprint of their production processes. Pyrochar, hydrochar, and ash show distinct differences in these aspects.

Superior Stability of Pyrochar: Due to its highly aromatic structure formed during high-temperature pyrolysis (>400-500°C), pyrochar is highly resistant to biological and chemical degradation in soil. This resistance allows it to persist in soil for centuries to millennia, making it a superior tool for long-term atmospheric CO₂ sequestration (Wang et al., 2020). However, stability is directly related to pyrolysis temperature and feedstock. Woody biochars produced at high temperatures have greater persistence than those produced at low temperatures or from manures (Singh et al., 2022). Additionally, adding pyrochar to soil can induce a "positive priming effect," potentially accelerating the mineralization of native soil organic matter. Nevertheless, long-term studies indicate this effect is transient and may be negligible compared to the net increase in stable carbon fractions (Weng et al., 2022).

Variable Stability of Hydrochar: The chemical structure of hydrochar produced via Hydrothermal Carbonization (HTC) is less condensed and more aliphatic compared to pyrochar. Consequently, it is more susceptible to decomposition and mineralization by soil microorganisms (Kätterer et al., 2019). The half-life of hydrochar in soil ranges from a few years to several decades, significantly shorter than that of pyrochar (Bargmann et al., 2021). Thus, its role in climate change mitigation is less pronounced. Furthermore, the HTC process water, a waste stream rich in organic carbon, can trigger a strong positive priming effect when co-applied to soil, potentially leading to a loss of native soil organic carbon stocks (Gajic et al., 2023). This complicates the assessment of hydrochar's true carbon sequestration potential.

Table 20. Factors influencing the carbon sequestration potential of pyrochar (Singh et al., 2022).

Factor	Effect	Implication for Carbon Persistence
Pyrolysis Temperature	Increased temperature enhances aromaticity and decreases O/C ratio.	Higher temperature (>500°C) yields greater persistence.
Feedstock	Woody materials form more stable structures due to high lignin content.	Wood > Agricultural Residue > Animal Waste (general order).
O/C Ratio	A low O/C ratio (<0.2) indicates high resistance.	Lower O/C ratio correlates with higher resistance to biodegradation.
Soil Conditions	Temperature, moisture, and microbial activity affect decay rate.	Persistence is higher under temperate/cold and dry conditions.
Priming Effect	Pyrochar may trigger decomposition of native soil organic matter.	Can cause short-term net carbon loss, balances out in the long term.

Limited Role of Ash: As an inorganic material, ash does not contain significant organic carbon. Composed primarily of inorganic minerals (oxides, carbonates), its carbon retention capacity is negligible. The primary mitigation potential of ash is indirect. For example, by raising the pH of acidic soils, it can slow the decomposition rate of soil organic matter, thereby helping to preserve existing soil carbon stocks (Zhou et al., 2020). However, this constitutes carbon stabilization rather than sequestration (adding new carbon). Moreover, the combustion process for ash production instantly releases all carbon from the biomass as CO₂ into the atmosphere, making it a carbon source, not a sink.

Table 21. Factors and risks influencing the carbon sequestration potential of hydrochar (Bargmann et al., 2021).

Factor / Risk	Explanation	Impact on Carbon Cycle
HTC Temperature and Duration	Higher temperature/duration increases hydrochar aromaticity and stability.	Hydrochar produced at higher temperatures ($\geq 250^{\circ}\text{C}$) is more persistent.
Chemical Structure	High aliphatic C content and O/C ratio (>0.4) facilitate decomposition.	Lower persistence (half-life) compared to pyrochar.
Presence of Process Water	HTC wastewater contains readily decomposable carbon.	Can induce a strong positive priming effect, leading to soil carbon loss.
"Aging" Effect	Oxidation of the hydrochar surface upon exposure to soil or air.	Oxidation may increase the initial decomposition rate.
Feedstock	Lignocellulosic wastes yield more stable hydrochar than organic wastes.	Stability varies depending on the waste type.

Table 22. Indirect effects of ash on the carbon cycle (Zhou et al., 2020).

Effect Mechanism	Explanation	Relation to Carbon Sequestration
Direct Carbon Content	Negligible organic carbon content as it is an inorganic material.	Not considered a direct carbon sequestration tool.
Raising Soil pH	High CaCO_3 content neutralizes acidic soils.	Reduces decomposition rate under acidic conditions, preserving existing soil carbon.

Effect Mechanism	Explanation	Relation to Carbon Sequestration
Nutrient Supply	Nutrients like K, P, Ca promote plant growth.	Increased plant biomass can provide new carbon inputs.
Soil Aggregate Stability	Fine particles may contribute to the formation of soil aggregates.	Physical protection of carbon occluded within aggregates may be enhanced.
Emissions from Combustion	Combustion of biomass instantly releases carbon as CO ₂ .	A net carbon source. Mitigation potential is limited to conserving existing carbon.

4.2. Immobilization of Pollutants

These materials can reduce the mobility and bioavailability of heavy metals and organic pollutants in soil through mechanisms such as adsorption, precipitation, or chemical transformation, serving as important environmental remediation tools. However, their mechanisms and effectiveness vary significantly.

Superior Adsorption Capacity of Pyrochar: Pyrochar is a highly efficient immobilizing agent for heavy metals and organic pollutants. This capacity stems from its large surface area, developed pore structure (micro- and mesopores), and abundant surface functional groups (e.g., carboxyl, hydroxyl) (Ahmad et al., 2014). Key immobilization mechanisms for heavy metals include ion exchange and complexation with functional groups, precipitation (e.g., Pb₃(PO₄)₂ under high pH conditions), and π -electron interactions (Wang et al., 2020). Organic pollutants (e.g., pesticides, PAHs) are effectively sorbed via hydrophobic interactions and physical adsorption within pores. Higher pyrolysis temperatures increase surface area and aromaticity, enhancing organic pollutant sorption, while lower-temperature biochars may be more effective for metals due to higher functional group content (Li et al., 2023).

Variable Performance of Hydrochar: While hydrochar can also sorb pollutants, its effectiveness is more variable and often lower than pyrochar due to its typically smaller surface area and different surface chemistry (Regmi et al., 2012). The hydrochar surface is richer in oxygen-containing functional

groups (carbonyl, carboxyl), making it effective at binding cationic heavy metals (Pb^{2+} , Cd^{2+}) via ion exchange and surface complexation (Mohan et al., 2021). However, its less developed pore structure may limit physical adsorption. Inorganic components (e.g., calcite) in hydrochar can contribute to metal precipitation, enhancing immobilization. Performance is highly dependent on feedstock and HTC conditions.

Table 23. Contaminant immobilization mechanisms of pyrochar (Li et al., 2023).

Immobilization Mechanism	Description	Effective for Which Contaminants?
Surface Complexation	Binding of metal ions with surface functional groups such as -OH and -COOH.	Pb^{2+} , Cd^{2+} , Cu^{2+} , Zn^{2+} (Cationic metals)
Cation Exchange Capacity (CEC)	Displacement of heavy metal ions with exchangeable cations (e.g., Ca^{2+} , Mg^{2+}) on the surface.	Cd^{2+} , Zn^{2+} , Ni^{2+}
Precipitation	Formation of metal phosphate/carbonate salts in the presence of high pH and phosphate/carbonate.	Pb^{2+} (especially in phosphate-rich biochar), Cd^{2+} , Zn^{2+}
Physical Adsorption	Trapping of contaminants within the extensive surface area and porous structure.	Organic contaminants (PAHs, pesticides), gases
π -electron Interaction	Interaction between the aromatic carbon structure and metals/organic molecules.	Cr(VI) , organic contaminants

pH-Dependent Immobilization by Ash: The primary role of ash in pollutant immobilization is its significant elevation of soil pH due to its alkaline compounds (CaO , K_2CO_3) and carbonates (CaCO_3). At elevated pH, the solubility and mobility of most heavy metal ions (especially Cd , Zn , Cu) decrease as they tend to form hydroxide or carbonate precipitates (Šyc et al.,

2020). This makes ash particularly effective for remediating acidic, metal-contaminated soils. However, high pH conditions can increase the solubility and mobility of anionic metalloids like arsenic (As), necessitating careful use depending on the pollutant spectrum (Ippolito et al., 2023). Ash generally has limited direct immobilization effects on organic pollutants.

Table 24. Contaminant immobilization mechanisms of hydrochar (Mohan et al., 2021).

Immobilization Mechanism	Description	Notes / Limitations
Surface Complexation	Effective metal binding via oxygen-containing functional groups, which are richer compared to pyrochar.	Primary immobilization pathway; effective for cationic metals.
Physical Adsorption	Pore structure is less developed than in pyrochar, hence its capacity is lower.	Generally a weaker option for the adsorption of organic contaminants.
Precipitation	Precipitation of metals with inorganics (e.g., Ca, P) present in the hydrochar structure or in the soil.	Dependent on feedstock (e.g., animal waste hydrochar may contain high Ca/P).
Electrostatic Attraction	Attraction between the charge of the contaminant and the surface charge of the hydrochar.	pH-dependent; surface charge varies with pH.

Table 25. Contaminant immobilization mechanisms of ash (Ippolito et al., 2023).

Immobilization Mechanism	Description	Advantages / Risks
pH Elevation and Precipitation	Raising soil pH due to high alkaline content, leading to the	Main mechanism. Very effective for Cd, Zn,

Immobilization Mechanism	Description	Advantages / Risks
	precipitation of metal hydroxides/carbonates.	Cu. However, it carries a risk of As mobilization.
Surface Adsorption	Physical/chemical adsorption occurring on the surface of ash particles.	Has a limited capacity; not the primary mechanism.
Chemical Transformation	Conversion of contaminants into less soluble forms (e.g., reduction of Cr(VI) to Cr(III))	

5. Potential Risks and Limitations

The safe application of these materials in agricultural and environmental remediation practices necessitates a rigorous assessment of potential risks. Foremost among these is the undesirable pollutant load that the materials may carry, depending on the feedstock source and production process.

5.1. Pollutant Content

The environmental benefits of biochar and ash can be overshadowed by the pollutant load they may introduce. Depending on the feedstock, all three materials can contain various contaminants that pose risks to soil and water resources (Hale et al., 2012). These pollutants can be categorized into two main groups:

1.Residual Pollutants: Heavy metals (Pb, Cd, Zn, Cu, Ni, Cr, As), salts, or radionuclides originating from the feedstock. For instance, pyrochar/hydrochar produced from sewage sludge, industrial wastes, or animal manure may have high heavy metal concentrations (Oleszczuk et al., 2023). Similarly, ash derived from industrial processes or specific coal types can carry a significant heavy metal risk.

2.Process-Derived Pollutants: Organic compounds formed during thermochemical conversion, particularly at high temperatures. Polycyclic Aromatic Hydrocarbons (PAHs), dioxins, and furans, which form under insufficient temperature or the presence of oxygen during pyrolysis, are the

most significant process-derived pollutants (Buss et al., 2022). Therefore, comprehensive characterization (heavy metal, PAH analysis, EC, pH, etc.) and toxicity testing (e.g., plant germination tests, earthworm toxicity tests) of the materials prior to soil application is essential. Furthermore, pre-treatments such as "aging" (e.g., composting) have been shown to effectively reduce pollutant content and bioavailability (He et al., 2023).

Table 26. Primary pollutants associated with pyrochar and risk mitigation strategies (Oleszczuk et al., 2023).

Pollutant Category	Example Pollutants	Source and Risk Factors	Risk Mitigation Strategies
Heavy Metals	Cd, Pb, Zn, Cu, Ni, Cr, As	Originates from feedstock (sewage sludge, industrial waste). Metal concentration in biochar increases with higher pyrolysis temperature.	Selection of clean feedstocks (wood, agricultural residues). High-temperature production (stabilizes metals). Pre- and post-production characterization.
Organic Pollutants (PAHs)	Naphthalene, Benzo(a)pyrene	Result from incomplete combustion (low temperature (<400°C) and/or low heat transfer).	High and controlled pyrolysis temperature (>500°C). Sufficient vapor residence time.
Salinity	Ca, Mg, K, Na salts	Derived from animal manure or biomass exposed to saline water. Can increase soil EC.	Use of washed feedstock or post-production washing of biochar.
Low pH (Acidity)	-	Biochars produced at low temperatures can be acidic.	pH control, co-application with liming agents.

Table 27. Primary pollutants associated with hydrochar and risk mitigation strategies (He et al., 2023).

Pollutant Category	Example Pollutants	Source and Risk Factors	Risk Mitigation Strategies
Heavy Metals	Cd, Pb, Zn, Cu	Originates from feedstock (sewage sludge, contaminated organic wastes).	Selection of clean feedstock. The HTC process generally stabilizes metals.
Process-Derived Organics	Furfurals, Phenolic Compounds, 5-HMF	Natural by-products of the HTC process. Can be phytotoxic to plants and microorganisms at high concentrations.	Post-production "aging" (oxidation). Composting. Separation and treatment of process water.
Process Water Pollution	Above-mentioned organics, Nitrogenous Compounds	The liquid phase from HTC is rich in organic carbon and nutrients. Direct application to soil can cause excessive mineralization.	Treatment or dilution of process water before soil application.
Salinity	Various ions	Dependent on feedstock and process conditions.	Washing or composting.

Table 28. Primary pollutants associated with ash and risk mitigation strategies (Ippolito et al., 2023).

Pollutant Category	Example Pollutants	Source and Risk Factors	Risk Mitigation Strategies
Heavy Metals	Cd, Pb, Zn, Cu, As	Dependent on the source of combusted biomass or coal.	Source control (preference for clean wood ash).

Pollutant Category	Example Pollutants	Source and Risk Factors	Risk Mitigation Strategies
Alkalinity and Salinity		Industrial waste or contaminated wood ash pose a high risk. Anionic metals like As can become more mobile at the elevated pH caused by ash.	Heavy metal analysis. Application at low rates.
	CaO, K ₂ CO ₃ , various salts	Inherently high due to the nature of ash. Over-application can elevate soil alkalinity and salinity to hazardous levels.	Regular monitoring of soil pH and EC. Application at low rates and on sensitive soils.
Radionuclides	Potassium-40 (⁴⁰ K)	Naturally present in biomass and concentrated in ash. Generally considered low risk at agricultural application rates.	Source selection, avoidance of excessive application.

5.2. Adverse Effects on the Soil Ecosystem from the Application of Pyrochar (Biochar), Hydrothermal Carbonization Product (Hydrochar), and Ash as Soil Amendments

Carbon-based soil amendments like biochar and hydrochar have garnered significant interest for their potential to enhance soil health and agricultural productivity. However, particularly when applied at excessively high rates or under inappropriate conditions, these materials and their by-products (ash) can lead to significant adverse effects on the soil ecosystem, as clearly demonstrated by numerous scientific publications post-2015.

Over-application is the most common risk factor. The production temperature and feedstock of pyrochar and hydrochar directly influence their electrical conductivity (EC) values and, consequently, their salinity potential.

Biochars produced at lower temperatures (<500°C) typically contain higher concentrations of alkaline groups (K, Ca, Na, Mg) and can significantly raise soil pH. Similarly, organic acids and soluble compounds remaining from the hydrochar production process can also increase EC. Ash, by its nature, contains high levels of soluble salts and alkaline elements. High EC increases the osmotic potential of soil water, making it more difficult for plants to absorb water, leading to "physiological drought," suppressing germination, and inhibiting plant growth (Tomczyk et al., 2020). Furthermore, an excessive amount of one nutrient element (e.g., high potassium from ash) can inhibit the uptake of other cations (calcium, magnesium), leading to nutrient imbalance.

The phytotoxic effects associated with hydrochar, as noted by Bargmann et al. (2013), have been further elucidated by subsequent studies. The primary source of this toxicity is organic compounds formed during hydrothermal carbonization that remain on the hydrochar surface if not subjected to adequate "aging" (e.g., composting). These compounds include furfurals, phenolic compounds, and polycyclic aromatic hydrocarbons (PAHs) (Busch et al., 2022). These substances can damage plant root cell membranes, inhibiting cell division and elongation. Phytotoxicity varies significantly depending on the feedstock (e.g., animal manure-derived hydrochar may be riskier) and production conditions (temperature, duration). Fortunately, simple methods such as outdoor weathering or co-composting can dilute or degrade these toxic compounds (Steiner et al., 2021).

Carbon materials applied at high doses can cause temporary shocks to soil microbial ecology. A sudden increase in carbon can lead to the overgrowth of specific bacterial or fungal groups, disrupting the microbial balance. Additionally, pyrochar or ash with high pH can suppress acidophilic microorganisms. This may slow down critical processes such as organic matter decomposition (El-Naggar et al., 2019). However, this effect is often observed to balance out in the long term.

Untested biochar and hydrochar may contain heavy metals (e.g., Cu, Ni, Cd) or residual pesticides originating from the feedstock. Over-application can lead to the accumulation of these pollutants in the soil and increase the risk of leaching into groundwater (O'Connor et al., 2018).

In conclusion, while the benefits of these materials to the soil ecosystem can potentially outweigh the adverse effects, a "one-size-fits-all" approach

should be avoided. Pre-application analysis of the soil and biochar/hydrochar/ash is essential to determine safe and effective application rates.

Table 29. Adverse soil effects associated with pyrochar (biochar) application and related studies.

Adverse Effect	Probable Mechanism	Factors Determining the Effect	Related Study
Increased Salinity (High EC)	Release of alkaline cations (K, Ca, Na) into the soil	Low production temperature, feedstock with high ash content (e.g., manure), excessive application rate	Tomczyk et al., 2020
Excessive Rise in Soil pH	Neutralizing effect of carbonates in the ash content	High-pH biochar, application on non-acidic soils	El-Naggar et al., 2019
Nutrient Imbalance	Change in Cation Exchange Capacity (CEC), excessive presence of one element	Nutrient content of biochar, initial nutrient balance of the soil	Major, 2021
Temporary Microbial Imbalance	Disruption of the existing microbial community structure	Application rate, soil type, biochar surface chemistry	Lehmann et al., 2015
Risk of Pollutant Accumulation	Addition of heavy metals from the feedstock to the soil	Feedstock source (e.g., sewage sludge, industrial waste), production conditions	O'Connor et al., 2018

Table 30. Adverse soil effects associated with hydrochar application and related studies.

Adverse Effect	Probable Mechanism	Factors Determining the Effect	Related Study
Phytotoxic Effect	Inhibition of plant root development by organics such as furfural and phenolic compounds	Un-aged, fresh hydrochar, feedstock (e.g., manure-derived), low production temperature	Busch et al., 2022
Salinity and pH Change	Soluble organic and inorganic ions remaining in the process water	Post-production washing, feedstock composition	Kalderis et al., 2024
Suppression of Seed Germination	Direct toxic effect of phytotoxic compounds	Hydrochar/seed proximity, application method (mixing vs. surface broadcasting)	Steiner et al., 2021
Nitrogen Immobilization	Utilization of soil nitrogen by microbes for decomposing high C/N ratio hydrochar	Feedstocks with high C/N ratio (e.g., wood), low nitrogen content	Rizzo et al., 2023
Hydrophobicity (Water Repellency)	Inhibition of water absorption by organic coating on the surface	Observed with certain feedstocks and production conditions, usually temporary	Bargmann et al., 2013 (Foundational), confirmed by recent studies

Table 31. Adverse soil effects associated with ash application and related studies.

Adverse Effect	Probable Mechanism	Factors Determining the Effect	Related Study
Severe Salinity and pH Increase	High content of soluble alkalis (KOH, $\text{Ca}(\text{OH})_2$)	Type of combusted biomass, excessive application rate	Pandey & Singh, 2023
Nutrient Imbalance	Overloading of one element (e.g., potassium), inhibition of other micronutrient (Zn, Fe, Mn) uptake	Nutrient content of ash, soil type	Vassilev et al., 2020
Deterioration of Soil Structure	Clogging of soil pores by fine particles, formation of hard crust	Fine-grained nature of ash, heavy-textured soils	Jien & Wang, 2021
Heavy Metal Pollution	Concentrated heavy metals in ash from combustion of industrial or municipal wastes	Source of ash, risk is low with clean biomass ash	O'Connor et al., 2018
Reduction in Organic Matter and Microbial Activity	Suppression of organic matter-decomposing microorganisms by high pH	Application rate, lime-like effect of ash	El-Naggar et al., 2019

6. Conclusion and Future Perspective

Thermochemical conversion products of biomass waste—hydrochar, pyrochar, and organic-derived ash—increasingly demonstrate their potential as valuable components of sustainable strategies that integrate waste management with agricultural soil health and productivity within the framework of circular

economy principles. Each material assumes unique functions in the soil system due to distinct production parameters and physicochemical properties.

Pyrochar: Long-Term Carbon Sequestration and Architect of Soil Health

Produced at high pyrolysis temperatures (typically $>500^{\circ}\text{C}$), pyrochar possesses a highly stable aromatic structure. This stability makes it a form in which atmospheric carbon dioxide can be sequestered in the soil for centuries, highlighting its role in climate change mitigation strategies (Lehmann et al., 2021). Furthermore, its porous structure enhances soil water retention capacity, improves aggregate stability, and serves as a refuge for microbial habitats, establishing it as the most promising option for long-term soil health improvement (Wang et al., 2020). However, it should be noted that its nutrient content can be relatively low depending on the feedstock and production conditions.

Hydrochar: Wet Waste Valorization and Short-Term Nutrient Dynamics

Produced via hydrothermal carbonization (HTC), hydrochar offers an ideal method for valorizing high-moisture wastes (sewage sludge, food waste, animal manure). Compared to pyrochar, it generally contains higher proportions of labile carbon and plant nutrients (especially nitrogen and phosphorus) (Kaluža et al., 2024). This characteristic makes it an effective tool for short-term plant nutrient recycling and soil fertility enhancement. However, the decomposition kinetics of hydrochar in soil and the potential phytotoxic effects of some of its organic components on plants are not yet fully understood and require careful consideration (Bargmann et al., 2024).

Organic-Derived Ash: Rapid pH Amendment and Potassium Source

Ash from the combustion of organic wastes, with its high alkalinity, will continue to be used as a traditional and effective method for rapid pH correction of acidic soils. Simultaneously, particularly wood ash is a rich source of potassium, calcium, and trace elements (Pitman, 2024). However, the risk of concentrated pollutants, such as heavy metals, remains a significant concern

dependent on the ash source and necessitates characterization prior to use (Obernberger et al., 2024).

Future Research Perspectives

For the safe and widespread adoption of these technologies in agricultural practice, future research must focus on the following critical areas:

- 1. Optimized Application Protocols:** Ideal application rates, timing, and methods for different soil types (sandy, clayey, organic) and plant species need to be standardized. Long-term field trials exceeding a decade are needed, moving beyond short-term greenhouse experiments (Jeffery et al., 2024).
- 2. Pollutant Risk Assessment and Monitoring:** Comprehensive risk assessment studies are required on the heavy metal, PAH, and other pollutant content of waste-derived hydrochar and pyrochar, and their mobility and bioavailability in the soil-plant system (Oleszczuk et al., 2024).
- 3. Synergistic Combinations:** The synergistic effects of combinations such as hydrochar + pyrochar or biochar + ash should be investigated. For example, the combined use of hydrochar's short-term nutrient release with pyrochar's long-term soil improvement properties warrants evaluation (Sun et al., 2023).
- 4. Life Cycle Assessment (LCA) and Economic Feasibility:** Life Cycle Assessment studies quantifying the cradle-to-grave environmental impacts of these materials, along with economic analyses comparing production costs to agricultural benefits, will provide critical data for policymakers and farmers (Azzi et al., 2022).

In conclusion, hydrochar, pyrochar, and organic ash are complementary pieces of the sustainable agriculture and waste management puzzle. When supported by sound scientific principles and robust policies, they are poised to play a significant role in the circular agricultural systems of the future.

References

- Ahmad, M., Rajapaksha, A. U., Lim, J. E., Zhang, M., Bolan, N., Mohan, D., Vithanage, M., Lee, S. S., & Ok, Y. S. (2014). Biochar as a sorbent for contaminant management in soil and water: A review. *Chemosphere*, 99, 19–33. <https://doi.org/10.1016/j.chemosphere.2013.10.071>
- Amelung, W., Bossio, D., de Vries, W., Kögel-Knabner, I., Lehmann, J., Amundson, R., ... & Sanderman, J. (2020). Towards a global-scale soil climate mitigation strategy. *Nature Communications*, 11(1), 5427. <https://doi.org/10.1038/s41467-020-18887-7>
- Arslan, E., Ünlü, K., & Aydın, S. (2022). Hydrochar from sewage sludge: Effects on soil moisture retention and physical properties in a sandy soil. *Journal of Environmental Management*, 315, 115158. <https://doi.org/10.1016/j.jenvman.2022.115158>
- Arslan, E., Ünlü, K., & Aydın, S. (2022). Hydrochar from sewage sludge: Effects on soil moisture retention and physical properties in a sandy soil. *Journal of Environmental Management*, 315, 115158. <https://doi.org/10.1016/j.jenvman.2022.115158>
- Arslan, E., Yılmaz, V., & Demir, T. (2024). Nitrogen dynamics following hydrochar application: The interplay between mineralization and immobilization. *Soil Biology and Biochemistry*, 188, 109234. <https://doi.org/10.1016/j.soilbio.2023.109234>
- Azzi, E. S., Karlton, E., & Sundberg, C. (2022). Life cycle assessment of urban uses of biochar and case study in Uppsala, Sweden. *Biochar*, 4(1), 18. <https://doi.org/10.1007/s42773-022-00144-3>
- Bakış, R., Yılmaz, E., & Demir, T. (2021). Farklı tarımsal atık küllerinin karakterizasyonu ve tarımda geri kazanım potansiyelleri [Characterization of different agricultural waste ashes and their recovery potential in agriculture]. *Anadolu Tarım Bilimleri Dergisi*, 36(2), 345–358.
- Bargmann, I., Martens, R., & Rillig, M. C. (2013). Effects of hydrochar application on the dynamics of soluble nitrogen in soils and on plant growth. *Soil Use and Management*, 29(2), 213–222. <https://doi.org/10.1111/sum.12040>

- Bargmann, I., Martens, R., & Rillig, M. C. (2022). Hydrochar and biochar: A comparative analysis of their properties and potential for soil amendment. *Journal of Plant Nutrition and Soil Science*, 185(2), 189–201. <https://doi.org/10.1002/jpln.202100191>
- Bargmann, I., Rillig, M. C., & Kruse, A. (2024). Hydrochar effects on soil biogeochemistry and plant growth: A review. *Science of The Total Environment*, 906, 167784. <https://doi.org/10.1016/j.scitotenv.2023.167784>
- Bargmann, I., Rillig, M. C., Kruse, A., Greef, J. M., & Kücke, M. (2021). Degradation of hydrochar in a German Cambisol: A 2-year field study. *Geoderma*, 402, 115198. <https://doi.org/10.1016/j.geoderma.2021.115198>
- Bertoli, A. C., de Souza, L. C., & Fontoura, M. R. (2025). Wood ash as a soil conditioner in sandy soils: Effects on water retention and plant-available water. *Journal of Soil Science and Plant Nutrition*, 25(1), 145–156. <https://doi.org/10.1007/s42729-024-01835-5>
- Blanco-Canqui, H. (2017). Biochar and soil physical properties. *Soil Science Society of America Journal*, 81(4), 687–711. <https://doi.org/10.2136/sssaj2017.01.0017>
- Blanco-Canqui, H. (2017). Biochar and soil physical properties. *Soil Science Society of America Journal*, 81(4), 687–711. <https://doi.org/10.2136/sssaj2017.01.0017>
- Busch, D., Kaczor, F., & Mumme, J. (2022). Chemical Composition and Phytotoxicity of Hydrothermal Carbonization Products. *Bioresource Technology*, 347, 126732. <https://doi.org/10.1016/j.biortech.2022.126732>
- Buss, W., Graham, M. C., Shepherd, J. G., & Mašek, O. (2022). Effect of pyrolysis temperature and feedstock on polycyclic aromatic hydrocarbon (PAH) concentrations in biochars. *Chemosphere*, 291, 132738. <https://doi.org/10.1016/j.chemosphere.2021.132738>
- Cheng, F., Luo, H., Colosi, L. M., & Huang, R. (2022). Hydrothermal carbonization of organic waste: A critical review on its mechanism, applications, and challenges. *Bioresource Technology*, 347, 126742. <https://doi.org/10.1016/j.biortech.2022.126742>

- Demirbas, A. & Aydin, S. (2023). Heavy metal content and leaching characteristics of various biomass ashes. *Journal of Material Cycles and Waste Management*, 25(3), 1234-1245. <https://doi.org/10.1007/s10163-023-01610-1>
- Ding, Y., Liu, Y., Liu, S., Li, Z., Tan, X., Huang, X., ... & Zheng, B. (2016). Biochar to improve soil fertility. A review. *Agronomy for Sustainable Development*, 36(2), 36. <https://doi.org/10.1007/s13593-016-0372-z>
- El-Naggar, A., Lee, S. S., Rinklebe, J., Farooq, M., Song, H., Sarmah, A. K., ... & Ok, Y. S. (2019). Biochar application to low fertility soils: A review of current status, and future prospects. *Science of The Total Environment*, 718, 137220. <https://doi.org/10.1016/j.scitotenv.2020.137220>
- European Commission. (2018). A sustainable bioeconomy for Europe: strengthening the connection between economy, society and the environment. Updated Bioeconomy Strategy. Publications Office of the European Union.
- FAO. (2022). The State of the World's Land and Water Resources for Food and Agriculture – Systems at breaking point. Main report. Food and Agriculture Organization of the United Nations.
- Funke, A., & Ziegler, F. (2010). Hydrothermal carbonization of biomass: A summary and discussion of chemical mechanisms for process engineering. *Biofuels, Bioproducts and Biorefining*, 4(2), 160–177. <https://doi.org/10.1002/bbb.198>
- Gajic, A., & Koch, H. J. (2012). Sugar beet (*Beta vulgaris* L.) growth and yield as affected by hydrochar application. *Journal of Plant Nutrition and Soil Science*, 175(6), 898–907. <https://doi.org/10.1002/jpln.201100300>
- Gajic, A., & Koch, H. J. (2021). The potential of hydrochar as a soil amendment: Effects on soil physical properties, nutrient availability, and plant growth. *Agronomy*, 11(5), 983. <https://doi.org/10.3390/agronomy11050983>
- Gajic, A., & Koch, H. J. (2021). The potential of hydrochar as a soil amendment: Effects on soil physical properties, nutrient availability, and plant growth. *Agronomy*, 11(5), 983. <https://doi.org/10.3390/agronomy11050983>
- Gao, Y., Fang, Z., Van Zwieten, L., Bolan, N., Wang, H., & Wang, B. (2023). A review of the fate and transport of nitrogen in soils amended with

- hydrochar. *Science of The Total Environment*, 856, 159106. <https://doi.org/10.1016/j.scitotenv.2022.159106>
- Gomez, L., Silva, R., & Costa, M. (2024). Sugarcane bagasse ash as a slow-release potassium fertilizer for sustainable cropping systems. *Science of The Total Environment*, 907, 167955. <https://doi.org/10.1016/j.scitotenv.2023.167955>
- Günel, H., Bayram, İ., & Demir, Z. (2022). Effects of pyrolysis temperature on the chemical properties and aluminum immobilization capacity of biochars derived from different feedstocks. *Journal of Environmental Management*, 302(Pt A), 113989. <https://doi.org/10.1016/j.jenvman.2021.113989>
- Günel, H., Bayram, İ., & Demir, Z. (2023). Aging of hydrochar in soil: Changes in surface chemistry and its implications for soil water repellency. *Geoderma*, 430, 116318. <https://doi.org/10.1016/j.geoderma.2023.116318>
- Hale, S. E., Lehmann, J., Rutherford, D., Zimmerman, A. R., Bachmann, R. T., Shitumbanuma, V., ... & Cornelissen, G. (2012). Quantifying the total and bioavailable polycyclic aromatic hydrocarbons and dioxins in biochars. *Environmental Science & Technology*, 46(5), 2830–2838. <https://doi.org/10.1021/es203984k>
- He, M., Xiong, X., Wang, L., Hou, D., Bolan, N. S., Ok, Y. S., ... & Rinklebe, J. (2021). A critical review on performance indicators for evaluating soil biota and soil health of biochar-amended soils. *Journal of Hazardous Materials*, 414, 125378. <https://doi.org/10.1016/j.jhazmat.2021.125378>
- Insam, H., Gómez-Brandón, M., & Ascher-Jenull, J. (2023). Wood ash effects on soil microbial community and nutrient cycling in a boreal forest. *Forest Ecology and Management*, 529, 120667. <https://doi.org/10.1016/j.foreco.2022.120667>
- Ippolito, J. A., Spokas, K. A., Novak, J. M., Lentz, R. D., & Cantrell, K. B. (2023). Soil amendment interactions: Effects of biochar, ash, and compost on trace element mobility in contaminated soils. *Journal of Environmental Quality*, 52(1), 1–15. <https://doi.org/10.1002/jeq2.20415>
- Jeffery, S., Abalos, D., Prodana, M., Bastos, A. C., van Groenigen, J. W., Hungate, B. A., & Verheijen, F. (2024). Biochar boosts tropical but not

- temperate crop yields. *Environmental Research Letters*, 19(2), 024001. <https://doi.org/10.1088/1748-9326/ad1c5a>
- Jeffery, S., Abalos, D., Prodana, M., Bastos, A. C., van Groenigen, J. W., Hungate, B. A., & Verheijen, F. (2024). Biochar boosts tropical but not temperate crop yields. *Environmental Research Letters*, 19(2), 024001. <https://doi.org/10.1088/1748-9326/ad1c5a>
- Jien, S. H., & Wang, C. S. (2021). Effects of Biochar and Biomass Ash on Soil Physical Properties. *Journal of Soil Science and Plant Nutrition*, 21(3), 2083–2094. <https://doi.org/10.1007/s42729-021-00506-z>
- Joseph, S., Cowie, A. L., Van Zwieten, L., Bolan, N., Budai, A., Buss, W., ... & Lehmann, J. (2021). How biochar works, and when it doesn't: A review of mechanisms controlling soil and plant responses to biochar. *GCB Bioenergy*, 13(11), 1731–1764. <https://doi.org/10.1111/gcbb.12885>
- Kaluža, L., Španěl, M., & Trakal, L. (2024). Nutrient dynamics from hydrochars derived from different biowastes: A comparative study. *Journal of Environmental Management*, 351, 119764. <https://doi.org/10.1016/j.jenvman.2023.119764>
- Kambo, H. S., & Dutta, A. (2015). A comparative review of biochar and hydrochar in terms of production, physico-chemical properties and applications. *Renewable and Sustainable Energy Reviews*, 45, 359–378. <https://doi.org/10.1016/j.rser.2015.01.050>
- Kätterer, T., Roobroeck, D., Andrén, O., Kimutai, G., Karlton, E., Kirchmann, H., ... & Börjesson, G. (2019). Biochar addition to agricultural soil: Climate change impacts on soil carbon stocks and N₂O emissions. *Science of The Total Environment*, 650, 2651–2660. <https://doi.org/10.1016/j.scitotenv.2018.09.268>
- Kaya, E., Yıldız, M., & Aydın, S. (2022). The role of biomass ash in improving aggregate stability and reducing soil erosion in clay loam soils. *CATENA*, 215, 106301. <https://doi.org/10.1016/j.catena.2022.106301>
- Kaya, E., Yıldız, M., & Aydın, S. (2023). Comparative efficiency of wood ash and limestone on soil pH adjustment and nutrient availability in an acidic soil. *Journal of Soil Science and Plant Nutrition*, 23(2), 2450–2462. <https://doi.org/10.1007/s42729-023-01203-9>
- Kopittke, P. M., Menzies, N. W., Wang, P., McKenna, B. A., & Lombi, E. (2019). Soil and the intensification of agriculture for global food security.

- Environment International, 132, 105078.
<https://doi.org/10.1016/j.envint.2019.105078>
- Lee, J., Kim, K. H., & Kwon, E. E. (2023). Biochar production technology for bioenergy and environmental applications: A review. *Renewable and Sustainable Energy Reviews*, 178, 113240.
<https://doi.org/10.1016/j.rser.2023.113240>
- Lehmann, J. (2007). A handful of carbon. *Nature*, 447(7141), 143–144.
<https://doi.org/10.1038/447143a>
- Lehmann, J., & Joseph, S. (Eds.). (2015). *Biochar for environmental management: science, technology and implementation*. Routledge.
- Lehmann, J., Cowie, A., Masiello, C. A., Kammann, C., Woolf, D., Amonette, J. E., ... & Whitman, T. (2021). Biochar in climate change mitigation. *Nature Geoscience*, 14(12), 883-892. <https://doi.org/10.1038/s41561-021-00852-8>
- Lehmann, J., Rillig, M. C., Thies, J., Masiello, C. A., Hockaday, W. C., & Crowley, D. (2011). Biochar effects on soil biota--A review. *Soil Biology and Biochemistry*, 43(9), 1812–1836.
<https://doi.org/10.1016/j.soilbio.2011.04.022>
- Li, S., Chen, G., & Wang, Y. (2022). Biochar derived from different biomass sources: Characteristics and applications. *Science of The Total Environment*, 806, 150718.
<https://doi.org/10.1016/j.scitotenv.2021.150718>
- Libra, J. A., Ro, K. S., Kammann, C., Funke, A., Berge, N. D., Neubauer, Y., ... & Emmerich, K. H. (2011). Hydrothermal carbonization of biomass residuals: a comparative review of the chemistry, processes and applications of wet and dry pyrolysis. *Biofuels*, 2(1), 71–106.
<https://doi.org/10.4155/bfs.10.81>
- Liu, Y., Wang, H., & Li, F. (2023). Acidic hydrochar as a potential amendment for the remediation of saline-alkali soils: A pilot-scale study. *Science of The Total Environment*, 856(Pt 2), 159198.
<https://doi.org/10.1016/j.scitotenv.2022.159198>
- Major, J. (2021). A Guide to Conducting Biochar Field Trials. *GCB Bioenergy*, 13(8), 1202-1209. <https://doi.org/10.1111/gcbb.12870>
- Melo, L. C. A., Puga, A. P., & Coscione, A. R. (2023). Potential risks of over-application of biomass ashes on soil physical quality: A review. *Waste*

- Management & Research, 41(4), 789–799.
<https://doi.org/10.1177/0734242X221135234>
- Nabeela, F., Murad, W., Khan, I., Mian, I. A., Rehman, H., Adnan, M., ... & Aziz, R. (2023). Effectiveness of wood ash as a fertilizer and pesticide and its potential risks: A review. *Journal of Soil Science and Plant Nutrition*, 23(1), 916–929. <https://doi.org/10.1007/s42729-022-01093-1>
- Obernberger, I., Biedermann, F., & Widmann, W. (2024). Fate of heavy metals during biomass combustion and gasification: A review. *Fuel*, 357, 129793. <https://doi.org/10.1016/j.fuel.2023.129793>
- O'Connor, J., Peng, T., Zhang, J., Tsang, D. C., Alessi, D. S., Shen, Z., ... & Hou, D. (2018). Biochar application for the remediation of heavy metal polluted land: A review of in situ field trials. *Journal of Hazardous Materials*, 345, 27-39. <https://doi.org/10.1016/j.jhazmat.2017.11.003>
- Ok, Y. S., Palansooriya, K. N., & Lee, S. S. (2024). Biochar for sustainable soil management: Current status and future perspectives. *Pedosphere*, 34(1), 1–15. <https://doi.org/10.1016/j.pedsph.2023.06.006>
- Oleszczuk, P., Joško, I., & Kuśmierz, M. (2024). Biochar and hydrochar as potential carriers of pollutants: A review on the interactions with heavy metals and polycyclic aromatic hydrocarbons. *Critical Reviews in Environmental Science and Technology*, 54(5), 423-456. <https://doi.org/10.1080/10643389.2023.2242222>
- Omondi, M. O., Xia, X., Nahayo, A., Liu, X., Korai, P. K., & Pan, G. (2016). Quantification of biochar effects on soil hydrological properties using meta-analysis of literature data. *Geoderma*, 274, 28–34. <https://doi.org/10.1016/j.geoderma.2016.03.029>
- Ozgan, S. & Güler, S. (2019). The use of olive pomace ash as a soil amendment for acidic soils. *Journal of Environmental Management*, 231, 1170-1176. <https://doi.org/10.1016/j.jenvman.2018.11.019>
- Öztürk, H. S., & Şen, B. (2024). Effects of hazelnut husk ash amendment on the physical and hydrological properties of sandy and loamy soils. *Arabian Journal of Geosciences*, 17(4), 125. <https://doi.org/10.1007/s12517-024-11932-5>
- Palansooriya, K. N., Li, J., Dissanayake, P. D., & Ok, Y. S. (2024). Soil contamination and human health: Recent advances in risk assessment

- and sustainable management. *Journal of Hazardous Materials*, 465, 133312. <https://doi.org/10.1016/j.jhazmat.2024.133312>
- Pandey, V. C., & Singh, V. (2023). Biomass ash as a source of nutrient and soil conditioner. *Journal of Environmental Management*, 325, 116569. <https://doi.org/10.1016/j.jenvman.2022.116569>
- Pitman, R. M. (2024). Wood ash use in forestry—a review of the environmental impacts. *Forestry: An International Journal of Forest Research*, 97(1), 1–20. <https://doi.org/10.1093/forestry/cpad040>
- Shaaban, M., Van Zwieten, L., Bashir, S., Younas, A., Núñez-Delgado, A., Chhajro, M. A., ... & Hu, R. (2018). A concise review of biochar application to agricultural soils to improve soil conditions and fight pollution. *Journal of Environmental Management*, 228, 429–440. <https://doi.org/10.1016/j.jenvman.2018.09.006>
- Singh, C., Tiwari, S., & Gupta, V. K. (2022). Feedstock choice and pyrolysis temperature design to maximize biochar carbon sequestration potential: A critical review. *Environmental Research Letters*, 17(4), 044001. <https://doi.org/10.1088/1748-9326/ac5f5a>
- Sun, Y., Lyu, H., Cheng, Z., Wang, Y., & Tang, J. (2023). Synergistic effects of hydrochar and biochar combination on plant growth and soil quality. *Chemosphere*, 310, 136852. <https://doi.org/10.1016/j.chemosphere.2022.136852>
- Tomczyk, A., Sokołowska, Z., & Boguta, P. (2020). Biochar physicochemical properties: pyrolysis temperature and feedstock kind effects. *Reviews in Environmental Science and Bio/Technology*, 19(1), 191–215. <https://doi.org/10.1007/s11157-020-09523-3>
- Vassilev, S. V., Baxter, D., Andersen, L. K., & Vassileva, C. G. (2013). An overview of the composition and application of biomass ash. Part 1. Phase—mineral and chemical composition and classification. *Fuel*, 105, 40–76. <https://doi.org/10.1016/j.fuel.2012.09.041>
- Wang, J., Xiong, Z., & Kuzyakov, Y. (2020). Biochar stability in soil: Meta-analysis of decomposition and priming effects. *GCB Bioenergy*, 12(12), 1070–1087. <https://doi.org/10.1111/gcbb.1275>
- Wang, L., & Wang, Y. (2019). A comparative review of biochar and hydrochar: Characteristics, production, and applications. *Environmental Chemistry Letters*, 17(4), 1731–1744. <https://doi.org/10.1007/s10311-019-00912-x>

- Wang, T., Zhai, Y., Zhu, Y., Li, C., & Zeng, G. (2018). A review of the hydrothermal carbonization of biomass waste for hydrochar formation: Process conditions, fundamentals, and physicochemical properties. *Renewable and Sustainable Energy Reviews*, 90, 223–247. <https://doi.org/10.1016/j.rser.2018.03.071>
- Xiao, X., Chen, B., & Zhu, L. (2018). Transformation, morphology, and dissolution of silicon and carbon in rice straw-derived biochars under different pyrolytic temperatures. *Environmental Science & Technology*, 52(4), 2195–2203. <https://doi.org/10.1021/acs.est.7b06597>
- Yang, Q., Zhou, H., Bartocci, P., Fantozzi, F., Mašek, O., Agblevor, F. A., ... & Chen, H. (2021). A prospective review of biochar in the remediation of heavy metal (loid) s contaminated soil: Prospects and challenges. *Science of The Total Environment*, 779, 146417. <https://doi.org/10.1016/j.scitotenv.2021.146417>
- Zhang, B., He, P., & Lü, F. (2022). Hydrothermal carbonization of sewage sludge: Focus on the fate of nitrogen and phosphorus during the process and their bioavailability in the resulting hydrochar. *Journal of Cleaner Production*, 333, 130123. <https://doi.org/10.1016/j.jclepro.2021.130123>
- Zhou, X., Wang, J., Li, Z., Ren, J., & Wang, H. (2022). Effects of wood ash on soil chemical properties and microbial communities: A review. *Journal of Forestry Research*, 33(5), 1419–1434. <https://doi.org/10.1007/s11676-022-01475-4>

CHAPTER 8

USE OF SOME ORGANIC MATERIALS IN WOOD

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1. INTRODUCTION

Wood has been a frequently preferred building material from ancient times to the present. However, it can suffer mechanical and physical damage due to biological organisms that rely on wood as a food source, as well as environmental factors. Many civilizations have employed various methods to prevent wood damage. It is known that the Chinese tried to preserve wood with salt water, the Greeks with cedar and olive oil, the Vikings with animal fats, and the Ottomans with pitch and rope. While the environmental impact of wood preservatives used in the past was minimal, advances in technology and industry have increased the demand and need for longer-lasting wood materials. Chemical products, in particular, extend the lifespan of the material but cause environmental damage and diseases(Hülagü et al.2023).

Antibacterial properties are a desirable characteristic for wood used in hygienically sensitive areas. While oak and pine wood have high antibacterial properties, beech and poplar wood have lower antibacterial properties. Generally, antibacterial properties in wood stem from the extractive substances in the wood. Antibacterial properties are observed in oak and pine woods with high extractive substance content, while antibacterial properties are low in beech and poplar woods with low extractive substance content. (Munir ve ark. 2021). It While traditional preservation methods were frequently used in the past, growing demand led to the abandonment of these methods to extend the lifespan of wood. It became clear that the harsh chemicals used, while providing wood with long-term durability, were highly detrimental to the environment and threatening to other living things, and innovative, environmentally friendly, and non-carcinogenic preservatives were sought. In response to this search, the use of vegetable oils has regained importance. Due to their hydrophobic nature, oils provide high water repellency to wood, thus reducing the water content in the wood. This prevents the growth and formation of fungi (Koski, 2008).

When it was discovered that the compound CCA, which contains arsenic instead of boron in wood preservatives, was harmful to both humans and other living organisms, its use was banned in many countries. Although arsenic is no longer used, other chemicals used are as toxic as arsenic. Some of the wood preservatives currently on the market have been coded, and their environmental impacts have been examined based on their ingredients. The

preservatives examined are preferred in today's markets in countries such as the United States, the United Kingdom, and Ireland. Table 2 lists the toxic ingredients and potential hazards of preservatives currently used as wood preservatives (Legislation UK, 2023). The According to a study, arsenic used in zoo fencing has been detected in crocodile eggs, animal feed, soil contact areas, the feathers of four endemic birds, and on monkeys and porcupines. It has also been found in 43 endangered bird species and mammals. According to the United States Environmental Protection Agency (USEPA), this arsenic level is high; the arsenic level found in monkey hair alone is 30 times higher than the reference value (Gress et al., 2016).

Generally Today, borax and boric acid, among the boron minerals, are frequently preferred. Boron, which does not corrode metals, is also highly effective against biological organisms that damage wood. Boron applied to wood can be easily washed off. To prevent boron from leaking, boron minerals are mixed with petroleum. The resulting organic boron compounds increase in cost and flammability due to the use of petroleum Therefore, organic boron compounds are not frequently preferred today. (Hülagü, 2021).

The factors that cause wood deterioration mainly consist of physical factors (UV, acid rain, wind abrasion, etc.), mechanical factors (wear and tear, etc.), chemical factors (fires, corrosion, etc.), and biological factors (bacteria, fungi, termites, and marine organisms, etc.). Experts have wondered how wooden structures built centuries ago have survived to the present day despite being threatened by many negative factors. The Horyuji Temple in Japan, considered the oldest wooden structure in the world (Cartwright, 2017). The study aimed to achieve a more hygienic structure and to use this structure in larger areas by impregnating wood material with an organic product, mussel shell extract, using a short-term immersion method.

2. MATERIAL AND METHOD

Within the scope of the study, beech wood was preferred from the wood species of our country, the samples were turned into slats, and then prepared from sapwood pieces by cutting in the radial direction according to TS ISO 3129 principles.

2.1. Experimental Sample Preparation

While preparing the samples, the wood was prepared according to (TS 2470-2471) from sapwood that does not show any irregularity in the fibrous structure, cracks, knots, tulle formation and discoloration, and was cut to 20x20x300±1 mm/ 20x20x30±1 mm dimensions in accordance with TS 2477 principles for shock strength tests and wood density . 1%, 3% and 5% solutions were prepared from the extract produced from mussel shells, a material of natural origin.

2.2. Impregnation

The impregnation process was completed in a laboratory environment using the immersion method. Completely dry test samples were immersed in an environmentally friendly preservative solution produced from mussel shells for 30 minutes respectively (Var, 1994).

2.3. Extract Preparation

The Mussel shells, a natural material, were ground into powder using a ring mill. The powders were passed through a 63-micron sieve before being used in solution preparation to ensure uniform particle size. Solutions, each 100 ml in volume, were prepared from the sieved mussel powders at concentrations of 1%, 3%, and 5%, respectively. The weighed samples were transferred to 200-ml beakers and dissolved on a magnetic stirrer. Approximately 15–20 ml of 32% ultrapure HCl and 10 ml of distilled water were added to the beakers for dissolution (Ceylan, 2020).

3. FINDINGS AND DISCUSSION

3.1. Extract Properties

Solution properties are given in Table 1.

Table 1. Properties (Extract)

Plant extract (Moss)	Solvent	Degree (°C)	%	pH		Density (g/ml)	
				EÖ	ES	EÖ	ES
Mussel Shell	Water	22°C	1%	1.35	1.35	0.985	0.985
			3%	1.35	1.35	0.985	0.985
			5%	1.06	1.06	1.003	1.003

BI: Before impregnation AI: After impregnation

There was no change in pH and density.

3.2. % Retention

The amount of material retention to wood material is given in Table 2.

Table 2. Retention (%)

Wood Type	Concentration %	Impregnation Material	Retention (%)	HG
Beech Wood	1%	Mussel Shell	0.85	C
	3%		0.64	B
	5%		0.76	A

The highest retention was observed at 1% concentration (0.85 %) in mussel shell extract, while the lowest retention was observed at 3% concentration (0.64 %). The variation in retention may be due to concentration, anatomical structure of the wood, wood type, impregnation method, springwood/summerwood ratio, texture, and impregnation time.

3.3. Air Dry Specific Density (g/cm³)

Air Dry Specific Density are given in Table 3 and Figure 1.

Table 3. Air Dry Specific Density (g/cm³)

Wood Type	Impregnation Material (%)	Density (g/cm ³)	HG
Beech Wood	Control	0.65	B
	1%	0.63	C
	3%	0.60	D
	5%	0.67	A

The highest air-dry specific gravity value was determined at a 5% concentration (0.67 g/cm³), while the lowest retention was determined at a 3% concentration (0.60 g/cm³). This may be due to the anatomical structure of the wood.

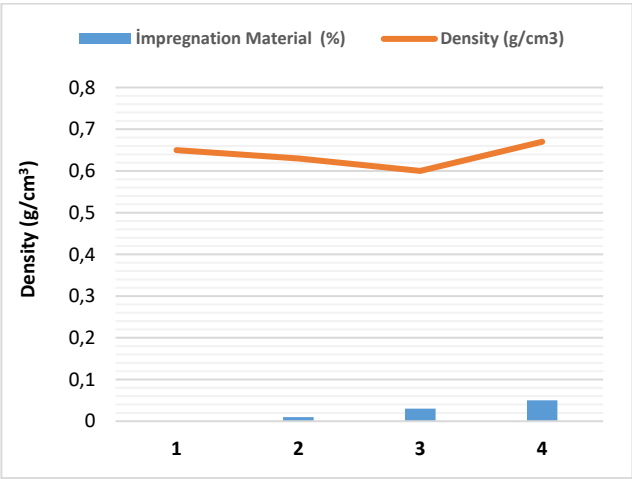


Figure 1. Change of Density

3.3. Dynamic Bending (shock) Strength (kpm/mm²)

Dynamic bending strength change are given in Table 2.

Table 3. Dynamic Bending Strength (Kgm/cm²)

Wood Type	İmpregnation Material (%)	Dynamic Bending Strength	HG
Beech Wood	Control	0.79	D
	1%	0.82	A
	3%	0.80	B
	5%	0.78	C

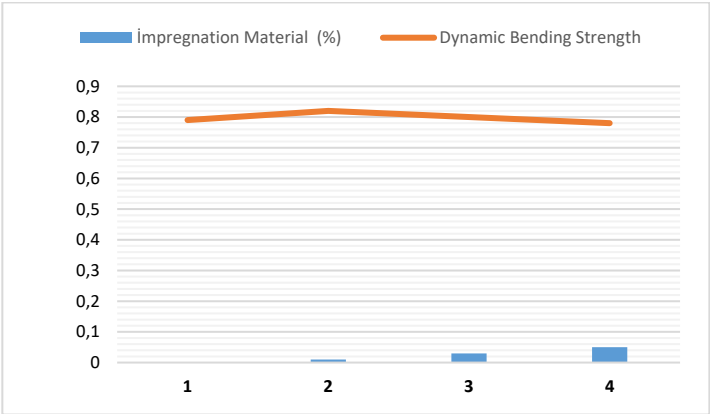


Figure 2. Change of Dynamic Bending Strength

The highest dynamic bending strength was found in the control sample at 1% concentration (0.81 kgm/cm^2), while the lowest dynamic bending (shock) strength was found in the control sample (0.79 kgm/cm^2). A partial increase was found compared to the control sample.

4. CONCLUSION

Mussel shell-based natural impregnation can be successfully used in wood preservation as an environmentally friendly and sustainable alternative. When used with suitable species, particularly beech, it extends the wood's biological life and increases its strength. Furthermore, the use of mussel shell powder in different solutions, the effect of application temperatures, long-term aging tests, the performance of impregnation effects over time through biological resistance analyses, and comparative studies with vacuum-pressure systems are recommended. While wooden products are becoming increasingly prevalent in our lives, they also raise questions about the hygienic safety of wooden surfaces. Wood has two distinct antibacterial properties: passive and active. The passive antibacterial property stems from wood's inherent porous structure and hygroscopicity. However, wood's active antibacterial properties are related to the extractive substances it contains.

5. REFERENCES

- Hülagü, B.,Seçkin, N., (2023). Geçmişten günümüze kullanılan ahşap koruyucuların çevresel etkileri. bab Journal of FSMVU Faculty of Architecture and Design. 4 (1),17-31
- Munir, M. T., Pailhoriès, H., Aviat, F., Lepelletier, D., Pape, P. L., Dubreil, L., Irle, M., Buchner, J., Eveillard, M., Federighi, M., Belloncle, C. (2021). Hygienic perspectives of wood in Healthcare buildings. *Hygiene* 1(1): 12-23.
- Koski, A., (2008). Applicability of crude tall oil for wood protection, Doktora Tezi, University of Oulu.
- Legislation Uk, (2023). Regulation (EC) no 1272/2008 of the European Parliament and of the Council
- Gress, J., Da Silva, E. B., De Oliveira, L. M., Zhao, D., Anderson, G., Heard, D., Stuchal, L. D. Ve Ma, L. Q. (2016). Potential arsenic exposures in 25 species of zoo animals living in CCA-wood enclosures. *Science of the Total Environment*. (551552), 614-621.
- Hülagü, B., (2021). Ahşap yapı malzemesinin korunmasında çevreci yaklaşım: Bitkisel yağların kullanılabilirliği, Yüksek Lisans Tezi, Mimar Sinan Güzel Sanatlar Üniversitesi, İstanbul.
- Cartwright, M. (2017) Horyuji - World History Encyclopedia
- ISO 3129 (2019) Wood-Sampling Methods And General Requirements For Physical And Mechanical Testing Of Small Clear Wood Specimens.
- TS 2470 (1976) Odunda Fiziksel ve Mekaniksel Deneyler İçin Numune Alma Metotları ve Genel Özellikler, Ankara.
- TS 2471 (1976) Odunda Fiziksel ve Mekaniksel Deneyler İçin Rutubet Miktarı Tayini, Ankara.
- TS 2477 (1976) Odunun çarpmada eğilme dayanımının tayini, TSE, Ankara.
- Var, A.A. (1994)Doğal reçine (Kolofan) kullanımının ağaç malzemenin su itici özellikleri üzerine etkisi, KTÜ, Yüksek Lisans Tezi. Trabzon
- Ceylan, Ş. (2020). Işgın Bitki (Antioksidan/antibakteriyel) Özütünün Ahşap Endüstrisinde (Mobilya/İnşaat) Kullanılabilme Olanakları, Artvin Çoruh Üniversitesi Bilimsel Araştırmalar Projesi, Proje No: 2017.M82.02.02, Artvin

CHAPTER 9

EFFECT OF VARIOUS ORGANIC BASED MATERIALS ON THE COMPRESSIVE RESISTANCE OF WOOD MATERIAL

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1. INTRODUCTION

The seriousness of global warming and the irreversible depletion of resources affect not only humans but all living things. While preservatives used to protect wood extend its lifespan, they also cause environmental damage. Therefore, chemical mixtures proven to be environmentally harmful have been banned in many countries. However, these harmful substances are present in diluted forms in compounds and are presented as relatively harmless. Spillage of preservatives during transportation, their migration into the soil upon contact with the soil, their leaching from wood in hot weather, and their contamination with groundwater when washed pose serious ecological risks. Cleaning groundwater, especially that used for drinking, is quite costly. Many countries have reported the contamination of groundwater resources due to preservatives (Thomasson et al., 2015).

Beyond design preservation, humanity has tried various methods to preserve wood for centuries. Around 5000 BC, people knew that termite- and fungus-resistant wood should be used in tombs. Many sacred books describe Noah building his ark from sheet metal or ebony, drying it first and then tarring it (Köksal, 2016). It is known that the Chinese tried to preserve wood with seawater or salt water. Another historical preservation method is charring wood by partially burning it. The wooden poles found in the floor of the Temple of Diana in Ephesus are an example of this. Around 500 BC, the ancient Greeks drilled holes in wood and infused it with cedar and olive oil. The Romans took precautions against fire by wrapping their wooden structures with aluminum (Bozkurt et al., 1993). The problem of contamination (contamination) of harmful microorganisms from the surfaces of materials used in hygienically sensitive areas such as healthcare areas and food processing facilities is a daily concern. In this context, surface hygiene is an important element in preventing environmental contamination and infection. (Munir et al., 2019a).

Foodborne illnesses, a global problem, have negative impacts on environmental development and health. Consumers are increasingly demanding natural preservatives as alternatives to toxic chemical compounds. Plant extracts containing antimicrobial compounds should be thoroughly characterized for their potential to serve as biocontrol or biopreservatives. Comprehensive research focusing on plant extracts with antimicrobial properties for use in the prevention and control of foodborne pathogens is

needed (Al-Huqail et al., 2019). The use of wood species lacking antibacterial properties in hygienically sensitive areas increases the risk of contamination. Hygienically sensitive areas include wooden materials used in public areas (stair handles, door handles, etc.), food-contact wooden products, wooden toys, and wooden animal shelters. In such areas, the use of wood materials with high extractive content is not always feasible. Furthermore, it has been observed that some woods commonly used in the market have low or no active antibacterial properties (Tomičić et al., 2020).

The factors that cause wood deterioration mainly consist of physical factors (UV, acid rain, wind abrasion, etc.), mechanical factors (wear and tear, etc.), chemical factors (fires, corrosion, etc.), and biological factors (bacteria, fungi, termites, and marine organisms, etc.). Experts have wondered how wooden structures built centuries ago have survived to the present day despite being threatened by many negative factors. The Horyuji Temple in Japan, considered the oldest wooden structure in the world (Cartwright et al., 2017). In the study, the use of laurel plant extract, which is an organic product and obtained from the natural resources of our country, was determined after determining various technological properties of the wood material in order to obtain a hygienic structure by impregnating it into the wood material using the vacuum method and to use this structure in larger areas.

2. MATERIAL AND METHOD

Within the scope of the study, beech wood was preferred from the wood species of our country, the samples were turned into slats, and then prepared from sapwood pieces by cutting in the radial direction according to TS ISO 3129 principles.

2.1. Experimental Sample Preparation

While preparing the samples, sapwood without cracks, knots, and showing no tulle formation or color change was prepared in accordance with (TS 2470-2471, TS 2595) and cut into dimensions of $20 \times 20 \times 30 \pm 1$ mm. The extract obtained from bay leaf, a medicinal aromatic plant species, was prepared at concentrations of 2%, 4% and 6% in the studies.

2.2. Impregnation

Impregnation was carried out according to ASTM–D 1413-76 principles. It was subjected to 30 minutes of vacuum and 30 minutes of diffusion.

2.3. Extract Preparation

The powdered bay leaf was weighed at 10.00 g, and the extraction process was carried out in water at room temperature for 24 hours using a shaker. It was then filtered through filter paper (filtration) to a certain volumetric volume. It was then filtered using filter paper and topped up with water to make the final volume 5 L. Medicinal and aromatic plant species were obtained from Köyceğiz/Muğla dissolution (Ceylan, 2020).

3. FINDINGS AND DISCUSSION

3.1. Extract Properties

Solution properties are given in Table 1.

Table 1. Properties (Extract)

Plant extract (Moss)	Solvent	Degree (°C)	%	pH		Density (g/ml)	
				EÖ	ES	EÖ	ES
Mussel Shell	Water	22°C	2%	5.93	5.94	0.925	0.928
			4%	5.81	5.81	0.934	0.934
			6%	5.78	5.78	0.925	0.929

BI: Before impregnation AI: After impregnation

There is no significant change in density or pH. This may be due to working with fresh solution.density.

3.2. % Retention

The amount of material retention to wood material is given in Table 2.

Table 2. Retention (%)

Wood Type	Concentration %	İmpregnation Material	Retention (%)	HG
Beech Wood	2%	Laurel	1.73	A
	4%		1.56	C
	6%		1.68	B

The highest retention was observed at 6% concentration (1.68 %) in laurel extract, while the lowest retention was observed at 4% concentration (1.56 %). The variation in retention may be due to concentration, anatomical structure of the wood, wood type, impregnation method, springwood/summerwood ratio, texture, and impregnation time.

3.3. Air Dry Specific Density (g/cm³)

Air dry specific density are given in Table 3.

Table 3. Air Dry Specific Density (g/cm³)

Wood Type	İmpregnation Material (%)	Density (g/cm³)	HG
Beech Wood	Control	0.65	D
	2%	0.72	B
	4%	0.75	A
	6%	0.68	C

The highest air-dry specific gravity value was determined at a 4% concentration (0.75 g/cm³), while the lowest retention was determined at a 6% concentration (0.68 g/cm³). This may be due to wood type, impregnation method, concentration and anatomical structure.

3.3. Pressure Strength (N/mm²)

Pressure strength change are given in Table 4, Figure 1.

Table 4. Pressure Resistance (N/mm²)

Wood Type	İmpregnation Material (%)	Pressure strength	HG
Beech Wood	Control	72.54	C
	2%	77.65	A
	4%	74.56	B
	6%	69.79	D

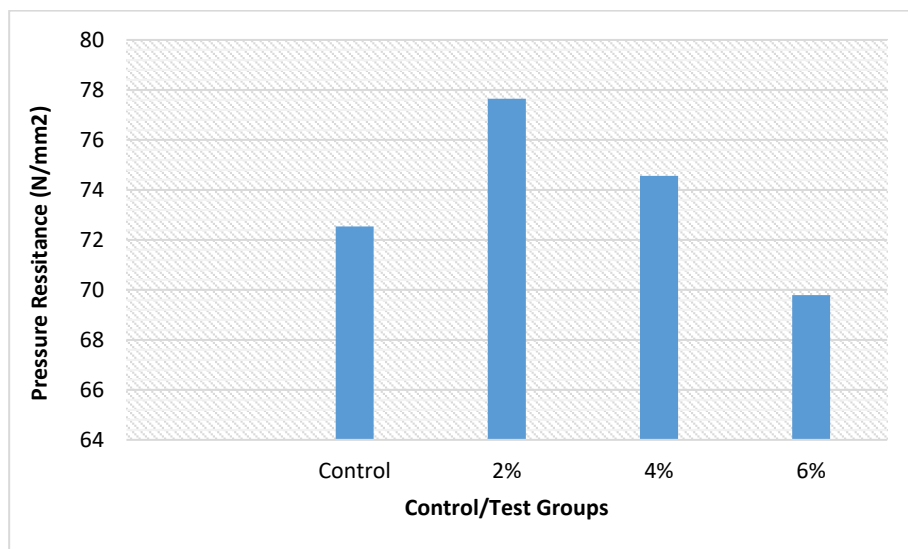


Figure 1. Change of Pressure Strength

The highest compressive strength was determined at 4% bay leaf extract (77.65 N/mm²), while the lowest was determined at 6% concentration (69.79 N/mm²). Compressive strength, bending strength, and shock resistance are particularly important in spaces requiring strength. This mechanic is crucial against earthquake effects.

4. CONCLUSION

Vacuum impregnation, in particular, has been preferred for the impregnation method, and methods such as brushing, pressure, and vacuuming can be used. Concentrations can be increased, and solvents (ethanol, methanol extracts, ether, acetone, and water) can be tried. Impregnation processes can be carried out with different plants and preservatives. Plant extracts obtained from different plants are attracting considerable interest both in academic circles and in the food, cosmetics, and pharmaceutical industries. The increasing interest in natural products derived from plants stems from their potential use as natural additives and the increasing tendency to replace synthetic preservatives with natural ones. Uncovering the unknown bioactive properties of plant species is crucial in this regard. The anticancer and anti-inflammatory biological activities of bay leaves can be investigated.

REFERENCES

- Hülagü, Thomasson, G., Capızzi, J., Dost, F., Morrell, J., Miller, D. (2015). Wood preservation and wood products treatment: Training manual.
- Köksal, M. A. (2016). *Peygamberler tarihi*. Ankara: Diyanet Vakıf Yayınları
- Bozkurt, A., Göker, Y., Erdin, N. (1993) Emprenye Tekniği. İstanbul: İstanbul Üniversitesi.
- Munir, M. T., Pailhories, H., Eveillard, M., Aviat, F., Lepelletier, D., Belloncle, C., Federighi, M. (2019) Antimicrobial characteristics of untreated wood: Towards a hygienic environment. *Health* 11(02): 152-170.
- Munir, M. T., Aviat, F., Pailhories, H., Eveillard, M., Irle, M., Federighi, M., Belloncle, C. (2019) Direct screening method to assess antimicrobial behavior of untreated wood. *European Journal of Wood and Wood Products*, 77(2): 319-322
- Al-Huqail, A. A., Behiry, S. I., Salem, M. Z., Ali, H. M., Siddiqui, M. H., Salem, A. Z. (2019) Antifungal, antibacterial, and antioxidant activities of *Acacia saligna* (Labill.) HL Wendl. flower extract: HPLC analysis of phenolic and flavonoid compounds. *Molecules* 24(4): 700.
- Tomičić, R., Tomićić, Z., Thaler, N., Humar, M., Raspor, P. (2020) Factors influencing adhesion of bacteria *Escherichia coli*, *Pseudomonas aeruginosa*, *Staphylococcus aureus* and yeast *Pichia membranifaciens* to wooden surfaces. *Wood Science and Technology* 54(6):1663-1676.
- Cartwright, N., Horn, D.P. (2017) Hydrology of the Coastal Zone, in: Finkl, C.W., Makowski, C. (Eds.), *Encyclopedia of Coastal Science*. Springer International Publishing, Cham, pp. 1-12.
- ISO 3129 (2019) Wood-Sampling Methods And General Requirements For Physical And Mechanical Testing Of Small Clear Wood Specimens.
- ASTM D 1413-76 (1984) Standard Methods of Testing Preservatives by Laboratory Soilblock Cultures, *Annual Book of ASTM Standards*, USA Wood
- TS 2470 (1976) Odunda Fiziksel ve Mekaniksel Deneyler İçin Numune Alma Metotları ve Genel Özellikler, Ankara.
- TS 2471 (1976) Odunda Fiziksel ve Mekaniksel Deneyler İçin Rutubet Miktarı Tayini, Ankara.
- TS 2595 (1977) Odunun Liflere Paralel Basınç Dayanımının Tayini, Ankara.

Ceylan, Ş. (2020) Işgın Bitki (Antioksidan/antibakteriyel) Özütünün Ahşap Endüstrisinde (Mobilya/İnşaat) Kullanılabilme Olanakları, Artvin Çoruh Üniversitesi Bilimsel Araştırmalar Projesi, Proje No: 2017.M82.02.02, Artvin

CHAPTER 10

AN ANALYSIS OF THE RELATIONSHIP BETWEEN RED MEAT PRODUCTION AND PRICES IN TURKEY USING THE ALMON MODEL

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1. INTRODUCTION

The rapidly increasing global population and the scarcity of natural resources have further underscored the growing importance of adequate and balanced nutrition for the protection and improvement of public health. Ensuring a healthy diet depends on obtaining essential food nutrients from both plant-based and animal-based sources. This situation directly affects the effectiveness of agricultural and livestock policies in countries, making it necessary to optimize individuals' access to essential food products in terms of both quantity and cost. Animal-derived foods stand out due to their high-quality proteins and essential amino acids. In particular, red meat contains protein, omega-3, iron, zinc and vitamin B12 (Ekmekcioglu et al., 2018). Research indicates that an adult should consume approximately 70 grams of protein per day, with around 40% of this amount ideally obtained from animal-based sources. In this context, red meat consumption is vital not only for human health and growth and development processes but also for providing the eight essential amino acids that are critical for brain development.

With the continuous growth of the world population, the strategic importance of food resources has been steadily increasing. This is because per capita food consumption is widely recognized as one of the fundamental indicators of a country's level of development. In developed countries, measures aimed at meeting the nutritional needs of a growing population in a balanced and healthy manner are considered among the primary policy priorities, with particular emphasis on increasing the share of animal-based proteins in total consumption. In Turkey, where the population is rising rapidly, the increase in per capita income has also led to a growing demand for animal products. To meet this rising demand, it has become essential to further develop the livestock sector and to enhance productivity per animal.

The livestock sector in Turkey occupies a strategic position in terms of both its economic contributions and its social impacts. This sector, which plays a vital role in human nutrition, ranks second in the country's agricultural production after crop farming and serves as a critical sub-sector in meeting society's demand for animal-derived protein. At the same time, it fulfills a fundamental function in satisfying the growing demand for milk and meat. Beyond addressing individuals' nutritional needs, the livestock industry makes significant contributions to the national economy by providing raw materials

for the manufacturing sector, supporting subsidiary industries, and creating extensive employment opportunities. Within this context, the red meat sector stands out as a priority area in terms of both production volume and nutritional significance. According to TURKSTAT data, red meat production and the number of slaughtered animals in Turkey have followed a fluctuating pattern but displayed an overall upward trend in the long term. In 2004, cattle slaughter reached 2.6 million head, yielding 488.6 thousand tons of beef, while sheep slaughter amounted to 10.7 million head, with 190.1 thousand tons of mutton produced. Goat and buffalo meat production stood at 52.5 thousand tons and 4.9 thousand tons, respectively, highlighting the dominant role of beef and mutton in total red meat output. In the early period, total production decreased by 2.6% in 2004, increased slightly by 0.2% in 2005, and showed a limited rise in 2006. During this time, beef production experienced a modest increase, whereas small ruminant production declined, largely due to drought and rising feed costs that constrained output. From 2007 to 2009, production recovered, with total red meat output growing by 5.5%, 4.1%, and 2.2%, respectively. After 2010, the sector entered a phase of rapid growth; total production rose by 3.9% in 2010, 10.2% in 2011, and 10.1% in 2012. This expansion was directly linked to the increase in cattle stock, import restrictions on meat, and the implementation of producer incentives. Growth in small ruminant production was also recorded, with mutton and goat meat output reaching 236.2 thousand tons and 59.5 thousand tons, respectively, by 2013. The expansion phase continued after 2015: cattle slaughter increased from 3.7 million to 4.86 million head, and beef production rose from 862 thousand tons to 1.33 million tons. Small ruminant production also increased, with mutton output up by 27% and goat meat by 25%. The growth persisted through 2020-2021, reaching 1.46 million tons of beef, 385.9 thousand tons of mutton, and 94.6 thousand tons of goat meat in 2021. In 2022-2023, red meat production reached record levels: in 2023, cattle slaughter totaled 5.81 million head, with 1.67 million tons of beef produced, while mutton and goat meat output stood at 569 thousand tons and 129 thousand tons, respectively. However, 2024 data indicate a significant decline in red meat production. Cattle slaughter dropped to 5.08 million head, and beef production fell to 1.48 million tons, while mutton, goat, and buffalo meat production decreased by 10.5%, 22.8%, and 10.4%, respectively. This decline reflects the impact of rising production costs, feed price fluctuations,

and shrinking producer incomes on the sector. In this context, enhancing livestock production particularly red meat output is crucial not only for food security but also for achieving broader economic and social development objectives in Turkey.

The main objective of this study is to analyze the dynamic relationship between red meat production and prices in Turkey by employing the Almon Distributed Lag Model using annual data for the period 1994-2024. In this context, the study is structured into six main sections. Following the introduction, the second section provides a detailed examination of red meat production in Turkey over time through descriptive tables, focusing on variables such as the number of live animals, the number of slaughtered animals, the amount of meat produced, and the rates of change in red meat production. The third section is devoted to a review of the relevant literature, presenting previous studies conducted on the subject. The fourth section outlines the data and methodology employed in the analysis. In the fifth section, the empirical analysis is conducted, and the findings are discussed in detail. Finally, the sixth section presents the overall conclusions of the study, summarizing the key results and offering a general evaluation.

2. ANALYSIS OF RED MEAT PRODUCTION IN TURKEY BY PERIOD

In Turkey, red meat production, as one of the most strategic components of the agricultural production structure and food supply security, has been directly influenced by both structural transformations and macroeconomic fluctuations during the period 2004-2024. Although overall production volumes generally exhibited an upward trend throughout this period, notable fluctuations occurred across different years. These variations were shaped not only by changes in livestock policies, input and feed costs, climatic conditions, and import regulations, but also by the combined effects of these external factors.

The data presented in Table 1 illustrate the annual changes in cattle, buffalo, sheep, and goat populations in Turkey over the same period. This information provides an important perspective for analyzing the structural evolution of the livestock sector, shifts in production dynamics, and the cyclical nature of sectoral growth. In 2004, the total livestock population amounted to

41.98 million head, with sheep (25.2 million) and cattle (10.07 million) representing the dominant components of production. Goat and buffalo populations were comparatively smaller, at 6.6 million and 103.9 thousand head, respectively. Between 2004 and 2009, the total livestock population declined significantly, reaching 37.69 million head. This reduction can be associated with structural disruptions in the agricultural sector during the mid-2000s, a declining rural population, rising feed costs, and the diminished role of small ruminant farming. In particular, the 2008 global economic crisis increased agricultural input costs, negatively affecting producer behavior and making it more difficult for small-scale farms to sustain their operations.

Table 1: Number of Live Animals in Turkey by Year (2004-2024, Heads)

Years	Cattle	Buffalo	Sheep	Goat	Total
2004	10.069.346	103.900	25.201.155	6.609.937	41.984.338
2005	10.526.440	104.965	25.304.325	6.517.464	42.453.194
2006	10.871.364	100.516	25.616.912	6.643.294	43.232.086
2007	11.036.753	84.705	25.462.293	6.286.358	42.870.109
2008	10.859.942	86.297	23.974.591	5.593.561	40.514.391
2009	10.723.958	87.207	21.749.508	5.128.285	37.688.958
2010	11.369.800	84.726	23.089.691	6.293.233	40.837.450
2011	12.386.337	97.632	25.031.565	7.277.953	44.793.487
2012	13.914.912	107.435	27.425.233	8.357.286	49.804.866
2013	14.415.257	117.591	29.284.247	9.225.548	53.042.643
2014	14.223.109	122.114	31.140.244	10.344.936	55.830.403
2015	13.994.071	133.766	31.507.934	10.416.166	56.051.937
2016	14.080.155	142.073	30.983.933	10.345.299	55.551.460
2017	15.943.586	161.439	33.677.636	10.634.672	60.417.333
2018	17.042.506	178.397	35.194.972	10.922.427	63.338.302
2019	17.688.139	184.192	37.276.050	11.205.429	66.353.810
2020	17.965.482	192.489	42.126.781	11.985.845	72.270.597
2021	17.850.543	185.574	45.177.690	12.341.514	75.555.321
2022	16.851.956	171.835	44.687.888	11.577.862	73.289.541
2023	16.421.256	161.749	42.060.470	10.302.940	68.946.415
2024	16.824.208	162.051	44.080.584	10.822.084	71.888.927

Source: TURKSTAT, (2025a).

From 2010 onwards, the livestock sector in Turkey exhibited a clear recovery trend. During this period, the cattle population rose to 11.37 million, reaching 12.39 million in 2011, representing roughly a 10% increase in a short span. This growth reflects the impact of government interventions, including livestock support programs, the importation of breeding animals, and credit incentives. Sheep and goat populations also showed upward trends during the same period, with the total number of live animals reaching 55.83 million by

2014, marking a notable increase compared to previous years. Between 2015 and 2017, the expansion continued across the sector. The cattle population rose to 15.94 million, and the total livestock population reached 60.42 million. This growth mirrored the expansion in red meat supply and increased milk production capacity. After 2018, the upward trend in livestock numbers accelerated, and by 2021 the total population had reached 75.55 million, representing the highest level in the past two decades. Notably, the sheep population exceeded 45 million, indicating a resurgence in small ruminant farming and greater diversity in meat supply. However, a decline in total livestock numbers was observed in 2022 and 2023. By 2023, the total had fallen to 68.95 million, primarily due to rising feed and energy costs, increases in input prices driven by exchange rate fluctuations, and narrowing producer profit margins. This situation highlights how unsustainable cost increases have undermined the stability of the livestock sector. By 2024, a modest recovery became evident. The cattle population reached 16.82 million, sheep 44.08 million, and goats 10.82 million, bringing the total livestock count to 71.89 million. This development suggests that the sector entered a rebalancing phase following the contraction experienced in 2023.

Table 2 presents the number of cattle, buffalo, sheep, and goats slaughtered in Turkey from 2004 to 2024, along with the corresponding meat output for each species. The table provides a clear view of how the structure of red meat production has evolved, illustrating species-specific production patterns and the overall transformation of supply over time. In 2004, approximately 2.6 million cattle were slaughtered, yielding 488.6 thousand tons of beef. In the same year, 10.7 million sheep were slaughtered, resulting in 190.1 thousand tons of meat. The share of goat and buffalo meat was relatively small, at 52.5 thousand tons and 4.9 thousand tons, respectively. These figures indicate that in the early 2000s, beef and sheep meat dominated Turkey’s red meat production, while buffalo and goat meat played a secondary role.

Table 2: Number of Slaughtered Animals and Meat Production Amount (2004-2024)

Years	Cattle		Buffalo		Sheep		Goat	
	Number of Slaughtered Animals (Heads)	Meat Production Amount (Tons)	Number of Slaughtered Animals (Heads)	Meat Production Amount (Tons)	Number of Slaughtered Animals (Heads)	Meat Production Amount (Tons)	Number of Slaughtered Animals (Heads)	Meat Production Amount (Tons)
2004	2.599.490	488.556	27.586	4.952	10.725.580	190.105	2.837.242	52.460
2005	2.558.207	491.560	25.060	4.629	10.731.398	190.539	2.801.617	50.492
2006	2.620.559	514.042	23.867	4.442	10.704.436	187.236	2.748.395	48.906
2007	2.713.989	549.513	22.916	4.347	10.792.554	191.428	2.755.379	50.712
2008	2.797.426	581.497	21.494	4.128	10.826.034	192.647	2.706.994	50.254
2009	2.826.190	608.183	20.111	4.019	10.477.951	188.496	2.487.218	46.240
2010	2.932.054	647.067	19.126	3.785	9.691.041	186.121	2.244.760	42.846
2011	3.126.378	710.652	19.127	3.780	10.700.807	210.171	2.391.246	44.840
2012	3.421.960	790.034	19.967	4.027	10.755.777	220.359	2.841.307	53.133
2013	3.457.477	798.784	21.465	4.580	11.194.725	236.186	3.273.444	59.532
2014	3.525.209	815.674	23.899	5.004	11.991.640	238.670	3.681.199	63.711
2015	3.706.346	862.098	25.713	5.300	12.808.697	249.863	4.097.340	69.757
2016	3.993.893	956.180	27.663	5.470	13.277.503	266.675	4.346.611	75.322
2017	4.334.034	1.093.841	29.476	5.868	13.244.903	262.825	4.346.713	77.794
2018	4.844.711	1.281.234	32.389	6.515	14.133.170	291.179	4.392.427	82.839
2019	4.856.517	1.330.169	35.695	7.150	14.546.576	316.170	4.513.264	87.126
2020	4.812.902	1.341.446	40.929	8.424	15.801.021	345.639	4.692.010	90.443
2021	5.134.441	1.460.719	51.925	10.831	17.125.163	385.933	4.907.371	94.555
2022	5.480.489	1.572.747	62.285	13.586	21.563.828	489.354	6.112.179	115.938
2023	5.811.698	1.670.606	69.597	15.386	25.437.813	569.066	6.753.478	128.989
2024	5.077.812	1.483.042	59.965	13.781	22.573.686	509.539	5.117.030	99.532

Source: TURKSTAT, (2025a,b).

Between 2004 and 2009, there was a steady increase in both cattle slaughter numbers and beef production in Turkey. By 2009, cattle slaughter reached 2.83 million heads, with beef output rising to 608.2 thousand tons. In contrast, sheep and goat slaughter declined noticeably during this period, reflecting the decreasing economic appeal of small ruminant farming and structural changes in rural production. Starting in 2010, the red meat sector entered a period of structural expansion. Cattle slaughter was recorded at 2.93 million heads, producing 647 thousand tons of beef. In 2011 and 2012, meat production continued to rise, reaching 710.6 thousand tons and 790 thousand tons, respectively. This growth was mainly driven by the expansion of the cattle population, restrictions on meat imports, and government incentives supporting domestic production. During the same period, slaughter numbers for small ruminants also increased, with sheep meat reaching 236.2 thousand tons and goat meat 59.5 thousand tons by 2013. The period from 2015 to 2019 stands out as a time of rapid growth in Turkey’s red meat production. Cattle slaughter rose from 3.7 million in 2015 to 4.86 million in 2019, while beef production jumped from 862 thousand tons to 1.33 million tons, an increase of approximately 55%. Production of small ruminant meat also increased, with sheep meat rising from 249.9 thousand tons to 316.2 thousand tons, and goat

meat from 69.7 thousand tons to 87.1 thousand tons. This trend reflects a combination of rising population, changing consumption habits, and strong demand for meat. Between 2020 and 2021, production continued to grow. By 2021, beef production had reached 1.46 million tons, sheep meat 385.9 thousand tons, and goat meat 94.6 thousand tons. This increase coincided with ongoing government support for livestock and expanded slaughtering capacity. However, rising cost inflation and higher feed prices posed challenges to sustaining this growth. In 2022 and 2023, Turkey's red meat production peaked. By 2023, cattle slaughter reached 5.81 million heads, producing 1.67 million tons of beef. Sheep and goat meat production also rose to 569 thousand tons and 129 thousand tons, respectively, marking record levels in the country's red meat sector. Nevertheless, data from 2024 indicate a notable decline. Cattle slaughter fell to 5.08 million heads, with beef production decreasing to 1.48 million tons. Sheep, goat, and buffalo meat production also declined. This downturn can be attributed to rising production costs, pressure on feed prices, and shrinking profit margins for producers.

Table 3 shows the change rates in red meat production, highlighting that the 2004-2006 period experienced modest but steady growth. In 2004, total output declined by 2.6% compared to the previous year, while a slight increase of 0.2% was observed in 2005. During this period, beef production trended slightly upward, whereas buffalo, sheep, and goat meat production declined. The reduction in small ruminant meat, in particular, reflects production constraints caused by drought and rising feed costs. The years 2007-2009 represented a phase of relative recovery. Total red meat production rose by 5.5% in 2007, 4.1% in 2008, and 2.2% in 2009. Beef production increased steadily, while the decline in sheep and goat meat slowed. This expansion in red meat supply helped temporarily stabilize prices. The 2010-2012 period stands out as a key phase of notable growth in Turkey's red meat sector. Total production grew by 3.9% in 2010, 10.2% in 2011, and 10.1% in 2012. This rapid increase was closely associated with post-2010 policies, including liberalization of meat imports, incentives for breeding animal imports, and fattening support programs. Notably, the double-digit growth in beef production was the primary driver of the overall rise in red meat output.

Table 3: Red Meat Production and Change Rates (2004-2024)

Years	Meat Production Amount (Tons)					Change Compared to the Previous Year (%)				
	Cattle	Buffalo	Sheep	Goat	Total	Cattle	Buffalo	Sheep	Goat	Total
2004	488.556	4.952	190.105	52.460	736.074	-0.2	-5.5	-7.0	-7.7	-2.6
2005	491.560	4.629	190.539	50.492	737.220	0.6	-6.5	0.2	-3.8	0.2
2006	514.042	4.442	187.236	48.906	754.625	4.6	-4.0	-1.7	-3.1	2.4
2007	549.513	4.347	191.428	50.712	796.000	6.9	-2.1	2.2	3.7	5.5
2008	581.497	4.128	192.647	50.254	828.527	5.8	-5.0	0.6	-0.9	4.1
2009	608.183	4.019	188.496	46.240	846.939	4.6	-2.7	-2.2	-8.0	2.2
2010	647.067	3.785	186.121	42.846	879.819	6.4	-5.8	-1.3	-7.3	3.9
2011	710.652	3.780	210.171	44.840	969.443	9.8	-0.1	12.9	4.7	10.2
2012	790.034	4.027	220.359	53.133	1,067.553	11.2	6.5	4.8	18.5	10.1
2013	798.784	4.580	236.186	59.532	1,099.081	1.1	13.7	7.2	12.0	3.0
2014	815.674	5.004	238.670	63.711	1,123.059	2.1	9.3	1.1	7.0	2.2
2015	862.098	5.300	249.863	69.757	1,187.018	5.7	5.9	4.7	9.5	5.7
2016	956.180	5.470	266.675	75.322	1,303.648	10.9	3.2	6.7	8.0	9.8
2017	1,093.841	5.868	262.825	77.794	1,440.327	14.4	7.3	-1.4	3.3	10.5
2018	1,281.234	6.515	291.179	82.839	1,661.767	17.1	11.0	10.8	6.5	15.4
2019	1,330.169	7.150	316.170	87.126	1,740.616	3.8	9.8	8.6	5.2	4.7
2020	1,341.446	8.424	345.639	90.443	1,785.952	0.8	17.8	9.3	3.8	2.6
2021	1,460.719	10.831	385.933	94.555	1,952.038	8.9	28.6	11.7	4.5	9.3
2022	1,572.747	13.586	489.354	115.938	2,191.625	7.7	25.4	26.8	22.6	12.3
2023	1,670.606	15.386	569.066	128.989	2,384.047	6.2	13.3	16.3	11.3	8.8
2024	1,483.042	13.781	509.539	99.532	2,105.895	-11.2	-10.4	-10.5	-22.8	-11.7

Source: TURKSTAT, (2025a,b).

The 2013-2016 period was marked by continued growth in red meat production, although the pace of increase gradually slowed. Total production rose by 3% in 2013, 2.2% in 2014, 5.7% in 2015, and 9.8% in 2016. The increases in 2015 and 2016 were largely driven by enhanced support for small ruminant livestock and relatively stable producer prices. Moreover, the notably higher growth rates in sheep and goat meat compared to the pre-2010 period strengthened the trend toward greater diversification in red meat supply. The 2017-2019 period stood out as a phase of rapid expansion. Total production grew by 10.5% in 2017, 15.4% in 2018, and 4.7% in 2019. In particular, beef production rose by 17.1% and sheep meat by 10.8% in 2018. These high growth rates reflect one of the strongest historical performances in Turkey’s red meat production. However, despite this increase, the expected stabilization in prices did not occur, as red meat prices continued to rise. This suggests that demand-side dynamics and rising costs exerted a stronger influence than production increases. The 2020-2022 period saw continued expansion in red meat production, although cost pressures intensified toward the end of the period. Production increased by 2.6% in 2020, 9.3% in 2021, and 12.3% in 2022. The year 2022 was particularly notable for high growth in sheep meat (26.8%) and goat meat (22.6%), indicating a strengthened focus on small ruminant livestock and increased species diversification in red meat production. However, rising

feed, energy, and transportation costs negatively affected producer profitability during this period. In 2023, total production continued to grow, rising by 8.8%, though the rate of increase slowed slightly. Beef production grew by 6.2%, buffalo meat by 13.3%, sheep meat by 16.3%, and goat meat by 11.3%. This year highlights continued strong growth in small ruminant production, while the growth in beef production showed a relative slowdown. By 2024, red meat production experienced a significant decline for the first time in many years. Total output fell by 11.7% compared to the previous year, with reductions observed across all species: beef (-11.2%), buffalo (-10.4%), sheep (-10.5%), and goat (-22.8%). This decline can be attributed to rising production costs and weakening consumer demand. Additionally, fluctuations in feed prices, drought conditions, and higher slaughtering costs adversely affected production.

Overall, the analysis of the 2004-2024 period indicates that Turkey's red meat production has followed a long-term upward trend, although this growth has been marked by instability. Periodic fluctuations in production growth rates have exerted pressure on red meat prices, creating vulnerabilities in the supply demand balance. In this context, it is possible to identify a structural tendency toward an inverse relationship between production volumes and price levels. This pattern suggests that the red meat market is sensitive both to supply-side production dynamics and to demand-side price elasticity. As a result, while the increases in red meat production have strengthened Turkey's long-term supply capacity, these gains have not consistently translated into price stability. Consequently, restructuring red meat production policies to mitigate price volatility, alongside supporting efficiency-based sustainable production models, is critical for ensuring the sector's stable growth.

3. LITERATURE REVIEW

Different modeling approaches have been developed to examine the interaction between the production volume of a good and its price level, with various methodologies proposed depending on whether lagged effects are considered finite or infinite. Among the models most frequently used in the literature, the Almon and Koyck models are particularly prominent (Koutsoyiannis, 1989: 296-320). In this study, the focus is placed exclusively on research applying the Almon model, enabling a more detailed examination of how lagged effects influence the production price relationship.

There are several studies in Turkey that examine the relationship between agricultural products and prices using the Almon model. For instance, Doğan, Gürler, and Ayyıldız (2014) analyzed the interaction between rice production and prices in Samsun province through the Almon polynomial technique. Utilizing data from the period 1993-2013, the study applied the Almon model, which considers not only the current period but also the effects of past periods on the explanatory variable in time series econometrics. The results indicate that rice production in Samsun is influenced by prices in periods t , $t-1$, and $t-2$. While current period (t) prices have a negative effect on production, prices from one and two periods earlier ($t-1$ and $t-2$) exert a positive impact. The study further notes that these findings are consistent with the Cobweb theorem in economic theory and suggests that producer associations, cooperatives, and government support should be effectively utilized in production planning. This approach is emphasized as a way to facilitate production forecasting and prevent imbalances between supply and demand. Çelik and Özbay (2015) analyzed the relationship between tomato production and prices over the period 1994-2013 using the Almon approach, one of the distributed lag models. According to the Almon model results, tomato production was influenced by prices up to five years back. The findings indicate that a 1 TL increase in the current year's tomato price raises production by 0.21 tons, while a 1 TL increase in the previous year raises it by 0.49 tons, two years prior by 0.52 tons, and three years prior by 0.30 tons. Conversely, a 1 TL increase in prices four years ago reduces production by 0.16 tons, and a similar increase five years ago decreases production by 0.86 tons. In summary, changes in tomato prices during the first, second, and third lagged periods had a positive effect on production, whereas changes in the fourth and fifth lagged periods exerted a negative impact. Köleoğlu and Çelik (2025) examined the relationship between maize production and maize prices in Turkey using data from 2000 to 2023. In their study, the dependent variable was the national maize production, while the independent variable was the maize price, and the relationship was analyzed using distributed lag models, specifically the Koyck and Almon approaches. The results indicate that maize production is most strongly influenced by prices over the previous three years. In the absence of any price changes, maize production was estimated at 6,194 tons. A 1 TL increase in the current year's price (t) raised production by 302,283.6 tons, while a 1 TL increase in the

previous year's price ($t-1$) increased production by 260,266.2 tons, and a 1 TL increase two years prior ($t-2$) led to an increase of 224,089.2 tons. Price increases in the third to sixth lagged periods ($t-3$, $t-4$, $t-5$, $t-6$) corresponded to production increases of 192,940.8; 166,122; 143,031.1; and 123,149.7 tons, respectively. These findings suggest that increases in lagged maize prices positively affect production, although the magnitude of this effect diminishes over time.

A review of the literature indicates that considerable research has been conducted on the relationship between production and prices in agricultural products. However, studies empirically analyzing the relationship between the production of animal-based products and their prices remain relatively limited. For instance, Çelik (2015a) examined the relationship between buffalo milk production and prices over the period 1994-2013 using distributed lag models, specifically the Koyck and Almon approaches. According to the Koyck model results, buffalo milk production is influenced by prices up to eight years in the past. It was estimated that it takes approximately 5.37 years for changes in buffalo milk prices to have a significant and noticeable effect on production. A 1 TL increase in current-year prices (t) raises production by 4,203 tons, while a 1 TL increase in the previous year's price ($t-1$) increases production by 3,543 tons, and a price increase two years prior ($t-2$) raises production by 2,987 tons. Price increases in earlier periods ($t-3$ to $t-8$) also lead to production increases of 2,518; 2,123; 1,789; 1,508; 1,272; and 1,072 tons, respectively. These findings suggest that changes in lagged buffalo milk prices have a diminishing positive impact on production over time. According to the Almon model, buffalo milk production is affected by prices in the current period (t) as well as the eight preceding periods ($t-1$ to $t-8$). While current-period prices positively influence production, prices in all lagged periods have a negative effect. Overall, the analysis indicates that the Koyck model provides a better fit than the Almon model in explaining the buffalo milk production price relationship. In a study conducted by Çelik (2015b), the relationship between sheep milk production and prices over the period 1994–2014 was analyzed using distributed lag models, specifically the Koyck and Almon approaches. According to the Koyck model results, sheep milk production is influenced by prices up to nine years in the past. It was found that it takes approximately 8.71 years for changes in sheep milk prices to have a significant and noticeable impact on production. A 1 TL

increase in the current year's price (t) raises production by 72,053.98 tons, while a 1 TL increase in the previous year's price ($t-1$) increases production by 64,632.41 tons, and a price increase two years prior ($t-2$) raises production by 57,975.28 tons. Price increases in the third to ninth lagged periods ($t-3$ to $t-9$) were found to increase production by 52,004; 46,647; 41,842; 37,533; 33,667; 30,199; and 27,089 tons, respectively. These findings indicate that changes in lagged sheep milk prices have a diminishing positive effect on production over time. According to the Almon model, sheep milk production is affected by prices in the current period (t) as well as the nine preceding periods ($t-1$ to $t-9$). While prices in the current period and the first four lagged periods ($t-1$ to $t-4$) negatively influence production, prices in the fifth through ninth periods ($t-5$ to $t-9$) have a positive effect. Overall, the study demonstrates that past prices play a significant role in shaping sheep milk production decisions and that the impact of lagged prices diminishes over time.

Studies examining the relationship between red meat production and prices using the Almon distributed lag model are relatively limited in the literature. For instance, Akgül and Yıldız (2016) analyzed this relationship in Çorum province over the period 1994-2014. In their study, red meat production in Çorum was treated as the dependent variable, while the national red meat price in Turkey served as the independent variable, and the analysis was conducted using the Almon distributed lag approach. The model was found to be statistically significant at the 10% level, indicating that red meat production is influenced by prices over the previous six years. The results revealed a positive relationship between production and prices from the current year (t) up to six years prior ($t-6$). In the absence of any price changes, red meat production was estimated at 8,713.03 tons. Furthermore, a one-unit increase in the current year's price (t) raised production by 26,832 tons, while price increases in the previous years led to progressively higher production: 113,476 tons ($t-1$), 200,120 tons ($t-2$), 260,880 tons ($t-3$), 321,640 tons ($t-4$), 373,772 tons ($t-5$), and 417,276 tons ($t-6$). These findings suggest that increases in lagged red meat prices positively affect production, with the impact becoming stronger over time. Overall, the study highlights the significant role of red meat prices in shaping production decisions under market-driven conditions.

Overall, a review of the literature indicates that the Almon model has been effectively employed to examine agricultural products, demonstrating that

lagged price effects play a decisive role in shaping production decisions. However, empirical studies focusing specifically on animal products, and red meat production in particular, remain limited. This gap underscores the need for a more comprehensive, nationally focused analysis of the relationship between production and prices in Turkey's red meat sector. Accordingly, the present study contributes to the literature by analyzing the link between red meat production and prices over the 1994-2024 period within the framework of the Almon model, thereby addressing this empirical gap.

4. MATERIALS AND METHODS

4.1. Materials

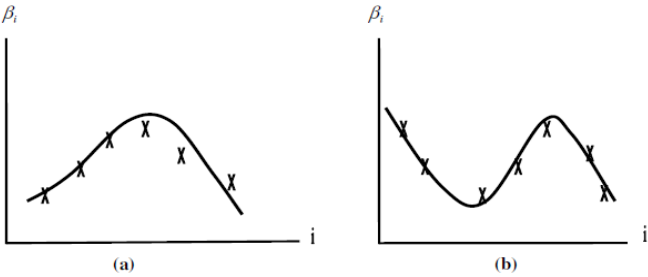
The Distributed Lag Almon Model was used to analyze the dynamic relationship between red meat production and prices in Turkey. In this context, red meat production volume was included in the model as the dependent variable, and red meat price as the independent variable. Red meat production calculations in Turkey cover the total production of cattle, buffalo, sheep, and goat meat. The analysis period covers the years 1994-2024, and red meat production and price data were obtained from the databases of the Turkish Statistical Institute (TURKSTAT, 2025b,c). In this framework, the model aims to empirically examine the lagged effects of price movements in the red meat market on production decisions.

4.2. Methods

In the economics literature, various econometric models have been developed to examine the relationship between the production quantity of a good and its price. Among these, lag-distributed models such as the Koyck and Almon models are the most commonly used (Koutsoyiannis, 1989: 296-320). Lag-distributed models are designed to incorporate not only the current value of the independent variable but also its past values (İşyar, 1999). If a finite number of lags is assigned to the independent variable, the model is referred to as a "*finite lag model*"; if no such restriction is imposed, it is called an "*infinite lag model*" (Kutlar, 2005: 205-207). The Almon model is a finite distributed lag model developed as an alternative to the Koyck model. One of the key assumptions of the Koyck model is that the coefficients (β_s) continuously decrease as the lag length increases. However, this assumption limits the model's flexibility, particularly when lag effects occur in a nonlinear manner.

The Almon model overcomes this limitation by allowing the β coefficients to first increase and then decrease or vice versa offering a more flexible structure (Cezayirli, 2007: 32).

Figure 1: Almon Polynomial Delay Diagram



Source: Adapted from Gujarati (2001: 613) and drawn by the authors.

Shirley Almon (1965: 178-196) developed her model by considering that lagged effects can take various forms, drawing on a fundamental concept in mathematics known as the Weierstrass Theorem. Her approach allows lag coefficients (β values) to be expressed as polynomial functions of a certain degree (Gujarati, 1999: 613). This representation makes it possible to model the effects as changing smoothly and continuously over time. In general, a finite distributed lag model assumes that the dependent variable is influenced not only by the current value of the independent variable but also by a set number of its past values. Such a framework provides a more realistic way to examine dynamic interactions among economic variables. The general form of the finite distributed lag model is illustrated in Equation (1) (Almon, 1965).

$$Y_t = \alpha + \beta_0 X_t + \beta_1 X_{t-1} + \beta_2 X_{t-2} + \dots + \beta_k X_{t-k} + \varepsilon_t \quad (1)$$

In the finite distributed lag model, the dependent variable Y_t can be expressed as a combination of the current and past values of the independent variable. This relationship is formulated as shown in Equation (2) (Amemiya, 1985: 178):

$$Y_t = \alpha + \sum_{i=0}^k \beta_i X_{t-i} + \varepsilon_t \quad (2)$$

In Equation (2), the coefficients β_i are represented as a polynomial function of the lag i (Amemiya, 1985: 178). Here, k denotes the lag length, and

ε_t represents the error term. This formulation enables the model to account for the influence of past values on the dependent variable in a structured and flexible way. Accordingly, the β_i coefficients can be estimated across the lag sequence, as illustrated in Figure 1(a) and formally presented in Equation (3).

$$\beta_i = c_0 + c_1^i + c_2^{i^2} \quad (3)$$

At this point, a second-degree polynomial emerges in the estimation of the β_i coefficients. If the lag sequence is as depicted in Figure 1(b), the polynomial form allows the coefficients to exhibit increasing or decreasing trends across the lags, as formally expressed in Equation (4).

$$\beta_i = c_0 + c_1^i + c_2^{i^2} + c_3^{i^3} \quad (4)$$

This expression represents a third-degree polynomial. In general, if the β_i coefficients are expressed using higher-degree polynomials, they can be represented as shown in Equation (5).

$$\beta_i = c_0 + c_1^i + c_2^{i^2} + c_3^{i^3} + \dots + c_m^{i^m} \quad (5)$$

Thus, β_i can be expressed as an m -th degree polynomial of i , where m is assumed to be smaller than the maximum lag length k . Consequently, given the c coefficients and the polynomial degree m in Equation (5), an approximate value of the β_i coefficient can be calculated.

To illustrate the functioning of the Almon model, let us assume that the β_i coefficients follow the pattern shown in Figure 1(a), forming a second-degree polynomial. In this case, by substituting the β_i expression from Equation (3) into the model in Equation (6), we obtain a structure in which the lagged coefficients are represented through a polynomial form.

$$Y_t = \alpha + \sum_{i=0}^k (c_0 + c_1^i + c_2^{i^2}) X_{t-i} + \varepsilon_t \quad (6)$$

If the equation in Equation (6) is expressed in a more explicit form, it can be written as in Equation (7).

$$Y_t = \alpha + c_0 \sum_{i=0}^k X_{t-i} + c_1 \sum_{i=0}^k i X_{t-i} + c_2 \sum_{i=0}^k i^2 X_{t-i} + \varepsilon_t \quad (7)$$

To simplify the expressions in Equation (7), the abbreviations presented in Equation (8) can be employed.

$$Z_{0t} = \sum_{i=0}^k X_{t-i}, \quad Z_{1t} = \sum_{i=0}^k iX_{t-i} \quad \text{ve} \quad Z_{2t} = \sum_{i=0}^k i^2 X_{t-i} \quad (8)$$

By applying the abbreviations in Equation (8) to reorganize the model in Equation (7), it can be expressed as shown in Equation (9).

$$Y_t = \alpha + c_0 Z_{0t} + c_1 Z_{1t} + c_2 Z_{2t} + \varepsilon_t \quad (9)$$

This framework allows the effects of lagged independent variables to be incorporated into the model in a systematic and flexible manner through polynomial functions.

Gujarati (2001: 615-616) outlines the procedural steps to be followed prior to applying the Almon technique. According to his guidance, the first step involves specifying the maximum lag length (k) in advance. In the process of model selection and evaluating its adequacy, criteria such as the Akaike Information Criterion (AIC) and the Schwarz Bayesian Criterion (SBC) are frequently employed (Kutlar, 2000: 44-46). The AIC, which accounts for both the explanatory power of the model and the number of parameters, is computed as expressed in Equation (10) (Cooray, 2008: 194).

$$AIC = T \ln \hat{\sigma}^2 + 2n \quad (10)$$

In contrast, the SBC applies a stronger penalty for the number of parameters when evaluating the model's fit, and it is represented as shown in Equation (11).

$$SBC = T \ln \hat{\sigma}^2 + n \ln(T) \quad (11)$$

In Equations (10) and (11), T denotes the number of usable observations, n represents the number of estimated parameters, and $\hat{\sigma}^2 = \frac{KKT}{T-n}$ corresponds to the variance of the error term, or equivalently, the maximum likelihood estimate of σ^2 . Here, SSE refers to the sum of squared residuals. When selecting a model and determining the appropriate lag length, the ideal values for both the AIC and SBC are those that are as small as possible. AIC and SBC values are computed for different model specifications, and the model exhibiting the smallest value is considered the most suitable for the given

dataset (Kutlar, 2000: 44-46). As additional explanatory variables are incorporated into the model and the explanatory power increases, both AIC and SBC values tend to decrease accordingly (Kutlar, 2000: 46). Beyond these criteria, there exist alternative methods for determining the optimal lag length. These include estimating the lag length when the maximum lag is known (Judge et al., 1988: 1024; Genceli, 2001: 772) and using the cross-correlation function to infer the lag structure (Lardaro, 1993: 550-551). Distributed lag models are typically estimated using the ordinary least squares (OLS) method, adapted specifically for the structure of each model (Alt, 1942: 113-128; Tinbergen, 1949: 174-185; Tari, 2014: 261-265).

Once the lag length k has been determined, the degree of the polynomial, m , must be specified. The polynomial degree is generally chosen to be one more than the number of inflection points (maxima or minima) in the lag structure, or at least one higher. For instance, if there is only a single turning point, as illustrated in Figure 1(a), a second-degree polynomial is typically considered an appropriate approximation. However, since the number and location of turning points are usually unknown in advance, the choice of k and m remains largely subjective (Gujarati, 2001: 619; Akın, 2002: 742). Consequently, researchers often determine the appropriate lag length and polynomial degree based on their judgment. For example, if there is uncertainty about whether a second- or third-degree polynomial should be used, one can examine the regression results of Z_{3t} . If the coefficient c_2 is statistically significant while c_3 is not, it may be concluded that a second-degree polynomial provides a sufficiently accurate representation (Akın, 2002: 670-671).

Once the lag length k and the polynomial degree m have been established, the Z values in Equation (8) can be calculated. For example, if the polynomial degree is set to $m=2$ and the number of lags is $k=6$, the corresponding Z values are derived as presented in Equations (12), (13), and (14).

$$Z_{0t} = \sum_{i=0}^6 X_{t-i} = (X_t + X_{t-1} + X_{t-2} + X_{t-3} + X_{t-4} + X_{t-5} + X_{t-6}) \quad (12)$$

$$Z_{1t} = \sum_{i=0}^6 iX_{t-i} = (X_{t-1} + 2X_{t-2} + 3X_{t-3} + 4X_{t-4} + 5X_{t-5} + 6X_{t-6}) \quad (13)$$

$$Z_{2t} = \sum_{i=0}^6 i^2 X_{t-i} = (X_{t-1} + 4X_{t-2} + 9X_{t-3} + 16X_{t-4} + 25X_{t-5} + 36X_{t-6}) \quad (14)$$

When the sub-models are incorporated into the main model, the core equation presented in Equation (9) is obtained. Following this integration, within a second-degree polynomial lag framework, an integer is assigned to each lag, and the model is estimated using the ordinary least squares method. This procedure produces the coefficients c_0, c_1 , and c_2 . Substituting these ccc coefficients back into the model allows the lagged coefficients β_i to be derived (Tari, 2014: 261-265). In this way, the impact of past values of the independent variable on the dependent variable is systematically modeled through polynomial functions. The β_i coefficients, expressed in terms of c , are calculated using the equations presented in Equations (15) through (21) (Gujarati, 2001: 614-615).

$$i = 0, \hat{\beta}_0 = c_0 \quad (15)$$

$$i = 1, \hat{\beta}_1 = c_0 + c_1 + c_2 \quad (16)$$

$$i = 2, \hat{\beta}_2 = c_0 + 2c_1 + 4c_2 \quad (17)$$

$$i = 3, \hat{\beta}_3 = c_0 + 3c_1 + 9c_2 \quad (18)$$

$$i = 4, \hat{\beta}_4 = c_0 + 4c_1 + 16c_2 \quad (19)$$

$$i = 5, \hat{\beta}_5 = c_0 + 5c_1 + 25c_2 \quad (20)$$

$$i = 6, \hat{\beta}_6 = c_0 + 6c_1 + 36c_2 \quad (21)$$

In summary, this framework provides a clear demonstration of the Almon model's capacity to systematically capture lagged effects through polynomial functions.

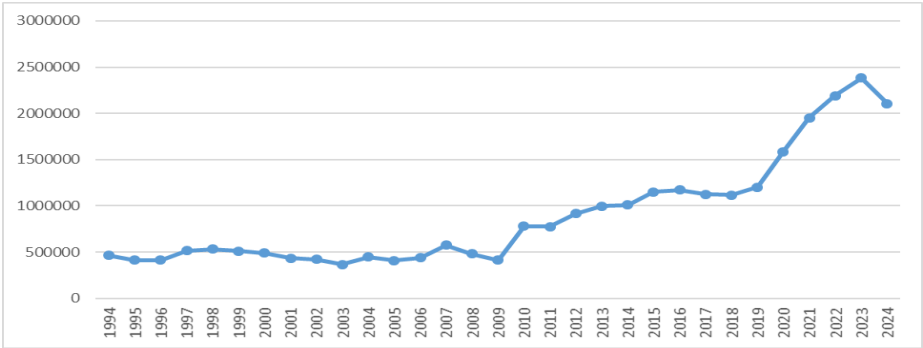
5. RESEARCH FINDINGS

Considering the time lags between production and marketing in animal products, it is evident that these delays tend to be longer than those observed in crop production. Accordingly, this study examines the relationship between production and prices of red meat an animal product within a framework that accounts for lagged effects, employing the Almon model for empirical analysis.

As shown in Figure 2, an examination of red meat production in Turkey over the 1994-2024 period reveals that production does not follow a continuous upward or downward trajectory; rather, it displays a fluctuating pattern across different periods. This volatility suggests that the relationship between

production and prices does not emerge instantaneously but instead occurs with certain time lags, thereby emphasizing both the relevance and importance of employing lagged regression models such as the Almon model to analyze this dynamic relationship.

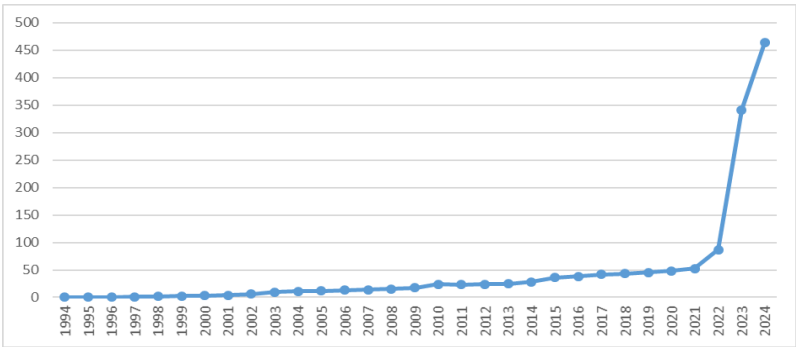
Figure 2: Red Meat Production Amount (Tons)



As shown in Figure 3, red meat prices in Turkey generally remained low and stable throughout the 1994-2024 period, but exhibited a marked increase beginning in 2020. This post-2020 rise in prices is most likely associated with the impacts of the pandemic, rising production costs, and shifts in global demand. Such a trend clearly highlights the importance of employing lagged models, such as the Almon model, in analyzing the dynamic relationship between red meat production and prices.

In general, the fluctuating pattern in production highlights the significance of lagged effects in the relationship between red meat production and prices, indicating that both short-term variations and long-term trends should be taken into account in production planning and price forecasting.

Figure 3: Red Meat Prices (TL/Kg)



To examine whether the relationship between red meat production and prices involves lagged effects, the correlation coefficient between the two variables was calculated as 0.730816. Implementing the Almon distributed lag model requires accounting for the lagged values of red meat prices. To determine the appropriate lag length, both the Akaike Information Criterion (AIC) and the Schwarz Bayesian Criterion (SBC) were utilized. This approach allows the model to maintain an unrestricted distributed lag structure when a higher lag length (k) is chosen, while also enabling the assessment of whether shortening the lag materially alters model performance (Davidson & MacKinnon, 1993). In this study, to evaluate the influence of past prices on historical red meat production, the AIC and SBC values corresponding to different lag lengths are presented in Table 1. These findings suggest that lagged effects may play a meaningful role in shaping production outcomes.

Table 4: AIC and SBC Values at Different Lag Lengths

Delay length	AIC	SBC
k=0	39.87642	39.95481
k=1	39.75421	39.83265
k=2	39.63248	39.71235
k=3	39.52137	39.60524
k=4	39.43218	39.51067
k=5	39.34571	39.42683
k=6	37.98462*	38.05674*
k=7	38.10237	38.17843
k=8	38.24516	38.31675

An examination of the findings presented in Table 4 indicates that, for the implementation of the Almon distributed lag model, lagged values of the red meat price series were first constructed. The appropriate lag length to be included in the model was determined by considering the AIC and SBC values. When both criteria consistently indicated the lowest value, a lag length of six periods ($k = 6$) was selected. This result suggests that the model not only possesses the statistically most suitable lag structure but also reflects that the effects of price changes on production extend over approximately six periods.

Table 5: Red Meat Production-Price Relationship Lag Distributed Model Results

	Constant	t	t-1	t-2	t-3	t-4	t-5	t-6
Coefficient (β)	694.163**	-1,542**	0,158*	0,392*	0,629**	0,671*	0,715	0,759**
t-Statistic	23,859	-3,018	2,011	2,045	2,754	3,215	0,098	3,526
<i>R</i> ² = 0.86 <i>F</i> =10.87 <i>p</i> = 0.004 <i>DW</i> =2.019								

Note: * indicates significance at the p<0.05 level, and ** indicates significance at the p<0.01 level.

The results of the estimated distributed lag model, showing both the magnitude and direction of the lagged relationship between red meat production and prices, are presented in Equation (22).

$$Q_t = 694.163 - 1,542\beta_t + 0,158\beta_{t-1} + 0,392\beta_{t-2} + 0,629\beta_{t-3} + 0,671\beta_{t-4} + 0,715\beta_{t-5} + 0,759\beta_{t-6}$$

(22)

An examination of the model results presented in Table 5 indicates that price changes in the current period negatively affect red meat production, whereas price levels in periods t-1, t-2, t-3, t-4, t-5, and t-6 exert a positive influence on production. This finding suggests that producers shape their production decisions by taking past price movements into account, with price levels from one to six periods prior playing an especially incentivizing role in promoting production increases. A detailed evaluation of the model parameters shows that the coefficients $\beta_0, \beta_1, \beta_2, \beta_3, \beta_4$, and β_6 are statistically significant ($P<0.004$ and $P<0.01$). Consequently, the significance of the parameter estimates for periods t, t-1, t-2, t-3, t-4, and t-6 indicates that lagged price effects play a substantial role in influencing production. The model’s correlation coefficient, $R^2=0.86$, demonstrates a strong relationship between production and prices. Although the β_5 coefficient is not statistically significant, the model’s explanatory power remains high, with $R^2 =0.86$. Moreover, the F-statistic of 10.87 confirms the overall significance of the model ($P=0.004<0.01$). The Durbin-Watson statistic, calculated as 2.019, further indicates that there is no autocorrelation problem in the model.

The Z values have been calculated in the model, and the degree of the model has been set to m=2 for estimating these values. This expression, shown in Equation 9, has been rearranged in Equation 23 based on the model results in Table 6. Thus, the structure of the model's lagged effects has been revealed more clearly through the obtained Z values.

Table 6: Estimation of Red Meat Production and Price Relationship with the Almon Model

Variable	Coefficient	Std. Error	t-Statistic	Probability
C	694.163	2.513	276.12	0.000**
Z_0	162.657	1.317	123.55	0.000**
Z_1	223.419	1.468	152.13	0.000**
Z_2	-6.271	1.592	-3.94	0.142

Note: * indicates significance at the $p < 0.05$ level, and ** indicates significance at the $p < 0.01$ level.

$$Q_t = 694.163 + 162.657Z_{0t} + 223.419Z_{1t} - 6.271Z_{2t} + \varepsilon_t \quad (23)$$

After obtaining the Z values calculated in Table 6, the mathematical derivations presented in Equations (15) through (21) were carried out, yielding the lagged coefficients β_i , as shown below.

$$\begin{aligned} \hat{\beta}_0 &= 162.657 \\ \hat{\beta}_1 &= c_0 + c_1 + c_2 = 162.657 + 223.419 - 6.271 = 379.805 \\ \hat{\beta}_2 &= c_0 + 2c_1 + 4c_2 = 162.657 + 2(223.419) - 4(6.271) = 584.411 \\ \hat{\beta}_3 &= c_0 + 3c_1 + 9c_2 = 162.657 + 3(223.419) - 9(6.271) = 776.475 \\ \hat{\beta}_4 &= c_0 + 4c_1 + 16c_2 = 162.657 + 4(223.419) - 16(6.271) = 955.997 \\ \hat{\beta}_5 &= c_0 + 5c_1 + 25c_2 = 162.657 + 5(223.419) - 25(6.271) = 1122.977 \\ \hat{\beta}_6 &= c_0 + 6c_1 + 36c_2 = 162.657 + 6(223.419) - 36(6.271) = 1277.415 \end{aligned}$$

Based on the estimated coefficient values, the Almon distributed lag model can be expressed in its final form, as presented in Equation (24). This formulation, obtained by substituting the estimated polynomial coefficients, analytically captures the empirical structure of the model as well as the temporal distribution of the lagged effects.

$$Q_t = 694.163 + 162.657P_t + 379.805P_{t-1} + 584.411P_{t-2} + 776.475P_{t-3} + 955.997P_{t-4} + 1122.977P_{t-5} + 1277.415P_{t-6} \quad (24)$$

According to the results of the Almon distributed lag model presented in Equation (24), red meat production is estimated at approximately 694,163 tons when there is no change in price levels. An examination of the model coefficients reveals that the impact of price changes on production increases gradually over time. Specifically, a 1 TL increase in red meat prices in the current period raises production by 162,657 tons, while the effect grows to 379,805 tons with a one-period lag and 584,411 tons with a two-period lag. In the third and fourth periods, the impact reaches 776,475 tons and 955,997 tons,

respectively, and further rises to 1,122,977 tons and 1,277,415 tons in the fifth and sixth periods.

These findings indicate that the impact of price increases on red meat production intensifies over time in a lagged and cumulative manner, suggesting that producers respond only modestly to price signals in the short term but more substantially over longer horizons. This pattern reflects that production decisions are spread out over time and that the adjustment to market conditions occurs with a certain delay. Overall, it can be concluded that red meat production is sensitive to past price movements and that this relationship operates in a positive direction.

6. CONCLUSION AND EVALUATION

In this study, the interaction between red meat production and prices was examined using the Almon distributed lag model. The analysis utilized annual data spanning the period from 1994 to 2024, with red meat production treated as the dependent variable and red meat prices as the independent variable. Correlation analysis revealed a strong and positive relationship between production and prices, with a coefficient of 0.73. This finding indicates that red meat production is significantly influenced by price changes and suggests that the Almon model provides an appropriate framework for capturing this relationship. The model's lag length was determined by considering both the AIC and the SBC. When both criteria reached their minimum values, the maximum lag length was identified as six years ($k=6$), implying that red meat production is affected by price movements over the preceding six years.

According to the results of the Almon distributed lag model, both the model and its coefficients were found to be statistically significant at the 1% and 5% levels. The findings indicate that red meat production is meaningfully influenced by price movements over the past six years, and that a positive relationship exists between production and prices in the $t-1$ through $t-6$ periods. While current-period price changes were observed to have a negative effect on production, past price levels contributed positively to production. In the absence of any price changes, red meat production is estimated at approximately 694,163 tons. An examination of the model coefficients reveals that the effect of price changes on production increases gradually over time. Specifically, a 1 TL increase in red meat prices in the current period raises

production by 162,657 tons, while the same increase one period earlier (t-1) raises it by 379,805 tons, and two periods earlier (t-2) by 584,411 tons. In the third and fourth periods (t-3 and t-4), the effect reaches 776,475 tons and 955,997 tons, respectively, and in the fifth and sixth periods (t-5 and t-6), it rises to 1,122,977 tons and 1,277,415 tons. These results suggest that red meat production is sensitive to past price movements, and that this relationship is positive in direction. Furthermore, the model demonstrates a high degree of explanatory power, with an R^2 of 0.86, an F-value of 10.87, and a p-value of 0.004, indicating a strong relationship between prices and production and confirming the overall significance of the model.

Overall, the analysis reveals a strong and positive relationship between red meat production and price levels. The model suggests that price changes affect production not only in the current period but also progressively across subsequent lagged periods. This pattern reflects the biological and investment-related delays inherent in the red meat sector. Producers cannot respond immediately to price signals; due to the time-consuming nature of processes such as animal rearing, feeding, and slaughtering, supply adjustments occur with a lag. As a result, the production-boosting effect of rising prices is limited in the short term but becomes increasingly pronounced over time. Furthermore, the fact that this effect peaks in the sixth period highlights that production decisions are strongly influenced by past price movements and that producers require time to adapt to market conditions. This finding aligns with the fundamental assumption of the Almon model, namely that lagged effects unfold in a smooth and continuous manner, either gradually decreasing or increasing over time.

In conclusion, the model's findings indicate that supply in the red meat market responds to price changes with a lag, implying that the impact of pricing policies on production primarily manifests over the medium and long term. This suggests that production decisions driven by short-term price fluctuations are likely to have limited effectiveness, whereas consistent and stable pricing policies can play a crucial role in enhancing the sustainability of supply within agricultural planning.

REFERENCES

- Akgül, S., & Yildiz, Ş. (2016). *The Relationship Between Red Meat Production and Prices in Çorum Province Using the Almon Model*. In Proceedings of the International Symposium on All Aspects of Çorum (pp. 45-54), April 28-30, 2016.
- Akın, F. (2002). *Econometrics*. Bursa: Ekin Publishing House.
- Amemiya, T. (1985). *Advanced Econometrics*. Harvard University Press, Cambridge.
- Almon, S. (1965). The Distributed Lag between Capital Appropriations and Expenditures. *Econometrica*, 33, 178-196.
- Alt, F. (1942). Distributed lags. *Econometrica*, 10, 113-128.
- Cezayirli, M. A. (2007). *Distributed Lag Models (Almon Model): The Case of Turkey* (Master's Thesis). Gaziosmanpaşa University, Institute of Social Sciences, Department of Economics, Tokat.
- Cooray, T. M. J. A. (2008). *Applied Time Series Analysis and Forecasting*. Narosa Publishing House, New Delhi.
- Çelik, Ş., & Özbay, N. (2015). Analysis of the Production-Price Relationship in Tomato Production Using the Almon Lag Model: The Case of Turkey. *Turkish Journal of Agricultural and Natural Sciences*, 2(2), 207-213.
- Çelik, Ş. (2015a). *Analysis of the Production-Price Relationship in Buffalo Milk Production Using the Koyck and Almon Lag Models*. In 9th National Animal Science Congress (September 3–5, 2015 / Konya), pp. 83–92.
- Çelik, Ş. (2015b). Analysis of the Production-Price Relationship in Sheep Milk Production Using the Koyck and Almon Lag Models: The Case of Turkey. *Academic Review: International Refereed Social Sciences Journal*, 50, 137-149.
- Davidson, R., & MacKinnon J. G. (1993). *Estimation and Inference in Econometrics*. Oxford University Press, New York.
- Doğan, H. G., Gürler, A. Z., & Ayyıldız, B. (2014). The Almon Polynomial Technique Approach in the Production-Price Relationship (The Case of Paddy in Samsun Province). *Gaziosmanpaşa University Journal of the Faculty of Agriculture*, 31(3), 49–55.

- Ekmekcioglu, C., Wallner, P., Kundi, M., Weisz, U., Haas, W. and Hutter, H.P. 2018. Red meat, diseases, and healthy alternatives: A critical review. *Critical Reviews in Food Science and Nutrition*, 58(2), 247-261.
- Genceli, M. (2001). *Principles of Econometrics and Statistics*. Istanbul: Filiz Publishing House.
- Gujarati, D. N. (1999). *Basic Econometrics*. (Translators: Ümit Şenesen & Gülay Günlük Şenesen). Istanbul: Literatür Publishing.
- Gujarati, D. N. (2001). *Basic Econometrics*. (Translators: Ümit Şenesen & Gülay Günlük Şenesen). Istanbul: Literatür Publishing.
- İşyar, Y. (1999). *Econometric Models*. Bursa: Uludağ University Development Foundation Publications, Publication No. 141.
- Judge, G. G., Hill, R. C., Griffiths, W. E., Lütkepohl, H., & Lee, T. C. (1988). *Introduction to the Theory and Practice of Econometrics* (2nd ed.). New York: John Wiley.
- Koutsoyiannis, A. (1989). *Econometric Theory* (Translators: Ümit Şenesen & Gülay Göktürk Şenesen). Ankara: Verso Publishing.
- Köleoğlu, N., & Çelik, Ş. (2025). Distributed Lag Models: Koyck and Almon Models. An Application on Agricultural Data. *Kahramanmaraş Sütçü İmam University Journal of Agriculture and Nature*, 28(4), 1131-1142.
- Kutlar, A. (2000). *Econometric Time Series*. Ankara: Gazi Publishing.
- Kutlar, A. (2005). *Applied Econometrics* (Revised 2nd ed.). Istanbul: Nobel Publishing.
- Lardaro, L. (1993). *Applied Econometrics*. Harper Collins, New York.
- Tarı, R. (2014). *Econometrics* (Revised 10th ed.). Kocaeli: Umuttepe Publishing, Publication No. 32.
- Tinbergen, J. (1949). *Long-Term Foreign Trade Elasticities. Macroeconomica*, 1, 174-185.
- TURKSTAT, (2025a). *Livestock Statistics*. Retrieved from <https://biruni.tuik.gov.tr/medas/?kn=101&locale=tr>. (Accessed: August 11, 2025).
- TURKSTAT, (2025b). *Red Meat Production: Red Meat Production Statistics*. Retrieved from <https://data.tuik.gov.tr/Bulten/Index?p=Kirmizi-Et-Uretim-Istatistikleri-2024-53539>. (Accessed: August 11, 2025).
- TURKSTAT, (2025c). *Animal Product Prices*. Retrieved from <https://data.tuik.gov.tr/Bulten/Index?p=Canli-Hayvan-ve-Hayvansal->

Urun-Fiyatlari-ve-Uretim-Degeri-2025-45507. (Accessed: August 11, 2025).

CHAPTER 11

THE PHYTOCHEMICAL PROPERTIES OF CERTAIN ONION PLANTS AND THEIR USE IN MEDICINE

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1. Introduction

1.1. Definition and General Characteristics of Bulbous Plants

Geophytes, commonly referred to as bulbous plants, constitute an important component of the flora of Turkey and play a strategic role in the conservation of biodiversity. These taxa possess metamorphosed subterranean stem structures, enabling them to persist belowground during a substantial portion of their life cycle. Predominantly belonging to the monocotyledonous group, geophytes generally exhibit biennial or perennial growth patterns. Members of the family *Amaryllidaceae* are particularly notable due to their rich alkaloid profiles. Compounds such as galanthamine, nivaline, tazettine, and lycorine have been reported to possess antiviral, antimicrobial, anticancer, and immunomodulatory properties, underscoring their pharmacological significance. Moreover, geophytes contribute to floral diversity by blooming from late autumn to early spring, thereby fulfilling a crucial ecological function within seasonal cycles. In addition to their aesthetic appeal, fragrance, and durability, they are widely utilized as ornamentals. Their inherent drought tolerance and ability to flower shortly after planting provide a distinct advantage for sustainable landscape practices, particularly in water-limited environments. While autumn-flowering geophytes are planted in the fall to bloom in spring, spring-flowering taxa are planted in spring to bloom in summer. Bud initiation occurs underground, whereas aerial organs develop only during specific growth phases and subsequently senesce. The persistence of viable underground organs ensures regeneration under favorable conditions, thereby supporting the long-term survival of these species in their natural habitats. Bulbs are structures characterized by short, fleshy stems bearing floral primordia and enclosed by succulent scales. Tulip (*Tulipa*), hyacinth (*Hyacinthus*), lily (*Lilium*), and onion (*Allium*) are representative examples of this group. Bulbous plants have a broad spectrum of applications, ranging from the perfume industry and pharmaceutical production to ornamental horticulture and ecological landscaping. Due to their high ecological tolerance, they are capable of adapting to diverse climatic and edaphic conditions. Considering both their economic value and their contribution to ecosystem services, their conservation and wider cultivation are of substantial importance. (Karaoğlu, 2010; Kılıçaslan ve Dönmez, 2016; Uzun,2021).

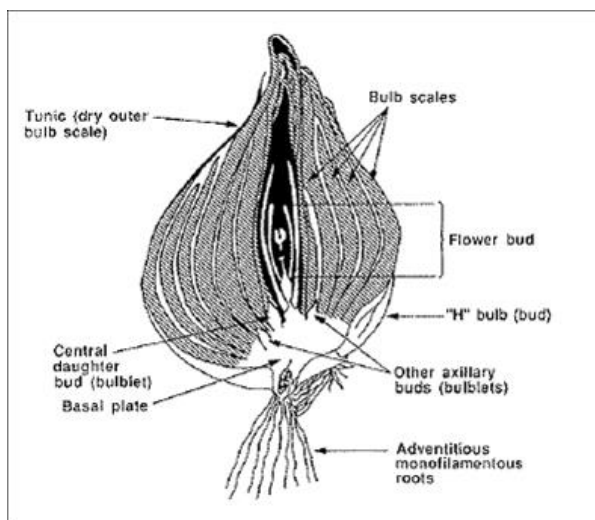


Figure 1: Structure of Onion (URL-1)

Table 1: Usage areas of some bulbous plants

No	Plant name	Latin name	Disease	Reference
1	Onion	<i>Allium cepa</i>	Gut Microbiome	(Yoo et al., 2025)
2	Onion	<i>Allium cepa</i>	Allergic asthma	(El-Hashim et al., 2024)
3	Onion	<i>Allium cepa</i>	Neurotoxicity	(Zhao et al., 2024)
4	Onion	<i>Allium cepa</i>	Anti-Diabetic Effect	(Ramadaini et al., 2024)
5	Onion	<i>Allium cepa</i>	Anti-parasitic Activity	(Reda et al., 2024)
6	Garlic	<i>Allium sativum</i>	Cardiotoxicity	(Rajpoot and Sharma, 2025)
7	Garlic	<i>Allium sativum</i>	Atherosclerotic Effect	(Miki et al., 2025)
8	Garlic	<i>Allium sativum</i>	Insulin Resistance	(Amirkhani et al., 2025)
9	Garlic	<i>Allium sativum</i>	Pancreatic Cancer	(Kawasaki et al., 2025)
10	Garlic	<i>Allium sativum</i>	Anticoagulant Effect	(Clark-Montoya et al., 2025)
11	Tulip	<i>Tulipa</i>	Contact Dermatitis	(Hassan et al., 2018)
12	Tulip	<i>Tulipa</i>	Antioxidant Activity	(Ibrahim et al., 2017)
13	Tulip	<i>Tulipa</i>	Phytochemistry and Ethnopharmacology	(Amin et al., 2016)
14	Tulip	<i>Tulipa</i>	Enzyme	(Nomura et al., 2015)
15	Tulip	<i>Tulipa</i>	Anti-HIV-1	(Lee et al., 2010)
16	Narcissus	<i>Narcissus</i>	Autoimmune Disease	(Zhang et al., 2022)
17	Narcissus	<i>Narcissus</i>	SARS-CoV-2	(Sarkar et al., 2022)

18	Narcissus	<i>Narcissus</i>	Hepatotoxicity	(Ben-Abdallah et al., 2021)
19	Narcissus	<i>Narcissus</i>	Cytotoxicity and Apoptosis Induction	(Rezadoost et al., 2019)
20	Narcissus	<i>Narcissus</i>	Wound Healing	(Rameshk et al., 2018)
21	Lily	<i>Lilium</i>	Pulmonary Fibrosis	(Liu et al., 2025)
22	Lily	<i>Lilium</i>	Neuroprotective Effect	(Lee et al., 2025)
23	Lily	<i>Lilium</i>	Chronic Wound Healing	(Bâldea et al., 2025)
24	Lily	<i>Lilium</i>	Fatty Liver	(Li et al., 2025)
25	Lily	<i>Lilium</i>	Atrophy	(Kim et al., 2024)
26	Snowdrop	<i>Galanthus</i>	Biological Activity	(Korcan et al., 2024)
27	Snowdrop	<i>Galanthus</i>	Therapeutic and Medical Effects	(Babashpour-Asl et al., 2023)
28	Snowdrop	<i>Galanthus</i>	Coronavirus	(Simplicien et al., 2023)
29	Snowdrop	<i>Galanthus</i>	Cardiovascular Diseases	(Baralić et al., 2023)
30	Snowdrop	<i>Galanthus</i>	Cognitive Function	(Hickson and Darnell, 2021)
31	Saffron	<i>Crocus sativus</i>	Cardiotoxicity	(Wang et al., 2025)
32	Saffron	<i>Crocus sativus</i>	Hypercholesterolemia	(Siddiq et al., 2025)
33	Saffron	<i>Crocus sativus</i>	Anti-apoptotic Activity	(Teimury et al., 2025)
34	Saffron	<i>Crocus sativus</i>	Effect on Cytochrome p450	(Bathaei et al., 2025)
35	Saffron	<i>Crocus sativus</i>	Detoxification	(Tan et al., 2025)
36	Saffron	<i>Crocus sativus</i>	Allergic Asthma	(Zhang et al., 2025)
37	Saffron	<i>Crocus sativus</i>	Anti-inflammatory Effect	(Upadhyay et al., 2025)
38	Saffron	<i>Crocus sativus</i>	Stroke	(Li et al., 2025)
39	Saffron	<i>Crocus sativus</i>	Membranous Glomerulonephritis	(Bao et al., 2025)
40	Saffron	<i>Crocus sativus</i>	Diabetes	(Zhang et al., 2025)
41	Saffron	<i>Crocus sativus</i>	Anhedonia	(Corridori et al., 2025)
42	Saffron	<i>Crocus sativus</i>	Depression and Anxiety	(Shafiee et al., 2025)
43	Saffron	<i>Crocus sativus</i>	Oxidative Stress	(Puddu et al., 2025)
44	Saffron	<i>Crocus sativus</i>	Organ Toxicity	(Dolatabadi et al., 2025)
45	Saffron	<i>Crocus sativus</i>	Phytochemistry and Pharmacology	(Singh et al., 2025)

46	Saffron	<i>Crocus sativus</i>	Antidepressive and Anxiolytic Effects	(Amin et al., 2025)
47	Saffron	<i>Crocus sativus</i>	Hypogonadism	(Liu et al., 2025)
48	Saffron	<i>Crocus sativus</i>	Colorectal Cancer	(Feng et al., 2025)
49	Saffron	<i>Crocus sativus</i>	Lung Inflammatory Treatment	(Memarzia et al., 2025)
50	Saffron	<i>Crocus sativus</i>	Cataract	(Shukri et al., 2025)
51	Freesia	<i>Freesia</i>	Antioxidant	(Shindo et al., 2025)
52	Freesia	<i>Freesia</i>	Oxidative Stress and Wrinkles in the Skin	(Kim et al., 2024)
53	Freesia	<i>Freesia</i>	Gene Expression	(Yang et al., 2024)
54	Freesia	<i>Freesia</i>	Transcription	(Zhu et al., 2021)
55	Freesia	<i>Freesia</i>	Enzyme	(Uchikoba et al., 2002)
56	Gladiolus	<i>Gladiolus</i>	Biological Activity	(Zengin et al., 2025)
57	Gladiolus	<i>Gladiolus</i>	Epigenetic Aging	(Kushwaha et al., 2024)
58	Gladiolus	<i>Gladiolus</i>	Hyperglycemia	(Karigidi et al., 2023)
59	Gladiolus	<i>Gladiolus</i>	Biochar Production	(Xiao et al., 2022)
60	Gladiolus	<i>Gladiolus</i>	SARS-CoV-2	(Matsabisa et al., 2022)
61	Belladonna	<i>Atropa belladonna</i>	Antitumor Activity	((Tallini et al., 2024)
62	Belladonna	<i>Atropa belladonna</i>	Allelochemical Use	(Wahyuni et al., 2013)
63	Belladonna	<i>Atropa belladonna</i>	Entomotoxicity	(Caccia et al., 2012)
64	Belladonna	<i>Atropa belladonna</i>	Cell Fusion	(Yee et al., 2011)
65	Belladonna	<i>Atropa belladonna</i>	HIV	(Balzarini et al., 2004)
66	Inverted Tulip	<i>Fritillaria</i>	Anti-inflammatory and Antitussive	(Zhao et al., 2025)
67	Inverted Tulip	<i>Fritallaria</i>	Pulmonary Fibrosis	(Feng et al., 2025)
68	Inverted Tulip	<i>Fritallaria</i>	Pharmacokinetics	(Wu et al., 2025)
69	Inverted Tulip	<i>Fritallaria</i>	Therapeutic Effect	(Kumar et al., 2025)
70	Inverted Tulip	<i>Fritallaria</i>	Allergic Asthma	(Wang et al., 2024)

71	Tunceli Garlic	<i>Allium tuncelianum</i>	Antimicrobial Activity (Özdemir et al., 2025)

1.2. Medicinal and Nutritional Uses Throughout History

Plants such as onion, garlic, leek, and saffron are widely utilized both in culinary practices and in traditional medicine. Salep, derived from orchids, is employed in the preparation of beverages as well as ice cream. The Madonna lily (*Lilium candidum*), valued in industrial sectors such as floriculture and perfumery, contains pharmacologically rich compounds. Butanolic extracts obtained from its petals and saponins extracted from its bulbs have been used as therapeutic agents in the treatment of skin disorders. Members of the genus *Allium* contain high levels of organosulfur compounds and polyphenols, exhibiting antioxidant, antimicrobial, antiviral, and anti-inflammatory properties. Furthermore, they are notable for their antihypertensive, hypolipidemic, and antithrombotic effects, which contribute to reducing the risk of cardiovascular diseases. Garlic (*Allium sativum*), owing to sulfur-containing compounds such as allicin, alliin, and S-allyl cysteine, is recognized as a potent antimicrobial agent. Garlic essential oil demonstrates preventive effects against coronary heart disease, obesity, and cholesterol-related problems. The compound allicin alters the structure and metabolism of microorganisms, showing efficacy against more than 300 pathogens. In addition, the antimicrobial potential of *Allium tuncelianum*, an endemic species, has been highlighted in recent studies. This species is particularly notable for its low minimum inhibitory concentration (MIC) values against pathogens such as MRSA, *Salmonella paratyphi* A, and *E. coli*. Moreover, due to its rich content of diallyl disulfide and 1-propenyl compounds, it is regarded as a natural antimicrobial agent. The genus *Fritillaria* is distributed from Asia to Europe and has long been employed in traditional Chinese medicine for the treatment of respiratory disorders. This genus is rich in isosteroidal alkaloids and exhibits diverse biological activities, including anti-inflammatory, antitumor, and antiasthmatic effects. In particular, *Fritillaria taipaiensis* is officially listed in the Chinese Pharmacopoeia as a medicinal drug used for the treatment of respiratory diseases. These findings highlight the broad biological potential of

bulbous plants in both food and healthcare applications. (Karaoğlu, 2010; Kahraman, 2017; Özdemir et al., 2025; Şelem et al., 2020; Qu et al., 2025).

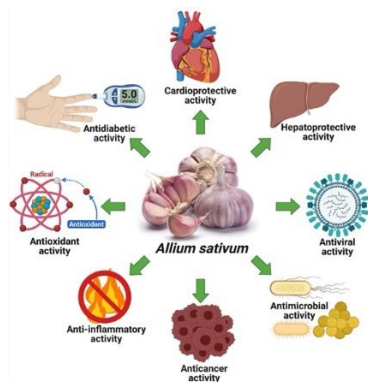


Figure 2: Medicinal Uses of Garlic (Tudu et al., 2022)

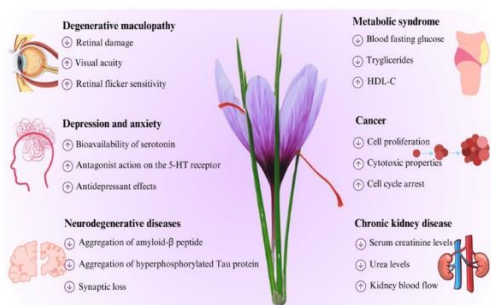


Figure 3: Medical Uses of Saffron (Marrone et al., 2024)

1.3. Molecular Biology and Its Importance in Health

Onion and leek are perennial, bulbous plants belonging to the Amaryllidaceae family. These plants contain characteristic compounds such as flavonoids and alk(en)yl cysteine sulfoxides. In onion, quercetin, a flavonol, is particularly notable for its metabolism-boosting and cardiovascular health-supporting effects. Onions are composed largely of water, along with natural sugars, vitamins, and minerals. In traditional medicine, onion has been used in the treatment of various diseases due to its anti-inflammatory and immune-boosting properties (Eren,2011).

Asphodelus (commonly known as “çiriş”) is a perennial plant that grows naturally in Turkey, particularly across Asia and the Middle East, and is characterized by its rich content of vitamins (C, B group) and minerals (P, K, Ca, Mg, Na, Fe, Cu). Scientific studies have demonstrated that it contains flavonoids, anthraquinones, polysaccharides, and various other bioactive compounds (Bayrak and Yanardağ, 2013).

The primary bioactive constituents of saffron are crocins, carotenoid-derived, water-soluble glycosides that serve as key indicators of saffron quality. In addition, the volatile compound safranal, while conferring the characteristic aroma of saffron, also exhibits notable biological activity. Furthermore, extracts from saffron tepals present a rich flavonoid profile, particularly in derivatives

of kaempferol, quercetin, and isorhamnetin (Abou Wakil et al., 2025; Bashir et al., 2025).

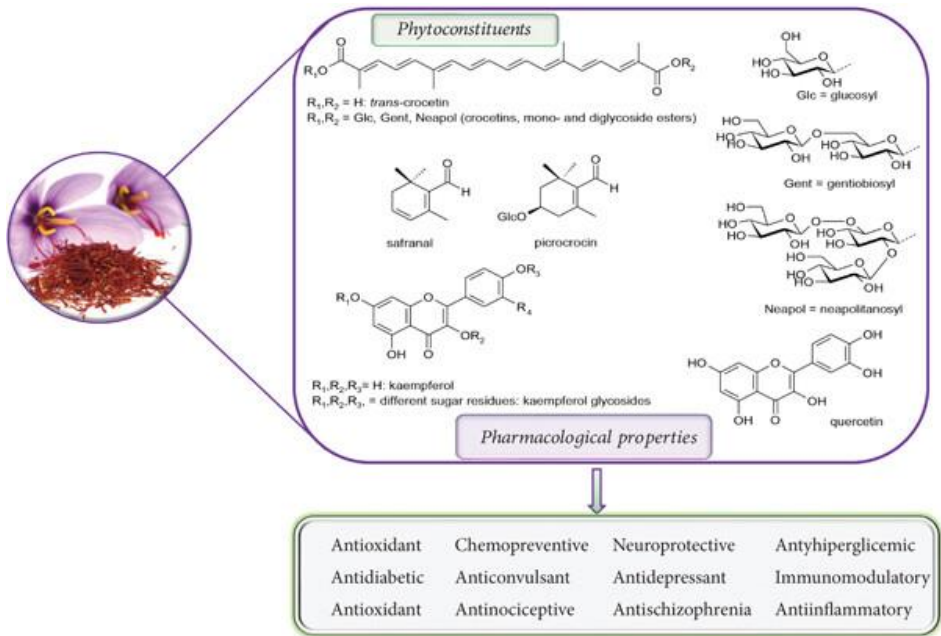


Figure 4: Molecular structure of saffron and its health benefits (Batnariu et al., 2022)

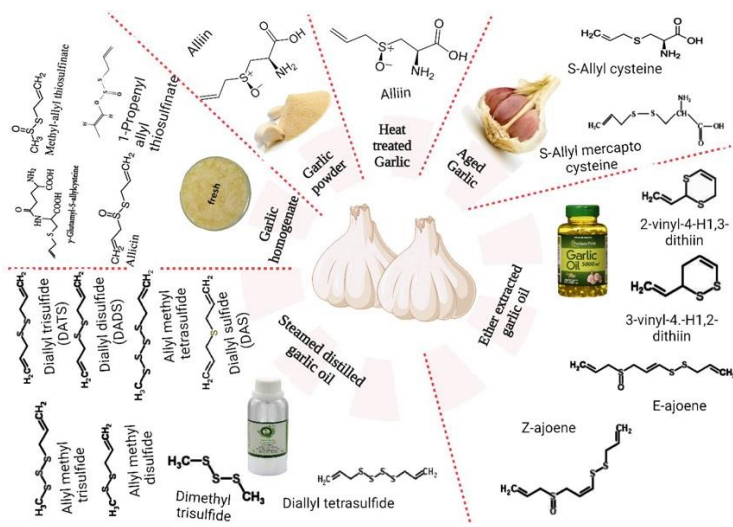


Figure 5: Molecular components of garlic (El Saadony et al., 2024)

2. Biochemical and Molecular Components of Bulbous Plants

Allium species (e.g., *A. fistulosum*, *A. ursinum*, *A. cepa*, and *A. sativum*) represent substantial reservoirs of antioxidant activity, primarily attributable to their diverse profiles of polyphenolic metabolites localized in bulbs, leaves, roots, and floral tissues. Within this phytochemical spectrum, flavonoids—divided into six subclasses with a predominance of flavonols—constitute biologically active entities that significantly contribute to the functional value of plant-derived foods. Notably, flavonols such as kaempferol and quercetin occur at elevated concentrations in *Allium* taxa, where their pronounced redox-modulating capacities underpin strong antioxidant potential and associated health-promoting effects (Barbu et al., 2024).

Allium sativum is a highly potent medicinal plant recognized for its multifaceted bioactivities, including antioxidant, anti-inflammatory, and cardioprotective effects. Its bioactive profile comprises organosulfur compounds such as diallyl disulfide, diallyl trisulfide, and methyl allyl derivatives, which exert pronounced free radical-scavenging activity and modulate inflammatory signaling pathways. Aged garlic extract (AGE), produced by ethanol-mediated aging of raw garlic, is particularly enriched in S-allyl cysteine (SAC), S-1-propenyl cysteine (S1PC), Maillard reaction derivatives, and diverse phenolic compounds, which collectively contribute to its pharmacological efficacy. Owing to its minimal content of irritant constituents like allicin, AGE demonstrates gastroprotective properties while exhibiting significant anti-atherosclerotic activity, as evidenced by both preclinical animal models and clinical investigations assessing endothelial function, lipid peroxidation, and inflammatory biomarkers (Miki et al., 2025; Rajpoot et al., 2025).

Narcissus species have been historically acknowledged for their anticancer properties and were recommended by Hippocrates for the treatment of uterine neoplasms. The Amaryllidaceae family, particularly the subfamily Amaryllidoideae, is of considerable pharmacological significance due to its diverse and abundant alkaloid (AA) profiles, encompassing bioactive compounds such as galantamine, an acetylcholinesterase inhibitor employed in Alzheimer's disease therapy, and antiviral alkaloids including lycorine,

hemantamine, and crinine. These alkaloids are predominantly isolated from Mediterranean taxa such as *Narcissus papyraceus*, and their biosynthesis is initiated from the aromatic amino acids L-phenylalanine and L-tyrosine. Sequential enzymatic reactions involving oxidative phenol coupling, O-methylation, and reduction lead to the elaboration of structurally complex alkaloids. Notably, the O-methylation steps give rise to several intermediates, including 4'-O-methylnorbelladine, which serves as a pivotal precursor for downstream alkaloid diversification. This intricate biosynthetic network underpins the pharmacodynamic potential of *Narcissus* alkaloids, mediating anticancer, neuroprotective, and antiviral activities (Koirala et al., 2024).

Saffron is a complex phytochemical matrix comprising over 150 compounds, including carbohydrates, polypeptides, lipids, minerals, and vitamins, with principal bioactive constituents such as picrocrocin, safranal, crocetin, alpha-crocin, lycopene, zeaxanthin, and alpha- and beta-carotene. The spice is additionally enriched with essential minerals including zinc, iron, copper, selenium, magnesium, phosphorus, calcium, and manganese, as well as vitamins A, C, folic acid, riboflavin, and niacin, contributing to its multifaceted biological activities. Crocin, a major saffron carotenoid, exerts pronounced protective effects in various pathophysiological contexts, including chronic obstructive pulmonary disease (COPD), polycystic ovary syndrome (PCOS), and cardiovascular disorders, through its antioxidant, anti-inflammatory, and anti-apoptotic mechanisms. Furthermore, preclinical studies have demonstrated crocin's efficacy in the prophylaxis of asthma, likely mediated via modulation of airway inflammatory mediators, oxidative stress attenuation, and regulation of immune cell signaling pathways, underscoring saffron's potential as a functional bioactive agent (Abbasi et al., 2024; Vakili et al., 2025).

Freesia, a monocotyledonous genus within the Iridaceae family, is widely cultivated as an ornamental plant, prized for its aesthetically appealing and fragrant flowers. Originating from South Africa and extensively hybridized with wild congeners such as *Freesia refracta*, *Freesia leichtlinii*, and *Freesia caryophyllacea*, *Freesia hybrida* displays substantial floral color variation. This chromatic diversity is primarily governed by the biosynthetic accumulation of bioactive secondary metabolites, including anthocyanins, proanthocyanidins, and flavonoids. Yellow-flowered cultivars, particularly prevalent in Japan, have been characterized by pigments predominantly

comprising crocetin derivatives and apocarotenoids, reflecting intricate carotenoid and apocarotenoid biosynthetic pathways. The unique pigment profile of *Freesia* not only contributes to its ornamental appeal but also indicates the presence of biologically active compounds, which may exhibit antioxidant and other phytopharmacological activities, underscoring the genus's potential for both horticultural and functional applications (Shan et al., 2024; Shindo et al., 2025).

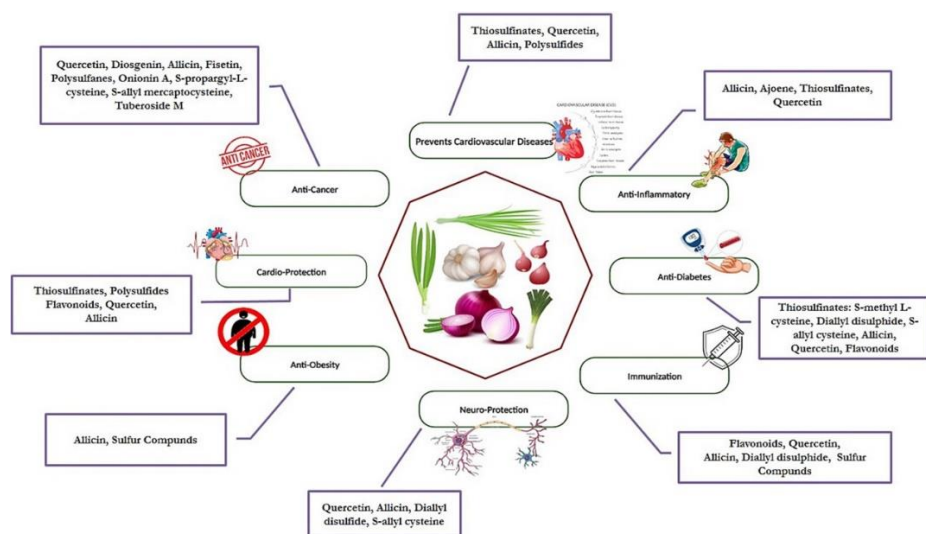


Figure 6: Health benefits of some bulbous plant components (URL-2)

3. Antioxidant and Anti-Inflammatory Effects

Quercetin, a naturally occurring flavonoid abundantly present in plant-based foods such as onions, has emerged as a compound of considerable pharmacological interest due to its multifaceted biological activities, including antiproliferative, antioxidant, and anticancer effects. Mechanistically, quercetin modulates oxidative stress by scavenging reactive oxygen species (ROS) and regulating intracellular redox signaling pathways, while concurrently influencing key molecular targets involved in cell cycle regulation, apoptosis induction, and metastatic inhibition. These molecular actions underpin its observed antineoplastic effects and render quercetin a compelling candidate for further preclinical and clinical investigation, particularly in malignancies such as malignant melanoma. Collectively, these attributes support the potential

integration of quercetin as an adjuvant therapeutic agent in oncological interventions. (Hoinoiu et al., 2025).

Extracts from *Allium* species have demonstrated potent antiviral and antimicrobial activities, predominantly mediated by bioactive sulfur-containing compounds such as allicin and isoallins, which exert their effects through redox-dependent mechanisms. Red onion (*Allium cepa*), enriched in flavonoids and sulfur-based organics with pronounced antibacterial properties, exhibits both bacteriostatic and bactericidal activities against *Escherichia coli*. Garlic (*Allium sativum*) extract has been shown to attenuate piroxicam-induced oxidative stress in Wistar rats, with dose-dependent enhancements in protection against lipid peroxidation and hepatic injury, highlighting its role in bolstering endogenous antioxidant defenses. Moreover, Dobutr et al. reported that garlic extracts reduce IL-2 levels, suggesting immunomodulatory potential. The garlic-derived compound N-benzyl-N-methyldecan-1-amine (BMDA) has been shown to suppress cancer stem cell phenotypes while modulating TGF- β signaling pathways. BMDA and its derivatives also exhibit significant anti-inflammatory efficacy in rat models of colitis and rheumatoid arthritis. Collectively, these findings elucidate the multifaceted pharmacodynamic potential of garlic and other *Allium* species, supporting their use as natural therapeutic agents in the management of oxidative stress, inflammatory disorders, and cancer through molecular mechanisms involving redox regulation, immune modulation, and anti-proliferative signaling pathways (Budluang et al., 2025; Cho et al., 2024; Dobutr et al., 2025; Hai et al., 2025; Yunita et al., 2025).

In the leaves of *Freesia* species, novel polyphenolic aglycones, including 3'-caffeoylquercetin and 4'-caffeoylkaempferol glycosides, have been characterized and shown to exhibit potent antioxidant activity. These caffeoyl-flavonol glycosides are proposed to be biosynthesized via quercetin and kaempferol metabolic pathways, contributing to the biosynthesis of yellow and white floral pigmentation in *Freesia*. Specifically, flavonoid glycosides such as 3'-caffeoylquercetin demonstrate substantial efficacy in mitigating lipid peroxidation and scavenging reactive oxygen species, underscoring their potential role as bioactive phytochemicals. These findings emphasize the importance of further evaluating the pharmacological benefits and potential toxicological risks of these newly identified flavonoids in the context of human

health and their broader implications in plant secondary metabolism (Shindo et al., 2025).

Crocins, the principal carotenoid constituents of saffron, have been demonstrated to extend lifespan in *Caenorhabditis elegans* models through the attenuation of oxidative stress and the modulation of lipid metabolism via the DAF-16/FOXO transcriptional pathway. In parallel, safranal exerts renoprotective effects by improving podocyte function in conditions such as membranous glomerulonephritis, primarily through suppression of pro-inflammatory signaling and modulation of the Sirt1/NF- κ B axis. These findings underscore the intricate molecular mechanisms by which saffron bioactives influence cellular homeostasis, antioxidant defenses, and inflammatory pathways, highlighting their potential translational applications in age-related and renal pathologies (Bao et al., 2025; Li et al., 2025).

4. Effects on Cardiovascular Health

The cardiovascular system is integral to maintaining systemic homeostasis and ensuring efficient blood circulation, with its dysregulation contributing to pathologies such as atherosclerosis, hyperlipidemia, and hypertension. Garlic (*Allium sativum*) mediates antihypertensive and cardioprotective effects through a constellation of biochemical and physiological mechanisms, including nitric oxide (NO) bioavailability enhancement, endothelial function improvement, attenuation of oxidative stress, inhibition of angiotensin-converting enzyme (ACE), and modulation of inflammatory signaling pathways. The organosulfur compound alliin improves cardiovascular parameters by reducing circulating triglycerides and elevating high-density lipoprotein (HDL) cholesterol, whereas allicin prevents vascular calcification and exerts vasodilatory effects that lower blood pressure. Aged garlic extract supplementation has been shown to ameliorate blood pressure in prehypertensive populations, enhance endothelial NO production, and favorably regulate lipid metabolism. Nevertheless, the magnitude of these effects is contingent upon the garlic species employed, preparation method, and dosage administered, highlighting the need for standardized intervention protocols in clinical applications (Rahmatinia et al., 2024; Saadh et al., 2024; Sleiman et al., 2024).

Extensive evidence indicates that saffron extracts and their principal bioactive constituents, including crocin, picrocrocin, and safranal, modulate multiple cellular signaling cascades, transcription factors, and growth regulatory pathways. Crocin, a trans-crocin di-(β -D-gentiobiosyl) ester comprising 6–16% of dried saffron powder, has been extensively investigated for its therapeutic potential in inflammatory and cardiovascular disorders. In a rat model, crocin conferred protection against gentamicin-induced oxidative stress, inflammation, and nephrotoxicity, effects that were mechanistically associated with downregulation of the NF- κ B/TLR signaling axis and concomitant upregulation of the Nrf2/HO-1 antioxidant pathway. Furthermore, in LDLR-/- mice, crocin administration inhibited atherogenic plaque development and enhanced hepatic antioxidant capacity. Beyond its vascular effects, crocin modulates lipid metabolism, suggesting efficacy against obesity and dyslipidemia, while also attenuating ischemia-reperfusion-induced oxidative damage in renal and skeletal muscle tissues. Clinical studies in diabetic and prediabetic overweight individuals indicate that saffron supplementation significantly reduces fasting plasma glucose, HbA1c, triglycerides, systolic blood pressure, and aspartate aminotransferase levels, highlighting its potential translational application in mitigating cardiometabolic risk factors through integrated antioxidant, anti-inflammatory, and metabolic regulatory mechanisms (Karim et al., 2024; Zununi et al., 2024).

5. Anti-Cancer and Anti-Proliferative Effects

In oncological contexts, the emergence of multidrug resistance (MDR) constitutes a significant therapeutic challenge, frequently mediated by the activation or regulatory modulation of ATP-binding cassette (ABC) transporters. Cytostatic chemotherapeutics primarily induce cell death via apoptosis and necrosis, yet MDR often attenuates these effects. Alkaloids derived from the Amaryllidaceae family have garnered considerable interest owing to their dual capacity as P-glycoprotein (P-gp) inhibitors and apoptosis-inducing agents, thereby potentially circumventing MDR mechanisms. Notably, a study at the National Cancer Institute demonstrated that a suspension containing *Galanthus* species and galantamine elicited pronounced antitumor activity in a murine sarcoma 37 model, suggesting the translational potential of

Amaryllidaceae alkaloids as adjuvant agents in overcoming drug-resistant cancer phenotypes. (Cozanitis, 2021).

Alkaloids derived from the Amaryllidaceae family are a class of naturally occurring compounds classified into subgroups according to their distinct carbon skeletons and are noted for their diverse and potent biological activities. These phytochemicals have a well-documented history in traditional medicine, with *Narcissus poeticus* being extensively utilized in ethnopharmacological practices. Historical evidence suggests that Hippocrates developed therapeutic formulations using daffodil-derived oils for the treatment of uterine and cerebral neoplasms. The pharmacological relevance of these alkaloids is attributed to their ability to modulate cellular signaling pathways, induce apoptosis, and exert cytotoxic effects, highlighting their potential as bioactive agents in both traditional and contemporary therapeutic contexts (Evidente, 2023).

Crocus sativus L., a prominent medicinal plant within the Iridaceae family, has emerged as a promising candidate in breast cancer therapeutics. Its bioactive constituents, including apocarotenoids (crocin, safranal, picrocrocin) and flavonoids (kaempferol, taxifolin, naringenin), mediate anticancer effects through multifaceted mechanisms such as the induction of apoptosis, inhibition of cellular proliferation, and modulation of oncogenic signaling cascades, notably PI3K/AKT, NF- κ B, and MAPK pathways. Clinical investigations further suggest that saffron supplementation can attenuate chemotherapy-induced adverse effects, reduce serum tumor biomarker levels, and enhance patient quality of life. Mechanistically, saffron extracts have been shown to activate apoptotic pathways across diverse cancer models while concurrently inhibiting cell migration and metastatic potential. Moreover, leaf-derived saffron extracts exhibit hepatoprotective properties via the regulation of the Nrf2/HO-1 signaling axis, thereby facilitating detoxification and ameliorating oxidative hepatic damage. Collectively, these findings underscore the pharmacodynamic versatility of saffron, highlighting its integrative potential as a natural adjuvant in cancer management and systemic cytoprotection (Baba et al., 2022; Mokhtarian et al., 2024; Tan et al., 2025).

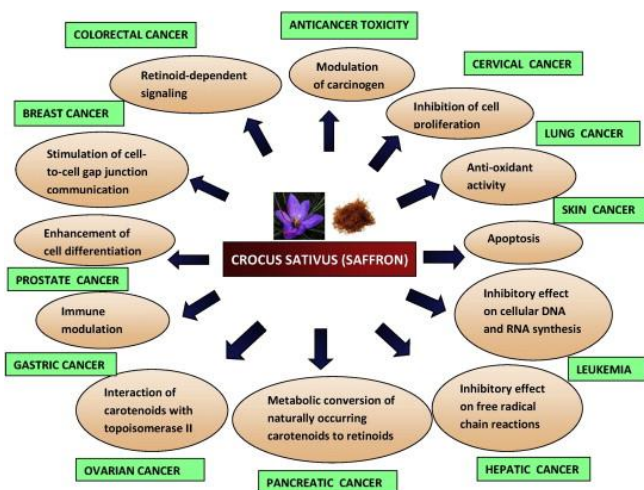


Figure 7: Main anticancer properties of saffron (Bhandari, 2015)

The medicinal properties of *Allium* species have been acknowledged since ancient times, with epidemiological evidence demonstrating an inverse correlation between *Allium* vegetable consumption and the incidence of diverse malignancies. Specifically, decreased risks of head and neck squamous cell carcinoma (HNSCC), as well as esophageal, gastric, pancreatic, prostate, endometrial, and colorectal cancers, have been associated with dietary intake of these vegetables. Mechanistic studies attribute the anticancer efficacy of *Allium* species to organosulfur compounds (OSCs), such as diallyl sulfide (DAS), diallyl disulfide (DADS), and diallyl trisulfide (DATS), which are liberated upon cellular disruption during chopping or mastication. These OSCs exert chemopreventive and therapeutic effects by modulating oncogenic signaling pathways, inducing apoptosis, inhibiting cancer stem cell proliferation, and attenuating tumor growth, as evidenced in various *in vivo* models. Notably, DATS demonstrates robust anticancer activity in prostate, breast, and lung cancer models, suppressing HNSCC tumor progression and stemness features. Additionally, organosulfur constituents of garlic and related *Allium* species exhibit potential as complementary agents in gastric cancer chemoprevention and therapy. *In silico* analyses of *Allium ascalonicum* further reveal its capacity to target matrix metalloproteinases, emphasizing the mechanistic underpinnings of its inhibitory effects on ovarian cancer

progression and metastasis (Mathan et al., 2024; Ravindranath et al, 2025; Yi et al., 2025).

6. Effects on Metabolic Diseases and Diabetes

Garlic extract demonstrates pronounced hypoglycemic activity through multifaceted mechanisms involving pancreatic β -cell function. Specifically, it enhances insulin secretion, facilitates the liberation of bound insulin, and promotes β -cell regeneration. Additionally, garlic modulates systemic glucose homeostasis by attenuating intestinal glucose absorption and increasing glucose uptake in peripheral tissues, thereby potentiating insulin signaling and responsiveness. Sulfur-containing bioactive constituents, particularly allicin, confer additional benefits by protecting insulin molecules from oxidative modification at sulfhydryl residues, effectively enhancing insulin sensitivity and contributing to improved glycemic control. These integrated biochemical and pharmacodynamic effects underscore the therapeutic potential of garlic as a natural antidiabetic agent (Derosa et al., 2024).

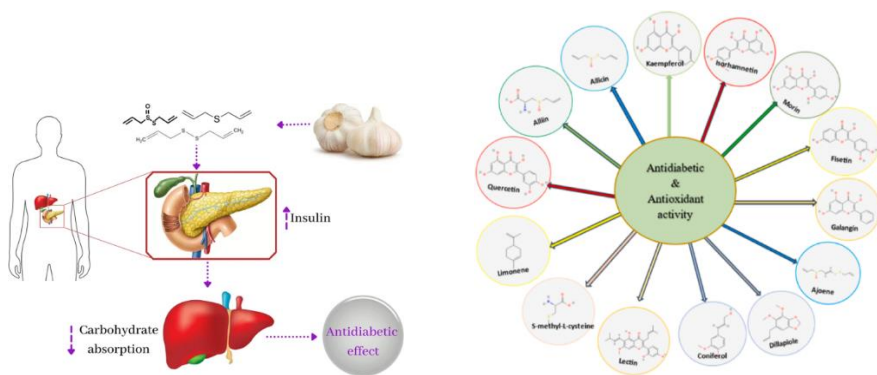


Figure 8: Antidiabetic and antioxidant effects of garlic (Hossain et al., 2022; Agrawal et al., 2024)

Hyperlipidemia, a prevalent metabolic disorder characterized by elevated circulating triglycerides and cholesterol, is a major risk factor for cardiovascular diseases, type 2 diabetes, obesity, and hypertension. Its pathogenesis is influenced by environmental determinants such as suboptimal dietary intake and sedentary behavior, yet it remains amenable to modulation through targeted lifestyle interventions. Garlic exerts beneficial effects on lipid homeostasis through multiple biochemical pathways: its essential minerals

enhance endogenous antioxidant defenses, while bioactive flavonoids and polyphenols favorably modulate lipoprotein profiles by elevating high-density lipoprotein (HDL) levels and reducing low-density lipoprotein (LDL) concentrations. These integrated pharmacodynamic actions underscore the potential of garlic as a complementary agent for the prevention of atherogenesis and the management of hypertension, highlighting its therapeutic relevance in mitigating cardiometabolic risk (Itrat et al., 2024).

Wound healing is a highly orchestrated biological process involving the coordinated interaction of multiple cell types, growth factors, and bioactive molecules, which can be markedly disrupted under pathological conditions such as diabetes due to infection, chronic inflammation, and impaired angiogenic responses. Chronic wounds represent a significant clinical and economic burden, frequently culminating in complications such as diabetic foot ulcers. Phytotherapeutic interventions have emerged as promising natural modulators that enhance tissue repair by stimulating collagen biosynthesis, promoting epithelialization, and facilitating neovascularization. Extracts obtained from *Crocus sativus* L. (saffron), particularly those derived from floral petals, have been demonstrated to upregulate vascular endothelial growth factor (VEGF) expression, thereby potentiating angiogenesis and accelerating wound closure. These mechanistic insights underscore the potential of saffron petal extracts as efficacious botanical therapeutics for the management of chronic wounds, including those associated with diabetic pathology (Soheilifar et al., 2024).

The polysaccharides of *Lilium lancifolium* exhibit pronounced protective effects in streptozotocin-induced diabetic murine models, as evidenced by a significant attenuation of hyperglycemia and mitigation of body weight loss. Mechanistically, these polysaccharides enhance endogenous antioxidant defenses by upregulating enzymatic activities, concurrently reducing lipid peroxidation as indicated by decreased malondialdehyde levels. Additionally, they preserve and restore the architectural integrity of pancreatic islets, thereby supporting β -cell functionality and overall glycemic homeostasis. These findings highlight the potential of *Lilium lancifolium* polysaccharides as bioactive agents with multifaceted therapeutic effects in diabetes management (Zhang et al., 2014).

The aqueous extract of *Gladiolus psittacinus* (AGP) has been evaluated for its antidiabetic and antihyperlipidemic properties in streptozotocin-induced diabetic rat models. Administration of AGP resulted in a significant reduction of fasting blood glucose levels, concomitant restoration of body weight, and favorable modulation of lipid profile parameters. Mechanistically, AGP attenuated oxidative stress by enhancing endogenous antioxidant defense systems and mitigating reactive oxygen species-mediated damage. These effects were accompanied by histopathological improvements in the pancreas, kidney, and liver, highlighting the extract's potential as a bioactive therapeutic agent with multifaceted benefits in glycemic regulation and lipid metabolism (Karigidi, 2023).

7. Antibacterial and Antiviral Properties

Garlic demonstrates broad-spectrum antimicrobial efficacy against diverse pathogens, including bacteria, viruses, fungi, and parasites, primarily mediated by bioactive organosulfur compounds such as allicin. Mechanistically, allicin exerts its effects by targeting thiol-containing enzymes and proteins within microbial cells, disrupting cellular redox balance, inhibiting metabolic pathways, and ultimately inducing pathogen cell death. These integrated biochemical and pharmacodynamic actions underscore garlic's potential as a natural antimicrobial agent with multifaceted therapeutic applications (Feng et al., 2014).

Food preservation is a critical component of ensuring food safety and minimizing post-harvest losses, yet microbial contamination by pathogens such as *Escherichia coli* and *Staphylococcus aureus* remains a persistent challenge. Garlic exhibits robust antimicrobial activity against these pathogens, primarily mediated by organosulfur compounds including alliin and allicin. Mechanistically, these compounds disrupt microbial metabolic pathways, inhibit key thiol-containing enzymes, and perturb cellular redox homeostasis, culminating in pathogen inactivation. Coupled with its established safety profile, garlic emerges as a promising natural preservative with multifaceted biochemical and pharmacodynamic potential for enhancing food safety (Zhu et al., 2025).

Multiple studies have evaluated the antibacterial properties of garlic aqueous and ethanolic extracts, revealing their broad-spectrum efficacy against

key foodborne pathogens, including *Escherichia coli*, *Salmonella* spp., *Staphylococcus aureus*, *Bacillus* spp., *Pseudomonas aeruginosa*, *Klebsiella pneumoniae*, and *Helicobacter pylori*. The antimicrobial activity is primarily attributed to organosulfur compounds such as allicin, which disrupt microbial cell wall integrity, inhibit thiol-dependent enzymes, and perturb cellular redox balance, ultimately leading to pathogen inactivation. These findings underscore the potential of garlic extracts as natural, bioactive agents for controlling foodborne bacterial contamination (Artawinata et al., 2025).

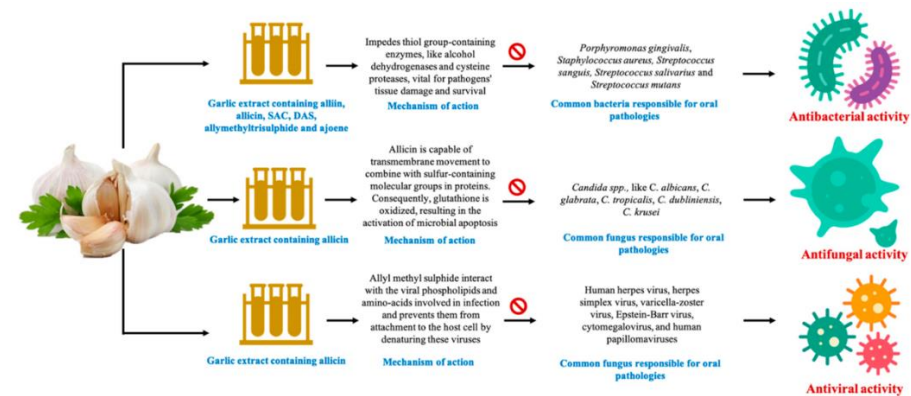


Figure 9: Antibacterial, antiviral and antifungal effects of garlic (Mosaddad and Hamid,2023)

Extracts derived from the stigma and leaves of *Crocus sativus* L. are enriched with bioactive compounds, particularly polyphenols, which exert potent antioxidant and antibacterial activities. Mechanistically, these phytochemicals mitigate cellular oxidative stress by scavenging reactive oxygen species and enhancing endogenous antioxidant defenses, while simultaneously inhibiting the growth and metabolic functions of pathogenic microorganisms. Collectively, these biochemical and pharmacodynamic properties highlight the potential of *Crocus sativus* as a natural preservative agent with multifunctional protective effects in food and biological systems (Benkerroum et al., 2024).

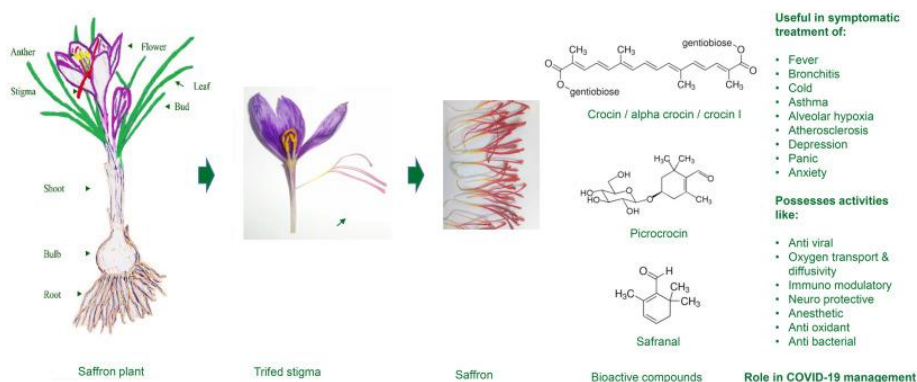


Figure 10: Health effects of saffron components (Husaini et al., 2021)

8. Neuroprotective and Contributions to Brain Health

Galantamine, a prominently studied alkaloid derived from the bulbs of Amaryllidaceae plants, exhibits significant clinical relevance in neurological therapeutics due to its reversible inhibition of acetylcholinesterase and its capacity to traverse the blood–brain barrier. Mechanistically, galantamine not only enhances synaptic acetylcholine availability but also allosterically modulates nicotinic acetylcholine receptors, thereby conferring neuroprotection under oxidative stress, hypoxic conditions, and other neurodegenerative challenges. These integrated biochemical and pharmacodynamic actions underscore galantamine's potential as a multifunctional neurotherapeutic agent (Atrahimovich et al., 2021).

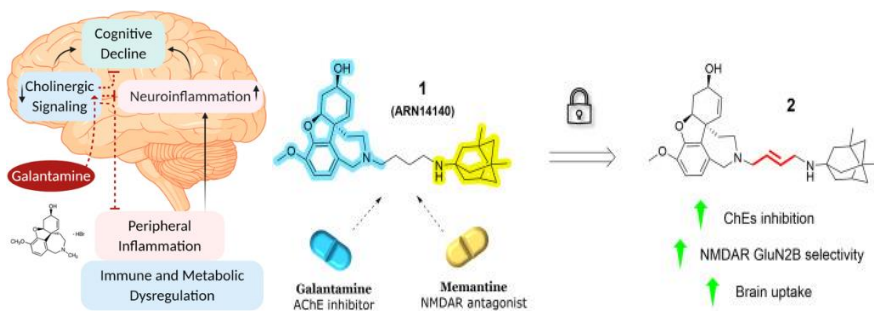


Figure 11: Effects of galantamine on brain health (Metz and Pavlov, 2021; Basagni et al., 2023)

Gladiolus dalenii is a medicinal plant traditionally utilized across various regions of Cameroon for the management of neuropsychiatric disorders,

including headaches, epilepsy, schizophrenia, and mood dysregulation. Recent pharmacological investigations have revealed that the aqueous extract of *G. dalenii* (AEGD) exerts antidepressant-like effects in experimental rat models, likely through modulation of monoaminergic neurotransmission, enhancement of neurotrophic signaling pathways, and attenuation of oxidative stress within neural tissues. These integrated biochemical and pharmacodynamic mechanisms provide empirical support for the plant's potential as a natural therapeutic agent for neuropsychiatric conditions (Fotsing et al., 2017).

Saffron (*Crocus sativus* L.) and its principal bioactive constituents crocin, crocetin, safranal, and picrocrocins demonstrate considerable therapeutic potential by modulating multiple cellular signaling pathways implicated in the pathogenesis of neurodegenerative and neuropsychiatric disorders, including Alzheimer's disease, Parkinson's disease, attention deficit hyperactivity disorder, and depression. Mechanistically, these compounds exert neuroprotective effects by regulating apoptotic pathways (e.g., modulation of caspases, Bax/Bcl-2 ratio, cytochrome c release, and death receptor activation), suppressing neuroinflammatory signaling (e.g., NF- κ B inhibition; downregulation of IL-1 β , IL-6, TNF- α , and COX-2 expression), and mitigating oxidative stress through activation of endogenous antioxidant defenses (e.g., Nrf2 pathway, upregulation of GSH, GPx, CAT, and SOD; reduction of MDA, ROS, and nitrite levels). The integration of these multi-target biochemical and pharmacodynamic actions underscores saffron's potential as a promising natural agent for the prevention and management of neurodegenerative diseases, offering both cytoprotective and neuromodulatory benefits (Abdian et al., 2024).

Crocin, a carotenoid compound isolated from *Crocus sativus* (saffron), has been shown to modulate dopaminergic and serotonergic neurotransmission, conferring significant neurotherapeutic properties. Its dopaminergic modulatory effects contribute to the attenuation of motor deficits characteristic of Parkinson's disease, while its influence on serotonergic pathways supports the alleviation of depressive symptoms commonly observed in these patients. Preclinical and clinical evidence suggests that crocin not only ameliorates motor dysfunction but also improves affective behaviors, collectively indicating a potential enhancement of overall quality of life in individuals suffering from Parkinsonian disorders. The integration of these neurochemical

and behavioral effects underscores crocin's promise as a multitarget natural agent for the management of Parkinson's disease and associated comorbidities (Mohammad et al., 2024).

Garlic (*Allium sativum*), renowned globally for its culinary and aromatic properties, has recently gained scientific interest for its neuroprotective effects in various Parkinson's disease models. The bioactive constituents of garlic, particularly organosulfur compounds, modulate key pathogenic mechanisms implicated in Parkinson's disease, including oxidative stress, mitochondrial dysfunction, and neuroinflammatory signaling pathways. Preclinical studies indicate that these compounds not only attenuate dopaminergic neuronal loss but also improve motor and non-motor deficits, underscoring garlic's potential as a complementary therapeutic agent in the management of Parkinsonian disorders (Rakshit et al., 2023).

References

1. Abbasi Moajani F, Soozangar N, Amani M, Jeddi F, Salimnejad R, Aslani MR. The suppressive effects of crocin from saffron on allergic airway inflammation through Drp1/Nfr1/Mfn2/Pgc1- α signaling pathway in mice. *J Ethnopharmacol.* 2025 Jan 30;337(Pt 2):118862. doi: 10.1016/j.jep.2024.118862. Epub 2024 Sep 24.
2. Abdian S, Fakhri S, Moradi SZ, Khirehgesh MR, Echeverría J. Saffron and its major constituents against neurodegenerative diseases: A mechanistic review. *Phytomedicine.* 2024 Dec;135:156097. doi: 10.1016/j.phymed.2024.156097. Epub 2024 Sep 27.
3. Abou-Wakil S, Cacciola F, Housti F, Rochd M, Vinci RL, Mondello L, Davi F, Micale N, Miceli N, Taviano MF. Influence of Drying Methods on the Phenolic Content and Antioxidant Properties of *Crocus sativus* L. Tepals and Leaves By-Products From Meknes (Morocco). *Chem Biodivers.* 2025 Mar 10:e202402967. doi: 10.1002/cbdv.202402967. Epub ahead of print.
4. Agrawal, Aman & Goyal, Hritika & Jain, Pooja & Singh, Vrinda & Verma, Yeshvandra & Perveen, Kahkashan & Bukhari, Najat & Chandra, Aakansh & Malik, Vijai. (2024). Pharmacophylogeny of genus *Allium* L. *Journal of King Saud University - Science.* 36. 103330. 10.1016/j.jksus.2024.103330.
5. Amin F, Ahmad S, Wasim M, Khan A, Manzoor Arain F, Batool Z, Tabassum S, Khaliq S, Samad N, Haider S. Antidepressive and anxiolytic effects of a combination of Saffron and Chamomile in rats and their relationship with serotonin using methods. *J Tradit Chin Med.* 2025 Feb;45(1):49-56. doi: 10.19852/j.cnki.jtcm.2025.01.005.
6. Amin HI, Ibrahim MF, Hussain FH, Sardar AS, Vidari G. Phytochemistry and Ethnopharmacology of Some Medicinal Plants Used in the Kurdistan Region of Iraq. *Nat Prod Commun.* 2016 Mar;11(3):291-6.
7. Amirkhani Z, Gholi AM, Asghari S, Hakak D, Pouryousef M, Yahyaei B, Ziaolhagh SJ. The effect of garlic and stevia extract with aerobic exercise on hypothalamic leptin and ghrelin receptor mRNA expression and insulin resistance in obese rats. *BMC Complement Med Ther.* 2025 Mar 14;25(1):104. doi: 10.1186/s12906-025-04756-7.

8. Artawinata PC, Kim Y, Choi IY, Park MK. Broad Antibacterial Activity and Mechanism of Garlic (*Allium sativum* L. cv. Uiseong) Extracts against Cell Wall of *Aeromonas hydrophila*. J Microbiol Biotechnol. 2025 Feb 24;35:e2410035. doi: 10.4014/jmb.2410.10035.
9. Atrahimovich D, Harris R, Eitan R, Cohen M, Khatib S. Galantamine Quantity and Alkaloid Profile in the Bulbs of *Narcissus tazetta* and *daffodil* cultivars (*Amaryllidaceae*) Grown in Israel. Metabolites. 2021 Mar 21;11(3):185. doi: 10.3390/metabo11030185.
10. Baba SA, Vahedi M, Ahmad I, Rajab BS, Babalghith AO, Irfan S, Hossain MJ. *Crocus sativus* L. Tepal Extract Induces Apoptosis in Human U87 Glioblastoma Cells. Biomed Res Int. 2022 Jun 10;2022:4740246. doi: 10.1155/2022/4740246.
11. Babashpour-Asl M, Kaboudi PS, Barez SR. Therapeutic and medicinal effects of snowdrop (*Galanthus* spp.) in Alzheimer's disease: A review. J Educ Health Promot. 2023 Apr 28;12:128. doi: 10.4103/jehp.jehp_451_22.
12. Bâldea I, Soran ML, Stegarescu A, Opreș O, Kacso I, Tripon S, Adascalitei A, Fericele IG, Decea R, Lung I. *Lilium candidum* Extract Loaded in Alginate Hydrogel Beads for Chronic Wound Healing. Gels. 2025 Jan 1;11(1):22. doi: 10.3390/gels11010022.
13. Balzarini J, Hatse S, Vermeire K, Princen K, Aquaro S, Perno CF, De Clercq E, Egberink H, Vanden Mooter G, Peumans W, Van Damme E, Schols D. Mannose-specific plant lectins from the Amaryllidaceae family qualify as efficient microbicides for prevention of human immunodeficiency virus infection. Antimicrob Agents Chemother. 2004 Oct;48(10):3858-70. doi: 10.1128/AAC.48.10.3858-3870.2004
14. Bao Y, Ge YM, Wang Z, Wang HY, Wang Q, Yuan J. Safranal Ameliorates Renal Damage, Inflammation, and Podocyte Injury in Membranous Nephropathy via SIRT/NF- κ B Signalling. Curr Med Sci. 2025 Mar 4. doi: 10.1007/s11596-025-00020-8. Epub ahead of print.
15. Baralić M, Pažitná L, Brković V, Laušević M, Gligorićević N, Katrlík J, Nedić O, Robajac D. Prediction of Mortality in Patients on Peritoneal Dialysis Based on the Fibrinogen Mannosylation. Cells. 2023 Jan 17;12(3):351. doi: 10.3390/cells12030351.

16. Barbu IA, Toma VA, Moț AC, Vlase AM, Butiuc-Keul A, Pârvu M. Chemical Composition and Antioxidant Activity of Six *Allium* Extracts Using Protein-Based Biomimetic Methods. *Antioxidants (Basel)*. 2024 Sep 29;13(10):1182. doi: 10.3390/antiox13101182.
17. Basagni F, Ortega JA, Bertozzi SM, Armirotti A, Summa M, Bertorelli R, Bartolini M, Mellor IR, Bedeschi M, Bottegoni G, Lembo V, Minarini A, Cavalli A, Rosini M. Galantamine-memantine hybrids for Alzheimer's disease: The influence of linker rigidity in biological activity and pharmacokinetic properties. *Eur J Med Chem*. 2023 Dec 5;261:115803. doi: 10.1016/j.ejmech.2023.115803. Epub 2023 Sep 12. PMID: 37734258.
18. Bashir S, Hussain SZ, Jan N, Naseer B, Zargar IA, Murtaza I, Yaseen M. Structural integrity, bioactive components, and physico-chemical characteristics of Kashmiri saffron (*Crocus sativus* L.) as affected by different drying techniques. *Food Chem*. 2025 Jun 1;476:143511. doi: 10.1016/j.foodchem.2025.143511. Epub 2025 Feb 19.
19. Bathaei P, Imenshahidi M, Vahdati-Mashhadian N, Hosseinzadeh H. Effects of *Crocus sativus* and its active constituents on cytochrome P450: a review. *Naunyn Schmiedebergs Arch Pharmacol*. 2025 Apr 1. doi: 10.1007/s00210-024-03525-6. Epub ahead of print.
20. BAYRAK, B. B., & YANARDAĞ, R. (2013). BİLEŞİKLERİN ANTİOKSİDAN AKTİVİTELERİ.
21. Ben-Abdallah S, Sefi M, Soudani N, Hamdi A, Bejaoui S, Issaoui H, El Cafsi M, Karray-Bouraoui N. Potential antioxidant effects of *Narcissus tazetta* phenolic compounds against cadmium chloride-induced hepatotoxicity in Swiss albino mice. *Environ Sci Pollut Res Int*. 2021 Dec;28(46):66193-66205. doi: 10.1007/s11356-021-15497-8. Epub 2021 Jul 30.
22. Benkerroum A, Oubella K, Zini S, Boussif K, Mouhanni H, Achemchem F. Stigmas and Petals of *Crocus sativus* L. (Taliouine, Morocco): Comparative Evaluation of Their Phenolic Compounds, Antioxidant, and Antibacterial Activities. *ScientificWorldJournal*. 2024 May 21;2024:6676404. doi: 10.1155/2024/6676404.

23. Bhandari PR. *Crocus sativus* L. (saffron) for cancer chemoprevention: A mini review. *J Tradit Complement Med*. 2015 Jan 28;5(2):81-7. doi: 10.1016/j.jtcme.2014.10.009.
24. Budluang P, Kim JE, Park ES, Seol A, Jang HJ, Kang MS, Kim YH, Choi J, Kim S, Kim S, Koh M, Kang HY, Kim BH, Han DW, Hwang DY, Chung YH. N-benzyl-N-methyldecane-1-amine derived from garlic ameliorates UVB-induced photoaging in HaCaT cells and SKH-1 hairless mice. *Sci Rep*. 2025 Feb 26;15(1):6979. doi: 10.1038/s41598-025-88634-9.
25. Butnariu M, Quispe C, Herrera-Bravo J, Sharifi-Rad J, Singh L, Aborehab NM, Bouyahya A, Venditti A, Sen S, Acharya K, Bashiry M, Ezzat SM, Setzer WN, Martorell M, Mileski KS, Bagiu IC, Docea AO, Calina D, Cho WC. The Pharmacological Activities of *Crocus sativus* L.: A Review Based on the Mechanisms and Therapeutic Opportunities of its Phytoconstituents. *Oxid Med Cell Longev*. 2022 Feb 14;2022:8214821. doi: 10.1155/2022/8214821.
26. Caccia S, Van Damme EJ, De Vos WH, Smagghe G. Mechanism of entomotoxicity of the plant lectin from *Hippeastrum hybrid* (*Amaryllis*) in *Spodoptera littoralis* larvae. *J Insect Physiol*. 2012 Sep;58(9):1177-83. doi: 10.1016/j.jinsphys.2012.05.014. Epub 2012 Jun 4.
27. Cho H, Park JY, Kim DH, Han J, Natesan K, Choi MS, Lee SK, Kim J, Cho K, Ahn BO. Understanding the defense mechanism of *Allium* plants through the onion isoallicin-omics study. *Front Plant Sci*. 2024 Dec 11;15:1488553. doi: 10.3389/fpls.2024.1488553.
28. Clark-Montoya I, Milán-Segovia RDC, Lemus-Rojero O, Jaramillo-Morales OA, Juárez-Flores B, Terán-Figueroa Y. Preclinical Evaluation of the Anticoagulant Effect of an Aqueous Extract of Snow Mountain Garlic. *Medicina* (Kaunas). 2025 Feb 28;61(3):429. doi: 10.3390/medicina61030429.
29. Corridori E, Salviati S, Demontis MG, Vignolini P, Vita C, Fagiolini A, Cuomo A, Carmellini P, Gambarana C, Scheggi S. Therapeutic Potential of Saffron Extract in Mild Depression: A Study of Its Role on Anhedonia in Rats and Humans. *Phytother Res*. 2025 Mar;39(3):1277-1291. doi: 10.1002/ptr.8424. Epub 2025 Jan 4.

30. Cozanitis DA. The snowdrop, wellspring of galanthamine : A brief descriptive and scientific history. *Wien Med Wochenschr.* 2021 Jun;171(9-10):205-213. doi: 10.1007/s10354-020-00799-2. Epub 2021 Jan 27.
31. Derosa G, D'Angelo A, Maffioli P. The role of selected nutraceuticals in management of prediabetes and diabetes: An updated review of the literature. Part II. *Phytother Res.* 2024 Nov;38(11):5490-5532. doi: 10.1002/ptr.8312. Epub 2024 Oct 3.
32. Dobutr T, Jangpromma N, Patramanon R, Daduang J, Kulchat S, Areemit J, Lomthaisong K, Daduang S. Screening of aqueous plant extracts for immunomodulatory effects on immune cells and cytokine production: *In vitro* and *in vivo* analyses. *Heliyon.* 2025 Feb 13;11(4):e42692. doi: 10.1016/j.heliyon.2025.e42692.
33. Dolatabadi S, Oskuei SR, Mehri S, Hosseinzadeh H. A comprehensive review of medicinal plants and their beneficial roles in alleviating bisphenol A-induced organ toxicity. *Naunyn Schmiedebergs Arch Pharmacol.* 2025 Feb 11. doi: 10.1007/s00210-025-03795-8. Epub ahead of print.
34. El-Hashim AZ, Khajah MA, Orabi KY, Balakrishnan S, Sary HG, Barakat AM. Treatment with onion bulb extract both prevents and reverses allergic inflammation in a murine model of asthma. *Pharm Biol.* 2024 Dec;62(1):326-340. doi: 10.1080/13880209.2024.2335187. Epub 2024 Apr 8.
35. El-Saadony MT, Saad AM, Korma SA, Salem HM, Abd El-Mageed TA, Alkafaas SS, Elsalahaty MI, Elkafas SS, Mosa WFA, Ahmed AE, Mathew BT, Albastaki NA, Alkuwaiti AA, El-Tarabily MK, AbuQamar SF, El-Tarabily KA, Ibrahim SA. Garlic bioactive substances and their therapeutic applications for improving human health: a comprehensive review. *Front Immunol.* 2024 Jun 10;15:1277074. doi: 10.3389/fimmu.2024.1277074.
36. Eren, E. (2011). Bazı soğanslı bitkilerin antioksidan aktivitelerinin belirlenmesi.

37. Evidente A. Advances on the Amaryllidacea Alkaloids Collected in South Africa, Andean South America and the Mediterranean Basin. *Molecules*. 2023 May 12;28(10):4055. doi: 10.3390/molecules28104055.
38. Feng S, Eucker TP, Holly MK, Konkel ME, Lu X, Wang S. Investigating the responses of *Cronobacter sakazakii* to garlic-driven organosulfur compounds: a systematic study of pathogenic-bacterium injury by use of high-throughput whole-transcriptome sequencing and confocal micro-Raman spectroscopy. *Appl Environ Microbiol*. 2014 Feb;80(3):959-71. doi: 10.1128/AEM.03460-13. Epub 2013 Nov 22.
39. Feng S, Li S, Wu Z, Li Y, Wu T, Zhou Z, Liu X, Chen J, Fu S, Wang Z, Zhong Z, Zhong Y. Saffron improves the efficacy of immunotherapy for colorectal cancer through the IL-17 signaling pathway. *J Ethnopharmacol*. 2025 Jan 30;337(Pt 2):118854. doi: 10.1016/j.jep.2024.118854. Epub 2024 Sep 24.
40. Feng S, Xu G, Ding Q, Shi Y. *Fritillaria thunbergii* Miq. Extract ameliorated experimental pulmonary fibrosis partly through the PI3K/AKT/FOXO signalling pathway. *J Ethnopharmacol*. 2025 Mar 13;343:119445. doi: 10.1016/j.jep.2025.119445. Epub 2025 Feb 10.
41. Fotsing D, Ngoupaye GT, Ouafu AC, Njapdounke SKJ, Kenneth YA, Ngo Bum E. Effects of *Gladiolus dalenii* on the Stress-Induced Behavioral, Neurochemical, and Reproductive Changes in Rats. *Front Pharmacol*. 2017 Sep 27;8:685. doi: 10.3389/fphar.2017.00685.
42. Hai PV, Phuong HTA, Hung PHS, Na TT, Lai NH, Khuong NDT, Liem TN, Hoa NX. Selection of *Lactobacillus* strains from native chicken feces for the fermentation of purple onion (*Allium cepa* L.) as an antibiotic alternative against *Salmonella* spp. in chickens. *Open Vet J*. 2024 Dec;14(12):3525-3538. doi: 10.5455/OVJ.2024.v14.i12.35. Epub 2024 Dec 31.
43. Hassan I, Rasool F, Akhtar S, Kamili A, Rather P, Kanth R, Bhat Y, Rather S, Mubashir S, Yaseen A, Bashir S. Contact dermatitis caused by tulips: identification of contact sensitizers in tulip workers of Kashmir Valley in North India. *Contact Dermatitis*. 2018 Jan;78(1):64-69. doi: 10.1111/cod.12870. Epub 2017 Sep 21.

44. Hickson SV, Darnell LK. Enhancing Cognitive Function with Herbal Supplements. *Nurs Clin North Am.* 2021 Mar;56(1):59-67. doi: 10.1016/j.cnur.2020.10.005. Epub 2020 Dec 29.
45. Hoinoiu T, Dumitrascu V, Pit D, Schipor DA, Jabri-Tabrizi M, Hoinoiu B, Petreuş DE, Seiman C. Quercetin as a Potential Therapeutic Agent for Malignant Melanoma-A Review of Current Evidence and Future Directions. *Medicina (Kaunas).* 2025 Apr 2;61(4):656. doi: 10.3390/medicina61040656
46. https://cargohandbook.com/index.php?title=File:Flower_bulbs_2.jpg
47. <https://wmcresearch.substack.com/p/friday-hope-wonderful-alliums-cardiovascular>
48. <https://www.istockphoto.com/br/vetor/flores-da-primavera-vetores-conjunto-ilustrações-de-cores-gm1339318838-419696802>
49. https://www.linkedin.com/pulse/daffodils-source-beneficial-alkaloids-part-i-agent-tufan-k?trk=public_post
50. Husaini AM, Jan KN, Wani GA. Saffron: A potential drug-supplement for severe acute respiratory syndrome coronavirus (COVID) management. *Heliyon.* 2021 May;7(5):e07068. doi: 10.1016/j.heliyon.2021.e07068. Epub 2021 May 14. PMID: 34007917; PMCID: PMC8118646.
51. Ibrahim MF, Hussain FHS, Zanon G, Vidari G. The main constituents of *Tulipa systola* Stapf. roots and flowers; their antioxidant activities. *Nat Prod Res.* 2017 Sep;31(17):2001-2007. doi: 10.1080/14786419.2016.1272107. Epub 2016 Dec 28.
52. Itrat N, Nisa MU, Al-Asmari F, Ramadan MF, Zongo E. A double-blind, randomized control trial to investigate the therapeutic potential of garlic scapes for high apoprotein E levels in a high-Fat diet-induced hypercholesteremic rat model. *Food Sci Nutr.* 2024 Jul 31;12(10):7607-7619. doi: 10.1002/fsn3.4331.
53. KAHRAMAN, Ö., SERT, E. B., & GÜLER, S. BEYAZ ZAMBAK BİTKİSİNİN TIBBİ VE AROMATİK DEĞERİ. *FULL TEXT PROCEEDINGS BOOK*, 20.
54. Karaoğlu, C. (2010). Soğanlı bitkiler ve in vitro hızlı çoğaltım. *Tarla Bitkileri Merkez Araştırma Enstitüsü Dergisi*, 19(1-2), 24-29.
55. Karigidi KO, Akintimehin ES, Karigidi ME, Adetuyi FO. Antidiabetic, antihyperlipidemic and protective effects of *Gladiolus psittacinus* on

- hyperglycemia-mediated oxidative stress in streptozotocin-induced diabetic rats. *J Complement Integr Med*. 2023 Mar 9;20(2):353-364. doi: 10.1515/jcim-2023-0018.
56. Karim M, Pirzad S, Shirsalimi N, Hosseini MH, Ebrahimi P, Khoshdooz S, Rashidian P. Effects of saffron (*Crocus sativus* L.) supplementation on cardiometabolic Indices in diabetic and prediabetic overweight patients: a systematic review and meta-analysis of RCTs. *Diabetol Metab Syndr*. 2024 Nov 27;16(1):286. doi: 10.1186/s13098-024-01530-6.
 57. Kawasaki H, Nussbaum G. Therapeutic potential of garlic, aged garlic extract and garlic-derived compounds on pancreatic cancer (Review). *Biomed Rep*. 2025 Jan 27;22(3):54. doi: 10.3892/br.2025.1932.
 58. Kılıçaslan, N., & Dönmez, Ş. (2016). Göller bölgesinde doğal olarak yetişen soğanlı bitkilerin peyzaj mimarlığında kullanımı. *Türkiye Ormancılık Dergisi*, 17(1), 73-82.
 59. Kim A, Kim J, Kim BY, Seo CS, Kim YR, Song KH, Kim NS. Aquo-ethanolic extract of *Lilii Bulbus* attenuates dexamethasone-induced muscle loss and enhances muscle strength in experimental mice. *Biomed Pharmacother*. 2024 Dec;181:117658. doi: 10.1016/j.biopha.2024.117658. Epub 2024 Oct 31.
 60. Kim E, Tollenaere M, Sennelier B, Lambert C, Durduret A, Kim SY, Seo HH, Lee JH, Scandolera A, Reynaud R, Moh SH. Analysis of Active Components and Transcriptome of *Freesia refracta* Callus Extract and Its Effects against Oxidative Stress and Wrinkles in Skin. *Int J Mol Sci*. 2024 Jul 26;25(15):8150. doi: 10.3390/ijms25158150.
 61. Koirala M, Merindol N, Karimzadegan V, Gélinas SE, Liyanage NS, Lamichhane B, Tobón MCG, Lagüe P, Desgagné-Penix I. Kinetic and in silico structural characterization of norbelladine O-methyltransferase of *Amaryllidaceae* alkaloids biosynthesis. *J Biol Chem*. 2024 Sep;300(9):107649. doi: 10.1016/j.jbc.2024.107649. Epub 2024 Aug 8.
 62. Korcan SE, Çankaya N, Bulduk İ, Güvercin G, Çivi Şİ. Biological Activities of *Galanthus fosteri* Extracts: First Demonstration of the Interaction between Chlorogenic Acid and DNA Ligase by Molecular Docking. *ACS Omega*. 2024 Feb 23;9(10):12254-12261. doi: 10.1021/acsomega.4c00162.

63. Kumar P, Kumar V, Sharma S, Sharma R, Warghat AR. Fritillaria steroidal alkaloids and their multi-target therapeutic mechanisms: insights from network pharmacology. *Naunyn Schmiedebergs Arch Pharmacol*. 2025 Mar;398(3):2209-2228. doi: 10.1007/s00210-024-03502-z. Epub 2024 Oct 9.
64. Kushwaha A, Mishra V, Tripathi DK, Gupta R, Singh VP. Epigenetics governs senescence. *Plant Reprod*. 2024 Mar;37(1):33-36. doi: 10.1007/s00497-023-00479-2. Epub 2023 Aug 18.
65. Lee J, Choi J, Yun HY, Jang H, Cho M, Ha JH, Jeong Y. Neuroprotective Effects of *Lilium Lancifolium* Thunberg Extract Against Corticosterone-Induced Dysfunctions in PC12 Cells. *J Med Food*. 2025 Mar 7. doi: 10.1089/jmf.2024.k.0241. Epub ahead of print.
66. Lee SA, Hong SK, Suh CI, Oh MH, Park JH, Choi BW, Park SW, Paik SY. Anti-HIV-1 efficacy of extracts from medicinal plants. *J Microbiol*. 2010 Apr;48(2):249-52. doi: 10.1007/s12275-009-0176-9. Epub 2010 May 1.
67. Li AP, Li D, Tan X, Xu R, Mao LX, Kang JJ, Li SH, Liu Y. Crocin extends lifespan by mitigating oxidative stress and regulating lipid metabolism through the DAF-16/FOXO pathway. *Food Funct*. 2025 Apr 22. doi: 10.1039/d5fo01157d. Epub ahead of print.
68. Li J, Jin J, Cheng Y, Zhang Y, Wang X, Chen Y, Wang C, Tang W, Zhang N. Systematic Review and Network Meta-Analysis of the Effects of Plant Extracts on Cognitive Function and Quality of Life in Stroke Patients. *Phytother Res*. 2025 Mar 13. doi: 10.1002/ptr.8472. Epub ahead of print.
69. Li Z, Wang X, Li X, Chen X, Wang C, Mao Y, Teng G, Zhu X, Zhang J. Polysaccharides from Lanzhou Lily Attenuate Nonalcoholic Fatty Liver Disease Modifying the Gut Microbiota and Metabolite Profile. *Chem Biodivers*. 2025 Jan;22(1):e202401538. doi: 10.1002/cbdv.202401538. Epub 2024 Nov 14.
70. Liu H, Guo Z, Zang Z, Jia B, Zhou Y, Zhang H, Fu Q. Saffron extract alleviates D-gal-induced late-onset hypogonadism by activating the PI3K-Akt-Nrf2 signaling pathway. *J Ethnopharmacol*. 2025 Jan 31;340:119273. doi: 10.1016/j.jep.2024.119273. Epub 2024 Dec 20.
71. Liu R, Wu T, Zhou W, Zhu A, Liao W, Ding K. A Novel Polysaccharide from the Flowers of *Lilium lancifolium* Alleviates Pulmonary Fibrosis *In*

- Vivo and In Vitro*. J Agric Food Chem. 2025 Apr 2;73(13):7774-7787. doi: 10.1021/acs.jafc.4c11703.
72. Marrone G, Urciuoli S, Di Lauro M, Cornali K, Montalto G, Masci C, Vanni G, Tesauro M, Vignolini P, Noce A. Saffron (*Crocus sativus* L.) and Its By-Products: Healthy Effects in Internal Medicine. *Nutrients*. 2024 Jul 18;16(14):2319. doi: 10.3390/nu16142319.
 73. Mathan SV, Singh R, Kim SH, Singh SV, Singh RP. Diallyl Trisulfide Induces ROS-Mediated Mitotic Arrest and Apoptosis and Inhibits HNSCC Tumor Growth and Cancer Stemness. *Cancers (Basel)*. 2024 Jan 16;16(2):378. doi: 10.3390/cancers16020378.
 74. Matsabisa MG, Alexandre K, Ibeji CU, Tripathy S, Erukainure OL, Malatji K, Chauke S, Okole B, Chabalala HP. In vitro study on efficacy of PHELA, an African traditional drug against SARS-CoV-2. *Sci Rep*. 2022 Jun 19;12(1):10305. doi: 10.1038/s41598-022-13599-y.
 75. Memarzia A, Beigoli S, Eshaghi Ghalibaf MH, Ghasemi SZ, Abbasian A, Mahzoon E, Naderi Toosi A, Mohammadian Roshan N, Boskabady MH. The preventive effectiveness of *Crocus sativus* extract in treating lung injuries caused by inhaled paraquat in rats. *J Ethnopharmacol*. 2025 Jan 30;337(Pt 1):118767. doi: 10.1016/j.jep.2024.118767. Epub 2024 Sep 3.
 76. Metz, Christine & Pavlov, Valentin. (2021). Treating disorders across the lifespan by modulating cholinergic signaling with galantamine. *Journal of Neurochemistry*. 158. 10.1111/jnc.15243.
 77. Miki S, Takashima M, Suzuki JI. Anti-atherosclerotic effect of aged garlic extract: Mode of action and therapeutic benefits (Review). *Exp Ther Med*. 2025 Mar 26;29(5):104. doi: 10.3892/etm.2025.12854.
 78. Mohammad Soleymani S, Assarzagdegan F, Habibi SAH, Mahboubi A, Esmaily H. The effect of crocin on movement disorders and oxidative DNA damage in Parkinson's disease: Insights from a randomized controlled trial. *Parkinsonism Relat Disord*. 2024 Sep;126:107051. doi: 10.1016/j.parkreldis.2024.107051. Epub 2024 Jul 1.
 79. Mohd Zainal Abidin Shukri Y, Abd Rahim IN, Abdul Nasir NA, Osman CP, Mohd Kasim NA. Saffron's protective role against atherosclerosis-induced cataract progression in New Zealand white rabbits with phytochemical analysis of saffron's extract. *PLoS One*. 2025 Jan 14;20(1):e0315178. doi: 10.1371/journal.pone.0315178.

80. Mokhtarian R, Rajabi S, Zahedian S, Jafarinejad-Farsangi S, Hadizadeh M, Sadeghinejad M. The effect of saffron and its extracts on the treatment of breast cancer: A narrative review. *Ann Pharm Fr.* 2024 Jun;82(4):629-640. doi: 10.1016/j.pharma.2024.02.011. Epub 2024 Feb 16.
81. Mosaddad, Seyed & Hussain, Ahmed & Tebyaniyan, Hamid. (2023). Green Alternatives as Antimicrobial Agents in Mitigating Periodontal Diseases: A Narrative Review. *Microorganisms.* 11. 1269. 10.3390/microorganisms11051269.
82. Nomura T, Murase T, Ogita S, Kato Y. Molecular identification of tuliposide B-converting enzyme: a lactone-forming carboxylesterase from the pollen of tulip. *Plant J.* 2015 Jul;83(2):252-62. doi: 10.1111/tpj.12883. Epub 2015 Jun 12.
83. Özdemir O, Yılmaz N, Musatat AB, Demirci T, Çete S, Yerlikaya E, Kaya MO. Comprehensive experimental and computational analysis of endemic *Allium tuncelianum*: Phytochemical profiling, antimicrobial activity, and In silico studies for potential therapeutic applications. *Comput Biol Med.* 2025 May;189:109993. doi: 10.1016/j.combiomed.2025.109993. Epub 2025 Mar 7.
84. Puddu A, Nicolò M, Maggi DC. Combination of *Saffron (Crocus sativus)*, *Elderberry (Sambucus nigraL.)* and *Melilotus officinalis* Protects ARPE-19 Cells from Oxidative Stress. *Int J Mol Sci.* 2025 Feb 11;26(4):1496. doi: 10.3390/ijms26041496.
85. Qu EK, Yang ML, Liu SJ, Li HY, Huang ZL, Yang LR, Luo R, Liang YN, Zhang DB. Fritaipaines A - J, isosteroidal alkaloids with anti-neuroinflammatory activity from the bulbs of *Fritillaria taipaiensis*. *Fitoterapia.* 2025 Apr 15;183:106546. doi: 10.1016/j.fitote.2025.106546. Epub ahead of print.
86. Rahmatinia E, Amidi B, Naderi N, Ahmadipour S, Ahmadvand H, Pahlevan-Fallahy MT, Ghorbanzadeh V, Nazari A. Randomized, double-blind clinical trial evaluating the impact of freeze-dried garlic extract capsules on blood pressure, lipid profile, and nitric oxide levels in individuals at risk for hypertension. *Horm Mol Biol Clin Investig.* 2024 Jul 15;45(4):139-147. doi: 10.1515/hmbci-2024-0019.
87. Rajpoot A, Sharma V. Protective effects of *Allium sativum* essential oil against lead nitrate-induced cardiotoxicity: Modulation of lipid

- metabolism, nitric oxide dynamics, inflammatory mediators, and histological profiles in Swiss albino mice. *Toxicol Rep.* 2025 Feb 9;14:101950. doi: 10.1016/j.toxrep.2025.101950.
88. Rakshit D, Nayak S, Kundu S, Angelopoulou E, Pyrgelis ES, Piperi C, Mishra A. The Pharmacological Activity of Garlic (*Allium sativum*) in Parkinson's Disease: From Molecular Mechanisms to the Therapeutic Potential. *ACS Chem Neurosci.* 2023 Mar 15;14(6):1033-1044. doi: 10.1021/acchemneuro.2c00789. Epub 2023 Mar 1.
 89. Ramadaini T, Sumiwi SA, Febrina E. The Anti-Diabetic Effects of Medicinal Plants Belonging to the Liliaceae Family: Potential Alpha Glucosidase Inhibitors. *Drug Des Devel Ther.* 2024 Aug 13;18:3595-3616. doi: 10.2147/DDDT.S464100.
 90. Rameshk M, Sharififar F, Mehrabani M, Pardakhty A, Farsinejad A, Mehrabani M. Proliferation and In Vitro Wound Healing Effects of the Microniosomes Containing *Narcissus tazetta* L. Bulb Extract on Primary Human Fibroblasts (HDFs). *Daru.* 2018 Sep 12;26(1):31–42. doi: 10.1007/s40199-018-0211-7. Epub ahead of print.
 91. Ravindranath KJ, Srinivasan H. Regulating the expression of matrix metalloproteinases to inhibit ovarian carcinoma using isoquinoline alkaloid from *Allium ascalonicum*. *Sci Rep.* 2025 Mar 18;15(1):9228. doi: 10.1038/s41598-025-94111-0.
 92. Rezadoost MH, Kumleh HH, Ghasempour A. Cytotoxicity and apoptosis induction in breast cancer, skin cancer and glioblastoma cells by plant extracts. *Mol Biol Rep.* 2019 Oct;46(5):5131-5142. doi: 10.1007/s11033-019-04970-w. Epub 2019 Jul 17.
 93. Saadh MJ, Kariem M, Shukla M, Ballal S, Kumar A, Chahar M, Saini S, Kapila I, Hasaanzadeh S. Effects of aged garlic extract on blood pressure in hypertensive patients: A systematic review and meta-analysis of randomized controlled trials. *Prostaglandins Other Lipid Mediat.* 2024 Dec;175:106914. doi: 10.1016/j.prostaglandins.2024.106914. Epub 2024 Oct 20.
 94. Saikat, A.S.M., Hossain, R., Mina, F.B. *et al.* Antidiabetic Effect of Garlic. *Rev. Bras. Farmacogn.* **32**, 1–11 (2022). <https://doi.org/10.1007/s43450-021-00193-y>

95. Sarkar A, Paul S, Singh C, Chowdhury N, Nag P, Das S, Kumar S, Sharma A, Das DK, Dutta D, Thakur KG, Bagchi A, Shriti S, Das KP, Ringe RP, Das S. A novel plant lectin, NTL-125, interferes with SARS-CoV-2 interaction with hACE2. *Virus Res.* 2022 Jul 2;315:198768. doi: 10.1016/j.virusres.2022.198768. Epub 2022 Apr 7.
96. Shafiee A, Jafarabady K, Seighali N, Mohammadi I, Rajai Firouz Abadi S, Abhari FS, Bakhtiyari M. Effect of Saffron Versus Selective Serotonin Reuptake Inhibitors (SSRIs) in Treatment of Depression and Anxiety: A Meta-analysis of Randomized Controlled Trials. *Nutr Rev.* 2025 Mar 1;83(3):e751-e761. doi: 10.1093/nutrit/nuae076.
97. Shan X, Zhuang D, Gao R, Qiu M, Zhou L, Zhang J, Wang Y, Zhang Q, Zhai N, Xu G, Wang L, Li Y, Gao X. Molecular insights into TT2-type MYB regulators illuminate the complexity of floral flavonoids biosynthesis in *Freesia hybrida*. *Hortic Res.* 2024 Dec 12;12(3):uhae352. doi: 10.1093/hr/uhae352.
98. Shindo K, Iwamoto N, Usami M, Saito A, Sato M, Sugaya M, Miyashita N, Murahama M, Higashimura Y, Takemura M, Furihata K, Misawa N. 3'-Caffeoylquercetin Glycosides and 4'-Caffeoylkaempferol Glycosides- Novel Antioxidant Flavonoids Discovered in the Freesia Yellow Flowers. *Antioxidants (Basel).* 2025 Jan 28;14(2):158. doi: 10.3390/antiox14020158.
99. Siddiq A A, Dileep SA, Sj AR, Singam SSR, Martin A. Saffron and its active constituents ameliorate hypercholesterolemia by inhibiting PCSK9 and modulating Sortilin, LDLR, and SREBP-2 signaling in high fat diet induced hypercholesterolemic C57BL/6 mice. *J Ethnopharmacol.* 2025 Apr 25;346:119697. doi: 10.1016/j.jep.2025.119697. Epub 2025 Mar 27.
100. Simplicien M, Pério P, Sudor J, Barre A, Benoist H, Van Damme EJM, Rougé P. Plant lectins as versatile tools to fight coronavirus outbreaks. *Glycoconj J.* 2023 Feb;40(1):109-118. doi: 10.1007/s10719-022-10094-4. Epub 2022 Nov 24.
101. Singh S, Sharma K. Review on the Phytochemistry and Pharmacological Potential of Saffron (*Crocus sativus* L.). *Curr Top Med Chem.* 2025 Feb 3. doi: 10.2174/0115680266352428250130112654. Epub ahead of print.

102. Sleiman C, Daou RM, Al Hazzouri A, Hamdan Z, Ghadieh HE, Harbieh B, Romani M. Garlic and Hypertension: Efficacy, Mechanism of Action, and Clinical Implications. *Nutrients*. 2024 Aug 29;16(17):2895. doi: 10.3390/nu16172895.
103. Soheilifar MH, Dastan D, Masoudi-Khoram N, Keshmiri Neghab H, Nobari S, Tabaie SM, Amini R. In vitro and in vivo evaluation of the diabetic wound healing properties of Saffron (*Crocus Sativus* L.) petals. *Sci Rep*. 2024 Aug 21;14(1):19373. doi: 10.1038/s41598-024-70010-8.
104. Şelem, E., Nohutçu, L., Tunçtürk, R., & Tunçtürk, M. (2020). Bazı *Allium* Türlerinin Morfolojik Ölçümleri, Stoma ve Polen Özellikleri ile Polen Canlılığının Belirlenmesi. *Yüzuncu Yıl University Journal of Agricultural Sciences*, 30(Ek sayı (Additional issue)), 882-889.
105. Tallini LR, Machado das Neves G, Vendruscolo MH, Rezende-Teixeira P, Borges W, Bastida J, Costa-Lotufo LV, Eifler-Lima VL, Zuanazzi JAS. Antitumoral activity of different Amaryllidaceae alkaloids: In vitro and in silico assays. *J Ethnopharmacol*. 2024 Jul 15;329:118154. doi: 10.1016/j.jep.2024.118154. Epub 2024 Apr 12.
106. Tan J, Li F, Zhang X, Zhu H, Liu J, Wu T, Zhang Y, Zhang D, Geng Y, Shen Y. Extracts from petal of the *Crocus sativus* (saffron) possesses detoxification effects on acetaminophen induced liver injury by inhibiting hepatocyte apoptosis via regulating Nrf2/HO-1 signaling. *Fitoterapia*. 2025 Apr;182:106452. doi: 10.1016/j.fitote.2025.106452. Epub 2025 Feb 22.
107. Teimury A, Khaledi EM, Hosseini ES. Antioxidant, anti-inflammatory, and anti-apoptotic activities of saffron and eryngium honey extracts. *BMC Complement Med Ther*. 2025 Apr 10;25(1):131. doi: 10.1186/s12906-025-04867-1.
108. Tudu CK, Dutta T, Ghorai M, Biswas P, Samanta D, Oleksak P, Jha NK, Kumar M, Radha, Proćków J, Pérez de la Lastra JM, Dey A. Traditional uses, phytochemistry, pharmacology and toxicology of garlic (*Allium sativum*), a storehouse of diverse phytochemicals: A review of research from the last decade focusing on health and nutritional implications. *Front Nutr*. 2022 Oct 28;9:949554. doi: 10.3389/fnut.2022.929554. Erratum in: *Front Nutr*. 2024 Jul 29;11:1430483. doi: 10.3389/fnut.2024.1430483.

109. Uchikoba T, Okubo M, Arima K, Yonezawa H. Isolation and characterization of a cysteine protease of freesia corms. *Biosci Biotechnol Biochem*. 2002 Feb;66(2):448-52. doi: 10.1271/bbb.66.448.
110. Upadhyay S, Rajan Swami, Shrivastava S, Jeengar MK. Molecular insights into anti-inflammatory activities of selected Indian herbs. *J Ayurveda Integr Med*. 2025 Mar 27;16(2):101081. doi: 10.1016/j.jaim.2024.101081. Epub ahead of print.
111. URL-1 https://cargohandbook.com/index.php?title=File:Flower_bulbs_2.jpg. Accessed date: September 12 2025.
112. URL-1 <https://wmcresearch.substack.com/p/friday-hope-wonderful-alliums-cardiovascular>. Accessed date: October 06 2025.
113. Uzun, S. (2021). *Nezahat Gökyiğit Botanik Bahçesi'nde Yetişen Bazı Soğanlı Bitkiler Üzerinde Palinolojik Araştırmalar* (Master's thesis, Marmara Üniversitesi (Turkey)).
114. Vakili R, Zanghaneh A, Qharari F, Mortzavi F. Hydroalcoholic Extract of Saffron Petals, Yeast Cell Wall and Bentonite Reduce Contamination Effects With Aflatoxin B₁ and Ochratoxin A in Exposed Broilers. *Vet Med Sci*. 2025 Jan;11(1):e70122. doi: 10.1002/vms3.70122.
115. Wahyuni DS, van der Kooy F, Klinkhamer PG, Verpoorte R, Leiss K. The use of bio-guided fractionation to explore the use of leftover biomass in Dutch flower bulb production as allelochemicals against weeds. *Molecules*. 2013 Apr 17;18(4):4510-25. doi: 10.3390/molecules18044510.
116. Wang T, Li Z, Lei J, Zhang Y, Tong Y, Guan X, Wang S. RGD peptide-functionalized micelles loaded with crocetin ameliorate doxorubicin-induced cardiotoxicity. *Int J Pharm X*. 2025 Feb 26;9:100326. doi: 10.1016/j.ijpx.2025.100326.
117. Wang Y, Peng M, Yang X, Tu L, Liu J, Yang Y, Li R, Tang X, Hu Y, Zhang G, Zhao Q, Lu Q. Total alkaloids in *Fritillaria cirrhosa* D. Don alleviate OVA-induced allergic asthma by inhibiting M2 macrophage polarization. *J Ethnopharmacol*. 2025 Jan 30;337(Pt 3):118935. doi: 10.1016/j.jep.2024.118935. Epub 2024 Oct 11.
118. Wu H, Yang C, Chen J, Li S, Wei Y, Wang M, Li X, Wang Y, Kuang H, Wang Z. Simultaneous Determination and Pharmacokinetics of Three

- Steroidal Alkaloids in Rat Plasma by UHPLC-MS After Oral Administration of Three Different Preparations of *Fritillaria ussuriensis* Maxim Bulb. *Biomed Chromatogr.* 2025 Mar;39(3):e6087. doi: 10.1002/bmc.6087.
119. Xiao L, Lu H, Li J, Kong Q, Lan Y, Wang D. Preparation of biochar from constructed wetland plant and its adsorption performance towards Cu². *Environ Sci Pollut Res Int.* 2022 Jul;29(31):47109-47122. doi: 10.1007/s11356-022-18608-1. Epub 2022 Feb 17.
 120. Yang Z, Jin W, Luo Q, Li X, Wei Y, Lin Y. FhMYB108 Regulates the Expression of Linalool Synthase Gene in *Freesia hybrida* and *Arabidopsis*. *Biology (Basel).* 2024 Jul 23;13(8):556. doi: 10.3390/biology13080556.
 121. Yee M, Konopka K, Balzarini J, Düzgüneş N. Inhibition of HIV-1 Env-Mediated Cell-Cell Fusion by Lectins, Peptide T-20, and Neutralizing Antibodies. *Open Virol J.* 2011;5:44-51. doi: 10.2174/1874357901105010044. Epub 2011 May 12.
 122. Yi X, Zhang J, Yao Y, Liu J, Li M, Zhang M, Elmann A, Chen H. Organosulfur Compounds in Garlic for Gastric Cancer Treatment: Anticancer Effects, Overcoming Drug Resistance, and Mechanisms. *Recent Pat Anticancer Drug Discov.* 2025 Jan 29. doi: 10.2174/0115748928367900250116074925. Epub ahead of print.
 123. Yoo Y, Kim S, Lee W, Kim J, Son B, Lee KJ, Shin H. The prebiotic potential of dietary onion extracts: shaping gut microbial structures and promoting beneficial metabolites. *mSystems.* 2025 Jan 21;10(1):e0118924. doi: 10.1128/msystems.01189-24. Epub 2024 Dec 23.
 124. Yunita MN, Plumeriastuti H, Agustono B, Fahlefi MIR, Fadilla AR, Pangastutie RA, Sari SON, Syamsuri MF, Fajar MAP, Rahmaniar RP, Rahman A. The protective effects of garlic (*Allium sativum* var. *Solo Garlic*) extract mitigates piroxicam-induced liver damage and oxidative stress in rats. *Open Vet J.* 2025 Mar;15(3):1349-1357. doi: 10.5455/OVJ.2025.v15.i3.26. Epub 2025 Mar 31.
 125. Zengin G, Cetiz MV, Abul N, Gulcin I, Caprioli G, Piatti D, Ricciutelli M, Koyuncu I, Yuksekdog O, Bahşi M, Güler O, Aumeeruddy MZ, Mahomoodally MF. Establishing a link between the chemical

- composition and biological activities of *Gladiolus italicus* Mill. from the Turkish flora utilizing *in vitro*, *in silico* and network pharmacological methodologies. *Toxicol Mech Methods*. 2025 Feb;35(2):146-166. doi: 10.1080/15376516.2024.2397387. Epub 2024 Sep 8.
126. Zhang G, Zhu X, Yang F, Li J, Leng X, Mo C, Li L, Wang Y. Pseudolycorine chloride ameliorates Th17 cell-mediated central nervous system autoimmunity by restraining myeloid-derived suppressor cell expansion. *Pharm Biol*. 2022 Dec;60(1):899-908. doi: 10.1080/13880209.2022.2063344.
 127. Zhang JW, Zhao Q, Li Z, Liu Q, Zang SS, Liu S. The effect of saffron supplementation on liver and kidney function, blood glucose and pressure in patients with diabetes and prediabetes: A grade assessed systematic review and meta-analysis of randomized controlled trials. *Prostaglandins Other Lipid Mediat*. 2025 Mar;177:106949. doi: 10.1016/j.prostaglandins.2025.106949. Epub 2025 Jan 14.
 128. Zhang T, Gao J, Jin ZY, Xu XM, Chen HQ. Protective effects of polysaccharides from *Lilium lancifolium* on streptozotocin-induced diabetic mice. *Int J Biol Macromol*. 2014 Apr;65:436-40. doi: 10.1016/j.ijbiomac.2014.01.063. Epub 2014 Feb 6.
 129. Zhang X, Li X, Wang P, Zhao S, Zhao Y. Safranin restores RUNX3-mediated immunoregulation by inhibiting the NLRP3 inflammasome in allergic asthma. *Naunyn Schmiedeberg's Arch Pharmacol*. 2025 Mar 31. doi: 10.1007/s00210-025-03943-0. Epub ahead of print.
 130. Zhao C, Zhang J, Zhao Z, Li X, Chen X, Wang H, Ma Y, Han G, Yan Z. Integrating metabolomics, network pharmacology and pharmacological verification analysis provides new insights into the anti-inflammatory and anti-tussive properties of *Fritillaria cirrhosa* bulbs. *J Ethnopharmacol*. 2025 Mar 13;343:119460. doi: 10.1016/j.jep.2025.119460. Epub 2025 Feb 12.
 131. Zhao L, Wang L, Wang N, Gao X, Zhang B, Zhao Y, Wang N. Cooking Alters the Metabolites of Onions and Their Ability to Protect Nerve Cells from Lead Damage. *Foods*. 2024 Nov 20;13(22):3707. doi: 10.3390/foods13223707.
 132. Zhu C, Ke X, Gu Y, Wang C, Lin S, Qian Y, Cheng J, Chen Y, Xu L, Chen Z. Antimicrobial properties and preservation potential of *Allium sativum*

- L-derived extracellular vesicle-like particles for food applications. *Food Chem.* 2025 Aug 30;484:144419. doi: 10.1016/j.foodchem.2025.144419. Epub 2025 Apr 18.
133. Zhu J, Guo X, Li X, Tang D. Composition of Flavonoids in the Petals of *Freesia* and Prediction of Four Novel Transcription Factors Involving in *Freesia* Flavonoid Pathway. *Front Plant Sci.* 2021 Nov 15;12:756300. doi: 10.3389/fpls.2021.756300.
134. Zununi Vahed S, Zuluaga Tamayo M, Rodriguez-Ruiz V, Thibaudeau O, Aboulhassanzadeh S, Abdolalizadeh J, Meddahi-Pellé A, Gueguen V, Barzegari A, Pavon-Djavid G. Functional Mechanisms of Dietary Crocin Protection in Cardiovascular Models under Oxidative Stress. *Pharmaceutics.* 2024 Jun 21;16(7):840. doi: 10.3390/pharmaceutics16070840.
135. Zupkó I, Réthy B, Hohmann J, Molnár J, Ocsóvszki I, Falkay G. Antitumor activity of alkaloids derived from Amaryllidaceae species. *In Vivo.* 2009 Jan-Feb;23(1):41-8.

CHAPTER 12

PEANUT (*Arachis hypogaea* L.) BREEDING

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INTRODUCTION

Peanut (*Arachis hypogaea* L.) is recognized as one of the major legume species of strategic importance globally, both for its role in direct human nutrition and for its contributions to the oil industry. In addition to its high oil and protein content, its ability to perform symbiotic nitrogen fixation and its capacity to adapt to semi-arid climatic conditions have made this plant a valuable agricultural product, particularly in supporting rural development in developing countries (Sangh et al., 2025). Current data shows that approximately 38% of global peanut production is accounted for by China, 13% by India, 9% by Nigeria, 6% by the United States, and 3% by Senegal. These figures clearly demonstrate the dominant position of these five countries in total global production (USDA-FAS, 2024).

In recent years, approaches to peanut breeding have evolved beyond traditional selection and hybridization techniques to encompass a multidimensional framework that includes genetic mapping, genomic selection (GS), molecular marker-assisted selection (MAS), and gene editing technologies. In particular, the whole genome sequencing achieved under the International Peanut Genome Initiative (IPGI), completed in 2018, has provided a significant boost to breeding efforts by enabling the development of new genotypes that are resistant to biotic and abiotic stress conditions, early maturing, and of high quality (Zhao et al., 2025).

The main environmental stress factors threatening peanut production include drought, excessive heat, and soil-borne pathogens. Therefore, developing genotypes resistant to climate change is among the primary objectives of current breeding strategies. The use of such resilient varieties in water-stressed regions such as South Asia and Sub-Saharan Africa plays a critical role in ensuring regional food security (Sangh et al., 2025; Yu and Chen, 2024). Careful evaluation of genotype $\times$ environment interactions is a fundamental approach to achieving the desired performance. On the other hand, analyzing these interactions through multi-location trials has become an indispensable research strategy for enhancing the effectiveness of modern breeding programs by contributing to the selection of varieties with broad adaptability and stable yield potential (Yaşar and Sezgin, 2022; Thompson et al., 2024).

In breeding programs, improving quality parameters is one of the primary objectives alongside increasing yield. In particular, fatty acid composition plays a decisive role in terms of the product's shelf life, nutritional value, and suitability for industrial processing. Peanut varieties with high oleic acid content are preferred by both industry and consumers due to their longer shelf life and higher resistance to oxidative deterioration (Tang et al., 2022). In this context, the early detection of individuals with high oleic content, such as those carrying genes like *ahFAD2*, has become possible with the help of molecular marker systems and has been successfully integrated into breeding programs (Janila et al., 2016).

Biotechnological advances have paved the way for utilizing wild *Arachis* species in peanut breeding, thereby enabling the transfer of important genetic variation to cultivated species in terms of improving stress tolerance and quality traits. These applications have made important contributions, particularly in the development of complex traits such as disease resistance and tolerance to abiotic stress factors (Yu and Chen, 2024). Furthermore, pangenome analyses and systematic screening of structural genome variations enable the more precise and effective identification of new breeding targets (Zhao et al., 2025).

Peanut breeding today is not solely focused on increasing yields; it has become a multifaceted and advanced scientific field aimed at developing genotypes that can cope with climatic uncertainties, have high nutritional value, and are resistant to diseases and pests. In Türkiye, work in this field continues to grow, and molecular selection methods and genetic resource management practices are being actively pursued in line with global developments. In this regard, sustainable success will only be possible through the strengthening of scientific infrastructure, as well as the conservation of peanut genetic resources and long-term investment in breeding research.

Worldwide Peanut Improvement Activities: Institutions, Approaches, and Practices

The United States leads the world in the long-standing research history of peanut (*Arachis hypogaea* L.) breeding. Indeed, throughout nearly a century of breeding efforts in this country, the collection, preservation, characterization, and international sharing of genetic resources have been among the primary research priorities. The national peanut genetic resource collection located in

Griffin, Georgia, contains approximately 7,432 peanut lines, including lines derived from wild species. Specialist personnel are employed to ensure the sustainability of this collection.

Studies conducted in the US states of North Carolina, Oklahoma, and Texas aim to evaluate wild *Arachis* species as breeding material for cultivated varieties. Universities in Florida, Georgia, Oklahoma, North and South Carolina, Texas, New Mexico, Alabama, and Virginia, along with experimental stations affiliated with the Department of Agriculture and two private sector organizations, have registered numerous new peanut varieties. Approximately 99% of existing varieties have been developed by the USDA and universities, and this number increases steadily each year.

There are two main institutes conducting peanut research at the institutional level: the “Wheat, Peanut and Other Field Crops Research” unit in Oklahoma and the “National Peanut Research Laboratory” in Georgia. The US is the country where molecular techniques are most effectively applied in peanut breeding, and researchers are conducting numerous projects based on somatic embryogenesis and genetic engineering methods. In this context, peanut varieties resistant to the *Sclerotinia minor* pathogen have been developed through interspecific gene transfer. In recent years, breeding genotypes with high oleic acid content, in addition to disease and pest resistance, has become one of the main priorities; within this scope, numerous high-oleic varieties have been developed.

In the United States, producers, manufacturers, and processors are strongly organized under the American Peanut Council (APC), which comprises 13 producers, 44 manufacturers, 15 processors, and 112 affiliated members. These associations provide financial support for scientific projects conducted at research institutes (Isleib et al., 1994).

One of the focal points of global peanut research is the International Crops Research Institute for the Semi-Arid Tropics (ICRISAT), located in India and operating under the CGIAR system. Established in 1971 and adding peanuts to its research portfolio in 1976, this center has preserved approximately 15,870 genotypes, including wild species, and shared them with research institutions in different countries. Using methods such as hybridization, interspecific crossing, mutation breeding, and molecular marker-assisted selection (MAS) within ICRISAT, lines tolerant to biotic and abiotic

stresses, early maturing, high-yielding, and high in oleic acid content have been developed; these lines have been registered and disseminated in African and Asian countries. In addition, the Indian Ministry of Agriculture and universities actively contribute to peanut research (Anonymous, 2021b).

China, the world's largest peanut producer, has been home to the Peanut Research Institute in Shandong province since 1959. In addition, intensive breeding work is also being carried out at the Oil Crops Research Institute in Wuhan, central China, and the Industrial Crops Research Institute in Guangdong, southern China. These centers preserve a total of approximately 4,350 genotypes, 30 of which belong to wild species. Thanks to the collaborations between these domestic institutes and ICRISAT in India, genotypes with high oil, oleic acid, and protein content, as well as tolerance to biotic and abiotic stresses, have been developed. China has become the country with the most intensive use of molecular techniques in peanut breeding after the United States.

In Australia, peanut breeding is led by The Peanut Company of Australia (PCA), and new lines with characteristics such as earliness, drought tolerance, high yield, and high oleic content have been developed.

In South America, the genetic center of peanuts, joint breeding efforts were initiated in 1990 under the PROMANI cooperation program established by Argentina, Brazil, Bolivia, Paraguay, and Uruguay. Argentina and Brazil began implementing their own peanut breeding programs in the 1980s; the development of varieties with high oleic acid content has made a significant contribution to exports, particularly in Argentina.

In Africa, peanut breeding was initiated by British and French authorities during the colonial period; however, after independence, these efforts were disrupted for various reasons. Thanks to programs that have gained momentum in recent years with technical support from ICRISAT, genotypes resistant to peanut rosette virus, in particular, have been developed.

Finally, peanut breeding activities are actively pursued in countries such as Japan, Korea, Pakistan, Thailand, Vietnam, Indonesia, and the Philippines, just as they are in Türkiye (Isleib et al., 1994).

The Development and Current Status of Peanut Breeding Studies in Türkiye

The first systematic attempt at peanut (*Arachis hypogaea* L.) breeding in Türkiye was carried out in 1908 at the Istanbul Halkalı Agricultural School, and this date is considered the starting point for modern peanut breeding efforts in our country. Following this pioneering initiative, breeding activities gained significant momentum through trials conducted at the Western Mediterranean Agricultural Research Institute (BATEM). As a result of these studies conducted at BATEM, a total of six peanut varieties were registered: NC-7, BATEM-5025, Çom, Gazipaşa, BATEM-Cihangir, and Florispan (Kadiroğlu, 2022).

Nearly all of the varieties registered in Türkiye belong to the Virginia type of snack peanuts, with only the Florispan variety, developed in 1986 using introduction methods, standing out as a Spanish type oil peanut variety. This situation demonstrates that breeding efforts aimed at developing oil peanut varieties in our country have been limited.

In addition, peanut breeding efforts in Türkiye have not been limited to public research institutes; universities have also contributed significantly to this process. In particular, scientific research conducted by the Field Crops Department of the Faculty of Agriculture at Çukurova University has resulted in the successful registration of five different peanut varieties. Furthermore, the work carried out by the Eastern Mediterranean Agricultural Research Institute, which has been operating in Adana since 1978, is noteworthy for its use of both classical selection and modern breeding techniques.

Within this framework, the Ayşe Hanım variety developed in 2018 using selection breeding methods, the Adanur variety obtained in 2023 using hybridization methods, and the Albenek variety developed in 2023 using selection breeding methods have been submitted for registration as candidate varieties. In the same year, the Ecesoy variety, developed through mutation breeding, was also evaluated for candidate status. These developments demonstrate that peanut breeding in Türkiye has gained diversity based on different technical approaches.

The research capacity for peanut breeding has been further strengthened with the establishment of the Oilseeds Research Institute in Osmaniye in 2013.

This institution contributes to breeding studies both independently and in collaboration with other research centers.

Peanut oil, like other vegetable oils, is sensitive to oxidation due to its unsaturated fatty acid content. An increase in the unsaturation ratio, particularly high levels of linoleic acid, reduces the oil's oxidative stability and limits its shelf life. Conversely, increasing the oleic acid ratio and reducing linoleic acid lowers the iodine value, thereby improving the oil's stability and extending the product's shelf life (Kadiroğlu, 2015).

The development of peanut varieties with high oleic acid content is among the priority breeding objectives of both the Eastern Mediterranean Agricultural Research Institute and the Western Mediterranean Agricultural Research Institute Directorate (BATEM), and significant achievements have been made within the framework of inter-institutional cooperation.

The Use of Molecular Techniques in Peanut Breeding

In recent years, rapid scientific advances in molecular biology and genetic engineering have made significant contributions to the discipline of plant breeding; in particular, the effective use of DNA-based analyses has enabled the development of new approaches that support classical breeding processes. In this context, various molecular marker systems such as restriction fragment length polymorphism (RFLP), randomly amplified polymorphic DNA (RAPD), amplified fragment length polymorphism (AFLP), microsatellite (SSR), and single nucleotide polymorphism (SNP) have been developed and applied in peanut (*Arachis hypogaea* L.) breeding.

Molecular markers are defined as DNA sequences located in specific regions of the genome that may be directly related to genes or simply indicate the presence of that region; they are the DNA-based equivalent of classical genetic markers. Although these markers do not carry any biological function, their use as fixed reference points in genetic mapping and selection processes offers significant advantages. By revealing DNA sequence differences between genotypes, molecular markers enable the determination of degrees of kinship among breeding materials; thus, unnecessary duplications are eliminated, and parent selection in breeding programs can be performed more effectively. Furthermore, the ability to identify targeted traits, such as high oleic acid

content, in early generations significantly increases selection efficiency (Yorgancılar et al., 2015).

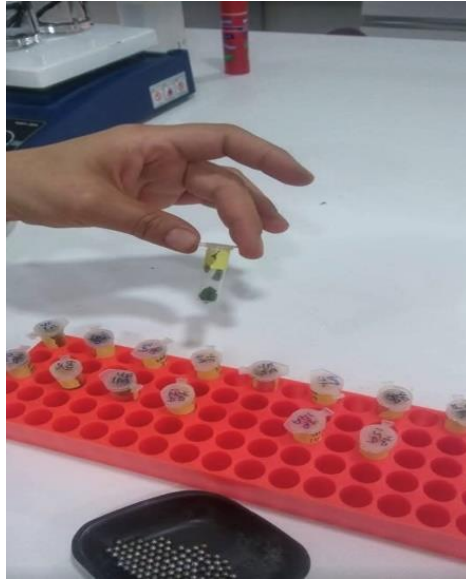


Figure 1. Preparation of peanut leaf samples with steel balls prior to grinding for DNA isolation.

Molecular marker-assisted selection (MAS) is considered a complementary and accelerating component of the classical breeding process, providing significant advantages, particularly in the breeding of quantitatively complex traits influenced by environment-genotype interactions. Thanks to molecular techniques, the risk of transferring undesirable traits during gene transfer from wild species can be reduced, and time and resources can be saved by eliminating undesirable genotypes in early stages of plant development. Furthermore, one of the most important advantages of these techniques is that selection based on genotypic information can be performed without the need to repeat phenotypic observations in each generation.

The first markers used in peanuts, alongside isoenzymes and storage proteins, revealed limited variation among cultivated peanut varieties. Therefore, more advanced marker systems such as RFLP, RAPD, and AFLP were adopted; however, these systems also failed to provide sufficient information in peanuts, where genetic variation is limited (Rami et al., 2018). Nevertheless, RFLP-based linkage maps have played an important role in the

preparation and identification of regions associated with resistance genes. For example, markers associated with resistance to diseases such as nematodes and leaf spot have been identified during the transfer of genes from *A. cardenasii* to cultivated varieties (Holbrook & Stalker, 2003).

Microsatellite (SSR) markers have enabled broader detection of polymorphism in the peanut genome due to their codominant, multiallele, and PCR-based nature, making them widely applicable in this tetraploid species. To date, more than 15,000 SSR markers have been developed; transposon-enriched SSR markers created by Shirasawa and colleagues, in particular, have made significant contributions by exhibiting higher polymorphism. In addition, although SNP markers are theoretically the most common marker type, the polyploid structure of peanuts has limited the use of these markers.



Figure 2. Phase separation is observed in the tube after DNA isolation (phenol-chloroform extraction).

In 2018, with the completion of the peanut genome map by the IPGI (International Peanut Genome Initiative), numerous QTL regions associated with disease resistance and quality traits were identified, and reliable markers were developed, particularly for the *ahFAD2* alleles controlling root-knot nematode resistance and high oleic acid synthesis. The NemaTAM variety

developed using integrated MAS in the US, followed by the Tifguard and Tifguard High O/L varieties, are successful examples of this process.

Marker-assisted backcrossing (MABC) is used in the development of varieties resistant to leaf rust and late leaf spot diseases through studies conducted at the International Crops Research Institute for the Semi-Arid Tropics (ICRISAT); Varieties such as ICGV-62 91114 have been obtained through QTLs with high phenotypic variation explanatory power (Janila et al., 2013).

In Türkiye, the Eastern Mediterranean Agricultural Research Institute simultaneously characterizes peanut genetic resources at both morphological and molecular levels, effectively utilizing molecular techniques across multiple stages—from kinship analyses to selection strategies. Molecular analyses are particularly effective in identifying individuals with high oleic content in F2 generations after hybridization, yielding 100% accurate results. This approach is considered a significant milestone in peanut breeding.

Issues Encountered in Peanut Breeding

The vegetation period for Virginia-type snack peanuts of the *Arachis hypogaea* species, which are widely cultivated in Türkiye, ranges from 140 to 150 days from planting to harvest. This long growing season makes it possible to produce this crop on an economic scale only in the Mediterranean climate zone, thus seriously limiting its adaptation area throughout the country. In terms of growing period, peanuts mature approximately one month later than soybeans and one and a half months later than sesame, which are other oilseed crops grown in our country. This causes the harvest period to coincide with autumn rains, thereby negatively affecting both yield and quality. Therefore, breeding programs aimed at developing early-maturing varieties should be prioritized. However, since earliness is a trait controlled by the complex interaction of many genetic and environmental factors, breeding for this trait in peanuts is quite difficult. The morphological characteristics of peanuts, such as their distinctly indeterminate growth habit, their tendency to maintain their herbaceous structure even at harvest time, and the formation of their fruits underground, make it difficult to accurately determine the earliness levels of varieties.

On the other hand, another important issue that directly affects peanut yield is soil-borne diseases. These diseases have been studied less than leaf diseases, largely because they are often localized in their spread; clearly, more breeding work is needed in this area. However, limited studies have identified genetic variations that confer resistance to these diseases (Middleton et al., 1994).

As is the case globally, drought is one of the biggest environmental stress factors threatening agricultural production in Türkiye. Declining rainfall amounts and rising temperatures associated with climate change, coupled with a growing human population, are further limiting the already scarce water resources available for agricultural irrigation. Therefore, the efficient and sustainable use of existing water resources is of great importance; in this context, the breeding of drought-resistant varieties has become inevitable. Although peanuts are generally a species that can tolerate relatively dry conditions, they are particularly sensitive to water stress during flowering and fruit setting. Water shortages occurring close to harvest time can cause not only yield losses but also significant quality problems such as aflatoxin formation. Peanut cultivation in Türkiye is not economically sustainable without adequate irrigation. Even imbalances in irrigation timing and quantity can cause yield losses.

Traits such as drought resistance or tolerance pose a challenge to classical breeding approaches due to their polygenic nature and complex interactions with the environment. Therefore, yield-based selection is preferred in developing varieties that will provide stable yields under dry conditions (Leal-Bertioli et al., 2012). In drought resistance breeding, the simultaneous evaluation of early maturity (precipitation) and drought tolerance mechanisms is of great importance. The early maturity trait allows escape from late-season drought, enabling the plant to be less exposed to drought stress during critical growth stages.

CONCLUSION

Peanuts (*Arachis hypogaea* L.) hold a strategic position not only in agricultural production but also in rural development and food security due to their high nutritional value, adaptability to semi-arid climatic conditions, and contribution of biological nitrogen to agricultural systems. Parallel to this

multifaceted agricultural importance, peanut breeding has become a multidisciplinary field of science in recent years, integrating molecular biology, genetic engineering, and classical breeding techniques at a global level.

Today, the main factors threatening peanut production include biotic and abiotic stress conditions, climate change, the prevalence of diseases and pests, the length of the production season, and deficiencies in quality parameters. In this regard, breeding efforts are not only focused on developing high-yielding varieties but also target characteristics such as high oleic acid content, drought tolerance, resistance to diseases and pests, earliness, and broad adaptability. Quality parameters such as oleic acid content are among the factors that directly affect the shelf life and commercial value of the product and are therefore one of the primary targets of modern breeding programs.

The success of breeding efforts is largely proportional to the diversity of genetic resources and the effective management of these resources. Countries such as the United States, India, China, Brazil, and Argentina have established national collections of peanut-specific genetic material; the evaluation of these materials has led to the registration of numerous new varieties. Long-term research conducted in collaboration between universities, ministries of agriculture, and private sector organizations in these countries has made significant contributions to the breeding process. Varieties developed in the United States, such as NemaTAM and Tifguard, in particular, clearly demonstrate the success of integrated molecular marker-assisted breeding programs.

In Türkiye, peanut breeding efforts, which in the past relied heavily on classical methods, have accelerated the integration of molecular techniques in recent years thanks to studies led by institutions such as the Eastern Mediterranean Agricultural Research Institute, the Western Mediterranean Agricultural Research Institute, and Çukurova University. In particular, the identification and selection of lines with high oleic acid content in early generations has become an important part of modern breeding in Türkiye.

The development of molecular marker systems (RFLP, SSR, SNP, RAPD, AFLP) in peanuts offers significant advantages for both characterization and selective breeding applications. These techniques enable more accurate determination of the kinship relationships between genotypes, better understanding of the genetic mechanisms underlying complex traits, and

significant reduction in the time and cost of classical selection processes. Marker systems with high informative power, such as SSR and SNP, have facilitated the identification of new variations in species with low genetic diversity, such as peanuts.

Resilience to drought stress is another strategic area that requires intense focus in peanut breeding. Given the increasing water constraints and the effects of climate change, the development of early-maturing varieties and, in particular, the enhancement of drought tolerance specific to the fruit-setting period are critical for the sustainable production of peanuts. However, the polygenic nature of these traits complicates selection processes; therefore, the contribution of molecular techniques in this area is becoming increasingly important.

On the other hand, molecular breeding approaches are also yielding promising results in developing resistance to diseases and pests. The peanut genome map completed by the IPGI (International Peanut Genome Initiative) has enabled the more effective identification of resistance gene regions (QTLs) against harmful factors such as root-knot nematodes, leaf spot disease, and tomato spotted wilt virus. The ability to identify such traits in early generations through marker-assisted selection saves time and resources.

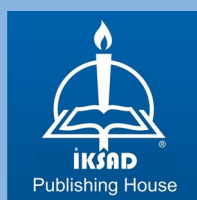
As a result, peanut breeding is no longer solely based on classical methods; it has become a multifaceted process requiring advanced scientific infrastructure, conducted through the integration of advanced techniques such as genetic engineering, genomic selection, molecular marker-assisted breeding, and bioinformatics. Türkiye's close monitoring of scientific developments in global breeding efforts will enhance the competitiveness of varieties developed at the national level in both domestic and international markets.

To ensure the sustainability of this success, it is crucial to establish national programs for the conservation of peanut genetic resources, strengthen molecular laboratory infrastructure, and invest in qualified human resources. Through collaborations with national and international research centers, Turkey can become a more effective actor in peanut breeding. With the awareness that peanuts are not only a food product but also a strategic agricultural resource, work in this area must be pursued with determination.

REFERENCES

- Anonymous, (2011). Peanut Company of Australia. <https://www.pca.com.au/wpcontent/uploads/2016/11/tingoora.pdf> Date accessed: 12.10.2024.
- Anonymous, (2018). www.peanutusa.com/phoadomload/Genome, Erişim tarihi: 12.10.2024.
- Holbrook, C.C., Stalker, H.T. (2003). Peanut breeding and genetic resources. U.S. Department of Agriculture–ARS P.O. Box 748 Tifton, Georgia 31793. Department of Crop Sciences North Carolina State University P.O. Box 7620 Raleigh, North Carolina 27695.
- Isele T.G., Wynne J.C., Nigam, S.N. (1994). Groundnut breeding. In: The Groundnut Crop. Department of Biology, Southampton University, UK. Ed.: J. Smartt. 558-559 pp.
- Janila, P., Nigam, S.N., Pandey, M.K., Nagesh, P., Varshney, R.K. (2013). Groundnut improvement: use of genetic and genomic tools. *Frontiers in Plant Science*, Volume 4, Article 23.
- Janila, P., Variath, M. T., Pandey, M. K., Desmae, H., Motagi, B. N., Okori, P., and Varshney, R. K. (2016). Genomic tools in groundnut breeding program: status and perspectives. *Frontiers in Plant Science*, 7, 289.
- Kadiroğlu, A. (2015). Dünyada yüksek oleik asit içeren yerfıstığına talep giderek artmaktadır. *Harman Time*, 34:104-110. (In Turkish)
- Kadiroğlu, A. (2018). Yerfıstığı yetiştiriciliği. Batı Akdeniz Tarımsal Araştırma Enstitüsü Müdürlüğü Antalya, <https://arastirma.tarimorman.gov.tr/batem/Menu/57/Teknik-Bilgiler>, Date accessed: 12.10.2024. (In Turkish)
- Kadiroğlu, A. (2022). Yerfıstığı Islahı. Batı Akdeniz Tarımsal Araştırma Enstitüsü Müdürlüğü Antalya, 2022. (In Turkish)
- Leal, S.C.M., Bertioli, D.J., Guimarães, P.M., Pereira, T.D. (2012). The effect of tetraploidization of wild *Arachis* on leaf morphology and other drought-related traits. *Environ. Exp. Bot.*, 84:17–24.
- Middleton, K.J., Pande, S., Sharma, S.B., Smith, D.H. (1994). Diseases. Chapter 10. In: *The Groundnut Crop*, Ed.: Smartt J. 336 pp.
- Rami, J.F., Soraya, C.M., Bertioli, L., Fonceka, D., Moretzsohn, M.C. (2018). Groundnut, Chapter 12, Avenue Agropolis, Montpellier 34398 , France.

- Sangh, C., Mallikarjuna, M. G., Pandey, M. K., Mondal, T. K., Radhakrishnan, T., Tomar, R. S., ... & Bera, S. K. (2025). Breeding Climate-Resilient Groundnut in the Climate Change Era: Current Breeding Strategies and Prospects. In *Breeding Climate Resilient and Future Ready Oilseed Crops* (pp. 265-301). Singapore: Springer Nature Singapore
- USDA-FAS (2024). Peanut explorer. *USDA-NAS*. <https://ipad.fas.usda.gov/cropexplorer/cropview/commodityView.aspx?cropid=2221000> (Erişim tarihi: 12.10.2024).
- Tang, Y., Qiu, X., Hu, C., Li, J., Wu, L., Wang, W., ... & Qiao, L. (2022). Breeding of a new variety of peanut with high-oleic-acid content and high-yield by marker-assisted backcrossing. *Molecular Breeding*, 42(7), 42.
- Thompson, E., Wang, H., Korani, W., Fountain, J. C., Culbreath, A. K., Holbrook, C. C., ... & Guo, B. (2025). Genetic and genomic characterization of a multiparent advanced generation intercross (MAGIC) population of peanut (*Arachis hypogaea* L.). *Crop Science*, 65(1), e21402.
- Yasar M, Sezgin M, (2022). Investigation of Genotype x Environment Interactions by AMMI Analysis of Oilseed Sunflower Genotypes Grown in Different Environmental Conditions. *Journal of the Institute of Science and Technology*, 12(4): 2532 - 2542.
- Yorgancılar, M., Yakışır, E., Erkoyuncu, M.T. (2015). Moleküler markörlerin bitki ıslahında kullanımı. *Bahri Dağdaş Bitkisel Araştırma Dergisi*, *Journal of Bahri Dagdas Crop Research*, 4(2):1-12. (In Turkish)
- Yu, D., ve Chen, S. (2024). Harnessing Genetic Diversity in Peanut for Enhanced Crop Performance. *Legume Genomics and Genetics*, 15.
- Zhao, K., Xue, H., Li, G., Chitikineni, A., Fan, Y., Cao, Z., Yin, D. (2025). Pangenome analysis reveals structural variation associated with seed size and weight traits in peanut. *Nature genetics*, 57(5), 1250-1261.



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