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Phytochemical diversity and nutraceutical potential in biointensive complementary cropping systems under garden-land ecosystems

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Abstract

The growing global burden of micronutrient deficiencies and diet-related chronic diseases has intensified the need to transition from yield-oriented agriculture to nutrition-sensitive and health-centered food systems. Biointensive complementary cropping systems (BICCS) practiced in garden-land ecosystems offer a promising approach to enhance phytochemical diversity and nutraceutical quality while maintaining ecological sustainability. This review synthesizes current knowledge on the conceptual, biochemical, ecological and analytical dimensions linking diversified cropping systems with phytochemical expression and functional food potential. The paper examines the mechanistic basis of secondary metabolite modulation through plant metabolic pathways, including phenylpropanoid, terpenoid and alkaloid biosynthesis and highlights how ecological drivers such as light quality, soil-plant-microbiome interactions, nutrient dynamics and moderate stress conditions influence phytochemical accumulation. Special emphasis is placed on phenolics, flavonoids, tannins, anthocyanins, terpenoids, alkaloids, saponins, carotenoids and organosulfur compounds, which collectively contribute to antioxidant, anti-inflammatory, antidiabetic, cardioprotective, antimicrobial and anticancer properties. The role of rhizosphere engineering, mycorrhizal associations, plant growth promoting rhizobacteria and organic inputs in modulating secondary metabolism is critically discussed. Analytical approaches ranging from conventional extraction and chromatographic techniques to advanced metabolomics are reviewed to provide methodological guidance for assessing phytochemical diversity in diversified systems. The review also identifies key constraints, including variability in metabolite expression, lack of standardization, limited market integration and insufficient policy support. Future directions emphasize omics-driven systems biology, breeding for phytochemical-rich varieties, climate-resilient nutraceutical agriculture and integration with functional food industries. Overall, biointensive complementary cropping systems in garden-land ecosystems emerge as integrative models that unite biodiversity conservation, soil health enhancement, ecosystem services and nutraceutical production. By linking agroecological design with metabolic optimization, these systems offer a sustainable pathway toward health-oriented agriculture capable of addressing both environmental and nutritional challenges in the twenty-first century.

1. Introduction

Modern agriculture stands at a critical crossroads. Although, global food production has increased significantly over the past half century, improvements in caloric availability have not translated into proportional gains in nutritional well-being. Large segments of the global population continue to suffer from micronutrient deficiencies, while others face rising incidences of obesity, diabetes, cardiovascular disorders and other non-communicable diseases linked to poor dietary quality. This paradox reveals a structural limitation of production systems that prioritize quantity over nutritional integrity. The contemporary challenge is therefore not simply to produce more food, but to produce food that nourishes, protects and promotes long-term health. Nutrient-dense food systems emphasize the availability of diverse foods rich in essential vitamins, minerals, dietary fiber and bioactive compounds. Among these, plant-derived phytochemicals have emerged as crucial contributors to health promotion. Once regarded merely as secondary metabolites with limited agronomic relevance, phytochemicals are now recognized for their antioxidant, anti-inflammatory, antimicrobial, anticancer, antidiabetic and cardioprotective properties. Compounds such as phenolic acids, flavonoids, carotenoids, glucosinolates, alkaloids and terpenoids play significant roles in modulating oxidative stress, immune responses and metabolic regulation in humans. Their presence in fruits, vegetables, spices, medicinal plants and underutilized crops underscores the importance of diversified agricultural systems in enhancing dietary quality.

Historically, agricultural development strategies have largely focused on maximizing yields of staple cereals through monocropping, improved varieties, irrigation expansion and synthetic inputs. While these strategies improved food security in quantitative terms, they also simplified agroecosystems and reduced on-farm biodiversity.

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Simplified systems tend to produce uniform outputs but may lack the nutritional diversity associated with mixed cropping and horticultural integration. As consumer awareness of functional foods increases, there is a growing demand for crops that provide health benefits beyond basic nutrition. This demand has catalyzed the expansion of the nutraceutical sector, encouraging research on cultivation practices that can enhance the bioactive content of crops. The transition from yield-centered to nutrition-centered agriculture reflects a broader shift in societal priorities. Success is no longer defined solely by output per unit area, but also by the capacity of farming systems to deliver nutritionally rich, safe and environmentally sustainable food. This shift has stimulated interest in agroecological approaches, sustainable intensification and biodiversity-based production systems. Diversified cropping systems are increasingly recognized not only for their ecological benefits but also for their potential to enhance the phytochemical composition of crops. Environmental conditions strongly influence plant metabolic processes. Secondary metabolites are often synthesized in response to environmental cues such as light intensity, temperature fluctuations, water availability and biotic interactions (Sogoni *et al*., 2021). Moderate stress can stimulate the production of defense-related compounds, leading to increased concentrations of antioxidants and other bioactives. Thus, cropping systems that create dynamic and heterogeneous microenvironments may inadvertently promote phytochemical enrichment. Understanding these interactions provides an opportunity to design agricultural systems that align ecological function with human health outcomes.

Garden-land ecosystems represent small-scale, highly diversified production systems that integrate multiple plant species within a limited spatial area. These systems are commonly found in tropical and subtropical regions, where climatic conditions support year-round cultivation of diverse crops. Managed primarily by smallholder households, garden lands often combine fruit trees, vegetables, tubers, medicinal plants, spices, pulses and ornamental species in multilayered arrangements. The result is a complex and resilient agroecosystem that supports continuous harvests and dietary diversity. A defining feature of garden-land ecosystems is structural diversity. Vertical stratification allows tall trees to coexist with understory shrubs and herbaceous crops, maximizing light interception and reducing unused ecological niches. Horizontal diversity further enhances productivity by distributing species across different microhabitats. Root systems vary in depth and architecture, enabling efficient exploitation of soil nutrients and water (Aæimoviæ *et al*., 2025). Such complementarity minimizes direct competition while enhancing total biomass production. In many tropical regions, garden lands function as a primary source of household nutrition. Fresh produce harvested daily supplies vitamins, minerals and phytochemicals essential for balanced diets. These systems often include indigenous and underutilized crops that possess unique bioactive profiles. Traditional leafy vegetables, tubers and medicinal plants are frequently rich in phenolics, flavonoids and other secondary metabolites that contribute to disease prevention. By conserving local plant genetic resources, garden lands also preserve valuable biochemical diversity.

Socially, garden-land ecosystems empower rural households, particularly women, who often manage these systems. Income generated from surplus produce supports household livelihoods, while the availability of diverse foods enhances food sovereignty. Environmentally, these systems contribute to soil health through organic matter recycling, composting and minimal chemical input use. High biodiversity levels promote natural pest regulation and pollinator activity, reducing dependence on synthetic pesticides. The ecological interactions within garden-land systems are intricate. Plant-plant interactions may involve facilitation, resource partitioning, allelopathy, or induced resistance. Soil microorganisms form symbiotic associations with plant roots, enhancing nutrient uptake and influencing metabolic pathways. Such interactions can shape secondary metabolite production. For example, mycorrhizal colonization has been associated with enhanced phenolic synthesis in certain crops. Similarly, exposure to mild interspecific competition may stimulate defensive metabolite accumulation. Therefore, garden-land ecosystems provide a dynamic context in which phytochemical diversity can be naturally amplified. From a sustainability perspective, these systems embody the principles of resilience and circularity. Nutrient recycling, diversified species composition and reduced reliance on external inputs make garden lands adaptable to climatic variability. Their capacity to integrate ecological complexity with food production positions them as promising models for nutrition-sensitive and climate-resilient agriculture.

Biointensive complementary cropping systems represent an advanced strategy for harnessing biodiversity to improve productivity, ecological balance and crop quality. The term “biointensive” emphasizes the reliance on biological processes rather than synthetic inputs to sustain soil fertility and crop performance. Compost application, green manuring, biological nitrogen fixation and microbial inoculants are central components of such systems. Complementary cropping refers to the deliberate combination of species with distinct functional traits to optimize resource use. Complementarity occurs when crops utilize resources differently in space or time. For instance, a deep-rooted species may access subsoil nutrients that shallow-rooted crops cannot reach. Leguminous plants can fix atmospheric nitrogen, benefiting neighboring non-legumes. Crops with contrasting canopy architectures can capture sunlight at different heights, increasing overall photosynthetic efficiency. Temporal complementarity, such as relay cropping, allows successive crops to exploit residual nutrients and moisture. By designing cropping patterns that emphasize complementarity, farmers can reduce competition and enhance system productivity (Ebrahimi *et al*., 2026). Ecological intensification is a guiding principle of BICCS. Instead of increasing yields through greater chemical input use, ecological intensification seeks to optimize ecosystem services such as nutrient cycling, biological pest control and soil microbial activity. Diverse plant assemblages foster beneficial insect populations and suppress pest outbreaks. Root exudates influence microbial communities, which in turn affect nutrient availability and plant health.

These biological interactions may also influence phytochemical expression. Plants synthesize secondary metabolites as adaptive responses to environmental stimuli. In diversified systems, interactions among species, soil microbes and microclimatic variations can trigger signaling pathways that regulate phenolic, terpenoid and alkaloid biosynthesis. Mild competition or shading may alter light quality, affecting flavonoid accumulation. Root interactions may induce allelochemical production. Organic nutrient sources may modulate carbon-nitrogen balance, influencing metabolite partitioning. Importantly, BICCS align productivity with sustainability. By reducing reliance on synthetic fertilizers and pesticides, these systems minimize environmental contamination and support soil biodiversity. Enhanced soil organic matter improves water retention and resilience to drought. The integrated management of crops within complementary frameworks contributes to stable yields while potentially enhancing nutritional quality. While the agronomic and ecological benefits of diversified cropping systems are increasingly recognized, their influence on phytochemical diversity and nutraceutical potential remains insufficiently synthesized (Soppelsa *et al*., 2025). Most research on intercropping and biointensive agriculture emphasizes yield, soil fertility, or pest management outcomes. Comparatively fewer studies systematically examine how cropping system design affects secondary metabolite profiles and health-related attributes of produce.

Phytochemical diversity is central to the emerging concept of food as medicine. Variations in agricultural practices can significantly influence the concentration and composition of bioactive compounds. Factors such as soil fertility management, plant density, light exposure and species interactions all affect metabolic pathways. Therefore, cropping systems represent an underutilized lever for enhancing the nutraceutical value of crops without genetic modification. Garden-land ecosystems, characterized by structural and species diversity, provide an ideal context for exploring these relationships. Their integration of multiple functional groups, reliance on organic inputs and dynamic microenvironments may foster conditions conducive to enhanced secondary metabolism. Yet comprehensive reviews linking biointensive complementary cropping with phytochemical outcomes are limited. This review aims to bridge that gap by synthesizing current knowledge on how diversified, biointensive systems within garden-land ecosystems influence phytochemical diversity and nutraceutical quality. It seeks to integrate insights from agronomy, plant physiology, soil science and nutrition to develop a holistic understanding of biodiversity-based production systems. By identifying knowledge gaps and research priorities, the review intends to support the development of agricultural models that simultaneously address ecological sustainability, nutritional security and human health.

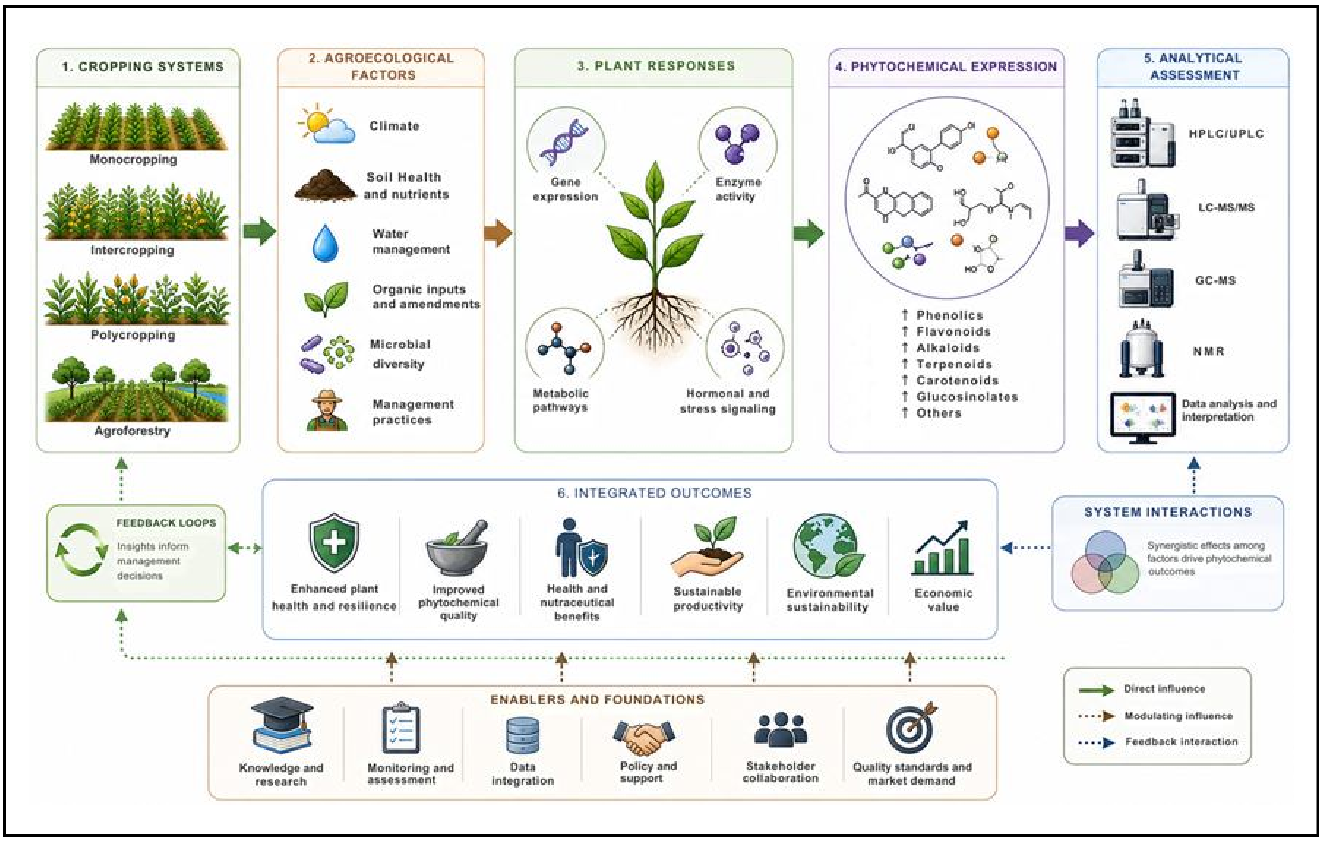

Figure 1: Framework illustrating the interaction between cropping systems and phytochemical modulation.

2. Conceptual framework: Linking cropping systems and phytochemical expression

Understanding how cropping systems influence phytochemical composition requires an integrative framework that connects plant metabolic processes with ecological interactions in the field. Plants are not passive entities; they continuously perceive and respond to environmental cues through complex biochemical networks. These responses are expressed through changes in growth, physiology and secondary metabolite production. In diversified agroecosystems particularly biointensive complementary cropping systems plants experience dynamic microenvironments shaped by neighboring species, soil biota, nutrient flows and fluctuating stress levels. Such ecological complexity can significantly influence the synthesis and accumulation of phytochemicals. This section presents a conceptual foundation for linking cropping system design with plant secondary metabolism, emphasizing the biological pathways and ecological drivers that shape phytochemical diversity (Figure 1).

2.1 Plant secondary metabolism overview

Plant metabolism can be broadly categorized into primary and secondary metabolic processes. Primary metabolism encompasses the essential biochemical pathways necessary for growth, development and reproduction. These include photosynthesis, respiration, carbohydrate metabolism, protein synthesis and lipid biosynthesis. Primary metabolites such as sugars, amino acids, nucleotides and fatty acids are directly involved in energy production and structural formation. Without these compounds, plant survival is impossible. Secondary metabolism, in contrast, produces compounds that are not directly required for basic cellular survival but confer adaptive advantages. These metabolites function in defense, signaling, competition, pollinator attraction and stress tolerance. Although, termed "secondary," these compounds are far from insignificant; they are central to ecological interactions and play a pivotal role in determining crop quality, flavor, color, aroma and therapeutic value. Many phytochemicals valued for human health originate from secondary metabolic pathways. Among the major biosynthetic routes responsible for phytochemical production, the phenylpropanoid pathway is particularly significant. This pathway begins with the amino acid phenylalanine and leads to the synthesis of phenolic acids, flavonoids, tannins, lignins and anthocyanins. Enzymes such as phenylalanine ammonia-lyase (PAL) act as key regulatory points in this pathway, often responding rapidly to environmental stimuli. Flavonoids produced through the phenylpropanoid pathway contribute to UV protection, pigmentation and antioxidant activity. In human nutrition, these compounds are associated with reduced oxidative stress and chronic disease risk (Fyie *et al*., 2024). The terpenoid pathway represents another extensive branch of secondary metabolism. Terpenoids are synthesized through the mevalonate and methylerythritol phosphate pathways, producing compounds ranging from simple monoterpenes to complex diterpenes and carotenoids. These metabolites serve diverse ecological roles, including defense against herbivores, attraction of pollinators and communication with beneficial organisms. Carotenoids, for instance, not only participate in photosynthetic processes but also function as antioxidants and precursors of vitamin A in human diets.

Alkaloids constitute a third major class of secondary metabolites. Derived primarily from amino acids, alkaloids often exhibit potent biological activity. They function as chemical defenses against herbivores and pathogens due to their toxicity or deterrent properties. In medicinal contexts, alkaloids have been utilized for their analgesic, antimicrobial and pharmacological effects. The biosynthesis of alkaloids involves intricate enzymatic steps influenced by developmental stage and environmental conditions. Secondary metabolism is tightly regulated and interconnected with primary metabolic fluxes. The allocation of carbon and nitrogen resources between growth and defense reflects a trade-off influenced by environmental cues. When plants encounter stress or competition, metabolic pathways may shift toward increased synthesis of protective compounds. Thus, the structure and management of cropping systems can alter the balance between primary and secondary metabolism, ultimately shaping phytochemical profiles.

2.2 Ecological drivers of phytochemical diversity

Phytochemical expression does not occur in isolation; it is shaped by ecological variables that vary across cropping systems. Light quality and intensity represent one of the most influential drivers. In diversified systems with multi-tier canopy arrangements, light spectra reaching understory plants differ from those in monocultures. Changes in red to far-red light ratios, ultraviolet exposure and shading patterns influence gene expression associated with flavonoid and anthocyanin synthesis. Reduced light intensity may stimulate certain protective compounds to optimize photosynthetic efficiency and shield tissues from photodamage. Conversely, moderate UV exposure often enhances phenolic accumulation as a protective response. Soil microbial interactions form another crucial driver of phytochemical diversity. The rhizosphere hosts complex communities of bacteria, fungi and other microorganisms that interact intimately with plant roots. Beneficial microbes such as mycorrhizal fungi enhance nutrient uptake and influence hormonal signaling pathways. Plant growth-promoting rhizobacteria can trigger systemic responses that increase secondary metabolite production. For example, microbial elicitation may stimulate phenolic biosynthesis through activation of defense-related enzymes. In biointensive systems enriched with organic matter and reduced chemical inputs, microbial diversity tends to be higher, potentially amplifying these effects. Nutrient availability also modulates secondary metabolism. According to the carbon-nutrient balance hypothesis, plants growing under limited nitrogen conditions may allocate more carbon toward secondary metabolites such as phenolics and tannins. Conversely, excessive nutrient availability may favor rapid growth over defense compound accumulation (Moyo *et al*., 2024). In complementary cropping systems, nutrient dynamics differ from monocultures due to biological nitrogen fixation, differential root exploration and organic amendments. Such variability can alter carbon allocation patterns, influencing phytochemical concentrations.

Biotic stress, including herbivory and pathogen attack, is another powerful regulator of secondary metabolism. Plants possess sophisticated defense mechanisms involving inducible chemical responses. When attacked, they produce phytoalexins, phenolics and volatile compounds that deter pests or attract natural enemies. In diversified cropping systems, pest pressure may be moderated by natural enemies, yet low-level herbivory can still occur, potentially inducing beneficial increases in secondary metabolites. This dynamic balance between defense activation and ecological regulation contributes to phytochemical heterogeneity. The interplay of these ecological drivers creates a mosaic of environmental signals within diversified cropping systems. Unlike monocultures with uniform conditions, biointensive complementary systems generate microhabitats characterized by variable light exposure, soil nutrient gradients and microbial diversity. Such heterogeneity fosters metabolic plasticity, enabling plants to express diverse phytochemical profiles.

2.3 Stress-induced phytochemical enhancement in diversified systems

A central concept linking ecological complexity to phytochemical enrichment is hormesis. Hormesis describes a biological phenomenon in which low to moderate levels of stress stimulate adaptive beneficial responses. In plants, mild stress conditions such as partial shading, limited nutrient availability, or controlled water deficit can enhance secondary metabolite production. Rather than impairing growth, these moderate stressors activate protective pathways that increase antioxidant and defense compound synthesis. Diversified cropping systems often create conditions conducive to hormetic responses. For example, intercropping may lead to subtle competition for light or nutrients, prompting plants to strengthen defensive metabolism without experiencing severe stress. The resulting increase in phenolics or flavonoids can improve both plant resilience and nutritional value. Allelopathy represents another mechanism influencing phytochemical dynamics. Certain plant species release chemical compounds into the soil or atmosphere that affect neighboring plants. These allelochemicals may inhibit or stimulate growth, alter root architecture, or induce defensive pathways. In complementary cropping systems, interactions between species can lead to reciprocal metabolic adjustments. Exposure to allelopathic signals may trigger enhanced synthesis of protective secondary metabolites, contributing to phytochemical diversity at the system level. Signaling molecules such as jasmonic acid, salicylic acid and ethylene play pivotal roles in stress-induced metabolic modulation. These phytohormones regulate defense responses and coordinate systemic acquired resistance. In intercropping scenarios, volatile organic compounds released by one species may prime neighboring plants for enhanced defense readiness. This priming effect can elevate baseline levels of certain phytochemicals, increase resilience and potentially enhance nutraceutical properties.

Intercropping-induced metabolic modulation extends beyond direct stress responses. Changes in microclimate, root exudation patterns and nutrient partitioning collectively shape metabolic pathways. For instance, legume-cereal intercropping may alter nitrogen availability, influencing alkaloid and phenolic synthesis. Multi-tier systems may modify light penetration, affecting anthocyanin accumulation in understory crops. Organic amendments used in biointensive systems may enrich soil microbial communities that act as elicitors of secondary metabolism (Li *et al*., 2021). The cumulative effect of these processes is a dynamic metabolic landscape shaped by ecological interactions. Rather than viewing phytochemical production as a fixed trait, it can be understood as a responsive outcome of system design. Diversified cropping systems, through their inherent complexity, provide multiple stimuli that activate and regulate secondary metabolic pathways.

Linking cropping systems with phytochemical expression requires recognition of the intricate relationships between plant metabolism and ecological context. Primary and secondary metabolic processes are interconnected and sensitive to environmental signals. Light quality, nutrient dynamics, microbial associations and biotic stress collectively influence biosynthetic pathways such as phenylpropanoid, terpenoid and alkaloid routes. Diversified systems, particularly biointensive complementary cropping arrangements, create moderate and varied stress environments that can enhance phytochemical accumulation through hormesis, allelopathic signaling and metabolic modulation. By understanding these mechanisms, agricultural design can be strategically aligned with nutritional and functional quality objectives, advancing the integration of agroecology and nutraceutical science.

3. Biointensive complementary cropping systems in garden lands

Garden-land ecosystems are uniquely suited for the implementation of biointensive complementary cropping systems (BICCS) due to their small scale, high species diversity and close management. In such systems, farmers strategically combine crops with differing functional traits to maximize productivity, ecological balance and nutritional value within limited land areas. Unlike monoculture-based production, which relies heavily on external inputs and simplified plant arrangements, biointensive complementary cropping harnesses biological interactions to improve resource-use efficiency and system resilience. The integration of multiple crop types within garden lands not only enhances overall productivity but also creates a dynamic ecological environment that influences soil health, microclimate, pest regulation and phytochemical expression. The fundamental principle underlying BICCS in garden lands is complementarity rather than competition. By selecting species that differ in growth habits, phenology, nutrient requirements and rooting depth, farmers can reduce resource overlap and promote synergistic interactions. These systems rely on careful spatial and temporal design to optimize light capture, water utilization, nutrient cycling and biological pest control. Moreover, the inclusion of indigenous and underutilized crops broadens the functional and nutritional spectrum of garden systems, strengthening both ecological sustainability and dietary diversity.

3.1 Types of complementary cropping

Biointensive complementary cropping encompasses several arrangements, each tailored to specific ecological and socioeconomic contexts. Among these, intercropping is one of the most widely practiced methods. Intercropping involves cultivating two or more crop species simultaneously in the same field with a defined spatial arrangement. Crops may be planted in alternate rows, paired rows, or specific geometric patterns. The goal is to combine species with contrasting growth characteristics so that they utilize resources differently. For example, legumes intercropped with non-legumes enhance nitrogen availability through biological fixation, benefiting companion crops. Similarly, tall and short crops may be combined to optimize vertical light distribution. Intercropping reduces pest incidence, improves yield stability and enhances overall land productivity compared to sole cropping. Mixed cropping represents a less structured form of diversification in which multiple crops are grown together without distinct row arrangements. Seeds are often broadcast or sown randomly, allowing species to coexist naturally (Brooker *et al*., 2021). Although, management may be less precise than in intercropping, mixed cropping increases biodiversity and reduces the risk of total crop failure. In garden lands, mixed cropping is common for leafy vegetables, pulses and minor cereals, where farmers prioritize continuous harvests and dietary diversity over uniformity. The coexistence of species with varied maturation periods ensures staggered production and resource sharing.

Relay cropping introduces a temporal dimension to complementarity. In this system, a second crop is sown before the first crop is harvested, allowing partial overlap in their growth cycles. This approach maximizes land use efficiency and reduces fallow periods. Relay cropping is particularly beneficial in garden lands where space is limited and year-round productivity is essential. By carefully timing crop establishment, farmers can exploit residual soil moisture and nutrients, enhancing system sustainability. Relay systems also distribute labor requirements more evenly across seasons. Multi-tier cropping is a defining feature of many garden-land ecosystems, especially in tropical and subtropical regions. This system involves cultivating crops at different vertical levels to mimic natural forest structures. Tall perennial trees form the upper canopy, medium-height fruit or spice crops occupy the middle layer and herbaceous vegetables or medicinal plants grow beneath. Such stratification maximizes sunlight utilization while moderating microclimatic extremes. Multi-tier arrangements create shaded environments suitable for certain understory crops and reduce evapotranspiration losses. The vertical diversity enhances habitat complexity, supporting beneficial insects and soil organisms. Crop-livestock integration represents another dimension of biointensive complementary systems. In garden lands, small ruminants, poultry, or cattle may be integrated into cropping areas. Livestock contribute manure, which improves soil fertility and microbial activity, while crop residues serve as feed. This cyclical exchange strengthens nutrient recycling and reduces dependency on synthetic fertilizers. Livestock grazing can also aid in weed management. By integrating animal and plant components, garden systems become more self-sustaining and resilient. Each of these complementary cropping types contributes to ecological intensification by leveraging biological processes. Together, they form a diversified production matrix capable of sustaining productivity while enhancing soil health and crop quality.

3.2 Spatial and temporal complementarity

The effectiveness of biointensive complementary cropping systems depends on the strategic management of spatial and temporal complementarity. Spatial complementarity refers to the arrangement of crops in space to minimize competition and maximize synergistic interactions. Temporal complementarity involves staggering crop growth cycles to optimize resource utilization across seasons. Root architecture complementarity is a critical aspect of spatial design. Different crop species possess varying root depths, branching patterns and nutrient absorption capacities. Deep-rooted crops can access subsoil water and nutrients, while shallow-rooted species exploit topsoil resources. When combined, these species reduce direct competition and enhance total nutrient extraction efficiency. For instance, pairing a deep-rooted tuber crop with a shallow-rooted leafy vegetable allows efficient partitioning of soil resources. Root exudates released by different species also influence rhizosphere microbial communities, potentially enhancing nutrient availability and stimulating beneficial interactions. Canopy stratification plays an equally important role. By combining crops of different heights and leaf architectures, farmers can maximize photosynthetically active radiation interception. Tall crops capture intense sunlight, while lower layers utilize filtered light. This layered arrangement reduces shading stress and increases overall system productivity. In tropical garden lands, canopy stratification often resembles natural forest ecosystems, promoting microclimatic stability. Lower temperatures and higher humidity beneath the canopy can protect sensitive crops and reduce water loss.

Nutrient niche differentiation further enhances complementarity. Crops differ in their nutrient demands and uptake patterns. Legumes contribute nitrogen through symbiotic fixation, benefiting adjacent crops with high nitrogen requirements. Some species are efficient phosphorus mobilizers, releasing organic acids that solubilize otherwise inaccessible forms. By combining crops with complementary nutrient acquisition strategies, farmers reduce nutrient depletion and improve soil fertility over time. Organic amendments and compost applications in biointensive systems support these processes by enriching microbial communities that facilitate nutrient cycling (Martin-Guay *et al*., 2018). Temporal complementarity extends these principles across time. Early-maturing crops can be followed by late-maturing species, ensuring continuous ground cover and reducing erosion. Seasonal crop rotations disrupt pest and disease cycles while improving soil structure. In relay cropping, overlapping growth periods allow for seamless transitions, minimizing idle land. Such temporal planning ensures efficient use of rainfall, solar radiation and labor inputs. Collectively, spatial and temporal complementarity create a multidimensional system where ecological niches are fully utilized. This complexity not only enhances productivity but also fosters resilience against environmental fluctuations.

3.3 Role of indigenous and underutilized crops

A defining strength of garden-land biointensive systems is the inclusion of indigenous and underutilized crops. These species often possess unique nutritional and phytochemical attributes that are absent or less pronounced in mainstream crops. Tubers, traditional leafy greens, medicinal plants, minor millets and aromatic herbs enrich garden biodiversity and dietary diversity. Tubers such as yam, taro and lesser-known root crops provide carbohydrates along with fiber, resistant starch and bioactive compounds. Many indigenous leafy greens are rich in iron, calcium, vitamins and phenolic antioxidants. Medicinal plants cultivated within garden lands supply essential oils, alkaloids and other therapeutic compounds. Their inclusion supports both household healthcare practices and local markets. Functional crop diversification refers to the deliberate inclusion of species that provide ecological or nutritional benefits beyond yield. Aromatic herbs may repel pests or attract pollinators. Flowering plants support beneficial insects. Shade-tolerant medicinal species thrive under multi-tier canopies. By integrating crops with diverse functional traits, farmers enhance ecosystem services while broadening the nutritional spectrum available to households. Underutilized crops are often adapted to local environmental conditions, exhibiting tolerance to drought, poor soils, or temperature extremes (Wezel *et al*., 2020). Their resilience contributes to climate adaptation strategies in garden lands. Moreover, these crops often require fewer external inputs, aligning with biointensive principles. Conserving and promoting such species helps preserve genetic diversity and traditional knowledge.

The integration of indigenous crops also strengthens cultural identity and food sovereignty. Many traditional crops hold culinary and medicinal significance within communities. By maintaining these species in garden systems, farmers safeguard both biodiversity and heritage. Biointensive complementary cropping systems in garden lands represent a holistic approach to sustainable agriculture. Through diverse cropping arrangements, spatial and temporal complementarity and inclusion of indigenous species, these systems optimize ecological interactions and enhance productivity. Their capacity to integrate functional diversity with nutritional value positions them as promising models for resilient and health-oriented food production.

4. Phytochemical diversity in complementary cropping systems

Phytochemical diversity represents one of the most significant quality attributes of crops cultivated under diversified agricultural systems. In complementary cropping systems, plants grow in close association with multiple species, experience variable microclimatic conditions and interact with diverse soil biota. These ecological dynamics influence metabolic allocation patterns, often enhancing the synthesis and accumulation of secondary metabolites. Unlike monoculture systems that provide relatively uniform environmental conditions, complementary cropping arrangements create heterogeneous niches that can stimulate biochemical plasticity (Figure 2). As a result, crops grown in such systems may exhibit altered concentrations and profiles of phenolics, terpenoids, alkaloids, pigments and other bioactive compounds.

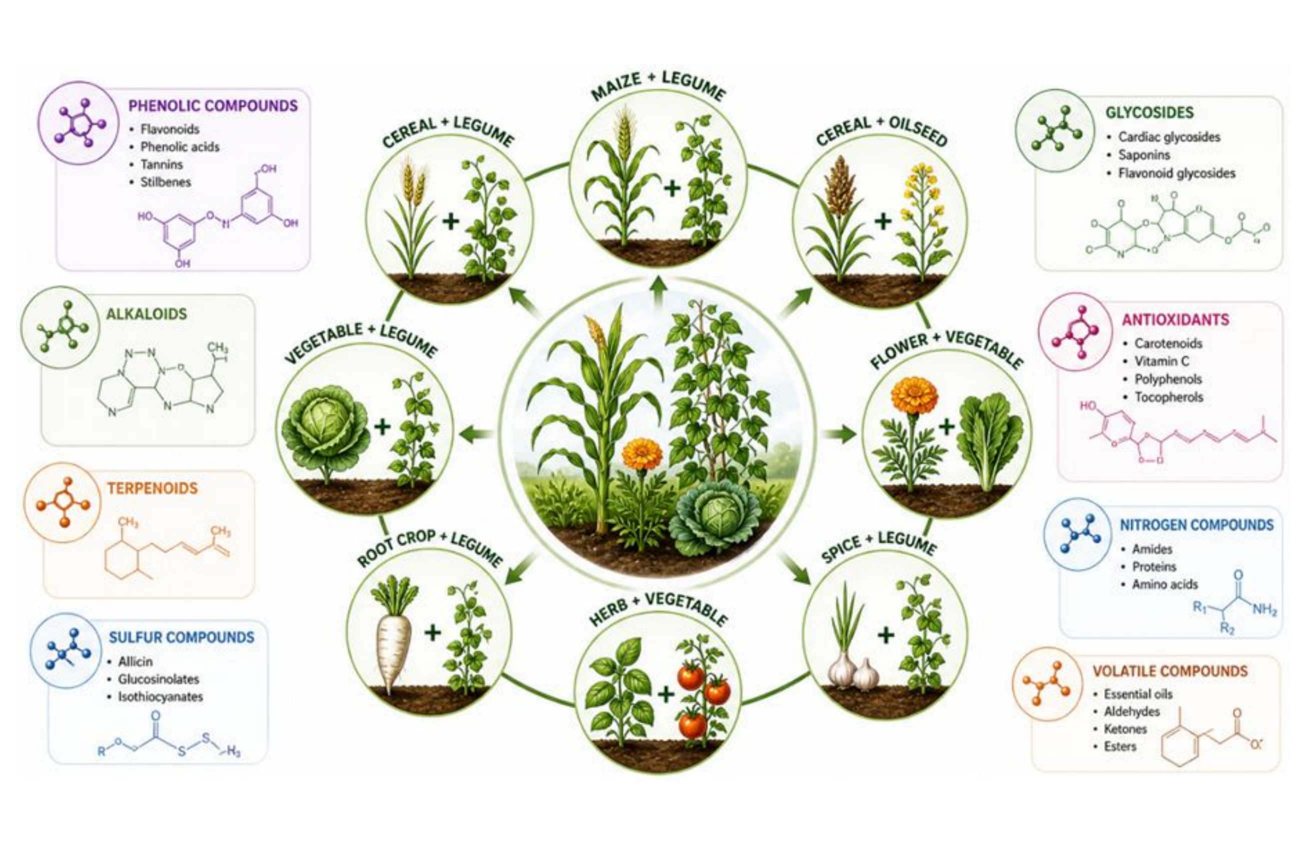

Figure 2: Phytochemical diversity in different complementary cropping systems.

The diversity of phytochemicals in complementary cropping systems is shaped by multiple factors including light modulation through canopy layering, nutrient interactions among species, root exudate signaling and mild stress induction (Zhang *et al*., 2020). These factors interact to influence biosynthetic pathways such as the phenylpropanoid, terpenoid and alkaloid pathways. The following sections examine major phytochemical classes commonly affected by diversified cropping practices and their functional significance.

4.1 Phenolic compounds

Phenolic compounds constitute one of the largest and most studied groups of plant secondary metabolites. They are primarily synthesized *via* the phenylpropanoid pathway and serve protective, structural and signaling functions in plants. In complementary cropping systems, phenolic accumulation is often influenced by light intensity, interspecific competition and microbial associations. Flavonoids are a prominent subclass of phenolics and include flavonols, flavones, flavanones, catechins and isoflavones. These compounds contribute to pigmentation, UV protection and defense against pathogens. In intercropped systems where shading patterns vary, changes in light quality particularly ultraviolet and blue light exposure can stimulate flavonoid biosynthesis. Flavonoids act as powerful antioxidants, scavenging reactive oxygen species generated under environmental stress. In human nutrition, they are associated with anti-inflammatory and cardioprotective effects. The microclimatic heterogeneity of complementary cropping systems may therefore enhance flavonoid content in leafy vegetables, fruits and medicinal plants (Gaba *et al*., 2018). Phenolic acids, including hydroxycinnamic and hydroxybenzoic acids, are widely distributed across plant tissues. They function in structural reinforcement, pathogen defense and allelopathic interactions. In diversified systems, root exudates from neighboring plants may induce phenolic acid production as part of competitive or defensive responses. Organic soil amendments commonly used in biointensive systems can further modulate phenolic synthesis by influencing carbon-nitrogen balance and microbial activity.

Tannins represent high-molecular-weight phenolics that contribute to plant defense by deterring herbivores and inhibiting microbial growth. In systems with moderate herbivore presence or biotic signaling, tannin levels may increase as part of inducible defense mechanisms. Although, excessive tannins can reduce palatability, moderate levels contribute antioxidant benefits and antimicrobial activity in human diets. Anthocyanins are pigmented flavonoids responsible for red, purple and blue coloration in fruits and vegetables.

Their synthesis is highly responsive to environmental cues such as temperature fluctuations and light exposure. Multi-tier cropping systems that modify light penetration may influence anthocyanin accumulation in understory crops. Beyond their ecological roles in pollinator attraction and photoprotection, anthocyanins possess significant antioxidant and anti-inflammatory properties beneficial to human health. Collectively, phenolic compounds illustrate how complementary cropping systems can shape phytochemical profiles through environmental modulation and plant-plant interactions.

4.2 Terpenoids and essential oils

Terpenoids constitute another extensive class of phytochemicals synthesized through the mevalonate and methylerythritol phosphate pathways. They include volatile and non-volatile compounds that play essential roles in plant defense, signaling and adaptation. Monoterpenes are volatile compounds commonly found in aromatic herbs and spices. They contribute to characteristic fragrances and possess antimicrobial, insect-repellent and antioxidant properties. In diversified systems, interspecific interactions may stimulate volatile emission as part of plant communication. For example, when one plant experiences herbivory, neighboring plants may respond by increasing monoterpene production as a pre-emptive defense strategy. Environmental factors such as temperature and light, modified by canopy structure, also influence monoterpene synthesis. Sesquiterpenes, larger and often less volatile than monoterpenes, serve defensive functions against pests and pathogens. They may accumulate in response to mechanical damage or microbial attack. Complementary cropping systems that enhance biodiversity can create complex signaling networks, potentially elevating baseline levels of certain sesquiterpenes. Some sesquiterpenes also exhibit anti-inflammatory and anticancer properties in pharmacological studies. Diterpenes encompass a diverse group of compounds, including some with significant medicinal value (Cong *et al*., 2020). They participate in plant growth regulation and defense. Stress-induced activation of diterpene biosynthetic genes may occur in systems where moderate environmental variation stimulates adaptive responses. Essential oil-bearing crops grown under diversified arrangements may therefore display modified oil composition and concentration. The production of terpenoids in complementary cropping systems underscores the importance of ecological interactions in shaping aromatic and therapeutic qualities of crops.

4.3 Alkaloids

Alkaloids are nitrogen-containing secondary metabolites derived primarily from amino acid precursors. They are often associated with potent biological activity and play crucial roles in plant defense. The synthesis of alkaloids is influenced by nitrogen availability, environmental stress and developmental stage. Defense-related alkaloids serve as deterrents against herbivores and pathogens due to their bitterness or toxicity. In diversified cropping systems, mild herbivore presence or competitive signaling may stimulate alkaloid production. Nitrogen dynamics in complementary systems, especially where legumes contribute biologically fixed nitrogen, can influence alkaloid biosynthesis. Balanced nitrogen availability may support optimal synthesis without excessive vegetative growth. Medicinal alkaloids have long been utilized in traditional and modern pharmacology. Their concentration in plants can vary depending on ecological conditions. Crops grown in biointensive systems enriched with organic matter and diverse microbial communities may experience metabolic modulation that affects alkaloid profiles (Dias *et al*., 2020). However, careful management is necessary, as excessively high alkaloid levels can pose safety concerns. Overall, alkaloid diversity in complementary cropping systems reflects the interplay between nutrient availability and ecological signaling.

4.4 Saponins and glycosides

Saponins are glycosylated compounds known for their soap-like foaming properties and defensive roles against pests and pathogens. They disrupt cell membranes of invading organisms and contribute to plant resilience. In diversified systems where plants encounter varied biotic stimuli, saponin accumulation may increase as part of adaptive defense responses. From a nutritional perspective, saponins exhibit cardioprotective, cholesterol-lowering and immunomodulatory effects when consumed in moderate quantities. They may enhance immune function and contribute to metabolic health. The inclusion of legumes, medicinal herbs and certain tubers in complementary cropping systems can enrich dietary saponin intake. Other glycosides, including cardiac and phenolic glycosides, also possess therapeutic potential (Tiwari and Cummins, 2018). Their biosynthesis may be influenced by carbon allocation patterns and environmental stress levels. Biointensive management practices that promote balanced nutrient supply and moderate stress may support favorable glycoside accumulation.

4.5 Carotenoids and pigments

Carotenoids are lipid-soluble pigments involved in photosynthesis and photoprotection. They include β-carotene, lycopene, lutein and other compounds that contribute to yellow, orange and red coloration in plant tissues. β-carotene functions as a precursor of vitamin A and plays a critical role in vision and immune function. Its synthesis is influenced by light exposure and temperature. In multi-tier cropping systems, variations in light intensity can modulate carotenoid accumulation in leafy vegetables and root crops. Lycopene, predominantly found in red fruits such as tomatoes, exhibits strong antioxidant properties and has been associated with reduced risk of certain chronic diseases. Environmental conditions including temperature stress and light quality affect lycopene biosynthesis. Diversified cropping systems that moderate extreme temperatures through canopy effects may influence pigment stability and concentration (Sarker and oba, 2020). Lutein, important for eye health, accumulates in green leafy vegetables. Its synthesis is linked to chloroplast development and light absorption. Complementary cropping arrangements that optimize light distribution may enhance lutein content by supporting balanced photosynthetic activity. The modulation of carotenoid biosynthesis in diversified systems illustrates how environmental heterogeneity can shape nutritional quality.

4.6 Organosulfur compounds

Organosulfur compounds are characteristic of certain plant families and contribute to pungency, defense and health benefits. Glucosinolates, found in cruciferous vegetables, are hydrolyzed into biologically active isothiocyanates upon tissue damage. These compounds possess anticancer and antimicrobial properties. Soil sulfur availability and plant stress influence glucosinolate synthesis. Complementary cropping systems with balanced nutrient cycling and moderate stress conditions may affect their concentration. Allyl sulfides, commonly associated with allium crops such as garlic and onion, exhibit cardioprotective and antimicrobial effects. Their formation depends on enzymatic reactions triggered by tissue disruption. Environmental conditions and soil fertility management influence the precursor compounds involved in allyl sulfide biosynthesis. In diversified garden-land systems, the inclusion of sulfur-rich crops contributes to dietary diversity and functional food potential. Interactions among species and soil microbes may further modulate sulfur metabolism and associated phytochemical expression (Kumar *et al*., 2021). Complementary cropping systems foster a rich tapestry of phytochemical diversity by integrating ecological complexity with plant metabolic responsiveness. Phenolics, terpenoids, alkaloids, saponins, carotenoids and organosulfur compounds each respond to environmental cues shaped by crop arrangement and management. By understanding these interactions, agricultural practices can be designed not only to enhance productivity but also to optimize nutraceutical value and functional food quality.

5. Influence of biointensive cropping on nutraceutical quality

Biointensive cropping systems are increasingly recognized not only for their agronomic and ecological advantages but also for their capacity to enhance the nutraceutical quality of agricultural produce. Nutraceutical quality refers to the health-promoting attributes of foods that extend beyond basic nutritional value. These benefits arise primarily from bioactive phytochemicals, including phenolics, flavonoids, carotenoids, terpenoids, alkaloids, saponins and organosulfur compounds. The concentration and composition of these compounds are influenced by environmental factors, cropping system design, soil health and plant-plant and plant-microbe interactions. Biointensive complementary cropping systems create dynamic agroecological conditions characterized by moderate stress, improved nutrient cycling, enhanced microbial diversity and diversified canopy structures. These factors can modulate plant metabolic pathways, often stimulating the accumulation of bioactive compounds. As a result, crops grown under diversified systems may exhibit improved antioxidant capacity and other health-related functional properties. The following sections examine how biointensive cropping influences key nutraceutical attributes.

5.1 Antioxidant capacity

Antioxidant capacity is one of the most widely studied indicators of nutraceutical quality. Reactive oxygen species (ROS) generated during normal metabolic processes or environmental stress can cause oxidative damage in biological systems. Antioxidants neutralize these reactive molecules, protecting cellular components from oxidative stress. In human health, diets rich in antioxidants are associated with reduced risk of chronic diseases, including cardiovascular disorders, neurodegenerative conditions and certain cancers. Laboratory assessment of antioxidant capacity commonly employs assays such as DPPH (2,2-diphenyl-1-picrylhydrazyl radical scavenging activity), FRAP (ferric reducing antioxidant power) and ABTS (2,22 -azino-bis (3-ethylbenzothiazoline-6-sulfonic acid) radical cation decolorization assay). These assays measure the ability of plant extracts to donate electrons or hydrogen atoms, reflecting their free radical scavenging potential. Numerous studies indicate a strong positive correlation between total phenolic content and antioxidant capacity measured through these assays. Therefore, cropping systems that enhance polyphenol accumulation often led to improved antioxidant performance (Yang *et al*., 2019).

Biointensive complementary cropping systems may promote antioxidant enrichment through several mechanisms. Moderate environmental stress resulting from interspecific interactions or microclimatic variation can stimulate phenylpropanoid pathway activity, leading to increased synthesis of phenolics and flavonoids. Enhanced soil microbial diversity in organic and biointensive systems can further influence metabolic processes, potentially upregulating antioxidant compound production. In addition, diversified canopy structures may modify light exposure, affecting flavonoid and anthocyanin biosynthesis (Chen *et al*., 2022). Polyphenol enrichment under complementary cropping arrangements is particularly significant. Polyphenols act as potent antioxidants and contribute to color, flavor and shelf stability of horticultural crops. Vegetables, fruits and medicinal plants cultivated in diversified systems often demonstrate improved total phenolic content compared to monocultures, suggesting that agroecological management can serve as a natural strategy for enhancing antioxidant potential.

5.2 Anti-inflammatory and immunomodulatory potential

Chronic inflammation is a key contributor to the development of numerous non-communicable diseases, including arthritis, diabetes, cardiovascular disease and neurodegeneration. Phytochemicals with anti-inflammatory properties can modulate inflammatory pathways by inhibiting pro-inflammatory mediators and reducing oxidative stress. Compounds such as flavonoids, terpenoids and certain alkaloids exhibit the capacity to suppress inflammatory cytokines and regulate signaling pathways associated with immune responses. Biointensive cropping systems influence anti-inflammatory potential primarily through their effects on phytochemical profiles. Plants exposed to mild biotic or abiotic stress in diversified environments may synthesize higher levels of protective compounds with anti-inflammatory activity. For instance, intercropping arrangements that induce moderate competition can activate defense-related metabolic pathways without severely limiting growth. The resulting increase in flavonoid and phenolic concentrations enhances anti-inflammatory capacity. Immunomodulatory effects of plant-derived compounds are also linked to diversified cropping systems. Saponins, polysaccharides and certain glycosides are known to stimulate immune function. Garden-land systems incorporating medicinal and underutilized crops may provide a broader spectrum of such bioactives (Patra *et al*., 2018). Furthermore, soil health improvements associated with organic amendments and crop diversity can influence plant nutrient composition, indirectly affecting immune-supportive phytochemicals. Thus, biointensive cropping not only enhances antioxidant activity but may also strengthen the anti-inflammatory and immunomodulatory properties of crops, contributing to improved dietary health outcomes.

5.3 Antidiabetic and antiobesity effects

The global prevalence of type 2 diabetes and obesity has increased dramatically in recent decades, highlighting the need for dietary strategies that support metabolic health. Phytochemicals play an important role in regulating glucose metabolism, lipid profiles and insulin sensitivity. Compounds such as flavonoids, phenolic acids, alkaloids and saponins have demonstrated inhibitory effects on carbohydrate-digesting enzymes, modulation of glucose transporters and improvement in insulin signaling pathways. Biointensive complementary cropping systems may enhance antidiabetic potential by promoting higher concentrations of these bioactives. For example, polyphenol-rich crops grown under diversified conditions may exhibit stronger α-amylase and α-glucosidase inhibitory activities. Moderate nutrient stress or shading can influence carbon allocation toward secondary metabolites, thereby increasing the functional properties of plant tissues.

Antiobesity effects are also associated with phytochemicals that regulate lipid metabolism and adipogenesis. Carotenoids, flavonoids and organosulfur compounds have been shown to influence lipid oxidation and inflammatory responses linked to obesity (Choudhary *et al*., 2020). Diversified cropping systems that improve carotenoid accumulation through optimized light distribution and soil fertility management can enhance these protective effects. Additionally, inclusion of high-fiber tubers, leafy greens and legumes in garden-based biointensive systems contributes to glycemic control and satiety, complementing phytochemical-mediated mechanisms. Thus, cropping system design can indirectly support metabolic health by influencing both nutrient composition and bioactive content.

5.4 Cardioprotective properties

Cardiovascular diseases remain among the leading causes of mortality worldwide. Diets rich in plant-based foods are consistently associated with reduced cardiovascular risk, largely due to the presence of antioxidants, fiber and bioactive phytochemicals. Phenolics, carotenoids, saponins and organosulfur compounds contribute to cardioprotection by reducing oxidative stress, improving endothelial function, lowering cholesterol levels and inhibiting platelet aggregation. Biointensive cropping systems may enhance cardioprotective properties by increasing the accumulation of relevant compounds. For example, saponins found in legumes cultivated in diversified systems can reduce cholesterol absorption in the intestine. Organosulfur compounds in allium crops may exhibit antihypertensive and antithrombotic effects. Enhanced carotenoid levels, including β-carotene and lycopene, contribute to reduced oxidative damage to blood vessels. The ecological conditions in complementary cropping systems such as moderate stress and improved nutrient cycling may promote higher synthesis of these cardioprotective phytochemicals (Sharma *et al*., 2021). Additionally, diversified systems often reduce pesticide residues and chemical inputs, improving overall food safety and supporting cardiovascular health.

5.5 Antimicrobial and anticancer potential

Phytochemicals with antimicrobial properties play a vital role in plant defense and human health. Terpenoids, phenolics, alkaloids and organosulfur compounds exhibit inhibitory effects against bacteria, fungi and viruses. Crops grown in diversified systems may experience mild pathogen exposure, stimulating the production of antimicrobial compounds as part of inducible defense responses. This natural elicitation can enhance the antimicrobial efficacy of plant extracts. Anticancer potential is closely linked to antioxidant activity, detoxification enzyme modulation and apoptosis induction by certain phytochemicals. Flavonoids, glucosinolate-derived isothiocyanates and carotenoids have demonstrated anticancer properties in experimental studies. Complementary cropping systems that influence glucosinolate and phenolic accumulation may therefore enhance the anticancer potential of cruciferous and other functional crops. Moreover, diversified garden-land systems often incorporate medicinal plants with known anticancer bioactives. By integrating such species into cropping systems, farmers contribute to the availability of plant-based therapeutic resources. Biointensive complementary cropping systems exert a multifaceted influence on nutraceutical quality (Nair *et al*., 2019). Through ecological interactions, moderate stress induction, improved soil health and biodiversity enhancement, these systems can stimulate the accumulation of antioxidant, anti-inflammatory, antidiabetic, cardioprotective, antimicrobial and anticancer phytochemicals. The integration of agroecological principles with nutritional objectives positions biointensive cropping as a promising strategy for producing health-promoting foods within sustainable agricultural frameworks.

6. Soil-plant-microbiome interactions and phytochemical modulation

The soil-plant-microbiome continuum represents a foundational axis through which biointensive complementary cropping systems influence phytochemical expression and overall crop quality. In diversified garden-land ecosystems, soil is not merely a growth medium but a biologically active environment teeming with microorganisms that interact dynamically with plant roots. These interactions regulate nutrient cycling, hormonal signaling, stress responses and ultimately the biosynthesis of secondary metabolites. Increasing evidence suggests that phytochemical accumulation is not determined solely by plant genetics or above-ground environmental conditions, but is strongly modulated by rhizosphere processes and microbial associations. Biointensive cropping systems, characterized by organic amendments, crop diversity and reduced synthetic input reliance, foster complex soil microbial communities (Singh *et al*., 2020). These communities influence metabolic pathways within plants through signaling molecules, nutrient exchange and stress priming. Understanding how rhizosphere engineering, mycorrhizal associations, plant growth-promoting rhizobacteria (PGPR) and organic inputs shape phytochemical production is critical for aligning agroecological management with nutraceutical quality enhancement.

6.1 Rhizosphere engineering

Rhizosphere engineering refers to the deliberate management of root-zone interactions to optimize plant growth, nutrient acquisition and metabolic performance. The rhizosphere the narrow region of soil directly influenced by root exudates acts as a biochemical interface between plants and microorganisms. Roots release sugars, amino acids, organic acids, phenolics and signaling molecules that shape microbial communities. In turn, microorganisms influence nutrient availability, phytohormone production and plant defense responses. In biointensive complementary cropping systems, rhizosphere engineering occurs naturally through species diversity. Different crops exude distinct chemical compounds, creating heterogeneous microenvironments in the soil. This diversity promotes microbial richness and functional diversity, enhancing nutrient solubilization and stress tolerance. For instance, some plants release organic acids that mobilize phosphorus, while others secrete flavonoids that signal symbiotic microbes. Such interactions can influence carbon allocation within the plant, potentially increasing the synthesis of phenolic and flavonoid compounds. Moreover, root-root interactions in intercropping systems may induce chemical signaling that activates secondary metabolic pathways (Zuza *et al*., 2024). Exposure to allelochemicals or volatile compounds from neighboring roots can stimulate defense-related gene expression. This rhizosphere-mediated modulation may enhance phytochemical diversity at the system level. Thus, rhizosphere engineering in diversified systems supports not only productivity but also metabolic complexity.

6.2 Mycorrhizal associations

Mycorrhizal fungi form symbiotic relationships with plant roots, facilitating nutrient uptake in exchange for photosynthetically derived carbon. Arbuscular mycorrhizal fungi (AMF), in particular, are widespread in agricultural soils and play a crucial role in phosphorus acquisition, water absorption and stress tolerance. In biointensive cropping systems where synthetic fertilizers are minimized, mycorrhizal associations become especially important for maintaining nutrient balance. Beyond nutrient exchange, mycorrhizal colonization influences plant secondary metabolism. Research indicates that AMF can stimulate phenylpropanoid pathway activity, leading to increased accumulation of phenolics, flavonoids and lignin. This enhancement may result from improved nutrient status or from activation of systemic defense signaling pathways. Mycorrhizal symbiosis often primes plants to respond more effectively to environmental stress, a phenomenon known as mycorrhiza-induced resistance (Bhar *et al*., 2018). In complementary cropping systems, plant diversity supports greater mycorrhizal diversity. Different plant species host varying fungal communities, creating a network of interconnected roots through common mycorrhizal networks. These networks facilitate nutrient sharing and communication among plants. The resulting cooperative interactions can stabilize nutrient supply and modulate metabolic responses, contributing to enhanced phytochemical accumulation. Additionally, mycorrhizal associations may influence terpenoid and alkaloid biosynthesis by affecting hormonal pathways such as jasmonic acid signaling. Through these mechanisms, mycorrhizae serve as critical mediators linking soil biodiversity with nutraceutical quality.

6.3 PGPR influence on secondary metabolites

Plant growth-promoting rhizobacteria (PGPR) represent another important component of the soil microbiome. These beneficial bacteria colonize the rhizosphere and enhance plant growth through nitrogen fixation, phosphate solubilization, phytohormone production and disease suppression. In biointensive systems enriched with organic matter and diverse crops, PGPR populations tend to be abundant and functionally diverse. PGPR influence secondary metabolite production through multiple mechanisms. Certain bacterial strains produce elicitor molecules that activate plant defense pathways, leading to increased synthesis of phenolics, flavonoids and terpenoids. Others modulate hormone levels such as auxins, cytokinins and ethylene, indirectly affecting metabolic allocation patterns. Induced systemic resistance triggered by PGPR can enhance the baseline production of protective compounds, improving both plant resilience and bioactive content. Nitrogen-fixing bacteria associated with legumes in complementary cropping systems contribute to balanced nutrient availability. Adequate nitrogen supply influences alkaloid biosynthesis, while carbon availability determines phenolic accumulation. By stabilizing nutrient dynamics, PGPR help maintain optimal conditions for secondary metabolite production (Koley *et al*., 2022). Furthermore, PGPR can enhance antioxidant enzyme activity in plants, reducing oxidative damage and supporting the synthesis of health-promoting compounds. The synergistic interaction between PGPR and diversified cropping arrangements underscores the importance of microbiome management in nutraceutical agriculture.

6.4 Organic inputs and biofertilizers

Organic inputs such as compost, green manure, farmyard manure and crop residues form the backbone of biointensive cropping systems. These materials improve soil structure, increase organic matter content and provide slow-release nutrients. Unlike synthetic fertilizers, organic amendments support microbial proliferation and enzymatic activity, fostering a biologically active soil environment. Organic inputs influence phytochemical modulation through both direct and indirect pathways. Improved soil fertility enhances plant vigor, while moderate nutrient limitation under organic management can stimulate secondary metabolism according to carbon-nutrient balance principles. Additionally, compost-derived humic substances may act as biostimulants, influencing root growth and metabolic processes. Biofertilizers containing beneficial microorganisms further strengthen soil-plant interactions. Inoculation with nitrogen-fixing bacteria, phosphate-solubilizing bacteria, or mycorrhizal fungi enhances nutrient uptake efficiency and stress tolerance. These biological inputs reduce dependency on chemical fertilizers while promoting phytochemical enrichment (Gupta *et al*., 2022). In diversified garden-land systems, regular application of organic inputs contributes to sustained soil health and metabolic stability. The combined effect of improved microbial activity, balanced nutrient supply and moderate environmental stress creates conditions favorable for enhanced phytochemical diversity and nutraceutical quality.

7. Analytical approaches for phytochemical assessment

Accurate evaluation of phytochemical diversity and nutraceutical quality in biointensive complementary cropping systems requires robust analytical methodologies. Because secondary metabolites vary widely in polarity, volatility, molecular weight and stability, no single method is sufficient to capture the full phytochemical spectrum. A comprehensive assessment therefore integrates extraction procedures, chromatographic separation, spectrophotometric quantification and advanced metabolomics tools. The reliability of conclusions regarding phytochemical enhancement under diversified cropping systems depends largely on methodological precision, reproducibility and standardization.

7.1 Extraction techniques

Extraction is the first and most critical step in phytochemical analysis. The objective is to efficiently isolate bioactive compounds from plant matrices while preserving their structural integrity. Selection of extraction method depends on the chemical nature of the target compounds, plant tissue type and analytical goals. Conventional solvent extraction remains widely used for phenolics, flavonoids, alkaloids and glycosides. Polar solvents such as methanol, ethanol and aqueous mixtures are typically employed for phenolic compounds, whereas less polar solvents like hexane are used for carotenoids and terpenoids. Soxhlet extraction provides exhaustive recovery but may expose samples to prolonged heating, potentially degrading heat-sensitive compounds. Maceration and reflux methods offer simpler alternatives but require optimization of solvent ratio, time and temperature. Advanced extraction technologies have gained prominence for improving efficiency and selectivity. Ultrasound-assisted extraction enhances mass transfer by disrupting cell walls, reducing extraction time and solvent consumption. Microwave-assisted extraction accelerates solvent penetration and compound release through rapid heating. Supercritical fluid extraction, particularly with carbon dioxide, is valuable for isolating essential oils and non-polar compounds without solvent residues (Shrma *et al.*, 2024). Pressurized liquid extraction and enzyme-assisted extraction further expand methodological options. In studies comparing cropping systems, standardized extraction protocols are essential to ensure comparability. Factors such as drying method, particle size, solvent composition and extraction duration can significantly influence measured phytochemical content. Therefore, harmonization of protocols is necessary when assessing the impact of biointensive cropping on nutraceutical quality.

7.2 Chromatographic methods

Chromatographic techniques are indispensable for separation, identification and quantification of individual phytochemicals. High-performance liquid chromatography (HPLC) is commonly used for non-volatile and thermally unstable compounds such as phenolic acids, flavonoids, alkaloids and glycosides. Coupled with ultraviolet-visible (UV-Vis) or diode array detection, HPLC allows precise quantification of specific metabolites. Gradient elution systems enable separation of complex mixtures present in plant extracts. Gas chromatography-mass spectrometry (GC-MS) is particularly suitable for volatile and semi-volatile compounds such as essential oils, monoterpenes, sesquiterpenes and certain organosulfur compounds. Prior derivatization may be required for non-volatile metabolites. GC-MS provides structural information through mass fragmentation patterns, facilitating compound identification. In complementary cropping studies involving aromatic herbs or allium crops, GC-MS is valuable for profiling essential oil composition. Liquid chromatography-mass spectrometry (LC-MS) combines the separation capability of liquid chromatography with high-resolution mass analysis. This technique is highly sensitive and capable of detecting low-abundance metabolites. LC-MS is widely used for targeted and untargeted phytochemical profiling, enabling comprehensive evaluation of metabolic shifts under diversified cropping systems. Tandem mass spectrometry (LC-MS/MS) further enhances structural elucidation and quantitative accuracy (Dini-Andreote, 2020). Chromatographic fingerprinting approaches can be used to compare metabolite profiles between monoculture and biointensive systems. Such comparisons reveal qualitative and quantitative differences attributable to cropping system design, providing insights into phytochemical modulation mechanisms.

7.3 Spectrophotometric assays

Spectrophotometric assays provide rapid and cost-effective estimation of total phytochemical groups and antioxidant capacity. These assays are particularly useful in screening large numbers of samples derived from field experiments. The Folin-Ciocalteu method is widely employed for estimating total phenolic content, expressed as gallic acid equivalents. Aluminum chloride colorimetric assays quantify total flavonoids, while vanillin or butanol-HCl methods are used for tannin estimation. Carotenoid content can be measured spectrophotometrically following solvent extraction. Antioxidant capacity assays, including DPPH, ABTS and FRAP, assess the radical scavenging or reducing power of plant extracts. Although, these assays do not identify individual compounds, they provide functional insights into overall antioxidant potential. Correlation analysis between total phenolics and antioxidant activity often reveals strong associations, highlighting the contribution of polyphenols to nutraceutical quality (Van der and Hartmann, 2018). Spectrophotometric assays are valuable for comparative analysis across cropping treatments, particularly when evaluating the impact of biointensive complementary systems on overall antioxidant enrichment. However, they should ideally be complemented by chromatographic methods for detailed characterization.

7.4 Metabolomics and omics approaches

Metabolomics represents a transformative advancement in phytochemical assessment. Unlike targeted approaches that focus on specific compounds, metabolomics aims to profile the complete set of metabolites within a biological sample. Using high-resolution LC-MS, GC-MS, or nuclear magnetic resonance (NMR) spectroscopy, metabolomics enables comprehensive evaluation of metabolic alterations induced by cropping systems. Untargeted metabolomics identifies patterns and biomarkers associated with ecological conditions, while targeted metabolomics quantifies specific metabolites of interest. Data analysis involves multivariate statistical tools such as principal component analysis and hierarchical clustering to distinguish treatment effects. Integration of metabolomics with other omics disciplines transcriptomics, proteomics and genomics facilitates systems-level understanding of metabolic regulation (Ahmed *et al.*, 2019). For example, linking gene expression data with metabolite profiles can reveal how intercropping influences phenylpropanoid pathway activation. Such integrative approaches are essential for unraveling the complex mechanisms underlying phytochemical modulation in biointensive systems.

8. Constraints and challenges

Despite promising evidence regarding phytochemical enhancement in complementary cropping systems, several constraints hinder widespread adoption and scientific validation. Standardization issues pose a major challenge. Variability in extraction methods, analytical protocols and reporting units complicates cross-study comparisons. Differences in sampling stage, plant part analyzed and post-harvest handling further influence phytochemical measurements. Establishing standardized protocols is crucial for generating reliable datasets. Variability in phytochemical expression presents another limitation. Secondary metabolite production is influenced by genotype, environmental conditions, soil fertility and management practices. Seasonal fluctuations and microclimatic differences within garden systems can result in inconsistent outcomes. Long-term and multi-location studies are necessary to account for this variability. Market limitations also constrain expansion of nutraceutical-oriented cropping systems. While consumer demand for functional foods is increasing, smallholder farmers may lack access to value-added processing, certification and marketing channels. Without economic incentives, adoption of diversified systems aimed at enhancing phytochemical quality may remain limited. Lack of policy support further impedes progress. Agricultural policies often prioritize yield and commodity production rather than nutritional quality. Incentives for biodiversity conservation, organic inputs and nutraceutical crop cultivation are insufficient in many regions. Strengthening policy frameworks to support agroecological and nutrition-sensitive agriculture is essential for scaling biointensive systems.

9. Future research directions

Future research should adopt omics-based systems biology approaches to deepen understanding of cropping system metabolism interactions. Integrating metabolomics with transcriptomic and proteomic analyses can elucidate regulatory networks controlling phytochemical biosynthesis. Such knowledge will enable targeted optimization of cropping designs for enhanced nutraceutical outcomes. Breeding programs should incorporate phytochemical traits as selection criteria. Developing crop varieties responsive to complementary cropping environments and capable of high bioactive accumulation will strengthen nutraceutical agriculture. Participatory breeding involving smallholder farmers may ensure adaptability to garden-land contexts. Climate-resilient nutraceutical agriculture represents another priority. Investigating how diversified systems buffer climate stress while maintaining phytochemical quality is critical under changing environmental conditions. Research should focus on adaptive species combinations, stress-tolerant varieties and sustainable soil management strategies. Integration with the functional food industry offers significant opportunities. Establishing value chains linking biointensive production systems with processing industries can enhance farmer income and consumer access to nutraceutical products. Certification schemes emphasizing biodiversity-based cultivation may further promote market recognition.

10. Conclusion

Biointensive complementary cropping systems in garden-land ecosystems represent a holistic strategy for enhancing phytochemical diversity and nutraceutical quality. Through ecological interactions involving plant diversity, soil microbiomes, moderate stress induction and organic nutrient cycling, these systems stimulate the biosynthesis of phenolics, terpenoids, alkaloids, carotenoids and other bioactive compounds. Analytical advances from spectrophotometric assays to metabolomics have enabled detailed assessment of these enhancements, confirming the potential of diversified systems to produce health-promoting foods. Beyond crop quality, biointensive systems deliver multiple ecosystem services, including biodiversity conservation, soil health improvement, carbon sequestration and pest regulation. By integrating ecological intensification with nutrition-sensitive objectives, these systems bridge the gap between sustainable agriculture and public health. In the context of rising chronic diseases, climate challenges and declining biodiversity, aligning cropping system design with phytochemical enrichment offers a transformative pathway. Biointensive complementary cropping in garden lands exemplifies how biodiversity-driven agriculture can support sustainable nutraceutical production while fostering resilient and health-oriented food systems.

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Conflict of interest

The authors declare no conflicts of interest relevant to this article.

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